2020
SID Annual Meeting Virtual Conference
MAY 13-16, 2020

Abstract Booklet

Thank you to those who submitted abstracts to the SID 2020 Annual Meeting. With the cancellation of the SID Annual Meeting originally scheduled for May 13 - 16, 2020, in Scottsdale, the meeting has been converted to provide select content in a virtual setting.

Abstract presenting authors were contacted to confirm their intent to publish their accepted abstract. On the following pages is a listing of the abstracts that have confirmed publication (as of May 8, 2020). Abstracts will be published in the July 2020 Supplement of the JID.

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*Category Name Amended (February 2020)

2020 SID Annual Meeting Virtual Conference

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Abstract Booklet

Adaptive and Auto-Immunity

001

Lymph node-fibroblastic reticular cells regulate differentiation of CD4 T cells through CD25

D. Kim[1], T. Kim[1], S. Lee[1], K. Shin[1, 2, 3]

[1]Biomedical Research Center, KAIST Institute for the Bio-Century, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea (the Republic of), [2]Department of Dermatology, Pusan National University Yangsan Hospital, Yangsan, Korea (the Republic of), [3]Department of Dermatology, Pusan National University Hospital, Busan, Korea (the Republic of)

Lymph node-fibroblastic reticular cells (LN-FRCs) not only provide functional structure to LNs, they also play an important role in the interaction between T cells and antigen-presenting cells. However, their role in the differentiation of naive CD4 T cells remains unclear. Here, we report that of the three distinct subunits of the IL-2 receptor (IL-2R), LN-FRCs express IL-2R alpha chain, CD25, only. LNFRCs provide naive CD4 T cells with IL-2 in a trans-presentation manner through CD25, thereby facilitating early IL-2-mediated signaling and regulating CD4 T cell differentiation. CD25-deficient FRCs exhibited attenuated phosphorylation of STAT5 due to diminished IL-2 signaling in naive CD4 T cells. CD25-deficient FRCs promoted Th17 cell differentiation and induced expression of genes involved in Th17-mediated immune responses. Hence, Th17 cell-mediated inflammatory disease was markedly enhanced in mice lacking CD25 on FRCs. Therefore, our results suggest that CD25 expression on FRCs regulates CD4 T cell differentiation by modulating early IL-2-mediated signaling of neighboring, naive CD4 T cells, and thus influences the overall character of the immune response.

003

Dextran-based acitretin nanoparticle ameliorates imiquimod-induced psoriasis-like skin inflammation

J. Lan[1], H. Du[1], Y. Li[2], Y. Zhang[1], J. Tao[1]

[1]Dermatology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (HUST), Wuhan, China., Wuhan, Hubei, China, [2]School of Chemistry and Chemical Engineering, Huazhong University of Science and Technology, Wuhan, China

Acitretin (Act) is one of the first-line treatments for moderate to severe psoriasis, while the systemic side effects caused by Act greatly restrict its clinical application. Herein, we developed the Dextran-based Act nanoparticles (DANPs) and proved their enhanced therapeutic effects in the mice models of imiquimod-induced psoriasis. Dextran and Act molecules were linked by ester bonds to form a spherical nanostructure, and extra free Act molecules were encapsulated. When injected *in vivo*, free Act will be soon released while the slow dissociation of linked Act will help keep an effective blood concentration for a long time. We treated the psoriasis mice with dextran, acitretin and DANPs by daily intraperitoneal injection for 6 days. Then, the skin lesions were evaluated by Psoriasis Area and Severity Index (PASI) scoring, H&E staining and immunohistochemical staining. Our results *in vivo* showed a significant reduction of PASI score and epidermal thickness after DANPs (1mg/kg/day) treatment , Ki-67 expression in keratinocytes also decreased (P<0.01). While the psoriasis mice treated with acitretin (1mg/kg/day) or dextran (1mg/kg/day) showed no obvious improvement in skin inflammation. *In vitro* study showed that the uptake of DANPs by HaCaT cells increased gradually with incubation time and DANPs concentration, reaching a maximum at 4h. Furthermore, we compared the *in vitro* effect of dextran, acitretin and DANPs on HaCaT cells. Compared to dextran and acitretin groups, DANPs showed more significant cytotoxicity to HaCaT cells (P<0.01) and induced more significant apoptosis of HaCaT cells (P<0.01). Overall, our work provided a new choice for psoriasis systemic treatment, with enhanced therapeutic efficiency and reduced the side effects.

004

Localized administration of methotrexate regulates psoriasis-like skin inflammation and protects from secondary sensitization at a distant site

H. Du[1], J. Lan[1], P. Liu[2], J. Zhu[2], J. Tao[1]

[1]Department of Dermatology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China, [2]School of Chemistry and Chemical Engineering, Huazhong University of Science and Technology, Wuhan, Hubei, China

Methotrexate (MTX), a first-line systemic agent for the therapy of psoriasis, is often associated with adverse effects. Localized transdermal delivery of MTX using dissolving microneedles (MNs) was demonstrated to be both more efficacious and better tolerated than oral administration in our previous work. This study aims to elucidate the detailed therapeutic mechanism of MTX-loaded MNs and to explore whether topical MTX treatment can affect the formation of skin lesion at distant parts of the body while attenuating local inflammation. In vitro experiments suggested that MTX effectively reduced the production of proinflammatory cytokines in IL-17A-treated HaCaT cells and imiquimod (IMQ)-treated mouse bone marrow-derived dendritic cells (P<0.001). Psoriasiform dermatitis was then induced on the left ear skin of mice by topical IMQ application. Histological and immunohistochemical (Ki67, CD11c and CD3) examinations showed much milder epidermal hyperplasia and inflammatory infiltration upon MTX-loaded MNs treatment. We also found that MTX-loaded MNs substantially downregulated leukocyte chemotaxis and migration, T cell proliferation and activation as well as the development and keratinization of epidermis by RNA sequencing. Additionally, flow cytometric analysis indicated that the accumulation of IL-17A-producing $V\gamma4^+$ γδT cells in MNs-treated left ear skin and the draining lymph nodes were both significantly inhibited (P<0.001). Furthermore, the subsequent generation of psoriatic phenotype on the right ears by IMQ rechallenge was markedly restrained compared with untreated mice. In conclusion, MTX-loaded MNs can not only ameliorate local skin inflammation by regulating immune and stromal components but also show a concomitant antipsoriatic effect against recurrent immune disorders at a distant site, emerging as a potential alternative topical treatment for psoriasis.

005

Respiratory activity in psoriatic circulating T cells predicts the efficacy of apremilast

H. Koguchi-Yoshioka[1, 2], R. Watanabe[2], Y. Matsumura[2], T. Matsuzaka[3], H. Shimano[3], M. Fujimoto[1, 2]

[1]Dermatology , Osaka University, Suita, Osaka, Japan, [2]Dermatology, University of Tsukuba, Tsukuba, Japan, [3]Internal Medicine, Metabolism and Endocrinology, University of Tsukuba, Tsukuba, Japan

While psoriasis is a Th17-mediated disease related with metabolic skews, the relation between T-cell reactivity and cellular metabolism has not been fully evaluated in psoriasis. In this research, the metabolic condition of blood T cells were evaluated in 35 psoriatic patients by use of an extracellular flux analyzer. The impact of apremilast on cellular metabolic condition was also investigated in total 71 patients with psoriasis both *in vivo* and *in vitro*. The oxygen consumption rate (OCR) was significantly higher in blood T cells of psoriasis patients compared to healthy controls (p = 0.0014), and the patients with higher OCR achieved higher % PASI improvement by apremilast (r = 0.6182, p = 0.0478). The increase of cAMP is regarded to upregulate the mitochondrial activity via activation of protein kinase A. However, apremilast paradoxically decreased the T-cell OCR (p = 0.0322). The decrease of OCR was presumably attributed to the reactive oxygen species (ROS) because the cellular ROS level was higher in psoriatic blood T cells compared to healthy controls (p = 0.0022) and the *in vitro* inhibition or elimination of ROS led to the decline of cellular OCR. The superoxide dismutase was also upregulated by co-culturing the psoriatic blood T cells with apremilast (p = 0.0313). Of note, the T-cell OCR showed a mild correlation with the serum level of LDH in the patients with psoriasis (r = 0.3454, p = 0.0454), and the patients with higher serum LDH levels tended to be benefitted more by apremilast (r = 0.4602, p = 0.0206). Our results indicate that the circulating T cells in psoriatic patients have higher mitochondrial activities with more oxygen consumption and ROS production. Our results also suggest that the OCR and possibly the serum LDH levels can be a predictor of treatment preference.

006

The association of platelet activation markers, neutrophil extracellular traps and anti-mitochondrial autoantibodies with cutaneous manifestations in Systemic Lupus Erythematosus

L. Mainville[1], I. Melki[1], Y. Becker[1], A. Julien[2], N. Cloutier[1], E. Rollet-Labelle[1], C. Lood[3], É. Boilard[1], P. Fortin[1]

[1]Centre de recherche du CHU de Québec – Université Laval, Quebec, Quebec, Canada, [2]Département de mathématiques et statistique, Université Laval, Quebec, Quebec, Canada, [3]University of Washington, Seattle, Washington, United States

Systemic Lupus Erythematosus (SLE) is an autoimmune disease associated with platelet activation, formation of neutrophil extracellular traps (NETs) and development of anti-mitochondrial autoantibodies (AMA). In the current study, we investigated the clinical utility of these markers and autoantibodies in cutaneous lupus manifestations. Clinical data on cutaneous manifestations were obtained in a large SLE cohort (n=73) through review of SLE questionnaires, SLICC Classification Criteria and revised ACR Criteria for SLE. Three main outcome measures; cutaneous manifestations reported by physicians, by patients, and additional lupus manifestations, were constructed to analyse their association with the studied markers. In logistic regression analyses, NETs and antibodies to La/SSB were protective against “cutaneous manifestations reported by patients” ($0.02 \leq P \leq 0.04$) due to their effect on “rash other than on cheeks”. No statistically significant association was found between predictor variables and “cutaneous manifestations reported by physicians”. IgG-bound platelets were associated with “additional cutaneous manifestations” due to the periungual erythema item ($0.01 \leq P \leq 0.02$). Based upon these findings, we suspect that “rash other than on cheeks” is of limited value to discriminate cutaneous manifestations of SLE. We therefore reanalyzed the data excluding non-specific cutaneous manifestations of SLE but were not able to find any further significant results. Our results demonstrate no significant association between our predictors and specific SLE cutaneous manifestations, as our definitions for skin manifestations were crude. This work will lead us to implement better physician’s evaluation of skin lesions in lupus, including CLASI, and information derived from skin biopsies.

007

Indoleamine 2,3-dioxygenase 2 knockout exacerbates imiquimod-induced psoriasis-like skin inflammation

K. Fujii[1], Y. Yamamoto[2], K. Saito[2], M. Seishima[1]

[1]Dermatology, Gifu University Graduate School of Medicine, Gifu, Japan, [2]Disease Control and Prevention, Fujita Health University Graduate School of Health Sciences, Toyoake, Japan

Psoriasis is a common chronic autoimmune disease and the pathogenesis is reported to be due to the activation of the interleukin-23/interleukin-17 (IL-23/IL-17) pathway. Indoleamine 2,3-dioxygenase 1 (IDO1) is known to be an inducing enzyme that suppresses immune responses and there are several reports showing the association with psoriasis. On the other hand, IDO2 is an isoform of IDOs, recently identified catalytic enzyme in the tryptophan-kynurenine pathway. Recent studies reported that IDO2 is expressed in dendritic cells and monocytes. The expression of IDO2 in immune cells suggests that IDO2 contributes to immune function. However, the role of IDO2 in the pathogenesis of psoriasis remains unclear. In this study, to elucidate the role of IDO2 in psoriasis, we assessed imiquimod-induced psoriasis-like dermatitis in IDO2 knockout (KO) mice. Wild-type (WT) and IDO2 KO female mice at 8–10 weeks old received a daily topical dose of 62.5 mg commercially available imiquimod cream on ears for 7 consecutive days. Skin inflammation in IDO2 mice evaluated with erythema, scaling and ear thickness was significantly worse than that in WT mice. In addition, we measured cytokine production by real-time PCR. The mRNA expression levels of TNF-α, IL-23p19 and IL-17A, key cytokines involved in the development of psoriasis, were increased in IDO2 KO mice than in WT mice on day 7. These results suggest that IDO2 suppresses the skin inflammation in imiquimod-induced psoriasis-like dermatitis.

008

Genome-wide DNA methylation analysis in lupus keratinocytes identifies differential methylation of genes that regulate apoptosis

G. Hile[1], P. Coit[1], C. Zeng[1], R. Wasikowski[1], A. Tsoi[1], A. C. Billi[1], J. E. Gudjonsson[1], A. Sawalha[2], J. Kahlenberg[3]

[1]Dermatology, University of Michigan, Ann Arbor, Michigan, United States, [2]Rheumatology, Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania, United States, [3]Rheumatology, University of Michigan, Ann Arbor, Michigan, United States

Cutaneous lupus erythematous (CLE) is a disfiguring manifestation of systemic LE (SLE). The pathophysiology of CLE is unclear. However, regulation of skin inflammation and apoptosis contribute, potentially in a female-biased manner. Differential DNA methylation is important in the organ-specific manifestations of SLE but has not been studied in skin. Thus, we explored the genome-wide DNA methylation changes in keratinocytes (KC) to investigate the functional relevance in CLE. Analysis of cultured KC (at passage 2) from 8 patients with SLE and 8 age, sex, and ethnicity matched controls was performed using the Infinium MethylationEPIC array and investigated biological significance using DAVID database. We identified 1443 differentially methylated sites with 924 hypomethylated genes and 519 hypermethylated genes in lupus KC compared to controls. The top canonical pathway was Hippo signaling, which is key in promoting apoptosis. TEA Domain Transcription Factor 1 (TEAD1), a transcription factor in the Hippo pathway, was significantly hypomethylated in lupus compared to control ($\Delta\beta$ =-0.17, P= 4.36 X 10^{-9}), which could promote cell death. Further, methylation of LATS1/2, which inactivates TAZ and YAP, members of the Hippo signaling pathway, was significantly increased ($\Delta\beta$= 0.11, P= 3.82 X 10^{-4} possibly leading to increased YAP/TAZ inhibition and increased apoptosis. YAP and TAZ were both hypermethylated ($\Delta\beta$= 0.11 (P= 1.53 X 10^{-3}) and 0.12 (P= 2.20 X 10^{-4})), thereby increasing TEAD1 activity. Vestigial like family member 3 (VGLL3) has been identified as a putative transcription factor and orchestrator of sex bias in autoimmune diseases. Importantly, VGLL3 operates through TEAD1 in other organs, and may be an upstream regulator in the skin. Overall, these results suggest that differential methylation in KC may underly dysregulated apoptosis and female bias of CLE.

009

Psoriasis patient serum biomarkers may be useful for separating response to therapy endotypes

S. Chu[1], K. P. Schrom[2], B. Richardson[1], M. Cameron[1], T. McCormick[1], K. Cooper[2]

[1]Case Western Reserve University, Cleveland, Ohio, United States, [2]University Hospitals Cleveland Medical Center, Cleveland, Ohio, United States

Identification of individual psoriasis patient characteristics, or groups of patients that share similar phenotypic traits (also referred to as endotypes), will be a critical step toward advancing precision (personalized) medicine. The psoriasis Center of Research Translation (CORT) has initiated an in-depth analysis of individual psoriasis patients with the aim of identifying various psoriasis patient endotypes that can be applied to predictive analytics for development of more advanced treatment and treatment algorithms for psoriasis patients. Transcriptomic analyses were performed on whole blood (Paxgene) from psoriasis patients and healthy controls (N= 79, N=16, respectively). In order to identify potential psoriasis endotypes, a UMAP driven multi-modal model was generated by combining the whole blood gene signatures of psoriasis and psoriatic arthritis patients and the signatures of the regressions of PASI, age, and the percentage of circulating intermediate monocytes. Using this method 6 distinct endotypic groups of psoriasis patients were identified. A further refinement using a Pathway/hierarchical-based definition identified 4 unique endotypes. Patient treatment and response were recorded in the case report form for all CORT psoriasis patients. Examination of ustekinumab treated patients revealed that of the four unique endotypes identified by hierarchical clustering only three contained ustekinumab treated patients. Eleven patients were previously treated with ustekinumab, four of whom discontinued treatment due to inadequate response. Five of the eleven patients who previously tried ustekinumab were started on guselkumab, achieving clear/almost clear results. Among Responders, hs-CRP values were significantly lower (median 1.5 vs. 7.1, p=0.0381) compared to Non-responding patients, suggesting that serum protein values may be useful as potential biomarkers for separating psoriasis patient endotypes.

010

Correlation of psoriasis severity with burden of disease cost in psoriatic patients

S. Chu[1], G. Damiani[1], B. Richardson[1], X. Gao[1], M. Cameron[1], T. McCormick[1], K. Cooper[2]

[1]Case Western Reserve University, Cleveland, Ohio, United States, [2]University Hospitals Cleveland Medical Center, Cleveland, Ohio, United States

Access to an electronic data warehouse (EDW) of psoriasis patient data is a rich source of information for big data analytics. University Hospitals Cleveland Medical Center (UHCMC) employs a central data archive obtained from various primary sources (inpatient, outpatient, billing etc…). Psoriasis is increasingly being regarded as a systemic inflammatory disorder with various comorbid diseases associated with a high burden of disease. We examined whether or not severe psoriasis patients have a greater associated cost-of-illness and determined if any unique patient cohorts (endotype) could be associated with psoriatic patients with differing burden rates. An EDW query was used to identify psoriasis patients who have seen providers at UHCMC and associated hospitals and clinics. The EDW pulls data from EMR billing systems and scheduling systems and includes data such as charges, diagnoses, medications, patient demographics, payer information, lab results, and provider orders. Charge data from 52 psoriasis patients was analyzed and separated into four categories: procedures, ambulatory, testing, and hospitalizations. Extensive transcriptomic (blood), flow cytometry, and clinical information were collected from these patients as part of their participation in the Psoriasis Center of Research Translation (CORT). Total cost per patient was significantly correlated (r=0.35, p=0.0115) with the percentage of circulating proinflammatory intermediate monocytes. In addition, total costs were also significantly correlated with the number of therapies patients failed (r=0.378, p=0.0056) and total burden of disease (r=0.394, p=0.0035). Thus, total charges may accurately reflect correlative biomarkers with psoriasis severity.

013

Fcγ receptor IIb-dependent blockade of immunoglobulin class-switch is crucial to prevent pemphigus onset in desmoglein 3-sepecific B cell receptor knock-in mouse

H. Nomura[1], N. Wada[1], H. Takahashi[1], S. Egami[1, 2], J. Yamagami[1], T. Watanabe[2], O. Ohara[2], S. Koyasu[2], M. Amagai[1, 2]

[1]Dermatology, Keio University School of Medicine, Tokyo, Tokyo, Japan, [2]RIKEN Center for Integrative Medical Sciences, Yokohama City, Kanagawa, Japan

B cell tolerance is crucial for regulating harmful autoantibody production. Representative B cell tolerances such as anergy and receptor editing are achieved by modifying or utilizing crucial processes of fundamental B cell activities, such as B cell receptor activation and gene rearrangement, respectively, to avoid autoimmunity. However, it remains unclear whether class switch recombination (CSR) is associated with tolerance mechanism. Pemphigus vulgaris (PV) is an autoimmune bullous disease caused by anti-desmoglein (Dsg) 3 IgG. We previously isolated pathogenic anti-Dsg3 IgG monoclonal antibody, AK23, that caused acantholysis *in vivo*, but IgM form of AK23 couldn't, suggesting importance of Ig subclass in PV pathogenesis. To elucidate mechanism regulating Dsg3-specific B cell, we generated AK23, knock-in (KI) mice that allow CSR *in vivo*. Each stage of KI B cell development to $IgM^{+}CD21^{int}CD93^{-}$ follicular B cell was normally observed in bone marrow and spleen of KI mice. However, KI mice produced only AK23 IgM and no IgG, and did not develop PV phenotype. In contrast, immunization with Dsg3, but not with vehicle, KI mice produced AK23 IgG and developed PV phenotype after CSR. Comparison analysis of $CD19^{+}$ B cell transcriptome between pemphigus patients and healthy controls showed that not only *FCGR2B* expression, but also its signaling pathway were attenuated in pemphigus patients. To imitate patients' immunological condition, AK23KI mice were crossed with *FcγrIIb*$^{-/-}$ mice. There was no difference in amount of serum total IgG between *FcγRIIb*$^{-/-}$ and WT mice at the age from 6 to 16 weeks. However, *FcγrIIb*$^{-/-}$-AK23KI mice spontaneously produced anti-Dsg3 IgG from early age afte 6 weeks and developed PV phenotype. The results demonstrated that pathogenic autoantibody production is prevented at CSR level and FcγRIIb crucially maintains this tolerogenic condition to avoid autoimmunity.

014

IL-27 induces IL-15 production to facilitate T cell survival in allergic contact dermatitis

J. Suwanpradid[1], M. Lee[1], P. Hoang[1], J. Kwock[1], L. Floyd[1], J. Smith[1], Z. Yin[2], A. Atwater[1], S. Rajagopal[1], R. Kedl[3], D. Corcoran[1], A. S. MacLeod[1]

[1]Duke University, Durham, North Carolina, United States, [2]Jinan University, Guangzhou, China, [3]University of Colorado, Aurora, Colorado, United States

Re-exposure to an allergen (Ag) initiates the efferent phase and clinical expression of allergic contact dermatitis (ACD) characterized by recruitment of Ag-specific T cells to the sites of Ag challenge. We observed that, in contrast to non-lesional skin of ACD patients, skin of (+) patch-tested individuals showed high expression of IL-15 in keratinocytes, and in monocyte/macrophage (MAC) within dermal hair follicle (HF)-associated immune cell clusters. Within these clusters, T cells with high BCL2 interacted with $CD14^{+}$ cells co-expressing IL-15 and iNOS and/or CD86 and IL-27. Moreover, in the murine allergic contact hypersensitivity (CHS) model using IL-$27p28^{eGFP}$ mice, we found increased IL-27 production in $CD172a^{+}$MAC. Functionally, IL-27p28fl/fl;LysM-cre mice demonstrated less DNFB-induced ear thickening and dermal HF-associated CD8 T cells compared to WT mice ($p < 0.05$), supporting a functional role for IL-27 in CHS. Mechanistically, IL-27 stimulated *IL15* in a STAT-1 dependent manner as evidenced by rapid p-STAT1 nuclear translocation and abrogation of this response by silencing STAT1 ($p < 0.05$). Given the relevance of these Ag-induced dermal T cell clusters for CHS responses, we tested the functional importance of IL-27-induced IL-15 signaling. In the CHS mouse model, administration of IL-27-neutralizing antibody (NAB) resulted in decreased *IL-15* expression associated with downregulation of BCL2 in T cells and overall decreased cutaneous CD8+ T cell numbers and T cell clusters ($p < 0.05$). The reduction of BCL2 in T cells was also found in CHS-mice treated with IL-15 NAB. Similarly, when treated with rhIL-15, human skin T cells increased BCL2 expression ($p < 0.05$). The functional relevance of IL-27 and IL-15 is further supported by the finding that IL-27 NAB-induced CHS suppression as shown by ear thickening was overcomed by administering IL-15 complex ($p \leq 0.05$). Overall, our findings implicate IL-27 as a potential therapeutic target in regulating cutaneous T cell immunity.

015

Interleukin-27 alleviates psoriatic inflammation by suppressing Interleukin-17A production from T17 cells

Z. Chen, Y. Shi

Tongji University School of Medicine, Irvine, California, United States

Interleukin (IL)-27 has been widely reported to inhibit IL-17A production in many diseases. Psoriasis is a IL-17A-related autoimmune skin disease and IL-17A antagonists show excellent efficacy in treating psoriasis. However, the role of IL-27 in psoriasis remains controversial. In this study, we measured the effect of IL-27 on the IL-17A production from αβT cells and γδT cells and used IL-27 receptor A deficiency (*Il27ra*$^{-/-}$) mice to determine the role of IL-27 in psoriasis. The expression of IL-27 in psoriasis was determined using immunofluorescence staining. The expression of IL-17A in T cells was determined using flow cytometry and enzyme-linked immunosorbent assay. Imiquimod (IMQ)-induced psoriasis model was used to investigate the role of IL-27 in psoriasis in vivo. Our results show that IL-27 is expressed in both epidermis and dermis. IL-27 expression is significantly decreased in the lesional skin of psoriasis patients in both epidermis and dermis. IL-27 downregulates IL-23-induced IL-17A expression in αβT cells and T cell receptor (TCR)-agonist-induced IL-17A expression in γδT cells from skin-draining lymph nodes. Supernatant level of IL-17A is also decreased in cultured skin-draining lymph nodes cells treated with IL-27. IL-27 also inhibits IL-17A production by peripheral blood mononuclear cells (PBMCs) from psoriasis patients. Deficiency of IL-27 signaling exacerbates IMQ-induced psoriatic inflammation. Epidermal thickness and dermal infiltrating γδT cells are increased in *Il27ra*$^{-/-}$ mice. The proportions of Th17 cells and IL-17A-producing γδT cells are up-regulated in the skin-draining lymph nodes of *Il27ra*$^{-/-}$ mice. In conclusion, IL-27 plays an anti-inflammatory role in psoriasis by suppressing IL-17A production.

017

Inflammatory monocyte-derived dendritic cells mediate autoimmunity in murine model of systemic lupus erythematosus

F. Miyagawa, H. Asada

Dermatology, Nara Medical University, Kashihara, Nara, Japan

Using a mouse model of systemic lupus erythematosus (SLE) induced by 2,6,10,14-tetramethylpentadecane (TMPD), we recently demonstrated that IRF7-deficient mice developed glomerulonephritis but failed to develop autoantibody production. We suggested that the two major manifestations of SLE are mechanistically independent because the type I interferon (IFN) pathway leads to the autoantibody production whereas the NF-kB activation is sufficient for the development of glomerulonephritis. To further advance our understandings on the molecular pathways regulating the development of SLE, we studied the role of IRF8 because it controls both type I IFN and NF-kB pathways and saw that IRF8-deficient mice failed to develop either glomerulonephritis or the autoantibody production. Furthermore, these genetically engineered mice prompted us to realize the important role of Ly6C^{high} inflammatory monocytes in the development of SLE. These monocytes migrate to the peritoneal cavity in WT and IRF7-deficient mice but not in IRF8-deficient mice, and there they produce both type I IFN and proinflammatory cytokines in WT mice, while in IRF7-deficient mice they only produce proinflammatory cytokines. Upon migration to the spleen, Ly6C^{high} inflammatory monocytes differentiate into dendritic cells (DCs) which are capable of producing proinflammatory cytokines in response to dsDNA autoantigen. Collectively, type I IFN produced from inflammatory monocytes/monocyte-derived DCs might be essential for autoantibody production whereas proinflammatory cytokines produced from them might mediate tissue damages in this model. Our study reveals a specialized role for monocyte-derived antigen presenting cells in autoimmunity. Plasticity of monocyte might play an important role not only in the pathogenesis of the disease but also in flare-ups of the disease.

023

Circulating serum amyloid A levels correlate with the severity of generalized pustular psoriasis

N. Yu[1, 2]

[1]Shanghai Skin Disease Hospital, Shanghai, China, [2]Institute of Psoriasis of Tongji University, Shanghai, China

Generalized pustular psoriasis (GPP) is a severe and rare variant of psoriasis, which presents as widespread sterile pustules on erythematous skin and is associated with systemic symptoms. Besides the cutaneous manifestations, the severity of GPP has also been evaluated using blood tests, such as neutrophil count and C-reactive protein (CRP) levels. Serum amyloid A (SAA) is one of the most prominent positive acute phase proteins, which is highly elevated in serum due to systemic inflammation. Here, we measured the levels of circulating SAA in patients with GPP and psoriasis vulgaris (PV) as well as healthy controls, and assessed its correlations with inflammatory markers like blood neutrophil count and CRP levels. Sera were obtained from 25 patients with GPP (17 males and 8 females) ranging from 16 to 69 years old (mean = 45.9), 40 patients with PV (28 males and 12 females, mean = 51.2, PASI score < 10), and 38 healthy controls (22 males and 16 females, mean = 48.8). Serum SAA levels were evaluated by ELISA (Human Serum Amyloid A1 DuoSet ELISA, R&D systems, Minneapolis, MN, USA). The serum CRP levels were measured by immunoturbidimetry [CRP (latex) High-Sensitivity, Roche Pharma & Diagnostics, Shanghai, China]. The mean levels of serum SAA in GPP and PV patients were significantly higher than healthy control subjects (764.03 ± 146.28 ng/mL, 191.14 ± 208.53 ng/mL vs. 83.85 ± 95.12 ng/mL), while the difference between GPP and PV groups was also significant. As for the correlation between SAA levels and markers for disease severity in patients with GPP, we observed that SAA presented a positive correlation with neutrophil count (r = 0.43, P = 0.03) and CRP levels (r = 0.40, P = 0.04). In summary, we described the elevation of circulating SAA levels in patients with GPP, and serum SAA levels might reflect the clinical severity of GPP, though the findings of this study should be confirmed in a prospective study of a larger number of patients.

025

Extracellular vesicles induce STING-mediated proinflammatory cytokines in Dermatomyositis

Y. Li, C. Bax, M. Bashir, K. Desai, M. Zeidi, V. Werth

University of Pennsylvania/VAMC, Philadelphia, Pennsylvania, United States

Dermatomyositis (DM) is an acquired inflammatory myopathy characterized by chronic skin inflammation. The pathogenesis of DM is still unclear. Extracellular vesicles (EVs) are lipid bilayer membrane vesicles existing in various bodily fluids and implicated in the pathogenesis of autoimmune diseases. As type I interferons, specifically IFN-b, are uniquely elevated in DM, and Stimulator of interferon genes (STING) works as a critical sensor and adaptor in type I IFN signaling, we hypothesized that EVs derived from DM patients' plasma might trigger STING-mediated proinflammatory effects. DM patients were recruited in the dermatology clinic at U Penn. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll gradient. EVs derived from plasma were isolated via ultracentrifugation. The supernatant was harvested for ELISA and the lysed cells were collected for Western blot after HC derived PBMCs were stimulated by EVs. We found that DM patients' plasma derived EVs triggered cytokines release (IFNb: (30.24±0.65) vs control (2.683±0.35); TNFα: (1451±98.40) vs control (16.75±1.407)pg/mL; n=6) with STING phosphorylation. Inhibition of STING significantly attenuated DM patients' plasma derived EVs-triggered cytokines production (IFNb: (21.58±2.22) vs (28.34±1.73); TNFα: (434.8±94.50) vs (919.1±133.0)pg/mL; n=6) via suppressing STING and its down-stream signal TBK1, IRF3, and NFκB phosphorylation. To further explore whether STING phosphorylation and the proinflammatory effects were caused by EVs-captured DNA, EVs were pretreated with Triton X-100 and DNase to digest DNA. Triton X-100 and DNase pretreatment decreased EVs-triggered cytokines release (IFNb: (4.113±2.08) vs (28.94±5.47); TNFα: (290.3±57.03) vs (1361±293.6)pg/mL; n=3-6) and STING activation. Thus we found EVs derived from plasma could trigger STING-mediated proinflammatory effects in DM. The STING phosphorylation during EVs triggering of proinflammatory effects was at least partially mediated by DNA captured by EVs. Targeting STING might provide insight into a potential therapeutic approach for DM.

026

Increased levels of high mobility group box-1 in the serum and skin in patients with generalized pustular psoriasis

T. Watanabe, Y. Yamaguchi, Y. Watanabe, N. Takamura, M. Aihara

Dermatology, Yokohama City University School of Medicine, Yokohama, Japan

High-mobility group box-1 (HMGB-1) is a highly abundant pro- inflammatory protein which is associated with the pathogenesis of inflammatory and autoimmune diseases, such as drug eruption, sepsis, and rheumatoid arthritis. HMGB-1 has a dual function: inside the cells, it plays a role in the transcriptional regulation. While outside the cells, it plays as an alarmin or a damage-associated molecular pattern. It has been reported that HMGB-1 expression levels of the serum and skin were increased in patients with psoriasis vulgaris (PV). However, HMGB-1 expression in patient with generalized pustular psoriasis (GPP) was unknown. In this study, we investigated the HMGB-1 levels in the serum and skin in GPP patient. To analyze the expression levels of HMGB-1, we performed ELISA and immunohistochemistry in the skin and serum obtained from patients with GPP, PV, atopic dermatitis (AD), and healthy controls (HC). Immunohistochemistry analysis revealed that HMGB-1 expression levels in epidermis were significantly increased in patients with GPP compared to that in patients with PV, AD and HC. In addition, GPP patients had elevated serum HMGB-1 levels compared to AD patients and HC. Furthermore, serum levels of HMGB-1 were significantly decreased after the systemic treatment compared to baseline levels. In the correlation analysis, a high positive correlation was detected between serum HMGB-1 levels and Japanese severity criteria for GPP in patients with GPP. In conclusion, our findings show that HGMB-1 might be involved in the pathogenesis of GPP and is a simple and attractive marker for the analysis of disease severity and the effectiveness of treatment in patients with GPP.

027

A pilot study of human salivary N- and O-glycan profiles in Bullous pemphigoid

X. Bai, L. Yang, E. Dang, G. Wang

Xijing Hospital, Fourth Military Medical University, Xi'an, China

Aberrant glycosylation is strongly correlated with the pathogenesis of various autoimmune diseases. However, the precise alterations of glycosylation remain largely unknown in Bullous pemphigoid (BP). Herein we aimed to evaluated changes of salivary N- and O-glycan profiles associated with BP. A total of 37 lectins were quantified in BP as compared to healthy donors by performing lectin microarray analysis. Among these quantified lectins, 9 and 13 lectins were up- or down-regulated in BP, respectively. The expression of 9 lectins increased by up to 1.5- to 3.3-fold in BP relative to that of controls, in which AAL, Jacalin and PNA showed significantly increased. Conversely, 13 lectins showed a 0.36- to 0.64-fold decrease in BP patients, in which the PTL-I, PWM, MAL-I, SNA, and PHA-E showed dramatically decreased NFIs. To facilitate the comparison of changes in salivary glycopatterns in BP, these differentially expressed lectins were classified into four categories according to their glycan specificity: (a) mucin type O-glycan recognizing lectins (MAL-I, SNA, PWM, PTL-I), (b) N-acetyllactosamine motif recognizing lectins T antigens (Jacalin and PNA), (c) fucose recognizing lectins (AAL), and (d) bi/tri/tetra antennary structure recognizing lectins (PHA-E). A decrease in O-glycosylation was observed in BP, on the other hand T antigen was diminished sharply for BP as compared to controls, suggesting biosynthesis of precursor of mucin-type O-glycan was activated in BP. Furthermore, we observed an increase in fucosylated saliva in patients with BP vs controls (for AAL) and a corresponding decrease of bisecting N-glycosylation (for PHA-E), which appeared to be associated with disease severity and activity in BP. In conclusion, we associated levels of saliva-glycosylation with BP compared to controls. These findings could increase our understanding mechanisms of BP pathogenesis and be used to develop diagnostics or guide treatment.

029

Transcriptome analysis suggests a role of IL-17-related genes in pemphigus

J. Holstein[1], F. Solimani[2], K. Meier[2], T. Tekath[3], K. Ghoreschi[2, 1]

[1]Department of Dermatology, University Medical Center Tübingen, Tübingen, Germany, [2]Department of Dermatology, Venereology and Allergology, Charité – Universitätsmedizin Berlin, Berlin, Germany, [3]Institute of Medical Informatics, University of Münster, Münster, Germany

Pemphigus is an autoimmune blistering skin disease characterized by autoantibodies (auto-ab) directed against the desmosomal proteins desmoglein (Dsg)1 and/or Dsg3. The exact pathogenesis of pemphigus is not fully understood. Some data suggest an association with Th2 immune responses, others with Th1 cells. More recently, IL-17 expression was also reported to be present in pemphigus skin. To better understand the immune signature of pemphigus, we performed transcriptome analysis of pemphigus skin. First, we performed a large scale analysis by whole transcriptome shotgun sequencing (RNA-seq) of six lesional pemphigus skin samples. We compared the data of lesional pemphigus skin to data obtained from healthy control skin samples (n=6). By biostatistics we identified an unexpected IL-17A-dominated immune signature in pemphigus skin with some similarities to psoriatic skin. KEGG pathway analysis revealed that the IL-17A pathway was upregulated in pemphigus with a positive feedback effect on functionally different genes varying from skin modelling genes, antimicrobial peptides as well as pro-inflammatory chemokines and cytokines. To confirm this initial data by conventional quantitative RT-PCR we analyzed lesional skin from 29 patients. In agreement with previous reports, we found some expression of IL-4 and IFN-g. Strikingly, we could confirm the RNA-seq data and detected high expression of IL-17A and associated mediators. In subsequent analysis, we compared the immune signature of lesional skin to perilesional skin and also the features of mucosal lesions compared to non-mucosal sites. Taken together, we found some expression of IL-4 and IFN-g, but dominant expression of certain mediators of the IL-17A pathway, strongly suggesting that pemphigus has a unique IL-17A-dominated immune signature that may be important for autoantibody formation and skin blistering at local tissue sites.

030

VGLL3, an orchestrator of female-biased autoimmunity, interfaces with the Hippo pathway to modulate genes involved in immunity and fibrosis

A. C. Billi[1], C. Zeng[1], M. Gharaee-Kermani[2], S. W. Stoll[1], M. J. Wilson[1], O. Plazyo[1], X. Xing[1], J. M. McCarthy[1], L. C. Tsoi[4, 5], J. Kahlenberg[2], A. A. Dlugosz[1, 3], J. E. Gudjonsson[1]

[1]Dermatology, University of Michigan, Ann Arbor, Michigan, United States, [2]Internal Medicine, Division of Rheumatology, University of Michigan, Ann Arbor, Michigan, United States, [3]Cell and Developmental Biology, University of Michigan, Ann Arbor, Michigan, United States, [4]Biostatistics, University of Michigan, Ann Arbor, Michigan, United States, [5]Computational Medicine & Bioinformatics, University of Michigan, Ann Arbor, Michigan, United States

Autoimmune disease is the leading cause of morbidity among women. For largely unknown reasons, many autoimmune diseases show a striking female bias. Fibrosis is a common feature of female-biased autoimmune diseases. This is exemplified by systemic sclerosis (SSc), a debilitating disease marked by progressive skin hardening and organ damage that affects women at ninefold the rate of men. We previously identified the transcriptional cofactor VGLL3 as an immune regulator enriched in female skin whose targets overlap significantly with genes dysregulated in SSc. We further showed that excess epidermal VGLL3 causes cutaneous and systemic autoimmune disease in mice. However, how VGLL3 promotes autoimmunity in the skin remains entirely unexplored. By combining IP-mass spectrometry, RNA-seq, and ChIP-seq approaches in cell culture and transgenic mice with epidermal VGLL3 overexpression, we have found that VGLL3 binds key factors in the Hippo signaling pathway to modulate both immune genes and established Hippo pathway targets. These targets include the pro-fibrotic factor CTGF and members of the TGF-β pathway, both of which have been implicated in SSc pathogenesis. Consistent with this, transgenic mice with epidermal VGLL3 overexpression show gross and microscopic features of skin fibrosis. These findings elucidate the molecular mechanisms by which VGLL3 promotes autoimmunity and leads to the hallmark fibrosis of many autoimmune diseases such as SSc. Furthermore, this reveals a previously unexplored connection between autoimmune disease and the Hippo signaling pathway, which has recently been linked to organ fibrosis.

031

Enhancement of Th2 cell differentiation by TRIM32 deficiency is negatively associated with PKCζ

Y. Liu[1], A. Yoo[1], R. De La Torre[1], C. Topham[1], J. Hanifin[1], E. Simpson[1], R. Messing[2], M. Kulesz-Martin[1], Z. Wang[1]

[1]Department of Dermatology, Oregon Health & Science University, Portland, Oregon, United States, [2]Department of Neurology, The University of Texas at Austin, Austin, Texas, United States

Atopic dermatitis (AD) is a chronic skin inflammatory disease characterized by skin barrier defects and the sustained activation of Th2-related inflammation. Despite the importance of Th2 activation in AD pathogenesis, the mechanism of Th2 activation in AD remains largely elusive. Tripartite motif-containing protein 32 (TRIM32) is an E3 ubiquitin ligase with innate antiviral activity. In our previous studies, we showed that *Trim32* null mice developed Th2 biased skin inflammation in response to imiquimod and associated low level of TRIM32 with AD. In this study, we provide evidence that TRIM32 deficiency contributes to enhanced Th2 cell differentiation *in vitro*. Analysis of TRIM32-associated proteins from public databases identified PKCζ as a TRIM32-associated protein that contributes to the regulation of Th2 signaling. We demonstrated that PKCζ was specifically ubiquitinated by TRIM32, and further, that the half-life of PKCζ was increased in the Th2 cells with *Trim32* null background. Furthermore, *Prkcz* null mice showed compromised AD-like phenotypes in the MC903 AD model. Consistently, the high PKCζ and low TRIM32 ratio were associated with $CD4^+$ cells in AD human skin and in Th2 cells differentiated *in vitro* from AD patients compared to healthy controls. Taken together, these findings suggest that TRIM32 functions as a regulator of PKCζ that controls the differentiation of Th2 cells important for AD pathogenesis. Because TRIM32 is an E3 ubiquitin ligase with innate antiviral activity, Th2 regulation by TRIM32 provides a potential connection between defective innate immunity and Th2 activation in AD pathogenesis.

034

Autoimmune blistering diseases accompanied with vitiligo

J. Wang, S. Zhou, M. Pan

Department of Dermatology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, Shanghai, China

Autoimmune blistering diseases (AIBDs), mainly including epidermolysis bullosa acquisita (EBA), pemphigus Vulgaris (PV) and bullous pemphigoid (BP), are a large class of autoimmune diseases presenting the blistering eruptions on the skin and mucosa membrane. Circulating autoantibodies play a critical role in the pathogenesis, antibody-specific B cells and CD4+T cells involved as well. While vitiligo, a common pigmentation disorder, is mostly considered as CD8+T cell-mediated, with multiple melanocyte-derived autoantibodies also detectable in part of the patients, although its pathogenicity remains undetermined. AIBDs concomitant with vitiligo have rarely been reported. We report 3 cases in our institution, highlighting to date the second case of EBA with vitiligo and the first case of vitiligo underlying PV, while in the literature the onset of AIBDs was mostly preceded by vitiligo. It is not clear whether the concomitant AIBDs and vitiligo may have some interaction in the pathogenic pathway or develop just as a mere chance occurrence. Some interesting correlation has been noticed in the onset, severity, and location of the two entities, which might indicate the interaction in their pathogenesis. We hypothesis that a probable undiscovered antigen-antibody crossover reaction or activation of auto-reaction after some component exposure caused by the cell destruction in the underlying diseases may explain the situation of the comorbidity. Genetic susceptibility may contribute to the occurrence of multiple autoimmune diseases in an individual while infection, trauma, and anxiety can act as a trigger. In a word, the clinicians should be aware of the possible coexistence of vitiligo and AIBDs, the inner immunopathologic interaction leaves more to explore.

035

Biogeographical differences in gene segment usage

S. Le[1], A. Marusina[1], A. Toussi[1], L. Downing[1], M. Tran[1], N. Maverakis[2], G. Luxardi[1], A. Tsoi[3], J. E. Gudjonsson[4], A. Merleev[1], E. Maverakis[1]

[1]Dermatology, UC Davis, Sacramento, California, United States, [2]New York University, New York, New York, United States, [3]Biostatistics, University of Michigan, Ann Arbor, Michigan, United States, [4]Dermatology, University of Michigan, Ann Arbor, Michigan, United States

Biogeography is known to shape the human skin metagenome, which in turn, helps to shape the adaptive immune system. Clearly, immune-mediated diseases (e.g. hidradenitis, lichen planus, atopic dermatitis, psoriasis, palmoplantar psoriasis/pustulosis) have predilections for specific anatomical sites (axillae, groin, and inguinal folds; dorsal hands, volar wrists, anterior lower legs and oral mucosa; antecubital fossa and popliteal fossa; elbows and knees; and palms and soles; respectively). A better understanding of how immune composition and function differs by anatomic location remains a major gap in our understanding of skin immunology. As a first step to investigate anatomical-specificity of the cutaneous immune response in humans, we have employed our in-house developed bioinformatics pipeline, *TCRminer*, to characterize intra-person differential TCR gene usage by body site. This analysis revealed differential expression of several TCR gene segments. Specifically, TRAV8-5 was overexpressed in hip skin in comparison to peripheral blood (FDR = 1.97e-35), a finding that was independent of HLA haplotype. The TCR repertoire of the blood was also found to be more diverse than that of the hip. The skin-resident repertoire of the palm was also compared to that of the hip. Results revealed that TRAJ39 was overexpressed (relative fold change = 2.74, FDR = 6.39e-03) in palmar skin, a finding that was independent of HLA haplotype. Palm skin also had significantly less T cell repertoire diversity. Analysis of BCR genes revealed that IgHA1 was poorly expressed in the palm when compared to the hip (relative fold change = 0.06, FDR = 2.02e-05). These findings are relevant because diseases such as palmoplantar psoriasis, hand dermatitis, and palmoplantar pustulosis have a predilection for palmar skin, which according to these results has differential expression of both TCR and BCR receptors.

039

Evaluating T cell activation and polarization impact in morphea

H. W. Chen, E. Burger, J. L. Zhu, G. Barber, S. M. Black, H. T. Jacobe

Dermatology, UT Southwestern Medical Center, Dallas, Texas, United States

Morphea is an idiopathic disorder that can result in functional impairment and long-lasting disfigurement from sclerotic plaque development. Insight into the immune phenotype of morphea patients may provide inroads into the development of new, targeted therapies. Based on preliminary studies, we hypothesize that the development and progression of sclerosis in morphea results from early dysregulation of the Th1 immune axis in the tissue microenvironment which triggers and/or maintains an inflammatory cytokine environment. We utilized multicolor flow cytometry to compare immune cells in peripheral blood of patients with active morphea (defined by clinical activity score, LoSSI ≥ 3, and no treatment with immunosuppressives for ≥ 3 months) (n=15) to matched healthy controls (n=11). Using a T-cell panel tagging extracellular (CD4, CD8, CD45RO, CCR7, CLA, HLA-DR, ICOS) and intracellular (IFNg, IL-4, IL-17) proteins, we identified subset populations within the T-cell population. These proteins served to delineate the following sub-populations: central vs. effector, skin-homing vs. systemic, mid vs. late-activation, and Th1 vs. Th2 vs. Th17. All experiments were conducted on an LSRFortessa and analyzed using FlowJo. Using a shotgun-approach Mann-Whitney U analysis of different subset populations of T-cells, we identified CD4+ Tem systemic late activation (*p=0.008*), CD4+ skin-homing Th17 (*p=0.037*), and cytotoxic CD8+ systemic IFNg-producing (*p=0.023*) population proportions to be significantly higher in active morphea patients compared to healthy controls. We have shown that morphea blood displays increased systemic and skin-homing T-cell activation in tandem with multi-cytokine polarization of IFNg/IL-17 predominance. Discovering an inflammatory milieu in morphea provides critical insights into the pathogenesis underlying lesion development and could serve as biomarkers for disease progression and severity as well as guidance for future targeted therapies.

041

miR-146a regulates the interleukin-17 inflammatory response to Cutibacterium acnes in human peripheral blood mononuclear cells

A. T. Pham, M. Qin, T. To, A. Baugh, J. Kim

Division of Dermatology, David Geffen School of Medicine at UCLA, Los Angeles, California, United States

Inflammation from the immune response targeting Cutibacterium acnes plays a significant role in the pathogenesis of acne vulgaris. Previous studies have shown that C. acnes is a potent inducer of T helper 17 (Th17) cells, a unique class of $CD4^+$ T cells characterized by production of the highly inflammatory cytokine interleukin-17 (IL-17). Emerging evidence has shown that microRNAs (miRNAs) play an important role in modulating the body's inflammatory response, including regulation of Th17 differentiation and IL-17 production. However, the role of miRNAs in acne pathogenesis has not been extensively looked at in prior studies. Here we investigated the role of miR-146a in the response of human peripheral blood mononuclear cells (PBMCs) to C. acnes stimulation. miR-146a has been shown to negatively regulate differentiation of Th17 cells in various autoimmune diseases, dampening production of IL-17. Increased expression of miR-146a was detected in C. acnes-stimulated PBMCs, with expression increasing 2 to 3-fold over time and positively correlated with IL-17 production. In the presence of miR-146a overexpression, production of IL-17 by C. acnes-stimulated PBMCs was reduced nearly 2-fold, as well as a 2-fold reduction in gene expression of Th17 promoting cytokines. A corresponding increase in IL-17 production was seen in the presence of a miR-146a inhibitor. Furthermore, we found that miR-146a expression was decreased 6-fold in human-derived monocytes (THP-1) with toll-like receptor 2 (TLR2) knocked out, suggesting the role of TLR2 in miR-146a induction. Finally, cells isolated from acne lesions showed increased expression of IL-17 related genes and proinflammatory precursors that lead to production of miR-146a. The role of miRNAs in Th17 development in acne lesions has not been previously studied and provides further insight into regulation of the body's inflammatory response. miRNAs represent a new angle in acne pathogenesis and highlight the potential for future miRNA-based therapeutics.

042

Tumor neoantigens and a novel hapten vaccine promote immune targeting of wild type tumor antigens and improve response to immune checkpoint blockade

M. Su[1], T. Erlich[1], J. Lo[1], M. LaFleur[2], M. Salomon[3], L. Kemeny[1], D. Hoon[3], G. Freeman[4], A. Sharpe[2], D. Fisher[1]

[1]CBRC, Department of Dermatology, MGH, HMS, Boston, Massachusetts, United States, [2]Department of Immunology, HMS, Boston, Massachusetts, United States, [3]Department of Translational Molecular Medicine, JWCI, Santa Monica, California, United States, [4]Department of Medical Oncology, DFCI, Boston, Massachusetts, United States

In metastatic melanoma, both tumor neoantigen load and development of vitiligo have been associated with favorable response to immunotherapy. We hypothesize that in the context of immune checkpoint blockade (ICB), neoantigens facilitate epitope spreading and immune targeting of tumor-lineage self-antigens. We also aim to harness this process with a hapten-based vaccine to improve ICB response. Previously, our lab demonstrated in a murine model of melanoma that tumors with high neoantigen load respond significantly better to ICB than syngeneic tumors with low neoantigen load. We show that this response is associated with increased immune recognition of a melanocyte self-antigen (gp100) and that long-term survivors develop a durable immune response against tumor-lineage self-antigens. We leverage the understanding that neoantigens can promote epitope spreading into a novel vaccine therapy by exogenously introducing “neoepitopes” into tumor cells with hapten treatment. Mice bearing melanomas with low neoantigen load responded significantly better to hapten vaccine plus anti-PD-1 compared to unhaptenated control vaccine plus anti-PD-1. Bulk tumor RNA-Seq revealed enhanced immune and T cell signatures with hapten vaccine treatment. Immunohistochemistry showed increased $CD8^+$ T cells and decreased $Foxp3^+$ Tregs intratumorally, and flow cytometry demonstrated elevated functional $CD8^+$ T cells targeting the melanocyte self-antigen gp100. Depletion of specific immune cell populations confirmed that $CD8^+$ T cells are required for treatment efficacy. Hapten vaccine treatment also increased the efficacy of combination immunotherapies and improved ICB response in a model of pancreatic ductal adenocarcinoma. This novel hapten-based vaccine may have broad clinical applications as a strategy to enhance ICB response.

045

Piglitazone, a PPAR γ Agonist, alleviates imiquimod-induced psoriasis-like skin lesions by regulating keratinocyte proliferation and differentiation through inhibiting STAT3

Y. Li[1, 2], P. Xu[3, 2], C. Guo[1, 2], X. Zhang[1, 2], N. Yu[1, 2], J. Lu[1, 2], Y. Ding[1, 2], Y. Shi[1, 2]

[1]Dermatology, Shanghai Skin Disease Hospital, Shanghai, China, [2]Institute of Psoriasis, Tongji University School of Medicine, Shanghai, China, [3]Dermatology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China

Psoriasis is a chronic autoimmune skin disease, characterized by abnormal keratinocyte proliferation and differentiation. Peroxisome proliferator activated receptor (PPAR) γ, is a ligand-activated nuclear transcription factor, which is involved in the regulation of inflammatory cytokines synthesis in immune cells and regulates keratinocyte proliferation and differentiation. Piglitazone, a PPAR γ agonist, has been shown to have significant improvements when treated patients with psoriasis. However, the mechanism is still unclear. In this study, we investigated the underlying mechanism. In vivo, we applied piglitazone to imiquimod (IMQ)-induced mouse model of psoriasis and found that piglitazone ameliorated IMQ-induced psoriasis-like dermatitis, with reduced inflammation, less Ki67 positive cells, and increased the K1 expression. Then, we studied the effect of piglitazone on the proliferation and differentiation of keratinocytes. Hacat cells was stimulated with IL-17A /TNF-α to model the skin inflammation in psoriasis. We found that piglitazone suppressed cell proliferation, promoted cell differentiation and inhibited the expression of inflammatory cytokines in Hacat cells. In addition, piglitazone significantly down-regulated the phosphorylation of signal transduction and activator of transcription 3 (p-Stat3). Therefore, piglitazone alleviates IMQ-induced psoriasis-like skin lesions possibly by regulating keratinocyte proliferation and differentiation via inhibiting Stat3 signaling pathway.

046

Effect to the adipose tissue by inflammation from sever skin dermatitis

K. Mizutani[2], E. Shirakami[1], S. Iida[2], Y. Matsushima[2], K. Okada[2], A. Umaoka[2], M. Kondo[2], K. Habe[2], K. Yamanaka[2]

[1]Dermatology, Shimane University Faculty of Medicine, Izumo, Japan, [2]Dermatology, Mie University Graduate School of Medicine, Tsu, Mie, Japan

Adipose tissue (AT) is the largest endocrine organ producing bioactive products called adipocytokines, which regulate several metabolic pathways, especially in the inflammatory condition. On the other hand, evidences were accumulated that chronic inflammatory skin disease is closely associated with AT abnormal remodeling; however, the mechanism is still unclear. We addressed this problem using keratin 14-specifically overexpressing caspase-1 transgenic mouse (KCASP1Tg) that shows severe erosive dermatitis from 8 weeks, followed by reepithelization and parakeratotic scale-crust formation with elevated circulating plasma IL-1 level derived from severe skin inflammation, causing vascular sclerotic change and severe systemic amyloidosis. In this study, we investigated the influence of the long-lasting dermatitis to AT pathological and functional change. Firstly we examined the influence of skin inflammation to the whole body and perigonadal white adipose tissue (GWAT) weight, and both of them were decreased in KCASP1Tg showing small “burn-out” adipocytes histopathologically with increased stromal cell and TLR (Toll-like receptor) 4/ CD11b positive monocyte infiltrates. Adipocytes isolated from KCASP1Tg had elevated levels of TNF-α and a tendency to increase monocyte chemotactic protein-1 (MCP-1) and adipokine. After 4°C cold challenge, the expression of thermogenic genes, uncoupling protein1 (UCP1) mRNA in adipocyte was elevated, but the body temperature decreased rapidly in KCASP1Tg, revealing the impaired thermogenesis ability of AT due to the AT atrophy. These AT disability was reproduced by cytokine intra-peritoneal administration, suggesting the influence of circulating skin-derived inflammatory cytokines. Our study suggested that tight control of inflammatory skin disease leads to the prevention of AT complications.

049

Generalized pustular psoriasis (GPP) and palmoplantar pustulosis (PPP) both show upregulation of the IL-36, neutrophil chemokine, and innate pathways that are modulated by spesolimab, an anti-IL-36 receptor antibody, treatment

P. Baum[1], S. Visvanathan[2], S. Garcet[3], J. Roy[4], R. Schmid[1], S. Bossert[1], B. Lang[1], H. Bachelez[5], R. Bissonnette[6], C. Thoma[1], J. Krueger[3]

[1]Boehringer Ingelheim GmbH & Co. KG, Biberach, Germany, [2]Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, Connecticut, United States, [3]Laboratory for Investigative Dermatology, Rockefeller University, New York, New York, United States, [4]Staburo GmbH, Munich, Germany, [5]UMR INSERM U1163, Institut Imagine, Paris, France, [6]Innovaderm Research Inc., Montreal, Quebec, Canada

Pustular psoriasis comprises a spectrum of inflammatory skin conditions, including GPP and PPP, characterized by neutrophilic infiltration of the epidermis and development of visible sterile pustules. GPP is a rare, multisystemic, potentially life-threatening, flaring disease, whereas PPP is a localized, relapsing, debilitating, chronic disease. Here, gene and protein expression of lesional and non-lesional skin from 7 patients with a moderate GPP flare, 8 patients with moderate-to-severe PPP, and skin samples from the thigh/back (n=10) or palm/sole (n=6) of healthy donors were compared. In lesional skin samples, 7,614 genes in GPP and 1,651 in PPP were found to be differentially expressed compared with healthy donors. In GPP and PPP, 1,287 transcripts were commonly up- or downregulated (adjusted $p \leq 0.01$, absolute fold-change ≥2). Markedly upregulated genes in lesional vs non-lesional skin include *IL36a*, *KRT6A*, *defensin4b*, *IL6*, *CXCL1*, *CXCL8*, *IL17A*, *IL23A*, and *IL1b*; common molecular pathways included increased Th1 and Th17 signaling, keratinocyte-driven inflammation, and a strong upregulation of neutrophil attractants, inflammatory mediators, and the IL-36 pathway. Treatment of 7 GPP patients with a single 10 mg/kg IV dose of spesolimab (BI 655130; NCT02978690) led to rapid clinical improvements and modulation of the transcriptomic profile and select proteins in the skin (NE and lipocalin 2) and blood (IL-6, CRP, CXCL1, IL1RN, CCL20) common to GPP and PPP.

050

Malignant melanoma cell growth is prevented in the systemic inflammatory environment

Y. Matsushima, K. Mizutani, S. Iida, A. Umaoka, K. Okada, M. Kondo, K. Habe, K. Yamanaka

Dermatology, Mie University Graduate School of Medicine, Tsu, Mie, Japan

A wide range of dermatitis such as psoriasis is considered as a systemic inflammatory disease. Here, we examined the effects of inflammation on malignant melanoma development in the skin-derived systemic inflammatory mice model. Melanoma cells were injected subcutaneously into the dorsal non-eczema skin region and the progress of the tumor growth was measured. Tumors grew more slowly with significance compared to wild-type mice. Serum from inflammation mice did not show a direct effect on melanoma cells compared to wild type mice. Tumor infiltrating lymphocytes (TILs) was also analyzed by flow cytometry. TILs from inflammation mice tended to release more inflammatory cytokines such as TNF-α and IFN-γ compared to wild-type mice, suggesting that systemic inflammation may inhibit tumor growth.

051

Non-steroidal anti-inflammatory drugs act as adjuvant in allergic sensitization

A. Eisenstein[3], B. Hilliard[1, 2], A. Wang[1, 2]

[1]Department of Medicine, Yale School of Medicine, New Haven, Connecticut, United States, [2]Department of Immunobiology, Yale School of Medicine , New Haven, Connecticut, United States, [3]Department of Dermatology, Yale School of Medicine, New Haven, Connecticut, United States

Atopic disease, including asthma, food allergies and atopic dermatitis, are on the rise. Concurrently, the use of non-steroidal anti-inflammatory drugs (NSAIDs) is also growing resulting in 30 billion American doses yearly. NSAIDs, which inhibit cyclooxygenase enzymes (COX) have been associated with worse allergic diseases and is thought to exacerbate allergic inflammation by shunting arachidonic acid metabolism towards leukotriene synthesis. We hypothesize that NSAIDs possess adjuvant properties and are sufficient to induce allergic sensitization, and thus may partially explain the exponential increase in the prevalence of allergic diseases. To test this, we sensitized mice to the model antigen ovalbumin (OVA) with commonly utilized NSAIDs. We found that certain NSAIDs, irrespective of COX specificity, but not other commonly used analgesics such as acetaminophen, were sufficient to generate immunologic memory to OVA. NSAID-treated animals, regardless of sex or strain, produced high levels of OVA-specific IgE and IgG1 during sensitization, and anaphylaxed to OVA, but not NSAID, challenge. This suggests that the widespread use of NSAIDs may be a major contributor to the development of allergic disease and has broad implications for their judicious use as supportive therapy in a wide variety of common clinical settings.

052

Targeting keratinocytes to potentiate non-viral DNA skin immunization

J. You[1], R. Hao[1], X. Hao[1], L. D. Falo[1], E. Kim[2], C. D. Carey[1], G. Erdos[1], A. Gambotto[2], Z. You[5], L. D. Falo[3, 4, 6]

[1]Dermatology, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [2]Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [3]Dermatology, Univ of Pittsburgh, Pittsburgh, Pennsylvania, United States, [4]Bioengineering, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [5]Immunology, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [6]Hillman Cancer Center, UPMC, Pittsburgh, Pennsylvania, United States

Skin is a uniquely accessible and responsive target for vaccine delivery. Emerging evidence suggests that keratinocytes can modulate skin immunity in response to diverse stimuli, producing either proinflammatory or immune suppressive mediators depending on the nature of the exogenous stress. To improve the immunogenicity of skin targeted vaccines, we engineered keratinocytes to support a proinflammatory local environment. Keratinocytes were genetically engineered to express the stress response transcription factor x-box binding protein 1 (XBP1). In a mouse model, keratinocyte-specific overexpression of XBP1 was transient and induced a proimmunogenic skin microenvironment characterized by increased expression of proinflammatory mediators, localized inflammatory infiltrates, including localized increases of dermal $CD103^+$ DCs, $XCR1^+$DCs, plasmacytoid DCs, $\gamma\delta$ T cells, and group 1 innate lymphoid cells. Simultaneous non-viral delivery of plasmids driving expression of XBP1 and antigen OVA resulted in increased antigen expression and increased the induction of antigen-specific cellular and humoral responses, including durable antigen-specific skin-resident memory CD8 T cells and efficacious protective immunity, compared to delivery of antigen encoding plasmid alone. This translated to increased titers of ZIKV-ENV antibody in a Zika virus model, and improved therapeutic immunity in a clinically reflective endogenous melanoma model. Further, overexpression of XBP1 in keratinocytes in human skin resulted in a proimmunogenic skin microenvironment. These findings support the feasibility of keratinocyte targeted DNA vaccines to induce a proinflammatory skin microenvironment for effective immunization.

053

The link between N^6-methyladenosine modification and psoriasis

L. Cui, J. Cai, C. Hu, Y. Yu, Y. Shi

Department of Dermatology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China

Psoriasis is a complex, chronic inflammatory skin disease characterized by inflammation and hyperproliferation of the epidermis. In recent years, the epigenetic mechanism has been revealed to play vital roles in the pathogenesis of psoriasis. N^6-methyladenosine (m^6A) is the most prevalent internal modification of messenger RNA (mRNA) in eukaryotes, and it is involved in gene expression regulation and various biological processes. To reveal the relationship between m^6A methylation and psoriasis, we analyzed the data from the GEO database and found that the expressions of m^6A regulatory enzymes have changed in psoriatic lesions compared to the healthy controls, which was also confirmed by performing real-time PCR. The m^6A methylation levels are significantly reduced in psoriatic lesions compared with the skin of healthy controls. The results of immunostaining showed that the expression of METTL3, which plays a central role in catalysis of m^6A, showed a declining tendency in psoriatic lesions, while the expression of ALKBH5, a main demethylase of m^6A, was upregulated in psoriatic lesions compared to the healthy controls. In the back skin of imiquimod-induced psoriatic mice, the expression of METTL3 decreased, while the expression of METTL14 and WTAP these two writers as well as both erasers, FTO and ALKBH5, were all upregulated with the increase of modeling days, and the results of immunofluorescence showed the same tendency. We also separated the epidermis and dermis from the back skin of imiquimod-induced psoriatic mice as well as normal mice, and we found that both of the reduced expression of METTL3 and increased expression of ALKBH5 mainly exits in the epidermis instead of the dermis. This study demonstrates the link between m^6A and its regulatory enzymes with psoriasis for the first time, and it provides a solid foundation for further research on the effect of m^6A modification in the pathogenesis of psoriasis.

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Highly Multiplexed Immunophenotyping of Dermatomyositis Skin Lesions

J. Patel[1, 2], S. Maddukuri[1, 2], C. Bax[1, 2], V. Werth[1, 2]

[1]CMCVAMC, Philadelphia, Pennsylvania, United States, [2]Derm, U Penn, Philadelphia, Pennsylvania, United States

Dermatomyositis(DM) is an autoimmune systemic disease that most often affects skin and muscle. The pathogenesis of cellular skin inflammation has yet to be investigated. Previous work revealed a type 1 interferon gene signature characterized predominantly by interferon-beta (β). To investigate the type 1 interferon signature, we identified pathways and cellular phenotypes in a subset of DM patients. 5 Healthy control (HC) and 5 DM formalin-fixed, paraffin-embedded (FFPE) samples obtained from trunk, arm, or leg were stained with a panel of 35 metal conjugated antibodies. Regions of interest (ROI) of 500x800µm were ablated at a frequency of 200Hz on the Hyperion Imaging System (Fluidigm). The resulting files were MCD files were exported to 16-bit TIFF files using MCD Viewer™ (Fluidigm). Cell segmentation was performed using an app-based algorithm in Visiopharm. Per object mean pixel intensity (MPI) was gathered and analyzed using histoCAT. Positive and negative cell populations were identified using a sliding scale for each channel. Phenograph algorithm was used for unsupervised clustering of cell populations after thresholding each channel. Statistical analysis between groups was performed using the Mann-Whitney test all values reported as mean ± SEM. Skin lesions of DM patients contain an increased number of CD163+ cells compared to normal skin from HC patients (14±4.7 vs 2±1 cells/ROI) p<0.05). CD163+ cells had increased MPI of key inflammatory pathways: pSTING (34.4±4.9 vs 3.9±1.9), IFNβ (8.2±0.8 vs 4.4±7.5), and IL17 (6.5±1.0 vs 1.3±0.3); all p<0.05. A population of CD4 cells was identified that produced higher IFNβ MPI compared to HC CD4 cells (16.4±4.5 vs 8.0±0.8; p<0.01). Lesional DM skin also contained more FOXP3+ CD4 cells when compared to HC (64±20.3 vs 6±1.3 cells/ROI; p<0.05). The function of these cells is unclear. Compared to HC CD163+ cells in DM appear to be an important source of IFNb via activation of the STING pathway. IFNβ is produced by both CD163+ and a subset of CD4 cells.

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Assessment of fidelity to the desmoglein compensation hypothesis in a large pemphigus cohort

L. Sielski, J. D. Baker, M. Depasquale, K. Seiffert-Sinha, A. A. Sinha

Dermatology, University at Buffalo, Buffalo, New York, United States

Pemphigus vulgaris (PV) and pemphigus foliaceus (PF) are autoimmune blistering diseases characterized by oral or mucosal lesions in the presence of autoantibodies (autoAb) targeting the cell-adhesion proteins desmoglein (Dsg)3 and Dsg1. Lesion location has been elegantly explained by the Desmoglein Compensation Hypothesis (DCH), which utilizes the epidermal distribution of Dsg subtypes as well as autoAb profiles. According to this theory, PF presents with subcorneal lesions in the presence of anti-Dsg1 Abs only, while lesions in PV are suprabasilar and accompanied by anti-Dsg3 only in mucosal PV, or anti-Dsg1 and -Dsg3 in mucocutaneous PV. While the validity of this hypothesis has been supported in the literature, logical inconsistencies have been noted and exceptions have been published in several small-scale studies. We sought to comprehensively assess how often patients contradict the DCH and characterize these contradictions in a large sample size of 289 pemphigus patients. We find that roughly half of the PV and PF patients with active disease at time of visit present with a combination of lesion morphology and anti-Dsg levels that contradict the DCH. The most common contradiction is cutaneous only PV at time of enrollment (n=34), including 7 patients who report no mucosal lesions at any time in their history. Other categories in which lesion morphology does not align with the predicted autoAb status include mucocutaneous disease in the absence of either Dsg1, Dsg3, or both (n=31) and mucosal disease in the absence of Dsg3 or presence of Dsg1 (n=23). We find stark differences based on ethnicity, with the highest proportion of patients that follow the DCH among the Ashkenazi Jewish population (63.5%) and the lowest for African Americans (25%). These findings demonstrate the need to expand our understanding of pemphigus morphology beyond the DCH, in particular for populations that have not been the focus of previous studies.

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Systems approach to evaluate disease factors in pemphigus

L. Li[2], K. Seiffert-Sinha[1], Y. Sun[2], A. A. Sinha[1]

[1]Dermatology, University at Buffalo, Buffalo, New York, United States, [2]Computer Science and Engineering, University at Buffalo, Buffalo, New York, United States

Pemphigus vulgaris (PV) pathogenesis is dependent upon HLA genetic susceptibility and multiple autoantibody (autoAb) reactivities to desmoglein (Dsg) and non-Dsg targets. The relative roles of specific disease-associated factors have yet to be determined. In this study, we integrated large scale genetic and proteomic data on (1) PV-associated HLA alleles, (2) 31 autoAb specificities detected by protein array, and (3) 4 autoAb detected by ELISA (anti-Dsg3, -Dsg1, -TPO and -Tg) in 200 PV patients and 121 healthy controls (CR). We built Random Forest (RF) models to predict the diagnosis of PV and to reveal the contribution of each pathogenic factor in different clinical groups. The predictive power of each combination was evaluated according to the area under the receiver operating curve. When comparing all patients vs. all CR, anti-Dsg3, followed by -Fc Fragment of IgE and -ANXA9, carried the highest predictive value. To further our understanding of the contribution of each factor regarding disease activity and HLA status, we retrained RF models in different clinical groups. When comparing subgroups that include patients carrying PV-associated HLA alleles (HLA+) or patients in active disease vs. CR, the top 3 factors that contribute most the prediction performance are anti-Dsg3, -Fc Fragment of IgE, and -ANXA9. However, when comparing HLA- PV vs. All CR and HLA- PV vs. HLA- CR, markers including anti-Fc Fragment of IgE and -ANXA9 instead of Dsg3 gain in importance. These results indicate that HLA association shapes autoAb selection. We further performed a co-occurrence network analysis to investigate the relationships between the pathogenic factors. We observe that the Dsg and thyroid function related antigens (Ags) are less connected to the remaining Ags, with Fc fragment of E serving as a bridge to anti-Dsg Ags and anti-Tg serving as a bridge to anti-TPO. Our study provides deeper insights into underlying disease mechanisms in PV and may help in the development of actionable next-generational tools for clinical decision-making support.

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Distinct Chromatin Accessibility Profiles of $CD8^+$ Tissue Resident Memory T Cells

J. Zhao, Y. Pan, K. Wu, C. A. Stingley, Y. Yan, T. S. Kupper

Dermatology, Brigham Health/Harvard Medical School, Boston, Massachusetts, United States

Tissue-resident memory T cells (T_{RM}) differ fundamentally from their circulating counterparts, central and effector memory T cells (T_{CM} ,T_{EM}). The epigenetic mechanisms and transcription factor networks by which they maintain their distinct differentiation states remain obscure. Here we compared genome-wide maps of chromatin accessibility of $CD8^+$ T_{RM} ,T_{CM}, T_{EM} generated by skin vaccinia virus (VACV) infection using Assay for Transposase-Accessible Chromatin sequencing (ATAC-seq). Principal component analysis (PCA) segregated T_{RM}, T_{CM}, T_{EM} into 3 clearly separate clusters which were also distinct from naïve CD8 T cells. We found 9627 and 9042 differentially accessible chromatin regions in T_{RM} compared to T_{CM} and T_{EM}, respectively. T_{RM} open chromatin regions included *Gzmb, Gzmk, Ifng,* and *Il2.* In addition, *Itgae* and *CD36* were exclusively open in $T_{RM,}$ which is consistent with their gene expression in these cells. Conversely, *S1pr1, Ccr7, Sell, Klf2* loci were closed in T_{RM} compared to T_{CM} and $T_{EM.}$, also consistent with their transcriptional downregulation in T_{RM}. Transcription factor networks controlling T_{RM} differentiation were inferred from differentially accessible motifs using HOMER motif analysis software. We found motifs for several members of the Basic Leucine Zipper Domain (bZIP) family as most significantly enriched at open chromatin regions in T_{RM}; top hits included Fra2, JunB, Atf3, and Batf. In contrast, Zinc Finger (ZF) and E-26 Transformation Specific (ETS) motifs were enriched at chromatin sites more open in T_{EM} and $T_{CM.}$ Taken together, our data indicated that T_{RM} have a unique epigenetic signature, with higher activity of bZIP transcription factor family members in T_{RM} compared to T_{CM} and T_{EM}. The factors responsible for this epigenetic signature are under investigation.

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[18F]FDG PET/CT-based imaging method to characterize the therapeutic effects of DMF in EAE

J. Brück[2], C. Calamanius[1], S. Hoffmann[1], M. Harant[1], K. Ghoreschi[3, 2]

[1]Werner Siemens Imaging Center, Tübingen, Germany, [2]Department of Dermatology, Erberhard Karls University Tübingen, Tübingen, Germany, [3]Department of Dermatology, Charité – Universitätsmedizin Berlin, Berlin, Germany

Data from clinical and preclinical studies have shown that fumarates like dimethylfumarate (DMF) - by suppressing the Th17 response - improve psoriasis and multiple sclerosis in human and experimental autoimmune encephalomyelitis (EAE) in mice. In our previous studies, we analyzed the anti-inflammatory effects of DMF on the immune response by methodologies like intracellular cytokine staining and flow cytometry or by performing quantitative mRNA expression from isolated cells. Here we aimed to establish an in vivo method to follow T cell activation and the anti-inflammatory properties of DMF in mice immunized for developing EAE. We decided to investigate whether it is possible to characterize the therapeutic effects of DMF treatment in vivo using a non-invasive imaging technique. As proliferating T cells have been shown to uptake the clinically-used radioactive glucose analogon [18F]FDG (18F-fluorodeoxyglukose), we applied this tracer to mice immunized for EAE. Our aim was to follow T cell activation in the lymphatic system at different time points. In addition, we established a PET/CT-based imaging method to characterize the therapeutic effects of DMF treatment after active EAE induction. The PET/CT imaging data was analyzed by region of interest (ROI)-based methodology and validated by biodistribution studies and *ex vivo* mRNA expression analysis. Our findings show that the [18F]FDG and PET/CT-based imaging methodology can be used to characterize the effects of therapeutic compounds in the disease course of actively induced EAE in mice.

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Characterization of a novel patient-derived antibody with sequence homology to antibodies directed against both desmosomal and non-desmosomal targets in Pemphigus vulgaris

K. Seiffert-Sinha, M. Leiker, A. A. Sinha

Dermatology, University at Buffalo, Buffalo, New York, United States

Historically, the primary autoantigenic targets in Pemphigus vulgaris (PV) have been considered to be the keratinocyte-associated adherens proteins desmoglein (Dsg)3 and Dsg1. We, and others have shown that PV patients additionally harbor autoantibodies to several non-Dsg antigens, including thyroid peroxidase (TPO). However, there remain major gaps in our knowledge regarding the scope, specificity, and functionality of non-Dsg autoantibodies present within and across individual PV patients. To investigate the broader repertoire of B-cell derived autoantibodies in PV we utilized Immune Repertoire Capture™ technology to generate natively paired heavy and light chain sequences of antibodies expressed by single B cells and to deliver fully human, recombinant monoclonal antibodies (rmAb) isolated directly from patients. Utilizing plasmablasts from a PV patient in active disease, we identified and isolated a series of B cell receptor sequences that clustered in clonal antibody families. One of these, AB003613, was found to bear 74% heavy-chain homology to anti-TPO antibody and 86% light-chain homology to an anti-desmosome antibody as per BLAST alignment. This antibody did not bind to Dsg3 or -1 by ELISA, did not stain intercellular regions on monkey esophagus by IIF, and did not bind TPO protein by Western Blot. However, AB003613 did bind a 55-60kDa protein in HaCaT keratinocyte lysates. Moreover, immunofluorescence revealed a cytoplasmic target within HaCaT keratinocytes but no co-localization with the cell membrane or any component thereof, including Dsg3. While the precise epitope target of this antibody is yet to be identified, we clearly show the existence of a patient-derived antibody targeting a non-Dsg target within keratinocytes in an active PV patient. The availability of this renewable patient-derived mAb will be useful in future systematic studies of specificity and function of non-Dsg autoantibodies in PV.

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Delivery of contact sensitizers and neurokinin 1 receptor antagonists by microneedle arrays targets different skin cells to abrogate contact dermatitis

M. Bandyopadhyay[1], A. E. Morelli[2], N. L. Ward[3], G. Erdos[1], T. Sumpter[1], S. Balmert[1], O. Tkacheva[1], W. Shufesky[2], L. D. Falo[1], A. Larregina[1]

[1]Dermatology, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [2]Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [3]Dermatology, Case Western Reserve University, Cleveland, Ohio, United States

Development of contact dermatitis (CD) relays on innate and adaptive immunity that promote the activation of CD4 T helper 1 (Th1) and CD8 T cytotoxic 1 (Tc1) biased cells. Signaling via the neurokinin 1 receptor (NK1R) by the proinflammatory neuropeptides substance P and/or hemokinin 1, triggers skin neuroinflammation and supports Th1 and Tc1 immunity. Thus, we hypothesized that limiting neuroinflammation during skin Ag entry induces an immune-suppressive environment that limits the function of activated T cells that cause CD. Using self-dissolving microneedle arrays, we efficiently co-delivered 2,4-dinitrocholorobencene (DNCB) or OVA and two different NK1R antagonists to the skin of C57/BL6 mice during sensitization (prevention) or between relapses (therapy) of CD. This approach resulted in significant decrease of local and systemic CD in an Ag specific manner. Using $NK1R^{KO}$ bone marrow chimeras and the Cre-Lox P system, we demonstrate that absence of functional NK1R in keratinocytes but not in leukocytes decreased IL-1β and IL-6, and inhibited the sensitization phase of CD. Absence of functional NK1R in either keratinocytes or dendritic cells, but not in mast cells abrogated the adaptive immunity of CD. Mechanistic studies using DNCB (polyclonal) or OVA OT1 and OT2 (monoclonal) models showed expansion of regulatory T cells, death of activated Th1 and Tc1 cells, decreased IFN-γ and, increased IL-10 in skin draining lymph nodes. Together these events resulted in a significant diminished number of CD8 T cells homing in the skin after re-exposure to OVA or DNCB. Our data demonstrate the possibility of preventing sensitization and relapses of CD by an immune-suppressive method based on restraining neuroinflammation during skin Ag penetration.

061

Tissue DC antigen capture is selectively regulated by type II Interferon

Q. Huang[1, 3], J. Hsu[2], N. Anandasabapathy[1, 3]

[1]Department of Dermatology, Weill Cornell Medical College, New York, New York, United States, [2]Weill Cornell Medical College, New York, New York, United States, [3]Meyer Cancer Center, Weill Cornell Medical College, New York, New York, United States

DCs are specialized antigen-presenting cells that serve as essential mediators of immunity and tolerance. DCs mature from bone marrow progenitors, that exit the bone marrow, circulate through the blood and seed lymphoid organs and non-lymphoid tissue such as the skin, forming a network of poised sentinels. It remains unknown how maturation and the distribution of DCs across tissues and lymphoid organs occurs *in vivo*. We previously reported a highly conserved program of semi-maturation occurs during DC (and other myeloid) development from the bone marrow out to the tissue, and upon migration from the tissue to the draining LN. We identified IFNg as a likely instructive cue. This study aimed to further interrogate how IFNg and IFNa/b signaling shapes the development and behavior of DCs during this developmental trajectory. Using 11-color multi-parametric flow cytometry to distinguish DC progenitors and subsets, we found that IFNGR1 expression varies during the course of differentiation with IFNGR1 levels largely dictated by both location and/or DC maturation status in most sites. Because transcriptome analysis of IFNGR1-/- vs WT migDCs revealed differences in molecules associated with antigen processing and presentation, we tested antigen uptake ability *in vivo* by testing anti-DEC205 antigen capture. Fluorescent anti-DEC205 antibody capture in WT, IFNGR1-/-, IFNGR1-/-IFNAR1-/- and IFNAR1-/- mice was compared. We did not see significant differences in the *in vivo* antigen capture by migDCs derived from WT and IFNAR1-/- mice. However, we identified enhanced early capture of anti-DEC205 in IFNGR1-/- and IFNGR1-/-IFNAR1-/- mice, suggesting the specific regulation of antigen capture by type II IFN (IFNg) signaling. Future studies in our lab will further interrogate the molecular mechanisms involved in how IFNg/IFNGR1 signaling conditions DC development and function in tissues.

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Hidradenitis suppurativa RNA-seq skin transcriptome overlaps with psoriasis vulgaris and reveals a marked upregulation of multiple targetable cytokines

S. Garcet, J. W. Frew, K. Navrazhina, J. Krueger

Laboratory of Investigative Dermatology, Rockefeller University, New York, New York, United States

Hidradenitis Suppurativa (HS) is a chronic inflammatory dermatosis of inguinal, axillary, and submammary skin. Using established consensus definitions, unaffected, perilesional and lesional HS skin samples were collected and RNA-sequencing performed. A mixed-effect model was estimated. Hypotheses were tested under the general framework for linear models in the R limma package. P values from t-tests were adjusted for multiple hypotheses using the Benjamini–Hochberg procedure. Statistical analysis revealed a HS transcriptome of approximately 5000 genes (FCH>1.5; fdr<0.05), with HS samples clustering distinctly away from control skin. Broad inflammatory signatures, including increased expression of T-cell, neutrophil and B-cell related pathways were seen in HS. Increased levels of calcium binding proteins cytokine/chemokine production related (S100A7, S100A8, S100A9, S100A12) transcripts were observed. Several chemokines (CCL11, CXCL1, CXCL6, CXCL8, CXCL13) and cytokines (IL1B, IL6, IL17A, IL17F, IL22, IL24, IL26, IL36A, IL36G) significantly up-regulated, with down-regulation of IL34 and IL37. Signatures associated with innate immune system (HRNR) and immunoglobulins were observed. Confirmatory RT-PCR demonstrated significant elevation of IL-17A, IL-17F, IL-22, IL-26, IL-36A and IL-36G in HS skin, with increasing expression between unaffected, perilesional and lesional skin. HS lesional skin had similar or higher levels of the pro-inflammatory cytokines seen in psoriasis. Unaffected HS skin had higher expression of these cytokines compared to healthy controls, suggesting that even normal-appearing HS skin is inflamed, establishing HS as a systemic disease. This is the largest comprehensive dataset of large-scale RNA-sequencing of an untreated HS cohort. This data establishes parallels between HS and psoriasis, suggesting that psoriasis-like feed-forward mechanisms may be involved in disease pathology.

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Therapeutic effects of Smad7-based protein on imiquimod-induced psoriatic lesions

Y. Ke, B. Li, J. Gao, B. Li, D. Wang, S. Wang, X. Wang

University of Colorado Denver Anschutz Medical Campus, Aurora, Colorado, United States

Smad7 structurally consists of N- and C- terminal domains linked with a PY motif. It shuttles between nuclear and cytoplasm to exert different biological functions. Smad7 was overexpressed in epidermis of clinical psoriatic lesions, which was thought to contribute to epidermal hyperplasia in psoriasis. To address if this is the case, we assessed the role of Smad7 and its functional domains in IMQ-induced psoriatic pathogenesis using genetic and pharmacological approaches. K5.Smad7 mice, which express Smad7 transgene(Tg) by a keratin-5 promoter, were resistant to IMQ-induced psoriatic lesions, suggesting an anti-inflammatory effect of Smad7 overexpression. K5.N-Smad7 Tg skin that expresses an N-terminal portion of Smad7, revealed a similar IMQ response to wildtype skin, suggesting this anti-inflammatory effect primarily attributed to the C-terminal domain of Smad7. To test this, we produced a recombinant protein containing PY and C domains of Smad7 fused with a cell permeable Tat tag (Tat-PY-C-Smad7). Topical application of this protein significantly alleviated IMQ-induced psoriatic lesions dose-dependently. N-Smad7 Tg immobilized in nuclear in normal and IMQ-treated skin. In contrast, Smad7 Tg and Tat-PY-C-Smad7 translocated to the cytoplasm in IMQ-treated skin, which ameliorated TGF-β-band NF-κB signaling in the epidermis and reduced proliferative keratinocytes, $CD45^+$, $F4/80^+$, $ly6G^+$ leukocytes and dermal $CD3^+$T cells. RNAseq analysis revealed that both K5.Smad7 Tg skin and Tat-PY-C-Smad7-treated skin significantly downregulated psoriasis-related genes, keratinocyte differentiation, inflammatory response and several other key pathological signaling pathways including IL-17 signaling pathway. Therefore, the PY-C-Smad7 may contain the functional domain of Smad7 that enable to dampen multiple pathogenic processes of IMQ-induced psoriasis. Our study provides evidence to support the feasibility of Tat-PY-C-Smad7 to be developed as an agent to treat psoriatic lesions.

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CDK7 inhibitor suppresses psoriasis inflammation via inhibiting glycolysis to modulate Th17/Treg balance

Y. Lin[1, 2], B. Li[1, 2], K. Xue[1, 2], G. Wang[2]

[1]Dermatology, Xijing Hospital, Xi'an, Shaanxi, China, [2]Xijing Hospital, Fourth Military Medical University, Shaanxi, Xi'an, China, Xi'an, China

Psoriasis is a chronic inflammatory disease characterized by hyper-activated Th17 and suppressive Treg cells, but the mechanism of Th17/Treg cells imbalance is still unclear. Cyclin-dependent kinase 7(CDK7) which is known as a cell cycle regulator has been reported an anti-inflammatory effect in immuno-cells. Here we firstly found $CD4^+$T cells of psoriasis patients expressed higher levels of CDK7 along with an increased glycolysis levels than those in healthy controls. The chemical inhibitor of CDK7 called THZ1 restricted glycolytic metabolism in $CD4^+$T cells of psoriasis patients as well as typical glycolysis related genes. More importantly, THZ1 could suppress Th17 cell differentiation and promote Treg cell differentiation even under Th17 polarizing condition in vitro. Intraperitoneal injection of THZ1 in mice exhibited an alleviated epidermal hyperplasia and alleviated inflammation caused by imiquimod(IMQ) treatment. THZ1-treated IMQ mice had significantly lower ratio of Th17 cells and higher ratio of Treg cells from the splenocytes, in comparison with vehicle-treated IMQ mice. Furthermore, we identified IL-23 as an upstream regulator that stimulated CDK7 expression and glycolysis through p-AKT-Hif-1α signalling pathway. Taken together, our results showed that abnormal CDK7 expression induced by IL-23 in $CD4^+$ T cells in psoriasis patients contributed to the enhanced glycolysis levels which lead to the imbalance of Th17/Treg cells. CDK7 inhibitor THZ1 may serve as an immuno-modulator for psoriasis therapy in the future.

066

In vivo tracking of antigen-specific skin-resident memory CD4+ T cells

Z. Sun[2], K. Zhang[2], H. Chu[2], T. S. Kupper[1], C. Park[2]

[1]Department of Dermatology & Harvard Skin Disease Research Center, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, United States, [2]Department of Dermatology & Cutaneous Biology Research Institute, Yonsei University, Seoul, Korea (the Republic of)

While development of skin-resident memory CD4+ T (TRM) cells homing to infected or inflamed skin has been well-characterized, in vivo tracking of antigen-specific CD4+ T cells in these processes remain undefined. In this study, we adoptively transferred both DNFB sensitized (Red) and OVA sensitized (Green) CD4+ T cells to naïve recipients, and challenged with DNFB and OVA, respectively, into the recipients' ears. Then, we performed two photon intravital imaging to monitor the in vivo tracking of antigen-specific CD4+ T cells during development of skin TRM cells in our allergen-induced mouse model. We observed that both DNFB sensitized and OVA sensitized CD4+ T cells infiltrated into the skin, very early at 6 h after DNFB and OVA challenge, respectively. On day 7, only antigen-specific CD4+ T (Red or Green) cells infiltrated into the antigen-challenged ear while non-specific CD4+ T cells were barely observed. Immobile antigen-specific CD4+ T cells were also visualized in the skin 30 days after antigen challenge, indicating the importance of antigen-specific CD4+ TRM cells. Consistently, OVA-specific DO11.10 CD4+ T cells also showed similar properties in our adoptively transferred mouse model. In summary, both antigen-specific and non-specific CD4+ T cells infiltrated into skin at day1, then only antigen-specific CD4+ T cells were visualized at day 7, finally developing sessile skin-resident memory CD4+ T cells at day 30 in our live imaging. These results suggest that antigen-specific TRM cells involve long-term memory of allergic inflammation while both antigen-specific and non-specific effector CD4+ T cells participate acute inflammation.

067

Development of allergen-specific Foxp3+RORγt+Treg cells during allergen-specific immunotherapy

K. Zhang[1], S. Kim[1], S. Lee[1], H. Kim[1], S. Kim[2], K. Lee[2], T. S. Kupper[3], C. Park[2]

[1]Dermatology, Yonsei University, Seoul, South Korea, Korea (the Republic of), [2]Department of Dermatology, Severance Hospital, Cutaneous Biology Research Institute, Yonsei University College of Medicine, Seoul, Korea (the Republic of), [3]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States

Allergen-specific immunotherapy (ASIT) is an effective treatment that can induce clinical and immunological tolerance to pathogenic allergens for atopic dermatitis (AD). The main mechanism of ASIT therapy is to induce the allergen-specific regulatory T (Treg) cells. Recently, transcriptomic and functional analyses of Treg cells have identified three specialized subsets based on RORγt (Rorc) and GATA3 expression along with Foxp3. Although skin Treg cells are composed largely of Foxp3+GATA3+ Tregs cells, recent studies have shown that Foxp3+RORγt+ Treg cells have immune-regulatory function in different peripheral and intestinal related organs. In our study, we enrolled AD patients with subcutaneous ASIT against house dust mite (HDM), and allergen-specific Treg cells were analyzed in peripheral blood samples before and after 3, 6, 12 months of ASIT. Treg cells were isolated from peripheral blood mononuclear cells to extract RNA and performed transcriptomic analyses. We observed that Foxp3+RORγt+ Treg cells were increased from 3 months through 12 months of ASIT compared to before ASIT. Foxp3+GATA3+ Tregs cells were not significantly induced after ASIT compared to before ASIT. We also found that serum levels of HDM-specific IgE and expression levels of Th1, Th2 and Th17-related genes were significantly decreased after ASIT while serum HDM-specific IgG4 levels were significantly induced after allergen-specific immunotherapy. Taken together, our results suggest that ASIT induce allergen-specific Foxp3+RORγt+ Treg cells to develop immune tolerance in atopic dermatitis.

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Levels of plasma total IgE and D-dimerand basophil FcεRIexpressionas potential predictors of response to autologous whole blood injection in chronic spontaneous urticaria

Q. Chen, J. Luo, H. Wang, Z. Song

Dermatology, Southwest Hospital, Third Military Medical, Chongqing, China

Background: The efficacy and mechanism of autologous whole blood injection (AWBI) in treating chronic spontaneous urticaria (CSU) are unclear, which may be explained by seeking appropriate biomarkers toeffectively predict the response of the affected patients. Objectives: To explore the possible mechanism of AWBI in treating CSU by investigating the correlation between IgE, D-dimer, anti-FcεRI IgG and basophil FcεRI expression and the clinical symptoms of CSU treated by AWBI. Methods: Eighty patients with autologous serum skin test (ASST)-positive CSUwere enrolled and randomly divided into AWBI treated group (receiving AWBI and antihistamine) and control group (only with antihistamine). Urticaria activity score (UAS7) and dermatology life quality index (DLQI) of the patientsbefore and after treatment were compared and analyzed. Levels of plasma total IgE, D-dimer,and anti-FcεRI IgG of 30 AWBI group patients before and after treatment were compared with those of 25 healthy controls.The basophil FcεRI expression in the peripheral venous blood of the patients was also analyzed. Results: A better clinical response was observed in the AWBI treatedgroup than in controls. ASST+ CSU patients had higher concentrations of baseline plasma IgE, D-dimer and anti-FcεRI IgG,as compared to health controls. IgE and D-dimer were differentially expressed between AWBI responders and non-responders, displaying good diagnostic value in predicting therapeutic response to AWBI. Basophil FcεRI expression was significantly higher in AWBI responders, withan obvious decline during AWBI treatment. Conclusion: This study supported the effectiveness of AWBI in CSU, withthe changes of plasma total IgE and D-dimer as good potential predictors of treatment response. A possible mechanism of AWBI in treating CSU is through the reduced expression of basophil FcεRI.

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There are two isoforms of BP180 in the mouse brain

J. Chen[1, 2], L. Chen[1], B. Hwang[2], N. Li[2], S. Burette[2], L. Lin[2], L. Lin[2], H. Zhang[1], D. Li[1], B. Dong[1], G. Matsushima[3], Z. Liu[2]

[1]Department of Dermatology, Wuhan No.1 Hospital, Wuhan, China, [2]Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States, [3]Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States

BP180 is a hemidesmosomal protein in the skin and other epithelial tissues. The extracellular domain NC16A of BP180 is the main target of pathogenic autoantibodies in bullous pemphigoid (BP). Anti-BP180 autoantibodies exist in both the sera and cerebrospinal fluids of patients with dementia or stroke, suggesting that BP180 could be a shared autoantigen of the skin and brain. The purpose of this study was to investigate the expression of BP180 in the brain and whether BP180 in the brain is the same as skin in mice. We confirmed that BP180 mRNA and protein exist in the brain. The expression level of BP180 mRNA in the skin is about 1000 times higher than that in the brain. The molecular weight of BP180 in the brain was 160 kd instead of the 180 kd in the skin. The reduced size of BP180 in the brain was produced by alternative splicing, resulting in deletion of exons 1 to 6 and the mRNA translated at the ATG initiator codon in exon 11. Further sequence analysis revealed that BP180 in the brain has two isoforms, isoform 1 lacking exons 1-6 (789 nucleotides) and isoform 2 lacking exons 1-6 and exon 48 (111 nucleotides). By immunoblotting we found that BP180 protein was expressed in the cerebral cortex, hippocampus, cerebellum and olfactory bulb. In conclusion, this study identified two isoforms of BP180 in the brain that are different in size from BP180 in the skin. Since these isoforms of BP180 have the NC16A domain, they are expected to be targets of anti-BP180 autoantibodies in BP patients with dementia or stroke.

075

Mast Cells participate in an imiquimod-induced mouse model of psoriasis

Y. Hao[1, 4], D. Che[3], B. Peng[1], Y. Zheng[1], L. He[3], S. Geng[2]

[1]Department of Dermatology, Northwest Hospital, The Second Hospital Affiliated to Xi'an Jiaotong University, Xi'an, Shaanxi, China, [2]Dermatology, The Second Hospital Affiliated to Xi''An Jiaotong University, Xi'an, China, [3]School of Pharmacy, Xi'an Jiaotong University, Xi'an, China, [4]Department of Dermatology, The Second Affiliated Hospital of Baotou Medical College, Baotou, China

Psoriasis is a chronic, inflammatory, polygenic disorder that is associated with both a physical and psychological burden. it is widely accepted that the IL23/Th17/IL17 axis is critical in the development of psoriasis, however, the pathogenesis of psoriasis is still not fully understood. Recent studies reported that mast cells increase and may be the main source of IL-17, IL22 in psoriatic lesions, whether mast cells participate in psoriasis need to be addressed. We used an imiquimod-induced mouse model of psoriasis-like dermatitis, the phenotype of which closely resembles the one observed in psoriasis patients. We found that wildtype mice treated with imiquimod had significantly increased and activated mast cells in their skin compared to control group, while the MrgprB2 knockout mice treated with IMQ had decreased and less activated mast cells, with decreased IL-17, IL-22, IL-23 and TNF-α levels. In vitro studies showed that imiquimod could activate MRGPRX2 in human mast cell and MrgprB2 in mouse mast cell. These results suggested that mast cells participated in the development of psoriasis-like dermatitis via MrgprB2 in mice, associated with the IL23/IL17 axis, which will help us understand the immunopathogenesis and provide new strategies for the prevention and treatment in psoriasis.

077

RNA interference screening for novel cytokine and chemokine factors regulating HIV-1 *trans*-infection from dendritic cells to CD4+ T-cells

S. Caucheteux[1], J. Wheeldon[2], R. Bayliss[2], V. Piguet[1]

[1]University of Toronto, Toronto, Ontario, Canada, [2]Cardiff University, Cardiff, United Kingdom

The propagation of HIV-1 is driven in part by cell-to-cell transmission most frequently observed in dendritic cells (DC) and their subsets - one of the earliest immune cells likely to encounter HIV-1 during acute infection at mucosal surfaces. DCs are capable of highly effective viral transfer to target CD4+ T-cells across the virological synapse, a specialised virus-induced cell junction, which enables wide-spread viral dissemination and accelerated disease progression. Our previous findings have implicated a major role for cytokines and chemokines in the infection and transmission of HIV-1 from DC subsets, though a global study of their molecular functions has yet to be completed. In this study, we screened 319 individual genes using the Human ON-TARGET *plus* SMARTpool cytokine & chemokine siRNA library to investigate the differential effects on HIV-1 transfer from monocyte-derived DCs (MDDC) to a CD4+ SupT1 T-lymphoblastic cell line. Using integrative, data-driven approaches we successfully identified several cytokine superfamilies with potent restrictive properties against HIV-1 *trans*-infection from MDDC to SupT1. The activities of these candidates were validated using three key loss-of-function assays including genetic downregulation, neutralisation by biologics and pharmacological inhibition in *trans*- to both SupT1 and autologous CD4+ T-cells. Disruption of specific cytokine-driven mechanisms in MDDC results in dramatic changes in the capacity for cell-to-cell transfer to CD4+ T-cells. These findings add to a growing body of evidence linking the cytokine network which will inform novel therapeutic strategies against early-stage HIV-1 infection and transmission

078

The effect of LPS on neutrophil extracellular trap-induced Th17 polarization is IL-6 and STAT3-Dependent

S. Lambert[1], R. Nair[1], S. Wang[3], J. T. Elder[1, 2]

[1]Dermatology, Univ of Michigan, Ann Arbor, Michigan, United States, [2]Ann Arbor VA Hospital, Ann Arbor, Michigan, United States, [3]Pharmacology, Univ of Michigan, Ann Arbor, Michigan, United States

Using a 7-day *in vitro* assay involving CD3-CD28 stimulation of peripheral blood mononuclear cells (PBMC), we have previously reported that neutrophil extracellular traps (NETs) exert a stimulatory effect on Th17 polarization from pre-existing memory T-cells (JID 139:125) and that the stimulatory effect of NETs is monocyte-dependent (JID 139:S69). To further investigate the nature of this response, we carried out single-cell RNA-seq analyses, confirming a large increase in the numbers of IL-17-expressing CD4+ T-cells between day 2 and day 7 cultures, which was enhanced by NETs. As assessed by qPCR of cell lysates and ELISA of culture supernatants after 24 hr of CD3/CD28 stimulation, expression of IL-6 mRNA and protein were significantly increased by treatment with 5 ng/ml LPS at the onset of NETosis ($p < 0.05$) but not by exposure to NETs alone (n = 8). In contrast, exposure to LPS after NETosis had no significant effect on IL-6 expression (n =3). Expression and production of IL-6 was totally dependent on monocytes, and did not occur when NETs were generated with 25 nM phorbol myristate acetate (PMA). As assessed by flow cytometry, IL-17 ELISA, and qPCR for IL17A and IL17F, Th17 polarization in the presence of NETs was dependent on STAT3, as shown by treatment with a potent and highly specific STAT3 degrader designed using the proteolysis targeting chimera (PROTAC) concept. The effect of the STAT3 degrader was more pronounced when NETs were formed in the presence of 5 ng/ml LPS, and an inactive congener of the degrader was ineffective. Notably, there was no effect of the STAT3 degrader on Th1 polarization. Taken together, our results from this in vitro system demonstrate that the effect of NETs on Th17 polarization is STAT3-dependent, and suggest that myeloid-cell-derived IL-6 is involved in LPS-mediated enhancement of Th17 polarization.

080

A novel disease-associated haplotype emerges from large scale HLA association analysis in bullous pemphigoid and mucous membrane pemphigoid

N. Khechen, K. Seiffert-Sinha, A. A. Sinha

Dermatology, University at Buffalo, Buffalo, New York, United States

Mucous Membrane Pemphigoid (MMP) and Bullous Pemphigoid (BP) are subepithelial mucosal and cutaneous blistering diseases, respectively. Cicatricial variants of MMP affect the ocular (OCP) and non-ocular mucosa (CP). As for other autoimmune diseases, an association with certain Human Leukocyte Antigen (HLA) alleles has been described before. The overwhelming majority of studies have reported a strong association with HLA-DQB1*03:01 in European, Iranian, and Brazilian population for both BP and MMP, while HLA-DRB1*11:01 has been noted in the Japanese BP population, and HLA-DQA1*05:05 in a portion of the Brazilian and Chinese Han populations. Only two studies have reported on HLA associations in OCP in the US in the early 1990s with 20 and 22 patients, respectively. The goal of our study was to identify the most prevalent BP/MMP HLA associations in North American population and discern whether the data correlates with worldwide associations. We enrolled 84 patients (35 BP, 49 MMP), majority female (69% BP, 78% MMP) and of Caucasian descent (88% BP, 88% MMP). The most frequent alleles in our population were DQA1*05:05 (47.6%), DQB1*03:01 (69.0%), and DRB1*11:01 (26.2%). All three alleles were also found to be significantly ($p<0.05$) overrepresented when compared to the general population (worldwide population frequency vs. frequency in our population: 0.7%, vs 47.6% for DQA1*05:05; 52.1% vs. 69.0% for DQB1*03:01; 10.9% vs. 26.2% for DRB1*11:01). 21.4% of our population inherited all three significant alleles together, with 97.5% of DQA1*05:05 patients also carrying DQB1*03:01 and 45% carrying DRB1*11:01. We also noted that 37.93% of DQB1*03:01 patients carried DRB1*11:01, and 67.24% typed for DQA1*05:05. Finally, when DRB1*11:01 was present, 100% of patients carried DQB1*03:01, and 81.81% carried DQA1*05:05. Our findings suggest that these three genes are in linkage disequilibrium. However, the extent to which each of these molecular subtypes directly impact the autoimmune response in pemphigoid disease remains to be determined.

081

Granzyme K: A potential mediator of psoriasis

K. Richardson[2, 1], C. Turner[2, 1], S. Hiroyasu[2, 1], R. Crawford[1, 3], A. Burleigh[3], D. Granville[2, 1]

[1]Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada, [2]International Collaboration On Repair Discoveries (ICORD), Vancouver, British Columbia, Canada, [3]Dermatology and Skin Sciences, University of British Columbia, Vancouver, British Columbia, Canada

Psoriasis is a common skin disease characterized by skin inflammation and epidermal proliferation forming thick scaly plaques. Current therapies are not ideal and often present with side effects. As such, a deeper understanding of the pathological mechanisms pertaining to the onset and progression of psoriasis are necessary. Granzyme K (GzmK) is a serine protease recently elucidated as a mediator of cutaneous inflammation. Elevated GzmK is observed in human psoriatic skin lesions. However, its role in psoriasis is unknown. We hypothesized that GzmK contributes to the onset and progression of psoriasis through the augmentation of inflammation and/or epidermal proliferation. GzmK expression was evaluated histologically in tissue from psoriasis patients and compared to healthy skin. The role of GzmK was investigated in a murine model of psoriasis, comparing GzmK-/- to WT mice. Psoriasis severity was assessed macroscopically using a modified psoriasis area and severity index (PASI). Psoriatic tissue was examined histologically for epidermal thickness, GzmK expression and inflammatory cell infiltrate. To elucidate a mechanistic role, human keratinocytes were incubated with GzmK. Cell proliferation, cytokine expression and the GzmK degradome were assessed. GzmK positive cells were markedly elevated in lesional human psoriasis skin compared to healthy skin. Lymphocytes and dendritic cells were identified as the predominant cell sources of GzmK. Psoriatic GzmK-/- mice had an average 2.5 point total reduction (modified PASI scale, $p<0.001$) in erythema and plaque formation compared to WT mice. In vitro, GzmK induced keratinocyte proliferation. Elucidation of the GzmK degradome is in progress. In summary, GzmK is elevated in psoriasis and may contribute to increased disease severity. As such, the present study will provide key rationale for pursuing GzmK-targeted inhibitors for the treatment of psoriasis.

082

Topical chemo-immunotherapy of melanoma

A. Mizes, C. D. Carey, B. Friedman, J. Zhang, E. Korkmaz, S. Balmert, G. Erdos, L. D. Falo

Dermatology, University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Anthracyclines, such as doxorubicin (Dox), eradicate tumors by inducing immunogenic cell death (ICD) that both kills tumor cells and facilitates tumor specific immune induction. However, Dox delivered intravenously is associated with systemic toxicity. We have developed dissolvable microneedle arrays (MNAs) that enable direct topical delivery of drugs specifically to the cutaneous tumor microenvironment (TME) without systemic exposure. We hypothesized that application of MNAs delivering Dox to cutaneous tumors, would induce an effective therapeutic antitumor immune response, and that inclusion of a potent TLR3-agonist would improve the tumor specific immune response. Tumors were established by ID injection of B16 cells into C57/Bl6J mice, or by 4HT of B6-Tyr-Cre^{ERT2}Braf^{CA}Pten$^{lox/lox}$ (BRAF) mice. Established B16 and BRAF melanoma tumors were treated two-three times weekly with MNA and the TME was evaluated for proinflammatory changes and immune infiltrates by flow cytometry, immunofluorescence, and RT-qPCR at various time points following MNA application according to established protocols. In both B16 and BRAF melanoma models, tumor bearing mice treated with MNAs demonstrated decreased tumor progression and increased survival, and treated B16 melanoma survivors demonstrated significantly decreased tumor and lung metastases on re-challenge compared to injected naïve mice. Inclusion of the TLR3-agonist improved therapeutic effects. Mechanistically, flow cytometry and fluorescence microscopy demonstrated MNA treatment induced early increases in Ly6G+ neutrophils in treated tumors, with subsequent increases in tumor infiltrating CD4+ and CD8+ T cells. Gene expression and protein analysis demonstrated therapy associated changes consistent with proinflammatory remodeling of the TME. These studies support the clinical development of MNA enabled localized chemo-immunotherapy strategies for the treatment of skin cancer.

083

Linear morphea with evidence of hair regrowth

K. Svigos, M. Criscito, J. Marji, N. K. Brinster, K. Lo Sicco

The Ronal O. Perelman Department of Dermatology, New York University School of Medicine, New York, New York, United States

A 32-year-old male presented for initial evaluation of alopecia on the frontal scalp of eight months duration. On initial clinical examination, he was found to have a 7.0cm x 1.2cm sclerotic plaque on the paramedian forehead extending to the scalp with associated alopecia. No ocular, neurological, or odontostomatologic complications were identified. A 4mm punch biopsy from the frontal scalp demonstrated marked follicular dropout with absent sebaceous glands and presence of perifollicular and perivascular lymphocytic inflammation. There was vacuolar alteration and clefting along the basement membrane with necrotic keratinocytes and evidence of dermal fibrosis. These findings were consistent with a diagnosis of early, inflammatory linear morphea. On dermoscopy, there was perifollicular scale scattered with loss of follicular openings on a pink-white surface. Pinpoint follicular black dots, broken hairs, several short vellus hairs and pili torti were appreciated. Minoxidil was initially trialed without resulting hair regrowth. The patient was subsequently treated with topical calcitriol, desonide cream and fluocinonide cream for one year. Thereafter, he exclusively used pimecrolimus cream twice daily with regrowth of hair noted on clinical and dermoscopic evaluation. Although linear morphea may result in scarring alopecia, our patient remarkably experienced hair regrowth throughout his treatment. Our report demonstrates the importance of dermoscopy in assisting diagnosis of linear morphea and highlights the utility of dermoscopy in monitoring progression or improvement of disease, as evidenced by the short vellus hairs on dermoscopy, as well as a tool to monitor progression or resolution of disease, as evidenced by short vellus hairs on dermoscopy.

084

T cell vaccination using conserved influenza proteins is highly protective against lethal challenge

T. Tian, J. Zhao, Y. Pan, Y. Yan, C. A. Stingley, T. Pan, T. S. Kupper

Dermatology, Brigham Health/Harvard Medical School, Boston, Massachusetts, United States

A possible future influenza A pandemic similar to the 1918 H1N1 outbreak remains an existential public health threat. A universal influenza A vaccine that protects regardless of hemagluttinin (H) and neuraminidase (N) subgroups remains elusive. The influenza genome encodes for a few proteins highly conserved among all flu strains, including NP and M1. We sought to generate a T cell vaccine broadly protective against all influenza A viruses based on this property. Using Modified Vaccinia Ankara (MVA), we created MVA-NP and MVA-M1 vectors. Based on previous experiments to generate lung TM, we administered each to mice through epidermal disruption (ed). After 60 days, lungs were assessed for memory T cells (T_M) using tetramers for NP and M1. Both MVA-NP and MVA-M1 generated tetramer specific lung T_M, with NP being more effective than M1. A second immunization further increased lung T_M numbers. With regard to protective immunity, immunized mice were given a lethal intranasal challenge with H1N1 PR8 influenza virus. Naïve mice uniformly lost weight and succumbed; however, MVA-NP mice immunized through epidermis showed 100% survival. In contrast, MVA-NP administered i.m. led to only 40% protection (consistent with previous studies on route of MVA administration). MVA-M1 was ineffective regardless of route. Since NP resides inside the influenza virion inaccessible to antibodies, protection against lethal influenza challenge induced by MVA-NP was T cell mediated. This approach to influenza vaccination does not depend on generation of antibodies to variable H and N epitopes. MVA vaccines have been safely administered to patients for many years, and we believe this is a promising approach to a universal influenza vaccine.

085

Neutrophils are critical in linear IgA bullous dermatosis in mice

N. Li[1], S. Burette[1], K. Jing[1, 2], E. Mulligan[1], J. Yanik[1], B. Yang[3], M. P. Marinkovich[4], L. Diaz[1], S. Feng[2], Z. Liu[1]

[1]Dermatology, University of North Carolina, Chapel Hill, North Carolina, United States, [2]Institute of Dermatology, Nanjing, China, [3]Shandong Institute of Dermatology, Jinan, China, [4]Stanford University, Stanford, California, United States

Linear IgA bullous dermatosis (LABD) is a skin autoimmune disease characterized by the linear deposition of IgA autoantibodies at the basement membrane zone (BMZ), subepidermal blisters, and neutrophil infiltration. These IgA autoantibodies specifically recognize the hemidesmosomal component BP180 (also called type XVII collagen) and its processed 120 kDa and 97 kDa extracellular regions. The main epitopes recognized by LABD IgA autoantibodies are in the NC16A domain of BP180. Since there is a lack of immune cross-reactivity between mouse and human BP180 in the NC16A domain and lack of receptors for human IgA in mice, we generated a double humanized mouse strain expressing human BP180 NC16A domain and human IgA receptor FcαRI (termed NC16A/FcαRI mice). NC16A/FcαRI mice, when injected with IgA from LABD patients' sera and not normal controls, developed subepidermal blisters 48 h post IgA injection. The lesional skin showed IgA deposition at the BMZ, extensive neutrophilic infiltration and significantly increased levels of proinflammatory cytokines and chemokines. NC16A/FcαRI mice deficient in neutrophils became resistant to anti-NC16A IgA-induced LABD, whereas reconstitution of neutrophils from NC16A/FcαRI mice or normal human subjects restored LABD disease in neutrophil-deficient NC16A/FcαRI mice. NC16A/FcαRI mice lacking the neutrophil homing receptor CXCR2, when injected with anti-NC16A IgA showed delayed and reduced disease. In vitro, anti-NC16A IgA in the presence of recombinant NC16A, activates human neutrophils, resulting in the release of the proteolytic enzymes neutrophil elastase, MMP-9 and inflammatory mediators. These findings suggest that neutrophils are critical for anti-NC16A IgA-induced LABD, and that anti-NC16A IgA-triggered neutrophil recruitment is mediated by both CXCR2-dependent and CXCR2-independent pathways.

088

MVA Vectors delivered through epidermis protect against pulmonary infections

Y. Pan, T. Tian, L. Liu, J. Zhao, R. Clark, T. S. Kupper

Dermatology, Brigham Health/Harvard Medical School, Boston, Massachusetts, United States

Delivery of Vaccinia virus (VACV) as well as VACV vectors by epidermal disruption/skin scarification (ed/ss) promotes optimal T cell and B cell immunity, generating both skin and lung T_{RM} as well as abundant circulating T_{EM} and T_{CM}. We asked whether ed/ss with non-replicating Modified Vaccinia Ankara (MVA) would be similarly effective. To demonstrate safety, MVA was delivered to Rag1-/- mice by ed/ss; all MVA infected mice survived without weight loss or morbidity. MVA, as well as MVA encoding for $OVA^{257-264}$ (MVA_{OVA}) by ed/ss at doses as low as $1.8X10^5$ pfu engendered powerful humoral and T cell responses as well as protective immunity from lethal pulmonary challenge. Optimal T cell responses required both Langerhans cells and dermal DC. MVA_{OVA} delivery by intramuscular (i.m.), intradermal, and subcutaneous routes was much less effective, and generated transcriptionally distinct effector and memory T cells. We compared ed/ss to intratracheal immunization, and found that T effector cells from lymph nodes draining skin and lungs had overlapping transcriptional profiles, respectively. Both routes of immunization generated both lung and skin T_M, indicating shared T cell homing pathways. Finally, we immunized mice via ed/ss or i.m. with MVA_{OVA}, and at 60 days gave a lethal pulmonary influenza infection ($PR8_{OVA}$). Complete protection was seen in ed/ss immunized mice, while 80% lethality and 100% lethality was seen in i.m. immunized and naïve mice. This raises the potential that MVA vectors delivered via ed/ss may be useful as T cell vaccines against pulmonary pathogens, including influenza A.

089

Modulation of the IL-23/Th17 immune axis by enhancement of adenosine A_{2A} receptor ($A_{2A}R$) signaling alleviates psoriasis (PsO)

A. Welihinda, P. Ravikumar, S. Yadav, G. Kang, E. Amento

Molecular Medicine Research Institute, Sunnyvale, California, United States

Dysregulation of the IL-23/Th17 immune axis is a major driver of PsO. The $A_{2A}R$ is a key regulator of both immunity and inflammation. Recently, we described a unique means to enhance $A_{2A}R$ function through a small molecule that acts as a positive allosteric modulator (PAM) of the receptor. The PAM has no intrinsic activity at the $A_{2A}R$ but enhances endogenous adenosine-mediated $A_{2A}R$ function. The PAM alters the function of both mouse and human monocytes, pDCs, cDCs, γδT and $CD4^+$ T cells as well as human epidermal keratinocytes to reduce expression/production of key cytokine mediators implicated in PsO. In imiquimod-induced PsO-like dermatitis, both oral and topical administration of the PAM reduces ear thickness, back skin erythema and scale formation as well as inflammatory cytokine expression in the skin, ear tissue and in plasma. As no single model recapitulates all features of human PsO, we evaluated the PAM in an IL-23-induced mouse model and in an ex vivo human skin model. We found that oral administration of the PAM reduced IL-23-induced ear edema as well as mRNA levels of CCR6, Defb4 and S100A7a. In an ex vivo human skin model in which activation of skin-resident naïve T cells under Th17-skewing conditions mimics the predominately Th17-polarized profile of lesional psoriatic skin, the PAM inhibited production/release of IL-17A, IL-22, IL-10, IFN-γ and TNF-α and attenuated IL-17A mRNA expression, supporting the notion that the PAM impacts directly the transcriptional activity of ROR-γt. In addition, the PAM inhibited IL-17-induced chemokine expression in, and IL-22-mediated proliferation of, human epidermal keratinocytes in vitro. In summary, our findings suggest that enhancement of $A_{2A}R$ responsiveness to endogenous adenosine, targeted through positive allosteric modulation, may be effective in altering the course of Th17 cell-driven diseases such as PsO by altering the immune cascade toward a homeostatic state.

091

B cells are required for development of skin lesions in a mouse model of cutaneous lupus erythematosus

C. Garelli[1], P. Mande[2], S. Moses[2], K. Pike[2], L. Wong[1], A. Marshak-Rothstein[2], J. Richmond[1]

[1]Dermatology, UMass Medical School, Worcester, Massachusetts, United States, [2]Medicine, UMass Medical School, Worcester, Massachusetts, United States

Cutaneous Lupus Erythematosus (CLE) is a spectrum of autoimmune skin diseases that are characterized by interface dermatitis and lupus band reaction, or inflammation and autoantibody deposition at the dermal-epidermal junction. Several triggers have been identified including UV light, drug reactions and smoking, but CLE immunopathogenesis remains to be fully elucidated. There is mounting evidence that B cells play a role in CLE development and in skin manifestations of systemic lupus. We used a mouse model of CLE to test the role of B cells in development of skin lesions. Mice in this model develop IgM and IgG1 autoantibodies, and exhibit T cell accumulation in the skin that positively correlates with severity of skin lesions. Mice lacking B cells are protected from lesion formation, and reintroduction of B cells restores skin disease. B cells from mice with severe skin disease exhibit increased costimulatory molecule expression, and ANA score does not correlate with amount of skin disease, implying that the major role they play is to present antigen to T cells. Taken together, our data support a mechanistic role for B cells in CLE development and support targeting B cell antigen presentation as a novel treatment strategy.

093

Comparison of skin autoimmune diseases by single-cell RNA sequencing

Y. Wang, K. Gellatly, J. Strassner, K. Essien, M. Ahmed, R. Murphy, A. Coffin-Schmitt, X. Fan, X. Ding, M. Frisoli, E. Kim, Z. Abbas, A. Derr, P. McDonel, M. Rashighi, J. Harris, M. Garber

University of Massachusetts Medical School, Worcester, Massachusetts, United States

A common mechanism of autoimmunity is the loss of self-tolerance due to the presence of autoantibodies and autoreactive lymphocytes that cause a continuous immune response. However, autoimmune diseases vary in their symptoms, affected organs, as well as the target cells. How different mechanisms of autoimmunity manifest into diverse symptoms is still not well understood. To address these questions, we have begun a comparative study on different skin autoimmune diseases in order to dissect the common and disease-specific mechanisms of autoimmune disorders. We employed a systems biology approach that combines suction blistering (a non-invasive sample collection method) with single-cell RNA sequencing followed by network analysis of inferred ligand-receptor interactions across epidermal cell types. Our initial analysis compares vitiligo, psoriasis, dermatomyositis (DM), and cutaneous lupus erythematosus (CLE). We find that (i) the upper spinous layer of epidermal keratinocytes are activated in psoriasis, DM, and CLE, indicating the presence of skin damage and repairment that is not found in healthy or vitiligo patients. (ii) Melanocytes, the primary target cell of vitiligo, shows similar alterations of functional gene sets in vitiligo, DM, and CLE, indicating that melanocytes may be a common target in these three diseases. (iii) We identified different patterns of cytokine up-regulation in each disease. Some cytokines are shared across all diseases while others are disease-specific, e.g., CXCL13 in $CD4^+$ T cells is significantly up-regulated in DM while slightly up-regulated in the other three diseases. Our approach combines the power of single-cell genomics and systems biology in order to reveal cell type-specific candidates for both diagnosis markers as well as potential therapeutic targets.

094

Molecular and cellular characteristics of nummular eczema

Y. Wu[1], G. Zhang[1], M. Su[1], X. Zhang[2], Y. Zhou[1]

[1]University of British Columbia, Vancouver, British Columbia, Canada, [2]Dermatology, Huashan Hospital, Shanghai, China

Nummular eczema is the most common non-atopic endogenous eczema of adult onset. However, to date there has been little understanding of its molecular and cellular characteristics. The goal of our study is to define the gene expression profiles and cellular infiltrates of nummular eczema. Biopsies from lesional (LS) and non-lesional (NLS) skin were obtained from patient volunteers with nummular eczema and volunteers with healthy skin (HS). The biopsies were examined histologically for diagnostic confirmation and used for RNA extraction. RNA sequencing was performed to characterize the gene expression profiles and cellular infiltrates using cellular deconvolution algorithms. More than 2000 genes were differentially expressed in lesions of nummular eczema, and these genes were involved in Th2 immune response and in regulation of keratinization. Cellular deconvolution showed that there is a dramatic increase in T cells as well as monocytes and macrophages in the lesional skin. Further, there is heterogeneity among nummular lesional skin both in differentially expressed genes and in the cellular infiltrates. Finally, milder gene expression and cellular infiltrate changes were found even in non-lesional skin of nummular eczema patients. In conclusion, nummular eczema is a heterogeneous systemic disease of the skin that involves global gene expression changes associated with increased infiltration of T cell subsets as well as monocytes and macrophages. These findings may provide useful information in future therapeutic development for nummular eczema and other eczematous conditions.

095

Single-cell Profiling Reveals a Highly Specific, Compartmentalized Functional Response in the Cutaneous Immune System

Y. Liu[2], C. Cook[2], P. Haririchian[2], R. Ricardo-Gonzalez[2], J. P. North[2], A. Charruyer[1], R. Ghadially[1], R. J. Cho[2], J. B. Cheng[1]

[1]Dermatology, University of California, San Francisco and Veterans Affairs Medical Center, San Francisco, California, United States, [2]Dermatology, University of California, San Francisco, San Francisco, California, United States

Effective inflammatory responses arise from coordinated reaction of diverse immune cell populations to exogenous stimuli. The considerable heterogeneity of these populations has impeded high-resolution dissection of their molecular behavior during activation. To systematically characterize cutaneous inflammatory response, we analyzed 52,086 immune cells isolated from mouse skin by single cell RNA sequencing (scRNA-seq), after induction of Toll-like receptor (TLR)-dependent inflammation using imiquimod (IMQ) or delayed-type hypersensitivity using oxazolone (OXA). Differentially expressed genes in these two conditions were identified using Model-based analysis of single-cell transcriptomics (MAST). We report striking induction by IMQ of *Ccl4* and *Ccl5* across disparate antigen-presenting cells, revealing an underappreciated mechanism underlying TLR mediated innate immunity, in addition to well established features, e.g. *Il17/Il22* induction in dermal γδT cells. In contrast, with OXA treatment, we detect sharp restriction of *Il4/Il6/Il13* induction to infiltrating basophils, and also broad priming of immune cell populations by induction of *Ilr4α* ($p<10^{-3}$). Curiously, we also detect pervasive upregulation of *Jak2* but not *Jak1/3* ($p<10^{-14}$), including in *Ilr4α*^{lo} lymphocyte populations such as Tregs and ILC2s ($p<10^{-4}$), suggesting a distinct, targetable pathway active in hypersensitivity reactions. We also discover a set of distinct transcriptional programs which are shared across cells from disparate CD45+ immune cell populations, suggesting a component of single-cell level inflammatory responses appear to be stochastic, rather than innate to cell type. Collectively, this work not only sheds new light on cutaneous inflammatory responses, but also yields systems-level insights into immune cell reactivity on the single cell level.

097

Reactivation of pyoderma gangrenosum associated with anti-programmed cell death 1 therapy

W. Haidari[1], K. Coerdt[1], G. Gibney[2], H. B. Pasieka[1]

[1]Dermatology, Georgetown University, Washington, District of Columbia, United States, [2]Medicine, Lombardi Comprehensive Cancer Institute, Georgetown University, Washington, District of Columbia, United States

Therapies targeting the programmed cell death 1 (PD-1) pathway are effective therapies for a variety of metastatic malignancies. Such therapies are associated with cutaneous eruptions among other immune-related adverse effects. We describe two patients with clinical and histological findings consistent with pyoderma gangrenosum (PG) that was reactivated after receiving PD-1 inhibitor therapy. We successfully treated our first patient with wound care with clobetasol ointment and triamcinolone acetonide injections. She is sustaining remission of her metastatic SCC despite the discontinuation of anti-PD-1 therapy. Our second patient was also successfully treated with intralesional triamcinolone acetonide injections; she remained on PD-1 inhibitor treatment for her Merkel cell carcinoma (MCC). Our cases raise a number of questions, in relation to the mechanism of PG reactivation in patients undergoing treatment with PD-1 blockade and the management of patients with PG while on anti-PD-1 therapy. We describe two novel cases of PG reactivation related to PD-1 inhibitor therapy. Our first patient developed PG at the site of her prior healed ulcer. We postulate that this “recall phenomenon” may be related to tissue-resident memory T (T_{RM}) cells. T_{RM} cells are non-recirculating immune cells residing in peripheral issues where they protect against local infections and cancer.<span style="font-size:10.8333px T_{RM} cells are enriched tumor-specific CD8+ T cells that may trigger neutrophil homing and their cytotoxic activity if activated by anti-PD-1 cancer immunotherapy. T_{RM} cells in the skin also appear to drive many inflammatory cutaneous diseases. We propose that patients with history of PG be closely followed for signs of PG flare when treated with PD-1 inhibition. Early initiation of diagnosis and treatment will minimize the morbidity, as the ulcer will be smaller and easier to heal, and may allow them to continue on their PD-1 inhibitor therapy.

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Comparative RNA-Seq profiling of oral lichen planus and mucous membrane pemphigoid patient samples reveal distinct and shared molecular pathways

S. Jariwala[1], P. Googe[2], D. Rajpal[1], J. Freudenberg[1], D. A. Culton[2]

[1]GSK, Collegeville, Pennsylvania, United States, [2]Dermatology, University of North Carolina, Chapel Hill, North Carolina, United States

Oral lichen planus (OLP) is an inflammatory condition affecting the skin and mucous membranes. While cutaneous lichen planus may self-remit, OLP is typically a chronic and debilitating condition with few effective treatment options. OLP is mediated by cytotoxic T lymphocytes that destroy the basal keratinocytes possibly in response to altered self-antigens or neo-epitopes expressed on lesional keratinocytes. However, the pathophysiology is poorly understood. To explore the underlying molecular pathways involved in OLP, total RNA was extracted from well characterized OLP samples (n=16) and compared to normal mucosal controls (n=7) and both lesional (n=8) and perilesional (n=5) mucous membrane pemphigoid disease controls. Paired-end RNA-Seq at relatively high sequencing depth was completed and differential expression and pathway enrichment analyses were performed. To the best of our knowledge, this is the first RNA-Seq study in OLP. A total of 4,020 genes were differentially expressed in OLP compared to mucosal controls. Most of these changes were driven by immune-mediated pathways, particularly adaptive immunity pathways, a response not broadly observed in the mucous membrane pemphigoid (MMP) samples. In contrast, extracellular matrix related pathways were differentially regulated in both diseases. Upregulation of genes involved in regulation and activation of T lymphocytes, cytokine mediated signaling pathways in response to external biotic stimulus, and dysregulation of genes within the IL23 pathway were noted in OLP. Overall, this study identifies differentially expressed genes in OLP and MMP, and the distinct functional enrichments of their underlying molecular mechanisms, suggesting possible therapeutic targets.

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Competition for active TGFβ eliminates bystander CD8$^+$ T_{RM} from the epidermal niche

T. Hirai[1], Y. Yang[1], Y. Zenke[1], H. Li[1], P. Y. Zhou[1], B. A. Nguyen[1], D. Masopust[2], D. Kaplan[1]

[1]Dermatology, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [2]Departments of Microbiology and Immunology, University of Minnesota, Minneapolis, Minnesota, United States

Epidermal resident memory CD8$^+$ T cell (T_{RM}) provide efficient host defense against neoplasia and pathogens. After antigen-driven expansion in lymph node, differentiation of T cell effectors into T_{RM} and their persistence in the epidermis is independent of cognate antigen but requires TGFβ. T_{RM} that do encounter cognate antigen in the skin outcompete 'bystander' T_{RM} that do not encounter antigen in the skin by unknown mechanisms. We examined the effect of antigen encounter in the skin by comparing Ova-specific OT-I T_{RM} recruited to skin by Vaccinia virus-Ova (VV-Ova) infection where antigen is present with 'bystander' T_{RM} recruited by epicutaneous application of the chemical sensitizer DNFB at a different site within the same animal. We found that mAb or small molecule inhibition of TGFβ activation efficiently depleted OT-I T_{RM} from the DNFB-treated 'bystander' site but not the VV-Ova infected site. Application of exogenous OVA peptide rendered 'bystander' OT-I T_{RM} in DNFB-treated site resistant to depletion by inhibition of TGFβ activation, confirming antigen encounter in skin determine the sensitivity to the deletion. Moreover, following challenge with unrelated antigen, T_{RM} that had originally encountered antigen in the skin during the primary challenge were better able than 'bystander' T_{RM} to compete with newly recruited cells. Alternatively, T_{RM} that received augmented TGFβ signaling outcompeted pre-existing T_{RM} as well as newly recruited cells. These data suggest that competition for limiting amounts of active TGFβ is a mechanism that rids the epidermis of 'bystander' T_{RM} and allows for the persistence and concentration of antigen-specific T_{RM} in the epidermis.

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Engineering the skin with microneedle arrays to induce immune tolerance

S. C. Balmert, C. D. Carey, G. Erdos, E. Korkmaz, S. R. Little, L. D. Falo

University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Aberrant immune responses to foreign and self-antigens can result in allergic contact dermatitis, autoimmunity, and transplant rejection. Current treatments often use anti-inflammatory drugs that broadly and transiently suppress inflammation, but fail to address the underlying immune dysfunction. Here, we present a translational, skin-centric approach to induce systemic antigen-specific tolerance, using dissolvable microneedle arrays (MNAs) to deliver antigens together with MC903, a vitamin D3 analog and tolerogenic immune modifier, to the cutaneous microenvironment. In murine models of protein- or hapten-mediated contact hypersensitivity (CHS), naïve or sensitized mice were treated with MNAs prior to sensitization and/or challenge. Hypersensitivity responses were then evaluated by ear swelling and histology, and T-cell responses assessed by flow cytometry. Supporting clinical translation, we also characterized murine and human skin microenvironments and migratory DCs by qRT-PCR and flow cytometry. Co-delivery of allergens with MC903 significantly reduced pro-inflammatory cytokine expression in both murine and human skin. Furthermore, treatment of murine and human skin with allergen+MC903 MNAs promoted migration of DCs with more tolerogenic phenotypes (e.g., reduced expression of costimulatory receptors and increased expression of inhibitory receptors). Function of human migratory DCs was also modulated by inclusion of MC903, and these DCs induced less T-cell proliferation in allogeneic mixed leukocyte reactions. Finally, tolerization or desensitization of mice with antigen+MC903 MNAs reduced subsequent hypersensitivity responses at distal sites in an antigen-specific manner, and significantly enhanced the ratio of regulatory to effector T cells in skin draining lymph nodes. These results suggest simultaneous introduction of antigen and tolerogen into the skin with MNAs can promote systemic antigen-specific tolerance, which may have broad implications for inflammatory disorders, autoimmunity, or transplantation.

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ATF6-MARCH5-MFN2 axis regulates melanoma cell survival upon ER stress through mitochondrial dynamics

H. Wang, W. Guo, T. Gao, C. Li

Department of Dermatology, Xijing Hospital, Xi'an, Shaanxi, China

Solid tumor microenvironment typically characterizes by hypoxia and nutrients deprivation, which generally results in severe endoplasmic reticulum (ER) stress in tumor cells. It has been shown that cancer cells are relatively resistant to environmental and pharmacological ER stress-induced cell death. However, the underlying mechanism is far from understood. Mitochondrial dynamics have been greatly implicated in determining cell fate in response to stress. Herein, we demonstrate that ATF6-MARCH5-MFN2 axis-mediated mitochondrial dynamics remodeling functions as a pro-survival mechanism in melanoma cells upon ER stress through the activation of mitophagy. We proved that exogenous ER stress significantly induced melanoma cell death and meanwhile mitochondrial fission. Moreover, the inhibitor of mitochondrial fission, Mdivi-1, rendered melanoma cells more sensitive to ER stress-induced cell death. While ER stress decreased the expression of MFN2, induced mitochondrial fragmentation and triggered mitophagy in melanoma cells, overexpression of MFN2 inhibited mitochondrial fragmentation and mitophagy, sensitizing melanoma cells to ER stress-induced cell death. Further, we found that the down-regulation of MFN2 protein accounted for ER stress-induced mitochondrial fission, and the proteasome inhibitor MG132 suppressed the down-regulation of MFN2 under ER stress inducers. More importantly, ER stress inducers treatment could activate ATF6 pathway and thereby up-regulated E3 ligase MARCH5, which was responsible for the ubiquitination and degradation of MFN2. The knockdown of ATF6, rather than IRE1α or eIF2α, rescued ER stress-induced MFN2 down-regulation. Overall, our results demonstrated that ATF6-MARCH5-MFN2 axis-mediated mitochondrial dynamics acted as an intrinsic protective signaling to alleviate ER stress-induced cell death through the activation of mitophagy. The inhibition of mitochondrial fission could be a potent synergic approach for melanoma treatment with ER stress inducers.

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The molecular context of vulnerability for CDK9 suppression in melanoma

S. M. Guhan[1, 2], M. Shaughnessy[2], A. Rajadurai[2], R. Kumar[2], Z. Ji[2], N. Gray[3], H. Tsao[2]

[1]Harvard Medical School, Boston, Massachusetts, United States, [2]Wellman Center for Photomedicine, Massachusetts General Hospital, Boston, Massachusetts, United States, [3]Dana Farber Cancer Institute, Boston, Massachusetts, United States

In unpublished studies, we recently found that a cyclin-dependent kinase 9 (CDK9) degrader (Thal-SNS-032, TS-032) selectively inhibited BRAF/NRAS wild type melanomas. We now confirm this finding using another competitive CDK9 inhibitor, NVP-2. To further explore the context of this vulnerability, we undertook a large RNA seq analysis of 9 pigment cell lines (1 triple wild-type melanoma line, 2 uveal lines, 2 BRAF-mutated, 2 NRAS-mutated, 1 NF1-mutated, and 1 immortalized melanocyte line) in order to uncover transcriptional programs which may be preferentially extinguished or activated by TS-032 and NVP-2. At 8 hours after treatment, NVP-2 more robustly depleted transcripts compared to TS-032 (2539 differentially expressed genes (DEG) versus 1 DEG) consistent with the immediate effects of a small molecule inhibitor versus a degrader. Unsupervised clustering showed separation among cell lines rather than between DMSO, NVP-2 and TS-032 suggesting that there is not a unique "drug" signature. Since NVP-2 exhibited a greater transcriptional effect at 8 hours, we performed a meta-analysis of pathways that are selectively impacted by NVP-2 in triple wild-type/uveal melanoma lines compared to BRAF/NRAS/NF1-mutated lines. ToppGene analysis indicated a strong "cell cycle" program (FDR B&H=4.778E-102) with the E2F transcriptional network being preferentially targeted (E2F Q4 transcription factor binding site FDR B&H=1.317E-15; E2F1 Q6 01=1.631E-14; E2F Q6=3.853E-14; E2F 03=6.220E-13; E2F1 Q6=6.220E-13). Overall, our results suggest that genes involved in the cell cycle lead to the unique vulnerabilities in triple wild-type and uveal cell lines against CDK9 inhibition. We may have further identified a unique "Achilles cluster" of genes anchored with the E2F pathway in triple wild-type and uveal melanomas.

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Trends in the interest of indoor tanning beds internationally

A. Misitzis[3, 1, 2], A. Fonseca[1, 2], M. A. Weinstock[1, 2], A. Stratigos[3]

[1]Dermatology, Brown University, Providence, Rhode Island, United States, [2]Center for Dermatoepidemiology, Providence Veteran Affairs Medical Center, Providence, Rhode Island, United States, [3]Dermatology, National and Kapodistrian University of Athens, Athens, Greece

Keratinocyte carcinoma and melanoma are associated with a history of indoor tanning bed use. Despite strong evidence for the effect of indoor tanning on skin cancer and photo-aging, people continue to use UV-emitting tanning devices. We sought to assess and compare interest in indoor tanning among countries with different indoor tanning regulations with *Google Trends.* From 2004 - 2019 we extracted search queries for "σολαριουμ" ("solarium") which is greek for indoor tanning and "indoor tanning" for the United States, "solarium" for Australia, France, Sweden, Belgium, Italy, Spain and Brazil. For the period studied there was a significant increase in the interest in "solarium" in Greece (β: 1.60, 95% Confidence Interval (CI): 1.18 - 2.01, p<0.001), Sweden (β: 1.45, CI: 1.05 - 1.84, p< 0.001) and Belgium (β: 0.77, CI: 0.37 - 1.17, p<0.001). The interest for "indoor tanning" experienced a significant decrease in the United States (β:-1.54, CI: -1.93 - -1.14, p<0.001). Likewise, decrease was noted for the term "solarium" in France (β: -1.53, CI: -2.24 - -0.82, p<0.001), Australia (β: -1.27, CI: -1.61 - -0.93, p<0.001) and Brazil (β: -2.33, CI: -2.71 - -1.95, p<0.001). No significant change was observed for Spain (β: 0.01, CI: -0.41 - 0.44, p:0.951) and Italy (β:-0.42, CI: -0.88 - 0.47, p:0.078). Many states in the US have banned minors' use of commercial tanning parlors. Furthermore, the American Academy of Dermatology and others are consistently advocating against indoor tanning with campaigns on media that reach younger, more susceptible to tanning age groups. Indoor tanning is illegal in Australia and Brazil for all age groups, which was associated with a decrease in interest. Systematic efforts and bolder measures may have a significant preventative effect against indoor tanning and if implemented in other countries could reduce tanning bed use.

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XPC splice site founder mutation in families with xeroderma pigmentosum from Dominican Republic and Honduras

S. G. Khan, D. Tamura, J. J. DiGiovanna, K. H. Kraemer

Lab Cancer Biol and Gen, NCI, Bethesda, Maryland, United States

Xeroderma pigmentosum (XP) is a rare autosomal recessive DNA repair disorder. XP is caused by mutations in 7 nucleotide excision repair genes, XP-A to XP-G, and *POLH* gene involved in trans lesion synthesis pathway by which cells replicate across UV-induced DNA lesions. XP-C is the most common in the U.S. XP patients are highly sun-sensitive with 10,000-fold increased risk of skin cancer. In a cohort of 123 XP patients and their families enrolled at the National Institutes of Health (NIH), we identified a founder *XPC* splice site mutation (c.2251-1G>C) in 8 XP patients (4 from the Dominican Republic, 2 from Honduras and 2 from Tanzania). They all had extensive sun exposure with multiple skin cancers and severe ocular damage (blindness or ocular cancer). Seven XP patients were homozygous for the splice mutation and one was a compound heterozygote. Microsatellite analysis surrounding the *XPC* founder mutation indicates that these patients might share a common ancestor. This splice mutation has been reported as very frequent in parts of Africa (1/5,000 in Mayotte). This African *XPC* founder mutation may have been brought to the Dominican Republic and Central America as part of the slave trade that flourished in the 16th to 18th centuries. Other founder mutations have been reported in XP patients in Japan (*XPA)* and Morocco (*XPC,* TG-deletion). The increase in frequency of *XPC* founder mutations in Dominican Republic and Honduras would have public health implications. The early detection and rigorous UV protection can improve XP patient clinical outcomes in these countries.

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Increased prevalence of thyroid nodules in xeroderma pigmentosum patients: A feature of premature aging

S. K. Kouatcheu[1, 2], J. Marko[3], C. R. Lee[4], D. Tamura[1], M. Rafeld[4], S. G. Khan[1], J. J. DiGiovanna[1], K. H. Kraemer[1]

[1]Lab Cancer Biol and Gen, NCI, Bethesda, Maryland, United States, [2]NIH Academy, Bethesda, Maryland, United States, [3]Radiology, NIH Clinical Center, Bethesda, Maryland, United States, [4]Pathology, NIH, NCI, Bethesda, Maryland, United States

Xeroderma pigmentosum (XP) is a rare autosomal recessive disease of DNA repair characterized by severe ultraviolet (UV) sensitivity resulting in a 10,000-fold increased risk for skin cancer. Symptoms include freckle-like pigmentation in sun exposed skin before age 2 years, severe burns after minimal sun exposure (50% of patients) and skin cancers in children. XP is also considered a premature aging disease because of its features such as: dry skin, increased sun-induced freckling in young patients, higher incidence of skin cancers in young patients and premature menopause. In our NIH XP cohort of 123 patients examined from 1971 till 2019, 29 patients were examined by thyroid ultrasound. The 18 (62%) patients with thyroid nodules had mutations in XP-C (14 patients), XP variant (2) and unknown (2). The median age of patients with thyroid nodules in our XP cohort (27 years) was younger than that of 3 control groups: 34 yr (UCSF – 208 subjects); 47 yr (Korea – 24,757 subjects) and 46 yr (NIH – 682 research subjects). There were 1 to 4 nodules per patient with TI-RADS scores (a measure of possible malignancy) from 1 to 4. Thyroid examination at autopsy of 8 additional XP patients in our cohort (2 XP-A, 4 XP-C, 1 XP-D and 1 XP-V) revealed follicular adenomas in the 4 XP-C patients. Two additional XP patients in the study had papillary thyroid cancer excised. One had a pathogenic mutation in the *PTEN* gene and the other had a rare fusion of *TFG-NTRK1* genes that may be amenable to drug therapy if the cancer recurs. XP patients may have an increased and early prevalence of thyroid nodules or cancer relative to the general population. This reveals another premature aging feature of XP. DNA repair may play a role in preventing malignancy in the thyroid gland.

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OVOL2/ZEB1 axis restricts the transition from actinic keratosis to cutaneous squamous cell carcinoma

M. Murata[1], T. Ito[1], Y. Tanaka[1], K. Yamamura[1], K. Furue[1], G. Tsuji[1, 2], M. Furue[1, 2]

[1]Dermatology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, [2]Research and Clinical Center for Yusho and Dioxin, Kyushu University Hospital, Fukuoka, Japan

Cutaneous squamous cell carcinoma (cSCC) is the second most common skin cancer and actinic keratosis (AK) is one of the precancerous lesion of cSCC. It is necessary to control the development because there are not enough treatments established for advanced cSCC. Most cases of AK are restricted to the epidermis for a long time through the suppression of epithelial-to-mesenchymal transition (EMT). While ovo like transcriptional repressor 1 (OVOL1) and ovo like zinc finger 2 (OVOL2) are important modulators of EMT in some tumors, little is known in skin tumors. The aim of this study was to elucidate the role of OVOL1 or OVOL2 in AK and cSCC. We investigated an A431 human SCC cell line, 30 AK and 30 cSCC clinical samples using molecular biological approaches and immunohistochemistry. Among EMT-related factors, knockdown of *OVOLs* increased the mRNA levels of *vimentin* and *zinc finger E-box-binding homeobox 1* (*ZEB1*). Vimentin protein was increased by OVOL1 knockdown. ZEB1 protein was increased by both OVOL1 and OVOL2 knockdown. Immunohistochemical analysis showed that the expression of OVOL1 and OVOL2 were upregulated in AK but downregulated in cSCC whereas vimentin and ZEB1 showed opposite patterns. In addition, the OVOL2-low tumors exhibited higher ZEB1 expression than that of OVOL2-high tumors. In this study, we have shown that OVOL1 and OVOL2, especially the latter, play roles as the important modulators, and there are an inverse association between OVOL2 and ZEB1 expression in the transition from AK to cSCC.

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Characterization of Polyomavirus encoded-circular RNAs in Merkel Cell Carcinoma
R. Yang[1], E. E. Lee[1], S. Willcox[4], J. Kim[2], C. Sullivan[5], J. Zhao[1], J. Griffith[4], R. Wang[3]
[1]Dermatology, UT Southwestern Medical Center, Dallas, Texas, United States, [2]Quantitative Biomedical Research Center,, UT Southwestern Medical Center, Dallas, Texas, United States, [3]Dermatology, UT Southwestern Medical Center, Dallas, Texas, United States, [4]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States, [5]Department of Molecular Biosciences, University of Texas, Austin, Texas, United States
Circular RNAs (circRNAs) are an emerging class of RNAs with diverse functions. We previously described human papillomavirus derived circRNAs with transforming activity. Here, we describe circular RNAs encoded by Merkel Cell Polyomavirus (MCPyV), including two that are circular forms of the previously described alternative open reading frame (ALTO) gene in the early region of MCPyV (circALTO1 and circALTO2). CircALTOs can readily be detected MCPyV-positive Merkel Cell Carcinoma (MCC) by both inverse PCR and northern blot. Both circALTOs contain the ALTO open reading frame and are able to ALTO peptides. CircALTOs are stable, predominantly located in the cytoplasm, and modified with N^6-methyladenosine (m6A). Thus, we describe the first known polyomavirus-encoded circRNAs (circALTO1/2), which encode for proteins, and may contribute to both MCPyV replication and MCC tumorigenesis.

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RNA expression analysis in stage IVA-B cutaneous T-cell lymphoma to identify novel biomarkers of prognosis and diagnosis
F. N. Mirza, S. Yumeen, J. M. Lewis, A. O. King, S. Kim, K. R. Carlson, F. M. Foss, M. Girardi
Department of Dermatology, Yale School of Medicine, New Haven, Connecticut, United States
Cutaneous T-cell lymphoma (CTCL) is a primary non-Hodgkin lymphoma and the most common primary cutaneous lymphoma. Due to its varied clinical and histological presentation, the accurate diagnosis of CTCL is difficult and therefore delayed; in advanced stages, CTCL is incurable and often fatal, though treatment-resistant phenotypes and survival time can be difficult to predict. There remains an unmet need for new and more effective diagnostic and prognostic indicators and novel therapies. To this end, malignant cells were isolated from the peripheral blood of 15 CTCL patients using antibody-magnetic bead or FACS sorting for CD3+CD4+ and CD7- and/or CD26- based on the known aberrant phenotype of the patient or positive selection for CD45RO+ CD4+ T cells in 3 healthy controls. Samples were submitted for RNA-Seq using the Illumina platform and differential gene expression analysis was completed with a cutoff of FDR ≤0.05 used for significance. A Cox-proportional hazards regression for survival was conducted on quantiles of differentially expressed genes. The median purity of cells was >98% and the median alignment was >95%. A principal component analysis successfully demonstrated two separate populations – CTCL patients and healthy controls. A total of 563 genes were found to be significantly differentially expressed, of which 426 were upregulated. At least 18 of these genes – *CCR4*, *KLHL42*, *IGFL2*, *CD70*, *CXCL13*, *DNM3*, *KIR3DL2*, *NEDD4L*, *PLS3*, *TLR9*, *TNSF11*, *TOX*, *TWIST1*, *IFI44*, *TGFBR2*, *DPP4*, *IDO1*, and *TUB4A* – have been described before in the literature in connection with CTCL. 67 upregulated cell surface biomarkers have been identified for further validation studies. In age, sex, comorbidity, and treatment adjusted models, 39 genes were identified as prognostic markers with a p ≤0.05; of particular interest include *DPP4* and *CD70*, which have previously been correlated with outcomes. Genes identified in this analysis may represent novel diagnostic or prognostic biomarkers in CTCL.

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Defining the immune tumor microenvironment in a genetic mouse model of multistep squamous cell carcinogenesis
N. Sakakibara[1], P. Clavijo[2], A. George[1], V. Gray[1], K. King[1], R. Ponnamperuma[1], C. Van Waes[2], C. Allen[2], W. Weinberg[1]
[1]CDER, FDA, Silver Spring, Maryland, United States, [2]NIDCD/NIH, Bethesda, Maryland, United States
Squamous cell carcinomas of the head and neck (HNSCC) frequently harbor amplification of chromosome 3q26-28, which encodes proto-oncogene *TP63* and is a feature common to SCCs of multiple organ sites. HNSCC are typically marked by a heavy immune cell infiltrate. The development and clinical response of these tumors reflect an imbalance between anti-tumor immune responses and mechanisms of immune evasion. Using lentiviral vectors to model the overexpression of ΔNp63α, we have established in primary murine keratinocytes that lenti-ΔNp63α enhances nuclear localization and activation of NF-kB/c-Rel, a known mediator of inflammatory responses. Using a nude mouse orthotopic grafting model, we showed that elevated levels of ΔNp63α cooperate with the oncogenic H-Ras pathway to drive malignant conversion of H-Ras-initiated papillomas. Here, we adapted the grafting model to immune competent syngeneic mice to profile changes in the tumor microenvironment (TME) during malignant conversion. Papillomas (H-Ras expressing) and carcinomas (H-Ras/ΔNp63α) were harvested 2, 3, and 4 weeks post-grafting. Neutrophilic myeloid-derived suppressor cells (PMN-MDSCs) significantly increased at 2 weeks in carcinomas compared to papillomas. Preliminary data indicate that this population decreased over the next 2 weeks in carcinomas, while increasing in papillomas. An increase in the number of CD4+ T cells, CD8+ T cells, and regulatory T cells was also seen in both groups at 2 weeks compared to grafts of normal primary keratinocytes, with a further increase in CD8+ T cells over time in carcinomas relative to papillomas. qPCR studies indicate that overall, carcinomas express higher levels of chemokines/receptors associated with an immunosuppressive TME. Thus, altered immune infiltrate by ΔNp63α/H-Ras-expressing carcinomas may establish an immunosuppressive TME at early time points post-engraftment, allowing outgrowth of the malignant phenotype.

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Differential molecular expression patterns and risk for metastasis in cutaneous squamous
cell carcinoma: A systematic revi
P. Mulvaney, P. Massey, J. Drinan, C. Schmults
Dermatology, Brigham & Women's Hospital, Boston, Massachusetts, United States
Cutaneous squamous cell carcinoma (CSCC) is a common malignancy with an estimated incidence of 1,000,000 U.S. cases per year. A vast majority of tumors are cured with surgical removal, however, up to 4% of CSCCs will go on to develop metastasis to regional lymph nodes or distant sites. Current risk stratification is performed using clinical and histopathologic tumor information. The role of molecular expression testing is poorly established but may provide improved accuracy in prognostication as well as therapeutic insights. Using PubMed/MEDLINE and EMBASE, a systematic review was performed to identify and examine studies published between January 1st, 2005 and September 1st, 2019 that reported risk of metastasis or death from CSCC in humans with respect to tumor protein or RNA expression. An initial search yielded 436 records; 335 abstracts and 81 full text articles were screened and reviewed. Inclusion criteria were met by 45 studies containing 81 evaluations of 44 distinct proteins and 25 miRNAs. For analysis, studies results were divided into two categories: primary CSCCs tracked for poor outcomes (n=62, 2,267 subjects), and primary CSCC compared to metastatic tissue (n=19, 479 subjects). Significant results (p-value<0.05) of tumors tracked for poor outcomes were found in 50% (27/54) of evaluations reporting metastasis and 41% (12/29) reporting death. In evaluations comparing primary to metastatic tissue, 32% (6/19) detected significant differences in molecular expression. On pooled analysis, high expression of PD-L1 (OR 2.34, 95% CI 1.09-5.02, p=0.030), EGFR (OR 2.57, 95% CI 1.24-5.33, p=0.011) and podoplanin (OR 2.33, 95% CI 1.00- 5.41, p=0.049) conferred increased odds for metastasis. PD-L1 expression tissue differed between primary and metastatic tissue (OR 3.13, 95% CI 1.00-9.75, p=0.049). These results indicate further studies are needed to determine if immunohistochemical staining for PD-L1, EGFR, and podoplanin expression can aid in CSCC prognostic estimations and/or predict
response to therapy.

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Aspirin protects melanocytes and keratinocytes from UV-induced DNA damage *in vivo*

H. Rahman[1], D. Kumar[1], S. Florell[2], N. Okwundu[1], C. E. Burd[3], D. Grossman[1, 2]

[1]Huntsman Cancer Institute, Salt Lake City, Utah, United States, [2]Dermatology, University of Utah, Salt Lake City, Utah, United States, [3]Molecular Genetics, Cancer Biology and Genetics, Ohio State University, Columbus, Ohio, United States

Ultraviolet (UV) radiation promotes skin cancer through multiple mechanisms, which can be secondary to inflammation, oxidative stress, and DNA damage. UV-induced DNA mutations may arise from disrepair of cyclobutane pyrimidine dimers (CPD) or 8-oxoguanine (8-OG). We investigated whether the anti-inflammatory activities of aspirin (ASA) could protect against UV-induced DNA damage and skin cancer in mouse models. Skin from adult C57BL/6 mice receiving 0.4 mg ASA daily by gavage had reduced inflammation (CD3+ T-lymphocytes, CD272+ lymphocytes/NK cells, Ly6G+ neutrophils, F4/80+ macrophages), fewer sunburn cells, and lower 8-OG levels than skin from control animals (gavaged with only water) 48 h after acute UVB exposure (600 J/m^2). Significant reductions in UV-induced sunburn cells and 8-OG lesions were also seen in the skin of both adult and neonatal melanoma-prone (Tyr:CreERT2;LSL-Nras^{61R}) TN^{61R} mice treated with ASA. ASA treatment also decreased plasma and skin prostaglandin (PG) E_2 levels, and lowered CPDs levels in melanocytes and keratinocytes from TN^{61R} mice as compared to control-treated animals. ASA did not, however, delay melanoma onset in *TN^{61R}* mice receiving a single, neonatal UVB exposure (1000 J/m^2), although treatment with ASA did lower tumor burden and tumor cell proliferation as compared to control-treated animals. Finally, ASA reduced DNA damage in skin and PGE_2 in plasma and skin of squamous cell carcinoma (SCC)-prone SKH1-E mice following acute UVB exposure (1200 J/m^2) and delayed SCC onset induced by chronic UVB (700-2100 J/m^2, three times weekly), compared to control-treated mice. These results indicate that ASA can protect against UV-induced inflammation in skin and reduce UV-induced DNA damage in both melanocytes and keratinocytes, although these effects translated into greater chemopreventive efficacy for UV-induced SCC than melanoma in mouse models.

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Analyzing the metabolic demands of genetic alterations observed in squamous cell carcinoma

M. Moses, A. George, N. Sakakibara, K. King, C. Sourbier, W. Weinberg

CDER, FDA, Silver Spring, Maryland, United States

Squamous cell carcinoma (SCC) of various organ sites is frequently characterized by amplification of *TP63*, leading to elevated levels of the ΔNp63α isoform. In a murine orthotopic grafting model of multistage carcinogenesis, overexpression of ΔNp63α cooperates with oncogenic ras (v-ras^{Ha}) to drive malignant progression *in vivo*. Metabolic changes are a hallmark of cancer, including SCC, and preliminary gene array data from our lab revealed altered mRNA levels of metabolic genes in primary murine keratinocytes expressing elevated levels of ΔNp63α and/or v-ras^{Ha}. Furthermore, both v-ras^{Ha} and ΔNp63α are known to play a role in regulating glycolysis and oxidative phosphorylation. As such, we evaluated the metabolic characteristics of primary murine keratinocytes using Seahorse technology to test if v-ras^{Ha} and elevated ΔNp63α levels cooperate to meet the energetic demands of SCC. v-ras^{Ha} and/or ΔNp63α were introduced by retro/lentiviral gene transduction and analyzed for basal metabolic activity using the Seahorse XFe96 Analyzer with CyQUANT data normalization. v-ras^{Ha} led to a dramatic increase in basal glycolysis and respiration, while ΔNp63α slightly decreased respiration. In combination, ΔNp63α mitigated the v-ras^{Ha}-dependent metabolic effects. We next tested if ΔNp63α imparts energy source flexibility to control or v-ras^{Ha}-expressing primary mouse keratinocytes and impacts their maximal energy output during stress. v-ras^{Ha}-expressing mouse keratinocytes displayed higher maximal respiration and glycolytic rates with increased spare energy capacity over control and ΔNp63α-expressing mouse keratinocytes during chemically induced stress, while co-expression of exogenous ΔNp63α and v-ras^{Ha} partially reversed the v-ras^{Ha}-dependent metabolic effects. Given that high metabolic activity can lead to excess reactive oxygen species, these data suggest the role of ΔNp63α may be multifaceted; *e.g.*, by controlling metabolic flux to a level that is permissive for cell survival in stressful environments associated with hallmarks of cancer.

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Dysregulated estrogen signaling through CYP1B1 contributes to Notch deficiency in squamous cell carcinoma

L. Yang[1, 2], X. Li[5, 3], Y. S. Brooks[3, 4]

[1]Division of Nephrology and Hypertension, Vanderbilt University Medical Center, Nashville, Tennessee, United States, [2]Radiation and Medical Oncology, Zhongnan Hospital of Wuhan University, Wuhan, Hubei, China, [3]Cutaneous Biology Research Center, Massachusetts General Hospital, Boston, Massachusetts, United States, [4]Department of Dermatology, Harvard Medical School, Boston, Massachusetts, United States, [5]King's Lab, School of Pharmacy, Shanghai Jiaotong University, Shanghai, China

Squamous cell carcinomas (SCCs) represent the most frequent human solid tumors and are a major cause of cancer mortality. It has been long recognized in the clinic that the incidence rates of SCC arising in different organs exhibit a gender disparity which cannot be attributed to lifestyle difference alone. Previously, we showed that ERβ mediated estrogen signaling activates the Notch pathway which confers cancer protection to females through inducing squamous differentiation. In this study, we further demonstrated that CYP1B1, a heme-containing monooxygenase that catalyzes multiple chemicals including estrogen is overexpressed in SCCs from multiple organs. Inhibition of CYP1B1expression or activity attenuated proliferation of SCC cells and promoted NOTCH1 expression and squamous differentiation both in vitro and in mouse xenotransplants. Overexpression of CYP1B1 in HKCs downregulated Notch1 expression as well as activation which was not observed using enzyme-dead CYP1B1. Tracing of metabolized estrogen compound by CYP1B1 revealed that the chemical bound to DUSP3 and consequently activated ERK signaling. Our study identified a link between dysregulated estrogen signaling and Notch downregulation in SCCs, with implications for novel cancer therapy.

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A dynamic panel of UV signature genes for risk stratification of cutaneous actinic keratosis and squamous cell carcinoma subtypes

D. Queen[1], Y. Shen[2], A. Lopez[1], M. H. Trager[1], F. Samie[2], J. Lewin[2], L. Geskin[2], L. Liu[3]

[1]Vagelos College of Physicians and Surgeons, Columbia University, New York, New York, United States, [2]Dermatology, Columbia University, New York, New York, United States, [3]The Hormel Institute, University of Minnesota, Austin, Minnesota, United States

Actinic keratoses (AKs) are premalignant cutaneous lesions, with a small percentage (<5%) eventually developing into squamous cell carcinomas (SCCs). SCC is generally curable except a subset of aggressive SCCs characterized by increased tendency for recurrence and metastasis. Currently, there is no test for identifying high-risk AKs and aggressive SCC subtypes. Biomarker-based molecular testing represents a promising tool for risk stratifying these cutaneous lesions to guide clinical diagnosis and treatment. Here, we evaluated the utility of a novel UV biomarker panel in identifying SCC-prone AKs and aggressive SCC subtypes. We utilized a custom NanoString CodeSet to perform paired mRNA expression analysis of 57 UV biomarker genes in 10 pairs of AK and matched normal skin (NS) and 31 pairs of SCC and NS. The fold change for each biomarker gene expression between every AK-NS and SCC-NS pair was derived and analyzed by statistical testing, unsupervised hierarchical clustering, and principal component analyses, which categorized the clinical samples into subgroups depending on the expression signature of selected biomarkers. The 57-gene signature divided the samples into two major groups, a UV-group and a non-UV group, indicating UV-independent factors that may contribute to a subset of cutaneous lesions. Based on the clinical and histological data, a 6-gene panel not only identified SCC-prone AKs but also more aggressive SCC subtypes. Furthermore, a 2-gene panel effectively classified these clinical samples as either low- or high-risk lesions with approximately 75% consistency with histopathologic information. These analyses highlight the interesting potential of the UV biomarker gene subsets as molecular classifiers for risk stratifying AK and SCC lesions, which will complement or improve histopathologic diagnosis in identifying high-risk patients.

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Dysregulated m6A methyltransferase METTL3 suppresses acral melanoma proliferation and migration through p38/ERK pathways

Z. Yue, Y. Wang, J. Sun

Institute of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College, Nanjing City, Jiangsu Province, China

Background: Although many biological processes are involved in the modification of N6-methyladenosine (m6A), the exact role of m6A in the development of malignant tumors remains unclear. Methyltransferase 3 (METTL3) is a major RNA n6-methyladenosine methyltransferase. We aimed to explore the role of METTL3 in Acral melanoma carcinogenesis and disease progression. Methods: In this study, Q-PCR, Western blots and immunohistochemistry were performed to identify the difference expression of METTL3 between acral melanoma tumor tissue and paratumor tissue. m6A content was analyzed by using RNA Methylation Quantification Kit. Q-PCR and Western blots were used to evaluate the expression of METTL3 in melanoma cells. The effect of METTL3 on cell proliferation, migration and invasion of melanoma cells was examined by cell count, CCK8 assay, wound healing assay and transwell assay, respectively. In vivo role of METTL3 was studied on xenograft models. Results: We found that the level of m6A in tumor tissues was significantly increased, and m6A methyltransferase METTL3 expression was significantly increased in tumor tissues, which was related to tumor stage. METTL3 was overexpressed in melanoma cell lines, together with increased m6A content. Functionally, silencing of METTL3 by shRNA in melanoma cell lines resulted in decreased m6A content, cell proliferation, migration, and invasion. Moreover, the inhibitors of p38 or Erk kinase could significantly reverse the effect of migration and invasion, which was induced by knockdown of METTL3. Moreover, depletion of METTL3 inhibited tumor growth in vivo. Conclusion: We concluded that the m6A methyltransferase METTL3 promotes the growth and motility of acral melanoma cells through P38/ERK pathways.

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In vivo cellular reprogramming facilitates viral T antigen-driven Merkel cell carcinoma development in adult mice

M. E. Verhaegen, J. Van Goor, J. Arche, P. Harms, D. Wilbert, H. Zabawa, M. Patrick, L. C. Tsoi, A. Chinnaiyan, A. A. Dlugosz

University of Michigan, Ann Arbor, Michigan, United States

Merkel cell carcinoma (MCC) is a rare and aggressive neuroendocrine skin cancer that frequently carries integrated Merkel cell polyomavirus (MCPyV) sequences and expresses viral small T antigen (sTAg) and a truncated large T antigen (tLTAg). MCC cells also express a complement of signature genes detected in normal, postmitotic Merkel cells (MCs), including Atoh1, which is required for MC development from epidermal progenitors. Although the link between MCPyV and MCC was discovered 12 years ago, the development of an adult mouse model of MCC has not yet been reported. We now present the successful use of *in vivo* cellular reprogramming as a powerful tool for facilitating full-blown murine MCC development from epidermal progenitors, mimicking the developmental biology of normal MCs. We generated mice to conditionally express MCPyV sTAg, tLTAg, and Atoh1 in K5+ epidermal cells and their progeny, yielding microscopic collections of proliferating MCC-like cells that failed to produce gross tumors. Immunostaining of these nascent tumor-like aggregates revealed a robust DNA damage response, p53 accumulation, and apoptosis. Deletion of one copy of p53 in this model led to the development of gross skin tumors which exhibited classic histologic features and multiple protein markers mimicking human MCC, including Sox2, Isl1, Insm1, dot-like Keratin 8 expression, as well as immune cell infiltration. Principal component analysis of RNA-seq data established close similarity of mouse and human MCCs at the global transcriptome level, which was reflected in hierarchical clustering showing consistent upregulation of multiple key MC signature genes. These data establish cellular reprogramming as a novel tool that has enabled, for the first time, the development of MCPyV TAg-driven murine tumors that are remarkably similar to human MCCs based on comprehensive analysis at the levels of histopathology, protein marker expression, and transcriptome.

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A systematic review provides insight into genes mutated in metastatic SCC

M. Lobl[1], D. Clarey[1], C. Schmidt[2], A. Wysong[1]

[1]Dermatology, University of Nebraska Medical Center, Omaha, Nebraska, United States, [2]Libraries, University of Nebraska Medical Center, Omaha, Nebraska, United States

Background: Cutaneous squamous cell carcinoma (SCC) is responsible for approximately 1 million cases annually in the United States. Metastatic SCC (3-5%) carries a poor prognosis. Genes that increase metastatic risk are not fully understood. Objective: To perform a systematic review of all cases of SCC with gene mutation data in the literature and statistical analysis to determine if there are genes that are mutated in significantly more metastatic SCCs compared to localized SCCs. Methods: A systematic review was performed in July 2019, yielding 3017 initial articles. After screening for inclusion and exclusion criteria, 127 full text articles remained. After screening for individual-level mutation data, 11 articles remained for inclusion. The Chi Square variance of proportions test was used. Results: Genes that had significantly different proportions of mutations in metastatic SCC compared to localized SCC were CDKN2A (44% (n=90) vs 28% (n= 189), p=0.0068), TERT (39% (n=33) vs 3% (n=38), p<0.0001), and TP53 (82% (n=90) vs 58% (n=189), p<0.0001). Genes that were mutated in more localized SCCs compared to metastatic SCCs were CREBBP (26% (n=46) vs 9% (n=76), p=0.013), EP300 (30% (n=46) vs 14% (n=76), p=0.04), MLL2 (45% (n=84) vs 26% (n=90), p=0.007), MLL3 (40% (n=180) vs 13% (n=48), p=0.0004), NF1 (23% (n=56) vs 9% (n=86), p=0.02), NOTCH2 (41% (n=170) vs 20% (n=80), p=0.001), SPEN (29% (n=134) vs 4% (n=75), and SPTA1 (56% (n=38) vs 27% (n=33), p=0.01). Discussion: Our findings support a recent study that found TERT mutations to be correlated with an elevated risk of metastasis (p<0.05). CDKN2A and TP53 mutations have been previously found to be increased in metastatic SCC compared to localized SCC. Mutations in MLL2 have been previously reported to occur more often in metastatic SCC than localized SCC, in contrast to what was observed in this study. Further research with larger sample sizes and investigation into mechanistic effects in SCC progression would be useful.

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Correlation between skin viscoelasticity and tumor progression during aging

S. Park[1], A. Chien[2], Y. Chen[1]

[1]Mechanical Engineering, Johns Hopkins , Baltimore, Maryland, United States, [2]Dermatology, Johns Hopkins Medical Institutes, Baltimore, Maryland, United States

Aging is accompanied by the decline of biological and physical functions in living organisms. It is widely acknowledged that increased prevalence of skin cancer in the elder population is primarily caused by accumulated DNA damage that is left unrepaired by overwhelmed and less efficient genomic maintenance machinery, which ultimately lead to harmful mutations. Recent evidences imply the additional possibility that mechanical properties of the aging skin, including altered stiffness, elastic modulus, and shear modulus, significantly contribute to the accelerated pace of tumor progression. However, systematic comparisons of young and aging skins across the body in humans or animal models were not performed. We developed a device to measure human or mouse skins *ex vivo* or *in vivo*. We observed that abdominal skins in 2.5 year-old female mice are 6-fold stiffer than 2 month-old mice; overall relaxation time of old skin is 2-fold higher. To examine whether stiffness and viscosity promote cancer in elderly patients, we first tested the activity of tumor suppressor p53, since p53 activity declines with age. Moreover, accumulated gain of function mutations (GOF) p53 mutations during aging further facilitate tumor progression. To evaluate the relation between p53 activity and skin mechanical properties, substrates with matching mechanical values based on measurement results are fabricated. Cancer cells are cultured on these skin-mimetic substrates. No change was observed in wildtype p53. But multiple GOF p53 mutants are more stable in stiffer substrates by avoiding protein degradation. Furthermore, the phenotype of carcinoma-associated fibroblasts was observed in fibroblasts in aging, stiffer skins, suggesting altered mechanical properties in aging skins correlate with tumor progression. Parallel experiments will be performed in the skin tissues collected from the elder patients with skin cancer.

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Identification of GAPDHS as a novel regulator of melanoma metastasis and metabolic plasticity

J. G. Gill[1, 2], V. Ramesh[2], S. Leef[2], A. Rao[2], T. Mathews[2], Z. Zhao[2], S. Morrison[2]

[1]Dermatology, University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Children's Research Institute, University of Texas Southwestern Medical Center, Dallas, Texas, United States

Cancer metastasis is a complex, multi-step process involving periods of proliferation, invasion, migration, dormancy, and tumor re-establishment. Successfully metastasizing cancer cells must display metabolic plasticity to fulfill the high bioenergetic demands of these cellular processes in a wide range of environmental contexts. The factors which regulate this metabolic plasticity in melanoma cells are largely unknown. To identify novel metabolic regulators of metastasis, we performed a large-scale RNA-sequencing of multiple ex vivo patient-derived xenograft primary melanomas and their associated metastases. Through this screen, we identified glyceraldehyde-3-phosphate dehydrogenase, spermatogenic (GAPDHS) as an enzyme consistently downregulated during melanoma metastasis. Although normally only expressed in spermatocytes, we found GAPDHS to be expressed in the majority of melanomas and not in other cancers. In addition, melanomas expressed a novel isoform of GAPDHS lacking the proline-rich N-terminal domain responsible for subcellular localization in spermatocytes. GAPDHS expression was highest in primary human melanomas and was downregulated in both metastases and conditions of higher oxygen tension. Through in vivo metastasis assays, we found that knocking down GAPDHS in melanomas accelerated metastasis and increased overall tumor burden. Conversely, maintaining expression of GAPDHS in melanomas limited their ability to metastasize to distant sites. Through in vitro and in vivo metabolomics and 13C-labeled isotope tracing, we have identified the impact GAPDHS expression has on both glycolysis and related pathways. Further studies are underway to determine whether GAPDHS expression can predict the metastatic efficiency and metabolic phenotype in human melanoma patients.

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In situ hybridization assay for detection of Merkel cell polyomavirus in Merkel cell carcinoma

C. Wang[1], M. Eid[2], H. Jiang[1], S. Hewitt[1], I. Brownell[1]

[1]National Institutes of Health (NIH), Bethesda, Maryland, United States, [2]University of Virginia, Charlottesville, Virginia, United States

Merkel cell carcinoma (MCC) is a rare and aggressive neuroendocrine skin cancer. In a majority of cases, MCC is associated with the Merkel cell polyomavirus (MCPyV). However, there is currently no gold standard for detecting MCPyV in MCC tumors. Common methods for detecting virus include polymerase chain reaction (PCR) and immunohistochemistry staining (IHC). Recently, in situ hybridization (ISH) and high throughput sequencing have been applied to determine MCC viral status. We compared a novel ISH viral detection assay to PCR and IHC for accurate detection of MCPyV in MCC samples. ISH probes using branched DNA colorimetric detection were designed to target MCPyV invariant early region DNA/RNA containing T antigen (MCV-T Ag E) and late region DNA (MCV 3075-5210). MCC tissue microarrays (TMA) from two institutions containing 43 cases (Heidelberg) and 67 cases (University of Washington) were assayed. True viral status of each case was defined as a consensus of the three tests with 90 (82%) cases being virus positive. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were determined for each assay. Sensitivity for ISH, PCR, and IHC were 98%, 93%, and 96%, respectively. Specificity was less robust with 70%, 85%, and 75%, respectively. Overall, ISH compared favorably with PCR and IHC for the detection of MCPyV in MCC tumor samples. Our data suggests that a multimodal approach can optimize sensitivity and specificity to detect the presence of MCPyV. Accurate detection of MCPyV in MCC will allow for further investigation of the virus and its role in guiding management in MCC patients.

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ROCK inhibition reduces Ras-induced senescence in primary mouse keratinocytes

L. Li, A. J. Lee, S. Yuspa

Laboratory of Cancer Biology and Genetics, National Cancer Institute, Bethesda, Maryland, United States

The Rho-associated protein kinase (ROCK) is responsible for the regulation of cell adhesion, shape and migration by altering cytoskeletal structure. The ROCK inhibitor, Y27632, protects cells from anoikis and enhances embryonic stem cell viability in culture and cryopreservation. Y27632 prolongs the life span of cultured primary mouse keratinocytes. Oncogenic RAS transduced primary mouse keratinocytes (RAS-keratinocytes) become senescent in vitro. Here, we show that chronic treatment of Ras transduced primary mouse keratinocytes (RAS-keratinocytes) with Y27632 reduces the number of senescence associated β-galactosidase positive cells which are flat and enlarged. Oncogenic RAS induces Cyclin D1 and p19Arf in RAS-keratinocytes which contribute to the cellular senescence. Y27632 attenuated RAS-induced Cyclin D1 and P19Arf. Y27632 treated RAS-keratinocytes also showed higher levels of Cyclin B1, indicating more cells undergoing mitosis. RAS-keratinocytes express many components of senescence-associated secretory phenotype, including MMP9, GM-CSF, and many other proinflammatory proteins which are suppressed by Y27632. Y27632 treatment of RAS-keratinocytes increased DNA synthesis measured by EdU incorporation and total cell numbers. We performed RNA-seq analysis to study the global changes and specific pathways associated with Y27632 treatment of RAS-keratinocytes and identified 113 genes that are commonly up or down regulated in cells treated with Y28632 for one and three weeks using Qlucore. Using Ingenuity Pathway Analysis (IPA), we found that the top IPA network is formed by 22 molecules among the 113 gene list. Within this network, downregulated TGFβ is one of the central nods. IPA upstream analysis also found that TGFβ1 is predicted to be inhibited in Y27632 treated cells. In vivo xenograft experiments show that Y27632 treated RAS-keratinocytes can form tumors. Our data indicate that ROCK inhibition may prevent oncogenic RAS-induced senescence to promote cell proliferation and extend the life span of cultured RAS keratinocytes via suppressing the TGFβ pathway.

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Small Non-coding RNAs Interact with ERK2 and Effect MAPK/ERK Pathway

Z. Siprashvili[1], R. M. Shenoy[1], L. Elcavage[1], P. Khavari[1, 2]

[1]Dermatology, Stanford University, Stanford, California, United States, [2]VA Palo Alto Healthcare System, Palo Alto, California, United States

Small non-coding RNAs play fundamental roles in biology. Among them are snoRNAs, which are best studied for their role in modifying RNA nucleosides. Of the two major snoRNA classes, H/ACA box snoRNAs are known to enable RNA pseudouridylation while C/D box snoRNAs facilitate RNA methylation.137/412 annotated snoRNAs do not have defined RNA targets, and are designated as orphan snoRNAs. Recent data suggest that snoRNAs may have broader impacts relevant to human disease, including in genetic disorders, metabolic stress, and neoplasia. To identify new roles for snoRNAs in disease, we hybridized all non-clustered orphan snoRNAs consist of 30 C/D and 26 H/ACA box snoRNAs to 9200 recombinant human proteins. Unexpectedly, 4/26 H/ACA box snoRNAs and 5/30 C/D box snoRNAs directly bound an essential kinase in the MAPK protein kinase cascade, ERK2 (MAPK1). MicroScale Thermophoresis (MST) analyzes confirmed one of the H/ACA box snoRNA SNORA12 interaction with EKR2 with Kd=362nM. A CRISPR/Cas9 dual-cut gRNA lentiviral vector was used to produce *SNORA12* snoRNA knockout clones from human cancer cell lines. SNORA12 deletion reduced ERK2 activation, the effect that was rescued by ERK2 forced expression. The analysis of the status of 104 phosphorylation sites on 62 proteins relevant to MAPK signaling in wild-type and *SNORA12* knockout cells supported SNORA12 effect on MAPK/ERK pathway, while in vivo tumorigeneses study showed reduced tumor growth in SNORA12 depleted cells. Taken together, the resulting data demonstrated that SNORA12 controls ERK2 kinase activity by modulating ERK2 protein-protein interactions in highly specific ways. These findings identify SNORA12 as a new essential regulator of MAPK signaling in cancer and further extend the biological roles for small non-coding RNAs to the control of cellular signal transduction.

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The effect of PM 2.5 from three rural USA areas on inflammatory markers in human keratinocytes

H. Sun[1], T. Kluz[1], M. Matsui[2], T. Carle[2], D. Gan[2], T. Gordon[1], A. Ortiz[1], M. Costa[1]

[1]Environmental Medicine, New York University School of Medicine, New York, New York, United States, [2]Mary Kay Skin and Clinical Research, Dallas, Texas, United States

Poor air quality in large cities has shown to have a negative effect on human health and to induce skin inflammation and premature aging. Less attention has been paid to rural environments, thus the effects on skin of specific rural pollutants are largely unknown. In addition, the combined effect of rural PM and solar radiation is also of interest. Here we investigate the effects of fine particulate matter ($PM_{2.5}$) collected from rural environments on interleukin (IL) and matrix metalloprotease (MMP) production in human neonatal keratinocytes in the absence or presence of solar simulated UVR (ssUVR). Human neonatal keratinocytes were exposed for 24 hours to $PM_{2.5}$ collected from three different rural areas (Goshen NY, Ann Arbor MI, and Davis CA) with or without pre-exposure to ssUVR (3J). The levels IL6 and MMP1 were analyzed by ELISA to assess the outcome of exposures to PM, UV, or both. IL6 production was induced by exposure to ssUVR or PMs alone, and exposure to both was more than either alone. Interestingly, the three sampled PMs displayed different IL6 induction potencies, with the highest induction from Goshen $PM_{2.5}$ and the least induction from Ann Arbor $PM_{2.5}$. These differing potencies were further reflected by MMP-1 induction, with the highest from Davis and the least from Ann Arbor. To explore the potential mechanism underlying IL6 induction by different PMs, the levels of NRF2, an upstream regulator of IL6, were analyzed in keratinocytes exposed to PMs, UV, or both. Our results indicated that, similar to IL6 production, NRF2 increased differently in response to PMs from different US areas, Inductively coupled plasma mass spectroscopy (ICP-MS) analysis revealed higher levels of Cr, As, Ni, Pb, and Cd in the Goshen $PM_{2.5}$ compared to the Davis and Ann Arbor, which may explain the greater activity of the Goshen $PM_{2.5}$. Taken together, these data suggest populations residing in rural environments are not immune to the damaging effects of air pollution on the skin.

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Merkel cell polyomavirus small t antigen activates non-canonical nf-κb signaling to promote tumorigenesis

J. Zhao[1], Y. Jia[2], S. Shen[2], J. Kim[3], E. E. Lee[1], I. Brownell[4], J. H. Cho-Vega[7], J. Homsi[6], R. Sharma[5], R. Wang[1]

[1]Dermatology, UT Southwestern Medical Center, Dallas, Texas, United States, [2]Children's Research Institute, UT Southwestern Medical Center, Dallas, Texas, United States, [3]Clinical Sciences, UT Southwestern Medical Center, Dallas, Texas, United States, [4]Cutaneous Development and Carcinogenesis, NIAMS, Bethesda, Maryland, United States, [5]Surgery, UT Southwestern Medical Center, Dallas, Texas, United States, [6]Medicine, UT Southwestern Medical Center, Dallas, Texas, United States, [7]Pathology, University of Miami, Miami, Florida, United States

Multiple human polyomaviruses (HPyVs) can infect the skin, but only Merkel Cell Polyomavirus (MCPyV) has been implicated in the development of a cancer, Merkel Cell Carcinoma (MCC). While expression of HPyV6, HPyV7, and MCPyV small T antigens (ST) all induced a senescence associated secretory phenotype (SASP), MCPyV ST uniquely activated non-canonical NF-κB (ncNF-κB), instead of canonical NF-κB signaling to evade senescence. MCPyV activated NFKB2 and RELB transcription through H3K4 trimethylation and promoted the post-translational stability and processing of NFKB2 by inhibiting FBXW7. In addition to inducing SASP gene expression and promoting cell proliferation, we identify unexpected roles for ncNF-kB in resolving endoplasmic reticulum stress, which prevents apoptosis, and in promoting PD-L1 expression. The ncNF-κB pathway is active in virus-positive patient MCCs, and its inhibition prevented the growth of MCC xenografts. Thus, we identify unique, tumorigenic roles for ncNF-κB signaling which are essential for tumorigensis in MCC.

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The relationship between FGFR1 and mevalonate in adiposity-associated cancer: Implications of statins for chemoprevention

V. Benham, W. Shoemaker, J. Bernard

Michigan State University, East Lansing, Michigan, United States

The relationship between excess adiposity and skin cancer risk is not fully understood. Studies demonstrate an inverse correlation between non-melanoma skin cancer (NMSC) and excess body weight, as assessed by body mass index (BMI). However, the Swedish Obese Subjects study demonstrated that bariatric surgery was associated with a reduced risk of both melanoma and NMSC. Based upon these opposing findings, further studies are warranted to understand how excess adiposity influences skin carcinogenesis. Our previous *in vitro* studies demonstrated that FGF2 secreted by visceral adipose tissue (VAT) activates FGFR1 leading to oncogenic Ras protein induction and malignant transformation of mammary epithelial cells (MCF-10A) and JB6 P^+ epidermal cells. Using a screen to identify agents that block FGFR1-stimulated transformation, we identified fluvastatin, a HMG-CoA Reductase (HMG-CR) inhibitor, as a potential chemopreventive agent. We hypothesize that FGFR1 activation by VAT promotes malignant transformation of epithelial cells by stimulating HMG-CR activity. HMG-CR is the rate limiting step in the mevalonate pathway and HMG-CoA is metabolized to intermediates that farnesylate Ras, enabling Ras activation. *In vitro* data demonstrate that VAT stimulates HMG-CR protein expression as well as its downstream isoprenoid synthases and that this associates with Ras protein expression. These effects are prevented with either fluvastatin or a FGFR1 inhibitor. FGFR1 somatic mutation has previously been implicated with unfavorable prognoses in metastatic melanoma. Moreover, analysis of gene-sequenced melanomas from the Cancer Genome Atlas (TCGA) database shows a significant correlation between FGFR1 gene copy number and increased mevalonate gene copy number (p=0.00122). Together, our data implicate the VAT-FGFR1-Mevalonate pathway and its downstream mediators, such as Ras, in malignant transformation, and suggest that they may serve as targets for cancer prevention in the context of excess adiposity.

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Systemic chemotherapy promotes HIF-1α mediated glycolysis and IL-17F pathways in Mycosis Fungoides

B. Wang[1], J. Zheng[2], X. Shen[2]

[1]Dermatology, University of Michigan, Ann Arbor, Michigan, United States, [2]Dermatology, Ruijin Hospital, Shanghai, China

Systemic chemotherapy is often the last resort of advanced Mycosis Fungoides (MF). Tumor reoccurrence and adverse effects of systemic chemotherapy are the main limitations. Here, we aim to investigate the metabolic alterations in tumor cells after CHOP chemotherapy. In advanced MF, CHOP chemotherapy has no survival benefit and the duration of response is significantly inferior to other canonical treatments. HIF-1α is significantly elevated in lesions of advanced MF patients as well as tumor cell line Hut78 and tumor xenograft mice model. CHOP therapy also increased glycolytic activities in a HIF-1α-dependent manner. In xenograft tumor mice model, lesional cells showed a significant increase in IL-17F after chemotherapy, shifting towards a Th17 phenotype, which process is also regulated by HIF-1α. Echinomycin, HIF-1α inhibitor, was co-administered in xenograft tumor mouse models with CHOP and showed a significant reduction in tumor growth. In conclusion, CHOP chemotherapy promotes glycolysis and IL-17 pathways in a HIF-1α-dependent fashion. Furthermore, HIF-1α blockade is promising as an accompanying agent in systemic chemotherapy for patients with advanced MF.

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Clonal dynamics and the earliest steps of carcinogenesis in chronically UV-exposed skin

L. Tordesillas, S. Avdieiev, M. Abdalah, O. Chavez Chiang, Z. Chen, L. S. Simoes, Y. A. Chen, R. A. Gatenby, E. R. Flores, J. S. Brown, K. Y. Tsai
H. Lee Moffitt Cancer Center, Tampa, Florida, United States

BACKGROUND: How chronic UV exposure changes clonal dynamics and expands genomic diversity in the initiation and progression of skin cancer remains unclear. Here we describe our initial findings characterizing clonal dynamics and transcriptional signatures in response to chronic UV exposure by multicolor lineage tracing. We interpret our results in a framework of carcinogenesis in which initial heritable genomic changes then require sufficient cell turnover and release from normal tissue constraints to form tumors. METHODS: To perform *in vivo* live imaging of clonal dynamics in the skin, we used "Confetti" mice (K14CreERT2; R26R-Brainbow2.1; Hairless compound mutant). We traced lineages of K14+ keratinocytes using multicolor fluorescent readouts generated by in-vivo confocal imaging following UV exposure. RESULTS: Following low-dose UV exposure, there were significantly fewer clones in exposed areas, but their mean sizes differed by some 15-fold, with an over 6-fold increase in variance. The outgrowth of larger clones in UV-exposed skin not only compensates for lost clones but suggests the initiation of clonal selection. Epidermal progenitor cell compartments constitute physical barriers to expansion and UV may disrupt space constraints by inducing cell death and permitting fitter cells to clonally expand into neighboring compartments. scRNAseq revealed the generation of transcriptional diversity with two distinct groups of signatures – transient ones only observed in the presence of UV-exposed skin (metabolism), and ones that persisted through tumors (differentation, inflammation). CONCLUSIONS: Our initial results validate the ability to assess the outgrowth of potentially malignant clones in the skin upon chronic UV exposure. Tissue damage reduces the number of progenitor cells, and permits a high variance in proliferation rates among neighboring clones that can then initiate further clonal selection. The "runway" for carcinogenesis therefore becomes much longer when skin is exposed to UV.

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Evolutionary and molecular determinants of resistance to UV-induced skin cancer in naked mole rats

S. Avdieiev[1], E. Vice[2], C. Whelan[1], R. A. Gatenby[1], E. R. Flores[1], T. Park[2], J. S. Brown[1], K. Y. Tsai[1]
[1]H. Lee Moffitt Cancer Center, Tampa, Florida, United States, [2]University of Illinois, Chicago, Chicago, Illinois, United States

Background. Naked mole rats (NMR) are unusually long-lived and unusually resistant to aging and spontaneous cancer development. NMR live colonially in underground tunnel systems. They are under no direct selection to develop and maintain adaptations against UV-induced tissue damage. Here we test NMR's resistance to UV-induced DNA damage and skin cancer, and we probe for potential anti-cancer mechanisms. Methods. We exposed NMR and SKH1 Hairless mice to low-dose UV for three months. We monitored animals for lesions, and sampled skin at multiple time points. Another group of animals received an hour long acute exposure to UV during which we took skin punches before, and 1, 6, and 24 hours after exposure. We used skin punches for histology, RNAseq, and IHC analyses. Results. No NMR developed tumors after chronic UV, with no apparent adverse effects after 12 months. All Hairless mice developed tumors within 2 months of UV exposure. Curiously, within 4 weeks the NMR exhibited skin sloughing akin to molting that covered 58% of the UV-exposed back. Relative to the mice, NMR had a blunted p53 response which took unusually long to stabilize (24 hours vs. 1 hour) and significantly less epidermal apoptosis. RNAseq revealed upregulation of TCA cycle, pyruvate metabolism, and mitochondria-related and oxidative phosphorylation metabolic pathways in acutely irradiated NMR epidermis. Relative to mice, we observed downregulation of DNA repair pathways and cell cycle checkpoints in NMR. Increased energy production might not be used for intra-cellular damage repair but instead for cell functioning and proliferation. Conclusions. Failure to induce skin cancer suggests that NMR have tumor-suppressor mechanisms operant in all tissues including for UV induced damage. Unlike elephants and humans, the molecular basis seems to include down-regulating p53-dependent responses, and at least temporarily tolerating DNA damage while upregulating cell metabolism for proliferation: "keep calm and carry on".

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Decreased cytotoxic T cells, decreased cytotoxic/regulatory T-cell ratio, and decreased TCR clonality are associated with increased numbers of primary cutaneous squamous cell carcinomas in solid organ transplant recipients

N. Doudican[2], N. Frazzette[2], A. Khodadadi-Jamayran[1], A. Tsirigos[1], J. Carucci[2]
[1]Applied Bioinformatics, NYU Langone Med Cntr, New York, New York, United States, [2]Dermatology, NYU Langone Med Cntr, NY, New York, United States

Background: We developed a novel analytical method to define tumor infiltrating lymphocyte (TIL) phenotype in immune competent vs. immune suppressed cSCC patients based on data obtained from single cell sequencing and gene expression. Methods: CD8+ TILs (n = 34,399 for cSCC, n = 14,902 for TSCC) obtained from fresh tumor specimens from immunocompetent (n = 5) versus organ transplant recipients (OTRs; n=6) were subject to single-cell RNA profiling matched with T-cell receptor (TCR) sequencing via barcoding. Data were analyzed using iCellR, a custom R packaged we developed for single cell sequencing analysis. Antigens recognized by the top 10 clonotypes for each sample were assessed using McPAS. Results: Gene expression analysis showed TSCC had fewer cytotoxic cells (56% vs. 66%, $p < 0.0001$), fewer naïve cells (18% vs. 26% $p < 0.0001$), similar numbers of regulatory cells (8% vs. 5%) and similar numbers of exhausted T cells (9% vs. 10%). CD8+ TILs from TSCC exhibited more homogeneous gene expression compared with immunocompetent patients. TILs from both OTRs and immunocompetent patients showed clonality. However, fewer TCR clonotypes were observed in immune suppressed transplant patients (mean = 544 vs. 1140, $p < 0.05$). Many of the TCR sequences represent antigens previously reported in melanoma, other carcinomas and viral infections. The majority of TCR sequences for the top 10 clonotypes of each sample have known antigens, but up to 24% of these sequences recognize putative neoantigens. Solid organ transplant recipients in our study showed increased numbers of cSCC events over 12 months (6.2 vs. 1.2, $p < 0.01$). Conclusion: TSCC TILs tend to show decreased cytotoxic CD8+ TIL, decreased cytotoxic/regulatory T cell ratio and increased exhausted and naïve T cells and decreased cytotoxic T cells. This may contribute to the higher numbers of primary cSCC seen in OTRs.

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Copy number gain at chromosome 7q21 potentiates the large cell transformation in cutaneous T cell lymphoma

F. Liu[1], X. Ren[2], Y. Wang[1]
[1]Department of Dermatology and Venereology, Peking University First Hospital, Beijing, China, [2]Biomedical Pioneering Innovation Center (BIOPIC), Peking University, Beijing, China

Mycosis fungoides (MF), the most common type of cutaneous T cell lymphoma (CTCL), may undergo large cell transformation (LCT), which is related to aggressive clinical courses and resistance to conventional treatments. However, the mechanisms of LCT remain largely unknown. To explore the molecular pathogenesis underlying the transformation of MF, we performed whole-transcriptome sequencing (RNA-seq) followed by integrated deep analyses on lesional biopsies from 49 tumor-stage MF patients, including 26 patients with LCT and 23 patients without LCT (NLCT). Transcriptome profiling revealed that LCT samples were enriched in pathways related to cell-cycle, metabolism, histone deacetylase and ubiquitin-proteasome system. Inferring copy number alterations from RNA-Seq data via InferCNV showed copy number gains at chromosome 7q21-7q22 in LCT. Paternally expressed 10 (*PEG10*), located at chromosome 7q21, was the most significantly upregulated gene in LCT samples compared with NLCT. *PEG10* gene was upregulated along with disease progression and related to poorer clinical prognosis. Silencing *PEG10* in CTCL lines inhibited cell proliferation and conferred increased sensitivity to bortezomib and SAHA. Further analysis on PEG10 silenced and over-expressed CTCL lines demonstrated a PEG10-KLF2-NF-kB axis responsible for the resistance to bortezomib and SAHA. Targeting PEG10 with a small-molecule E2F inhibitor, HLM006474, transcriptionally decreased PEG10 expression, reduced cell proliferation, and reversed the resistance to bortezomib and SAHA in CTCL cells. These results provide new insights into the mechanisms underlying transformed MF. PEG10 may serve as a novel therapeutic target in MF with LCT, which does not have a cure.

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Identification of long noncoding RNAs in mouse hair follicle stem cells using computational methods

N. Paidimukkala[1], R. Kuang[1], R. Morris[2]

[1]Bioinformatics and Computational Biology, University of Minnesota, Minneapolis, Minnesota, United States, [2]The Hormel Institute- University of Minnesota, Austin, Minnesota, United States

Gene expression profiling, especially deep sequencing, has contributed significantly to our understanding of hair follicle stem cell biology; however, roles played by non-coding RNAs, especially the long non-coding RNAs (lncRNAs) for the most part remain undefined. We performed Illumina paired end sequencing and received raw reads for stem- and non-stem cell samples. FASTQC quality control was performed and adaptors were trimmed using Trimmomatic. We aligned the reads to the reference genome using Tophat. For mRNAs, we used USCS mm10 mouse annotated genome and GENCODE for lncRNA. Differential gene expression was calculated using DESeq between stem cell and non-stem cell. From DeSeq analysis, we found 2776 mRNA and 829 lncRNA that were statistically significant (Bonferroni *p*-value < 0.05 and fold change >2). Among the top-ranked genes, we found known keratinocyte stem cell genes (KSC): CD34, Keratin-15 (Krt15) and S100 family members. LncRNAs identified included known (Pvt1) and novel lncRNAs. Interestingly, we found an antisense lncRNA near a cluster of differentially expressed histone genes. To query function, we used parameters such as genomic location, co-expression analysis by Pearson coefficient correlation, and GO terms associated with co-expressed protein coding genes. After identifying the lncRNA pairs using bioinformatics, further biological validation involves RNA scope, qRTPCR, knock down of lncRNA-mRNA pairs *in vitro*, and *in vivo* effects on cell proliferation and carcinogenesis. These studies will determine the role of lncRNAs in hair follicle stem cells. Thus, we sequenced lncRNAS in conjunction with nearby mRNAs and compiled a unique data set focused on enriched mouse CD34+/CD49f+ hair follicle stem cells. We conclude that this approach furthers understanding of lncRNAs roles in KSCs and cancer, and will enable tissue specific patterns observed in human homologs of lncRNA-mRNA pairs to validate them as potential biomarkers and targets for treatments in mouse and human.

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AP-1 and TGFß cooperativity drives non-canonical Hedgehog signaling in resistant basal cell carcinoma

C. Yao[1], D. Haensel[1], S. Gaddam[1], T. Patel[1], S. Atwood[2], K. Sarin[1], S. McKellar[1], S. Aasi[1], K. Rieger[1], A. E. Oro[1]

[1]Program in Epithelial Biology, Stanford University, Stanford, California, United States, [2]UC Irvine, Irvine, California, United States

Tumor heterogeneity and lack of knowledge about resistant cell states remain significant barriers to effective targeted cancer therapies. Basal cell carcinomas (BCCs) uniformly depend on Hedgehog (Hh)/Gli signaling for cell growth. We previously identified a nuclear myocardin-related transcription factor (nMRTF) resistance pathway that amplifies Gli1 activity, but definition of the nMRTF cell state, key factors driving its accumulation, and a therapeutic strategy targeting this cancer cell state, remain unknown. Here, we use single cell sequencing of patient tumors to demonstrate that the nMRTF cell state resembles transit-amplifying cells of the hair follicle matrix, and identify three surface markers (LYPD3, TACSTD2, and LY6D) which reliably correlate with activity and serve as robust biomarkers to highlight Smo-inhibitor-resistant tumor cell populations. Epigenetic analysis of isolated nMRTF human tumor subpopulations demonstrates that cooperative AP-1 and TGFß signaling drive nMRTF activation. Mechanistically, JunD/AP-1 signaling drives changes in chromatin accessibility leading to differential Smad3 DNA binding and a transcriptional program of upstream activators of Rho, including RhoGEFs that facilitate nMRTF activity. Importantly, we observe that small molecule AP-1 inhibitors selectively target LYPD3+/TACSTD2+/ LY6D+ nMRTF human BCCs explants. Overall our work for the first time defines tumor subpopulations based on sensitivities, opening an avenue for improved combinatorial therapies.

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All-trans Retinoic Acid Inhibits Cell Proliferation through Upregulation of TET2 in Squamous Cell Cancer

X. Zhang, L. Shi, Z. Chu, S. Geng

Dermatology, The Second Hospital Affiliated to Xi''An Jiaotong University, Xi'an, China

Cutaneous squamous cell carcinoma (cSCC) is the second most frequent non-melanoma skin cancer. All-trans retinoic acid (ATRA) has been previously proposed anti-tumor potential via inhibiting cancer cell growth by binding to the intracellular retinoic acid receptors (RAR). The ten-eleven translocation 2 (TET2) proteins oxidize the epigenetic mark 5-methylcytosine to 5-hydroxymethylcytosine (5hmC) and loss-functions of TET2 leading to hypermethylation have been established in various cancers. However, its role in cSCC remains unknown. In the present study, we detected the expression of 5hmC, TET2 and retinoic acid receptor B (RARb) in normal skin, actinic keratosis (AK), Bowen disease and cSCC clinical samples by immunohistochemical staining. It's significant downregulation of 5hmC and TET2 were observed in the AK, Bowen disease and cSCC tissues compared with normal skin, while expression of RARb was reduced. Human primary keratinocytes and cSCC cell lines (Scl-1 and HSC-1) were cultured *in vitro*, receiving stimulation of retinoic acid. The results showed that treatment with ATRA inhibited the proliferation of cSCC cell lines and increased the expressions of TET2 and RARb in cSCC cell lines. Moreover, ectopic overexpression of TET2 in cSCC cell lines induce upregulation of RARB while no obvious expression changes of TET2 were observed in RARB overexpressed cSCC cell lines. Our results suggest that TET2 and 5hmC were decreased in cSCC tissue and cSCC cell lines compared to normal tissues and human primary keratinocytes. In addition, ATRA promotes the expression of TET2 in cSCC cell lines, which is closely related to RARb. This study provides evidence that 5hmC and TET2 have the potentials to serve as biomarkers that identified presence and progression of cSCC. It identified a novel function of ATRA in promoting a TET-mediated epigenetic regulation suggesting that the availability of ATRA in cancer cells will have various effects on different epigenetic targets.

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Epidermal integrin α3β1 is essential to maintain tumor growth and promotes a tumor-supportive keratinocyte secretome

W. M. Longmate[1], S. Varney[1], D. Power[1], R. Pandulal Miskin[2], K. E. Anderson[1], L. DeFreest[1], L. Van De Water[1, 2], C. DiPersio[1, 2]

[1]Surgery, Albany Medical College, Albany, New York, United States, [2]Regenerative & Cancer Cell Biology, Albany Medical College, Albany, New York, United States

As extracellular matrix receptors that mediate both 'inside-out' and 'outside-in' signaling, integrins on tumor cells control reciprocal interactions with the tumor microenvironment (TME) that drive tumor growth and progression. However, therapeutic strategies to target integrins are hindered by incomplete understanding of their complex roles in TME modulation. Mice with constitutive *Itga3* deletion in epidermis show greatly reduced skin tumors in the 2-step chemical tumorigenesis model, indicating a pro-tumorigenic role for integrin α3β1. We generated mice with floxed *Itga3* alleles that express tamoxifen-inducible Cre from a K14 promoter, allowing ablation of α3β1 specifically in epidermis during 2-step tumorigenesis through topical tamoxifen application. Strikingly, loss of α3β1 from growing tumors caused their rapid regression over 2 weeks. Interestingly, while reduced proliferation and increased apoptosis occurred in α3β1-deficient tumor cells, these changes followed a robust increase in apoptosis of stromal cells. Furthermore, macrophages and fibulin-2 deposition were reduced in the stroma of α3β1-deficient tumors. Mass spectrometry of medium conditioned by wild type or α3-null immortalized keratinocytes showed that α3β1 regulates a substantial fraction of the secretome, including proteins that mediate matrix remodeling or stimulate stromal cells (macrophages, fibroblasts, endothelial cells). RNA *in situ* hybridization showed that gene expression for two such proteins, fibulin-2 and macrophage colony-stimulating factor 1 (CSF1), was reduced in α3β1-deficient tumor cells *in vivo*. GSEA showed that a core subset of the α3β1-dependent secretome is enriched in human squamous cell carcinomas with high *ITGA3* expression. Our findings identify a novel role for α3β1 in regulating the keratinocyte secretome and paracrine signals that promote a tumor-supportive TME, implicating α3β1 as a potential therapeutic target.

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Identification of actinic keratosis susceptibility loci

Y. Kim[1], H. Choquet[2], H. Huang[1], E. Jorgenson[2], M. Asgari[1]

[1]Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Kaiser Permanente Northern California, Oakland, California, United States

Actinic keratosis (AK) is a keratinocyte-derived precancerous lesion that arises on skin exposed to chronic ultraviolet radiation. AK is highly prevalent, afflicting >58 million Americans, and its treatment costs are estimated at >$1 billion per year. AK can progress to keratinocyte carcinoma, including cutaneous squamous cell carcinoma (cSCC). Genetic risk has been implicated in AK, as evidenced by a genome-wide association study (GWAS), which identified 3 susceptibility loci (*IRF4, TYR,* and *MC1R*) among Europeans. To identify additional AK-associated loci, we performed a GWAS in non-Hispanic white participants of the Kaiser Permanente Genetic Epidemiology Research on Adult Health and Aging cohort. Participants with a physician-rendered diagnosis of AK were classified as cases (n=16,251), and the non-cases (n=46,454) were assigned to the control group. Genome-wide genotype data were generated on the Affymetrix Axiom European array and imputed to the 1000 Genomes reference panel. Genetic association analysis with AK was performed using logistic regression adjusted for age, sex, and ancestry principal components, and we identified ten genome-wide significant loci for AK ($P<5\times10^{-8}$), including 8 novel loci. Identified loci are implicated in the pigmentation pathway (*IRF4, TYR, SLC45A2, BNC2,* and *HERC2*), the Notch signaling pathway (*FOXP1*), the TGF-ß pathway (*SPIRE2*), immune regulation (*HLA*), coactivating receptors including retinoid and vitamin D receptors (*NCOA6*), and an additional locus *DLGAP4* whose biologic relevance to AK risk is currently unknown. Interestingly, some of our AK-associated loci have been previously reported to be cSCC-associated loci (*IRF4, TYR, SLC45A2, BNC2, HERC2, FOXP1,* and *HLA*), suggesting common biological pathways in keratinocyte carcinogenesis. Study findings provide new insight into the genetic basis of AK susceptibility and may help identify subjects at higher risk for developing AK and cSCC. Further studies replicating our findings in an independent cohort are needed.

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Increased normal tissue telomere length is associated with decreased survival in melanoma patients

Y. Ni[1], T. Romigh[3], P. Funchain[4], J. Arbesman[2, 3]

[1]Quantitative Health Sciences, Cleveland Clinic, Cleveland, Ohio, United States, [2]Dermatology, Cleveland Clinic, Cleveland, Ohio, United States, [3]Cancer Biology, Cleveland Clinic, Cleveland, Ohio, United States, [4]Hematology/Oncology, Cleveland Clinic, Cleveland, Ohio, United States

Normal tissue telomere length (nTL) varies within the population, affected by inherited genetics and environmental risk factors. Previous work has investigated whether telomere length interplays with overall cancer survival. We aim to determine whether nTL was predictive of melanoma patient survival. Both normal and tumor tissue telomere lengths from The Cancer Genome Atlas (TCGA) skin melanoma dataset, along with other cancers, were obtained from supplementary data of the Barthel et al. study (Nat Genet 49, 349–357 (2017)), which were estimated from whole genome/whole exome sequencing data. Univariate and multivariate Cox proportional hazard regression models were applied to identify variables predicting survival, adjusting for age, gender and stage. nTL from individuals with melanoma did not demonstrate a normal distribution. The majority of patients (56%) had short nTL, defined as lower than one-third of the range, while only 8% of patients demonstrated nTL in the intermediate range. Analyzed as a continuous variable, increased nTL was negatively correlated with both overall (OS) and progression free survival (PFS) for melanoma patients (p=0.005, p=0.042), even after adjusting for age, gender and stage. In contrast, tumor telomere length did not demonstrate an association with survival. When stratified into groups, those with longer than median nTL showed worse OS and PFS compared to those with shorter nTL (p<0.001, p=0.007). We then expanded this analysis to 30 other cancers in TCGA. When stratified into groups, longer nTL correlated with worse OS within bladder and colorectal cancers. However, unlike melanoma, no other cancer demonstrated a significant correlation between nTL and OS when nTL was treated as a continuous variable. In conclusion, normal tissue telomere length may represent a prognostic marker for melanoma survival.

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Loss of 5-hydroxymethylcytosine in CD4+ small /medium T-cell lymphoproliferative disorder

H. Jiahui, X. Zhang, S. Geng

Department of Dermatology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

Primary cutaneous CD4+ small /medium T-cell lymphoproliferative disorder (PC-SMTLD) has recently been considered as a distinct clinicopathological entity. Because of a considerable degree of overlap with pseudolymphoma, diagnosis of PC-SMTLD is often challenging. The methylation of DNA at position 5 of cytosine, and the subsequent reduction in intracellular 5-hydroxymethylcytosine (5-hmC) levels, is a key epigenetic event in several cancers, including systemic lymphomas. However, this epigenetic marker has never been studied in cutaneous lymphomas. Therefore, in this study we tried to analyze the expression of 5-hmC in primary cutaneous CD4+ small /medium T-cell lymphoproliferative disorder and compared it with a control group of pseudolymphoma. Retrospective case series study with immunohistochemical and immunofluorescence analysis of 5-hmC were performed in pseudolymphoma (PL) (n=15) and primary cutaneous CD4+ small /medium T-cell lymphoproliferative disorder (PC-SMTLD) (n=6) specimens. We found that Significant loss of 5-hmC nuclear staining was observed in PC-SMTLD when compared with PL (*p=0.001*). The averge positive expression rates of nuclear staining were 22.14% and 51.43% in PL and PC-SMTLD, respectively. By semi-quantitative grade integration, there were some differences in the distribution of 5-hmc scores among the two study groups. Based on these data, we concluded that 5-hmC is a useful marker in distinguishing PC-SMTLD from benign diseases, which will assist us in the differential diagnosis with PL.

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Global phosphoproteomic analysis of protein kinase C delta activation reveals signaling pathways involved in triggering keratinocyte growth arrest

M. Denning, J. Samantha, M. Bielski

Loyola University Chicago, Maywood, Illinois, United States

Activation of multiple protein kinase C (PKC) isoforms have been associated with keratinocyte (KC) cell cycle withdrawal during differentiation. To study how PKC activation may induce KC growth arrest, we evaluated the effects of the PKC agonist TPA on normal human epidermal KCs (NHEKs) and HaCaT cells, an immortalized human KC cell line. Treating NHEKs with 10 nM TPA for as little as 15 minutes induced complete growth arrest, while untreated KCs underwent a 93-fold increase in cell number over 7 days (p<0.005). HaCaT cells were completely resistant to TPA-induced growth arrest, indicating that immortalized KCs have defective PKC-dependent growth arrest signaling. To test if PKCδ activation was able to enforce growth arrest in HaCaT cells, we transduced HaCaT cells with retroviruses encoding either EGFP or a PKCδ-EGFP fusion and sorted EGFP positive cells. HaCaT-EGFP cells behaved similar to HaCaTs and were not growth arrested by TPA treatment, while HaCaT-PKCδ-EGFP cells were 80% growth inhibited by TPA over a 4 day period. TPA-induced growth inhibition in HaCaT-PKCδ-EGFP cells was due to arrest in G1 and G2/M, not apoptosis. Global phosphoproteomics analysis of HaCaT-EGFP and HaCaT-PKCδ-EGFP cells exposed to TPA for 30 minutes identified 667 proteins whose phosphorylation was significantly changed >2-fold, with 605 proteins having increased phosphorylation in TPA-treated PKCδ-EGFP cells. Surprisingly, only 54 of these proteins were phosphorylated at the PKCδ consensus motif, with phosphorylated motifs for the kinases MAPKAP2, AKT1 and RSK2 most abundant. Gene set enrichment analysis revealed increased phosphorylation of proteins involved in translation (eIF4, mTOR, eIF2 signaling), Rho, tight junctions and ERK/MAPK signaling. Thus, global phosphoproteomics identified PKCδ signaling pathways that can overcome the defective growth arrest in immortalized KCs and may have therapeutic benefit for the treatment of actinic keratosis and squamous cell carcinomas.

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The expression of 5-hydroxymethylcytosine, TET2 and retinoid acid receptor-β in male genital diseases

Q. Shang, B. Peng, Z. Chu, X. Zhang, S. Geng

Department of Dermatology, Northwest Hospital, The Second Hospital Affiliated to Xi'an Jiaotong University, Xi'an, Shaanxi, China

DNA methylation is an important epigenetic modification that is frequently altered in a wide range of diseases. 5-Hydroxymethylcytosine (5-hmC) acts as a positive transcriptional regulator in normal development and carcinogenesis. The expression and function of RAR-β are altered in most cancer cells. In this study, we investigated the expression level of 5-hmC, RAR-β and TET2 in a group of male genital diseases using immunohistochemistry. These diseases have very similar appearance and are very difficult to differentiate from each other. The aim of this study was to figure out whether these epigenetic makers can be used in differentiate these conditions. We performed Immunohistochemical staining on paraffin-embedded tissues of 14 penile psoriasis, 13 penile lichen planus, 14 erythroplasia of Queyrat, 6 penile verrucous carcinoma and 7 penile SCC. We found different expression level of 5-hmC,RAR-β and TET2 in epidermis among these five group of diseases. Compared with penile psoriasis and penile lichen planus, expression of 5-hmC was significantly lower in erythroplasia of Queyrat, penile verrucous carcinoma and penile SCC. Moreover, the expression of TET2 was significantly lower in penile SCC compared to other 4 groups of diseases. The expression of RAR-β was also lower in penile verrucous carcinoma and penile scc than that in penile psoriasis, penile lichen planus and erythroplasia of Queyrat. The expression of 5-hmC, TET2 and RAR-β was lower in malignant lesions(erythroplasia of Queyrat, penile verrucous carcinoma penile scc) than that in benign lesions(penile psoriasis, penile lichen planus), suggesting that 5-hmC, RAR-β and TET2 can be used in differentiating benign and malignant diseases with similar clinical manifestations in male genitalia and can help us detect malignant diseases early and reduce the difficulty of clinical biopsy sampling and misdiagnosis.

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Dysregulation of TOX1 and STAT3 in the pathogenesis of cutaneous T-cell lymphoma

A. M. Seffens[1, 2], S. B. Koralov[2], L. Geskin[3]

[1]Columbia University Vagelos College of Physicians and Surgeons , New York, New York, United States, [2]Pathology, New York University School of Medicine, New York, New York, United States, [3]Dermatology, Columbia University Irving Medical Center, New York, New York, United States

Development of novel therapies for CTCL has been hindered by a lack of mouse models for the disease. TOX1, a transcription factor that is required to establish the CD4+ lineage, is overexpressed in malignant T-cells found in the skin and blood of patients with CTCL. STAT3, a transcription factor critical for the differentiation of Th17 and follicular helper T cells, is also consistently overexpressed in CTCL. We hypothesize that both TOX1 and STAT3 are involved in CTCL pathogenesis, and that their upregulation contributes to the ability of malignant cells to survive, proliferate, migrate, and invade tissues. The Koralov lab has previously created a small animal model which overexpresses a hyperactive STAT3 allele, STAT3C, selectively in T lymphocytes. These animals demonstrate an accumulation of Th cells in the lymph nodes and in the skin, recapitulating several key features of early CTCL. I expect that simultaneous activation of TOX1 and STAT3 will result in a phenotype which more closely mimics aggressive forms of CTCL. To evaluate the contribution of TOX1 overexpression to CTCL pathogenesis, we have introduced *Tox1* cDNA downstream of a floxed stop cassette into the ubiquitously expressed Rosa26 locus of C57Bl/6J embryonic stem (ES) cells. We have generated R26Tox1^{stopfl} mice using tetraploid complementation to generate 100% ES cell derived animals. They have been crossed to CD4Cre and CD4Cre STAT3C^{stopfl} strains, giving us an opportunity to examine synergy between TOX1 overexpression and hyperactive JAK/STAT signaling. Here, we present the initial evaluation of this CTCL mouse model, including a 13-fold upregulation of *Tox1* cDNA in peripheral blood of both CD4 Cre Tox1^{stopfl}and CD4 Cre STAT3C^{stopfl} Tox1^{stopfl} mice. We hope that these mice will pave the way to a better understanding of this enigmatic malignancy.

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Shared CpG methylation defects in mycosis fungoides and Sézary syndrome

A. Moerman-Herzog[1], Y. Rahmatallah[2], G. V. Glazko[2], H. K. Wong[1]

[1]Dermatology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States, [2]Biomedical Informatics, University of Arkansas for Medical Sciences, Little Rock, Arkansas, United States

Mycosis fungoides (MF) and Sézary syndrome (SS) are related cutaneous T cell lymphomas thought to arise from distinct memory T cell populations. However, a subset of MF patients progress to SS, suggesting that these clinical variants can arise from a common lineage, and may share abnormal epigenetic imprinting with impact on clinical findings. To address this issue, we compared genome-wide CpG methylation profiles of T cells from SS, MF tumors (MFT), MF blood, and psoriasis blood as a disease control. Comparing all differentially methylated positions (DMPs) showed that while 15% of DMPs from SS T cells were shared by MFT-eluted cells, this shared fraction was 69% of all DMPs in MFT-eluted cells. DMPs annotated to CpG islands were more prevalent in the shared DMP group (49%) and non-shared MFT group (44%) compared to the non-shared SS group (7%). Conversely, intergenic DMPs were less prevalent in the shared DMP group (27%) and non-shared MFT group (31%) compared to the non-shared SS group (74%). DMPs located within 200 bp of transcription start sites and in first exons represented a smaller fraction of sites in the non-shared SS group compared to the shared group and non-shared MFT group. For the shared group, hypomethylated DMPs were primarily intergenic (78%), while most hypermethylated DMPs were in GpG islands (58%). Thus, a large majority of abnormally methylated CpG sites in MFT-eluted cells were also abnormally methylated in SS T cells, suggesting that MF and SS share many epigenetic defects. SS is a more advanced stage of disease, and bore many additional DMPs that were not highly affected in MF tumors.

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A biomarker function of HMGA2 in cutaneous squamous cell carcinoma development

W. Ha, L. Xie, A. Hinde, L. Liu

The Hormel Institute, University of Minnesota, Austin, Minnesota, United States

The high mobility group AT-hook 2 (HMGA2) gene encodes a transcription factor that is expressed during embryonic development but down-regulated in adult tissues. Re-expression of HMGA2 in adult tissues is often associated with both benign and malignant tumor formation. HMGA2 has been identified as a biomarker of melanoma progression and prognosis, but its role in non-melanoma skin cancer development remains controversial. In this study, we measured HMGA2 expression in normal human keratinocytes and found a significant decrease in HMGA2 mRNA expression in adult keratinocytes compared to neonatal keratinocytes. HMGA2 is highly expressed in human cutaneous squamous cell carcinoma (SCC) cell lines and primary human SCC tumors, but not detected in adjacent normal skin. In mouse skin, Hmga2 has been found to be translocated from the cell membrane into the nucleus during DMBA/TPA-induced skin tumorigenesis. While Hmga2 expression is absent in the hair follicle in non-UV-irradiated mouse, UV irradiation increased Hmga2 expression in both the epidermis and hair follicles. In agreement, we found enhanced Hmga2 expression in UV-induced mouse skin SCCs and also Hmga2 reactivation in the dermis from UV-irradiated mice. Furthermore, oncogene depletion such as FOXM1 and TRIP13 in human SCC cell lines using CRISPR/Cas9 decreased the expression of HMGA2 coupled with decreased cell proliferation and survival. Taken together, these results demonstrate that the upregulation of HMGA2 is an important biomarker of skin SCC tumorigenesis. Moreover, understanding of the mechanism for HMGA2 overexpression in skin hair follicle cells can develop the new drug for skin cancer treatment.

Cell-Cell Interactions in the Skin *Single-Cell Transcriptomics and Cell-Cell Interactions in the Skin

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IL-10$^+$ regulatory B cell migration into inflamed skin limits cutaneous inflammation

L. E. Aira, G. F. Debes

Immunology and Microbiology, Thomas Jefferson University, Philadelphia, Pennsylvania, United States

To maintain skin homeostasis several types of leukocytes exist in or are recruited into the skin. While most of these leukocytes are well characterized, the roles of skin-associated B cells remain largely unknown. Our lab previously demonstrated that a subset of IL-10$^+$ regulatory B cells preferentially migrates into the inflamed skin of mice, a process that required α4β1 integrin. Thus, we hypothesized that B regulatory cells suppress skin inflammation and that impaired migration of these cells into the skin exacerbates skin inflammation. To test this hypothesis, we used two mouse models of skin inflammation: (i) IL-17-dominated psoriasiform skin inflammation induced by imiquimod (IMQ) cream and (ii) IFN-γ-mediated cutaneous delayed contact hypersensitivity elicited by dinitrofluorobenzene (DNFB). Furthermore, we employed a mouse model with a B cell-specific tamoxifen-inducible deletion of α4-integrin (*Itga4*). Here, we found that inducing the deletion of *Itga4* in B cells led to a significant decreased in skin-associated IL-10$^+$ B cells in inflamed skin and included both conventional B2 (CD19$^+$B220^{high}CD43$^-$) and innate-like B1 (CD19$^+$B220$^{-/low}$CD43$^+$) cells. The decrease accumulation of IL-10$^+$ regulatory B cells was associated with a significant increase in the clinical and histopathological parameters of skin inflammation in both skin inflammation models. Thus, our data show a crucial function of skin-homing IL-10$^+$ regulatory B cells in the suppression of IL-17- and IFN-γ-dominated skin inflammation; supporting the notion that B regulatory cells are critical players in the cutaneous environment during inflammatory skin diseases.

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Single cell analysis of emigrating cells from psoriasis lesion identifies distinct IL-17A and IL-17F producing T-cell populations and other novel disease-associated alterations

J. Kim[1, 2], J. Lee[1], H. Kim[1], N. Kameyama[1], R. Nazarian[2], E. Der[2], S. Cohen[2], C. Putterman[2], J. G. Krueger[1]

[1]Rockefeller University, New York, New York, United States, [2]Albert Einstein College of Medicine, Bronx, New York, United States

Single-cell sequencing is transforming how we understand inflammatory cells, but previous single-cell analysis of skin has not been reliable enough to provide the firm foundation that this field requires - immune cells constitute only a small fraction of the overall cell population (leukocytes <5%), such that functional subsets may be difficult to ascertain. We have overcome these obstacles by harvesting inflammatory cells emigrating from a half of 6 mm punch biopsy skin after 48-hour incubation in culture medium without any enzyme, and then analyzing the harvested cells with single-cell sequencing and flow cytometry simultaneously. In parallel, we quantified immune cells in the skin by immunohistochemistry with the other half of biopsy tissues. By this strategy, we have sequenced 22,056 cells (leukocytes 48%) from 12 psoriasis lesions and 5 controls. Unsupervised clustering identified NK cells, CD161$^+$ T cells, CD8$^+$ T cells, CD4$^+$ T cells, regulatory T cells (Tregs), mature & semimature dendritic cells (DCs), melanocytes, corneocytes, suprabasal & basal keratinocytes. The average expression of IL-17A & IL-22 was the highest in CD4$^+$ T cells, while the average expression of IL-17F & IL-26 was the highest in CD161$^+$ T cells. An average ratio of Tregs to effector T-cells increased in psoriasis, but a subset of psoriasis Tregs expressed IL-17F. BDCA-3$^+$ DCs are known as "regulatory" DCs, but BDCA-3$^+$ semimature DCs showed high expression of IL-23 in psoriasis lesions. Psoriasis mature DCs showed increased IL-36G. Psoriasis basal keratinocytes showed decreased stem cell markers (KRT15 & CD34) and CCL27. In summary, our single-cell data showed two "regulatory" leukocyte subsets become dysfunctional in psoriasis skin: high production of IL-23 by BDCA-3$^+$ DCs and IL-17F production by Tregs and that IL-17F can be independent of IL-17A production, with major implications for therapeutic targeting of IL-17 isoforms.

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Downregulation of serum exosome miR-1305 contribute to the development of psoriasis through non-canonical Wnt signaling pathway

J. Yan, Q. Sun

Dermatology, Shandong University, Qilu Hospital, Jinan, Shandong, China

Background: The extensive involvement of microRNAs (miRNAs) in the pathogenesis of psoriasis is well documented. However, whether the serum exosome derived miRNAs play a role in psoriasis remains unclear. *Objectives:* To explore the role of serum exosome microRNA-1305 (miR-1305) in psoriasis. *Methods:* Exosomes were isolated from serum by differential ultracentrifugation. The morphology was identified by transmission electron microscope (TEM). Observation of serum exosomes uptake by keratinocytes though confocal fluorescence microscopy. MiRNA microarray was performed in serum exosomes from 4 patients with psoriasis and 4 controls. qRT-PCR were used to identify the differential expression miRNAs. Using bioinformatic predicted the signaling pathways related to mir-1305. Western blot was used to investigate the protein levels of Wnt5a and its downstream effectors. Normal human epidermal keratinocytes (NHEKs) and HaCat cells were transfected with miR-1305 mimic/Ctrl (50 nM) or miR-1305 inhibitor/Ctrl (100 nM); or siWnt5a/ctrl (60 nM) with Lipofectamine 2000. *Results:* The diameter of serum exosomes were around 100 nm. Serum exosomes could be uptake by keratinocytes after co-culture for 12h. Using miRNA microarray, we found 16 differentially expressed miRNAs in serum exosomes; 14 (87.5%) were downregulated and 2 (12.5%) were upregulated (fold change>2, P<0.05). Among these differentially expressed miRNAs, miR-1305 was down-regulated in serum exosomes from psoriasis patients (fold change=0.20, P<0.01, n=4). Through the methods of bioinformatics analysis, qRT-PCR and Western blot, we found that miR-1305 was closely related to non-canonical WNT signaling pathway (P<0.01), and miR-1305 regulated the expression of Wnt5a in Normal human epidermal keratinocytes (NHEKs) and HaCat cells. *Conclusions*: MiR-1305 was downregulated in serum exosomes from psoriasis patients. Downregulation of serum exosomes miR-1305 was involved in the pathogenesis of psoriasis through regulating non-canonical WNT pathways. However, much work remains to be done in the future to test our point of view.

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Defining a role for CREB in hair follicle stem cell metabolism

I. Avila, M. Miranda, J. Esparza, W. E. Lowry

Molecular Biology, UCLA, Los Angeles, California, United States

Hair follicle stem cells (HFSCs) are multipotent epithelial stem cells that undergo cyclical bouts of growth and rest. They reside in a niche called the bulge and aid in wound healing, regulate the hair cycle, and produce new hair shafts every round of the hair cycle. The transcription factor, cyclic AMP (cAMP)-responsive element binding protein (CREB) plays a role in cell proliferation, differentiation, and is involved in the G-protein-coupled receptor (GPCR) signaling pathway. In vivo experiments on murine dorsal skin revealed that the phosphorylated, active form of CREB can be induced in the bulge upon topical treatment with a variety of compounds that facilitate downstream CREB activity such as cAMP, forskolin, and phosphodiesterase inhibitors. These same experiments revealed that the expression of several glycolytic proteins such as lactate dehydrogenase A (LDHA) and Glucose transporter 1 (Glut1) could be induced in treated tissue, validating previous work where glycolytic shifts in HFSCs can promote hair growth. We hypothesize that the canonical GPCR pathway through CREB functions in HFSC homeostasis. Furthermore, research done on murine fibroblast-based cells indicated that altered levels of CREB influence cellular metabolism. Cells were treated with CREB inhibitors 666-15 or surfen, and demonstrated to have lower metabolic activity and lower expression levels of glycolytic proteins Glut1 and pyruvate. Our study reveal that GPCR/CREB signaling drives the hair cycle by influencing HFSC homeostasis via cellular metabolic states and provides novel methods to regulate HFSC fate.

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Single-cell RNA sequencing combined with interstitial fluid proteomics defines cell-type-specific immune gene regulation in atopic dermatitis

T. B. Rojahn[1], V. Vorstandlechner[1], T. Krausgruber[2], W. Bauer[1], N. Alkon[1], C. Bangert[1], N. Fortelny[2], K. Rindler[1], A. Elbe-Burger[1], C. Bock[2], M. Mildner[1], P. M. Brunner[1]

[1]Medical University of Vienna, Vienna, Austria, [2]CeMM Research Center for Molecular Medicine, Vienna, Austria

Atopic dermatitis (AD) is the most common chronic inflammatory skin disease, but its complex pathogenesis is still only partly understood. To comprehensively characterize AD on both transcriptomic and proteomic levels in humans, we used skin suction blistering, a painless and non-scarring procedure that can simultaneously sample skin cells and interstitial fluid, and compared results to conventional biopsies. Suction blistering captured epidermal and most infiltrating immune cells equally well as biopsies, except for non-migratory CD163+ macrophages that were only present in biopsy isolates. Using single-cell RNA sequencing, we found comparable transcriptional profiles of key inflammatory pathways between blister and biopsy AD, but suction blistering was superior in cell-specific resolution for high-abundant transcripts (KRT1/KRT10, KRT16/KRT6A, S100A8/S100A9), which showed background signals in biopsy isolates. Compared to healthy controls, we found characteristic upregulation of AD-typical cytokines such as IL-13 and IL-22 in Th2 and Th22 cells, respectively, but we also discovered these mediators in proliferating T-cells and NKT-cells, that also expressed the antimicrobial IL-26. Overall, not T-cells, but myeloid cells were most strongly enriched in our AD samples, and we found dendritic cell (CLEC7A, amphiregulin/AREG, EREG) and macrophage products (CCL13) among the top-upregulated proteins in AD blister fluid proteomic analyses. These data show that by using cutting-edge analysis methods, suction blistering offers several advantages over conventional biopsies, including better transcriptomic resolution of skin cells, combined with proteomic information from interstitial fluid, unraveling novel inflammatory players and pathways that shape the cellular and proteomic microenvironment of AD.

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Single cell analysis of human vitiligo lesions reveals a role for CCR5 in T regulatory cell function

K. Gellatly[1], J. Strassner[2], K. Essien[2], M. Ahmed[2], R. Murphy[1], A. Coffin-Schmitt[1], X. Fan[2], X. Ding[2], M. Frisoli[2], E. Kim[2], Z. Abbas[2], A. Derr[1], P. McDonel[1], M. Garber[1], J. Harris[2]

[1]Bioinformatics and Integrative Biology, UMASS Medical School, Worcester, Massachusetts, United States, [2]Dermatology, UMASS Medical School, Worcester, Massachusetts, United States

Vitiligo is an autoimmune disease of the skin mediated by CD8+ T cells that target melanocytes for destruction. In the interest of developing new treatments, we sought to dissect the complex molecular and cellular interactions that occur within the epidermis during vitiligo progression. We used suction blistering to isolate cells from affected and unaffected skin from 11 vitiligo patients and 7 healthy controls before performing single cell RNA sequencing (scRNA-seq). We found most of the cell types in the epidermis were represented in our data, including keratinocytes, melanocytes, innate immune cells, and both CD8+ and CD4+ T cells. From the scRNA-seq data, we were able to build a ligand and receptor signaling network of all annotated communications between epidermal cells. We found that a large number of communications were significantly disrupted (FDR < .01) in the skin from vitiligo patients compared to controls. Specifically, we found disruption in the complex chemokine circuits that influence the localization of T cells during vitiligo inflammation (CXCL9/10, CCL3/4/5) and increased antigen presentation through MHC-1 that may lead to attenuated recognition of self antigen. We then focused on T regulatory cells which are present in affected skin but down-regulate key cytokines for immune suppression such as TGFB1 and IL-10. In contrast, lesional Tregs induce the expression of CCR5, which was validated in both vitiligo patients and the mouse model. In order to determine the therapeutic potential of these findings, we performed mechanistic studies in the mouse model that revealed CCR5 is essential for Treg suppressive function in the skin, with KO leading to worse disease. Through our study we have identified novel pathways and chemokine axis that may enable the development of new therapeutics.

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Micro RNAs enriched in exosome derived from keratinocytes under oxidative stress contributes to melanocyte loss in vitiligo

J. Ma, Y. Yang, W. Zhang, T. Gao, C. Li, Z. Jian

Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi'an, China

Vitiligo is a skin disease characterized by the destruction of epidermal melanocytes. Oxidative stress is closely related to the development of vitiligo. As the main constituent cells in the epidermis, keratinocytes regulates the viability and the function of melanocytes, but the underlying mechanism is still not clear. We hypothesized that exosomes derived from keratinocytes under oxidative stress induce the destuction of melanocytes. We treated human keratinocytes (HaCaT) with H_2O_2 for 24h and extracted exosomes from culture media by ultracentrifugation. TEM images revealed that exosomes secreted by keratinocytes displayed double layer membranes and cup-like structure, and varied in size (30–200 nm), findings consistent with NTA result. The markers of exosomes, CD63 and Hsp70, can be detected by western blot. Interestingly, we found that the amount of exosomes secreted by keratinocytes was dramatically increased under H_2O_2 treatment, with a concentration dependent. H_2O_2 at 0.4mM enhanced exosomes secretion by 3-fold. Meanwhile, we found that exosomes secreted by keratinocytes were successfully intake by melanocytes after 24h co-culture. Importantly, exosomes derived from H_2O_2-treated keratinocytes (Exo. KC-H_2O_2) significantly suppressed the proliferation of melanocytes after 96h co-culture. Subsequently, micorRNAs (miRNAs)-seq data showed that the miRNAs expression profile of Exo.KC-H_2O_2 had distinct characteristics. The qRT-PCR results showed that there are six miRNAs enriched in Exo.KC-H_2O_2. Further, we proved that two of the six miRNAs inhibited proliferation through regulating MITF axis and induced caspase triggered cell apoptosis of melanocytes in both normal condition and oxidative stress. In conclusion, our data have proved that miRNAs enriched in exosomes derived from keratinocytes under oxidative stress contribute to melanocyte loss. The findings provide new insights for understanding the pathogenesis of vitiligo.

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Aryl hydrocarbon receptor and autophagy-related protein LC3 expression in psoriasis

B. Chung[1], J. Kim[2], H. Kim[1], S. Kang[1], M. Jung[1], H. Kim[1], C. Park[1]

[1]Department of Dermatology, Hallym University, Seoul, Korea (the Republic of), [2]Dermatology, Soonchunhyang University Cheonan Hospital, Soonchunhyang University College of Medicine, Cheonan, Korea (the Republic of)

Background: Aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor that sense environmental stimuli. Autophagy is an intracellular disintegrative process regarding as an endogenous defense mechanism. Both AhR and autophagy participate in maintaining skin homeostasis as well as development of skin disorders. Objectives: We aimed to study the immunohistochemical expression of AhR, CYP1A1, and autophagy-related marker, microtubule-associated protein IA/IB light chain 3 (LC3) in lesional skin of psoriasis patients and to investigate the correlation between AhR and autophagy-related protein. Methods: This study enrolled 20 psoriasis patients, 6 patients with atopic dermatitis (AD) and 10 healthy volunteers. Skin biopsies were taken from all subjects. We examined for AhR, CYP1A1, and LC3 antibody expression by immunohistochemistry. We next investigated the correlations between the expression levels of these markers and various clinicopathological factors. Results: The intensity of AhR and CYP1A1 immunohistochemical expression were increased in psoriasis lesions in comparison with AD lesional skin as well as normal control skin ($p<0.001$, $p<0.001$). LC3 expression in psoriasis lesions was decreased in psoriasis lesions compared to AD lesion and normal control ($p=0.001$). AhR and CYP1A1 expression in psoriasis lesions showed significant positive correlations with mean of epidermal thickness and inflammatory cell density. There were significant negative correlations between LC3 expression in psoriasis lesions and the mean of epidermal thickness or inflammatory cell density. There was a significant negative correlation between AhR and LC3 expression in psoriatic skin ($p=0.006$). Conclusions: AhR and autophagy could play a role in the pathogenesis of psoriasis through the modification of epidermal hyperproliferation and inflammation. AhR and autophagy regulation may be used as a novel therapeutic target in chronic inflammatory diseases.

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Skin infection generates two distinct subgroups of $CD8^+$ resident memory T cells

Y. Pan, N. Smith, Y. Yan, J. Zhao, T. Tian, C. A. Stingley, K. Wu, R. Clark, C. Villani, T. S. Kupper

Dermatology, Brigham Health/Harvard Medical School, Boston, Massachusetts, United States

Rapid and efficient protection from skin infection relies on local resident memory T cells (T_{RM}). In this study, we analyzed the transcriptional profile of CD8+ T_{RM} using single cell RNA seq. Mice were infected with Vaccinia virus (VACV) and skin and lymph node $CD8^+$ T cells were analyzed by scRNAseq at day 0, 5, 10, 15, 20, 25, 30, 45, and 60. Using force-directed layout embedding (FLE) analysis, skin T cells clearly clustered away from lymph node (and naive) T cells, while each followed distinct differentiation trajectory from day 0 to day 60. Skin T cells from days 20-60 showed strong connections to each other via partition-based graph abstraction,inferring that the overall formation of T_{RM} lineage likely happens at this time. A more detailed FLE cluster of T cells from skin showed unexpected heterogeneity of these cells. Surprisingly, two transcriptionally distinct clusters were found in skin T_{RM}. Both clusters expressed CD3 and CD8, confirming their identity as T cells. While cluster 1 T cells also express genes commonly associated with antigen presenting cells, cluster 2 cells express high levels of cytotoxic and mitochondrial related genes reminiscent of conventional cytotoxic T cells (CTLs). Expression of several genes demonstrating distinct transcriptional profiles between these 2 clusters were validated at the protein level by flow cytometry.While these two clusters showed equal number of cells at late time points (day 30- 60), cluster 1 T_{RM} appeared earlier (day 15) compared to day 25-30 for cluster 2 T_{RM}s. Overall, our study demonstrates the unexpected existence of two transcriptionally distinct subgroups of $CD8^+$ T_{RM} after VACV skin infection, revealing previously unappreciated heterogeneity of this memory T cell subset. The respective functions of these newly described T_{RM} subsets is under intensive investigation.

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Tranilast directly targets NLRP3 to protect melanocytes from keratinocyte-derived IL-1β under oxidative stress

S. Guo, P. Kang, S. Li, T. Zhuang, C. Li

Xijing Hospital, Xi'an, China

Vitiligo is a kind of oxidative-mediated inflammatory disease characterized by the death of melanocytes. Recent studies have indicated that the activation of NLRP3 inflammasome-IL-1β pathway in keratinocyte contributed to melanocytes death via disturbing the local immunity in the pathogenesis of vitiligo. Notably, as a safe small-compound drug employed frequently in clinic, tranilast (TR) is reported to block the activation of NLRP3 inflammasome in macrophage. Nevertheless, whether TR could improve the melanocyte damage exerted by microenvironment, especially constituted by keratinocytes, via inhibiting the NLRP3-IL-1β pathway of keratinocytes is not clear. In the present study, we initially found that the NLRP3-mediated IL-1β in keratinocytes could impair the melanosome synthesis by lowering the TYR activity and the expression of TYR and TYRP1, which could be impeded conspicuously by the pretreatment of TR in keratinocytes. we also found that TR pretreatment could significantly palliate the severity of inflammation in epidermis under oxidative stress, including the decreased secretion of inflammatory cytokines such as IL-6, IL-8, TNF-α and IL-18. In summary, our study firstly verified that TR disturbed the NLRP3 oligomerization in keratinocytes under the oxidative stress and further inhibited the secretion of IL-1β. Sequentially, we validated that TR pretreatment in keratinocytes could decrease the melanocyte apoptosis and improve the melanogenesis and melanosome translocation via attenuating the secretion of IL-1β. Additionally, we found that TR pretreatment could significantly palliate the severity of inflammation in epidermis under oxidative stress. Given the in vitro model only in this research, further studies performed in vivo are still needed to confirm the role of TR in protecting melanocytes via regulating keratinocytes. At last, our results underscored the promising therapeutic value of TR in vitiligo.

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Dermis-hair follicle communication: Extracellular microvesicles signaling for hair regeneration

B. Closs[1], A. Le Riche[2, 1], L. Marchand[1], C. Jahoda[3], I. Petit[2], E. Aymard[1], S. Bordes[1], D. Aberdam[2]

[1]R&D Department, SILAB, Brive la Gaillarde, France, [2]INSERM U976, Université de Paris, Paris, France, [3]Department of Biosciences, Durham University, Durham, United Kingdom

Dermal papilla cells play a pivotal role in the regulation of hair follicle growth, formation, and cycling, mainly through paracrine mechanisms. In the last decade, extracellular microvesicles (EVs) have been described for their interest in tissue regeneration. Indeed, through a new paracrine mechanism, by transferring biological material, they can modify the physiological state of recipient cells. This study aimed to investigate the effect of EVs isolated from stimulated dermal fibroblasts on hair follicles. For this purpose, dermal fibroblasts were stimulated with a combination of PDGF-AA and bFGF to mimic physiological context. Then, EVs secreted by fibroblasts and named st-EVs were isolated and their effect evaluated *ex vivo* on hair growth and *in vitro* on human dermal papilla cells as well as on human hair follicle keratinocytes. Results demonstrated that st-EVs enhanced hair follicle growth *ex vivo*. Comparative transcriptomic analysis on treated dermal papilla cells identified specific activation of the NDP gene, encoding the non-Wnt ligand norrin. We found that norrin was secreted by these st-EVs-stimulated dermal papilla cells and in turn induced the activation, in a non-cell autonomous manner, of β-catenin pathway in follicular keratinocytes resulting in hair growth *ex vivo*. Moreover, while norrin-specific receptor Frizzled4 was barely detected in human hair follicle keratinocytes, its presence was found on EVs isolated from dermal fibroblasts. Accordingly, dermal fibroblasts EVs provided Frizzled4 to potentiate norrin effects *ex vivo*. In conclusion, this study identifies dermal fibroblasts EVs as efficient activators of dermal papilla cells and norrin as a novel modulatory player in hair follicle regeneration.

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MrgprD-expressing peripheral sensory neurons may directly suppress mast cell function

J. Wu[1, 2], S. Zhang[3], D. Kaplan[1, 4]

[1]Departments of Dermatology and Immunology, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [2]School of Medicine, Tsinghua University, Beijing, China, [3]Dermatology, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [4]Pittsburgh Center for Pain Research, University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Cutaneous mast cells (MC) and sensory afferent neurons are well known to have close anatomical and functional associations. Pain-sensing peptidergic neurons that express TRPV1 can induce mast cell degranulation through release of the neuropeptide Substance P that interacts with the receptor MrgprB2 on MC. We have found that a MrgprD-expressing subset of nonpeptidergic neurons suppresses the expression of MrgprB2 on MC (see *Zhang et al.* abstract). Ablation of MrgprD-expressing neurons results in functionally increased MrgprB2 expression on MC while MrgprD-agonism functionally suppresses MrgprB2 expression. To determine whether MrgprD-expressing neurons directly interact with MC, we established a MC, neuron co-culture system. Peritoneal-derived mast cells (PMC) were cultured *in vitro* with IL-3 and SCF for 3 weeks to generate MC similar to those found in the dermis. Neuron cell bodies were then dissociated from dorsal root ganglia isolated from MrgprD-deficient or WT mice and co-cultured with PMC. After a 48-hour co-culture, PMC were stimulated with compound 48/80, a MrgprB2 agonist, and MC degranulation was determined based on the percentage of β-hexosaminidase released into the culture media. Preliminary data suggests that co-culture with neurons reduces MC sensitivity to compound 48/80. These data suggest that peripheral neurons may directly suppress MC activation.

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Comparative analysis of pruritus and intraepidermal nerve fiber density in atopic dermatitis and bullous pemphigoid

T. M. DeGrazia[1], E. M. Cole[1], Y. Xing[2], H. Hilley[2], L. Han[2], S. Chisolm[1, 3], S. C. Chen[1, 3], R. Feldman[1, 3]

[1]Emory University School of Medicine, Atlanta, Georgia, United States, [2]Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia, United States, [3]Regional TeleHealth Service, VISN 7, Atlanta, Georgia, United States

Chronic pruritus significantly impacts quality of life and is a unifying symptom in patients with the inflammatory skin diseases bullous pemphigoid (BP) and atopic dermatitis (AD). Limited data are available related to neurophysiologic mechanisms underlying both pruritic conditions. Five patients with BP (n=2) or AD (n=3) were followed over a 6-month period while receiving treatment. Disease severity was calculated with the BP Disease Area Index for BP patients or the Eczema Area and Severity Index (EASI) and SCORing Atopic Dermatitis (SCORAD) for AD patients. Itch intensity was measured using the ItchyQuant self-reported pruritus severity scale. Alterations in intraepidermal nerve fiber density (IENFD) were compared between BP patients, AD patients, and healthy controls (HC) (n=7). Severity scores for patients at baseline ranged from: 11-24 (BPDAI), 0-7.4 (EASI) and 14.91-62.16 (SCORAD). Itch intensity was not altered between baseline and 6-month follow-up (mean 5.2 at both time points). Both AD and BP demonstrated a decreased IENFD compared with HC (AD 4.04 fibers/mm, BP 6.95 fibers/mm, HC 9.34 fibers/mm) at baseline. While there was no significant difference between IENFD in AD and BP at baseline, there was a significant increase in IENFD in both conditions noted at 6-month follow-up, respectively (AD 4.0 fibers/mm, BP 6.9 fibers/mm vs AD 7.4 fibers/mm, BP 11.9 fibers/mm, p=0.002). These findings suggest that neuroanatomical alterations of epidermal nerve fibers seen in chronic pruritus may be a common phenomenon in seemingly divergent inflammatory skin diseases and reversible with appropriate treatment. Future studies to elucidate mechanistic details related to changes in IENFD may inform potential novel therapeutics.

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Glycation: Impact on skin cells' functions and ECM mechanical properties

D. Layman[1], M. Rafailovich[3], N. Pernodet[1, 2]

[1]Research & Development, The Estee Lauder Companies, Melville, New York, United States, [2]Estee Lauder Research Laboratories, Melville, New York, United States, [3]Materials Science & Engineering, Stony Brook University, Stony Brook, New York, United States

Skin aging is the result of the effects of intrinsic factors as well as external factors. External factors, such as sun exposure and pollution, have been well described and studied, showing the significant impact on skin cell aging through an increase of damage, such as DNA damage, oxidative damage and inflammatory mediators. Additional mechanisms contributing to skin aging also need to be considered. Recently, Glycation has been recognized as a critical parameter that accelerates signs of skin aging. This mechanism is even more significant on skin exposed to the environment, as UV significantly increases skin glycation. Glycation is the binding of sugar (glucose) to proteins, lipids and nucleic acids, forming Advanced Glycated End-products (AGEs). Glycation naturally increases with age. The appearance of glycated collagen is first observed in the skin of people in their twenties. Over time, the accumulation of AGEs, results in dysfunctional proteins, lipids and nucleic acids, which impair skin behavior. Glycation impacts both biomechanical and functional properties of skin. In this study, we measured the extracellular matrix mechanical properties, which are significantly altered through glycation. Dermal fibroblasts are unable to recognize this altered microenvironment and therefore change their mechanotransduction signals, i.e. their functions. Through our research using normal human dermal fibroblasts, we show that glycation impairs many different natural processes. Inflammatory mediators increased while cellular proliferation, migration and protein production were decreased.

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miR-146a and its importance in skin cell aging

N. Pernodet[1, 2], K. Stafa[1], K. Dong[1], D. Layman[1], K. Corallo[1], J. Trivero[1], W. Eagle[1], E. Goyarts[1]

[1]Research & Development, The Estee Lauder Companies, Melville, New York, United States, [2]The Estee Lauder Companies, Melville, New York, United States

microRNAs (miRNAs or miRs) have been identified as a class of epigenetics signaling molecules. miR research is still a relatively new field, with miRs having been first recognized as a distinct class of biological regulators in the early 2000s. However, it is extremely active in the medical field for diagnosis and precision medicine. miRs are small RNAs (containing, on average, 22 nucleotides), and they function as major players of gene regulation. They fine tune gene expression during many biological processes and in response to the environment. miRNAs have emerged as powerful regulators of tissue remodeling but they are extremely complex. Although they number in the hundreds, they are highly tissue-specific. Therefore, it is critical to study them in skin tissues and skin cells to understand how they contribute to skin behavior and health/youth. During our research, we have identified one micro-RNA of interest in skin, miR-146a. Here, we present how miR-146a expression changes with age and how the loss of mir-146a negatively impacts skin cells through an increase of inflammatory mediators, DNA damage, a decrease of Per-1 expression, and changes to structural proteins. We believe this is the first demonstration of the critical importance of miR-146a on multiple biological pathways in skin and its role in helping skin cells to resist damage.

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VE-cadherin endocytosis controls vascular integrity and patterning during development

C. M. Grimsley-Myers[1], R. Isaacson[1], C. Cadwell[1], J. Campos[1], M. Hernandes[2], K. Myers[1], T. Seo[1], W. Giang[1], K. Griendling[2], A. Kowalczyk[1, 3]

[1]Cell Biology, Emory University, Atlanta, Georgia, United States, [2]Medicine, Emory University, Atlanta, Georgia, United States, [3]Dermatology, Emory University, Atlanta, Georgia, United States

During blood vessel morphogenesis, endothelial cell-cell adhesions must be strong enough to maintain vascular integrity and prevent leakage, but flexible enough to allow cellular movements and intercalations. These competing requirements require a precise balance of stability and plasticity at cell-cell junctions, yet the molecular and cellular mechanisms that control this balance are poorly understood. We hypothesized that endocytosis of the cell-cell adhesion molecule vascular endothelial cadherin (VE-cad) may play a key role. VE-cad endocytosis is mediated by an internalization motif (DEE residues) within the p120-catenin (p120) binding domain of the VE-cad cytoplasmic tail. p120 binding blocks this motif and inhibits VE-cad internalization. To test the role of cadherin endocytosis and p120 binding in vascular morphogenesis, we used CRISPR/CAS9 to generate homozygous VE-cad mouse mutants with decreased internalization (VE-cad$^{DEE/DEE}$) or decreased p120 binding (VE-cad$^{GGG/GGG}$). The VE-cad$^{GGG/GGG}$ mutant mice exhibited reduced VE-cad levels, microvascular hemorrhaging, and decreased postnatal survival, suggesting p120 binding to the VE-cad tail is essential for vessel integrity and for vascular barrier function. By contrast, VE-cad$^{DEE/DEE}$ mutants exhibited normal vascular permeability but displayed angiogenesis defects in multiple vascular beds, suggesting cadherin endocytosis is needed for normal vessel patterning *in vivo*. *In vitro*, we found that VE-cad endocytosis is required for establishment of polarity during collective cell migration by permitting dynamic reorganization of the actin cytoskeleton. Together, these findings demonstrate that p120 regulation of cadherin endocytosis is an essential mechanism that governs both the stability and plasticity of cell-cell contacts during vertebrate development, and that cadherin endocytosis is integrated with polarity cues to regulate cell migration and angiogenesis.

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Wnt-induced excessive lipolysis drives fat loss in skin fibrosis

B. Zhang[1], A. Jussila[1], M. Steele[1], E. Hamburg-Shields[1], R. Atit[1, 2, 3]

[1]Biology, Case Western Reserve University, Cleveland, Ohio, United States, [2]Genetics, Case Western Reserve University, Cleveland, Ohio, United States, [3]Dermatology, Case Western Reserve University, Cleveland, Ohio, United States

Fibrotic disorders contribute to approximately 45% of deaths in Europe and North America. Fibrosis affects all soft tissues, and is characterized by the deposition of excessive extracellular matrix in all organs and the loss of lipid-filled cells in most organs. Dermal fibrosis is a good model for studying fibrosis-associated lipid

depletion due to the distinct dermal white adipose tissue (DWAT) compartment in the lower dermis, which modulates angiogenesis and immune cell recruitment. The mechanisms underlying fibrosis-associated lipid depletion are unknown. Although adipocytes homeostatically activate ATGL-dependent lipolysis in order to break down stored lipids into extracellular glycerol and fatty acids, stimulated lipolysis can lead to rapid lipid depletion. Wnt signaling is dysregulated in human fibrosis, and sustained Wnt signaling is sufficient to cause dermal fibrosis including DWAT lipid depletion in mice. We hypothesized that dermal Wnt signaling activation stimulates ATGL-dependent lipolysis. We developed a genetic mouse model of dermal Wnt activation allowing for inducible and reversible dermal ECM expansion, with which we were able to quantify the dynamics of dermal lipid depletion during the onset and progression of Wnt-induced fibrosis. Dermal Wnt activation led to an increase in the activated lipolytic enzyme downstream of ATGL, phosphorylated HSL, preceding DWAT loss *in vivo*, showing stimulated lipolysis as an early event in dermal fibrosis. Consistently, Wnt-activation in murine intradermal adipocytes *in vitro* released three times as much glycerol as untreated adipocytes, indicating that Wnt signaling has cell-autonomous lipolytic effects. Subsequently, we found that enzymatic inhibition of a key lipolytic enzyme, ATGL, is sufficient to rescue Wnt-induced lipolysis. Current studies are focused on the role of ATGL in Wnt-induced dermal fibrosis *in vivo*. Our results implicate lipolysis as a potential therapeutic target for fibrosis treatment.

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Altered Celsr1 adhesion disrupts planar cell polarity and junction asymmetry

S. N. Stahley, L. Basta, D. Devenport

Molecular Biology, Princeton University, Princeton, New Jersey, United States

Collective polarization of cells along a tissue plane, referred to as planar cell polarity (PCP), is fundamental to embryonic development and tissue organization in complex, multicellular organisms. The ordered alignment of body hairs along the mammalian skin is an excellent example of PCP. PCP is regulated by the asymmetric localization of core PCP components at cell junctions bridged by atypical cadherin Celsr1. The goal of this work is to elucidate how Celsr1 mediates extracellular adhesion to coordinate functional PCP and how these activities are altered by the PCP-disrupting mutation *Crash* (*Crsh*). *Crsh* maps to the cadherin repeats within the Celsr1 extracellular domain, suggesting it may perturb Celsr1 adhesion. Surprisingly, *Crsh* mediates cell aggregation, a read-out for trans-cell adhesion, comparable to wildtype (WT). However, *Crsh* and WT expressing cells sort into distinct aggregates when mixed, indicating this point mutation alters Celsr1 adhesive properties. *Crsh* displays a more diffuse cell surface distribution by confocal microscopy and reduced junctional stability as measured by FRAP, suggesting *Crsh* is defective in lateral clustering. Importantly, chemically induced *cis*-dimerization of *Crsh* rescues keratinocyte junctional enrichment and *trans*-interactions with WT, indicating a role for *cis*-interactions to stabilize Celsr1 adhesion. Finally, super-resolution microscopy imaging was used to investigate the subcellular organization of PCP junctions. dSTORM of cultured keratinocytes revealed altered organization of Celsr1 puncta at cell junctions, while *in vivo* SIM revealed altered lateral organization of core PCP proteins Frizzled and Vangl along cell borders in contrast to their asymmetric localization across cell borders in WT. Collectively, our results support a model in which *cis*-adhesive interactions play a critical role in stabilizing Celsr1 adhesion to coordinate PCP junction organization and functional asymmetry.

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Immune cell-derived growth factors drive fibrosis in scleroderma and graft-vs-host disease

I. Odell[1], R. Flavell[2, 3]

[1]Dermatology, Yale University, New Haven, Connecticut, United States, [2]Immunobiology, Yale University, New Haven, Connecticut, United States, [3]Howard Hughes Medical Institute, Chevy Chase, Maryland, United States

Scleroderma (Systemic sclerosis) is an autoimmune disease that leads to excess production of collagen and other matrix proteins in the skin and other organs. Graft-vs-host disease, a major complication of stem cell transplant, can cause similar scarring of the skin. We hypothesized that scleroderma and the sclerotic form of graft-vs-host disease have overlapping immune-mediated pathogenesis. To examine pathways that drive fibrosis, we performed single-cell RNA sequencing from skin biopsies of patients with diffuse scleroderma and sclerotic graft-vs-host disease compared to normal controls. We validated the findings from patients using immunohistochemistry and qRT-PCR, performed mechanistic studies in vitro with patient-derived fibroblasts and pericytes, and functional studies with knock-out mouse strains. We discovered increased expression of immune-derived growth factors and activation of their associated receptors in fibroblasts and pericytes. A subset of ligands and receptors were validated in published datasets of scleroderma interstitial lung disease. We confirmed that the RNA expression data corresponded to increased number of ligand-positive cells and increased receptor phosphorylation in biopsy specimens. We found that inhibition of the most upregulated ligand and its receptor in cultured fibroblasts decreased the expression of the extracellular matrix genes identified in the single-cell RNA sequencing. In mice, inhibition of the receptor with a small molecule inhibitor prevented the development of bleomycin-induced fibrosis. In knock-out mice, we found that there was significant functional redundancy by the family of ligands and that inhibition of multiple ligands was required to prevent fibrosis. These findings show that inhibition of immune cell-derived growth factors or their cognate receptor may provide an effective treatment strategy in patients with scleroderma or graft-vs-host disease and warrants consideration of clinical trials with inhibitors of this ligand and receptor family.

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Single cell transcriptomic and microarray analysis reveals the dynamic landscape of acne lesion

T. H. Do[1, 2], R. L. Modlin[3]

[1]Molecular Biology Institute at UCLA, Los Angeles, California, United States, [2]David Geffen School of Medicine, Los Angeles, California, United States, [3]Dermatology, David Geffen School of Medicine at UCLA, Sherman Oaks, California, United States

Acne is an inflammatory skin disease often results in the rupture of the sebaceous follicle due to increased sebum production. Although significant advances have been made in the last decade in identifying the pathophysiological mechanisms involved in acne, the cellular components and mechanisms that initiate and perpetuate inflammation at the site of disease are still unclear. Here, we used single cell RNA-sequencing analysis to study gene expression of inflamed lesions compared to non-lesional skin from the same patient (n=6). We identified several lesional specific cellular subclusters including (Triggering Receptor Expressed on Myeloid Cells 2) TREM2+ macrophages that has the ability to modulate the immune system through phagocytosis and lipid uptake. We also discovered a subset of LAMP3+ (DC-LAMP) mature dendritic cells that appears to be an active mediator of inflammation with high expression of chemokine receptors and ligands such as CCR7, CCL19, and CCL22 as well as CD274 (PD-L1) and PDCD1 (PD-L2). These results suggest TREM2+ macrophages may be involved in the detection of local cell death and lipid accumulation while LAMP3+ dendritic cells play a role in perpetuating the inflammatory response by activating the adaptive immune response. Taken together, our study provides new insights into the comprehensive changes in cellular networks involved in acne lesions at the single cell level, identifying potential therapeutic targets for inflammatory acne.

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scRNA-seq and RNA-seq for Stiff Skin Syndrome identify pericytes as a key pathogenic cell population and avenue for therapeutic targeting

J. E. Gudjonsson[1], L. C. Tsoi[2], A. C. Billi[2], O. Plazyo[1], R. Wasikowski[2], Y. Jiang[1], C. Zeng[2], J. Kirma[2], M. J. Wilson[1], M. Patrick[1], K. Raja[2], R. Lafyatis[3], J. Kahlenberg[1], D. Khanna[1]

[1]University of Michigan, Ann Arbor, Michigan, United States, [2]Dermatology, University of Michigan, Ann Arbor, Michigan, United States, [3]University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Stiff skin syndrome (SSS) is a rare (<100 reported cases), slowly progressive skin condition, caused by genetic mutation in the *FBN1*gene. It is characterized by thickened skin associated with muscle weakness and limited joint mobility. Currently there is no known treatment. While SSS is considered as scleroderma (Sc)-like disorder, there has not been any molecular study conducted to investigate the potential differences or similarities between the two diseases. Here, we report molecular profiling for the skin biopsies of 3 stiff skin syndrome and 3 scleroderma patients using bulk RNA-seq and single cell RNA-seq (scRNA-seq) techniques. We revealed >4,000 genes being differentially expressed in SSS skin that are highly correlated with genes dysregulated in scleroderma (Sc) skin. In stark contrast to scleroderma, which showed enrichment of myofibroblasts, there was marked enrichment of pericytes (distinguished by the expression of *RGS5*) in the dermal layer of SSS (10% in Sc versus >30% in SSS), confirmed by IHC. We also demonstrate expression of the therapeutically targetable adrenergic receptors (i.e. *ADRA1D*,*ADRA2B*) in pericytes, and *ABCA1*in fibroblasts in SSS. Previous work has suggested ADRA signaling as a promoter of fibroblast differentiation in smooth muscle. When restricting to SSS-dysregulated genes that are not differentially expressed in Sc, we identified fibroblast-expressing *FGFR4*(fibroblast growth factor receptor 4) as the most significantly up-regulated gene (FC=6.2; $p=1.2x10^{-15}$). Our transcriptomic study illustrates the largely similar but subtle molecular differences between SSS and Sc and demonstrates that identification of dysregulated disease specific pathways can potentially facilitate the identification of therapeutic targets.

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Highly efficient, massively-parallel single-cell RNA-seq analysis of leprosy skin lesions identifies IL-1β as a key regulator of an antimicrobial gene network linked to host defense

F. Ma[1], R. Teles[2], P. Andrade[2], B. Silva[2], M. Pellegrini[1], R. L. Modlin[3]

[1]MBI, UCLA, Los Angeles, California, United States, [2]Dermatology, UCLA, Los Angeles, California, United States, [3]Dermatology, David Geffen School of Medicine at UCLA, Sherman Oaks, California, United States

Reversal reactions (RR) in leprosy provide an opportunity to investigate mechanisms of host defense against bacterial infections in skin, as patients upgrade from the disseminated lepromatous (L-lep) form to the self-limiting tuberculoid (T-lep) form, often associated with significant tissue injury. Here we performed single-cell RNA-seq to study gene networks associated with host defense comparing lesions from RR vs. L-lep patients (n=10). We detected 43,363 genes in 21,318 cells, with an average of 741 genes and 3,556 transcripts per cell. The 11 subclusters of T cells, myeloid cells, keratinocytes (KC), endothelial cells (EC) and fibroblasts (FB) that were enriched in RR lesions contained 567 genes that encoded programs known to be involved in antimicrobial responses. In addition, pseudotime analysis identified differentiation of macrophages (MF) and KC in the progression from L-lep to RR. We identified IL-1β as a key upstream regulator of the antimicrobial gene network as well as the differentiation programs of both MF and KC. In addition to the direct regulation of genes involved in the vitamin D antimicrobial pathway (IFNG, CD40LG, CD40, IL32, VDR), IL-1β induced the antimicrobial chemokines (CCL2, CCL4, CCL8, CCL20; CXCL1, CXCL2, CXCL3, CXCL8, CXCL11; CX3CL1) and antimicrobial proteins (GBP2; MMP9, MMP14; S100A6, S100A10) in different cell types. IL-1β regulated Th17 cell responses including the antimicrobial protein IL-26. IL-1β coordinates the antimicrobial response from diverse cell types in leprosy as well as inducing differentiation of MF, KC and T cells, such that blockade of the IL-1 receptor could provide an opportunity to mitigate concomitant tissue damage that leads to permanent nerve injury in RR patients.

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Super-resolution imaging of desmosome architecture during assembly and maturation

R. R. Beggs[1], T. C. Rao[1], W. F. Dean[1], R. M. Albert[1], A. Kowalczyk[2], A. L. Mattheyses[1]

[1]Cell, Developmental, and Integrative Biology, University of Alabama at Birmingham, Birmingham, Alabama, United States, [2]Cell Biology, Emory University, Atlanta, Georgia, United States

Desmosomes are macromolecular cell junctions that play a critical role in adhesion and resisting mechanical stress in epithelial tissue. They have a complex architecture with transmembrane cadherins constituting the adhesive interface and intracellular proteins organized into plaques coplanar with the plasma membrane and integrated with keratin. Formation and remodeling of these junctions is essential in wound healing and development. However, changes in desmosome architecture during assembly and disassembly are not well characterized. To understand these dynamic processes, it is vital to relate the nanoscale arrangement of their protein constituents to desmosome maturation and adhesive function. The initiation of desmosome assembly was synchronized with a switch from low to high calcium and we tracked desmosome assembly for 36 hours over the course of maturation. Using super-resolution direct STochastic Optical Reconstruction Microscopy (dSTORM) we quantified the nanoscale organization of the desmosomal protein desmoplakin following the calcium switch. We found the distance between desmoplakin plaques decreased during the process of maturation. Additionally, we found an increase in desmoplakin plaque length, along the plasma membrane, over the same time frame. E-Cadherin associates more strongly with nascent desmosomes. The structural changes reported here correlated with a decrease of E-cadherin colocalization with desmoplakin. Together this suggests that the organization of desmoplakin within desmosomal plaques changes during maturation and the architecture of plaques in nascent desmosomes is not the same as that of mature desmosomes. Understanding desmosome architecture during assembly provides keen insight into an important aspect of maintaining epithelial integrity in healthy states such as wound repair as well as disease states such as skin blistering or cancer metastasis.

Epidermal Structure and Barrier Function

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N/TERTs replicate primary keratinocytes in barrier formation and viral infectivity

M. G. Brewer[1], M. Moran[2], R. P. Pandya[1], B. L. Miller[1], B. M. Ward[2], L. A. Beck[1]

[1]Dermatology, University of Rochester, Rochester, New York, United States, [2]Microbiology and Immunology, University of Rochester, Rochester, New York, United States

Atopic dermatitis (AD) is a skin disease characterized by an impaired epidermal barrier and increased susceptibility to cutaneous viral infections. We have previously observed that barrier disruption (caused by our tight junction disrupting peptide [TJDP®]), enhances vaccinia virus (VV) infection of primary human foreskin keratinocytes (PHFK). To evaluate the importance of AD-relevant tight junction proteins in epidermal viral infections, we sought to identify an epidermal cell line that we could genetically manipulate. We tested N/TERT cells (immortalized PHFK) to determine whether they faithfully recapitulated primary cells in both our barrier and VV infection models. We found that N/TERTs developed a robust barrier, as measured by transepithelial electrical resistance (TEER), with peak values of 300–400 ohms/cm^2 observed 4-5 days after treatment with high Ca^{+2} (1.8mM) containing media. After exposure to our TJDP®, N/TERTs exhibited a substantial reduction in barrier compared to media controls (e.g. TEER reduction: 46% ± 13 and 68% ± 11 at Days 2 and 3, respectively; n=3). We previously observed that PHFK are more susceptible to VV when treated with TJDP® during differentiation. With N/TERTs, we observed an increase in VV susceptibility as measured by % change in plaque number (179% ± 64 at Day 2; n=3) and % of the monolayer infected (27% ± 24 vs 42% ± 20 at Day 2 for media compared to TJDP® treated samples, n=3). These observations suggest that N/TERTs behave similarly to PHFK in both our infection and barrier experiments. Future studies will focus on determining how reduction in AD-relevant TJ barrier proteins (Claudin 1, 4, 23, occludin, and zonula occludens 1) using the CRISPR/Cas9 system impacts the susceptibility of N/TERTs to viral infections (VV and herpes simplex virus).

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Relationship between the physicochemical effect of compounds on phospholipid membranes and their influence on epidermal permeability barrier homeostasis

M. Denda[1], Y. Umino[1], S. Nakata[2], N. Kumazwa[3]

[1]Shiseido Global Innovation Center, Takashimacho Nishiku, Kanagawa, Japan, [2]3. Graduate School of Integrated Sciences for Life, Hiroshima University , Hiroshima, Japan, [3]2. Department of Biomolecular Functional Engineering, College of Engineering, Ibaraki University, Hitachi, Japan

Secretion of lipids from lamellar bodies into the intercellular space between stratum granulosum and stratum corneum is a crucial step in epidermal water-impermeable barrier homeostasis. Our previous work showed that topical application of fatty acids, sex hormones, hexoses, polyols and polymers influences barrier homeostasis, but the effects are highly dependent on even small variations of molecular structure. Therefore, we evaluated the effects of these molecules on the physicochemical properties of phospholipid monolayers and liposomes as models of the lamellar body membrane and cell membrane. Molecules that influenced the barrier recovery process also altered the stability of liposomes and the air-water surface pressure of phospholipid monolayers. Studies using attenuated total reflection Fourier-transform infrared spectroscopy (ATR-IR), differential scanning calorimetry (DSC) and ^{13}C NMR spectrometry suggested that molecules influencing barrier recovery interact specifically with phospholipids. The idea that molecules interacting with phospholipids may influence barrier homeostasis may open up new approaches to treat the barrier abnormalities observed in a variety of skin diseases.

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Altered epidermal function in uninvolved skin supports a pathogenic role of epidermal dysfunction in hand eczema

X. Wang[1], L. ye[1], Q. Lai[2], S. Wen[1], Z. Long[1], X. Qiu[2], P. Elias[3], B. Yang[1]

[1]Dermatology Hospital of Southern Medical University, Guangzhou, China, [2]Center of Chronic Disease Prevention, Puning, China, [3]University of California San Francisco, San Francisco, California, United States

Although compromised epidermal permeability barrier can contribute to the development of contact dermatitis, whether subjects with hand eczema display abnormalities in epidermal permeability barrier function in their uninvolved skin remains unknown. We assess here epidermal permeability barrier function in subjects with or without hand eczema. All volunteers were recruited from clothing manufacturers in Guangdong, China. Epidermal functions, including transepidermal water loss (TEWL) rates, stratum corneum (SC) hydration and skin surface pH were measured on the flexural surface of the left forearm in all volunteers. These epidermal functions were compared among cohorts of subjects with active hand eczema, a prior history of hand eczema and without any history of hand eczema. A total of 650 questionnaires were collected from 462 females and 188 males. Thirty five subjects (5.4%) currently had hand eczema, while 28 subjects (4.3%) reported a prior history of hand eczema that was inactive currently. Neither a prior personal nor a family history of allergies was associated with the prevalence of hand eczema. But certain occupations and frequent contact with disinfectants were independently associated with the prevalence of hand eczema. Males displayed higher TEWL rates and SC hydration levels than did females. Both skin surface pH and TEWL rates differed significantly among normal controls and subjects with active hand eczema, or a prior history of hand eczema (p<0.05).In conclusion, the uninvolved skin site of subjects with hand eczema exhibits abnormalities in epidermal permeability barrier, supporting a pathogenic role of epidermal dysfunction in hand eczema. Whether subjects with hand eczema in other occupations also display altered epidermal function on uninvolved skin remains to be explored.

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The effect of magnolol on the TNF-α/IL-23/Th-17 axis of the imiquimod induced psoriasis-like dermatitis mice

J. Guo[1, 2], Y. Cheng[3], Y. Ro[3], Z. Wu[3], S. Jee[3]

[1]Medical Research Institute, Cathay General Hospital, New Taipei City, Taiwan, [2]Fu Jen Catholic University, Ph.D. Program in Pharmaceutical Biotechnology, New Taipei City, Taiwan, [3]Department of Dermatology, Cathay General Hospital, Taipei, Taiwan

Background: Psoriasis is a common chronic, recurrent, immune-mediated disease of the skin and joints. In psoriasis, epidermal hyperproliferation, abnormal keratinocyte differentiation, angiogenesis with blood vessel dilatation and excess Th-1 and Th-17 infiltration can be observed. Magnolol is a polyphenolic compound that exerts its biological properties through a variety of mechanisms. It has been extensively documented and possesses a range of therapeutic properties such as on dermatology research such as anti-inflammatory, anti-tumor, and anti-free radical activity, and so on. However, there is less study about the effect of magnolol on psoriasis skin barrier. Objectives: The research is intended to clarify the effect of magnolol on psoriasis skin barrier and then develop an alternative therapeutic method for psoriasis treatment. Materials and Methods: BALB/c mice were topical application imiquimod to induced psoriasis-like skin and then randomly assigned to control, vehicle control, low and high dose of magnolol, and Esperson ointment (0.25% desoximetasone) treatment groups for barrier function, cytokines array, and histology assessment. Results: The high dose of magnolol treatment significantly inhibited the IL-23, IL-1β, IL-6, TNF-α, and INF-γ protein expression. However, both low and high doses of magnolol induced a further skin barrier disruption. In contrast, Esperson inhibited all the above cytokines expression, including IL-17A, as well as restore skin barrier functions. Conclusions: The results may imply simultaneous inhibition of IL-17 and IL-23 contributes to improve the barrier functions and therapeutic effect of psoriasis. The effect of topical magnolol combining with topical glucocorticosteroid is worthy of being further investigated on psoriasis treatment.

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Variation in skin barrier function among older adults in a large cohort of healthy aging

K. Abuabara[1], M. Ye[1], S. R. Cummings[1], T. Mauro[1], L. Ferrucci[2]

[1]UCSF, San Francisco, California, United States, [2]National Institute on Aging, Bethesda, Maryland, United States

Prior research on selected populations suggests that skin barrier function declines with age, which may contribute to increases in systemic inflammation known as inflammaging. To define barrier function among older adults and its association with two widely studied serum inflammatory markers (CRP and IL-6), we measured baseline TEWL and recovery after barrier perturbation within a large ongoing NIH cohort designed to study healthy aging, the Baltimore Longitudinal Study of Aging. Measurements were taken in a temperature- and humidity- controlled room as part of an annual 3-day study visit. Baseline TEWL was measured on the mid-volar forearm on day 2 of the study visit and repeated 24 hours after tape stripping to 4x the baseline value. Among 195 subjects ages 60-97 (mean 79 years, standard deviation 8 years), we found a great deal of variability in skin barrier function (baseline TEWL range 0.2-19.4 g/m^2/h, mean 7.1, SD 3.1; % recovery range -213% to 135%, mean 48%, SD 42%). Within this age group, there was no evidence of a linear decline in skin barrier function with age (beta from adjusted linear regression model for % recovery =-0.15, p=0.512); and sex, race/ethnicity, BMI, and smoking history were not significantly predictive of baseline TEWL or % recovery in multivariable regression models. Neither of these measures was significantly associated with serum CRP or IL-6 in adjusted linear regression models, and there was no evidence of an interaction (effect modification) by sex or age. These results fill an important gap in the literature on skin barrier function beginning in middle adulthood and suggest that age is not a major predictor of barrier function among the 60+ age-group in a large cross-sectional analysis. Additional research is needed to assess predictors of skin barrier function change over time and associated inflammatory biomarkers in this population.

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IL-17A suppresses the formation of the granular layer in a human epidermis model via regulation of terminal differentiation genes

E. Sato[1], N. Yano[2], Y. Fujita[2], S. Imafuku[1]

[1]Dermatology, Fukuoka University, Fukuoka, Japan, [2]Pharmaceutics, Fukuoka University, Fukuoka, Japan

Immunotherapies targeting IL-17 have a strong effect on psoriasis. However, most previous studies on IL-17 focused only on the Th17 immune response, and a few studies have reported that IL-17A may affect psoriatic epidermal structure. However, the detailed roles of IL-17A in epidermal keratinization are still unclear. Herein, we demonstrated that IL-17A directly suppressed the formation of the granular layer of an *in vitro* three-dimensional (3D) human epidermis model ($P < 0.0001$), whereas IL-17C did not. IL-17A significantly downregulated the gene expression of profilaggrin (*FLG*) which is a major component of keratohyalin granules in the granular layer by both microarray (Z-score < -6.0) and qRT-PCR ($P < 0.05$). Global gene expression analysis of this in vitro 3D epidermis model showed that both IL-17A and IL-17C upregulated *S100A7A* and type 1 interferon-related genes including *MX1*, *IFI44L*, *XAF1* and *IFIT1*. However, only IL-17A directly downregulated 'GO:0030216~keratinocyte differentiation' and 'GO:0001533~cornified envelope'-related genes including *FLG, LOR, C1ORF68, LCE1E, LCE1B, KRT10, CST6* and *RPTN*. On the basis of these results, we concluded that in addition to being immunologically important, IL-17A is involved in the construction of the epidermal granular layer. Targeting IL-17 may not only reduce inflammation but it may also directly affect epidermal differentiation in psoriasis

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Involucrin knockout mice exhibit decreased Vitamin D receptor expression and reduced Vitamin D agonist-induced skin inflammation

A. D. Schmidt, M. E. Mathyer, E. Brettmann, C. de Guzman Strong

Washington University School of Medicine, St. Louis, Missouri, United States

We previously discovered recent evolution in our human skin barrier for increased Involucrin (IVL) in European populations and relatively lower IVL levels in African populations suggesting an adaptive benefit for IVL dosage for cutaneous health. No defects in epidermal barrier development were observed in Ivl -/- mice. However, we have a poor understanding of the role of IVL for epidermal barrier maintenance in response to an inflammatory trigger. I hypothesized that Ivl deficiency would result in increased inflammation. To test this hypothesis, we examined the inflammatory response in ear skins of Ivl -/- and control wild-type mice upon vitamin D agonist (MC903, calcipotriol) treatment. Wild-type mice exhibited notable skin inflammation and hyperkeratosis in the MC903-treated ears as expected in comparison to ethanol-treated controls ($P<.001$). However, Ivl -/- mice revealed a surprising reduction in skin inflammation compared to wild-type mice. We further determined a dominant effect for Ivl reduced MC903-induced inflammation evident in Ivl -/-, Ivl +/- and Ivl +/- Flg +/- mice in contrast to Flg+/- and wild-type mouse skins which exhibited higher and high inflammatory responses, respectively. The dampened inflammatory response coincides with reduced expression of Tslp, a known mediator of MC903-induced inflammation, in Ivl -/- compared to wild-type mice. We next examined the potential mechanism by which MC903-induced inflammation was reduced in Ivl-/- mice and found reduced Vitamin D receptor (Vdr) expression in Ivl-/- skin that was not observed in wild-type mice. The data suggests a positive correlation for Ivl dosage to Vdr expressions that was further confirmed in primary human keratinocytes whereby darker pigmentation coincides with lower IVL and VDR. Together, our findings identify functional significance for IVL in the epidermis revealing an emerging paradigm for IVL dosage that has recently evolved in human adaptation to potentially regulate VDR expression in the epidermis.

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Purified Cannabidiol Isolate does not appear to be anti-inflammatory in post-treated UVB or ATP NLRP Inflammasome-activated Normal Human Epidermal Keratinocytes (NHEK) i*n vitro*.

J. V. Gruber[1], R. Holtz[2]

[1]JVG Innovative Consulting, Washington, New Jersey, United States, [2]Bioinnovation Laboratories, Inc, Lakewood, Colorado, United States

Recent work has suggested that Cannabidiol may possess anti-inflammatory properties, but frequently, these claims are based around testing of various mixtures of CBD-containing oils, particularly hemp oil. The results of the testing, therefore, could be related to other ingredients found with the CBD. Cannabidiol Isolate is a highly purified form of CBD that is isolated from the flowering bodies of *Cannabis sativa*. CBD Isolate typically has a Cannabidiol purity of greater than 99% with no residual Tetrahydrocannabinol (THC). Recently, a model for examining NLRP inflammasome-activated release of active Caspase-1 in UVB and ATP-activated Normal Human Epidermal Keratinocytes (NHEK) was reported [1]. This model allows for direct examination of the impact of topical treatments on activated NHEKs through measurement of release of active Caspase-1 (ACasp-1). This paper will examine the cellular cytotoxicity in non-exposed cells using the MTT assay and then examine the effects of cellular CBD treatment on UVB- and ATP-activated NHEKs. In addition, looking specifically at UVB treatments, NHEKs were examined as well for the release of inflammation markers including PGE2, IL-6 and IL-8. Results indicate that Cannabidiol Isolate does not appear to have anti-inflammatory benefits when post-added to UVB or ATP-activated NHEKs.[1] Gruber JV, Holtz R. In vitro expression of NLRP inflammasome-induced active Caspase-1 expression in normal human epidermal keratinocytes (NHEK) by various exogenous threats and subsequent inhibition by naturally derived ingredient blends. *J Inflamm Res* 2019

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Inducible nitric oxide synthase regulates epidermal permeability barrier homeostasis

E. Dang[1], G. Man[2], D. Lee[5, 2], J. Zhang[3, 2], Z. Li[4, 2], T. Mauro[2], P. Elias[2], M. Man[2]

[1]Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi, China, [2]Dermatology, VA Med Ctr/UCSF, San Francisco, California, United States, [3]Dermatology, Huadong Hospital/Fudan Univ, ShangHai, China, [4]Dermatology, 2nd Affil Hosp, Xi'an Jiatong Univ, Xi'an, Shaanxi Province, China, [5]Princeton University, Princeton, New Jersey, United States

Though a regulatory role for nitric oxide (NO) in diverse cutaneous functions is well known, whether NO impacts permeability homeostasis remains unexplored. Here, we utilized inducible nitric oxide synthase (iNOS) KO mice to explore the role of NO and iNOS in epidermal homeostasis. Although iNOS KO and wild type mice looked grossly similar, iNOS KO mice displayed a significant abnormality in permeability barrier recovery in comparison with wild type mice, and epidermal biophysical properties were comparable between these two groups at baseline. Consistent with delayed barrier recovery, the expression of genes strongly linked to permeability barrier homeostasis, including epidermal lipid synthetic enzymes (HMGCoA reductase, fatty acid synthase, serine palmitoyltransferase), and keratinocyte differentiation marker-related proteins (filaggrin, loricrin and involucrin) was significantly decreased in iNOS KO mice. Pertinently, topical applications of NO upregulated the expression of epidermal mRNA for lipid production and keratinocyte differentiation in parallel with accelerated barrier recovery in iNOS KO mice. Taken together, these results indicate that the generation of NO by epidermal iNOS is crucial for epidermal homeostasis.

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Association of epidermal dysfunction and constipation in the elderly

L. ye[1], C. Lv[2], Z. Wang[3], S. Wen[1], B. Yang[1], M. Man[1]

[1]Dermatology Hospital of Southern Medical University, Guangzhou, China, [2]Dalian Dermatology Hospital, Dalian, China, [3]Shenyang No.7 Hospital, Shenyang, China

Chronologically aged humans display epidermal dysfunction and high prevalence of constipation. Whether epidermal dysfunction and constipation are associated is unknown. In the present study, we compared the epidermal function in subjects with and without constipation. A total of 577 aged subjects, including 169 males and 408 females, were enrolled in this study. Epidermal biophysical properties, including transdermal water loss (TEWL) rates and stratum corneum hydration, on the flexor forearm, were measured with a Gpskin device, while skin surface pH was measured with a portable pH meter. Our results showed that the stratum corneum hydration levels were 14% lower in subjects with constipation than that without constipation. Moreover, both TEWL rates and skin surface pH were significantly higher in subjects with constipation than that without constipation ($p<0.05$). Furthermore, permeability barrier recovery was delayed in subjects with constipation in comparison with those without constipation. In contrast, epidermal biophysical properties were comparable in subjects with or without gastric disorders. Interestingly, twice-daily applications of an emollient for 6 months improved epidermal functions, such as stratum corneum hydration, TEWL rates and barrier recovery rates, along with marked reductions in constipation scores. These results suggest that epidermal dysfunction plays a pathogenic role in aging-associated constipation, and improvements in epidermal function can alleviate aging-associated constipation.

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Ability of mathematical models to predict human *in vivo* percutaneous penetration of steroids

A. Burli[1], H. I. Maibach[3], R. M. Law[2]

[1]University of Rochester School of Medicine , San Jose, California, United States, [2]Memorial University of Newfoundland, St. Johns, Newfoundland, Canada, [3]University of California, San Francisco, San Francisco, California, United States

Background: Human skin is a common route for topical steroids to enter the body. To aid with risk management of therapeutic steroid usage, the US Environmental Protection Agency estimates percutaneous penetration using mathematical models. Research Question: How accurate are mathematical models in estimating percutaneous penetration/absorption of steroids? Methodology: In this study, the accuracy of predicted flux (penetration/absorption) by the widely used Potts and Guy model based on in vitro data is compared to actual human in vivo data of percutaneous absorption of topical steroids. Results: For most steroids the flux was over- or underestimated by a factor 10-100. However, within the group itself, there was an association between the Potts and Guy model and experimental human in vivo data (Pearson Correlation= 0.786, $p=0.001$). Additionally, the physiochemical parameters used in the Potts and Guy equation, namely Kp, Koctanol, and molecular weight, did not correlate significantly with in vivo flux. Conclusion and Discussion: Current mathematical models used in estimating percutaneous penetration/absorption did not accurately predict in vivo flux of steroids. Why? Proposed limitations to mathematical models currently used include: not accounting for volatility, lipid solubility, hydrogen bond effects, drug metabolism, as well as protein binding. Further research is needed in order to increase the predictive nature of such models for in vivo flux.

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Regulated mitochondrial fission via NIX and DRP1 in keratinocytes drives epidermal differentiation

C. L. Simpson[1], M. Tokito[1], R. Uppala[2], M. Sarkar[2], J. E. Gudjonsson[2], E. Holzbaur[1]

[1]Univ. of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Univ. of Michigan, Ann Arbor, Michigan, United States

Epidermal keratinocytes initiate wholesale organelle degradation during cornification, but the mechanisms driving this process are poorly understood. Using live confocal microscopy, we imaged single organelle dynamics in organotypic human epidermis focusing on mitochondria, which are known to regulate keratinocyte differentiation. Mitochondria in the lower epidermis underwent cyclic fission and fusion, but in the upper layers, they became stably fragmented, depolarized, and acidified, leading us to investigate if they were targeted for autophagic degradation. We found differentiating keratinocytes up-regulate the autophagy receptor NIX, which is concentrated on granular layer mitochondria in both organotypic epidermis and human skin biopsies. Thus, we hypothesized NIX could serve as an initiation signal for mitochondrial degradation during cornification. Accordingly, ectopic expression of NIX in undifferentiated keratinocytes was sufficient to induce mitochondrial fragmentation and led to premature differentiation of epidermal cultures. To test the requirement for NIX in epidermal morphogenesis, we used CRISPR/Cas9 gene editing to generate NIX-deficient human organotypic epidermis. NIX-targeted cultures exhibited impaired expression of epidermal differentiation markers and aberrant mitochondrial degradation seen by electron microscopy. Delineating the underlying mechanism, we showed that NIX enhanced mitochondrial localization of FIS1, a receptor for the membrane fission GTPase DRP1. Further, over-expression of FIS1 induced fragmentation of keratinocyte mitochondria similar to NIX. Finally, we showed that compromising DRP1 function directly using a dominant-negative mutant blocked mitochondrial fragmentation in the upper epidermal layers, resulting in abnormal morphology and differentiation of suprabasal keratinocytes. In sum, we report a novel pathway in differentiating keratinocytes driving break-down of mitochondria via the autophagy receptor NIX and DRP1 in order to support proper epidermal morphogenesis.

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Increased 11β-hydroxysteroid dehydrogenase type 1 contributes to the impaired barrier in aged skin

B. Kim[1], N. Lee[1], C. Lee[1], Y. Lee[1], S. Lee[1, 2], H. Hwang[1], E. Kim[1], E. Choi[1]

[1]Dermatology, Yonsei University Wonju College of Medicine, Wonju, Korea (the Republic of), [2]Preventive Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea (the Republic of)

11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts cortisone, an inactive form of glucocorticoid (GC) into cortisol, the active form in human. It is expressed by several tissues including the skin. Excessive active GC deteriorates skin barrier function. Aged skin presents skin barrier impairment. This study was conducted in human and mice to determine if 11β-HSD1 affects the impaired barrier function of aged skin. For human study, elderly and young peoples were enrolled. Mice were divided into the wild aged mice, wild aged mice treated by topical 11β-HSD1 inhibitor, 11β-HSD1 knockout (KO) mice, wild young mice, and wild young mice treated by 11β-HSD1 inhibitor. Cortisol levels were elevated in the stratum corneum (SC) and oral epithelium of the elderly rather than the young. The 11β-HSD1 expression was increased in immunohistochemistry staining of aged mouse skin. Aged mice showed higher transepidermal water loss (TEWL) and lower SC hydration than young ones. Serum inflammatory cytokines such as interleukin-1α, -4, -31 and tumor necrosis factor-α were significantly increased in aged mice than in young mice. SC corticosterone was suppressed in aged 11β-HSD1 KO mice and topical 11β-HSD1 inhibitor applied wild-type mice. Horneodesmosome density was suppressed in aged wild mice, but elevated in aged 11β-HSD1 KO mice and in aged wild mice treated by topical 11β-HSD1 inhibitor. The number of lamellar bodies was increased in topical 11β-HSD1 inhibitor applied mice compared to wild aged mice. The amounts of SC lipids including ceramides, cholesterol, and fatty acids were increased in topical 11β-HSD1 inhibitor applied mice compared to wild aged mice. Expressions of mRNA of lipid synthesis related enzymes were increased in aged 11β-HSD1 KO mice and topical 11β-HSD1 inhibitor applied wild mice. Conclusively, 11β-HSD1 expression is elevated in the aged skin, that increases active GC and then deteriorates skin barrier function.

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Biological effects and skin bioavailability of Aphloïol

C. Laperdrix[1], V. Mariette[1], S. Guenin[2], D. Bonnel[3], F. Bernard[2], C. Lubrano[1]

[1]R&D, Yves Rocher, Issy Les Moulineaux, France, [2]Bioalternatives, Gencay, France, [3]Imabiotech, Loos, France

Aphloïol (syn Mangiferin) is a photostable C-glucoside xanthone from vegetal species (*Aphloïa theaformis, Mangifera indica*). It is well known for free radical scavenging and antioxidant effect. Previously, we patented protective effect against stresses maintaining cell viability, inhibiting dermal matrix degradation enzymes and pro-inflammatory mediator release. In this study, we focussed on skin architecture and on epidermal differentiation. The effect was evaluated using Affymetrix and RT-qPCR on monolayers and at the protein level (immunolabellings) on 3D-models. The effect of aphloïol was also assessed on lipid synthesis in (TLC separation). Secondly, we investigated skin penetration of aphloïol with the Franz cell method with UV and a new label-free method were tested; MALDI-FTICR (Matrix Assisted Laser Desorption Ionization Fourier Transform Ion Cyclotron Resonance) imaging. It allows detection and quantification of molecules on tissue section without any labeling, generating a specific molecular spectrum (*m/z)* for each laser impact. The results demonstrated that aphloïol strongly stimulated epidermal differentiation of keratinocytes by increasing the expression of genes coding for late differentiation keratins, transglutaminases, filaggrin, loricrin and proteins of junctions (desmosomal cadherins). These effects were confirmed at the protein level in 3D conditions and indicated that aphloïol was at least as effective as calcium on keratinocyte differentiation. In addition, aphloïol increased the production of several categories of lipids, especially ceramides and cerebrosides, thus corroborating the positive effect of aphloïol on epidermis differentiation, not only at early stages but also on late process. In addition to its protection properties against oxydation and inflammation, aphloïol is now described and patented as a potential and efficient keratinocytes differentiation inducer. Thanks to MALDI FTICR, we also demonstrated the specific and quantitative penetration of aphloïol into the different skin compartments.

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Comprehensive microbiota analysis of oily *versus* normal scalp

S. Leoty-okombi[1], A. Guinta[2], D. Nicolas Stefano[2], G. Kritikos[3], V. Andre-frei[1], P. Ludwig[2]

[1]BASF BCS, Lyon, France, [2]BASF Corp, Tarrytown, New York, United States, [3]BASF SE, Ludwigshafen, Germany

Scalp conditions management deserves a holistic approach considering the scalp environment (oily/non oily) and its microbiota composition and balance. This prompted us to conduct a comprehensive study-based DNA sequencing analysis to observe the microbiome diversity. The group with normal healthy scalp (non-oily) was characterized by a significant ($p<0.05$) higher diversity in: *Staphylococcus warneri* and *Staphylococcus pasteuri* commensal bacteria producing antimicrobial peptides to fight other pathogenic microorganisms; -*Streptococcus australis* able to antagonize pathogenic *Streptococcus mutans;* -*Veillonella parvula*, a commensal also found in the oral, gastrointestinal, respiratory, and female genital tract biota, coexisting with producing lactic acid bacteria; -*Rothia aeria* able to produce catalase after lactic acid metabolization; -*Actinomyces odontolyticus*, a gram+ facultative anaerobe known to produce a natural occurring antioxidant enzyme: the superoxide dismutase. These bacteria seem to be aerobic and low lipophilic bacteria, likely due to the lower sebum levels on the normal scalp. The volunteers with oily scalp had a high abundance of lipophilic and anaerobic bacteria, which are hypothesized to be thriving off the sebum rich scalp. We noticed in particular bacteria which are likely to be more pathogenic than commensal as: -*Propionibacterium granulosum* isolated from acneic skin; -*Anaerococcus vaginalis* involved in chronic wound; -*Finegoldia magna* described as an opportunistic skin pathogen; -*Corynebacterium kroppenstedtii* associated with extensive rosacea. This comparison between oily and normal healthy scalp microbiome composition will support us to evaluate substances which may help us to recover a normal and healthy scalp.

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Dry skin, altered barrier function & inflammation: The vicious circle of Inflamm'Dryness™

E. Valin[1], C. Bize[1], E. Le Gélébart[2]

[1]Innovation, Seppic, Castres, France, [2]Innovation, BiotechMarine, Pontrieux, France

Dry skin is the most important concern in skin care worldwide. Indeed, this phenomenon occurs in healthy individuals thus being a cosmetic target, but it is also a major dermatologic challenge to address atopic dermatitis and psoriasis prone skins for instance. Dry skin manifests itself in the daily life through tightness, itching, lack of elasticity, scaly microrelief and an overall discomfort. Dry skin is due to genetic factors and environmental parameters which contribute greatly (sun exposure, heat/cold, inappropriate cleansing routine…) and can lead to escalate the condition. In terms of biology, it can be correlated to several causes such as an impaired lipid production (loss of impermeability), a lack of water in the uppermost layers of the skin (dehydration), an over-proliferation of keratinocytes within the epidermal compartment and a disruption of the *stratum corneum*. In the past years, scientific studies have shown that an alteration of the barrier function could trigger, via the activation of different cell types (keratinocytes, Langerhans cells…), an inflammation signalling cascade. This biological response acts deeply within the tissue maintaining dry skin condition through a vicious circle, that we called Inflamm'Dryness™. Our scientific team has unveiled the biological mechanisms behind Inflamm'Dryness™ and identified interesting biomarkers of skin hydration giving rise to a new approach regarding skin homeostasis. Inflammation is a natural and physiological defence mechanism, but it needs to be controlled in order to return to tissue homeostasis. This resolution phase which involves specific proteins, is a key step in opposing the negative impact of inflammation on dry skin. Furthermore, to address this concept, we have developed an innovative product which has demonstrated its *in vitro* and *in vivo* efficacy. It allows to restore the barrier function, improves the three-dimensional structure of the tissue, helps the resolution of inflammation and in fine, increases epidermal moisturization thus bringing balance and relief to dry skin.

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Metabolomic identification of an essential glucose-IRF6 axis in differentiation

V. Lopez-Pajares[1], A. Bhaduri[1], A. Guerrero[1], G. Gowrishankar[1], A. Sanchez[1], S. Gambhir[1], P. Khavari[1, 2]

[1]Dermatology, Stanford University , Stanford, California, United States, [2]VA Palo Alto, Palo Alto, California, United States

Advances in high throughput metabolomics enable discovery of new essential roles for biomolecules in processes such as epidermal differentiation. Metabolomic analysis during keratinocyte differentiating using 5 types of mass spectrometry that detects >14,000 diverse analytes in all major metabolite classes was performed and unexpectedly, glucose was a top increased analyte of the 614 that changed significantly. Functional studies in epidermal tissue demonstrated that intracellular glucose elevation was essential for normal differentiation, where it was required both for physiologic cell cycle arrest and induction of terminal differentiation genes. Metabolites in glucose catabolic pathways were unchanged in differentiation, suggesting that the accumulated pool of glucose itself was required for differentiation. Consistent with this, decreasing cellular glucose levels, both by restricting available glucose as well as by increasing intracellular glucose catabolizing enzymes, HK1/2 and G6PD, blocked differentiation. The effects of glucose restriction were rescued by non-metabolizable glucose analogs indicating primary action by glucose itself. Systematic knockout and pharmacologic inhibition studies demonstrated that 3 glucose transporters, GLUT1, GLUT3 and SLGT1, were essential for both glucose accumulation and differentiation. Glucose affinity chromatography followed by mass spectrometry identified the IRF6 transcription factor as a glucose binding protein. IRF6 was essential for normal epidermal differentiation and was verified to bind glucose directly at high affinity. Glucose was found to enhances IRF6 binding to its cognate DNA binding sequences in vitro and by ChIP. Interestingly, an IRF6 mutant found in both ectodermal dysplasia and cancer displayed diminished glucose binding. These data support a model in which epidermal differentiation requires upregulation of specific glucose transporters that enable accumulation of glucose, which in turn binds to and enables IRF6-driven differentiation gene induction.

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Skin barrier, inflammation, and metabolism– connections through Ovol1/Ovol2

M. Dragan, P. Sun, D. Haensel, R. Vu, A. Verlande, A. Pham, Q. Nguyen, G. Gutierrez, S. Masri, X. Dai

Biological Chemistry, UC Irvine, Irvine, California, United States

Skin barrier dysfunction contributes to inflammatory diseases such as psoriasis and atopic dermatitis, which can lead to systemic defects (e.g. atopic march). The cellular and molecular events that regulate barrier-induced inflammation and its connection to whole-body physiology remain to be fully understood. SNPs in human *OVOL1*, which encodes a transcriptional repressor, are associated with many skin inflammatory diseases. Germline deletion of *Ovol1* leads to epidermal hyperproliferation and a transient delay in embryonic skin barrier acquisition. However, the function of *Ovol1* and its homolog *Ovol2* in adult skin homeostasis and inflammation is unknown. Here we show that skin epithelia-specific deletion of *Ovol1* results in radically aggravated barrier disruption and psoriasis-like inflammation upon stimulation with imiquimod. Using ChIP-seq we found that Ovol1 protein in epidermal cells directly binds to the promoters of not only genes involved in controlling proliferation and differentiation (e.g. *Id1*) but also genes involved in inflammation (e.g. *Cxcl1* and *Nfkb2*). We generated mice with both *Ovol1* and *Ovol2* inducibly deleted in adulthood specifically in epidermal cells and found the mice to rapidly develop skin barrier defects. Subsequently, they developed oversized paws with elongated and reddish toenails, and lower bodyweight and reduced fat deposits. Preliminary analysis using metabolic cages shows that these mice eat more and are metabolically more active than their control littermates. Additionally, they release more heat while maintaining a normal body temperature. Current experiments focus on characterizing potential inflammation in mutant mice and analyzing gene expression changes in skin and metabolic tissues such as brown adipose tissue of these mice. Our goal is to elucidate the mechanism by which Ovol1 and Ovol2 regulate skin barrier function and inflammation, and how cutaneous defects are linked to alterations in whole-body physiology and metabolism.

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The lipoxygenase inhibitor ML355 prevents covalent adduction of the corneocyte lipid envelope in a novel preclinical model of congenital ichthyosis

J. Meyer, T. Mauro, P. Elias

Dermatology, VA Med Ctr/UCSF, San Francisco, California, United States

Epidermal lipoxygenase (LOX) deficiency is a major cause of autosomal recessive congenital ichthyosis (ARCI), and it could also drive common skin diseases with reduced protein-bound ceramides. In this study, we validated a pharmacological model of epidermal LOX deficiency using the commercially available platelet 12S-LOX inhibitor ML355. ML355 inhibited methyl arachidonate LOX activity in differentiated human foreskin keratinocytes (HFK) lysates in a dose-dependent and noncompetitive manner, with an IC50 of ~30 μM and >75% inhibition at 100 μM. ML355 did not compromise keratinocyte viability (assessed by trypan blue exclusion in HFKs), but caused dose-dependent barrier dysfunction in human epidermal equivalent (HEE) cultures (assessed by transepithelial electrical resistance, TEER). Protein-bound ceramides and omega-hydroxy fatty acids (analyzed by thin-layer chromatography) were reduced by ML355, while free lipids were relatively preserved. The histopathology and ultrastructure of ML355-treated HEEs were characterized by hyperkeratosis, cytosolic lipid droplet accumulation, and defective lamellar lipid processing, despite normal lamellar body structure and no overt signs of cellular toxicity. Lipid membranes were present surrounding the corneocyte protein envelope in ML355-treated HEEs, but unlike the corneocyte lipid envelopes (CLEs) in control HEEs, these could be removed by organic solvent treatment, indicating that ML355 inhibited covalent adduction of the CLE but not the delivery of CLE lipids. These results demonstrate that ML355 inhibits keratinocyte LOX activity and causes an ichthyosis-like phenotype in HEE cultures. ML355-treated HEEs should be a useful model to test treatments for ARCI and other diseases associated with epidermal LOX deficiency.

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Desmoglein 1 deficiency in knockout mice impairs epidermal barrier formation and results in a psoriasis-like gene signature in E18.5 embryos

Q. R. Roth-Carter[1], L. Godsel[1], J. L. Koetsier[1], J. A. Broussard[1, 6], H. E. Burks[1], G. Fitz[4], A. L. Huffine[1], S. Amagai[1], S. Lloyd[3], J. Kweon[3], L. C. Tsoi[2], W. R. Swindell[2], G. Urciuoli[5], C. Missero[5], X. Bao[3], J. E. Gudjonsson[2], K. J. Green[1, 6]

[1]Department of Pathology, Northwestern University, Chicago, Illinois, United States, [2]Dermatology, University of Michigan, Ann Arbor, Michigan, United States, [3]Molecular Biosciences, Northwestern University, Evanston, Illinois, United States, [4]Vanderbilt, Nashville, Tennessee, United States, [5]CEINGE, Naples, Italy, [6]Dermatology, Northwestern, Chicago, Illinois, United States

Desmoglein 1 (Dsg1) is a desmosomal cadherin expressed in concert with a commitment to stratify and differentiate in multi-layered tissues. Its importance in epidermal function is underscored by human diseases such as SAM syndrome (Severe Skin Dermatitis, Allergies and Metabolic Wasting), caused by loss of function mutations. Dsg1 promotes differentiation and normal epidermal morphogenesis in human 3D epidermal models. To determine the systemic response to loss of Dsg1, we deleted the three, tandem a, b, and g genes in the mouse cadherin cluster using CRISPR/Cas9. Dsg1 knockout (KO) mice exhibit barrier impairment accompanied by peeling, denuded skin, and postnatal lethality. The basal desmosomal cadherin, desmoglein 3, is upregulated in Dsg1 KO skin, while differentiation associated proteins loricrin and filaggrin are decreased. Disrupted ZO1 localization, increased TEWL, and increased dye penetration are consistent with a defect in barrier formation. Whole transcriptome analysis of E18.5 skin was consistent with the observed alteration in differentiation and barrier formation, but also revealed an increase in pathways linked to psoriatic processes, including IL-17 signaling, MAPK activity, and ErbB signaling. There was significant overlap between the top 100 genes increased in human psoriasis lesions and genes increased in E18.5 Dsg1 KO animals. These data not only support an important role for Dsg1 in epidermal differentiation and barrier formation, they identify a potential role for Dsg1 in regulating inflammatory responses, with loss of Dsg1 in mouse embryo skin activating inflammatory pathways similar to those observed in patients with psoriasis.

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Skin controls gut immune function through innate immune ECM cross talk

T. Dokoshi[1], F. Li[1], M. Liggins[1], M. R. Williams[1], J. Seidman[2], R. Knight[1], B. C. Taylor[1], J. T. Chang[1], J. Olvera[1], R. L. Gallo[1]
[1]University of California San Diego, San Diego, California, United States, [2]University of California, San Diego, San Diego, California, United States

Inflammatory bowel diseases (IBD) are associated with several skin inflammatory diseases but the mechanism responsible for communication between organs is unknown. Local immune activation occurs in part by the action of induced hyaluronidase in the extracellular matrix (ECM) and subsequent recognition of soluble HA fragments. We hypothesized that such HA fragments may also act to enable organ crosstalk between skin and gut. To test this, the intestine was examined in mice expressing hyaluronidase in the skin (K14/Hyal1 mice). K14/Hyal1 mice do not show spontaneous skin inflammation. These mice were then compared with littermate controls or mice with incisional skin wounds (Wd) that induce the endogenous dermal hyaluronidase *Cemip*. Both groups showed HA digestion in the dermis. Remarkably, all skin specific interventions enhanced DSS-induced inflammation in the colon as seen by greater weight loss ($p<0.0001$) and lower survival rates (Control 100% survived, K14/Hyal1: 20%, Wd; 80%). Transcriptional profiling by single cell RNA Seq revealed that expression of hyaluronidase in skin promoted large changes in the abundance of colon fibroblast subsets; cluster 5 of 9 increased from 1.21% to 43.2%, and clusters 0, 2 and 7 decreased from 29.8 to 4.16%, 18.9 to 0% and 4.5 to 0%, respectively). Genes altered in these subsets were validated by whole tissue RNA Seq and qRT-PCR, and after DSS challenge showed activation of genes related to reactive adipogenesis in colon. FACS analysis also showed increase in PDGFRa and Thy-1 positive cell populations (K14/Hyal1: p=0.0001, Wd; p=0.07) as well as a shift in resident colon RORgt Tregs (vs K14/Hyal1: p=0.009, Wd; p=0.013). The fecal microbiome also significantly changed in K14-Hyal1 mice compared to cohoused littermate controls(vs K14/Hyal1: p=0.05). Taken together, these data show how disruption of skin ECM HA may explain associations between skin and gut disorders.

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Loss of EPHA2 represses GATA-3 function and causes a terminal differentiation defect

P. Kumar, Z. R. Murphy, S. Lenz, N. Kaplan, L. Lyass, S. Yang, S. Bhatt, B. E. Perez White
Northwestern University, Chicago, Illinois, United States

Epidermal morphogenesis and differentiation require the coordination of complex signal transduction networks. These signal relays are often initiated at the plasma membrane and transmitted to the nucleus to control gene expression. Receptor tyrosine kinases (RTKs) are integral in orchestrating communication cascades to induce differentiation. We report loss of EPHA2 RTK causes a terminal differentiation defect in 3D human skin equivalents (3D HSE) resulting in loss of stratification and ablation of the granular and cornified layers. In EPHA2-deficient (shEPHA2) 3D HSE, we show significant loss ($P<0.05$) of loricrin, filaggrin, and involucrin protein and mRNA, indicating EPHA2 signaling can impact keratinocyte differentiation at the transcriptional level. The transcription factor GATA-3 is a key driver of differentiation-associated gene expression in epidermis, although the upstream signals regulating its activity are unknown. GATA-3 is expressed in the nuclei of suprabasal keratinocytes in mature 3D HSE, mimicking the pattern in normal human skin ($R^2=0.99$). In 2D cultures, GATA-3 accumulates in the nucleus following 24 h exposure to 1.2 mM calcium. However, GATA-3 protein and transcript expression are lost in shEPHA2 3D HSE and 2D cultures ($P<0.001$). Further, there is a decrease of GATA-driven transcription indicated by a significant loss in GATA binding activity in a promoter luciferase reporter assay ($P<0.001$). Re-expression of nuclear GATA-3 using a retroviral construct in EPHA2-deficient 3D HSE restored differentiation. Taken together, these results indicate that EPHA2 promotes GATA-3 nuclear accumulation to positively regulate the transcription of terminal differentiation genes in epidermal keratinocytes.

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IRAK2 promotes abnormal epidermal differentiation during inflammatory states to facilitate and amplify immune responses in skin

S. Shao[1, 2], J. E. Gudjonsson[2], L. C. Tsoi[2], B. E. Perez White[3], B. Andersen[4], R. L. Modlin[5], S. Weidinger[6], J. Kahlenberg[7]
[1]Dermatology, Xijing Hospital, Fourth Military Medical University, Xi'an, China, [2]Dermatology, University of Michigan, Ann Arbor, Michigan, United States, [3]Northwestern University, Chicago, Illinois, United States, [4]Department of Biological Chemistry, University of California, Irvine, California, United States, [5]Division of Dermatology, Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, California, United States, [6]Department of Dermatology and Allergy, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany, [7]Division of Rheumatology, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, United States

Many inflammatory diseases of the skin including atopic dermatitis (AD) and psoriasis are characterized by altered epidermal differentiation, but the mechanisms for such changes have remained unclear, in particular given their diverse immunologic pathogenesis. Here we show that IRAK2, a member of the signaling complex downstream of IL-1/IL-36, correlates positively with disease severity in both AD and psoriasis. IRAK2 brings together pro-inflammatory and differentiation dependent responses and this function of IRAK2 is specific to keratinocytes. Using 3D epidermal raft models, we demonstrate that IRAK2 is responsible for epidermal thickening (acanthosis) and promotion of epidermal immune responses. Lastly, through RNA-sequencing and single cell RNA-sequencing analyses we show that the transcription factor ZNF750 is a critical downstream mediator of this role of IRAK2, influencing both epidermal differentiation and pro-inflammatory responses. Taken together, our findings suggest that IRAK2 plays a common critical role in promoting altered inflammatory epidermal differentiation that is responsible for the feed-forward amplification of inflammatory responses of otherwise diverse etiologies in skin. This work identifies IRAK2 as a novel potential therapeutic target for inflammatory skin diseases including AD and psoriasis, which likely broadens to other chronic inflammatory diseases involving epidermal hyperplasia.

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Analysis of corneodesmosomal proteins in nummular eczema skin

S. Montenegro, J. Oh, J. Mun, J. Chung
Dermatology, Seoul National University College of Medicine, Seoul, Korea (the Republic of)

Nummular eczema, a chronic dermatitis characterized by coin-shaped lesions, was first introduced by Devegie in 1857. In spite of its long history, data regarding its corneodesmosomal components are hardly available in the current literature. We performed this study to investigate whether the distribution of corneodesmosomal components and surface areas of corneocytes in nummular eczema skin lesions are different from those found in healthy skin. Tape-stripping was used to acquire corneocytes from a disease group (n = 24 cases both lesional and non-lesional skin) and a control group (n = 15). Western blotting and immunofluorescent microscopy were performed. Corneodesmosomal proteins and their regulators showed higher levels of expression in nummular eczema-affected skin compared to the skin of healthy subjects, including corneodesmosin, desmoglein-1, desmocollin-1, kallikrein-7, caspase-14, and the lympho-epithelial Kazal-type related inhibitor. The staining patterns in the tape stripped samples were abnormal in the lesional skin of nummular eczema. A dense diffuse pattern was prominent in lesional skin, while in the non-lesional and healthy group, the peripheral pattern with a small expression of the sparse diffuse pattern was noted. In summary, the tape stripping method revealed the abnormal expression of corneodesmosomal proteins and corneodesmosome staining patterns in the skin lesions of nummular eczema. The corneocytes in nummular eczema were found to be abnormally differentiated. In particular, an increase in the ~30 KDa form of LEKTI in lesional skin appeared to contribute to an increase in the amount of fragmented proteins and a delay in skin desquamation.

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Deficiency of Wnt5a in keratinocyte does not ameliorate the imiquimod-induced psoriasis-like dermatitis

F. Tian[1], Z. Li[2]

[1]Medical Research Center, North China University of Science and Technology, Tangshan, Hebei, China, [2]Dermatology, Xi'an Jiaotong University, Xi'an, Shaanxi Province, China

Objective Wnt5a activated signalling cascades has been proved to be involved in the development of Psoriasis. However, the importance of keratinocyte produced Wnt5a in Psoriasis remained to be tested. The present study aimed to determine the effect of keratinocyte-specific knockout of Wnt5a on psoriasis-like dermatitis in imiquimod-induced mice. Methods Eight weeks old mice,in keratinocyte-specific knockout of Wnt5a (Wnt5a-cKO) mice and control littermates, were randomly assigned to receive topical application of Imiquimod (5% cream, 50mg) (IMQ) or control vihicle (VEH) on their shaved back for 7 days. Phenotypical presentation and severity index (PASI) modified from the human PASI guidelines to score scales, erythema, skin thickness of mouse back skin. Skin barrier was assayed by gpskin.Spleen index was calculated fluorescence activated cell sorter (FACS) was used to assess therapies of CD11+, CD68 +, and CD4 +, γσTCR+ T cell subsets in peripheral blood. Expression of β-catenin and its phosphorylation were detected by western blot and Immunofluorescence staining. Results PASI score showed significantly higher score in IMQ treated mice, with no significant difference between cKO and control littermates.Skin barrier assay showed increased skin barrier score and TEWL in IMQ treated mice in both cKO and control littermates.IMQ induced mild splenomegaly with non-significant higher spleen index compared to VEH treated mice, FACS profiles showed increased levels or ratios of CD11+, CD68 +, and CD4 +, γσTCR+ T cell subsets by IMQ stimulation in both cKO and control.Western blot analysis indicated that phosphorylation of β-catenin was markedly enhanced in control littermates, but not cKO mice, after 1 week IMQ treatment. Conclusions These results indicate that keratinocyte produced wnt5a is not nessecary for psoriasis-like dermatitis development in imiquimod-induced mice,even though the enhanced phosphorylation of β-catenin by IMQ stimulation was repressed in wnt5a cKO mice.

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Novel chia seed extract (HyVia™) inhibits demethylation of PP2A and increases barrier repair markers, resulting in increased hydration of human skin

J. R. Fernandez[1], K. L. Huber[1], C. N. Webb[1], J. Healy[1], M. Tamura[1], M. Stock[1], J. B. Stock[2, 1], E. Perez[1]

[1]Signum Dermalogix, Inc., Monmouth Junction, New Jersey, United States, [2]Molecular Biology, Princeton University, Princeton, New Jersey, United States

Protein phosphatase 2A (PP2A), is a master regulator whose critical role and function has been mostly studied in neurodegeneration, cancer, and metabolic disorders. However, more recent studies demonstrate this heterotrimeric protein also plays a critical role for skin barrier function, oxidative stress signaling and inflammation. Oxidative stress has been shown to induce demethylation of PP2A in human fibroblasts, driving the disassociation of the fully active PP2A holoenzyme trimer to the less active dimer form. Chia (*Salvia hispanica*) seeds have been one of the basic foods of Central American civilizations for centuries dating back to 1500 BC. Recently, chia seeds also the highest known plant source of ω3 alpha-linolenic acid (ω3-ALA) (54%-67%) as well as high levels of ω6 linoleic (ω6-LA) (12-21%) polyunsaturated fatty acids, have gained importance and notoriety as a “super food”. While chia has been widely studied as a functional food and reported to have several clinical benefits including cardio-protection, weight loss and metabolic disorder, very little has been published on its benefits to skin when applied topically. Here, we demonstrate for the first time the identification and characterization of a novel chia seed extract called HyVia™. We report that in addition to HyVia™’s enhanced levels of (ω6-LA) and (ω3-ALA), it inhibits demethylation of PP2A and increases the expression of important hydration factors, Aquaporin-3 (AQP3) and Hyaluronic Acid Synthase 2 (HAS2) in cultured NHEKs. Lastly, human clinical testing demonstrates that , topical application of HyVia™ is well tolerated and significantly increases skin barrier and hydration properties over vehicle-only.

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The association of interleukin-36γ, claudin-1 and claudin-7 in psoriasis

Y. Pan, S. Tang, L. Xu, S. Zheng, J. Qiao, H. Fang

Dermatology, The First Affiliated Hospital, Zhejiang University, Hangzhou, Zhejiang, China

Background: To investigate the expression and determine their association of IL-36γ, claudin-1 and claudin-7 in psoriasis. Methods: This study included 42 patients as psoriasis group with 15 persons as control group. The expression of IL-36γ, claudin-1 and claudin-7 were detected by immunohistochemistry. Results: The expression level of IL-36γ in the psoriasis group was significantly higher than that in the control group(P=0.022). The expression levels of claudin-1 and claudin-7 in the psoriasis group were lower compared to control group (P=0.001, 0.001 for claudin-1 and claudin-7 respectively). The expression level of IL-36γ was negatively correlated with that of claudin-1(r=-0.344, P=0.025) and claudin-7(r=-0.320, P=0.039). Conclusion: The expression of IL-36γ increased in psoriasis skin lesions and it was associated with down-regulation of claudin proteins expression in the lesions.

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The importance of sirtuins in skin and new findings about sirt2 and its link to mechanobiology

J. Trivero[1], K. Dong[1], E. Goyarts[1], E. Pelle[1], D. Collins[1], K. Corallo[1], N. Pernodet[1, 2]

[1]Research & Development, The Estee Lauder Companies, Melville, New York, United States, [2]Estee Lauder Research Laboratories, Melville, New York, United States

Sirtuins (SIRTs) are a family of NAD+-dependent histone deacetylases that play a critical role in many diverse cellular processes including transcriptional signaling, gene silencing, metabolism, genomic stability, inflammation, energy, stress response and aging. The objectives of this study were to define the temporal role of SIRTs in skin by kinetically quantifying SIRT expression and to assess environmental impact by examining the response to environmental stressors via measuring the effect on energy (ATP) production and oxidative damage (ROS). Additionally, the effect on mechanical properties such as collagen production were measured. Finally, we demonstrate the importance of SIRT2 and its effect on age-induced changes in cell shape. Sirtuin expression levels by Normal Human Epidermal Keratinocytes (NHEK) were measured by RT-PCR. NHEK were exposed to low doses of UVB (10mJ/cm^2). Cellular energy (ATP) production as well as oxidative damage (H_2O_2) were measured. Finally, the effects of SIRTs on pro-collagen type I production in Normal Human Dermal Fibroblasts (NHDF) and cell spreading by aged NHDF were measured. Temporal differences in sirtuin expression levels were observed over time. Furthermore, SIRTs were impacted by environmental stressors such as UVB exposure and ozone, resulting in differences in ATP and H_2O_2 production. These data show that SIRTs 1,2,3 and 6 support cellular activity necessary for skin mechanical properties such as boosting collagen production and through SIRT-2 increasing cellular-spreading in aging skin cells. SIRTs exhibit temporal expression and are sensitive to environmental stressors. We show that SIRTs are beneficial for both cellular integrity and repair activity. Therefore, supporting their activity is imperative for maintaining optimal skin cell activity.

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Celastrol enriched extract modulates Th17 key-disease mediators by interrupting feed-forward inflammation and by restoring homeostasis in psoriasis epidermal skin

T. Nguyen, F. Lestienne, A. Cousy, N. Steward, P. Bianchi, V. Mengeaud, S. Bessou-Touya, N. Castex-Rizzi, H. Duplan
Pierre Fabre Dermo Cosmetique, Toulouse, France

Background: Psoriasis is an autoinflammatory skin disorder due to complex interaction between lymphocytes, DCs and keratinocytes (KCs). High IL-17 level produced by T cells, induces a feed-forward inflammatory response in KCs, resulting in the development of thickened skin lesions infiltrated with mixture of inflammatory cell population. Here, we evaluated modulator effect of Celastrol enriched extract (CEE), from *T. wilfordii* plant cell culture, in two Th17 psoriasis induced models *in vitro*. Methods: NHEK were stimulated by cytokines cocktail Mix 1 [IL-17, OSM and TNF-α] for 24 H after a preincubation for 4 H with: CEE (90 ng/ml), Celastrol (135 ng/ml) or JAK I inhibitor (10 µM). Anti-microbial peptides (AMPs), Cytokines and Chemokines key biomarkers gene were assessed by RT-qPCR. Multilayered micro-epidermis model (Cytoo™) were stimulated by cytokines cocktail Mix 2 [Mix 1 + IL-22 & IL-1α] for 3 days after preincubation for 24 H with: CEE (10 ng/ml) or Tazarotene (0.3 µM). The readouts assayed were IL-1β (ELISA) and Filaggrin (Flg) or S100A7 (immunostainings). CEE is expressed as Celastrol titer in all assays. Results and conclusion: In NHEK, CEE and Celastrol inhibited expression of biomarkers: AMPs (S100A7, PI3, DEFB4), Cytokines (IL-19 & -36 g) and Chemokines (CXCL-1 & -8, CCL-5 & -20). These biomarkers, up-regulated and self-induced by KCs, are described as key mediators in maintaining feed-forward inflammation in psoriasis. In micro-epidermis, CEE inhibited significantly IL-1β and S100A7 while up-regulated Flg expression. CEE, a high added-value product has effective modulator effects in Th17 mediated epidermal skin. It breaks the vicious psoriasis inflammation circle and restores epidermal native phenotype. This is the first time, a well-defined immune-modulator CEE natural of origin, has been proposed for dermo cosmetic in psoriasis.

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Evaluation of barrier properties of topical barrier formulation to common allergenic agents

F. Crepel[1], C. Jeanjean[1], C. Dejean[1], V. Mengeaud[2], S. Bessou-Touya[1], H. Duplan[1]
[1]Pharmacology, Laboratoires Pierre Fabre Dermo-cosmétique, Toulouse, France, [2]Laboratoires Ducray, Lavaur, France

Atopic or irritated skin needs a physical protection against external aggressions. Irritant chemicals such as acids, bases and metals represent the major external agents from which such a skin must be isolated. Barrier creams are designed to prevent or reduce the penetration and absorption of these hazardous substances into the skin, preventing skin lesions and/or other toxic effects from dermal exposure. The objective of our study was to evaluate the barrier properties of a formulation towards acids and bases, and nickel and chromium exposure. The penetration of acids and bases was assessed by the monitoring of HCl and NaOH solutions applied onto a cellulose membrane (mounted on a Franz cell) treated beforehand by the studied formulation. The quantities found in the receptor fluid were assessed by titrimetric assay to evaluate the barrier properties of the formulation. The results showed that 83.2% of acid and 73.3% of base were found in the receptor fluid in control membrane. After application of our product, only 0.9% of acid and 0.4% of base were found in the receptor fluid. Then, we evaluated the barrier properties of the same formulation towards chromium and nickel compounds by monitoring in-vitro dermal absorption of both compounds, applied onto the skin treated beforehand by the formulation. The study was performed using human skin mounted on static diffusion cells. All samples were analysed by ICP-MS. After 24 hours, 19.4 µg/cm^2 of chromium were bioavailable in the skin and the receptor fluid of the control explants. On the opposite, after application of barrier formulation, only 6.7 µg/cm^2 were bioavailable. In the same way, 6.0 µg/cm^2 of nickel were bioavailable on control explants, whereas only 2.5 µg/cm^2 were bioavailable, after application of the barrier formulation. Both studies enabled to evaluate the barrier properties of the formulation towards common irritating and contact allergic agents such as acid, base and metals.

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Retinol at a concentration of 0.3% restores fibrillin-rich microfibrils and modifies the epidermis in photoaged human skin *in vivo* in a manner similar to 1% retinol

K. T. Mellody[1], E. Bradley[2], M. Bell[2], P. Halai[1], L. Cotterell[1], C. Griffiths[1], R. Watson[1]
[1]The University of Manchester & Salford Royal NHS Foundation Trust, Manchester, Greater Manchester, United Kingdom, [2]Walgreens Boots Alliance, Nottingham, United Kingdom

Whilst retinol concentrations of 1% weight/volume (w/v) in skincare cosmetics are commonplace within the US market, lower percentage formulations are associated with fewer tolerance issues. Here we investigated the effect of retinol at 0.1%, 0.3% and 1% w/v upon epidermal barrier, keratinocyte proliferation and deposition of fibrillin-rich microfibrils (FRM) in a 12-day *in vivo* patch test. The vehicle control (VC) and retinol formulations were applied under occlusion to photoaged forearms of healthy volunteers (*n*=5; 66-84 years) prior to biopsy and analysis of key biomarkers of skin health. Epidermal thickening occurred in response to 0.1% retinol (mean ± SEM; 81.62 µm ± 8.5), 0.3% retinol (92.7 µm ± 8.2) and 1% retinol (122.3 µm ± 31.2) compared to untreated, occluded forearm (baseline; 42.3µm ± 4.8) and VC (42.36 ± 3.5). Statistical significance however, was only reached in response to 0.3% retinol ($p<0.05$). Deposition of papillary dermal FRM, known to be diminished in photoaged skin, was significantly restored after treatment with 0.3% (a.u., mean ± SEM; 3.36 ± 0.1; $p<0.05$) and 1% retinol (3.31 ± 0.2; $p<0.05$); 0.1% retinol (2.95 ± 0.26) and VC (3.06 mean ± 0.2) failed to induce significant changes above the baseline (2.32 ± 0.2). All retinol concentrations increased keratinocyte proline-rich protein deposition within the *stratum granulosum* and induced keratinocyte proliferation, concomitant with loss of e-cadherin expression at the basal layer. These data suggest that a retinol concentration of 0.3% appears to have similar efficacy to 1% retinol in its ability to modify the epidermis and restore the FRM network.

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Multi-functional *in vitro* and *in vivo* efficacy of *Tiliacora triandra*, natural ingredient with clinical anti-aging skin benefits

J. Idkowiak-Baldys, F. Liebel, J. R. Glynn
Avon Products, Inc., Suffern, New York, United States

Tiliacora triandra is a flowering plant also known as “bai yanang”. It is native to Southeast Asia and used particularly in the cuisines of northeast Thailand and Laos. Traditionally recommended for fever reduction and for relief from hangover. Pharmacological activities include anti-bacterial, anti-fungal, and anti-inflammatory properties, suggesting its adaptogenic function. The present study is to identify biological endpoints of proprietary extract of *T.triandra.* Testing *in vitro* in 3D skin model showed strong effect on hyaluronic acid production. After topical application of the ingredient *in vivo*, histological analysis of skin biopsy samples confirmed this observation. In addition effect on collagen production and epidermal turnover was also observed. *In vivo* bioinstrumentational analysis confirmed skin firming and wrinkle reduction benefits in a dermatologist-supervised, double-blind, randomized, placebo-controlled, split face clinical trial. Additionally, anti-inflammatory properties of *T.triandra* extract were confirmed by *in vivo* redness reduction assay. Interestingly, when tested in vitro, *T.triandra* was able to stimulate HSP70 pathway, confirming its potential not only as anti-aging but also adaptogenic ingredient, i.e. plant derived substance with protecting and balancing properties.

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In vitro and *in vivo* skin efficacy of CBD extract

J. Idkowiak-Baldys, F. Liebel, J. R. Glynn
Avon Products, Inc., Suffern, New York, United States

Cannabidiol (CBD) has been implicated as beneficial in variety of skin conditions, including acne, atopic dermatitis, psoriasis, scleroderma, and pruritis. CBD mediates its effect through skin's own endocannabinoid system composed of CB1 and CB2 receptors, but detailed mechanism of action in skin and clinical efficacy potential needs to be established. In this study we investigated the role of CBD on modulation of heat shock protein pathway in human dermal fibroblasts. Cells were treated with various concentration of CBD and amount of HSP-70 induced was evaluated by ELISA. In addition, clinical efficacy was assessed using an *in vivo* model. Erythema was induced by topical methyl nicotinate (MN), leading to prostaglandin release and vasodilatation of the peripheral blood capillaries of the skin. CBD was shown to significantly stimulate HSP70 protein level in human dermal fibroblast cells as well as reduce erythema in a clinical model of redness.

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C. Acnes IA1 Phylotype induces features of acneic skin when applied on 3D *in vitro model*

S. Bordes, M. Laclaverie, R. Jugé, C. Grimaldi, E. Aymard, B. Closs
R&D Department, SILAB, Brive la Gaillarde, France

Acne is an inflammatory skin disease of the pilosebaceous unit, involving 4 essential factors: hyperseborrhea combined to a modification of sebum composition, colonization by *Cutibacterium* (*C.*) *acnes*, yperkeratinization and secreted inflammation. Understanding and mimicking this compromised skin is essential for further development of therapeutic solutions. This study aims to develop new *in vitro* 3D models mimicking acneic skin, by combining two main factors involved in the physiopathology. Normal human keratinocytes were used to generate reconstructed epidermis (RE) that were either untreated (control) or treated in topic with a combination of: - peroxidized squalene to mimic the altered sebum composition and create an anaerobic environment; - 5.10^4 CFU of *C. acnes* to mimic the microbial colonization. To get as close as possible to the pathophysiology of acne, *C. acnes* strains were specifically isolated by way of using culturomics-inspired techniques on swabbing from acneic and healthy patients. This allowed investigating bacterial strains' behavior according to their original environment, as they retain their characteristics when used immediately after collection. 39 isolates were characterized at phylotype and strain-type level and 4 ones were selected: 2 isolates of IAI phylotype from acne and healthy skin (described as acne-associated phylotype in literature) and 2 isolates of II and IB phylotype, both healthy-associated skin. While both the IB and II strains did not impact RE, IA1 strains of *C. acnes* lead to hyperkeratinization and inflammation regardless of their origin (acneic *vs.* healthy patient), thus suggesting a role of ecosystem in controlling *C. acnes* virulence in healthy skin. In conclusion, by combining 2 main factors involved in the physiopathology of acne, we (1) succeeded to design *in vitro* 3D models mimicking this skin disorder, (2) highlighted how the phylotype of *C. acnes* strains can have an impact on epidermal physiology. These relevant models are suitable for the substantiation of therapeutic molecules dedicated to acne treatment.

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Protective properties of Avène thermal spring water on biomechanical, ultrastructural and clinical parameters of the human skin

C. Mias[1], A. Maret[1], E. Gontier[2], C. Carrasco[1], C. Satge[1], S. Bessou-Touya[1], H. Coubetergues[1], R. Dauskardt[3], H. Duplan[1]
[1]R&D, Pierre Fabre Dermocosmetique, Toulouse, France, [2]Bordeaux Imaging Center, Bordeaux, France, [3]Stanford University, Stanford, California, United States

The mineral content of thermal spring waters (TSW) applied at the surface of the skin can directly influence the skin barrier through changes in the skin biomechanical properties and ultrastructure; however, the mechanisms underlying these are not completely elucidated. The aim of this study was to compare the effects of Avène TSW with mineral-rich (MR) TSW on the biomechanical properties of the skin using *ex-vivo* and clinical studies. *Ex-vivo* studies included analyses on: the skin surface ultrastructure and mineral element deposit using scanning electron microscopy coupled to energy dispersing X-ray spectroscopy; and the drying stress profile of the stratum corneum (SC) when exposed to dehydration. Human clinical studies were performed to compare the soothing effect of TSW after a dermatological chemical peeling of face skin and determine the overall sensitive scale of consumers using Avène TSW. The *ex-vivo* studies showed that both TSW completely preserved surface skin ultrastructure; however, crystals formed from MR-TSW were needle-like and formed small grains, present in clusters heterogeneously spread over the surface and were mainly composed of calcium. By contrast, Avène TSW formed small crystals, composed of sodium and chlorine, regular and homogeneously distributed at the skin surface. Then, biomechanical studies demonstrated that peak stress of SC layers was increased by MR-TSW, while Avène TSW protected the SC from dehydration and stress. In clinical study, Avène TSW significantly decreased redness of altered skin barrier function after the first application, in contrast to MR-TSW. We also showed that it exhibited several beneficial clinical effects on skin tension, itch feeling, and well-being. In conclusion, this study reveals that Avène TSW protects the SC from dehydration, maintains its mechanical properties and fully respect skin surface integrity, providing related clinical benefits.

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RNAseq profiling highlights immune and barrier differences among ichthyoses

K. Malik[1], H. He[1], A. Pavel[1], T. Canter[2], T. Erickson[2], J. Krueger[3], S. Rangel[2], E. Guttman-Yassky[1], A. S. Paller[2]
[1]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [2]Dermatology and Pediatrics, Northwestern Univ. Feinberg School of Medicine, Chicago, Illinois, United States, [3]Laboratory for Investigative Dermatology, Rockefeller Univ., New York, New York, United States

Transcriptional analyses of a small sample of patients with rare forms of ichthyosis have suggested a shared Th17-skewed profile. To elucidate pathogenic differences among ichthyoses, we performed RNAseq on skin of a large cohort (n=56) ichthyosis patients (7 Netherton syndrome/NS, 16 lamellar ichthyosis/LI, 18 congenital ichthyosiform erythroderma/CIE, 13 epidermolytic ichthyosis/EI, and 2 ichthyosis with confetti/IWC) vs. 40 matched controls. Using threshold of fold change/FCH>2 and false discovery rate/FDR<0.05 criteria, we found increased markers of T-cell activation/migration (ICOS, CCR7) and Th17/Th22 (IL17A/F, IL36G, S100s, PI3) across ichthyoses. IL-17/TNF-α-induced markers (e.g., VNN3, KYNU, PI3, DEFB4) were highest in the more erythrodermic forms (NS, CIE, IWC) and lowest in EI. Th22/IL22 expression was more elevated in NS and CIE than other forms and CIE specifically showed very high increases in Th1/IFN-related responses (IL12B, IL1B, CXCL9/10). Th2 cytokines IL4 and IL13 markers were reduced in ichthyoses, but IL4R was particularly high in NS and CCL18 in NS and CIE. Terminal differentiation genes (e.g. FLG) were generally increased in ichthyoses, except LOR, which was decreased in NS, CIE, and IWC. Claudin gene expression was reduced universally, but reduction of lipid-related gene expression (ie, FADS1, ELOVL3, FAR2, FA2H) was greatest for LI and significant for NS and CIE, but not EI or IWC. Enrichment analyses highlighted cell cycle targets in all ichthyoses and pathways upregulated by IL6/STAT3 in CIE and EI. Our broad skin profiling of ichthyoses highlights immune and barrier-specific landscapes that may require alternative therapeutic modulation.

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Narcissus tazetta bulb extract delays cellular senescence by reducing mTOR pathway activation

F. Havas[2], S. Krispin[2], N. Borenstein-Auerbach[2], E. Loing[1]

[1]R&D, Lucas Meyer Cosmetics, Québec, Quebec, Canada, [2]R&D, IBR - Lucas Meyer Cosmetics, Yavne, Israel

Somatic mammalian cells enter cellular senescence after a *finite* number of cell divisions. Senescent cells are characterized by their inability to proliferate and the secretion of promoters of inflammation and tissue deterioration. Telomere shortening is a main underlying mechanism of replicative senescence – the molecular clock counting down towards a programmed limit on cell replications, ending in senescence. This contributes to intrinsic tissue aging, leading to dryness, wrinkling, loss of elasticity, weakened barrier function, and hyperpigmentation. The bulb of *Narcissus tazetta* is a storage organ enabling the plant to survive adverse conditions through dormancy. When dormant, the plant produces reversible dormancy-inducing cell proliferation inhibitors ("dormins").IFF/Lucas Meyer Cosmetics presents a natural aqueous extract of dormant *Narcissus tazetta* bulbs, capturing the plant dormancy concept for skin anti-aging. *In vitro*, treating a culture of aged human dermal fibroblasts with the extract showed: *Restrained cell proliferation* (by over 40%, from 0.01% extract); *Telomere length preservation*, significantly increasing telomere length in the aged cells; Significantly *reduced activation of the mechanistic target of rapamycin (mTOR) signaling pathway*, a central regulator of cellular senescence closely related to the DNA damage response, with influence on longevity and aging; *Improved cellular function*, significantly enhancing procollagen-1 production in aged cells. In a 28-day double-blind, placebo-controlled clinical study with 1% *Narcissus tazetta bulb* extract in formulation, the active significantly ($p<0.05$) improved key skin aging parameters, including: *Wrinkles and lines* (fine lines, wrinkle counts, wrinkle volumes); *Skin elasticity*; *Skin pigmentation*; and *Skin barrier function (TEWL)*. These results demonstrate the value of *Narcissus tazetta* bulb extract as an anti-aging active ingredient through delayed cellular senescence.

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CYP11A1-derived hydroxy-lumisterols act as agonists of LXRα and β

S. Qayyum[8], T. Kim[8], R. M. Slominski[2], Z. Janjetovic[8], Y. Song[8], A. Oak[8], C. Raman[2], P. Guroji[8], Y. Bilokin[4], Y. Song[5], A. Jetten[6], E. K Y Tang[7], R. Tuckey[3], A. T. Slominski[1]

[1]Dermatology, The University of Alabama at Birmingham, Birmingham, Alabama, United States, [2]Department of Medicine and Microbiology, Div. of Clinical Immunology and Rheumatology, The University of Alabama at Birmingham, Birmingham, Alabama, United States, [3]School of Chemistry and Biochemistry, The University of Western Australia, Perth, Western Australia, Australia, [4]Otava Ltd., Vaughan, Ontario, Canada, [5]Department of Biomedical Engineering, The University of Alabama at Birmingham, Birmingham, Alabama, United States, [6]Cell Biology Section, National Institute of Environmental Health Sciences, Durham, North Carolina, United States, [7]School of Molecular Sciences, The University of Western Australia, Perth, Western Australia, Australia, [8]Dermatology, UAB, Birmingham, Alabama, United States

Liver X receptors (LXRs) bind oxysterols as their endogenous ligands and are important regulators of cholesterol, fatty acid, and glucose homeostasis. To define the mechanism of action of metabolites of recently discovered pathways of lumisterol (L3) activation by CYP11A1, we investigated their interactions with LXRα and LXRβ. Discovery of these pathways challenged existing dogmas that lumisterol is biologically inactive and only oxysterols can control LXR activation. Molecular docking of LXRα and β revealed high docking scores for L3 analogs similar to those of the natural ligands, predicting good binding to the receptor. A series of L3 derivatives stimulated LXR-responsive element in CHO, hepatoma and keratinocytes cell lines. Western blot and qPCR analysis in cell lines and murine brain showing enhanced expression of genes downstream of LXR by L3 analogs. Importantly, L3 derivatives showed high affinity binding to the LBD of the LXRα and β using the LanthaScreen TR-FRET LXRα and β Coactivator assays and majority of L3 compounds acted as agonists. Molecular dynamics simulation results showed the conformational changes of LXRs by binding with L3 derivatives. Thus, our identification of L3 derivatives as ligands for LXRs opens up new possibilities for regulation of epidermal barrier functions.

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Optimized low pH niacinamide formulations show a significant induction of autophagy gene expression over neutral niacinamide control formula

J. Oblong, T. L. Laughlin, J. D. Sherrill, S. Zhao, B. B. Jarrold, Y. M. DeAngelis, J. C. Bierman, B. Fang, T. Hakozaki

The Procter & Gamble Company, Mason, Ohio, United States

Niacinamide (N, aka nicotinamide, Vitamin B_3) has been used in skin care formulations for decades and clinically proven to improve barrier integrity, appearance of skin color, and topographical attributes. Mechanistically it is believed that N can impact autophagy in skin by increasing cellular NAD+ and NADPH pools, which are known to decrease in skin with age. N based formulations have historically been practiced within a neutral pH range in order to maintain stability and thereby prevent formation of niacin, a known vasodilator. However, it has been previously shown that formulations at lower pH can have a positive impact on stratum corneum integrity and overall skin health. Thus, we were interested in testing whether N formulated in a more stabilized chassis system at lower pH would have an impact on overall N potency and mechanism of action in skin. Formulation optimization efforts established that lowering the pH to as low as 2.5 would still allow for usage of 2% N with minimal niacin formation. To test what effect lowering the pH range has on N mechanistic response, we utilized the Skinethic™ human pigmented epidermal 3D skin model system to compare niacinamide formulations at varying pH ranges. Bioinformatic analyses identified that 2% N formulations at pH 2.5 and 3.8 showed a statistically significant higher induction of the GO term of "positive regulation of autophagy" compared to a 2% N formula at pH 5.8 and control formulas at respective pH. In summary, we have identified that N based formulations at pH levels as low as 2.5 show a significant induction of autophagy related gene expression patterns in 3D skin models when compared with neutral pH N and pH control formulas. These findings support the potential for increased clinical efficacy response by low pH N formulas on skin aging appearance metrics.

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Combination of niacinamide, a tripeptide, and yeast extract show synergistic induction of autophagy and stress response biothemes in human epidermal keratinocytes

J. Oblong, T. L. Laughlin, J. D. Sherrill, L. A. Mullins, J. C. Bierman

The Procter & Gamble Company, Cincinnati, Ohio, United States

Niacinamide (N, aka nicotinamide, Vitamin B_3) has been used in skin care formulations for decades and clinically proven to improve skin's surface barrier integrity, appearance of skin color, and topographical attributes. To improve N's effectiveness, we screened for chemistries that would significantly increase protection against stress-induced processes when combined with N in *in vitro* models. Human hTERT keratinocytes were used to screen for inhibitors of UVB-induced PGE_2 synthesis (15 mJ/cm^2) and for inducers of autophagy. A hydrolyzed Saccharomyces cerevisiae yeast extract was found to reduce PGE_2 levels by 63%. A tripeptide Val-Try-Val (VYV) was found to increase autophagy by 9% as measured by CytoID, an assay that measures autophagic flux in cells. Gene expression profiling was performed to identify potential additive or synergistic effects from the combination of N, VYV, and yeast extract on canonical biothemes under basal and UV-stressed conditions. hTERT keratinocytes were treated with N, VYV, and yeast extract alone and in combination for 18 hrs prior to stress by UVA/B (18/2 mJ/cm^2) or unstressed conditions and processed for microarray chip analysis. Bioinformatic analyses identified the N, VYV, and yeast extract combination as inducing the highest number of differentially regulated probesets with the highest absolute fold change under basal and stressed conditions. Additionally, there was a synergistic activation of multiple autophagy-related pathways. Under non-stress conditions, the combination activated canonical pathways of epithelial junction/gap junction signaling and inhibited ER stress response. Under UV stress conditions, the combination showed inhibition of DNA damage and apoptosis of skin, as well as activation of fatty acid metabolism. In summary, we have identified a combination of N, VYV, and yeast extract that shows synergistic impact on stress associated pathways, including autophagy induction.

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Skin epidermal keratinocyte differentiation-associated processes regulate homeostatic antiviral protein expression

M. Lee[1, 2], J. Shannon[1, 3], V. Jain[4], S. Joost[5], M. Kasper[5], D. L. Corcoran[6], S. G. Gregory[4], A. S. MacLeod[1, 2, 3]

[1]Dermatology, Duke University, Durham, North Carolina, United States, [2]Molecular Genetics and Microbiology, Duke University, Durham, North Carolina, United States, [3]Immunology, Duke University, Durham, North Carolina, United States, [4]Molecular Physiology Institute, Duke University, Durham, North Carolina, United States, [5]Biosciences and Nutrition and Center for Innovative Medicine, Karolinska Institute, Huddinge, Sweden, [6]Center for Genomics and Computational Biology, Duke University, Durham, North Carolina, United States

Epidermal keratinocytes produce antimicrobial peptides and proteins, including antiviral proteins (AVPs) as innate defense against various pathogens. We made the novel observation that distinct genes encoding innate AVPs are transcriptionally activated upon calcium-induced epidermal differentiation of primary normal human epidermal keratinocytes (NHEK). The AVPs include, but are not limited to, members of oligoadenylate synthetase (OAS) family, myxovirus resistance proteins (MX), interferon-induced proteins with tetratricopeptide repeats (IFIT), interferon-induced transmembrane protein 1 (IFITM1), interferon stimulated gene 15 (ISG15), and guanylate-binding proteins (GBP) ($p < 0.05$). Our single-cell RNA-seq analyses of both calcium-differentiated NHEK and murine epidermis further stratified populations of keratinocytes with AVP-enriched gene signatures. Furthermore, AVP production of calcium-differentiated NHEK is abrogated when the calcium-calcineurin signaling is disrupted by the calcineurin inhibitor Cyclosporin A ($p <0.05$). Mechanistically, we found that calcium-mediated AVP production in keratinocytes is mediated by a subset of nuclear factors of activated T-cells (NFATs), downstream of the calcium-calcineurin signaling. Overall, we demonstrate that epidermal keratinocyte differentiation regulates homeostatic innate antiviral immunity potentially via calcium-calcineurin signaling. Understanding the process of how keratinocytes differentiate to constitute an innate antiviral barrier is a pivotal step towards developing new and improved methods of accelerating skin barrier function acquisition and reducing medical risks of viral infections in patients with impaired skin barriers.

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Targeting Multiple Hallmarks of Skin Aging through Growth Factors

G. Naughton

Histogen, Inc., San Diego, California, United States

Intrinsic and extrinsic aging of skin is associated with reduced levels of growth factors and extracellular matrix and the loss of key cell functions such as proteostasis and intercellular communication. The efficacy of a novel serum formulated with growth factors produced by neonatal fibroblasts under hypoxic conditions as well as botanicals, marine extracts, and peptides (GF serum) was assessed at the molecular biology level through gene expression analysis in EpiDermFT 3D full thickness human skin models. The in vitro skin models were irradiated with 200mJ/cm2 UV light with UB-B filter to induce extrinsic aging changes. GF serum or dH20 (control) were applied to the epidermal layer followed by incubation of the tissues for 24 hours. After incubation mRNA was extracted for quantitative PCR analysis. Several biomarker panels for proteasome (POMP, PSMB5, PSMB6), autophagy (ATG5, ATG7, ATG12, BECN1, MAP1LC3A), stemness (NES, ACTA2), connexins (Cx26, Cx30, Cx30.3, Cx31.1, Cx37) and epidermal barrier (IVL, SPRR1A, TGM1, TGM3) showed increased gene expression after GF serum application compared to non-treated skin models. In addition, expression of genes encoding extracellular matrix proteins such as collagen type I (COL1A1), collagen type III (COL3A1), elastin (ELN), and fibrillin 1 (FBN1), basement membrane proteins (COL4A1, COL7A1), and tight junction components (OCLN) were upregulated in treated skin models . Furthermore, human skin explants (ex vivo skin) treated with GF serum showed a reduction of the fibroblast senescence biomarker H2AFJ suggesting a potential anti-senescence benefit. This study has demonstrated that the GF serum containing growth factors from neonatal fibroblasts cultured under hypoxic conditions has the potential of reversing multiple hallmarks of aging including proteostasis, cellular senescence, stem cell exhaustion, and altered cellular communication as well as inducing new extracellular matrix to restore aging skin.

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RNA-seq profiling of tape strips from infants with atopic dermatitis show profound barrier and immune abnormalities

A. Diaz[1], H. He[1], A. Pavel[1], R. Lefferdink[2], M. Fang[2], T. Canter[2], S. Rangel[2], J. Krueger[3], A. S. Paller[2], E. Guttman-Yassky[1]

[1]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [2]Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [3]The Rockefeller University, New York, New York, United States

Atopic dermatitis (AD) onset usually occurs in children younger than 5 years old. While whole-skin profiling has been the gold standard for identifying AD-related biomarkers in adults, skin biopsies are not feasible in infants. An alternate minimally invasive technique to detect barrier and immune biomarkers in infants with AD is needed. We used RNA-seq to profile 20,000+ transcripts in lesional and non-lesional skin of 19 infants (0-5 y/o) with moderate-to-severe AD and 17 healthy controls. We achieved 100% detection rate of all samples, with 1,890 differentially expressed genes in lesional or non-lesional AD versus controls, using threshold of fold change>2 and false discovery rate (FDR)<0.05. Important barrier-related genes were significantly dysregulated in AD versus controls, primarily in lesional skin, including markers of terminal differentiation genes (FLG, LCEs), lipid metabolism (ELOVL3, GAL, FA2H), and claudins (CLDN8) (FDR<0.05). Robust up-regulation of inflammatory genes were shared by lesional and non-lesional skin versus controls, including markers of general inflammation (MMP12), T-cell/dendritic cells (CD1A, CD3E, CD86), Th2 (IL13, IL31, IL4R, CCL17), JAK signaling (JAK2/3), Th17 (IL8, IL19, IL36A/G, S100s), and innate immunity (IL1A/B) (FDR<0.05). Tape strips accurately capture barrier and immune alterations in early AD in infants, and provide a minimally invasive strategy for defining biomarkers of disease and of therapeutic response in longitudinal pediatric studies and large-scale clinical trials, where skin biopsies are not possible.

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AKR1B10 inhibition in keratinocytes as a strategy to improve retinaldehyde efficacy and increase endogenous atRA

L. Walker[1], J. Kreitinger[1], N. Wageling[1], K. Fields[2], K. Rodan[2], J. S. Craw[2], T. Falla[2], C. Crane[2], P. Diaz[1, 3]

[1]DermaXon, Missoula, Montana, United States, [2]Rodan and Fields, San Francisco, California, United States, [3]BMED, University Of Montana, Missoula, Montana, United States

Retinoic acid (RA) is a potent regulator of numerous physiological processes in the skin and controls keratinocyte proliferation and differentiation. The RA biosynthesis pathway is tightly regulated by enzymes that increase intracellular RA through oxidation of retinaldehyde to RA and decrease intracellular RA through reduction of retinaldehyde (RAL) to retinol (ROL), respectively. Aldo-keto reductase enzymes (AKRs), specifically AKR1B10, is responsible for the reduction of RAL to ROL thereby acting to limit the synthesis of intracellular RA. This makes the selective inhibition of AKR1B10 a highly promising mechanism for increasing endogenous RA. We hypothesized that a select panel of natural fatty acids and synthetic compounds evaluated through molecular docking studies would reduce AKR1B10 activity and increase intracellular atRA. Using healthy adult keratinocytes, we found that AKR1B10 RNA and protein is significantly upregulated differentiated keratinocytes, and AKR1B10 inhibitors modulate keratinocyte expression of differentiation and proliferation markers. Further studies using molecular docking simulations have identified optimized orientation of AKR1B10 ligands to generate potential adduct structures with increased AKR1B10 binding affinity, and LC/MS was used to measure the concentration of retinoids in keratinocytes following AKR1B10 inhibitor exposure. Together, this in-depth understanding of structural features and bioassay validation enables specific AKR1B10 inhibitors to increase endogenous atRA.

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Keratinocytes isolated from dark or light pigmented skin showed different degrees of tight junction impairment after PAR2 activation *in-vitro*
P. Nadeau, A. De Benedetto
Dermatology, University of Florida, Gainesville, Florida, United States
Protease activated receptor 2 (PAR2) is a transmembrane receptor with a tethered extracellular amino-terminus small peptide which acts as an activating ligand after cleavage. In the skin, PAR2 has extensively documented effects in promoting Th2-inflammation, skin barrier impairment and pruritus. Increased PAR2 expression was found in epidermal nerve fibers and keratinocytes in Atopic Dermatitis (AD) skin. In this study we aim to investigate the effect of PAR2 on Tight Junction (TJ) function and composition in primary human keratinocytes (PHK) isolated from dark or light pigmented foreskin. PHK were differentiated in high-Ca^{+2} media in the presence of the selective PAR2 agonist (SLIGKV-NH_2) or reverse peptide as control. TJ integrity was assessed by trans-epithelial electrical resistance (TEER) and permeability to Na-Fluorescein. Expression of PAR2 and TJ components was evaluated at the RNA level. We confirmed greater expression of PAR2 in dark vs light-PHK. Both dark and light PHK differentiating in the context of PAR2 activation (100 μM) had a reduced TJ function resulting in reduced TEER and increased permeability to Na-Fluorescein ($p \leq 0.05$, n=5/each group). However, the degree of perturbation was greater for light-PHK as it compared to dark-PHK. Reduction in TEER after PAR2 activation was 40.8% in light-PHK vs 16.6% in dark-PHK (AUC 72-144 hours, p=0.01, n=5/each group). Also, a dose response effect was observed only in light-PHK, with significant TEER reduction after 50 μM PAR2 (p=0.03 at 120 hours). Notably, we observed a greater effect of PAR2 activation on the downregulation of CLDN1 and occludin mRNA in light-PHK. Our data highlights differences between keratinocytes of different ethnic backgrounds in TJ regulation through PAR2 activation. As we transition toward a better understating of AD phenotype, it is mandatory to keep in consideration and investigate intrinsic ethnic difference in skin barrier regulation that could be relevant to AD pathogenesis/treatment.

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Treatment of Netherton syndrome with dupilumab
S. Blunder, M. Schmuth, R. Gruber
Dermatology, Innsbruck Medical University, Innsbruck, Austria
Netherton syndrome (NS) is a rare autosomal-recessive disease that is caused by loss-of function mutations in the SPINK 5 gene encoding for the serine protease inhibitor lympho-epithelial Kazal type inhibitor (LEKTI). LEKTI opposes the function of several epidermal serine proteases including kallikrein 5 (KLK5), kallikrein 7 (KLK7) and kallikrein 14 (KLK14). This results in an activation of the PAR2-TSLP axis and an increase of other Th2 polarizing mediators including CCL-17 and CCL-22. This presumably spurs a Th-2 response consequently leading to increased IgE levels. In NS effective treatment options are missing. In contrast, in atopic dermatitis treatment with dupilumab an antibody directed against the alpha unit of the IL-4 receptor leads to a remarkable success in controlling disease activity which is reflected in decreased IgE levels. Thus, dupilumab represents a worthwhile treatment strategy in NS. Three adult patients with genetically confirmed NS were individually treated with dupilumab 300mg injections every other week for 32 weeks. EASI scores at baseline were 30.0, 29.0 and 18.2. Clinical improvement was observed as early as at week 8 leading to continuously improved EASI scores (w32: -55.82%+/-16.69). In line, IGA scores enhanced in all three patients. DLQI clearly improved with ongoing dupilumab treatment. Serum IgE levels declined steadily (w32: -60.93%+/- 8.57). In contrast, LDH serum levels and blood eosinophil count were unchanged. Further analyses of serum cytokines are underway. Decrease of serum IgE levels clearly correlated with both EASI and DLQI reduction intra- and interindividually. Similarly, EASI improvement correlated with DLQI reduction. In summary, these data for the first time shows that dupilumab is effective in NS patients.

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A novel mutation of COL7A1 in a Chinese family with dystrophic epidermolysis bullosa pruriginosa
H. Tuo, Y. Zheng
Dermatology, The second affiliated hospital of Xi'an Jiaotong University, Xi'an, China
Background: Dystrophic epidermolysis bullosa pruriginosa (DEB-Pr) is a rare subtype of dystrophic epidermolysis bullosa (DEB) characterized by papules, pruritus and scratches. It was caused by the mutation of COL7A1 gene encoding type VII collagen fibers, resulting in the destruction of the anchoring structure of the epidermis and dermis. Methods: Histopathological examination and blood sample for the whole-exon sequencing (WES) were performed on the proband. Then we collected blood samples from other 3 affected family members, 4 unaffected members and 50 healthy controls to verify the mutation of COL7A1.
Result: Characteristic clinical manifestations such as papules, pruritus and scratches were found in 4 patients of the family with different degrees of severity. A novel heterozygous mutation of COL7A1 in exon 69 c.5765G>A, p.G1922E, which has never been reported before, was detected in all patients in the family, but not in the unaffected members of the family or healthy controls. Conclusion: Our study suggests that c.5765G>A may influence the phenotype of PEB, expanding the database of COL7A1 mutations, and providing more basis for the molecular diagnosis, precise treatment and disease prediction of DEB-Pr.

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Mosaicism in tuberous sclerosis complex detected by genome analysis
A. M. Cartron[2, 1], X. Zhang[3], A. Treichel[2, 1], D. Pithadia[2, 1], W. Steagall[2], A. Jones[2], P. Julien-Williams[2], M. Wilkerson[3], C. Dalgard[3], J. Moss[2], T. Darling[1]
[1]Dermatology, Uniformed Services University, Bethesda, Maryland, United States, [2]National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland, United States, [3]The American Genome Center & APG, Uniformed Services University, Bethesda, Maryland, United States
The skin phenotype of tuberous sclerosis complex (TSC) is less severe in those with mosaic disease and low variant allele fractions (VAFs) for *TSC1* or *TSC2*. Extremely low levels of mosaicism may account for lymphangioleiomyomatosis (LAM) occurring sporadically in adult women (sLAM), rather than in association with TSC (TSC-LAM). In a cohort of 167 patients (98% female, average age 49 years), 138/167 (83%) had sLAM, 25/167 (15%) had TSC-LAM, and 4/167 (2%) had only TSC. Examination of sLAM patients revealed 9 (7%) with a hypomelanotic macule and 3 (2%) with an angiofibroma or periungual fibroma. All patients had whole genome sequencing (GS) from PCR-free library preparations of peripheral blood DNA. Unique dual-indexed libraries were sequenced as pools on an Illumina NovaSeq 6000 generating 2x150 read pairs. Reads were mapped to reference genome hg19 using Isaac Aligner and variant calls were made by Strelka Germline Variant Caller. Sample-level data passed quality control assessments for yield, alignment, base quality and contamination metrics resulting in a mean coverage of ~45x across all samples. 16 pathogenic variants in *TSC2* and 2 pathogenic variants in *TSC1* were detected in 18/29 (62%) TSC patients. Pathogenic variants were germline in 11 TSC patients, including one with a large deletion of *TSC2* and polycystic kidney disease. In 7 patients *TSC2* VAFs ranged from 3.3-21%, with mosaicism validated in 5 patients to date. Two mosaic TSC patients with asymmetric angiofibromas had VAFs less than 6%. No unexpected recurrent mutations in other cancer-related genes were detected. No germline mutations in *TSC1* or *TSC2* were detected in sLAM patients, suggesting TSC diagnosis is not being missed in this population. Analysis of affected skin in sLAM patients may reveal mosaic disease that is not detectable in the blood.

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Coagulation factor XIII-A subunit missense mutation in the pathobiology of autosomal dominant multiple dermatofibromas

C. Supsrisunjai[1, 2], H. Chao-kai[3], M. Michael[4], C. Duval[5], J. Lee[1], H. Yang[3], H. Huang[3], T. Chaikul[1], A. Onoufriadis[1], R. Steiner[4], R. Ariens[5], O. Sarig[6], E. Sprecher[6], M. Eskin-Schwartz[6], C. Samlaska[8], M. Simpson[7], J. Calonje[1], M. Parsons[4], J. McGrath[1]

[1]Genetic and Molecular Medicine, St John's Institute of Dermatology, King's College London, London, United Kingdom, [2]Medical Services, Institute of Dermatology, Bangkok, Thailand, [3]Dermatology, National Cheng Kung University, Tainan, Taiwan, [4]Randall Cell & Molecular Biophysics, King's College London, London, United Kingdom, [5]Leeds Institute of Cardiovascular And Metabolic Medicine, Leeds, United Kingdom, [6]Dermatology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel, [7]Medical and Molecular Genetics, King's College London, London, United Kingdom, [8]Academic Dermatology of Nevada, University of Nevada, Henderson, Nevada, United States

Dermatofibromas are common benign skin lesions, the etiology of which is poorly understood. We identified two unrelated pedigrees in which there was autosomal dominant transmission of multiple dermatofibromas. Whole exome sequencing revealed a rare shared heterozygous missense variant in the *F13A1* gene encoding factor XIII subunit A (FXIII-A), a transglutaminase involved in hemostasis, wound healing, tumor growth, and apoptosis. The variant (p.Lys679Met) has an allele frequency of 0.0002 and is predicted to be a damaging mutation. Recombinant human Lys679Met FXIII-A demonstrated reduced fibrin crosslinking activity in vitro. Of note, the treatment of fibroblasts with media containing Lys679Met FXIII-A led to enhanced adhesion, proliferation, and type I collagen synthesis. Immunostaining revealed co-localization between FXIII-A and α4β1 integrins, more prominently for Lys679Met FXIII-A than the wild type. In addition, both the α4β1 inhibitors and the mutation of the FXIII-A Isoleucine-Leucine-Aspartate-Threonine (ILDT) motif prevented Lys679Met FXIII-A-dependent proliferation and collagen synthesis of fibroblasts. Our data suggest that the Lys679Met mutation may lead to a conformational change in the FXIII-A protein that enhances α4-integrin binding and provides insight into an unexpected role for FXIII-A in the pathobiology of familial dermatofibroma.

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Precision medicine: Exome sequencing adds complexity to genotype/phenotype correlation

S. Oska[1, 2], D. Tamura[2], J. E. Blau[3], K. H. Kraemer[2], J. J. DiGiovanna[2]

[1]Oakland University William Beaumont School of Medicine, Rochester, Michigan, United States, [2]Lab Cancer Biol and Gen, NCI, Bethesda, Maryland, United States, [3]MDB, NIDDK, Bethesda, Maryland, United States

With the increasing availability of exome sequencing (ES), we are recognizing the complexity of how genetic variation results in clinical disease. In the family presented in this case, ES identified multiple molecular diagnoses. A 3-year-old male, CS594BE presented to the NIH with delayed myelination, slow linear growth, microcephaly, photophobia, and photosensitivity. The patient's weight (10.9 kg) was in the 1st percentile, and his height (83 cm) and head circumference (44.6 cm) were below the 1st percentile. His cachectic appearance, early developmental delay, uncoordinated gait, and cutaneous photosensitivity are characteristic features of Cockayne Syndrome (CS). CS594BE's father had a history of MEN1 syndrome with hyperparathyroidism, pituitary prolactinoma, nonfunctioning pancreatic neuroendocrine tumors, plus cutaneous angiofibromas and collagenomas. CS594BE's paternal grandfather had MEN1 syndrome and had two affected siblings. Exome sequencing performed on CS594BE revealed a homozygous mutation in the *CSA (ERCC8)* (c.600dupT)(p.lle201Tyrfs*8) DNA repair gene. Both parents were heterozygous for this CSA mutation. The father also had a heterozygous *MEN1* mutation (c.781C>T)(p.Gln261*). CS594BE was also heterozygous for this *MEN1* mutation but had no clinical features as expected for his young age. In addition, CS594BE and his mother were heterozygous for a likely pathogenic mutation in the fumarate hydratase (FH) gene (c.1431_1433dupAAA)(p.Lys477dup), which is associated with hereditary leiomyomatosis and renal cell cancer (HLRCC). The mother did not have identifiable features of cutaneous or uterine leiomyomata at time of presentation. This case is significant for mutations in 3 different genetic diseases: CS, MEN1 and HLRCC. In a family with complex genetic history, it is important to evaluate for multiple abnormalities, which can lead to earlier identification of disease and the need for additional monitoring.

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Assessment of readthrough of premature termination codons in xeroderma pigmentosum group C patients by real-time PCR and immunohistochemistry

E. R. Heller[1], L. Al-Eryani[1], S. G. Khan[1], D. Tamura[1], C. R. Lee[2], R. M. Simpson[1], J. J. DiGiovanna[1], K. H. Kraemer[1]

[1]Lab Cancer Biol and Gen, NCI, Bethesda, Maryland, United States, [2]Pathology, NCI, Bethesda, Maryland, United States

Xeroderma pigmentosum (XP) is a rare autosomal recessive DNA repair disorder caused by mutations in 8 genes, XP-A to XP-G, and Variant with severe sun sensitivity and10,000-fold increased risk of skin cancer. XP-C is the most common type in the U.S. About 12% of XP-C patients have premature termination codon (PTC) mutations that can lead to XPC protein elongation arrest and *XPC* mRNA template degradation by nonsense mediated decay. Our previous studies have shown treatment with aminoglycosides promote readthrough of PTC in XP-C cells. Amlexanox, an anti-inflammatory, antiallergic and immunomodulator, was reported to increase readthrough in human cells with PTC associated diseases. We treated parallel pairs of fibroblasts and lymphoblasts derived from two unrelated XP-C patients along with 2 normal control cells with amlexanox and/or previously tested aminoglycosides. Levels of *XPC* mRNA increased in XPC fibroblasts exposed to both G418 and amlexanox to a different extent in different cells. We are also developing a more direct assessment of level of XPC protein in human skin by use of immunohistochemistry with XPC antibodies. Two mm skin punch biopsies are stained with IHC and assessed microscopically. The slides are then scanned and the intensity of the staining is assessed by computer algorithm. In skin from normal donors XPC staining was prominent in the nuclei of epidermal cells and sweat glands in the dermis. In contrast, only background XPC staining was observed in cells from XPC patients. These preclinical tests are a step towards precision medicine that should assist patient selection and determining which drug combination would be optimal to increase XPC function for each patient.

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UV-endonuclease and photolyase DNA repair enzymes increase cystatin gene expression after UVB induced downregulation

H. Kolli[1, 3], A. Rosenthal[2, 3], R. Moy[3]

[1]Virginia Commonwealth University School of Medicine, Los Angeles, California, United States, [2]Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, Florida, United States, [3]Research, Moy-Fincher-Chipps Facial Plastics & Dermatology, Beverly Hills, California, United States

Background: The purpose of this study is to determine gene expression changes induced by UVB light and assess the effect of topical UV endonuclease and photolyase in recovery from these changes. Methods: Non-invasive, adhesive patch skin biopsies were performed on the right and left post-auricular areas of 48 subjects before and 24-hours after UVB exposure using an excimer laser (300mJ). Subjects then applied DNA repair enzymes (UV-endonuclease from *Micrococcus luteus* or photolyase from Anacystis nidulans) to the right post-auricular area only daily for 2 weeks. Subjects returned 2 weeks later for repeat biopsies. RNA was isolated and assessed by reverse transcriptase followed by quantitative PCR to assess gene expression changes. Results: 7/18 assessed genes demonstrated significant downregulation (Vitamin A, Programmed Cell Death protein, Small Proline Rich Protein) or upregulation (Interleukin Families 1/2) 24-hours following UVB exposure. UV-endonuclease (p =.015) and photolyase (p =.039) DNA repair enzymes significantly reversed UVB-induced downregulation of the cystatin gene family. Conclusions: These results suggest that UVB exposure decreases or increases gene expression and that DNA repair enzymes demonstrate efficacy in reversing these changes. Topical DNA repair enzymes can increase cystatin gene expression following UVB-induced downregulation after only 2 weeks of application. Cystatins have been reported to be diminished or lost in both basal and squamous cell carcinomas and these findings suggest that topical DNA repair enzymes may hold the ability to repair UV-induced genetic changes and prevent future skin cancers.

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First in human use of a novel *in vivo* gene therapy for the treatment of autosomal recessive congenital ichthyosis: Results of a phase I/II placebo controlled trial
P. Agarwal[1], B. Agostini[1], A. Collin L'Hortet[1], P. Zhang[1], S. Krishnan[1], A. Paller[2]
[1]Krystal Biotech Inc, Pittsburgh, Pennsylvania, United States, [2]Northwestern University, Chicago, Illinois, United States
Autosomal recessive congenital ichthyosis (ARCI) is a rare, monogenic cornification disorder with erythema, epidermal scaling, ectropion, and impaired skin barrier function. Mutations in *TGM1* encoding transglutaminase 1 are the predominant cause of ARCI, affecting >55% of US ARCI patients. Current therapeutic options for treating ARCI provide only symptomatic relief, necessitating the development of targeted therapeutics. KB105 is a novel, convenient, first in class, off-the-shelf disease correcting topical gene therapy for the treatment of TGM1-deficient ARCI. An intra-patient placebo-controlled Phase I/II study (NCT04047732) has been initiated in the US, with safety and tolerability of repeat administration of KB105 as the primary outcomes. Secondary outcomes include TGM1 expression and activity and ichthyosis severity at the treated sites. Three adult subjects (ages 20, 24, and 39) with a confirmed genetic diagnosis received multiple KB105 or placebo treatments in the selected target areas. KB105 was well-tolerated by all three subjects with no reported drug related adverse events or immune response. A significant increase in correctly localized TGM1 *in situ* activity and expression as well as reduced ichthyosis severity was observed in KB105-treated areas in all three patients, demonstrating that topical application of KB105, the first and only corrective therapeutic candidate for TGM1-deficient ARCI was well tolerated and efficacious. With safety and preliminary efficacy established, the next phase of the study will evaluate KB105 safety and efficacy in larger target areas and expand enrollment to pediatric subjects in 1H 2020. A multi-center pivotal Phase 3 study is planned following completion of Phase I/II study.

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Interrogating altered enhancer landscapes to decode pathogenic changes in macrophages during chronic inflammatory disease
J. Seidman[1], T. Troutman[1, 2], M. Sakai[1], R. Li[1], Z. Ouyang[1], N. Spann[1], H. Bennett[1], C. Bruni[1], B. Vu[1], K. Ego[1], M. Pasillas[1], L. Texari[2], S. Heinz[2], C. Glass[1]
[1]Dept. of Cellular and Molecular Medicine, UC San Diego, La Jolla, California, United States, [2]Dept. of Medicine, UC San Diego, La Jolla, California, United States
Myeloid cells play essential roles in skin homeostasis as well as inflammation, fibrosis and wound healing. Genomics techniques such as scRNA-seq and ChIP-seq are powerful tools for understanding the regulation of cellular phenotypes in response to inflammation. We applied these techniques to study how chronic inflammation impacts the liver's highly abundant macrophage population, Kupffer cells (KCs). While LXRα, Spi-C and SMAD4 are known to be essential for KC identity and homeostasis, transcriptional regulation of KC inflammatory responses remains poorly understood. Using scRNA-seq, we found transcriptional diversity in mouse liver myeloid cells in a model of nonalcoholic steatohepatitis (NASH), and conservation with human "scar-associated macrophages" (SAMs) from patients with cirrhosis. SAMs are marked by expression of CD9 and TREM2, a critical regulator of phagocytosis and lipid metabolism. ChIP-seq for H3K27ac identified 4201 enhancers upregulated during NASH, which were enriched for ATF3/AP1 binding motifs. Additional ChIP-seq experiments revealed that Trem2, Cd9 and other NASH/SAM phenotype genes were hierarchically controlled by LXRs and ATF3, which is induced during NASH. LXRα/β-deficient mice failed to upregulate a significant portion of the NASH/SAM program, supporting their role in driving KC inflammatory pathways. NASH suppressed 2553 enhancers involved in the LXR-controlled KC identity and homeostatic program and downregulated Spi-C, Irf1 and Tfec, which likely collaborate with LXRs in healthy KCs. Thus, KC responses during NASH involve reprogramming of LXRs through changes with collaborative transcription factors, suggesting ATF3 inhibition may be a potential therapeutic strategy for controlling KC-mediated inflammation. This integrated genomics-based approach will yield insights into the mechanisms controlling immune cells in dermatologic diseases.

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Genome-wide association study of hidradenitis suppurativa in a multi-ethnic cohort
A. Khan[1], M. Hayes[2], J. Connolly[3], F. Mentch[3], P. Sleiman[3], H. Hakonarson[3], C. Wang[1], G. Hripcsak[1], K. Kiryluk[1], L. Petukhova[1]
[1]Columbia University, NY, New York, United States, [2]Northwestern University, Chicago, Illinois, United States, [3]Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States
Hidradenitis suppurativa (HS) is a prevalent inflammatory skin disease. HS patients suffer from deep, painful, recurrent abscesses that drain malodorous fluid and lead to disfiguring scars that can limit mobility. African Americans and females are at an increased risk. A lack of effective therapies and limited knowledge about HS pathogenesis contribute to unmet needs. Unlike other common inflammatory skin diseases, there has never been a genome-wide association study (GWAS) conducted for HS. Here, we performed a first GWAS for HS using data from the eMERGE network of electronic health record linked biorepositories (project NT227). We used HS diagnosis codes to identify cases and controls. We estimated ancestry with principal component analysis using a set of 40,156 SNPs. Our final cohort consisted of 600 HS cases and 82,611 controls with comparable multi-ethnic ancestry (λ=1.005). Our cohort recapitulated HS race and gender predilictions with genetically African female participants accounting for 35% of cases, but only 10% of controls. Genotype data for 40 million variants was tested for association, adjusting for five principle components. No locus exceeded our threshold for statistical significance. There was no evidence for HLA association supporting classification of HS as inflammatory rather than autoimmune. Several loci approached the significance threshold, suggesting that a moderate expansion in cohort size may provide adequate power to detect associations. Interestingly, the lead SNP at one of the most significant loci (rs11075745; $p=8x10^{-7}$) is an eQTL for NFAT5, a mediator of NOTCH signaling whose expression is downregulated in HS lesional skin relative to patient-matched nonlesional skin. The risk allele influences expression in tissue specific manner. Our group is constructing multi-ethnic replication cohorts that will allow us to expand this study in the near future.

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Trichothiodystrophy, a multisystem disorder with early onset debilitating hip degeneration
J. J. DiGiovanna, E. R. Heller, S. G. Khan, D. Tamura, S. Folarin, K. H. Kraemer
Lab Cancer Biol and Gen, NCI, Bethesda, Maryland, United States
Trichothiodystrophy (TTD) is a rare multisystem autosomal recessive disorder of DNA repair and transcription. Cut hair shafts have "tiger tail" banding with polarizing microscopy. Patients have multiple skeletal abnormalities, e.g. short stature, osteosclerosis of the central skeleton with osteopenia of the distal appendicular skeleton. They have a high risk of death before the age of 10 years, usually from infection. Between 2001 and 2019 we followed a cohort of 39 TTD patients, ranging in age from 1-36 years. Twenty-four had mutations in the *XPD [ERCC2]* gene, 5 in *TTDN1*, 3 in *TTDA*, 1 in *GTF2E2*, and 6 in unknown genes. Nine with *XPD* mutations developed rapidly progressive, debilitating hip degeneration. Typically, this started at mean age 8 yrs (range 5-12) as hip/leg pain that interfered with walking. Imaging showed degenerative changes, interpreted as avascular necrosis of the femoral head, by mean age 9 yrs (range 5-13). Of the 9 affected patients, all had lower extremity contractures/tightness on clinical examination and 6 had W sitting, suggesting joint laxity. In 6 of the 9 patients similar changes rapidly developed in the other hip, by mean age 10 yrs (6-17). Only 1 patient of the 9 affected was able to regain ambulation. Following bilateral hip replacements at age 17, he continues to be ambulatory at age 29 years. Three of the 9 died (aged 9, 9 and 15 yr), two from complications of hip surgery. The average age of the 30 TTD patients without hip degeneration was 14 (range 1-36 years). Seven of these patients died at mean age 10 yrs (range 2-36): 5 had *XPD* mutations, 1 *TTDA* and 1 unknown. Avascular necrosis can be caused by several collagen abnormalities, e.g., Legg-Calve-Perthes disease (*COL10A1* mutations), multiple epiphyseal dysplasia (type IX collagen mutations). The previously reported downregulation of *COL6A1* expression and/or over-expression of *matrix metalloproteinase 1* in fibroblasts from TTD patients with *XPD* mutations suggest that collagen abnormalities may contribute to these degenerative hip abnormalities.

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Mechanisms governing epigenetic regulation of apoptosis in CTCL: Implications for therapy with methotrexate, JAK inhibitors, and resveratrol

M. Nihal[1], G. Wood[1, 2]

[1]Dermatology, University of Wisconsin-Madison, Madison, Wisconsin, United States, [2]Dermatology, VAMC, Madison, Wisconsin, United States

We showed previously that the death receptor/ligand pair, FAS/FASL, is silenced at least partially by promoter methylation. MTX and analogs, as well as knockdown of DNMT1 and/or 3A, increased FAS/FASL expression in association with decreased promoter methylation. MTX also increased Caspases 3/8/9 and apoptotic cell death in CTCL lines HH, SZ4, Hut78, and MyLa. FASL promoter methylation was decreased by MTX, knockdown of DNMT1 and/or DNMT3 in these same CTCL lines with the most significant effects in FAS methylation-high HH and SZ4. Furthermore, MTX enhanced FASL upregulation induced by knockdown of either DNMT1 or DNMT3. Using pull-down experiments, we identified STAT3 as a DNMT binding partner. JAK2 is essential for STAT3 activation by phosphorylation at Tyr-705. The selective JAK2 inhibitor, Fedratinib (FED), was more effective than other mixed JAK inhibitors in reducing CTCL viability, spheroid formation, and STAT3 phosphorylation at Tyr-705. Interestingly, these effects were observed in Hut78 that has JAK1 and JAK3-activating mutations. MTX+FED enhanced apoptosis in HH and SZ4 in association with increased Caspase 8. However, FED did not enhance MTX-induced increases in FAS/FASL, suggesting another extrinsic apoptotic pathway was responsible for the enhanced killing of MTX+FED compared to MTX alone. In this regard, we found that MTX+FED enhanced TRAIL expression compared to either agent alone. The optimal functioning of STAT3 also involves acetylation. Therefore, we tested the efficacy of the antioxidant and histone acetyltransferase inhibitor, resveratrol (RES), as an anti-CTCL agent alone or in combination with MTX and/or FED. Although RES alone generally had no impact on CTCL apoptosis, MTX+FED+RES significantly increased apoptosis compared to MTX+FED and reduced STAT3 acetylation. If similar results are observed in ex-vivo studies of CTCL, then MTX+FED+RES might be a novel CTCL Rx option. MTX and RES are inexpensive with favorable toxicity profiles. FED was recently FDA-approved for myelofibrosis.

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Results from the largest whole-genome sequencing study of atopic dermatitis with extensive phenotyping

S. Smieszek

Genetics, Vanda Pharmaceuticals, Washington, District of Columbia, United States

Atopic dermatitis (AD) is a highly heritable disorder with estimates reaching 75%. The genetic background of chronic pruritus in Atopic Dermatitis (AD) is complex. We have conducted a whole-genome sequencing association analysis of 760 AD patients with chronic pruritus associated with AD and 750 controls. We investigated the frequency and effect of rare and common loss of function (LOF) variants within the cohort as compared to WGS controls as well as GNOMAD. The samples were obtained as part of a randomized, double-blind, placebo-controlled, multi-center study in patients with chronic pruritus associated with AD. The inclusion criteria included: chronic (≥ 6 weeks) itch related to AD, refractory to treatment by patient history, average itch score by visual analog score (VAS) of ≥ 70 mm (out of 100 mm), SCORAD: AD1 <80; Body surface area coverage: AD1 – N/A; AD2 ≥1%. In a GWAS case-control study, we report several interesting significant signals, MAF>5% including variants within IL12B and ATP2C1. We detect novel signals as well as confirm previously detected variants on chromosome 1. Furthermore, we conducted a whole-genome sequencing study of baseline itch associated with AD. We tested the association between the WGS and Worst Itch Visual Analog Scale (WI-VAS). We detect significant variants in *ABCA6* and *PRIM2* in addition to other signals. The *INADL* region contains the variant that is most significantly associated with change in WI-VAS, rs11207834 (p-value = 6.1E-5). Age of onset analysis shows not only the effect of the *FLG* and likely EDC variants in terms of heightened risk of AD but foremost enables to predict early-onset, lending further credence to the penetrance and causative effect of the identified variants. Understanding the genetic background and risk of early-onset is suggestive of primary skin barrier dysfunction etiology of a subgroup of AD patients. Pruritus has a large impact on AD patients' quality of life; thus the results of this analysis are relevant as they could stratify personalized therapeutic approaches based on the variants identified.

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Optimizing keratinocyte differentiation from induced pluripotent stem cells for the treatment of Epidermolysis Bullosa Simplex

M. Pavlova, M. H. Shivananda Murthy, J. Schipper, A. Rozhok, P. McGrath, K. C. Butterfield, S. McGarvey, D. R. Roop, I. Kogut, G. Bilousova

Dermatology, University of Colorado Denver , Aurora, Colorado, United States

Epidermolysis bullosa simplex (EBS) is an autosomal dominant skin fragility disorder caused by mutations in one of copolymeric keratins, keratin (K) 5 or K14. Current therapy for EBS is limited to wound care. However, the development of an experimental stem cell therapy using induced pluripotent stem cells (iPSCs) that can be genetically corrected, differentiated into new skin cells and administered back to the same patient as an autograft offers a promising new approach for EBS treatment. Toward this goal, we have previously combined our high-efficiency RNA-based reprogramming method with a Cas9-mediated gene correction strategy into a one-step procedure and successfully generated corrected EBS iPSC lines with a specific knock out of the mutant K14 allele carrying a heterozygous C373T mutation at codon 125. While keratinocytes differentiated from these corrected iPSCs do not show the EBS-associated phenotype and may be suitable for transplantation, the problem of heterogeneity of cell populations derived during differentiation of iPSCs into epidermal progenitors is still a significant safety hurdle toward clinical translation of iPSCs for the treatment of skin diseases. To address this hurdle, we have optimized our iPSC differentiation protocol and increased the yield of iPSC-derived keratinocytes (iPSC-KCs) to 60-80%. To improve our iPSC-KC enrichment strategy, we have also generated an iPSC line with a K5-specific knock-in of a fluorescent marker that allows us to trace the derivation and maintenance of $K5^+$ iPSC-KCs in culture. Using this line, we are currently evaluating different iPSC-KC enrichment approaches to obtain high-quality iPSC-KCs suitable for the generation of full-thickness skin grafts, thus paving the way toward developing a safe iPSC-based therapy for EBS.

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Mutation analysis of autosomal recessive woolly hair/ hypotrichosis in 72 Japanese patients

R. Hayashi[1], A. Fujimoto[1], H. Fujikawa[1], R. Yokoyama[1], S. Shinkuma[1], Y. Shimomura[2], R. Abe[1]

[1]Division of Dermatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan, [2]Division of Dermatology, Yamaguchi University Graduate School of Medicine, Ube, Japan

Woolly hair is a hair shaft anomaly characterized by tightly curled hair and is frequently associated with hypotrichosis. Autosomal recessive woolly hair/hypotrichosis (ARWH/H: OMIM 278150/604379) is a non-syndromic form of hereditary hair disorder characterized by short and tightly curled scalp hair with hypotrichosis. ARWH/H is hereditary hair disorder and is caused by mutations in either *LIPH* or *LPAR6* genes. In the Japanese population, two mutations in the *LIPH* gene, c.736T>A (p.Cys246Ser) and c.742C>A (p.His248Asn) are considered prevalent founder mutations. Therefore, most Japanese patients with ARWH/H are homozygous for either c.736T>A or c.742C>A, or compound heterozygous for c.736T>A and c.742C>A. Although past reports revealed that patients with homozygous c.742C>A mutation showed very severe phenotype, homozygous c.742C>A mutation had been identified in few patients. In this study, we performed mutation analysis of candidate genes and identified either c.736T>A or c.742C>A in 72 Japanese patients with ARWH/H who visited Niigata University Hospital. We also checked scanning electron microscope (SEM) in patients with either homozygous c.736T>A or c.742C>A, or compound heterozygous for c.736T>A and c.742C>A. Forty eight patients had homozygous c.736T>A mutation and compound heterozygous mutations were found in 22 patients. Only 2 patients carried homozygous c.742C>A mutation and their phenotypes were not so severe. We could not find the difference of morphology in SEM. We need further follow up because we have only two patients with homozygous c.742C>A mutation and they are still children. In summary, we did not find clear genotype/phenotype correlations and homozygous c.742C>A mutation was not severe.

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Targeted reactivation of a dormant tumor suppressor gene *CDKN2A* inhibits proliferation of skin cancer cells

J. W. Lee[1], D. Rokunohe[1], D. D. Walker[1], K. Tuttle[1], K. Bradwisch[1], O. Denisenko[2], K. Bomsztyk[2], M. Kawasumi[1]

[1]Dermatology, University of Washington, Seattle, Washington, United States, [2]Allergy and Infectious Diseases, University of Washington, Seattle, Washington, United States

Cutaneous squamous cell carcinoma (cSCC) is the second most common cancer in the U.S. cSCC frequently manifests with inactivation of the tumor suppressor gene *CDKN2A*, which encodes $p16^{INK4A}$, due to not only genetic but also epigenetic alterations. The loss of *CDKN2A* expression increases malignant phenotypes in cancer. Thus, we investigated whether $p16^{INK4A}$ transcriptional suppression in cSCC cells can be reversed and whether targeted transcriptional reactivation of $p16^{INK4A}$ inhibits cell proliferation. We transduced the cSCC cell line A431 with lentivirus encoding nuclease-deactivated Cas9 (dCas9) fused to the catalytic domain of histone acetyltransferase p300 or to transcriptional activator VP64. Each of these lentiviruses additionally encoded a puromycin resistance gene for drug selection. Subsequently, we performed lentiviral transduction with guide RNA (gRNA) directing dCas9 fusion proteins to the *CDKN2A* promoter where DNA methylation and histone deacetylation may occur in cSCC. After one week of puromycin selection for transduced cells, we measured $p16^{INK4A}$ expression via RT-qPCR and cell proliferation. Both dCas9-p300 and dCas9-VP64, each with gRNA targeting the *CDKN2A* promoter, increased $p16^{INK4A}$ mRNA expression relative to untransduced cells and transduced cells without gRNA. Increased $p16^{INK4A}$ expression correlated with decreased cell proliferation. These findings suggest that *CDKN2A* can be upregulated in cSCC cells by CRISPR-Cas9-based, targeted epigenetic modification or transcriptional activation, leading to inhibition of cell proliferation. Further investigations are needed to comprehensively assess epigenetic alterations in metastatic cSCC and their roles in driving malignant phenotypes. Targeting multiple cancer-related loci for epigenome editing may synergistically inhibit cancer phenotypes.

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Large scale functional inference for skin-expressing lncRNAs using expression and sequence information

M. Patrick[1], S. Sreeskandarajan[1], A. Shefler[1], R. Wasikowski[1], M. K. Sarkar[1], J. Chen[1], E. Prens[2], A. Hovnanian[3], S. Weidinger[4], J. T. Elder[1], C. Kuo[5], J. E. Gudjonsson[1], L. C. Tsoi[1]

[1]University of Michigan, Ann Arbor, Michigan, United States, [2]Erasmus University, Rotterdam, Netherlands, [3]Paris Descartes University, Paris, France, [4]University of Kiel, Kiel, Germany, [5]Aachen University, Aachen, Germany

Long non-coding RNAs (lncRNA) play important roles in gene regulation and have been associated with different human conditions. Our previous study revealed skin-expressed lncRNAs are dysregulated in the lesional skin of psoriasis and atopic dermatitis (compared to non-lesional skin and controls). However, their biological roles can be difficult to determine due to limited prior information and generally lower expression levels compared to protein-coding transcripts. To address this, we compiled a catalog of 18,517 lncRNAs, and assessed their potential functions by profiling their expressions in skin from over 800 RNA-seq samples of different skin conditions and keratinocytes under cytokine stimulations. We applied machine learning to predict the involvement of lncRNAs in 4,247 biological functions/pathways, achieving AUROC >0.7 for 2,168 of them, including the pathway for T-cell activation. Among the lncRNAs most highly predicted to belong to this pathway, *MALAT1* was up-regulated in differentiated keratinocytes from psoriatic epidermis in scRNA-seq and has previously been suggested to be involved in T-cell lymphoma, while *RMRP* is associated with defective T-cell proliferation. Our algorithm also considered that lncRNAs may be negatively correlated with the protein-coding genes in the same pathway. Overall, we assigned 4,487 lncRNAs to at least one of 619 biological functions/pathways, and 13% of pathways had at least one negatively correlated lncRNA. In addition, when applying SEEKR to cluster their 6-bp sequence motifs, we found the distance to the overall cluster proportions to be significantly greater than expected by chance ($p=1.30x10^{-17}$). By integrating large-scale information to infer the functions of lncRNAs, the findings can facilitate understanding of the roles lncRNAs play in different cutaneous conditions.

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Biotin Is required for the zinc homeostasis in the skin

Y. Ogawa, M. Kinoshita, T. Sato, S. Shimada, T. Kawamura

Dermatology, University of Yamanashi, Chuo, Yamanashi, Japan

Patients with biotin deficiency present symptoms that are similar to those in patients with acrodermatitis enteropathica (inherent zinc deficiency). However, the association between biotin and zinc deficiency remains unknown. We have previously shown that epidermal keratinocytes of mice fed zinc-deficient (ZD) diets secreted more adenosine triphosphate (ATP) than those of mice fed zinc-adequate (ZA) diets and that epidermal Langerhans cells are absent in ZD mice. Langerhans cells highly express CD39, which potently hydrolyzes ATP into adenosine monophosphate (AMP). Thus, a lack of Langerhans cells in ZD mice leads to non-hydrolysis ofATP, thereby leading to the development of ATP-mediated irritant contact dermatitis. In this study, we examined if biotin-deficient (BD) mice showed the same underlying mechanisms as those in ZD mice. BD mice showed reduced serum zinc levels, disappearance of epidermal Langerhans cells, and enhanced ATP production in the skin. Consequently, irritant contact dermatitis was significantly enhanced and prolonged in BD mice. In conclusion, the findings of our study showed that biotin deficiency leads to zinc deficiency because of which patients with biotin deficiency show similar symptoms as those with acrodermatitis enteropathica.

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In-progress validation of candidate rosacea genes by targeted interrogation of alleles and assessment of rosacea comorbidities

A. Kumar, Y. Shih, A. Chiou, S. Li, A. L. Chang

Stanford University, Stanford, California, United States

Rosacea is a chronic inflammatory facial condition. Current treatments vary in efficacy, reflecting ambiguity regarding rosacea's etiopathogenesis. Only recently have large scale genetic studies been performed in rosacea, despite known demographic associations and familial predispositions. Ten rosacea-associated single nucleotide polymorphisms (SNPs) have thus far been identified by genome wide association studies (GWAS). However, those GWAS were limited by use of patient self-reported rosacea diagnoses and allele imputation. Thus, we sought to validate the associated SNPs in a prospective cohort of dermatologist-verified rosacea cases and controls using targeted SNP sequencing, along with a secondary validation of published rosacea comorbidities. 306 participants with European ancestry (155 rosacea cases, 151 controls) were enrolled. Demographic and comorbidities data were collected via questionnaire, and DNA was provided via buccal swab. Comorbidities were assessed via conditional logistical regression for age matched pairs (n=83), adjusted for gender. Following DNA extraction and genotyping via commercial primers (6 SNPs), difference in frequency of SNPs between rosacea cases (n=136) and controls (n=150) was assessed. We found significantly increased ($p<0.05$) incidence of gastrointestinal reflux (20% vs. 13%), chronic diarrhea (7% vs 1%), hyperlipidemia (29% vs 28%), and sleep disorders (20% vs 12%) in rosacea cases over controls. These results support the connection between rosacea and systemic comorbidities from prior studies, suggesting this cohort should be useful for validating candidate genetic markers for rosacea. However, frequencies for all 6 genotyped SNPs did not differ statistically between cases and controls, even upon age and gender matched and combinatorial sensitivity analyses ($p>0.05$). Additional recruitment of rosacea cases and controls may be needed to achieve sufficient power to demonstrate statistical significance. Furthermore, non-commercially catalogued rosacea-associated SNPs remain to be genotyped in our samples.

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Autocrine IFN-κ restricts CRISPR-Cas9 Keratinocyte transfection through STING-APOBEC3G activation

M. Sarkar[1], R. Uppala[1], A. Tsoi[1], S. Shao[1], A. C. Billi[1], B. E. Perez White[2], A. Kidder[1], X. Xing[1], J. Kahlenberg[3], J. E. Gudjonsson[1]

[1]Dermatology, University of Michigan, Ann Arbor, Michigan, United States, [2]Dermatology, Northwestern University, Chicago, Illinois, United States, [3]Rheumatology, Internal Medicine, University of Michigan, Ann Arbor, Michigan, United States

CRISPR-Cas9 is a unique genome-editing tool but is limited in regards to low transfection efficiency in keratinocytes (KCs) (1-2%) in stark contrast to HEK293T cells (>60%). The cause of the limited transfection in KCs is poorly understood. We have recently identified a role for IFN-κ, an endogenous type I IFN in KCs, in mediating KC resistance to CRISPR/Cas9 transfection. Thus, CRISPR knockout (KO) KCs have significant suppression of *IFNK* expression ($p<0.001$) as well as type I IFN responses ($P<0.001$) in all KC KOs. The *IFNK* suppression in the CRISPR KO lines is associated with CpG hypermethylation in the *IFNK* promoter. DNMT3B, a DNA methyltransferase, was inversely correlated with *IFNK* expression, suggesting that this enzyme is responsible for inducing CpG methylation in the *IFNK* promoter. Notably, KOs with suppressed *IFNK* expression had significant increase in transfection efficiency compared to WT KCs($p<0.001$). Similarly, baricitinib, a JAK1/2 inhibitor, enhanced transfection efficiency ($P<0.001$) in KCs through suppression of *IFNK* ($p<0.001$). CRISPR/Cas9 transfection was associated with activation of antiviral responses with increased *IFNK* through STING (TMEM173) activation, and induction of *APOBEC3G*, a cytidine deaminase, in KO KCs. Notably, plasmid stability was increased in KC KOs as well as in STING KC KO compared to WT ($p<0.01$). In summary, our data suggest that CRISPR/Cas9 transfection efficiency in KCs is dependent upon CRISPR plasmid activation of STING-dependent pathway and activation of endogenous type I IFN signaling through induction of APOBEC3G, and that the activation of *IFNK* to CRISPR/Cas9 plasmids is dependent upon DNMT3B activity. These findings will have major implications for CRISPR research and its future use to correct epithelial defects.

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A novel deletion mutation in FERMT1 gene in a Kindler Syndrome patient and a literature review

L. Meng[2], X. Wang[1], G. Zhang[1]

[1]Institute of Photomedicine, Shanghai Skin Disease Hospital, Tongji University School of Medicine, Shanghai, China, [2]Shanghai Skin Disease Hospital, Shanghai, China

Background: Kindler Syndrome (KS) is a rare subtype of inherited epidermolysis bullosa characterized by blistering, photosensitivity, extensive epidermal atrophy, poor wound healing and increased risk of cancer. KS is inherited in an autosomal recessive manner, and the FERMT1 gene has been identified as the pathogenic gene of it. Many mutations of this gene have been detected in KS patients. Materials and methods: Peripheral blood was obtained from a KS patient and his parents. Multi gene panel including the FERMT1 gene was performed using blood sample from the patient and his parents. All pathogenic mutations identified in the FERMT1 gene were summarized by searching for literatures using PubMed. Result: One novel homogenous mutation c.1885_1901del (p.Val629fs) on exon 15 in the FERMT1 gene was identified in the patient, and KS diagnose was further confirmed based on clinical features and the gene detection findings. To date, together with our data, a total of 81 different pathogenic mutations in the FERMT1 gene have been identified in patients with KS. These mutations are scattered throughout the FERMT1 gene, with no evident hotspots or clustering. Conclusion: This study expanded the range of known FERMT1 sequence variants in KS.

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A quantitative xenograft model to validate causative mutations in patients with Ehlers-Danlos Syndrome

N. Diette, A. Frieman, A. Rozhok, D. Roop, I. Kogut, G. Bilousova

Gates Center for Regenerative Medicine, University of Colorado, Aurora, Colorado, United States

The Ehlers-Danlos Sydrome (EDS) is a connective tissue disease typified by cutaneous fragility and joint hypermobility, usually caused by mutations in fibrillar collagens or genes involved in the synthesis of collagen. In many cases, however, the causative genetic defects of EDS have not been identified and patients' treatment options are limited to symptomatic care. Study of EDS mechanisms and the development of therapeutic strategies for the disease is radically impeded by the lack of appropriate *in vitro* and *in vivo* EDS models that faithfully recapitulate the clinical phenotype of the disease. To establish a clinically relevant model for EDS, we focused on a cohort of patients with the hypermobility type of EDS who present classical symptoms, but do not harbor any known EDS-associated mutations. We grafted patient skin-derived fibroblasts with healthy keratinocytes onto an immunocompromised mouse to recapitulate the EDS skin phenotype in a xenograft model. We found that grafts formed with EDS fibroblasts exhibit a disorganized collagen network, which has previously been observed in the skin of EDS patients. With image analysis software, we have quantified the collagen present in control and EDS grafts to reveal significant differences between healthy and diseased skin. Using RNA sequencing analysis, we compared transcriptional profiles of EDS fibroblasts with that of control cells and identified several mutations and transcript variants that may be causing the phenotype in our patients. To validate the candidate mutations, we have reprogrammed EDS fibroblasts into induced pluripotent stem cells (iPSCs) and are correcting these candidate mutations using CRISPR/Cas9. The corrected EDS iPSCs will be differentiated into fibroblasts and grafted in our xenograft assay. If we no longer observe a disorganized collagen network in the xenograft, the candidate mutation is the cause of EDS in our patients, validating the usefulness of our model in identifying the mechanisms of EDS.

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Differential gene expression and chromatin accessibility reveal Th17 polarization in skin-homing T cells

Z. Zhang[1], L. C. Tsoi[1, 2], R. Nair[1], H. Zhang[2], P. Stuart[1], X. Wen[2], J. T. Elder[1]

[1]Dermatology, Univ. Michigan, Ann Arbor, Michigan, United States, [2]Biostatistics, Univ. Michigan, Ann Arbor, Michigan, United States

To further characterize genomic features of skin-homing T cells of relevance to psoriasis, we generated PBMC from 155 individuals (82 normal, 73 psoriatic) by Ficoll-Hypaque centrifugation, either unstimulated (0h) or 24h post-stimulation with anti-CD3/CD28 beads (24h). CD4 and CD8 memory T cells were purified using immunomagnetic beads followed by flow sorting (CD3+CD45RO+ CD4/8) as a function of skin-homing (CLA+/CLA-). The sorted cells were profiled by RNA-seq and ATAC-seq using established methods. We present data on 243 paired RNA-seq and ATAC-seq libraries from 47 individuals (27 normal, 20 psoriatic), using DESeq2 for differential gene expression and chromatin accessibility analyses as functions of CD4/CD8, CLA+/-, and CD3/CD28 activation (FDR<0.05, $|\log2\ FC|>0.585$). Across the resulting 8 comparisons, we found from 97 to 2384 differentially expressed genes (DEGs) as a function of CLA, and from 5817 to 7502 DEGs as a function of activation. KEGG analysis of up-regulated genes revealed enrichment for "osteoclast differentiation" ($p = 4.3$ E-11) and "Th17 differentiation" ($p = 2.4$ E-5) in CLA+ vs. CLA- CD4 T-cells at 0h and at 24h ($p = 2.3$ E-8 and 5.8 E-10, respectively), with IL17A, IL17F, IL23R, and IL22 appearing as DEGs only after activation. CD8+ T-cells manifested similar enrichments as a function of CLA at 0h ($p =3.0$ E-8 and 4.2 E-4, respectively) but not at 24h, likely due to low power (only 97 DEGs at 24h). Proteasomal genes comprised the most highly enriched annotation for CD3/CD28-upregulated genes in all 4 subsets ($p < 10E-20$). ATAC-seq confirmed previous findings (JID 139 Suppl1: 571, 2019) of enrichment for "Th17 cell differentiation" in genes +/- 100 kb of differentially-accessible regions in CLA+ vs. CLA- CD4 T-cells. This expanding dataset provides further evidence for the Th17 polarization potential of skin-homing T-cells, and suggests a possible connection to psoriatic arthritis via T-cell effects on osteoclast differentiation.

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Larger wounds in recessive dystrophic epidermolysis bullosa patients associated with worse quality of life: Results of a global cross-sectional survey

E. Gorell[5], V. Eng[5], D. Solis[1], S. Choi[5], J. Nazaroff[2], S. Li[5], M. de Souza[3], D. Murrell[4], M. P. Marinkovich[5], J. Tang[5]

[1]University of California, Riverside, Riverside, California, United States, [2]University of California, Irvine, Irvine, California, United States, [3]deSouzaTech, LLC, Berkeley, California, United States, [4]University of New South Wales, Sydney, New South Wales, Australia, [5]Dermatology, Stanford School of Medicine, Redwood City, California, United States

A disease severity spectrum exists in patients with recessive dystrophic epidermolysis bullosa (RDEB). We aimed to characterize how disease severity relates to wounds, comorbidities, and quality of life (QOL) in RDEB patients. RDEB patients were surveyed online via the EBCare website. QOL was measured with the Quality of Life in Epidermolysis Bullosa (QOLEB) instrument. 85 RDEB patients self-reported on 1,226 wounds: 937 recurrent wounds and 289 chronic open wounds. 52% of recurrent wounds and 30% of chronic open wounds were <19cm^2. 10% of recurrent wounds and 34% of chronic open wounds were > 40cm^2. Disease severity was self-reported as mild (26%, 22/83), moderate (48%, 40/83), or severe (25%, 21/83). Worsening severity was associated with larger wounds ($p<0.01$), history of squamous cell carcinoma ($p=0.04$), history of anemia ($p<0.01$), gastrostomy tube use ($p=0.02$), osteoporosis ($p=0.03$), and routine opiate use ($p=0.02$). The average QOLEB score (n=39) was 20.0±9. Larger wound size was associated with worse QOL ($p=0.02$). This study shows important correlations between larger wound size and worse QOL in RDEB. Chronic open wounds were larger, but recurrent wounds were more frequent, as seen in previous studies. Patients who self-reported more severe overall disease had larger wounds and more frequent extracutaneous manifestations. This study also revealed increased opiate usage in patients with more severe skin disease.

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Possibility of therapeutic application to autosomal dominant dystrophic epidermolysis bullosa using large deletion genome editing with CRISPR-Cas3

H. Morisaka[1], K. Yoshimi[2], Y. Okuzaki[3], A. Hotta[3], J. Takeda[4], T. Mashimo[2], S. Sano[1]

[1]Dermatology, Kochi Medical School, Kochi University, Kochi, Japan, [2]Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [3]Center for iPS Cell Research and Application (CiRA), Department of Clinical Application, Kyoto University, Kyoto, Japan, [4]Research Institute for Microbial Diseases, Osaka University, Osaka, Japan

Clustered regularly interspaced short palindromic repeats (CRISPR)- associated Cas systems are widely used for genome editing in eukaryotic cells. We recently reported programmable CRISPR-Cas3 genome editing in human cells. Cas3, which possesses helicase and nuclease activity, predominantly triggered several thousand base pair deletions upstream of the 5-ARG protospacer adjacent motif (PAM) (Morisaka, et al. Nat Commun, 2019). Knowing that genome editing with large deletion produced by CRISPR-Cas3, but not by CRISPR-Cas9, allowed us to hypothesize that it would be relevant to therapeutic benefit for dominant negative diseases if the mutated allele is successfully skipped. To assess whether the CRISPR-Cas3 system was applied to therapy for autosomal dominant dystrophic epidermolysis bullosa (DDEB), we targeted COL7A1 gene in primary fibroblasts with CRISPR-Cas3. Co-transfection of primary fibroblasts with the CRISPR-Cas3 expression plasmid and crRNA expression plasmid resulted in generation of several genome edited clones, which were detected by PCR genotyping and sanger sequencing. We are now under the progress in looking at the feasibility of this editing system to the diseased fibroblasts from patients with DDEB. These findings broaden our understanding of CRISPR systems, which may serve as a novel therapy for dominant negative diseases, including DDEB.

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Identification of psoriasis-protective chemokine, *FAM19A5*, and *IL17D* expression in psoriatic skin

A. Toussi[1], A. Merleev[1], D. Yamada[1], Z. Shi[1], S. Le[1], A. Marusina[1], G. Luxardi[1], I. Adamopoulos[2], S. Hwang[1], J. E. Gudjonsson[3], E. Maverakis[1]

[1]Dermatology, University of California, Davis, Sacramento, California, United States, [2]Rheumatology, Allergy, and Clinical Immunology, University of California, Davis, Sacramento, California, United States, [3]Dermatology, University of Michigan, Ann Arbor, Michigan, United States

Psoriasis is a chronic inflammatory skin disease with an estimated heritability of 80%. Susceptibility loci identified by GWAS studies account for a small fraction of this heritability. While gene expression analyses have identified thousands of differentially expressed genes in psoriasis, only a small fraction are likely involved in psoriasis pathophysiology. *FAM19A5* is of special interest because it is a chemokine-like molecule that is highly expressed in normal skin but downregulated in psoriatic plaques ($p= <2x10^{-16}$). We therefore sought to mine psoriasis RNA-Seq datasets to identify other genes associated with *FAM19A5* and to determine if this gene is related to psoriasis susceptibility. Correlative analysis identified a dependent relationship between *FAM19A5* and *IL17D* ($r= 0.64$, $p= 3.2x10^{-13}$). *IL17D* was also found to be downregulated in psoriasis ($p= <2x10^{-16}$). A nonlinear dimensionality reduction strategy illustrated a close spatial relationship between *FAM19A5* and *IL17D,* which mapped away from the proinflammatory cytokine cluster of *IL17A*, *IL23A*, *IL36,* and *IL1B.* We also identified an *IL17D*-associated allele (rs9509353) that was protective against psoriasis (OR= 0.20, $p= 5.9x10^{-7}$) and a protective variant of *FAM19A5* (rs131959; OR= 0.29, $p= 8.8x10^{-5}$). Both protective variants were associated with increased *FAM19A5* expression ($p= 6.1x10^{-4}$ and $7.8x10^{-5}$). Similar to the results in the setting of human psoriasis, IL-23 minicircle-induced psoriasis-like dermatitis in B6 mice demonstrated striking downregulation of *FAM19A5* compared to controls (>20 fold decrease). These results highlight a putative regulatory role for *FAM19A5* and *IL17D* in psoriasis and *IL17D* as an upstream regulator of *FAM19A5* expression.

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Premature thymic involution and oropharyngeal blistering cause early lethality in generalized severe junctional epidermolysis bullosa

V. K. Yenamandra[1], J. Dolorito[1], I. Gurevitch[1], E. Godoy[2], K. Casey[1], M. P. Marinkovich[1]

[1]Dermatology, Stanford University, Redwood City, California, United States, [2]Comparative Medicine, Stanford University, Stanford, California, United States

Generalized severe junctional epidermolysis bullosa (JEB-GS) due to laminin-332 mutations is a lethal and incurable autosomal recessive blistering skin disease. The average survival age in human JEB-GS is 6 months but the reasons for this poor prognosis remained obscure. In this study, we aimed to discover the underlying causes of this premature lethality using a tetracycline operator-regulated *LAMC2* transgenic mice, in which laminin-332 expression can be regulated by doxycycline administration in drinking water. Upon doxycycline withdrawal for two weeks, the mutant mice presented with only 2 distinct clinical symptoms: failure to thrive (10.8±2.8 vs 20.0±1.4; gm) and an inspiratory stridor as compared to age-matched doxycycline treated mice (controls). However, comprehensive necropsy analysis revealed numerous consequences in several organs: marked thymus and spleenic atrophy (-85.5%, as compared to controls), lymphocyte apoptosis, oropharyngeal ulceration associated with submucosal inflammation and neutrophilic infiltrate, intralesional bacteria and tissue edema causing pharyngeal airway narrowing. These findings support our preliminary observations of thymic atrophy, reduced peripheral lymphocytes and lung abnormalities in two JEB-GS patients. Further, we also demonstrate that reintroduction of doxycycline in untreated sick mutant mice for two weeks reversed these injuries as evidenced by gain in weight (21.0±3.0 vs 23.2±2.9; gm), healed oropharyngeal mucosa and thymus regeneration (+16.3%, as compared to controls). Taken together, our observations implicate that acute oropharyngeal blistering and/or premature thymic involution are indeed contributing to the poor prognosis of JEB-GS. We also provide proof-of-concept for therapeutic intervention studies to improve the prognosis and perhaps cure this lethal disease. Further studies are being undertaken to evaluate functional implications of thymus atrophy in these mice and patients.

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Secreted frizzled-related protein 5 (SFRP5) inhibits the melanin synthesis of melanocytes via Wnt/β-catenin signaling pathway in vitiligo

D. Zou, Y. Chen, L. Zhang, Y. Zhang, J. Chen

The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

Vitiligo is an acquired chronic depigmentation disorder characterized by progressive depigmentation due to the destruction of epidermal melanocytes. The dysfunction of melanocytes plays an important role in the pathogenesis of vitiligo. In this study, we found that secreted frizzled-related protein 5 (SFRP5) is overexpressed in the skin lesions of vitiligo patients. Compared with normal epidermal melanocytes (PIG1), the expression of SFRP5 is increased in vitiligo melanocytes (PIG3V). To investigate the effect of SFRP5 on melanin synthesis, PIG1 cells were infected with recombinant SFRP5 adenovirus (AdSFRP5), and PIG3V cells were infected with recombinant siSFRP5 adenovirus (AdsiSFRP5). It was found that SFRP5 inhibits melanin synthesis through MITF and its target proteins down-regulation via Wnt/β-catenin signaling. Besides, the inhibitory effect can be reversed by β-catenin agonist SKL2001. The inhibitory action of SFRP5 in pigmentation was also confirmed in vivo nude mice model. The results above indicate that SFRP5 can inhibit melanogenesis of melanocytes and play a vital role in the development of vitiligo, which could be a prospective therapeutic target for vitiligo.

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ATAC-Seq analysis reveals a widespread increase of chromatin accessibility in psoriasis

F. Zhou, L. Tang

Institution of Dermatology, Anhui Medical University, Hefei, Anhui, China

Psoriasis is a common skin disease characterized by hyperproliferative keratinocytes and turbulent immune response, the exact aetiology remains essentially unknown. Genetic, epigenetic and several transcription factors have been implicated in the modulation of chromatin accessibilities. To better understand underlying regulatory network for psoriasis, we performed comparative analysis of chromatin accessibility in 19 psoriatic (PP), 15 non-psoriatic (PN) and 21 normal (NN) skin tissues via ATAC-seq. More than 40% of ATAC-seq peaks in our dataset overlap the open regions of primary keratinocyte cells and skin tissues from the ENCODE project. We identified 4,915 significantly differential peaks shared in both PP vs. PN and PP vs NN comparisons, nearly all of which are predominately more accessible in psoriatic skin tissues. The psoriasis-associated accessible peaks are highly enriched in hypomethylated regions and negatively correlated with expression of proximate genes, suggesting DNA methylation might play roles in modulating accessibility in psoriasis. Our data also indicated disease susceptibility variations might act as active regulatory elements and modulate gene expression. FRA1/AP-1 was identified as a key transcription factor in modulating expression of psoriasis-associated genes.Immunohistochemistry and immuunofluoresence analysis showed upregulated FOSL1 expression appeared in both basal and suprabasal epidermal skins, with more pronounced significance in suprabasal layer.To better understand molecular changes underlying these biological processes, we firstly overexpressed FRA1 in normal human epidermal keratinocytes (NHEKs) and performed RNA-seq analysis. The highly differentially expressed genes included *CXCL1*, *IL23A*, *TNFAIP3*, *NFKB1*, *NFKBIB* and *IL17D*, most of them previously implicated in immune disturbance for Ps.These findings revealed that global increases in chromatin accessibility may play a critical role in the development and progression of psoriasis.

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Highly efficient RNA-based reprogramming of renal epithelial cells derived from recessive dystrophic epidermolysis bullosa patients into induced pluripotent stem cells

A. Frieman, N. Diette, P. McGrath, I. Kogut, G. Bilousova

Gates Center for Regenerative Medicine, University of Colorado, Aurora, Colorado, United States

Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a severe skin blistering disease caused by mutations in type VII collagen, resulting in impairment of the anchoring fibrils at the dermal-epidermal junction. There is no cure for RDEB, and current therapy is limited to wound care. Induced pluripotent stem cells (iPSCs) have immense value in furthering the study of this disease and developing new treatment options. The use of iPSCs, however, is limited by the availability of primary cells for reprogramming, the length of transfection regimens, and low reprogramming efficiency. iPSCs can be generated from a variety of cell sources including skin punch biopsies, blood and urine samples. However, due to the severe fragility of RDEB patients' skin, it is incredibly difficult to draw blood or obtain skin biopsies from these patients. Exfoliated renal epithelial cells (RECs) found in urine samples can provide an alternative, non-invasive and easily accessible source of RDEB somatic cells for reprogramming. Previously published methods for REC reprogramming rely on electroporation of non-integrating plasmids or the use of the Sendai viral vector. These methods require time-consuming regimens and result in poor efficiency of reprogramming. Here, we report a highly efficient, non-integrating RNA-based method for reprogramming of disease-associated RECs into high quality iPSCs with an efficiency and kinetics that surpass all previously published studies. The approach depends on highly – tuned transfections of RECs with reprogramming modified mRNAs and mature miRNA mimics in combination with optimized culturing conditions. The resulting renal-derived iPSCs exhibit normal karyotypes and display a pluripotent phenotype. Thus, our approach is ideal for generating high quality patient-derived iPSCs from a non-invasive source of somatic cells to be used for modeling RDEB and other diseases and for potential clinical applications.

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Functional characterization of *ABCC6* missense variants implicated in pseudoxanthoma elasticum

L. Kowal[1], J. Huang[1], I. Jacobs[1], H. Luo[1, 2], J. Uitto[1], Q. Li[1]

[1]Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, United States, [2]Dermatology, The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, China

Pseudoxanthoma elasticum (PXE), a prototype of heritable ectopic mineralization disorders, is caused by inactivating mutations in the *ABCC6* gene. The majority of mutations in *ABCC6* are missense variants causing a single amino acid substitution which can result in loss-of-function of ABCC6 protein through changes in its transport activity, cellular trafficking, or conformational stability. As ABCC6 has a specialized efflux function in the liver by contributing to plasma PPi levels, we characterized the potential pathogenicity of six missense variants via intravenous administration of recombinant adenovirus expressing the human *ABCC6* cDNA carrying each variant to the liver of *Abcc6*$^{-/-}$ mouse model of PXE. The consequences of the variants were compared to the wild type protein expressed by an adenovirus which was previously shown to reconstitute ABCC6 in the basolateral plasma membrane of hepatocytes, the physiologic location for ABCC6. Expression of the wild type protein raised plasma levels of PPi, and consequently counteracted the ectopic calcification phenotype in *Abcc6*$^{-/-}$ mice. In contrast, variant p.L420V showed mixed plasma membrane and cytoplasmic expression, failed to normalize plasma PPi levels and had no effects on ectopic calcification. Variant p.R1138W showed very little expression while p.T364R was expressed mostly intracellularly in the liver, suggesting their pathogenicity. In contrast, p.R391G, p.S400F, and p.R760W variants are less likely to be pathogenic mutations since they resulted in expression similar to the wild type protein, restored plasma PPi levels, and prevented ectopic calcification in the *Abcc6*$^{-/-}$ mice. These results suggest that adenovirus-mediated liver-specific ABCC6 expression in *Abcc6*$^{-/-}$ mice can be used as an experimental system to elucidate the functional consequences of human *ABCC6* missense variants identified in PXE patients.

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A retinol-*Myrtus* extract complex induced beneficial epigenetic and transcriptome changes related to human skin aging

D. Meza[1], W. Li[1], M. Chang[1], S. Walsh[1], V. Bardey[2], C. Reymermier[2], M. Rushing[3], A. Brillouet[1], M. Southall[1], R. Parsa[1]

[1]Johnson and Johnson, Skillman, New Jersey, United States, [2]BASF Beauty Care Solutions, Lyon, France, [3]BASF Corporation, Tarrytown, New York, United States

A facial moisturizer incorporating the extract of *Myrtus* and retinol (ROL) was developed, and previously showed that this ROL complex enhancing ROL activity helps to clinically delay and reduce the signs of skin aging. ROL complex exerts its many skin benefits through transcriptional activation. Recent studies suggest epigenetic regulation through micro-RNAs (miRNAs), specific inhibitors of targeted gene translation, may also play a role in the regulation of skin aging. However, no studies demonstrated retinol's rejuvenating skin benefits could be also associated with epigenetic regulation of miRNAs in human skin. Studies were conducted to discover whether ROL complex's support in the stimulation of anti-aging biomarkers could be associated with transcriptomics and epigenetic changes in miRNA expression in human skin cells. Human adult fibroblasts were treated with either ROL and ROL complex for up to 72 hours. mRNA, miRNA and protein expressions were evaluated. Differentially expressed genes (DEG) induced by ROL were identified and analyzed using Gene Ontology (GO) to identify enriched biological processes. GO enrichment analysis of ROL-treated fibroblasts revealed enrichment for processes related to human skin aging such as extracellular matrix organization. In addition, ROL Complex helped induce *ELN* gene expression and type I pro-collagen protein production. Concomitantly, ROL Complex also caused epigenetic changes by reducing the expression of multiple miRNAs known to inhibit collagen and elastin genes expression.Thus, ROL complex may also exert its rejuvenating skin benefits through epigenetic regulation of both collagen and elastin that supports increases of extracellular matrix proteins. In conclusion, ROL complex may exert anti-aging skin benefits through a pleiotropic mode of action, uncovering epigenetics as an additional mechanism to explain and enhance its benefits.

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Individuals with pseudoxanthoma elasticum have a significantly increased incidence of kidney stones

D. Ralph[1], R. Allawh[1], L. Kowal[1], I. Terry[2], S. Terry[2], J. Uitto[1], Q. Li[1]

[1]Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, United States, [2]PXE International, Washington, District of Columbia, United States

Kidney stone disease (nephrolithiasis) has an incidence of 9.2% in the general U.S. population above the age of 20, according to a cumulative analysis of the National Health and Nutrition Examination Survey (NHANES) from 2007-2016. Pseudoxanthoma elasticum (PXE), a genetic disorder caused by inactivating mutations in the *ABCC6* gene, is characterized by progressive calcification of connective tissues in the skin, blood vessels, and retina. Two sisters, aged 11 and 13 years, presented with skin lesions characteristic of PXE, and histopathology of the lesions revealed mineral deposition in the reticular dermis, confirming the PXE diagnosis. Genetic analysis identified compound heterozygous mutations in *ABCC6* for both patients; c.2787+1G>T in intron 21, and c.3774_3775insC in exon 27. Both mutations were previously reported in patients with PXE. Parents were clinically normal and carriers of the respective mutations. The younger patient has been suffering from recurrent kidney stones since the age of 8. While kidney stones are common in the older population, they have been reported in sporadic cases with PXE. We hypothesized that a PXE diagnosis may correlate with an increase in kidney stone incidence. A survey of 553 individuals with PXE revealed that 23.3% had previously had a kidney stone, a significant increase compared to 9.2% in the general population ($P < 0.05$). In addition, the PXE cohort passed about 2.6 times more kidney stones, on average, than the general population. These observations support the hypothesis that a PXE diagnosis correlates with increased risk of developing kidney stones with considerable morbidity and health-care cost.

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Recessive mutations in *AP1B1* cause ichthyosis, deafness, and blindness

L. Boyden, L. Atzmony, J. Zhou, Y. Lim, R. Hu, R. Lifton, K. Choate

Yale University, Northford, Connecticut, United States

We describe unrelated subjects with ichthyosis, failure to thrive, thrombocytopenia, hearing loss, and profound photophobia, progressing to complete deafness and near-complete blindness in one subject. Exome sequencing reveals that each have novel and deleterious biallelic mutations in *AP1B1*, the gene encoding the β subunit of heterotetrameric adapter protein 1 (AP-1) complexes. There are two types of AP-1 complex, ubiquitous AP-1A and epithelium-specific AP-1B, which function in clathrin-coated vesicle budding within the trans-Golgi network and endosomes and in polarized transport of proteins to the basolateral membrane. AP-1 complexes are composed of β, γ, μ, and σ subunits; both AP-1A and AP-1B share the same β subunit, encoded by *AP1B1.* In keratinocytes with pathogenic *AP1B1* mutations the AP-1 β subunit is lost and the γ subunit is greatly reduced, demonstrating destabilization of the AP-1 complex. Affected cells and tissue contain an abundance of abnormal vesicles observed via histology and electron microscopy. Staining for proliferation marker Ki67 is greatly increased. Keratin 14 is absent from the basal layer and has patchy suprabasal expression, and keratin 10 demonstrates abnormal focal staining. Intercellular junction proteins E-cadherin and β-catenin are disrupted, and dispase dissociation assays show significantly increased tissue fragmentation after mechanical stress, highlighting the vital role of *AP1B1* in cellular polarization and adhesion. Transduction of affected cells with wild-type *AP1B1* completely rescues the vesicular phenotype, conclusively establishing that loss of *AP1B1* function causes this previously undescribed disorder. Surprisingly its loss is not lethal, demonstrating that partial compensation for the AP-1 β subunit is possible. Furthermore, while intellectual disability is a feature of all other known disorders caused by AP subunit mutations, there is no apparent intellectual impairment in our subjects with recessive mutations in *AP1B1*, suggesting variability in residual function and/or other compensatory mechanisms is likely both subunit- and tissue-specific.

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Recombination efficiency and expression of fibroblast-specific Cre drivers in mouse skin

T. He, T. Quan, W. Xia, A. A. Dlugosz, J. J. Voorhees, G. J. Fisher

Dermatology, University of Michigan, Ann Arbor, Michigan, United States

Cell-type specific genetic recombination is a powerful strategy for advancing understanding of cellular function in genetically-modified mouse models. This approach relies on cell-type specific gene promoters to drive genetic recombination in target cells. Although the utility of many cell-type specific promoters has been well characterized, thorough characterization of fibroblast-specific promoters is lacking. Therefore, we employed the mTmG two-color fluorescent reporter system to examine the genetic recombination efficiency and expression patterns of three fibroblast-specific promoters in mouse skin. mTmG mice express the red fluorescent protein tdTomato (mT) constitutively and ubiquitously. Genetic recombination catalyzed by Cre recombinase silences mT expression and induces expression of enhanced green fluorescent protein (mG). Thus, cells that express Cre recombinase appear green, while all other cells appear red. We generated mTmG mice that express Cre recombinase under the control of promoter elements in three fibroblast-specific genes 1) fibroblast specific protein-1 (FSP-1), 2) type Iα2 collagen (COL1A2), or 3) platelet-derived growth factor receptor-α (PDGFRα). Dorsal skin from mTmG/Cre positive and control mice were analyzed by immunofluorescence microscopy. Surprisingly, mTmG/FSP-1 Cre mice exhibited recombination largely in keratinocytes throughout all the layers of the epidermis and follicular epithelium. Recombination was also observed in scattered fibroblast-like cells in the dermis. mTmG/COL1A2 Cre mice displayed recombination that was specific for dermal cells with fibroblast-like morphology throughout the interstitial dermis and in dermal papillae. mTmG/PDGFRα Cre mice displayed recombination that was similar to that of COL1A2-driven recombination, although the number of positive dermal cells and the intensity of mG expression was approximately five-fold greater. These data provide a foundation for the use of Cre drivers to study fibroblast gene expression in genetically-modified mouse models.

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Induction and evaluation of an oxidative stress response in the EpiDermFT *in vitro* human skin model
K. Marengo[1], J. Oldach[1], A. Armento[1], A. Langerveld[2], E. Lehigh[2], S. Ayehunie[1]
[1]MatTek Corporation, Ashland, Massachusetts, United States, [2]Genemarkers, Kalamazoo, Michigan, United States
As the main barrier to the outside world, human skin is constantly impacted by environmental stressors such as air pollution. A major source of air pollution is vehicular exhaust which can cause oxidative stress and induce immune responses. Air pollution can negatively affect the skin causing premature skin aging and other skin problems. In this study, a full-thickness *in vitro* human skin model (EpiDermFT) was exposed topically to varying concentrations of Diesel Exhaust Particles (DEPs) for 24 hours. Tissue structure, viability, lipid oxidation, cytokine release, and gene expression were evaluated. No major effects in tissue structure or viability were observed in the DEP treated tissues. Lipid oxidation, determined by 8-Isoprostane release, increased significantly following DEP exposure by at least 2-fold. Using Clariom S Human microarrays, 213 genes were identified whose expression was upregulated or down-regulated by at least 2-fold in n=2 experiments. Genes related to immune signaling and response and cellular senescence were elevated suggesting tissue exposure to DEPs mimics human skin responses to air pollution including inflammation and skin aging. In additional experiments, tissues were pre-treated for 4 hours with the antioxidant, Resveratrol, and release of the inflammatory cytokine, IL-8, was measured. Tissues exposed to DEPs showed at least a 2-fold increase in IL-8 release, but baseline levels of IL-8 were observed when the tissue was pre-treated with Resveratrol. These findings support the utility of the EpiDermFT model to study environmental pollution responses *in vitro* and to evaluate active ingredients and other molecules for the prevention of the human skin oxidative stress response.

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Induction of an atopic dermatitis-like phenotype in a full-thickness *in vitro* human skin model
J. Molignano, J. Oldach, K. Marengo, S. Ayehunie, M. Klausner
MatTek Corporation, Ashland, Massachusetts, United States
Atopic dermatitis (AD), a common type of eczema, is the most prevalent inflammatory disease of the skin and is characterized by defects in keratinocyte differentiation and skin barrier dysfunction. AD is estimated to affect 15-30% of children and 2-10% of adults, or approximately 20% of the population worldwide. Despite its prevalence, an in vitro, commercial model of AD is not currently available to facilitate basic research and therapeutic development. The current work utilizes EpiDermFT, a reconstructed full thickness human epidermal model, cultured in the presence of a cocktail of Th2 cytokines (IL-4, IL-13, and IL-31) to induce an AD-phenotype in vitro. Treatment with IL-4, IL-13 and IL-31 induced significant changes in markers of differentiation and skin barrier integrity characteristic of AD. Histological analysis revealed treatment with the Th2 cytokine cocktail reduced the presence of the stratum granulosum and induced spongiosis in the epidermis. At the gene and protein level, upregulation of pro-inflammatory mediators, such as IL-8, and down-regulation of differentiation markers, such as keratin 1, keratin 10, involucrin, and filaggrin, were observed. These findings demonstrate that induction of the EpiDermFT tissue model with Th2 cytokines produces an in vitro tissue that is consistent with the hallmark characteristics of AD. We anticipate that the model described here will be useful for screening and evaluating active ingredients and other molecules used in the treatment of atopic dermatitis.

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Using single cell technique to reveal the expression landscape of lncRNAs in psoriasis
R. Wasikowski, A. C. Billi, H. Zhang, S. Sutharzan, A. Shelfler, M. Patrick, J. Chen, J. E. Gudjonsson, L. C. Tsoi
Dermatology, University of Michigan, Toledo, Ohio, United States
Long-noncoding RNAs (lncRNAs) have been shown to regulate epidermal differentiation in skin, and bulk RNA-seq data have illustrated the tissue and cell type specificity for skin-expressing lncRNAs. In this study, we assessed the ability to use single cell RNA-seq (scRNA-seq) obtained from 9 psoriasis lesional skin (PP) and 6 uninvolved skin (PN) samples to characterize the expression profiles for lncRNAs in cell-level resolution. Despite the limitation of scRNA-seq in detecting low-expressing transcripts, we identified 4,785 expressing lncRNAs (using threshold of >=2 reads in at least one cell) in majority of the cells. LncRNAs were detected in the basal/differentiated/keratinized epidermal layers and other major cell types including dendritic cells, T cells, and fibroblasts. The majority of LncRNAs were detected in keratinocytes, which constituted >50% of all lncRNA expressing cells. Differential gene expression analysis identified 14 lncRNAs significantly (FDR<=10% and $|\ln FC| >= \ln(1.5)$) dysregulated in psoriatic skin, with the differentiated/keratinized layers harboring 7 and 10 dysregulated lncRNAs, respectively. The most significantly up-regulated lncRNAs in PP were MALAT1 (FC=1.42 and FDR<$1x10^{-16}$) and NEAT1(FC=2.88 and FDR<$1x10^{-16}$) in differentiated keratinocytes. Notably, NEAT1 has been shown to play role in innate immunity and promote activation of inflammasome, whereas MALAT1 has been implicated in various pathological processes including alternative splicing, nuclear organization, and regulation of autophagy. Our findings will facilitate understanding of the regulatory mechanism of lncRNA in keratinocytes and provide new potential markers of psoriasis at the single cell level.

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Whole exome sequencing in AA patients identifies a hotspot mutation in the type II hair keratin gene, KRT82
S. O. Erjavec[1], S. Gelfman[2], A. R. Abdelaziz[1], E. Y. Lee[1], L. Petukhova[1], A. Christiano[1]
[1]Dermatology Department, Columbia University, New York, New York, United States, [2]Institute of Genomic Medicine, Columbia University, New York, New York, United States
Alopecia Areata (AA) is a complex genetic disease, in which we previously identified common variation at 14 loci contributing to disease risk using GWAS. However, rare variants with moderate effect size are becoming increasingly important determinants in the genetic landscape of complex diseases. In order to identify novel rare variants contribute to AA risk burden, we performed whole exome sequencing (WES) on 849 AA patients compared to 15,640 controls using an unbiased genome-wide approach. Gene-level burden analyses (genome wide signficance, p=2E-7) identified a novel gene, KRT82, as the gene containing the most variants in AA patients compared to controls. The allelic series of KRT82 variants included 2 nonsense mutations (18 AA patients), 1 frameshift (1 patient), 7 missense (28 patients), and 1 splicing mutation (4 patients), for a total of 51 out of 849 AA patients carrying rare damaging heterozygous mutations in KRT82 (6.01%). The most common variant among our AA cohort (15 AA patients; 1.77%) was a nonsense mutation at arginine position 47 (R47X), a hotspot mutation that arose independently on several haplotypes, likely via spontaneous deamination of C to T. Interestingly, KRT82 is a type II hair keratin that is exclusively expressed in the hair shaft cuticle during anagen phase, when AA attack on the HF occurs. Using gene expression analysis and immunofluorescence, we found that AA patient scalp exhibits decreased expression of KRT82 in the skin and HF. Loss of KRT82 in the hair shaft cuticle may prevent dimerization of KRT82 with its potential partners (KRT32/39/40), and previous SEM studies showed morphological defects in the hair shaft cuticle in AA patients. Our WES data identified rare pathogenic variants in KRT82 as a novel mechanism implicating loss of structural integrity of the hair shaft in the pathogenesis of AA.

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Allele specific accessibility analysis to decipher molecular mechanism of psoriasis-associated loci
L. C. Tsoi[1], Z. Zhang[1], P. Stuart[1], Y. Zhang[2], M. Patrick[1], H. Zhang[2], X. Wen[2], R. Nair[1], J. T. Elder[1]
[1]Dermatology, University of Michigan, Ann Arbor, Michigan, United States, [2]Biostatistics, University of Michigan, Ann Arbor, Michigan, United States
Genome-wide association studies for psoriasis have identified >65 susceptibility regions, and our previous work has revealed these genetic signals overlap significantly with active enhancers in T-cells. To better understand the molecular mechanisms underlying the regulatory roles of these genetic signals, we conducted ATAC-seq to profile the chromatin accessibility patterns of resting and 24h CD3/CD28-activated T-cell subsets from >100 individuals. By tallying ATAC-seq read counts as a function of allelic variation, we assessed allele specific accessibility (ASA) for genetic variants mapping within +/- 100 kb of the 95% credible set of causal variants

for the psoriasis-associated loci. By considering the directionality of the accessibility of the allele and information from multiple samples, we identified >700 sites with heterozygous genotypes and mapping to accessible chromatin in the ATAC-seq experiments. Genome-wide, we found stronger ASA in open chromatin regions depending on their proximity to psoriasis-associated signals. We further identified at least 9 psoriasis-associated loci encompassing markers exhibiting significant (FDR<=10%) ASA, including *IL28RA*and *ZNF365*, both of which manifested ASA in activated but not resting T-cells. By jointly leveraging sequence variation and chromatin accessibility information, our results provide novel insights to the molecular mechanisms of psoriasis-associated genetic variation. Ongoing sample size expansion and GWAS genotyping will add to the catalog of psoriasis-associated variants manifesting context-dependent ASA in T-cells, generating new hypotheses for functional and mechanistic studies.

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Type VII collagen NC2 domain expression differentiates severe from milder recessive dystrophic epidermolysis bullosa subtypes

A. Ponakala[1], V. K. Yenamandra[1], C. E. Teng[1], M. Barriga[1], J. Dolorito[1], E. Gorell[2], N. Nguyen[1], S. Tufa[3], K. Rieger[1], D. Keene[3], J. Tang[1], M. P. Marinkovich[1]
[1]Dermatology, Stanford University, Redwood City, California, United States, [2]Dermatology, Stanford School of Medicine, Redwood City, California, United States, [3]Shriners Hospital, Portland, Oregon, United States

Recessive dystrophic epidermolysis bullosa (RDEB) results from type VII collagen (C7) deficiency due to *COL7A1* gene mutations. C7 contains collagenous domains (COL) flanked by amino (NC1) and carboxyl (NC2) non-collagenous domains. The genotype spectrum of RDEB is well studied but does not entirely explain the highly variable clinical phenotype. In this retrospective study, we aimed to identify a disease severity and prognostic indicator, from an extensively characterized RDEB cohort. 41 RDEB patients were studied with mild, intermediate and severe clinical phenotypes in 7, 11 and 23 patients respectively. 22 of 23 severe RDEB patients showed negative NC2 expression, with variable NC1 staining and variable AF morphology. 7 of 7 mild RDEB patients showed at least one allele with missense mutations in COL domains and positive NC2 and NC1 expression. 5 of 11 intermediate patients showed positive NC2 expression with missense and/or splice site COL domain mutations. 6 intermediate patients with negative NC2 expression showed bi-allelic PTC mutations and very low NC1 staining. Given their paucity of C7 NC2 expression, its possible these latter 6 patients may evolve into a more severe clinical phenotype over time or other factors outside of C7 expression may be playing a role in dictating disease severity. This remains to be further studied. We conclude that genotype or anchoring fibril morphology do not entirely explain the phenotypic variability and C7 NC1 expression did not prove to be a good indicator of disease severity. However, of all the tests utilized, C7 NC2 expression proved the most useful in distinguishing mild from severe RDEB clinical phenotypes.

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In vivo correction of recessive dystrophic epidermolysis bullosa (RDEB) by direct cutaneous COL7A1 gene replacement: Results of a phase 1-2 trial

M. P. Marinkovich[1], S. Vinzant[2], V. Karkala[1], K. Sridhar[1], I. Gurevitch[1], J. Dolorito[1], P. Agarwal[2], S. Krishnan[2]
[1]Dermatology, Stanford University, Redwood City, California, United States, [2]Krystal Biotech Inc, Pittsburgh, Pennsylvania, United States

The use of direct gene transfer to correct genetic skin disease has been a longstanding goal in the gene therapy field. Here we demonstrate correction of the genetic disorder RDEB by the application of a replication defective herpes simplex virus-1 vector encoding the type VII collagen (C7) gene *COL7A1*, bercolagene telserpavec (BVEC), directly to skin via a phase 1-2 clinical trial. This trial was an intra-patient comparison of recurrent and chronic wounds administered with either topical BVEC or topical placebo applied every other day until healing. The first-in-human Phase I-2 protocol enrolled 12 patients (7 adult, 5 pediatric). Subjects were monitored for safety, wound closure, and molecular correction in a placebo controlled clinical trial. We report successful demonstration of a novel in vivo approach to RDEB gene correction. B-VEC was well-tolerated treated topically to wounds every other day and no BVEC-related safety events have been reported in all treated patients. Over the 12-week treatment period, BVEC-treated wounds showed a median reduction in wound area from baseline of approximately 91%, compared to a median reduction of approximately 1% for the placebo-treated wounds. Duration of closure for B-VEC administered wounds exceeded 6 months in many cases and was significantly longer than durability of closure of placebo treated wounds. Robust linear C7 NC1 and NC2 expression by IF and IEM and presence of anchoring fibrils at the basement membrane zone was shown in biopsies of the BVEC treated sites, demonstrating molecular correction. Overall, results from the Phase I/II clinical studies demonstrate a novel, safe and effective approach to RDEB molecular correction and in promotion of durable wound healing, which can be easily administered in basic clinic facilities worldwide. A multicenter Phase 3 trial is planned for 2020.

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Topical QR-313, an Antisense Oligonucleotide, in the Treatment of Dystrophic Epidermolysis Bullosa

M. P. Marinkovich[1], K. Sridhar[1], V. Karkala[1], V. K. Yenamandra[1], I. Gurevitch[1], J. Dolorito[1], I. S. Bagci[1], C. O'Mara[2], D. Ramsdell[2], H. Landy[2]
[1]Dermatology, Stanford University, Redwood City, California, United States, [2]Wings Therapeutics, Berkeley, California, United States

Dystrophic epidermolysis bullosa is caused by COL7A1 mutations encoding collagen VII (C7), comprised of 118 exons. In-frame exon 73 accounts for 9% of recessive (RDEB) and 55% of dominant (DDEB) cases. QR-313 is a 21-mer modified 2'O-methyl ribose phosphorothioate single strand antisense oligonucleotide which binds exon 73 and prevents its incorporation into mature mRNA. *In vitro*, QR-313 shows tissue uptake by fluorescent *in situ* hybridization (FISH) and generation of mRNAs lacking exon 73 by ddPCR. The shortened, exon-skipped protein effectively binds laminin-332 and collagen IV. Toxicology studies in mice and minipigs showed typical but reversible class effects in kidney and liver. We administered QR-313 1 mg/cm^2 qod or vehicle for 8 weeks in a blinded fashion to 2 wounds in two patients (female, 13 years; male, 12 years) with RDEB due to mutations in exon 73 in at least one allele. There were no adverse events, no perturbations in renal or liver function or in coagulation studies. and both patients completed the study. Pharmacokinetics showed minimal systemic exposure. The wounds treated with QR-313 both showed a small amount of tissue uptake by FISH analysis and modest evidence of exon skipping in one of the patients on biopsies obtained at Week 2 or 4. Biopsies at Week 8 showed no difference between QR-313 and vehicle-treated wounds in C7 by indirect immunofluorescence staining or in anchoring fibrils by transmission electron microscopy, consistent with the limited tissue uptake of study drug. Both wounds in both patients waxed and waned in size during the study. In conclusion, QR-313 was safe and well-tolerated. Low tissue uptake at the study dose was likely responsible for the limited exon skipping observed and the lack of demonstrable C7 and anchoring fibrils on biopsy. Future studies will explore higher and/or more frequent dosing.

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Safety and clinical effects of systemic allogeneic UCB-MSCs therapy for patients with RDEB
S. Lee[1], K. Kim[2], B. Cho[2], K. Roh[3], S. Kim[1], S. Lee[1]
[1]Dermatology, Yonsei University College of Medicine, Seoul, Korea (the Republic of), [2]Cellular Therapeutics Team, Daewoong Pharmaceutical, Co., Ltd, Yongin, Korea (the Republic of), [3]Department of Clinical Development, Kangstem Biotech Co., Ltd., Seoul, Korea (the Republic of)
Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a severe genodermatosis caused by mutations in COL7A1 and characterized by mucocutaneous blistering after minor trauma. Bone marrow–mesenchymal stromal cells (MSCs) have shown therapeutic potential for RDEB patients. Recent preclinical study demonstrated that a systemic infusion of human umbilical cord blood (UCB)-derived nonhematopoietic stem cells correct RDEB murine model. In this study, we wanted to determine the safety and possible clinical efficacy of systemic allogeneic UCB-MSCs therapy for RDEB patients. Six Korean RDEB patients (4 adults and 2 children) were included in this clinical trial. Each participant received three intravenous infusions of allogeneic UCB-MSCs (1–3× 10^6 cells/kg) with no HLA matching. Change in mean disease severity measured by Birmingham Epidermolysis Bullosa Severity Score (BEBSS) was -16 point at 60 days. Mean BEBSS total body surface area (%) was significantly reduced (-15 point) from baseline to day 60. Blister count and blister area/body surface area (%) were reduced by 50% at day 60 compared to baseline. Pain and pruritus score (VAS) were also reduced by 43% and 13% at day 60 compared to baseline. We also found the increased number of c-kit+ mast cells and CD68+ macrophages in the patient's skin at baseline, but the number of both cells were markedly reduced at day 60. No significant increase in C7 deposition was observed at day 60. There were no severe adverse events during day 180. The results suggest that administration of allogeneic UCB-MSCs in patients with RDEB is safe and provide indications of possible clinical benefits, to be confirmed in further clinical trials.

Innate Immunity, Microbiology, and Microbiome

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Dissemination of cutaneous staphylococcus aureus infection is limited by early neutrophil recruitment regulated by ECRG4
R. A. Dorschner[1], A. Baird[2], B. Eliceiri[2]
[1]Dermatology, University of California, San Diego, San Diego, California, United States, [2]Surgery, University of California, San Diego, La Jolla, California, United States
Neutrophils are a critical component of the innate response to cutaneous infection, limiting the dissemination of microbes through the delivery of antimicrobial molecules and cytokines that mediate the inflammatory response. Esophageal Cancer Related Gene 4 (ECRG4) encodes a cell surface protein that is highly expressed on circulating leukocytes in humans and mice and has been shown to amplify early neutrophil recruitment to cutaneous injury. Given the importance of a rapid innate response for limiting cutaneous infection, we hypothesized that ECRG4 may be important for preventing bacterial dissemination through its ability to regulate early neutrophil recruitment. Using an intradermal Methicillin Resistant Staphylococcus Aureus (MRSA) infection model, we found that ECRG4 KO mice developed lesions that were over 2 times larger and persisted twice as long as WT controls ($P<0.001$). Quantification of bacteria per gram of tissue at 24 hours after infection demonstrated no difference between WT and KO mice, but KO mice had 100 times more bacteria recovered from the spleen, suggesting increased dissemination. There was no significant difference in leukocyte numbers or the antimicrobial capacity of whole blood from ECRG4 KO mice. Evaluation of gene expression identified 10-fold higher IL-1β expression in infected skin from KO mice ($P<0.01$), which is important for staph abscess formation. Immunophenotyping of the 24-hour infiltrate demonstrated that the ECRG4 KO mouse had a ~3-fold decrease in the number of neutrophils ($P<0.05$). This suggests that local production of chemotactic signals is increased in the ECRG4 KO mouse lesion, yet leukocyte recruitment is impaired and bacteria are able to disseminate. These results support the hypothesis that ECRG4 amplifies neutrophil recruitment to cutaneous infection and limits bacterial dissemination, which positions it as a therapeutic target for antimicrobial therapies addressing the growing threat of antibiotic resistance.

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Activation of AhR in Langerhans cells by a microbial metabolite of tryptophan maintains skin homeostasis
L. Xiaochun[1], X. Zhang[2], Y. Luo[1], B. Xu[1], S. Ling[1], Y. Zhang[1], W. Li[3], X. Yao[1]
[1]Department of Allergy and Rheumatology, Institute of Dermatology, Chinese Academy of Medical Sciences, Peking Union Medical College, Nanjing, China, [2]The First Medical Center, Chinese PLA General Hospital, Beijing, China, [3]Department of Dermatology, Huashan Hospital, Fudan University, Shanghai, China
Skin commensal bacteria play important roles in maintenance of skin homeostasis, and Langerhans cells (LCs) are critical in induction of peripheral tolerance; however, the role of LCs' response to the skin microbiota, specifically the microbial metabolites, in skin homeostasis is not clear. In the present study, LCs that derived from $CD34^+$ hematopoietic stem cells in the cord blood cells were utilized, and we found that a microbial metabolite of tryptophan (Trp), indole-3-aldehyde (IAId), induced the production of indoleamine 2,3-dioxygenase and IL-10 in LCs through activation of aryl hydrocarbon receptor (AhR). Moreover, IAId promoted the expression of receptor activator of NF-κB and receptor activator of NF-κB ligand on LCs and keratinocytes in an AhR-dependent manner respectively, which might result in the activation of NF-κB signaling and production of IL-10. Additionally, despite a mature phenotype of LCs induced by IAId, IAId-activated LCs inhibited $CD4^+$ T cell proliferation and induced IL-10 secretion. Our study revealed a negatively regulatory function of a Trp microbial metabolite on LCs through activation of AhR, and microbial metabolites could be developed as a new strategy for the treatment of inflammatory skin diseases.

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An oral decaherb formula suppressed Th2 inflammation and improved gut microbiota profile with 16S rRNA sequencing in mice model with atopic dermatitis
S. K. Loo[1], H. Li[1], Y. Xian[1], J. C. Tsui[2], S. K. Tusi[2], Z. Lin[1]
[1]School of Chinese Medicine, The Chinese University of Hong Kong, Hong Kong, China, [2]School of Biomedical Science, The Chinese University of Hong Kong, Hong Kong, China
Recent studies highlighted the T helper (Th)-2 and systemic nature of atopic dermatitis (AD). In addition, gut microbiota is found to be important in the immune development and regulation in AD. Despite the recent biologics evolution, there are still huge unmet needs in the systemic therapies. Oral herbal medicine has been used in the management of AD for centuries in Asia. An oral decaherb (ten herbs) formula is commonly used as an integrative treatment for AD patients in our center. However, its mechanism of action is unknown. We aimed to investigate its mechanism of action and effect on the gut microbiota in mice model with AD. 2,4-dinitrochlorobenzene (DNCB) was applied over the shaved back of mice to induce atopic dermatitis like skin inflammation. A total of 60 female BALB/c mice were randomly divided into positive/negative control groups and low/mid/high dose decaherb groups. Blood and skin biopsy samples were collected on Day 21. Fecal bacterial DNA were sequenced with V4-V5 region of 16S rRNA gene using MiSeq platform. Raw sequence data were analyzed using QIIME2. Histopathological analysis of the lesional skin revealed a decrease in epidermal thickening, eosinophil and mast cell infiltration in dermis in mid/high dose decaherb groups. Serum IgE and CCL17 were lowered in mid/high dose decaherb groups. Level of mRNA expression of Th2 cytokines including interleukin(IL)-4, IL-13, CCL-17 and IL-31 were reduced in the lesional skin of mid/high decaherb groups. Relative abundance of anti-inflammatory short-chain fatty acids (SCFAs) producing bacteria (*Ruminococcaceae, Coprococcus and Bifidobacterium)* were increased in mid/high dose decaherb groups. This study suggested that oral decaherb formula suppressed the Th2 inflammation and improved the gut microbiota profile in mice model with AD. Further clinical trial is needed to establish and optimize its therapeutic benefit in our AD patients.

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Dilute vinegar baths inhibit beneficial commensal skin microorganisms

J. Y. Cheng, R. L. Gallo, T. R. Hata

Dermatology, University of California, San Diego, San Diego, California, United States

Atopic dermatitis patients sometimes use dilute bleach and vinegar baths as adjunct therapies in atopic dermatitis, despite lack of high-quality clinical evidence for the benefit of these bath additives. Recent publications have shown that dilute bleach baths and dilute vinegar baths are not directly antimicrobial against *S. aureus* (SA) in-vitro. To better understand the relative effects of dilute bleach or vinegar baths on SA and the commensal microbiome, strains of SA or five commensal Staphylococcal species in stationary phase were bathed in the clinically recommended 0.005% concentration of commercially available 6% bleach (one-half cup 6% bleach in a 40-gallon bathtub of water) and in the popularly recommended 0.04% concentration of food-grade apple cider vinegar (one cup in a 40-gallon bathtub of water) for ten minutes. Effect on bacterial survival was assessed via overnight growth in nutrient-rich media after the ten-minute bath exposure. At the 0.005% bleach bath concentration, overnight growth compared to negative control occurred for SA ($p<0.001$), *S. capitis* ($p<0.01$), *S. epidermidis* ($p<0.001$), *S. hominis* ($p<0.001$), *S. lugdunensis* ($p<0.001$), and *S. warneri* ($p>0.05$). At the 0.04% concentration of apple cider vinegar, overnight growth of SA compared to negative control occurred after dilute vinegar bath ($p<0.001$), as did overnight growth of *S. capitis* ($p<0.01$) and *S. lugdunensis* ($p<0.001$). Overnight growth compared to water control of antimicrobial peptide-producing commensal species *S. epidermidis* ($p<0.05$), *S. hominis* ($p<0.001$), *and S. warneri* ($p<0.05$) did not occur after dilute vinegar bath, suggesting that these species are sensitive to growth inhibition by a ten-minute dilute vinegar bath. Taken together, this work confirms that the addition of clinically recommended dilutions of bleach or vinegar do not have antimicrobial effect on SA. However, dilute vinegar baths did inhibit some beneficial commensal microorganisms. This may have negative long-term clinical consequences.

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Lipid accumulation in epidermal Langerhans cells correlate with aberrant immunofunctions in psoriasis-like inflammation

X. Zhang[1], X. Li[2], Y. Ding[1], Y. Shi[1], J. Gu[3]

[1]Dermatology, Shanghai Skin Disease Hospital, Shanghai, China, [2]Dermatology, Longhua Hospital, Shanghai, China, [3]Shanghai Tenth People's Hospital, Shanghai, China

Psoriasis is a chronic, inflammatory, systemic disease characterized by abnormal lipid metabolism. Dendritic cells (DCs), an essential mediator in the pathogenesis of psoriasis, are recently discovered to be functionally regulated by intracellular lipid content. Being the sole DC subset within the epidermis, Langerhans cells (LCs) are potent regulators of immune surveillance and tolerance. Previous studies indicate a varying role of LCs in psoriasis with underpinning mechanism remains elusive. Here, we demonstrated aberrant maturation, enhanced phagocytosis and interleukin-23 (IL-23) over-secretion of LCs in imiquimod (IMQ)-induced psoriasis-like mouse skin. Remarkably, disease-associated LCs possessed an elevated level of neutral lipid content probably due to impaired autophagy of lipids other than altered lipid engulfment. Moreover, the inhibition of fatty acids synthesis, which resulted in a decrease in cellular neutral lipids, would significantly impair LC maturation and their production of IL-23. Further LC-MS analysis revealed a group of triglycerides (TG), diglycerides (DG), phosphatidylethanols (PEt) and phosphatidylcholines (PC) contributed to the increased neutral lipid level of LCs within IMQ-induced psoriasis-like skin. In accordance, low-input mRNA sequencing analysis uncovered the expressions of multiple genes involved in lipid metabolism, autophagy along with immunofunctions were correlatively dysregulated in murine disease-associated LCs. Single-cell RNA sequencing of psoriasis patients' LCs demonstrated an immune activation status of lesional LCs, which confirmed their pro-inflammatory role in the pathogenesis of psoriasis. Notably, hyperlipidemia does not directly influence LC homeostasis and immunofunction. In summary, lipid accumulation in epidermal Langerhans cells correlate with their aberrant immunofunctions in psoriasis-like inflammation.

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Dupilumab effects on the circulating ILC2 population and ILC2/3 repertoire in patients with atopic dermatitis

Y. Imai[1], N. Nagai[1], K. Yasuda[2], M. Kusakabe[1], Y. Inoue[1], M. Natsuaki[1], K. Yamanishi[1]

[1]Dermatology, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan, [2]Immunology, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan

Group 2 innate lymphoid cells (ILC2s) are thought to contribute to the pathogenesis of atopic dermatitis (AD). Previously, we reported that IL-4 stimulates ILC2s to proliferate and produce type-2 cytokines in mice overexpressing IL-33 (Imai Y, *JID*, 2019). Dupilumab, an antibody against the IL-4 receptor, is widely used in AD therapy. We speculated that its efficacy in AD treatment might involve blocking the proliferation and activation of ILC2s via IL-4. In this study, we examined the proportion and number of circulating ILC2s ($Lin^- CD127^+ CRTH2^+$) and ILC3s ($Lin^- CD127^+ CRTH2^- c\text{-}kit^+$) in 15 Japanese patients with AD before and after administration of dupilumab. Before treatment, the number of circulating ILC2s was significantly correlated with the number of eosinophils ($r = 0.62$, $P < 0.05$). Between 0 and 16 weeks after administration of dupilumab, percentage of ILC2s (among all ILCs) and the number of circulating ILC2s decreased from 29.9±20.3% to 21.1±15.0% (mean ± standard deviation; $p<0.001$) and from 2829±3935 ILC2s/mL to 1780±2024 ILC2s/mL ($p<0.05$), respectively. Conversely, in the same time frame, the proportion and number of circulating ILC3s were increased from 18.4±12.8% to 30.1±14.7% ($p<0.001$) and from 1141±868 ILC2s/mL to 1900±1223 ILC2s/mL ($p<0.001$), respectively. These results suggest that dupilumab might suppress the circulating ILC2 population and alter the ILC2/3 repertoire through inhibition of IL-4/13 signaling in AD patients.

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Short-term exposure to Western diet (WD) predisposes mice to psoriasis-like skin and joint inflammation

Z. Shi[1], Y. Wan[2], S. Hwang[1]

[1]Dermatology, UC Davis, Sacramento, California, United States, [2]Medical Pathology and Laboratory Medicine, UC Davis, Sacramento, California, United States

We have previously shown that long-term feeding (>3 month) with a high sugar, moderate fat diet (i.e., Western diet, WD) enhances susceptibility of mice to imiquimod-induced psoriasiform dermatitis (PsD), suggesting that specific components of diet increase susceptibility to PsD. Herein, we determined that short-term feeding (<1 month) with WD alone was sufficient to induce clinically and molecularly-detectable PsD. After weaning, C57BL/6 mice were fed a WD or an otherwise nutritionally matched, control diet (CD) for 1 month. WD-fed mice developed a subtle dermatitis that was characterized by skin edema, redness and mild scaling. Furthermore, we observed epidermal hyperplasia, parakeratosis, accumulation of IL-17A-producing γδ-low T cells, and elevated gene expression of characteristic Th17 cytokines (e.g. IL-17A by 300-fold) and Th1 cytokines. Notably, WD-induced skin inflammation was blocked by gut microbiota depletion with antibiotic treatment. Although no clinically visible inflammation was observed, WD-fed mice also exhibited higher levels of Th17 cytokines in joint tissue. In addition, a IL-23 minicircle DNA (IL-23 MC)-based murine model with features of PsD and psoriatic arthritis (PsA) was used to determine whether the WD exacerbated IL-23-mediated skin and joint inflammation. IL-23-MC-treated WD-fed mice had markedly enhanced skin inflammation versus CD-fed mice, as measured by increased ear thickness, epidermal thickness and mRNA levels of Th17 cytokines. Strikingly, joint inflammation was also exacerbated in WD-fed mice as evidenced by higher incidence of dactylitis and increased mRNA levels of cytokines such as TNF-α, IL-1β and Th17 cytokines (≥10-fold increase) in paw tissue. Furthermore, switching from a WD to CD after IL-23 MC delivery remarkably improved skin and joint inflammation. Taken together, diet influences inflammatory signaling in the skin and joint, supporting a critical role of dietary component in the pathogenesis of PsD and PsA.

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The sweat gland antimicrobial peptide dermcidin is downregulated in hidradenitis suppurativa and non-healing skin wounds but upregulated in healing-wounds

P. Rosa Coutinho Goulart Borges Mariottoni[1], M. Coates[1], D. Corcoran[2], H. F. Kirshner[2], T. Jaleel[1], D. A. Brown[3], A. S. MacLeod[1]

[1]Dermatology, Duke, Durham, North Carolina, United States, [2]Center for Genomic and Computational Biology, Duke, Durham, North Carolina, United States, [3]Plastic Surgery, Duke, Durham, North Carolina, United States

Hidradenitis suppurativa (HS) presents with recurrent abscesses and sinus tracts leading to chronic non-healing wounds (NHW) in areas rich in hair follicles and sweat glands (SG). Given this clinical presentation, we used biocomputational approaches to study transcriptional similarities between HS and healing and non-healing wounds. We analyzed microarray gene expression of patient-matched HS lesional and non-lesional skin samples (Blok *et al.* 2016), defined differentially expressed genes (DEGs) in HS lesional vs. non-lesional and compared these to DEGs from punch-biopsy skin of healing wounds vs. normal skin (Iglesias-Bartolome *et al.* 2018) and to DEGs from diabetic foot ulcer (DFU) vs. diabetic foot skin (Ramirez *et al.* 2018). Several innate antimicrobial peptides and proteins and interferon (IFN)-stimulated antiviral genes were significantly upregulated in HS lesional and healing wounds. In contrast, the SG antimicrobial dermcidin (DCD) was among the most down-regulated genes in HS lesional (log_2FC=-4.90, p<0.001) and DFU (log_2FC=-5.16, p<0.001) but was up-regulated in healing wounds. Genes associated with SG function, such as secretoglobins, also showed statistically significant decreased expression in HS lesional skin and non-healing DFU, but increased expression in healing wounds. By calculating the Pearson correlation between DCD and all other genes in the HS dataset, we identified that type I IFN related genes were negatively correlated with DCD, such as STAT1 (correlation -0.88), IRF1(-0.83) and IFNAR2(-0.74). In agreement, we found that IFN-b(100U/ml), known to signal via STAT1, suppresses significantly DCD mRNA in human SG cells (p<0.001). Our finding that SG associated DCD was decreased in HS lesional and non-healing DFU, but not in healing wounded skin, suggests that impaired SG function and reduced DCD may play a key role in wound repair.

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Resident neuropeptide PACAP mediates potent cell-free infection defense in tissues

E. Y. Lee[1], L. C. Chan[2], H. Wang[2], J. Lieng[1], M. Hung[1], Y. Srinivasan[1], J. Wang[1], J. Waschek[3], A. L. Ferguson[4], K. Lee[5], N. Y. Yount[2], M. R. Yeaman[2], G. C. Wong[1]

[1]Dept. of Bioengineering, UCLA, Los Angeles, California, United States, [2]Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California, United States, [3]Semel Institute for Neuroscience and Human Behavior, UCLA, Los Angeles, California, United States, [4]Pritzker School of Molecular Engineering, University of Chicago, Chicago, Illinois, United States, [5]Salk Institute for Biological Studies, La Jolla, California, United States

The central nervous system (CNS) must defend itself against bacterial and fungal infection while also minimizing inflammatory infiltration and maintaining normal cognitive function. Since the CNS is an immune privileged site, inducible innate immune defense mechanisms endogenous to the CNS likely play a key role in this regard. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide that regulates neurodevelopment, emotion, and stress responses via G-protein coupled receptors. While PACAP is known to interact with the immune system, its significance in host defense in the brain or in other tissues like the skin and the kidney is not known. Here, we use a machine learning classifier to identify PACAP as a tissue resident neuropeptide optimized for “clean” CNS defense against pathogens that traditionally require clearance by Th17 neutrophil-based responses. Synchrotron x-ray scattering, antimicrobial assays, and mechanistic fingerprinting enable us to profile precisely how PACAP exerts bactericidal activity against drug-resistant bacteria via multiple synergistic mechanisms including membrane permeabilization, disruption of cellular energetics, and activation of cell death pathways. Most importantly, we find that PACAP is strongly and selectively induced up to 50-fold in the brain and other tissues in mouse models of *S. aureus* and *Candida*infections. Interestingly, PACAP is also expressed by cutaneous peripheral neurons and likely plays a role in host defense of the skin. We are currently exploring how PACAP modulates the skin microbiome and cutaneous inflammation.

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Cutaneous group 2 innate lymphoid cells migrate to draining lymph nodes in mice with IL-33-induced atopic dermatitis-like inflammation

M. Kusakabe[1], Y. Imai[1], K. Yasuda[3], K. Nakanishi[3], E. Kuroda[3], M. Tomura[2], K. Yamanishi[1]

[1]Dermatology, Hyogo College of Medicine, Nishinomiya, Japan, [2]Laboratory of Immunology, Faculty of Pharmacy, Osaka Ohtani University, Osaka City, Japan, [3]Immunology, Hyogo College of Medicine, Nishinomiya, Japan

We previously generated a transgenic mouse line expressing IL-33 driven by a keratin 14 promoter (IL33Tg) and showed that IL-33 elicits an atopic dermatitis (AD)-like itchy skin inflammation associated with group 2 innate lymphoid cell (ILC2) infiltration. ILC2s are believed to be tissue-resident cells under steady-state conditions, but the dynamics of ILC2 migration are not fully understood. In this study, we focused on the migration of ILC2s from skin to draining lymph nodes (dLN) in IL33Tg mice. We tracked ILC2 migration by crossing IL33Tg mice with Kikume Green-Red (KikGR) knock-in mice to generate KikGR IL33Tg mice. KikGR, a photoconvertible fluorescent protein, changes color from Kik-Green to Kik-Red upon exposure to violet light. Exposing the inflamed skin of KikGR IL33Tg mice to violet light allowed us to label ILC2s in the skin and track ILC2 migration from the skin to the dLN. The skin-derived Kik-Red ^{pos} ILC2s were an ST2 ^{low} and major histocompatibility complex (MHC) class II ^{high} population in the dLN ILC2s. ILC2s can also boost Th2 cell responses to antigens in an MHC class II-dependent manner; therefore, these results suggest that ILC2s are important for innate immunity as well as for the induction of adaptive immune responses.

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Tetrahydrocurcumin ameliorates skin inflammation and oxidative stress and induces autophagy in mice fed a high-fat diet

J. Kim[1], H. Kim[2], B. Chung[2], J. Hong[1], E. Chung[1], J. Lee[1], S. Lee[1]

[1]Dermatology, Soonchunhyang University Cheonan Hospital, Soonchunhyang University College of Medicine, Cheonan, Korea (the Republic of), [2]Dermatology, Kangnam Sacred Heart Hospital, College of Medicine, Hallym University, Seoul, Seoul, Korea (the Republic of)

Background: Curcumin has been known to have antioxidant and anti-inflammatory properties. Tetrahydrocurcumin (THC) is one of the major metabolites of curcumin. However, it is unclear whether administration of THC has beneficial effects on skin. In this study, we investigated skin inflammation, oxidative stress, and autophagy to assess beneficial effects of THC on cutaneous changes induced by high-fat diet in mice. Methods: Eight-week old C57BL/6J mice were divided into four groups and fed regular diet (RD), high fat diet (HFD, 60% of total calories from fat), HFD supplemented with THC 50mg/kg/day, and HFD supplemented with THC 100mg/kg/day orally for 12 weeks. Body weights, plasma glucose and lipid profiles were measured during the experimental periods. At the end of experiment, the skin samples of the mice were obtained. Western blot, real-time PCR and immunofluorescence analyses for inflammatory cytokines, oxidative stress markers, and autophagy markers were conducted. Results: Administration of THC decreased the expressions of inflammatory cytokines, such as tumor necrosis factor-alpha, interleukin (IL)-1 beta, and IL-6. Furthermore, THC significantly decreased NADPH oxidase 2 (NOX2) and NOX4 levels and activated the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. Also, administration of THC enhanced autophagy markers including LC 3-II, ATG5 and beclin-1. Also, immunofluorescence stainings with autophagy markers, LC 3-II showed that THC could induce autophagy. Conclusion: These findings reveal that the anti-inflammatory and antioxidant potential of THC in mice fed a high-fat diet through promoting autophagy in the skin. THC could be developed as a potential therapeutic agent against inflammatory skin diseases.

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Staphylococcus epidermidis protease EcpA is a deleterious component of the skin microbiome in atopic dermatitis

L. Cau[1, 2], M. Williams[1], A. Butcher[1], T. Nakatsuji[1], J. Cheng[1], T. Hata[1], J. Kavanaugh[3], C. Mainzer[2], B. Closs[2], A. Horswill[3], R. L. Gallo[1]

[1]Dermatology Department, University of California, San Diego, California, United States, [2]R&D Department, SILAB, Brive, France, [3]Immunology and Microbiology Department, University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States

S. aureus (SA) and *S. epidermidis* (SE) are 80% genetically related and the two most abundant bacterial species found in atopic dermatitis (AD). Although both species are often over-abundant, SA is considered to exacerbate AD while SE is thought to be a beneficial commensal. In this study, we hypothesized that the overabundance of SE might damage the epidermis by a proteolytic mechanism similar to SA. To test this, multiple strains of SE and other Coagulase-negative *Staphylococci* (CoNS) were screened for enzymatic activity. Several SE strains, but not other CoNS, had strong proteolytic activity. The enzyme responsible for activity was identified to be the cysteine protease EcpA by selective inhibitors and targeted gene deletion (ΔEcpA). Application of the WT strain to mouse skin disrupted the skin barrier (TEWL : 48.7 vs 13.2 g/h/m2, p<0.001) and induced inflammation (erythema; immune cell infiltration; IL6 mRNA: 40.1 vs 1.1, p<0.01) while the ΔEcpA strain had no effect. Application of purified EcpA alone also disrupted the epidermal barrier and induced inflammation (TEWL: 40.9 vs 8.8 g/h/m2, p<0.05; IL6 mRNA: 13.5 vs 1.13, p<0.05). Western blots showed EcpA degraded desmoglein-1 but not involucrin or corneodesmosin from human keratinocytes. EcpA also inactivated the antimicrobial activity of LL-37 against SA. qPCR analysis of *EcpA* mRNA on the skin of 14 healthy and 13 AD human subjects showed that EcpA expression was increased on subjects with AD and correlated with disease severity (p<0.03). EcpA expression is controlled by agr quorum sensing and could be inhibited by concomitant application of a *S. hominis* strain producing an SE inhibitory autoinducing peptide. These data change the current paradigm regarding the functions of microbes in the AD microbiome and show that SE can damage skin through the expression of EcpA.

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Automated analysis of nail clippings for onychomycosis using convolutional neural networks: A pilot study

J. Weed[1], A. Hatzimemos[2, 1], S. A. Meehan[1], N. K. Brinster[1], R. H. Kim[1]

[1]Ronald O. Perelman Department of Dermatology, NYU Langone Health, New York, New York, United States, [2]Harvard College, Cambridge, Massachusetts, United States

Nail clipping followed by periodic acid–Schiff with diastase (PAS-D) staining has become the gold standard for diagnosis of onychomycosis, with relative ease of performance and high sensitivity. However studies have suggested this method may be less cost effective than in-office alternatives due to the expense incurred by staining and pathologist interpretation. We trained a convolutional neural network (CNN) to classify digitized slides of nail clippings as positive or negative for onychomycosis. Specimens stained with both hematoxylin and eosin (H&E, n=134) and PAS-D (n=135) were digitized at 40X magnification and subsequently divided into training, validation, and test cohorts. Image preprocessing included tiling each composite image into 299 x 299 pixel patches and labeling non-empty patches as positive or negative based on prior diagnosis. Training was performed with the Inception-v3 CNN architecture with separate models developed for H&E- or PAS-D-stained slides. Initial classification of each stained specimen was obtained by averaging tile scores. For H&E-stained slides, using average tile score on test set specimens, the area under the curve (AUC) for the receiver operating characteristic curve was 0.86 (95% confidence interval [CI]: 0.74-0.96). For the model trained on PAS-D-stained slides, the analogous AUC was 0.88 (95% CI: 0.77-0.97). These results support the potential role of CNNs to automate diagnosis of onychomycosis, even on H&E-stained slides. Further studies to classify all imaged tissue per specimen rather than subcomponent tiles and to compare CNN performance to dermatopathologists are underway.

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Cathelicidin LL-37 facilitates damage-associated molecular patterns to be bioactive substances by intermediating between scavenger receptors

Y. Hagiya, R. Amagai, T. Takahashi, T. Fujimura, K. Yamasaki, S. Aiba

Dermatology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan

Damage-associated molecular patterns (DAMPs) are group of bioactive molecules, which are generated by damaged host cells following injury or infection. DAMPs provoke intracellular signaling leading to chemokines and cytokines production. However, the fundamental mechanism how host cells recognize DAMPs on cellular surface has not been fully elucidated. Human cathelicidin LL-37, a biphasic and cationic peptide, is an essential component of innate immune responses and acts as an alarmin. Inappropriate expression of cathelicidin is observed in inflammatory skin diseases such as psoriasis and rosacea, and cathelicidin provokes and exacerbates innate immune type skin inflammation in these dermatoses. Since increase in DAMPs expression is also observed in these dermatoses, we hypothesized that cathelicidin LL-37 intermediates DAMPs recognition on cellular surface. We incubated normal human epidermal keratinocyte (NHEKs) or human leukemia monocytic cell line THP-1 with synthetic dsRNA, dsDNA, High Mobility Group Box 1 (HMGB1), or Toll-like receptor 7 agonist imiquimod, and examined cytokine production. These molecules moderately increased interleukin (IL)-6 production from both NHEK and THP-1, and co-incubation with LL-37 significantly augmented the IL-6 production. The LL-37-dependent cytokine increase were abolished by pretreatment of the scavenger receptor inhibitor fucoidan. Although the cationic lipid lipofectamine also enhanced IL-6 production by dsRNA, fucoidan did not suppress lipofectamine-dependent cytokine increase. Since LL-37 is a biphasic and cationic peptide, these data suggested that LL-37 selectively interacts with scavenger receptors and intermediates between DAMPs and scavenger receptors to facilitate DAMPs signaling in epithelial cells and mononuclear cells. These data imply us that the blockage of the interaction among LL-37, DAMPs and scavenger receptors can be novel therapeutic targets of innate type skin inflammation.

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Pediatric microbiome in alopecia areata

L. Castelo-Soccio[1, 2], L. Catanoso[2], S. Rangu[2], J. Lee[3]

[1]Pediatrics- pediatric dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Pediatric Dermatology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States, [3]Microbiome Analytic Core, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States

A cross sectional study of 41 children (11 males and 30 females) ages 4-17 years (22% 4-7 years, 27% 8-11 years, 34% 12-15, 17% 16-17 years) with alopecia areata (AA) and 41 of their sibling ages 4-17 years without AA was conducted. Fecal microbiome was analyzed from stool DNA using shotgun metagenomics. Children were excluded if antibiotics were taken in last 6 months. AA severity ranged from mild to severe (51% had severity of alopecia tool scores in 0-25% range (mild), 12% between 26-49%, 36% between 75-100% (severe). Most common co-morbidity among AA subjects was atopic disease. Diet was predominantly Western/meat eaters (83%) but a small portion were vegan (2%), vegetarian (2%), gluten free (5%) or dairy free (7%). Alpha Diversity was assessed by richness and Shannon index and did not show differences between subjects with AA and their sibling controls. Beta diversity which looks at similarity between samples was assessed by Bray-Curtis and Jaccard distances and showed statistical differences. Based on Jaccard distances, taxa were searched using McNemar's test for differentially present or absent taxa between the study group and control subjects. 105 taxa showed statistical difference. These included families of *Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes and Proteobacteria*. Taxa were clustered by similarity and by p-values. When clustering by similarity a few specific bacteria were noted to be highly represented in subjects with alopecia including types of *Firmicutes* and *Proteobacteria.* Many more taxa were noted to be highly expressed in sibling controls. When taxa were sorted by P-values the differences showed more enrichment of bacteria in the sibling control group. Differences noted show that pediatric AA is a systemic disease that has effects on hair but also leads to internal changes. These differences may have impact as a diagnostic tool or as a target for therapy. To create either, larger studies will be needed.

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Genetic susceptibility to *Talaromyces marneffei* infection

C. Cao

The Department of Dermatology and Venereology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China

The dimorphic fungus *Talaromyces marneffei* is an emerging fungal pathogen which cause a life-threatening opportunistic infections, mostly in Southeast Asia and South China. *T. marneffei* characterized by its ability to escape immune killing, survive and replicate inside macrophages, then spread the whole body. Commonly, *T. marneffei* infections occur in patients infected with human immunodeficiency virus (HIV), but this infection can occur in HIV-negative individuals in the absence of immunosuppression. However, the host factors underlying susceptibility to this infection are unknown, but a potential immunodeficiency has been suspected. In the lecture, we comprehensively analyse the host genetic suspectibility to *Talaromyces marneffei* infection in HIV-negative patients. Our study provides evidence that anti-interferon gamma autoantibodies act as novel host factors accounting for the susceptibility to *T. marneffeii* in HIV-negative adult patients. The strong association between anti-IFN-γ autoantibodies and HLA-DRB1*16:02/DQB1*05:02, provides a possible pathogenic role of this HLA haploid type for anti-IFN-γ autoantibody generation. On the other hand, primary immunodeficiency is the main cause of TM infection in non-HIV-infected children, severe infections are associated with monogenic primary immunodefificiencies such as defects in *CD40L* or *CARD9*, gain-of-function mutations of *STAT1/ STAT3*, which recently were discovered as novel clinical entities associated with fungal infection.Our study indicated that anti–IFN-γ autoantibodies should be tested in adult non-HIV talaromycosis patient and exon sequencing should be carried out in non-HIV pediatric as early as possible, which can lead to a better understanding of the pathogenesis of the disease, as well as to the design of novel immunotherapeutic strategies.

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Impact of injury-induced inflammation on *ex vivo* skin culture as a preclinical model for atopic dermatitis

H. Zhou[1, 2], S. Engle[3], C. Chang[3], M. Daniels[3], A. J. Okragly[3], D. C. Gemperline[3], N. Perumal[3], R. M. Slominski[1], J. T. Sims[3], M. J. Turner[1, 2]

[1]Dermatology, Indiana University, Indianapolis, Indiana, United States, [2]Richard L. Roudebush VA Medical Center, Indianapolis, Indiana, United States, [3]Eli Lilly and Company, Indianapolis, Indiana, United States

Ex vivo skin culture holds promise as a preclinical model for understanding pathogenic mechanisms and testing therapeutics for skin diseases. This project studied the impact of *ex vivo* culture on the transcriptome profile and cytokine/chemokine production of nonlesional and lesional skin from adult atopic dermatitis (AD) patients (n=19) with moderate to severe disease. Punch biopsy (2mm) specimens were immediately processed for RNA (baseline) or cultured for 48 hours (37°C, 5% CO_2, DMEM-10% FBS), followed by RNA isolation and supernatant collection. Transcriptome analyses were performed with HTA2.0 arrays (Affymetrix), and supernatants were analyzed with multiplex immunoassays. Microarray data analysis (t-SNE) revealed cultured samples clustered separately from baseline samples, which suggested culturing the skin had a dominant effect on gene expression and obscured AD transcriptome profiles. Relative to baseline samples, cultured AD skin exhibited increased inflammatory cytokine and chemokine transcripts. Proteins encoded by these transcripts were also detected in culture supernatants. These data demonstrated AD skin culture activates an inflammatory response distinct from that seen *in vivo*. *Ex vivo* culture of healthy donor skin also activated this response, which was determined to be interleukin-1 alpha (IL-1α)-dependent. In *ex vivo* skin culture, this IL-1α-induced inflammatory response obscured the ability of selected recombinant cytokines to induce known target genes. In conclusion, IL-1α activates a defined inflammatory response during *ex vivo* skin culture, which can obscure the native (*in vivo*) transcriptome/secretome of skin specimens. These results suggest including IL-1α neutralization as part of *ex vivo* skin culture systems would create a better preclinical skin disease model for evaluating therapeutic compounds.

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Diet-induced obesity impairs the antimicrobial defense function of dermal adipocyte progenitors

L. Zhang[1, 2], C. Guerrero-Juarez[3], S. Chen[2], X. Zhang[1], M. Yin[1], F. Li[2], S. Wu[1], J. Cheng[2], Y. Liu[3], T. Hata[2], M. V. Plikus[3], R. L. Gallo[2]

[1]School of Pharmaceutical Sciences, Xiamen University, Xiamen, Fujian, China, [2]Department of Dermatology, University of California, San Diego, San Diego, California, United States, [3]NSF-Simons Center for Multiscale Cell Fate Research, University of California, Irvine, Irvine, California, United States

Obesity affects more than 20% of the world population and is a major risk factor for several skin disorders such as infection, delayed wound healing and psoriasis. Dermal white adipose tissue (DWAT) has been recognized as an important antimicrobial defense layer of the skin, but how obesity impacts this important element of skin innate immunity is unclear. Here, we used a diet-induced obesity (DIO) mouse model, single-cell RNA-seq analyses and adipocyte lineage-tracing to better understand how obesity impacts the innate immune functions of DWAT and dermal adipocyte precursors. Mice fed a 60% high-fat diet for 6-months had marked adipocyte hypertrophy compared to standard diet and lineage-tracing showed that >95% of adipocytes at 6 m of DIO were new adipocytes formed 1 week after start of HFD feeding. DIO mice also showed a drastic change in dermal fibroblasts (dFB) subclusters including depletion of 3 adipogenic progenitor populations. Loss of these populations was associated with a lack of pathogen-triggered reactive adipogenesis, impaired cathelicidin antimicrobial peptide production and increased susceptibility to *S. aureus* infection. Culture of primary dermal adipocyte progenitors with mature adipocytes promoted a loss of antimicrobial function in the precursor population. This negative feedback from mature adipocytes was due to secretion of TGFβ, and administration of a TGFBR inhibitor or a PPARγ agonist reversed this inhibition in cultured adipocyte progenitors and restored the capacity *in vitro and in vivo* to initiate reactive adipogenesis and to kill *S. aureus*. Together, these results suggest that dysfunction of dermal innate immune defense function may explain several disorders associated with obesity and highlights TGFβ and PPARγ as targets for intervention.

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Innate immune tolerance of the epidermis is mediated by epigenetic regulation of MAP2K3

Y. Sawada, T. Nakatsuji, T. Dokoshi, N. Kulkarni, J. Jones, G. Sen, M. Liggins, R. L. Gallo

Department of Dermatology, University of California, San Diego, La Jolla, California, United States

Appropriate immune function of the epidermis requires recognition of danger while also tolerating diverse environmental and microbial exposures. Previously, we observed tolerance of the skin to TLR2 and 3 ligands is inhibited by metabolic products of the microbiome that act to inhibit HDAC8 and HDAC9 in keratinocytes (*Science Immunol* 2016). In this study, we sought to gain a detailed mechanistic understanding of this newly recognized system of innate immune tolerance. We observed that the promoters of inflammatory cytokines suppressed by HDAC8/9 were not direct targets of these deacetylases, but RNA-seq analysis after siRNA silencing of HDAC8 or 9 identified MAP2K3 as a gene target that could indirectly control expression of multiple inflammatory cytokines. Western blot confirmed HDAC8 and 9 were regulators of MAP2K3 as chemical inhibition or gene silencing of HDAC8 or 9 increased MAP2K3 protein expression and increased phosphorylation of p38MAPK. Chip-qPCR showed direct binding of HDAC8 and 9 to the MAP2K3 gene (P value=0.008). H3K9 and H3K27 acetylation marks were also increased in the MAP2K3 promoter after HDAC8 or 9 silencing (P value=0.0162 and 0.0323). Immunoprecipitation and mass spec identified both HDAC8 and 9 were complexed with FACT proteins SSRP1 and SPT16H; genes that act in transcriptional elongation. Silencing of these FACT proteins in keratinocytes abolished the effect of HDAC8/9 inhibition to induce inflammatory cytokines. HDAC8/9 silencing in keratinocytes also resulted in IFN-β-dependent activation of dendritic cell function leading to increased T cell proliferation (P value=0.010). Finally, following UVB radiation, $HDAC8^{-/-}$ or $9^{-/-}$ mice showed a visible increase in skin inflammation and increased IFN-β mRNA (increase of 223.8% and 271.5%, respectively, P value=0.02). A similar increased inflammation occurred after imiquimod treatment of $HDAC8^{-/-}$ or $9^{-/-}$ mice (P value=0.04). Taken together, we provide a detailed understanding of a novel innate immune tolerance mechanism in the skin.

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Evolution of skin microbiome during puberty in individuals with acne
A. Nelson, A. Paine, B. Barros, Z. Cong, S. Gettle, A. Longenecker, Q. Pham, C. Straight
Dermatology, Penn State College of Medicine, Hershey, Pennsylvania, United States
The exact role of the skin microbiome in the development of acne is still unknown, but in addition to the well-known Cutibacterium spp., large populations of Staphylococcus spp. and Malassezia spp. are positively correlated with inflammatory acne. When microbial shifts occur during puberty or in relation to acne onset are still obscure. We performed a cross-sectional study with shotgun sequencing to identify the skin's surface microbiome as related to pubertal development, sebum output and presence of acne. Forty-eight individuals, males and females, ages 7-17, with or without acne were enrolled. Subjects with acne had their acne graded by a dermatologist using an IGA scale. The pubertal stage of all subjects was documented according to Tanner staging criteria. Both sebum output and collection of the skin microbiome were done on the forehead. Age, race, ethnicity, use of medications, and use of makeup or lotion on the forehead at the time of sampling was documented for each individual. Increased sebum output positively correlated with pubertal development (r_s = 0.901) in normal individuals, yet was not correlated in acne individuals. Globally, the skin microbiome was composed of 320 species comprising ~80% bacteria, ~15% virus and ~5% fungus in this young population. As expected, the relative abundance of *C. acnes* significantly increased with pubertal development, regardless of acne status ($p < 0.05$); however, *C. acnes* levels were not correlated with sebum output. Interestingly, individuals with acne in early puberty (Tanner Stage 1) had 3-fold more *C. acnes* and 20-fold more *Malassezia* compared with normal individuals at the same stage ($p < 0.01$). In contrast, the relative abundance of *Malassezia* significantly decreased ($p < 0.01$) and was negatively correlated with pubertal development (r_s = -0.53; $p < 0.01$). Understanding the microbiome composition as it changes throughout puberty in relation to the development of acne may identify novel targeted therapeutic interventions.

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Dynamic neutrophil and T cell TNF production protects against *S. aureus* skin infections
C. Youn, M. Alphonse, J. H. Rubens, D. Joyce, D. Dikeman, Y. Wang, R. Ortines, Q. Liu, M. Mazhar, N. Archer, L. S. Miller
Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States
Staphylococcus aureus (*S. aureus*) causes the majority of skin and soft tissue infections (SSTIs) with an annual burden of 14 million outpatient visits, 500,000 hospitalizations, and 4.2 billion dollars of inpatient costs in the U.S. alone. The widespread use of antibiotics to treat recurrent *S. aureus* has led to the emergence of methicillin-resistant *S. aureus* clinical isolates that have limited treatment options, creating a serious threat to public health. Better understanding of protective immunity to *S. aureus* SSTIs is imperative to develop effective therapies as an alternative to antibiotics. Tumor necrosis factor (TNF) is a proinflammatory cytokine that has been targeted to treat chronic inflammatory and autoimmune diseases. Interestingly, TNF inhibition decreases IL-17- and IFNγ-mediated bacterial host defense responses and results in an increased risk of *S. aureus s*kin infections, implicating TNF in anti-staphylococcal immunity. Therefore, we evaluated the host defense role of TNF with an intradermal *S. aureus* skin infection model in TNF-deficient ($TNF^{-/-}$) and wildtype (wt) mice, and discovered $TNF^{-/-}$ mice exhibited significantly increased lesion sizes and bacterial burdens compared to wt mice. Interestingly, neutrophils were the predominant source of TNF production early in the infection, whereas skin infiltrating CD4+ and γδ T cells increasingly contributed to TNF expression as well as IFNγ and IL-17 expression at later time points. Although CD4+ T cells constitutively expressed TNF and were the main source of IL-17 and IFNγ in the skin draining lymph nodes, there was a significantly higher number of TNF and IL-17 expressing γδ T cells than CD4+ T cells in the skin. Taken together, these findings suggest that TNF production has dynamic cellular and temporal components that regulate both innate and adaptive immunity against *S. aureus* skin infections.

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Age and circadian regulation of cutaneous innate antimicrobial immunity
V. Lei[1], J. Kwock[1], S. Kirchner[1, 3], X. Ling[2], A. S. MacLeod[1, 3, 4]
[1]Department of Dermatology, Duke University, Durham, North Carolina, United States, [2]Dermatology, Suzhou Ninth People's Hospital, Suzhou, China, [3]Department of Molecular Genetics and Microbiology, Duke University, Durham, North Carolina, United States, [4]Department of Immunology, Duke University, Durham, North Carolina, United States
Neonates and elderly are at increased risk for skin viral and bacterial infections that have the potential to cause systemic malaise and death. Notably, under healthy conditions, and predominantly previously studied in adult skin, a natural antimicrobial defense program protects the host against microbial infections. Interestingly, newborns and elderly have immature diurnal circadian rhythms. Unknown to us is whether age and/or circadian rhythm regulate innate antimicrobial immunity in the skin. Here, we aimed to address the question whether an immature perinatal and dysregulated circadian clock suppresses innate antimicrobial peptide and protein expression, which consequently allows for predisposition to infections. We hypothesized that age-related maturation of the daily circadian rhythm via CLOCK/BMAL1 facilitates transcriptional regulation of innate antimicrobial host defense molecules in the skin. Innate antimicrobial peptides and proteins (AMPs), including antiviral proteins, play a pivotal role in innate immune defense system to protect against invading pathogens. AMPs are expressed in most barrier tissues, including the skin. Here, we show that many cutaneous AMP mRNAs are not expressed at birth, but their expression induction instead coincide with maturation of the circadian clock in mice. We also show using *ex vivo* studies in adult human skin that homeostatic AMP mRNA expression changes with age. Our findings indicate that AMP expression varies with age in human and murine skin, possibly in relation to altered expression of CLOCK/BMAL1.

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Revealing of Pattern recognition receptors mediated macrophage immune response network induced by *Mycobacterium leprae*
H. Liu, Z. Mi, Z. Wang, F. Zhang
Shandong Provincial Hospital for Skin Diseases & Shandong Provincial Institute of Dermatology and Venereology, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, Shandong, China
Leprosy is an infectious skin disease caused by *Mycobacterium leprae* (*M. leprae*). The regulation of macrophage polarization has been suggested to play a key role leprosy onset and clinical manifestations. But, the intracellular immune network induced by *M. leprae* infection in macrophage is unclear. To understand the macrophage intracellular immune response network induced by *M. leprae,* we performed a transcriptome sequencing study of mRNA, long non-coding RNA (lncRNA) and microRNA in infected macrophage. *M. leprae* sonicate and heat killed *M. marinum* were separately used to stimulate human macrophage. Pathway enrichment analysis of differentially expressed mRNA demonstrated that TNF, NF-κB, NOD-like receptor and TLR receptor signaling pathway were involved in the immune response of macrophage to *M. leprae* sonicate stimulation. Pattern recognition genes *NOD2* and *TLR7* were found to be specifically induced by *M. leprae,* while *TLR8* was specifically induced by *M. marinum.* We also found granuloma formation related genes *MMP9*, *ICAM-1* and *VCAM-1* were up regulated in *M. leprae* treated macrophage. Differential expression analysis and target prediction suggested that miR-548a-3p may regulate response to *M. leprae* by targeting *TLR7* or *NFKBIA*. lncRNA-RNA interaction analysis of differential expressed lncRNAs suggested that LINC01215 was also a potential regulator of NF-κB signaling pathway in the response of macrophage to *M. leprae.* We revealed a pattern recognition receptors genes *NOD2* and *TLR7* mediated macrophage immune response network leading to the production of granuloma formation related genes, which may be regulated by miR-548a-3p and LINC01215, in response to *M. leprae* infection.

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Quorum Quenching: A promising and physiological microbial control strategy

M. Gault[1], L. Danoux[1], S. Leoty-okombi[1], V. Andre-frei[1], P. Ludwig[2]

[1]BASF BCS, Lyon, France, [2]BASF Corp, Tarrytown, New York, United States

Many pathogens rely on cell-to-cell communication mechanisms such as the Quorum Sensing system (QS) to synchronize microbial activities essential for infection and survival in the host. In the skin, *Pseudomonas aeruginosa* (*Pae*) and *Staphylococcus aureus* (*Sa*) respectively secrete autoinducers and cyclic autoinducing peptides to promote their virulence. However, on the top of increasing bacterial motility and spreading, these molecules also disturb the skin barrier. Quenching microbial Quorum Sensing or in short, Quorum Quenching suggests a promising and physiological microbial control strategy in certain skin dysbiosis. To provide a specific and physiological protection to the skin against opportunistic pathogens, we developed specific culture conditions to evaluate the secretion of QS molecules. We used an emerging high-resolution mass spectrometry method to quantify with pmol/µL sensitivity pseudomonas quinolone signal (PQS) and homoserine lactone (HSL) for *Pae* or thiolactone peptide for *Sa*. For validation purpose, we used QS reference inhibitors: a furanone derivative for P*ae* and benzbromarone for *Sa*. We also checked bacterial motility and secretion of lipase known to break-down the skin lipidic barrier and we evaluated preselected plant extracts that did not directly affect bacterial growth. We first evidenced that the furanone derivative decreased the secretion of PQS and HSL over 90% and *Pae* swarming by 67%. However, in our trials, the secretion by *Sa* of thiolactone was not reduced by benzbromarone. We hypothesized that this reference does not act on the secretion of thiolactone but inhibits its binding to the AgrC receptor. Conversely, one of the selected plants extracts significantly reduced the *Staphylococcus* secretion of thiolactone peptide (-39 %) and triacylglycerol lipase (-80%). In our study, we demonstrated that this method allows to select accurate Quorum Sensing modulators to promote skin microbiota homeostasis, which represents a key step towards improving damaged or sensitive skin patient lives.

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Longitudinal changes in skin microbial populations as a function of atopic dermatitis severity

F. Bawany, S. R. Gill, L. A. Beck, T. Yoshida

Dermatology, University of Rochester Medical Center, Rochester, New York, United States

Atopic dermatitis (AD) is an inflammatory skin disease associated with increased *Staphylococcus aureus* (SA) colonization. We and others have found an inverse relationship between levels of SA and a common skin commensal, *Cutibacterium acnes* (CA). As intra-individual changes in the quantity of CA and SA from the skin of human subjects have not been well studied, we initiated a single-center, pragmatic, longitudinal study to address this, the design of which we present here. We hypothesize that CA, which produces antimicrobial peptides including cutimycin, limits SA growth in nonatopic (NA) and psoriasis (PS) subjects with low SA levels. The corollary is that AD subjects have higher SA levels due to lower levels of CA and/or CA strains producing lower levels of antimicrobial peptides. We are enrolling subjects (13-65 years) with moderate to severe AD (n = 30) receiving standard of care, as well as age- and gender-matched subjects with moderate to severe PS (n = 30) and NA (n = 30) and following them for 3 years. At each visit, we assess disease severity, collect lesional and non-lesional skin swabs and serum, and measure skin barrier (transepidermal water loss). Staphylococcus species from swabs are cultured on CHROMagar Staphylococcus. SA is confirmed with mannitol salt agar and PCR of Protein A (*spa).* CA is cultured anaerobically on tryptic soy agar with 0.5% Tween80 and confirmed by PCR of CA recombinase A (*recA*). Our primary endpoint is relative abundance of SA, other *Staphylococcus* species, and CA, as measured by CFUs. Our secondary endpoint will be to determine if conditioned media from CA clinical samples inhibits SA growth. We will also identify if CA samples contain genes necessary for the synthesis of antimicrobial peptides (cutimycin or lantibiotics) by PCR. Finally, we will evaluate this data across disease states (AD, PS, NA), disease severity, and treatments. The intra-individual changes observed in CA and SA over time and as a function of disease will provide valuable insights into host-microbe relationships in inflammatory diseases.

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Effect of a balm containing rhealba® oat plantlet extract on inflammatory response in an immunocompetent epidermal model of atopic dermatitis

M. Galliano[1], S. Alvarez-Georges[1], M. Saint Aroman[2], H. Duplan[1], S. Bessou-Touya[1], N. Castex-Rizzi[1]

[1]Pierre Fabre Dermo-Cosmétique, Toulouse, France, [2]Laboratoires A-Derma, Lavaur, France

Atopic dermatitis (AD) is characterized by a massive cutaneous infiltration with immune cells, dominated by CD4+ T cells. Even though inflammation in lesional AD skin is predominantly studied, microinflammation in non-lesional AD skin remains of importance for AD treatment. In particular, IFNγ, IL-13 and IL-22 have been shown to be more expressed in non-lesional skin from AD patients compared to normal skin from healthy subjects. Thus, down-regulation of the secretion of these cytokines, by lowering microinflammation, would be beneficial for AD patients. Here, we developed a model consisting of RHE co-cultured with activated CD4+ T lymphocytes. This immunocompetent system model allows to consider both the epithelial and the immune components of AD and can be used to mimic microinflammation encountered in non-lesional skin areas of AD patients. A balm containing rhealba® oat plantlet extract was applied on RHE before activated CD4+ T cells were added. A control cream containing 1% hydrocortisone was evaluated in parallel. After 48h of incubation, analyses were processed for cytokine dosage and mRNA expression of genes of interest. The control hydrocortisone cream significantly reduced cytokines production by CD4+ T cells (IFNg, IL-13, IL-17, IL-22 and IL-2) and IP10, a chemokine marker produced by keratinocytes. The rhealba® oat plantlet extract containing-balm inhibited significantly lymphocyte cytokines production (IFNg, IL-13 and IL-22) and IP10. At mRNA level, the control hydrocortisone cream significantly down regulated CCL2, CCL7 and CXCL10, while up-regulating loricrin. The rhealba®-balm was also significantly efficient at down regulating CCL2, CCL7 and up regulating loricrin. Our data demonstrate the efficacy of rhealba®-balm to modulate inflammation on both the epithelial and the immune cell components and support its beneficial for treatment of microinflammation of AD non-lesional skin.

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Tacrolimus induced pseudo-allergic reaction via Mas-related G protein coupled receptor-X2

X. Du[1], Y. Zheng[1], B. Peng[1], D. Che[2], Y. Hao[1], S. Geng[1]

[1]Department of Dermatology, Northwest Hospital, The Second Hospital Affiliated to Xi'an Jiaotong University, Xi'an, Shaanxi, China, [2]School of Pharmacy, Xi'an Jiaotong University, Xi'an, China

Tacrolimus is widely used in atopic dermatisis (AD), but the side effects of topical application effect the patient's compliance with medication, such as itching and burning, of which the mechanism is not clear. Recent studies have revealed that Mas-related G protein-coupled receptor X2（MRGPRX2, a receptor on mast cells, mediating pseudo-allergic reaction in many contact dermatitis caused by topical drugs, which is similar to the side effects of tacrolimus. In this study, the mechanism in pseudo-allergic reaction caused by tacrolimus was investigated. Wild-type (WT) and MrgprB2-/- mice were used to observed local inflammation by Haematoxilin & Eosin and immunofluorescence staining. Release of tryptase, histamine and MCP-1 were measured in LAD2 cells with specific knockdown targeting MRGPRX2 by siRNA. We found WT mice exhibited inflammatory reaction in dorsal skin and footpad induced by tacrolimus, while MrgprB2-/- mice showed slighter reaction. The level of tryptase, histamin and other inflammatory cytokines were lower in mutated mice. Downregulation of MRGPRX2 resulted in the reduced degranulation of LAD2 cells. These results reveal tacrolimus could induce pseudo-allergic reaction via MRGPRX2/MrgprB2 in human/mice.

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Effects of Celastrol enriched plant cell culture extract in a 3D human skin equivalent model of psoriasis

M. Galliano[1], S. Alvarez-Georges[1], F. Lestienne[1], T. Nguyen[1], V. Mengeaud[2], H. Duplan[1], S. Bessou-Touya[1], N. Castex-Rizzi[1]

[1]Pierre Fabre Dermo-Cosmétique, Toulouse, France, [2]Laboratoires Ducray, Lavaur, France

Psoriasis is a chronic inflammatory skin disease mainly characterized by an exaggerated inflammation of the skin driven by TH17 immune cells and an abnormal proliferation of keratinocytes causing red patches and inflammatory plaques. To demonstrate the activities of Celastrol enriched plant cell culture extract (CEE) on psoriasis biomarkers, we used an integrative psoriatic three-dimensional (3D) skin equivalent model in which purified TH17 cells were added. Purified TH17 cells were isolated from peripheral blood mononuclear cells and subsequently amplified and stimulated with anti-CD3/CD28/CD2. CEE was added to the 3D model, either into the culture medium or topically, as formulations containing 0.1% or 0.3% CEE. Finally, a commercialized cosmetic cream for psoriasis-prone skin containing 0,3% CEE was evaluated. Psoriatic inflammation was assessed after 48h or 96h incubation using multiplex and ELISA analyses of the culture media of the 3D models. The experiments were repeated on 5 equivalent skin models. When added to the culture medium of the 3D models, CEE strongly inhibited the inflammatory cytokines IL-17A, IL-22, IFN-g as well as epidermal markers HBD2, IL-8 and IL-6. Inhibition by CEE was equivalent to the one with control cyclosporine. CEE-containing formulations topically applied inhibited as well, cytokines and HBD2 productions compared with control formulation, in a dose-dependent manner. The cosmetic cream containing 0,3% CEE strongly inhibited IL-17, IL-22 and HBD2 secretion. In conclusion, we developed a psoriatic 3D model with relevance for testing active ingredients and products. Our data showed that CEE inhibited Th17/Th22 cytokines, and psoriasis associated biomarkers involved in the physiopathology of this cutaneous disease, supporting the benefits of CEE for adjunctive psoriasis treatment.

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A case of phaeohyphomycosis caused by *Corynespora cassiicola,* a plant pathogen

H. Luo[1, 2], M. Tao[2], J. Zhang[2], J. Cao[2], H. Hong[2], Q. Li[1], J. Uitto[1], Y. Cao[2]

[1]Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, United States, [2]The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, China

Corynespora cassiicola is a common plant pathogen. Humans infected by *C. cassiicola* are extremely rare. We report a case of subcutaneous and pulmonary phaeohyphomycosis caused by *C. cassiicola* in a 68-year-old male Chinese. The clinical manifestations were ulcers on the upper arm skin with erosions, scabs, and infiltrative plaques. The pulmonary manifestations presented with cough, chest tightness, and fever. The organism was identified as *C. cassiicola* based on the morphology and the sequence of the internal transcribed spacer region of the ribosomal RNA gene. *CARD9* deficiency has been reported to underlie several fungal infections. By employing whole-exome capture, compound heterozygous sequence variants were found in the *CARD9* gene, c.106C>T (p.Gln36*) and c.1118G>C (p.Arg373Pro). The patient was cured by the antifungal drug voriconazole, based on repeated fungal microscopy and culture which were negative. The skin lesions gradually healed, leaving scar and erosive surface. There are less than 10 reports on subcutaneous infection by *C. cassiicola,* and this case is unique with concomitant subcutaneous and pulmonary phaeohyphomycosis highlighting the role of CARD9 in the human immune response in controlling fungal infections. In summary, this case emphasizes the importance of genetic susceptibility to certain microbial infections.

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S. aureus superantigens enhance viral pathogenesis of the skin epithelium

M. Moran[1], F. Bawany[2], M. G. Brewer[2], P. Schlievert[3], T. Yoshida[2], B. M. Ward[1], S. R. Gill[1], L. A. Beck[2]

[1]Microbiology & Immunology, University of Rochester, Rochester, New York, United States, [2]Dermatology, University of Rochester, Rochester, New York, United States, [3]Microbiology & Immunology, University of Iowa, Iowa City, Iowa, United States

Atopic dermatitis (AD) is characterized by skin barrier dysfunction, type 2 immunity, and an altered skin microbiota with high *S. aureus* abundance. Eczema vaccinatum (EV) is one of the most serious AD-associated viral infections and occurs following exposure to vaccinia virus (VV; smallpox vaccine). Previously we had observed that eczema herpeticum, another AD viral complication, was more commonly observed in *S. aureus* colonized patients. This led us to hypothesize that *S. aureus* virulence factors (e.g. superantigens [SAgs]) enhance epithelial cells susceptibility to viral infections. To test this, primary human foreskin keratinocytes (PHFK) were incubated with supernatants from multiple *S. aureus* strains that vary in secreted virulence factors (USA300, HG003, RN4220) and then infected with VV. Treatment of PHFK with USA300 supernatant, which contained the greatest number and quantity of SAgs, resulted in a significant increase in both plaque number and size. USA300 supernatant treatment also resulted in a significant reduction in transepithelial electrical resistance (e.g. barrier function). We have identified the *S. aureus* SAg, SE/Q, as a virulence factor of interest as it is highly produced by USA300 and was detected on the skin of 100% of AD patients in a pilot study. Treatment of PHFK with purified SE/Q decreased barrier function, enhanced viral permissiveness, and altered cellular integrity and metabolism. These changes were not observed in PHFK treated with the most homologous SAgs to SE/Q, namely SE/K and SE/M. Our findings suggest that *S. aureus* SAgs, and uniquely SE/Q, significantly alter PHFK and enhance their permissiveness to VV infection and spread. Based on these findings, *S. aureus* virulence factors are potentially key contributors to EV in AD patients, and this may extend to other viral infections afflicting this population.

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Histone demethylase LSD1 is required for LC embryonic development but dispensable for LC maintenance and repopulation

X. Wang, Y. Yao, Y. Wang, L. Zhou, Q. Mi

Center for Cutaneous Biology and Immunology Research, Department of Dermatology, Henry Ford Health System, Detroit, Michigan, United States

Langerhans cells (LCs), the sole dendritic cell subpopulation in the epidermis, are potent regulators of immune surveillance and tolerance. Unlike conventional DCs, LCs follow unique patterns of development and maintenance under steady and inflamed states. Lysine-specific demethylase 1 (LSD1), known to mediate the demethylation of lysine amino acids on histone proteins, plays a key role in the maintenance and differentiation of hematopoietic stem cells. However, the role of LSD1 in LC ontogeny and homeostasis remains unknown. To address this, we generated *Csf1r*^{Cre}*LSD1*$^{fl/fl}$ conditional knockout (Csf1r.LSD1-KO) mice in which LSD1 was deficient in macrophage/monocytes and LC precursors from embryonic stage. We found a robust reduction of LC precursors in Csf1r.LSD1-KO mice at embryonic day 18.5 and postnatal day 0, suggesting that LSD1 is required for LC ontogeny at late embryonic stage. To investigate whether LSD1 is involved in LC maintenance and repopulation, we created *CD11c*^{Cre}*LSD1*$^{fl/fl}$ conditional knockout (CD11c.LSD1-KO) mice in which LSD1 was deficient in all DC populations including epidermal LCs after birth. Flow cytometry and immunofluorescent staining of skin showed that the frequency and number of LCs were unaltered in 2-week-old and adult CD11c.LSD1-KO mice compared to wild-type mice. Using an ultraviolet C (UVC)-induced skin damage model, we found that long-term LCs were able to repopulate to the inflamed epidermis of CD11c.LSD1-KO mice 2 weeks after UVC treatment. Overall, our data suggests that LSD1 is critical for LC embryonic development, but not required for the LC maintenance at steady state and repopulation under inflammatory conditions.

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Innate lymphoid cells in the blood of untreated and dupilumab-treated patients with atopic dermatitis

W. Bauer, N. Alkon, C. Bangert, P. M. Brunner, G. Stingl

Department of Dermatology, Medical University of Vienna, Vienna, Austria

To investigate the driving force behind the increase of innate lymphoid cells (ILCs) in atopic dermatitis (AD) skin, we numerically and phenotypically compared ILC populations in the blood of untreated AD patients (AD-U), long term (≥16 weeks) dupilumab-treated AD patients (AD-D) and healthy controls (HC). PBMCs were isolated from clinically active AD patients (median age: 32.2 years; n=9), dupilumab-treated AD patients (median age: 33.3 years; n=6) and healthy individuals (median age:28.3 years; n=10), immunostained with a panel of subset-characterising antibodies and analysed with a BD FACSAria III sorter. Total ILCs were defined as viable $CD45^+CD3^-Lin^-CD127^+CD161^+$cells and expressed as percent of $CD45^+$cells (median). Subpopulations of total ILCs were defined as follows: ILC1 ($CD117^-$, $CRTH2^-$), ILC2 ($CRTH2^+$), and ILC3 ($CD117^+$, $CRTH2^-$). Results obtained show that the amount of total ILCs among PBMCs is significantly lower in AD patients than in healthy individuals(AD-U: 0.009, HC: 0.049(p=0.001)). This reduction was particularly prominent in the ILC2 subset(ILC2: AD-U: 41.88, HC: 68.20 (p=0.0279)). By contrast, ILC3 were significantly increased in AD patients as compared to HC (ILC3: HC: 18.88, AD-U: 39.23 (p = 0.0435)). ILC1 occurred at a comparable frequency in both populations. Interestingly, continued dupilumab treatment led to an increase of ILCs to levels similar to those in healthy persons (AD-U: 0.009, AD-D: 0.037 (p=0.036)). Furthermore, the distribution of the individual ILC subsets in dupilumab-treated patients was similar to that seen in untreated patients. In additional experiments we searched for the presence of skin-homing molecules on ILCs in HC and AD patients and found CCR10>CCR6≥CCR4>CLA to be present on substantial portions of ILCs. Our data support the concept that the reported increase of ILCs, particularly ILC2, in AD skin is at least partly due to emigration from blood and that this pathogenic loop could be a potential pharmacologic target.

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IL-34 differentiation of immunosuppressive macrophage

K. Kelly-Scumpia, R. Shirazi, R. L. Modlin

Medicine/Dermatology, UCLA, Los Angeles, California, United States

Myeloid cells from bone marrow are necessary to mount effective immune responses to infectious organisms in the skin. These cells enter the skin and respond to local factors that control their differentiation into specific effector myeloid cells, such as dendritic cells and macrophages (MΦ). The local and systemic factors responsible for these phenomena are poorly understood. Leprosy provides an ideal human model to study myeloid cells in the skin as exposure of patients to the same pathogen, *Mycobacterium leprae*, results in distinct disease manifestations. In self-limited, tuberculoid leprosy (T-lep), appropriate monocyte activation results in their differentiation into effector M1 MΦ and dendritic cells (DCs) that produce antimicrobial peptides and control *M. leprae*. In progressive, lepromatous leprosy (L-lep), the host develops an inadequate immune response, and fail to clear *M. leprae*. We previously found that interleukin (IL)-15 and IL-10 are key cytokines promoting effector M1 MΦ in T-lep and immunosuppressive M2 MΦ in L-lep patients, respectively. Whether other immunologic factors affect MΦ differentiation in the context of leprosy has not been fully investigated. Herein, we find a regulatory role of IL-34, a hematopoietic factor that activates c-fms and drives M2 MΦ development *in vitro*, as being increased in L-lep skin lesions. Differentiation of monocytes derived MΦ (MDM) with IL-34 results in increased IL-10 production upon *M.leprae* stimulation when compared to traditional M-CSF derived MDM or IL-15 derived MDM. Similar to IL-10 derived MΦ IL-34 derived MΦ demonstrated increased phagocytic activity, but surprisingly, were able to activate a vitamin D dependent antimicrobial response similar to IL-15 derived MΦ. These data indicate that IL-34 drives an alternative pathway of M2 MΦ development, whereby they can produce anti-inflammatory cytokines, but retain the ability to induce antimicrobial responses. These findings raise the potential of IL-34 or IL-34 derived MΦ to treat inflammatory skin disorders while retaining the ability to fight infection.

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A polylysine dendrigraft able to balance acneic and non acneic strains of *Cutibacterium acnes* to prevent acne and skin imperfections

J. Attia[1], M. Borel[2], M. Barreau[3], E. Le Toquin[4], O. Lesouhaitier[3], E. Loing[1]

[1]R&D, Lucas Meyer Cosmetics, Toulouse, France, [2]Marketing, Lucas Meyer Cosmetics, Champlan, Croatia, [3]Laboratoire de Mibrobiologie Signaux et Microenvironnement, Evreux, France, [4]N2S, Evreux, France

Acne is one of the most common skin diseases worldwide, affecting up to 85% of the population. At the pathophysiological level, two factors play a crucial role: the sebaceous gland and *Cutibacterium acnes (C. acnes)*. New genomic analysis tools have shown that *C. acnes*, former *P. acnes*, a major member of the normal skin microbiota was subdivided into heterogeneous species including acneic bacteria such as RT4, RT5 strains, and commensal bacteria such as the RT6 strain. Moreover, recent data indicated also that the loss of diversity of *C. acnes* species is associated with acne severity. Taking all this information, we have developed a green polylysine dendrigaft, the Dendrimer (G2), able to rebalance acneic and non acneic stains of *C. acnes* to protect skin from inflammation, imperfections and acne. *In vitro* studies revealed the capacity of the G2 to increase membrane fluidity of acneic strains RT4 and RT5 and decrease their biomass in contrast to the RT6 strain. Moreover, G2 showed also a strong anti-adhesion power of *C. acnes* on human keratinocytes. *Ex vivo* studies indicated also an anti-inflammatory effect by decreasing IL1α and TLR2 expressions. These data were confirmed *in vivo*: the study was conducted during 28 days on hemi-face, with a twice daily application of G2 (2 ppm) or Placebo cream on 23 volunteers. After 28 days, we observed a significant decrease of the sebo-regulating effect by 11%, retentional and inflammatory lesions by 31% and 63%, respectively. Interestingly, G2 application promoted also the diversity of *C. acnes* by increasing the expression of its no acneic strains compared to its acneic strains. To conclude, we have demonstrated that G2 could be the new skin care ingredient able to balance acneic and non acneic strains of *C. acnes* to improve skin microbiota and protect skin from inflammation, imperfections and acne.

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Innate lymphoid cells in healthy and atopic skin

N. Alkon[1], W. Bauer[1], C. Bangert[1], C. Staud[2], T. Krausgruber[3], C. Bock[3], P. M. Brunner[1], G. Stingl[1]

[1]Department of Dermatology, Medical University of Vienna, Vienna, Austria, [2]Department of Surgery, Division of Plastic & Reconstructive Surgery, Medical University of Vienna, Vienna, Austria, [3]Center for Molecular Medicine, Vienna, Austria

Atopic dermatitis (AD) is a type 2 inflammatory skin disease with an abundant contribution of Th2 cells. In the recent past it became clear that the skin, under both physiologic and pathologic conditions, contains additional lymphocyte populations. In this context, attention has focused on Innate Lymphoid Cells (ILCs) which, as has been shown by us and others, are present in very small numbers in normal, but in sizable amounts in AD skin. To investigate the differences between ILCs in NHS and AD skin, we performed 10x Genomics single-cell RNA sequencing (scRNA-seq) of samples enriched for ILCs from 3 NHS and 4 AD lesions. For this purpose, $CD45^+CD3^-Lin^-$ cells (including NK-cells) were FACS-sorted from enzymatically digested NHS sheets (≈10x10 cm^2) or 5 mm punch biopsies from AD lesions, and prepared for scRNA-seq. Due to overall low numbers, ILC isolates were further supplemented with autologous keratinocytes to reach optimal cell counts for sequencing. Analysis of scRNA-seq was performed using R package Seurat (v.3). Results obtained show that ILCs from both NHS and AD display a predominant ILC2 phenotype (e.g., expression of *GATA3*, *RORA*, *AREG*). The differences in gene expression between NHS and skin AD-derived ILCs were mainly quantitative rather than qualitative. Examples for this include not only “classic” type 2 cytokines such as *IL13*, but also *IL17A*, *IL22* and *IL26*. When investigated at a single cell level, transcripts of type 2 cytokine genes in ILCs compared favorably with those encountered in T cells. While the factors responsible for the acquisition of the “activation” phenotype of ILCs in AD skin remain yet to be determined, our data ascribe an important role to ILCs in disease pathogenesis.

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Shifts in the skin bacterial and fungal microbiomes of healthy children transitioning through puberty

J. Park[1], N. H. Schwardt[1], J. Jo[1], J. Portillo[1], J. Yanovski[2], J. Segre[3], H. H. Kong[1]

[1]Dermatology Branch, NIAMS, NIH, Bethesda, Maryland, United States, [2]Program in Developmental Endocrinology and Genetics, NICHD, NIH, Bethesda, Maryland, United States, [3]Translational and Functional Genomics Branch, NHGRI, NIH, Bethesda, Maryland, United States

Previous cross-sectional studies have shown that skin microbiomes of adults and older children are distinct from those of younger children. However, the influence of sexual maturation on the human skin microbiome in individuals followed over time has not been studied as extensively. We performed a prospective, longitudinal study to investigate puberty-dependent shifts in skin microbiota. Twelve healthy children (4 girls and 8 boys) were evaluated every 6-18 months for up to 6 years at the outpatient dermatology clinic in the National Institute of Health Clinical Center. Using 16s rRNA (v1-3) and ITS1 amplicon sequencing with DADA2 pipeline, we characterized and compared the bacterial and fungal communities of five different skin sites correlated with clinical metadata and serum hormone levels. We identified significant alterations in the composition of skin microbial communities, transitioning from 'childlike' to 'adultlike' microbiome, during puberty. The microbial shifts were associated with Tanner staging of the degree of sexual maturation, rather than chronological age. Among bacterial communities, sex-specific differences were noticeable at all sampled sites. Samples from female children demonstrated predominance of *Cutibacterium* with decreasing diversity with sexual maturation, while samples from male children trended toward decreased diversity which was driven by several taxa including *Corynebacterium, Staphylococcus*, and others. For fungal communities, *Malassezia* became more predominant at most sites in both sexes. The increasing abundance of lipophilic taxa (*Cutibacterium, Corynebacterium, Malassezia*) with lower microbial diversity were closely correlated with serum sex hormone levels that influence sebaceous gland activity. Taken together, our results confirm the relationship between sexual maturation, skin microbiome, and skin physiology.

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IL-1β induces rapid secretion of the antimicrobial protein IL-26 from Th17 cells

D. Weiss[1], M. Gilliet[2], M. Pellegrini[3], R. L. Modlin[1]

[1]Division of Dermatology, UCLA David Geffen School of Medicine, Los Angeles, California, United States, [2]Department of Dermatology, University Hospital CHUV, Lausanne, Switzerland, [3]Department of Molecular, Cell, and Developmental Biology, University of California, Los Angeles, Los Angeles, California, United States

Th17 cells play a fundamental role in both immunity and autoimmunity at mucosal surfaces, including skin. Recent work has implicated the Th17 cytokine IL-26 as directly antimicrobial to extracellular and intracellular bacteria, including *Mycobacterium leprae*, the causative agent of leprosy. IL-26 protein expression was greater in skin lesions from patients with the resistant vs. progressive form of the disease. To characterize the mechanism of IL-26 induction, we examined the kinetics of IL-26 secretion by PBMCs exposed to *Mycobacterium leprae* sonicate, which revealed significant release at 3 hours, reminiscent of an innate response. Pattern recognition receptor ligands induced IL-26 secretion from PBMC, which was mediated by IL-1β. Recombinant IL-1β alone was sufficient to stimulate IL-26 release from PBMC and memory $CD4^+$ T cells in the absence of T cell receptor (TCR) activation. IL-1β stimulated IL-26 secretion more rapidly from memory $CD4^+$ T cells as compared to TCR activation with crosslinking antibodies. Further investigation revealed that IL-1RI expression was necessary for IL-1β induced IL-26 from memory Th17 cells. IL-1β stimulation did not lead to secretion of other Th17 cytokines, unlike TCR activation. RNA sequencing of IL-1β treated $IL\text{-}1RI^+$ Th17 cells revealed enrichment for NF-κB regulated genes. Inhibition of NF-κB signaling with Bay 11-7082 abrogated IL-26 production in response to either stimulus. Finally, supernatants from IL-1β treated memory T cells showed antimicrobial activity against *E. coli* in an IL-26 dependent manner. Together, these results identify IL-1RI+ Th17 cells as an antimicrobial Th17 subpopulation with the ability to rapidly respond to IL-1β and induce IL-26 to kill extracellular bacteria.

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Toll-like receptor 2 partially dependent phagocytosis of cutibacterium acnes: Acne-associated strains phagocytosed more than healthy-associated strains

A. G. Baugh, M. Qin, J. Kim

Medicine, University of California, Los Angeles, Los Angeles, California, United States

Pathogen phagocytosis is a fundamental process in innate immunity and important for host response to injury and infection. Some pathogenic bacteria possess structural or biochemical characteristics that allow them to resist or avoid host phagocytic and immune responses. Acne vulgaris is a multi-factorial inflammatory skin disorder with phagocytosis of *Cutibacterium acnes* (*C. acnes*) as an important part in disease pathogenesis. Previous studies have indicated Toll-like receptor 2 (TLR2) as a mediator of *C. acnes*-induced cytokine secretion, and other studies have observed immune cell phagocytic ability associated with TLR2 expression. In this study, we aimed to observe the effects of TLR2 functionality on *C. acnes* phagocytosis. Stimulation of TLR2-wild-type (WT) and TLR2-knock-out (KO) THP1 monocytes with *C. acnes* showed a decreased secretion of pro-inflammatory cytokines IL-1β, IL-6, IL-12p70, and TNF-α in TLR2-KO cells. Flow cytometry analysis of TLR2-WT and TLR2-KO cells stimulated with fluorescently labeled *C. acnes* revealed decreased phagocytosis in TLR2-KO cells. However, phagocytosis was not completely prevented in TLR2-KO cells, suggesting involvement of other co-receptors in C*. acnes* phagocytosis. Single-cell RNA sequencing of acne lesions showed increased expression of ITGAM, ITGB2, and C5AR1 genes of the complement system when compared to non-lesional skin, suggesting that immune cell phagocytosis of *C. acnes* does not completely depend on TLR2 and may involve activation of the complement system. Further analysis revealed that in both TLR2-WT and TLR2-KO cells, monocytes phagocytosed more acne-skin associated *C. acnes* strains (C_A) than healthy-skin associated strains (C_H). While both C_A and C_H induce similar pro-inflammatory cytokine secretion levels, C_A are phagocytosed more than C_H, suggesting the possibility of a structural or biochemical difference between C_A and C_H that may influence immune cell pathogen recognition, phagocytosis, and anti-microbial activity.

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CBFβ2 is required for LC hemostasis and repopulation but not required for its embryonic development

Q. Yu[1, 3, 2], L. Zhou[1, 2], Q. Mi[1, 2]

[1]Center for Cutaneous Biology and Immunology, Department of Dermatology, Henry Ford Health System, Detroit, Michigan, United States, [2]Immunology Research Program, Henry Ford Cancer Institute, Henry Ford Health System, Detroit, Michigan, United States, [3]Department of Dermatology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China

Langerhans cells (LCs), established through the differentiation of embryonic LC precursors, are the sole dendritic cell subpopulation in the epidermis and potent regulators of immune surveillance and tolerance. Unlike conventional DCs, LCs follow unique patterns of development and maintenance under steady and inflamed states. Several transcription factors are essential for LC differentiation, including Runx3, which functions by forming heterodimers with the non-DNA binding β-subunit, CBFβ (Core-Binding Factor Subunit Beta) 1 or 2. However, the roles of CBFβ in LC ontogeny, maintenance after birth and LCs repopulation remain unknown. To address if CBFβ2 is required for LC development, we generated $Csf1r^{Cre}CBFb2^{fl/fl}$ conditional knockout (Csf1r.CBFb2-KO) mice in which CBFβ2 was deficient in macrophage/monocytes and LC precursors from embryonic stage. We found that the specific deletion of CBFβ in CSF1R expressing cells resulted in a significant decrease in the number of LCs in adult mice compared to WT littermates, while there was no significant difference on LC precursors at embryonic E17.5 and P0 between the CBFβKO and WT mice. Furthermore, the conditional deletion of CBFβ2 dramatically blocked BM-derived $MHCII^+$ $Langerin^+$ long-term LC repopulation after exposing to UVC treatment. Thus, our data highly suggest that CBFβ is required for LCs self-renewing at steady state and controls LC repopulation at inflammatory state, but not required for its embryonic development.

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Skin-induced IL-36 triggers plasma cell IgE class switching and allergic disease

G. J. Patrick[1], H. Liu[1], M. Alphonse[1], D. Dikeman[1], C. Youn[1], J. Otterson[1], Y. Wang[1], A. Ravipati[1], Q. Liu[1], E. Raymond[2], M. Ramanujam[2], N. Archer[1], L. S. Miller[1]
[1]Dermatology, Johns Hopkins School of Medicine, Baltimore, Maryland, United States, [2]Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, Connecticut, United States

Atopic dermatitis (AD) is an inflammatory, relapsing, itchy skin disorder that affects 15-30% of children and up to 5% of adults. AD patients exhibit elevated serum IgE and *Staphylococcus aureus* colonization on lesional skin that correlates with disease severity. We previously discovered that *S. aureus* induces IL-36-dependent AD-like skin inflammation. However, it is unclear what role *S. aureus* has on allergic IgE responses. Herein, we used a *S. aureus* epicutaneous infection model to mimic AD skin inflammation and examine the immune mechanisms of IgE production. Interestingly, epicutaneous *S. aureus* infection promoted IL-36-dependent IgE responses and plasma cell populations. Similarly, treatment with an anti-IL-36R mAb currently approved for generalized pustular psoriasis decreased both skin inflammation and IgE levels. B cells stimulated *in vitro* with rIL-36α showed enhanced IgE expression and plasma cell differentiation compared to rIL-4 stimulation alone. To examine the role of *S. aureus*-induced IgE on secondary allergic lung inflammation, mice were co-sensitized with epicutaneous *S. aureus* and cockroach antigen (CRE), followed by CRE intratracheal lung challenge. Mice co-sensitized with *S. aureus* and CRE demonstrated significantly increased weight loss, lung pathology, and bronchoalveolar lavage IgE levels when compared to mice sensitized with either *S. aureus* or CRE alone. Additionally, IL-36R -/- mice exhibited significantly decreased weight loss compared to wild type mice, suggesting skin-derived IL-36 can influence secondary allergic disease. Taken together, these data demonstrate that epicutaneous *S. aureus* can mediate IgE responses via IL-36 signaling, and present a potential therapeutic for AD and other allergic diseases.

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Psoriatic fungal and bacterial microbiomes identify patient endotypes

I. Salem[2], K. P. Schrom[1], S. Chu[2], M. Retuerto[2], B. Richardson[2], S. Margvicius[2], M. Cameron[2], M. Ghannoum[2], T. McCormick[2], K. Cooper[2, 1]
[1]University Hospitals Cleveland Medical Center, Cleveland, Ohio, United States, [2]Case Western Reserve University, Cleveland, Ohio, United States

Psoriasis (PSO) is a chronic, inflammatory disease characterized by immune-driven keratinocyte hyperproliferation. We report identification of pro- and anti-inflammatory intestinal microbiota clusters, bacterial and fungal, in psoriatic patients versus healthy controls. We also examined the association between disease severity and the microbiome of psoriasis skin. Fecal and skin samples were obtained from 67 psoriatic patients and 12 healthy controls. Disease severity was assessed by body surface area. 16S (bacterial) and ITS1 region (fungal) genes were amplified and then sequenced using an Ion Torrent S5 system. Relative abundance was determined using non-parametric comparisons. Interestingly, fecal samples showed a pattern of intestinal dysbiosis similar to that previously reported for Crohn's disease (CD) patients. Clusters of psoriasis endotypes were identified by intestinal dysbiosis favoring pro-inflammatory bacterial and fungal pathobionts. There was a significant increase in Staphylococcus, Serratia, and Enterobacter (p = 0.0267, 0.00092, 0.0076, respectively) as well as fungi including C. albicans and C. Parapsiosis (p=0.0286 and 0.0368, respectively) in the gut of psoriatic patients compared to healthy controls. Conversely, microbes known for their beneficial anti-inflammatory properties such as F. prausnitzii and Clostridium were markedly reduced. Similar clustering was observed at the skin level, where we report for the first time a positive correlation between disease severity and increased relative abundance of the pro-inflammatory microbes – S. marcescens, E. coli, and C. albicans. Increased abundance of these three organisms has also been described in the gut of CD patients suggesting a potential link between the microbiota of CD and PSO

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Systemic sphingosine 1-phosphate receptor 2 (S1PR2) deficiency facilitates dermal neutrophil infiltration against *S. aureus* infection

S. Igawa[1, 2], Z. Wang[2], A. Ohzono[2], T. Nakatsuji[2], A. Ishida-Yamamoto[1], A. Di Nardo[2]
[1]Dermatology, Asahikawa Medical University, Asahikawa, Japan, [2]Dermatology, University of California San Diego, La Jolla, California, United States

Background: Sphingosine 1-phosphate (S1P) is a bioactive lipid mediator generated in the skin when cell membrane or barrier components are damaged. S1P regulates a variety of cell activities via different S1P receptors (S1PR). Keratinocytes express S1PR1-5. We have already reported that, in normal human epidermal keratinocytes, S1PR1 and 2 control neutrophil migration-related proinflammatory cytokine expression and secretion, respectively, *in vitro*. As a next step, we decided to evaluate the role of S1PR2 in bacterial skin infection *in vivo* environment. *Methods*: A mouse model that lack S1PR2 (*S1pr2*$^{-/-}$ mouse) was used for the study and compared to wild type (WT); *S. aureus* was applied on the skin and *S. aureus* bacterial supernatant was injected intradermally; qPCR was used to evaluate cytokine gene expressions; filaggrin2 expression and neutrophil infiltration were evaluated by histologically. *Results:* Both *S. aureus* epicutaneous application and *S. aureus* supernatant intradermal injection on *S1pr2*$^{-/-}$ mouse skin showed more dermal neutrophil infiltration. *S1pr2*$^{-/-}$ mouse dermis showed more severe dermal inflammation with higher proinflammatory cytokine expressions. These data were accompanied by an increased skin barrier permeability and a decrease in filaggrin2. These data suggest that, although the lack of *S1pr2*$^{-/-}$ in the epidermis decreases the proinflammatory cytokines response, the increased barrier permeability drives a strong dermal inflammation due to bacteria penetration. *Conclusions*: The lack of *S1pr2* shows two different activities in the skin, while it decreases the epidermal proinflammatory response to S1P signal, it also increases permeability to pathogens and promotes infections.

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The MrgprB2/X2 is regulated by the neurokinin 2 receptor in mast cells in the skin

Y. Li[1], J. Zhang[1], L. D. Falo[2], A. Larregina[1], T. Sumpter[1]
[1]Dermatology, University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [2]Dermatology, Univ of Pittsburgh, Pittsburgh, Pennsylvania, United States

In the cutaneous microenvironment, interactions between mast cells (MCs) and sensory neurons play an important role in immune responses. Neuropeptides such as neurokinin A and substance P direct MC function by initiating signaling through neurokinin (NKRs) receptors, and Mas related G-protein receptors (Mrgpr)s. Recent studies highlight the importance of the MrgprB2 (mouse) and MrgprX2 (human) in the MC response to pseudo-allergens, secretagogues and substance P. To date, a relationship between NKRs and the MrgprB2/X2 has not been investigated. In this study, we hypothesize that MrgprB2/X2 is transcriptionally controlled by the NK2R and its high affinity ligand, neurokinin A. In mice, we show that administration of neurokinin A diminishes MrgprB2 RNA expression. Surprisingly, NK2R antagonism also downregulates MrgprB2 expression and MrgprB2 expression is markedly diminished in mice lacking the NK2R. In contrast, co-administration of neurokinin A and an NK2R antagonist increases MrgprB2 expression. The MC response to the canonical MrgprB2 ligand, compound 48/80, mirrored the changes in MrgprB2 transcript expression. In human skin explants, NK2R antagonism had minimal effect on MrgprX2 expression, but co-administration of neurokinin A and a NK2R antagonist markedly upregulated MrgprX2 expression, as seen in murine skin. These data demonstrate that the NK2R-signaling influences MrgprB2/X2 expression and, that in absence of the NK2R, neurokinin A interacts with an unknown receptor to increase MrpgrB2/X2 expression. Collectively, these data uncover a novel role for NK2R signaling in the regulation of MrgprB2/X2. These important findings have implications for patients with mast cell mediated cutanous inflammatory diseases.

Patient Population Research

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Psoriasis and co-morbidity

M. Amin[1], E. Lee[2], T. Tsai[3], J. Wu[4]

[1]Dermatology, Kaiser Permanente Los Angeles Medical Center, Los Angeles, California, United States, [2]Santa Barbara Cottage Hospital, Santa Barbara, California, United States, [3]Dermatology, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan, [4]Dermatology, Dermatology Research and Education Foundation, Irvine, California, United States

Psoriasis is associated with multiple co-morbid medical conditions. The purpose of this study is to evaluate the relationships between psoriasis and cardiovascular disease, psoriatic arthritis, mental health conditions, and immune-mediated diseases, respectively. A literature search was performed during the study period January 1, 2015 to December 18, 2018. Of 2499 records identified, 28 met our criteria selection and were included in this review. Based on these findings, the relationships between psoriasis and these multiple comorbid disease conditions are discussed. Psoriasis is associated with cardiovascular disease, and chronic inflammation likely plays a major role in this relationship. Treatment of psoriasis improves underlying inflammation and TNF inhibitor therapy may provide a protective effect against risk of MACE for patients with psoriasis, which would ultimately promote better health outcomes for these patients. Additionally, psoriatic arthritis is a common comorbid condition associated with psoriasis that can lead to permanent disability. Early treatment is imperative to help prevent complications of psoriatic arthritis. Autoimmune diseases have also been reported to be associated with psoriasis, which may suggest that the pathogenesis of psoriasis may involve autoimmune mechanisms. Moreover, it is important to address and treat comorbid psychiatric conditions among patients with psoriasis, including depression, suicidal behavior, and suicidal ideation. Early recognition and treatment of comorbid disease conditions is important to consider when developing the treatment plan and overall management of patients with psoriasis to help improve the quality of life for these patients.

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Low English proficiency is associated with decreased biologic access in psoriasis

V. S. Chat[1], J. Hekmatjah[2], T. Sierro[3], C. Read[3], A. Kassardjian[3], A. W. Armstrong[3]

[1]Medical College of Georgia at Augusta University, Augusta, Georgia, United States, [2]Western Michigan University School of Medicine, Kalamazoo, Michigan, United States, [3]University of Southern California, Los Angeles, California, United States

Language proficiency is an integral aspect of patient-provider communication, and it affects healthcare choices and access. Disparities in access to biologic medications in psoriasis patients exist, but the influence of English proficiency is unknown. We conducted a cross-sectional study using the Medical Expenditure Panel Survey (MEPS) from 2013 to 2017 to compare biologic medication use among psoriasis patients of differing English proficiency. We compared patients in exclusively English speaking households to patients with a family member who speaks a language other than English at home, which we defined as having as less than perfect English proficiency. Among 5,426,851 U.S. psoriasis patients (weighted) pooled during the 5-year period, 4,822,222 (88.9%) spoke English as their only language and 604,629 (11.1%) had less than perfect English proficiency. We conducted a multivariate analysis adjusting for age, gender, race/ethnicity, Charlson comorbidity index, insurance status, income, region, and education. This multivariate analysis showed that those less proficient in English had significantly less use of biologic medications (OR: 0.159, 95% CI: 0.081–0.312) compared to patients in households that spoke exclusively English. Exploratory analysis showed that there was no significant difference in biologic access by sex (OR: 0.59, 95% CI: 0.339–1.025). Blacks are less likely to be prescribed biologics compared to whites (OR: 0.462, 95% CI: 0.2196-0.9737). Psoriasis patients with decreased English proficiency are more than six times less likely to be prescribed biologics after adjusting for medical insurance and other factors. For optimization of psoriasis management, providers need to be aware of this difference in biologic access based on patients' English language proficiency. Strategies to improve patient-provider communication aimed at decreasing language barriers are critical in dermatology.

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Epidemiology and clinical outcomes of 151 patients with mycosis fungoides at the Kosin University Gospel Hospital: Retrospective 27-year review

M. Jang, S. Seong, D. Kwon, J. Jang, J. Park, K. Suh

Dermatology, Kosin University, Busan, Korea (the Republic of)

Mycosis fungoides (MF) is the most common form of cutaneous T-cell lymphoma with a variety of clinicopathological features. A single center-based large scale study with long-term follow up in Korea has not been reported. This study was conducted to investigate epidemiologic features and clinical outcomes of MF patients at the authors' hospital over a 27-year period. This is a pilot study conducted on 151 patients diagnosed as MF, from 1991 to 2018, with the retrospective review. Of 151 patients, 62.9% were male and 37.1% female. The mean age at the diagnosis was 44.2 years (range, 5-82). The mean duration of symptoms was 50.4 months (range, 0.25-360). The mean follow-up duration was 57.6 months (range, 2-251). Common subtypes were classic MF (45.0%), mycosis fungoides palmaris et plantaris (MFPP) (23.8%), and folliculotropic MF (7.9%). In early-stage MF (IA-IIA) of 143 patients (94.7%), the 10-year overall survival (OS) was 93.6%. In advanced-stage MF (IIB-IVB) of eight patients (5.3%), the 10-year OS was 23.4%. Complete remission (CR) and disease progression were found in 63.6% and 4.6% of the patients. The recurrence after CR was observed in 33 patients (21.9%) and the mean recurrence free-duration was 24.1 months (range, 1-118). In summary, clinical outcomes generally paralleled the previous reports with favorable prognosis in the early-stage MF. Recurrence was not uncommon, largely due to greater prevalence of MFPP.

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Pregnancy outcomes in female patients with alopecia areata: A nationwide population based study in Korea

D. Yu[1], S. Cho[1], S. Kim[2], S. Lee[2], O. Kwon[1]

[1]Department of Dermatology, Seoul National University Hospital, Seoul, Korea (the Republic of), [2]Department of Obstetrics and Gynecology, Seoul National University Hospital, Seoul, Korea (the Republic of)

Alopecia areata (AA) is a common hair loss disorder characterized by an autoimmune reaction against hair follicles. Several autoimmune disorders are associated with adverse pregnancy outcomes, but the effect of AA on pregnancy outcome is unknown. To investigate pregnancy outcomes in female patients with AA, we performed a nationwide, population-based retrospective case-control study using the Korean National Health Insurance claims database from 2011 to 2017. The pregnancy outcomes of 4552 female patients with AA and 508,345 control subjects were analyzed. We found that the AA patient group showed a high frequency of autoimmune thyroid diseases, systemic lupus, and pelvic inflammatory disease, compared to the control group. They showed a lower total live birth rate (odds ratio [OR], 0.876; 95% confidence interval [CI], 0.820-0.937) and higher rates of all kinds of abortion (OR, 1.145; 95% CI, 1.071-1.225) and ectopic pregnancy (OR, 1.261; 95% CI, 1.112-1.431) after adjustment. These data suggest that AA is associated with an increased risk of abortion, especially ectopic pregnancy. This study may contribute both to the understanding of obstetric comorbidities in AA and to a better management of pregnancy.

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Outcomes of patients with cutaneous squamous cell carcinomas treated with anti-PD1 inhibitors: A single-institution cohort study

E. Muradova[1, 2], L. Cohen[1, 3], R. Panchaud[1, 2], Y. Zhao[1], R. Amorrortu[1], D. Rollison[1], K. Y. Tsai[1]

[1]Moffitt Cancer Center, Tampa, Florida, United States, [2]Dermatology, University of South Florida, Tampa, Florida, United States, [3]Florida International University, Miami, Florida, United States

Background: Anti-programmed cell death (PD)-1 inhibitors are emerging as the new standard of care for patients with advanced and metastatic cutaneous squamous cell carcinoma (cuSCC). The FDA approved the first anti-PD1 inhibitor, cemiplimab, for the treatment of patients who are not candidates for curative surgery or curative radiation in 2018. Due to the limited available options for the management of advanced cuSCC and the promising data in cemiplimab trials, other PD-1 inhibitors are currently subject to the ongoing investigation in multiple clinical trials. This retrospective study aimed to evaluate the efficacy and outcomes of patients with advanced, recurrent, or metastatic cuSCC treated with ICIs. Methods: An IRB-approved retrospective data analysis of patients treated with ICI for advanced, recurrent, or metastatic cuSCC between December 1998 and March 2019 at Moffitt Cancer Center was performed. Patient demographics, clinical and histopathologic data, and therapy outcomes from patients were collected from review of medical records. Results: A total of 80 patients (M:F=64:16) were included in the study, with the mean age of 66 years. Patients were treated with cemiplimab (11%), nivolumab (9%), pembrolizumab (79%), and a combination of 2 or more ICIs (1%). Partial response in 32%, stable disease (SD) in 16%, complete response in 19%, and the progression of the disease was observed in 32%. The 5-year disease-specific survival (DSS) was 79%, with the median survival of 8.25 years. Patients that received ICI as the first-line therapy had longer DSS compared to patients that received ICI as a second or later therapy line treatment (100% vs. 77%); improved DSS was also seen in the use of platinum-based therapy and ICI without any chemotherapy. Our findings further support the results of clinical trials and confirm the efficacy of ICIs as a first-line treatment and in patients with prior platinum-based chemotherapy.

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Acne patients on isotretinoin experience less psychological distress and depression symptoms compared to those on oral antibiotics

J. Hekmatjah[1], V. S. Chat[2], T. Sierro[3], C. Read[3], A. Kassardjian[3], A. W. Armstrong[3]

[1]WMU Stryker School of Medicine, Kalamazoo, Michigan, United States, [2]Medical College of Georgia, Augusta, Georgia, United States, [3]USC Keck School of Medicine, Los Angeles, California, United States

Patients with acne often experience psychological distress and depression. However, little is known regarding differential effects of systemic anti-acne treatments and mental health outcomes. We sought to determine whether differences exist in mental health outcomes between patients treated with isotretinoin versus oral antibiotics. We performed a nationwide, cross-sectional study of acne patients on isotretinoin or oral antibiotics (doxycycline, minocycline, or tetracycline) pooled from the 2004-2017 Medical Expenditure Panel Survey (MEPS). Among 9,046,894 (weighted) adults (≥18) with moderate-to-severe acne pooled during the 14-year period, 7,875,279 (87%) were on oral antibiotics and 1,171,615 (13%) were on isotretinoin. Patients on isotretinoin or oral antibiotics completed validated mental health assessments using the Patient Health Questionnaire 2 (PHQ2) and the Kessler 6 (K6). Lower PHQ2 scores represents fewer depression symptoms. After adjusting for socio-demographic characteristics and comorbidities, patients on isotretinoin had lower PHQ2 scores (mean PHQ2 score 0.280, 95% CI: 0.170 to 0.390) compared to patients on oral antibiotics (mean PHQ2 score 0.656, 95% CI: 0.554 to 0.758), with a difference of 0.337 (95% CI: -0.503 to -0.171, p<0.01). Furthermore, lower K6 scores represents less psychological distress. The adjusted comparison also showed patients on isotretinoin had lower K6 scores (mean K6 score 2.493, 95% CI: 1.798 to 3.189) compared to patients on oral antibiotics (mean K6 score 3.433, 95% CI: 3.137 to 3.728), with a difference of 0.759 (95% CI: -1.493 to -0.024, p=0.043). In conclusion, isotretinoin is associated with less psychological distress and depression symptoms as compared to oral antibiotics in adults with acne.

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Do less satisfied patients utilize more healthcare resources? A population study among U.S. adults with psoriasis

C. Read[1, 2], J. F. Apperley[2], S. P. Hettiaratchy[2], A. W. Armstrong[1]

[1]Dermatology, University of Southern California, Los Angeles, California, United States, [2]Medicine, Imperial College London, London, United Kingdom

How patient satisfaction impacts healthcare utilization is rarely studied in adults with skin diseases. Healthcare utilization, such as hospitalizations, accounts for one-third of the annual economic burden of psoriasis. We sought to determine the impact of patient satisfaction on healthcare utilization among U.S. adult patients with psoriasis. One way that patient satisfaction is measured is through evaluation of patients' perception of patient-provider communication. We performed a cross-sectional study using the Medical Expenditure Panel Survey (MEPS) from 2000-2017. Among 10,013,506 (weighted) U.S. adults (≥18 years) with psoriasis pooled during the 18-year period, 428,786 (4%) reported low patient satisfaction, 5,673,170 (57%) reported medium patient satisfaction, and 3,911,550 (39%) reported high patient satisfaction. Healthcare utilization was measured using emergency room (ER) visit and overnight inpatient hospitalization frequencies. We adjusted for socio-demographic characteristics and comorbidities and used the validated patient-provider communication composite score. Compared to patients reporting high patient satisfaction, patients reporting low patient satisfaction had a 3.6 times greater likelihood of having an ER visit [Adjusted OR: 3.64 (95% CI: 1.85-7.16); p<0.001]. Compared to patients reporting high patient satisfaction, patients reporting low patient satisfaction had 3.1 more overnight inpatient hospitalizations [β=3.05 (95% CI: 2.32-3.78); p<0.001); and a 4.7 times greater likelihood of having an overnight inpatient hospitalization [Adjusted OR: 4.65 (95% CI: 1.77-12.25); p=0.002). In conclusion, low patient satisfaction with healthcare providers is associated with greater healthcare utilization among U.S. adults with psoriasis.

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Gender-related characterization of cutaneous sensory symptoms in Chinese with skin disorders

X. Wang[1], Q. Lai[2], B. Zheng[1], L. ye[1], S. Wen[2], Y. Yan[1], P. Elias[3], B. Yang[1]

[1]Dermatology, Dermatology Hospital of Southern Medical University, Guangzhou, China, [2]Center of Chronic Disease Prevention, Puning, Guangdong, China, [3]Dermatology, University of California San Francisco, San Francisco, California, United States

Background: Although development of cutaneous sensory symptoms in skin disorders is known, features of dermatosis-associated cutaneous sensory symptoms have not been characterized yet. Objective: To characterize cutaneous sensory symptoms in Chinese with skin disorders. Subjects and Methods: A questionnaire was given to outpatients to identify self-proclaimed sensory symptoms at dermatology clinic. Prevalence, clinical symptoms and severity of sensory symptoms were analyzed. Results: A total of 2144 patients, including 1254 females and 890 males, aged 13-94 years, included in this study. Prevalence of cutaneous sensory symptoms was higher in females than in males (p<0.0001). In general, inflammatory skin disorders, including atopic dermatitis, eczematous dermatitis, psoriasis, displayed higher prevalence of cutaneous sensory symptoms (47-82%) in comparison to non-inflammatory skin disorders, such as alopecia areata, verruca vulgaris and vitiligo (1-22%). Moreover, subjects with a family history of sensitive skin also exhibited higher prevalence of cutaneous sensory symptoms in comparison with those without family history (p<0.0001). Likewise, high prevalence of cutaneous sensory symptoms was observed in subjects with either dry or oily skin as compared with those with normal skin. The sensitive scales of all symptoms except pain and itching were higher in females than in males. Triggering factors were associated with both gender and type of skin disorders. Taken together, these results demonstrate that prevalence, sensitive scales and triggering factors of cutaneous sensory symptoms are associated with gender and type of skin disorders. Conclusion: Prevalence, triggering factors and symptoms of cutaneous sensation vary with gender and skin disorders.

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The association between antibiotics for acne and subsequent infection sequelae and antimicrobial resistance: A systematic review

K. Bhate[2], L. Lin[2], J. Barbieri[1], R. Mathur[2], S. Sinnott[2], S. Langan[2]

[1]University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom

Background: Antimicrobial resistance (AMR) is a major global health priority and antibiotics are the leading contributor to increasing AMR. Acne is highly prevalent and antibiotics are widely used in its treatment. Average antibiotic use for acne ranges from a few months to many years. The dominant role antibiotics play in the treatment of healthy people with acne leads to questions about associations with AMR. Aim: To systemically search for and synthesize evidence around whether long-term oral antibiotic use in the treatment of acne in those over 8 years of age contributes to increased risk of infection or other outcomes suggestive of AMR. Method: We searched the following databases: Embase, MEDLINE, Cochrane and Web of Science using strategies developed with a librarian. Searches ran in July 2019 and date back to database inception. Inclusion criteria: RCT, cohort or case-control studies investigating oral antibiotics for minimum of 28 days compared to those with acne not treated with oral antibiotics or the general population. Primary outcome: antibiotic treatment failure or infection caused by a resistant organism. Data extraction and bias assessment using ROBINS-I were undertaken by three reviewers with medical and epidemiological training. Results: 6996 abstracts and titles were screened for eligibility, 73 full text papers were reviewed and seven studies were included comprising one RCT, five cohort studies and one case-control study. Due to heterogeneity it was not possible to perform meta-analysis. Four studies investigated changes to flora (total across studies n=141,20-60) and three susceptibility of infection (total across studies n=35,019,102-34,623) including pharyngitis and respiratory infections. Studies had mixed findings and most had a serious or critical bias risk. Conclusion: The relationship between long-term antibiotics for acne and infectious or resistance sequelae needs to be urgently addressed with more rigorous studies.

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Estimation of cutaneous squamous cell carcinoma incidence attributable to arsenic in U.S. water supplies

J. W. Lee[1], K. Ratnakumar[1], K. Bomsztyk[2], M. Kawasumi[1]

[1]Dermatology, University of Washington, Seattle, Washington, United States, [2]Allergy and Infectious Diseases, University of Washington, Seattle, Washington, United States

Arsenic in water supplies potentially exposes millions of people to increased disease risk worldwide. Despite strengthened regulation, arsenic concentrations commonly found in U.S. water supplies may increase the incidence of cutaneous squamous cell carcinoma (cSCC). We estimated cSCC incidence attributable to arsenic in water supplies among U.S. non-Hispanic whites, a demographic with disproportionately high skin cancer incidence. We determined the number of people exposed to various concentrations of arsenic in water supplies by analyzing three datasets: urine samples from the National Health and Nutrition Examination Survey (2015–2016), public water supply data from the Environmental Protection Agency (EPA) Six-Year Review 3 (2006–2011), and private well user data from Ayotte et al. (2017). Among 743 representative patients, total arsenic concentrations in urine had a median (interquartile range) of 3.00 µg/l (2.25–4.02). Public water supplies serving 10 million out of 151 million non-Hispanic white users had detectable levels of arsenic. An estimated 1.6 million out of 33 million non-Hispanic white private well users had water arsenic concentrations above 10 µg/l (EPA limit for public water). These data were combined with published odds ratios for cSCC incidence with arsenic exposure, assuming that total arsenic concentrations in urine less than 4.76 µg/l do not affect cSCC risk. Based on urinary arsenic data, 32,512 out of 2,548,845 cSCCs annually in the U.S. are attributable to arsenic in water supplies. This estimate includes 26,228 cSCCs among public water users and 6,284 among private well users. Separately, water supply data suggest that at least 10,361 and 4,380 cSCCs are attributable to arsenic in public water supplies and in private wells, respectively. Overall, up to 1.3% of cSCC incidence in the U.S. may be attributed to arsenic in current water supplies. Thousands of cSCCs may be prevented by further restricting arsenic in U.S. water supplies.

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Factors associated with late initiation of adjuvant radiotherapy in Merkel cell carcinoma

A. O. King[1], V. Lee[2], F. N. Mirza[1], Y. An[2]

[1]Dermatology, Yale School of Medicine, New Haven, Connecticut, United States, [2]Therapeutic Radiology, Yale School of Medicine, New Haven, Connecticut, United States

Introduction: Merkel Cell Carcinoma (MCC) is a rare skin malignancy with risk for locoregional recurrence and metastasis after definitive surgery. Several retrospective studies have suggested a survival benefit to be associated with the use of adjuvant radiotherapy (RT) after primary surgical resection and adjuvant RT is considered standard of care for high risk patients. Ideally, adjuvant RT should be initiated promptly after resection as delays in adjuvant therapy may allow tumor to re-grow. Methods: We examined 4,615 cases of MCC in the National Cancer Database (NCDB). Patients that were diagnosed at age≥18, pathologic stage 1, and pathologic stage 2 were included. Patients with nodal involvement, no surgery, or palliative radiation were excluded. Patients who started RT more than 42 days after definitive surgery were considered to have received late adjuvant RT. Results: Factors associated with later initiation of adjuvant RT included a primary tumor site of the upper limbs and shoulder vs. the head and neck (Odds Ratio [OR] 0.73, 95% Confidence Interval [95% CI] 0.54-0.98, p<0.041), tumor size of 1-2 cm vs. tumor size <1 cm (OR 0.69, 95% CI 0.50-0.94, p=0.020), a tumor size >2 cm vs. tumor size <1 cm (OR 0.67, 95% CI 0.48-0.93, p=0.018), and lymphovascular invasion (OR 0.70, 95% CI 0.52-0.92, p=0.012). Discussion: An understanding of factors associated with a greater time interval following primary resection to initiation of adjuvant RT may shed light on how timeliness of RT initiation is prioritized for patients in practice. In line with treatment guidelines, this data suggests that clinicians are initiating adjuvant radiation treatment earlier in patients with more severe disease.

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Implementing a teledermatology patient-facing mobile application in the VA

A. Fonseca[1, 2], S. Peracca[4], O. Lachica[4], I. Morris[6], H. King[6, 5], G. Jackson[6, 5], J. Whited[6, 5], D. H. Oh[3, 4], M. A. Weinstock[1, 2]

[1]Dermatology, Brown University, Providence, Rhode Island, United States, [2]Center for Dermatoepidemiology, Providence Veterans Affairs Medical Center, Providence, Rhode Island, United States, [3]Dermatology, University of California, San Francisco, San Francisco, California, United States, [4]San Francisco Veterans Affairs Health care System, San Francisco, California, United States, [5]Duke University School of Medicine, Durham, North Carolina, United States, [6]Durham Veteran Affairs Health Care System, Durham, North Carolina, United States

Enhancing access to care is a general problem for health care and understanding how technological interventions are successfully implemented is increasingly important in a digital health care world. To identify key factors for implementing a teledermatology app in a large healthcare system, we report preliminary qualitative findings from evaluation of a patient-facing app that is being deployed by the U.S. Department of Veterans Affairs (VA) to provide dermatology care. The app allows patients to follow up with dermatologists remotely rather than in-person. Data analyzed consisted of 17 individual organizational readiness for change (ORC) semi-structured telephone interviews with implementing teams involved in app usage from 3 field test sites to assess factors that affect app adoption. We evaluated which factors were most salient to ORC. While participants at all field test sites agreed that the use of the app would increase Veterans' access to dermatologists and save both dermatologists' and Veterans' time, app usage was low amongst both providers and Veterans. Staff at all sites thought that younger or more tech savvy patients would have more benefit using the app. Additionally, staff expressed concerns regarding clinical workflow and staffing support. When implementing a new app, the results suggest that it is important to consider the following factors 1) providing in-person as well as remote training to dermatologists and providing easily accessible resources on use of the app, 2) considering clinician and staff workload and availability, 3) introducing additional incentives for providers, and 4) advertising the availability and requirements of the app to appropriate patients.

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Differences in topical corticosteroid prescribing patterns between primary care providers and dermatologists for atopic dermatitis

I. Singh[1], A. Borba[2], A. J. Uy[1], A. Y. Chen[1], A. W. Armstrong[1]

[1]Department of Dermatology, University of Southern California Keck School of Medicine, Los Angeles, California, United States, [2]Geisinger Commonwealth School of Medicine, Scranton, Pennsylvania, United States

Atopic dermatitis (AD) is a chronic inflammatory skin disease. Many patients with AD seek care from both primary care physicians and dermatologists. However, little is known regarding topical corticosteroid prescribing patterns between these two specialties. We sought to determine if differences exist in the topical corticosteroid (TCS) prescribing patterns between dermatologists, family medicine physicians, and internal medicine physicians. We conducted a population-based, cross-sectional analysis using data from the National Ambulatory Medical Care Survey (NAMCS) from 2006 to 2016. There were 5,071,158 (weighted) outpatient AD visits between 2006 and 2016 for adults who were seen by physicians from family medicine, internal medicine, and dermatology. There was not a statistically significant difference in the rate of TCS prescriptions for AD between family medicine physicians and dermatologists (39.1% vs. 52.2%; p=0.27). However, family medicine physicians had a higher rate of prescribing TCS for AD than internal medicine physicians (39.1% vs. 5.1%; p=0.002). Dermatologists had a significantly higher rate of prescribing TCS for AD compared to internal medicine physicians (52% versus 5%; p<0.001). Our findings demonstrate that dermatologists prescribe topical corticosteroids for atopic dermatitis more frequently compared to internal medicine physicians but not in comparison to family medicine physicians. It is important to understand the key differences in practice patterns among medical specialties for AD care and identify educational gaps.

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Pruritus tools for 6-7-year-old children

H. Kong[1], S. Francois[1], S. Smith[1], M. Spraker[1], L. Lawley[1], G. Lee[1], K. Chen[1], J. Roberts[2], S. C. Chen[1, 3]

[1]Emory School of Medicine, Atlanta, Georgia, United States, [2]School of Psychology, Georgia Institute of Technology, Atlanta, Georgia, United States, [3]Regional TeleHealth Service, VISN 7, Atlanta, Georgia, United States

Various tools have been developed to quantitatively assess the severity and the quality of life (QoL) impact of pruritus in adults. However, due to barriers of language and comprehension, such tools are not directly applicable to the children. We address this gap by developing instruments for the pediatric population. We report on the 14-item QoL (Kids_ItchyQoL) and a self-reported severity (Kids_ItchyQuant) tool that measure impact and severity of pruritus for the preceding week in patients aged 6-7 years. Items were derived from in-depth interviews with children by asking them to draw their itch, and using the drawings to elicit concepts. We found the cartoon-annotated Kids_ItchyQoL to be reliable (Cronbach's a = 0.846) and reproducible (Intra-class correlation coefficient, ICC=0.66). The Kids_ItchyQuant was reproducible as well (ICC= 0.47). With respect to construct validity, exploratory factor analysis (EFA) techniques suggested three dominant factors. Four 3-dimensional confirmatory factor analysis models were subsequently explored, and of these, a simple structure model with correlated factors and item assignments determined by an initial EFA proved best (AIC=-99.66 and BIC =-292.44). When the Kids_ItchyQoL was analyzed as a function of worsening, improvement, or no change at their final visit, we confirmed responsiveness: the mean total scores decreased the least for the worsening cohort; the mean change decreased the most for the improved and no change groups. For the Kids_ItchyQuant, there was a statistically significant difference in the score changes (p=0.005, GLM procedure) with the biggest decrease in mean itch in the improvement cohort, less in the no change, and slight increase in the worsening group. These results suggest a new set of reliable and valid instruments to assess QoL and severity of pruritus in 6-7-year-old children.

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Dupilumab treatment provides continued improvements in signs and symptoms through week 100 in patients with moderate-to-severe atopic dermatitis who did not achieve ≥75% reduction in eczema area and severity index at week 16

A. W. Armstrong[1], A. Blauvelt[2], E. Simpson[3], Z. Chen[4], M. Ardeleanu[4], A. B. Rossi[5], P. Tomondy[5]

[1]Keck School of Medicine, University of Southern California, Los Angeles, California, United States, [2]Oregon Medical Research Center, Portland, Oregon, United States, [3]Oregon Health and Science University, Portland, Oregon, United States, [4]Regeneron Pharmaceuticals, Inc., Tarrytown, New York, United States, [5]Sanofi Genzyme, Cambridge, Massachusetts, United States

Atopic dermatitis (AD) is a chronic inflammatory skin disease requiring long-term management. Here, we report dupilumab efficacy up to Week (Wk) 100 of an open-label extension (OLE) study (NCT01949311) in patients with moderate-to-severe AD who did not have an improvement of ≥75% in Eczema Area and Severity Index (EASI-75) or ≥50% (EASI-50) at Wk16 in two parent studies (PS). Patients not achieving Investigator's Global Assessment (IGA) 0/1 or ≥3-point improvement in pruritus Numerical Rating Scale (NRS) at Wk16 in PS were also assessed. Patients included in this analysis participated in PS LIBERTY AD SOLO 1&2 (NCT02277743, NCT02277769) and were subsequently enrolled in LIBERTY AD OLE assessing long-term safety and efficacy, where all patients received dupilumab 300mg weekly (qw). 462/457/460 patients enrolled in SOLO 1&2 in the dupilumab 300mg qw/every 2 weeks (q2w)/placebo (PBO) groups, respectively, of whom, 169/195/330 patients did not achieve EASI-75 at Wk16. Among them, 91.1%/91.0%/88.8% achieved EASI-75 at OLE Wk100, in the qw/q2w/PBO groups, respectively. In addition, among EASI-50 non-achievers in SOLO 1&2 (qw/q2w/PBO: 127/124/290), almost all patients reached EASI-50 at OLE Wk100 (97.4%/98.8%/98.3%, respectively). Similar results were observed for patients who did not achieve IGA 0/1 or ≥3-point NRS improvement at Wk16 of PS, but did so at Wk100. Among patients with moderate-to-severe AD not achieving EASI-75, EASI-50, IGA 0/1, or ≥3-point NRS improvement at Wk16 in SOLO 1&2, a large proportion of patients achieved these respective endpoints after 100 weeks of dupilumab 300mg qw, regardless of treatment received in the PS.

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Assessing deep learning artefact bias using global saliency

J. Pfau[1, 2], A. T. Young[3, 2], M. Wei[1, 2], M. J. Keiser[1]

[1]University of California, San Francisco, California, United States, [2]Veterans Affairs Medical Center, San Francisco, California, United States, [3]Dermatology, University of California, San Francisco, San Francisco, California, United States

Deep learning convolutional neural networks (CNNs) are the dominant method for automated visual assessment, including skin lesion diagnosis. CNNs are prone to over-reliance on spurious artefacts in the training data, such as surgical ink markings in dermatology or X-ray tags in radiology, and consequently they may fail to generalize to new patient cohorts. We developed a method to assess the degree to which a CNN has acquired such an artefact-induced bias, which we term "global saliency." Global saliency works by aggregating saliency map information across all images in the validation set. Specifically, global saliency involves computing overlap between saliency map pixels and artefact segmentation mask pixels in order to determine whether a CNN is erroneously paying attention to the artefact. For example, we found that while CNNs reach dermatologist-level performance on selected public datasets, models may nevertheless incorrectly confound ink markings for darkly pigmented lesions such as melanoma, nevus and seborrheic keratosis. We use saliency map information to anticipate model error and correlate undesired saliency on ink marking with prediction error, achieving Kendall's τ=-0.889, P<0.0001. To reduce the biasing effect of ink markings on the model, we used a training time sampling strategy in which we exposed the model to corrected ratios of inked and un-inked images across each disease, removing correlation between ink presence and disease. This strategy reduced the bias of the model as measured by global saliency and encouraged the model to reject samples which had excessive levels of inking. We conclude that global saliency offers a quantitative alternative to manual inspection for detecting and measuring artefact-induced bias in a CNN, enabling automation of rejection of CNN models or individual predictions that are likely to be in error.

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Dupilumab provides early and sustained, clinically-meaningful improvements in both adults and adolescents: A post hoc analysis of patients not achieving Investigator's Global Assessment of 0/1 in phase 3 trials, LIBERTY AD SOLO 1 and 2, and LIBERTY AD ADOL

E. Simpson[1], L. A. Beck[2], M. Boguniewicz[3, 4], Z. Chen[5], M. Ardeleanu[5], A. Bansal[5], D. Sierka[6]

[1]Oregon Health and Science University, Portland, Oregon, United States, [2]University of Rochester Medical Center, Rochester, New York, United States, [3]National Jewish Health, Denver, Colorado, United States, [4]University of Colorado School of Medicine, Denver, Colorado, United States, [5]Regeneron Pharmaceuticals, Inc., Tarrytown, New York, United States, [6]Sanofi Genzyme, Cambridge, Massachusetts, United States

This post hoc analysis investigated the proportion of patients with moderate-to-severe atopic dermatitis (AD) who achieved clinically-meaningful responses in ≥1 of the signs, symptoms, or quality of life (QoL) domains of AD, despite not achieving an Investigator's Global Assessment (IGA) score of 0/1. Data from three phase 3 clinical trials (SOLO 1/2 [adults]: NCT02277743/NCT02277769; ADOL [adolescents]: NCT03054428) were utilized. SOLO patients received 300mg dupilumab weekly (qw)/every 2 weeks (q2w), or placebo. ADOL patients received 200/300mg dupilumab q2w, 300mg q4w, or placebo. Clinically-meaningful responses in the AD domains were defined as: ≥50% reduction from baseline in Eczema Area and Severity Index; ≥3-point reduction from baseline in weekly average Peak Pruritus Numerical Rating Scale score; or ≥4-point or ≥6-point reduction from baseline in Dermatology Life Quality Index scores in adults and adolescents, respectively. Among patients not achieving IGA 0/1, clinically-meaningful outcomes in ≥1 of the 3 AD domains were observed early in patients receiving dupilumab and sustained at Week 16: in 52.7%/62.8%/28.3% adults receiving qw/q2w/placebo, respectively (*P*<0.0001 vs placebo), and in 74.2%/55.1%/21.7% adolescents receiving q2w/q4w/placebo, respectively (*P*<0.0001 vs placebo). The majority of patients treated with dupilumab who did not achieve an IGA score of 0/1 at Week 16 achieved clinically-meaningful improvements in AD signs, symptoms, and QoL. Dupilumab was generally well tolerated with an acceptable safety profile.

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Complementary and alternative medicine (CAM) use in patients with cutaneous lymphoma

H. M. Mahurin[1], E. Tarabadkar[2], D. S. Hippe[3], K. Lachance[4], M. M. Shinohara[4]

[1]University of Washington School of Medicine, Seattle, Washington, United States, [2]Department of Dermatology, Emory University, Atlanta, Georgia, United States, [3]Department of Radiology, University of Washington, Seattle, Washington, United States, [4]Division of Dermatology, University of Washington, Seattle, Washington, United States

Primary cutaneous lymphomas are a group of rare non-Hodgkin lymphomas that can cause significant morbidity and adversely affect patients' quality of life (QoL). Complementary and alternative medicine (CAM) includes therapies that are not part of traditional Western medical care. CAM therapies have demonstrated potential benefits in both cancer and skin disease, however, there are few studies examining patterns of CAM use among patients with cutaneous lymphoma (CL). We performed a cross-sectional study assessing CAM use and QoL in patients with CL via an electronic survey administered to patients via the Cutaneous Lymphoma Foundation from February-April 2019. A total of 300 patient responses (67% female, mean age 57y) were included in analysis. The most common type of CL among respondents was mycosis fungoides (84%, n=246), followed by Sézary Syndrome (12%, n=35). A majority (58%) of patients reported using CAM for their CL, with 48% using CAM to treat their disease and 46% using CAM to manage their symptoms. The most commonly used CAM in our cohort were vitamins/minerals (32%), prayer/meditation (26%), diet (24%), and exercise/yoga (22%). QoL as assessed by the Skindex-16 and Functional Assessment of Cancer Therapy-General (FACT-G) was worse among patients who reported CAM use; Skindex scores were 51 ± 27 among CAM users compared to 38 ± 26 for non-CAM users (p<0.001). Higher itch scores were reported by patients using CAM compared to non-users (37 ± 29 and 26 ± 26 respectively; p=0.002). CAM use is common among patients with CL, and CAM use is higher among those with worse itching and worse QoL.

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Biomarkers of alopecia areata in blood reveal systemic immune and cardiovascular abnormalities

J. Glickman[1], C. Dubin[1], D. Dahabreh[1], K. Auyeung[1], Y. Estrada[1], G. Singer[1], G. Kimmel[1], A. Pavel[1], J. Krueger[2], E. Guttman-Yassky[1]

[1]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [2]Laboratory for Investigative Dermatology, The Rockefeller University, NY, New York, United States

Alopecia areata (AA) is a common nonscarring hair loss disorder with a lifetime risk of 2%. Although AA is characterized by Th1/IFN-skewing, with additional Th2 and IL-23 activation in scalp tissues, little is known about its systemic profile in blood. To evaluate the blood proteomic signature of AA and determine serum biomarkers associated with increased disease severity, we assessed ~350 inflammatory and cardiovascular proteins using OLINK high-throughput proteomics in 35 moderate-to-severe AA patients (>30% scalp involvement, mean age=43.17 years; mean SALT=74.98), in comparison with age-matched healthy individuals, and as a point of reference also to moderate-to-severe psoriasis (n=19, mean PASI=20.43), and atopic dermatitis/AD patients (n=36, mean SCORAD=61.35). 74 proteins were significantly differentially expressed between AA and controls (FCH>1.3, FDR<.05) including innate immunity (IL-6, IL-8), Th1 (CXCL9/CXCL10/CXCL11/IFNG), Th2 (CCL13/CCL17/CCL7), and Th17 markers (CCL20/PI3/S100A12). 86 biomarkers were correlated with clinical severity in AA patients (P<.05) including Th1 (CCL3), Th2 (CCL11/CCL13), innate (IL8) and Th17 markers (S100A12). Many cardiovascular/atherosclerosis-related proteins were significantly higher in AA compared to controls, and also correlated with severity, including SELP, SRC, AXIN1, MPO, IL18, and OSM (P<.05). Pathway analysis showed significant increases in cardiovascular/atherosclerosis, and immune pathways compared to controls (FCH>4.0, FDR<.001), which also correlated with clinical severity (P<.05). This study defined the abnormalities of moderate-to-severe AA and associated circulatory biomarkers. It shows that AA is a systemic disease with immune, cardiovascular and atherosclerosis dysregulation, highlighting the need for systemic treatment approaches.

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Teledermatology-based inpatient consult rotations engender autonomy for trainees

R. Rolader[1], J. Cheeley[1], L. Lawley[1], C. Haydeck[1], J. Mackelfresh[1], S. C. Chen[1, 2]

[1]Emory University, Atlanta, Georgia, United States, [2]Regional TeleHealth Service, VISN7, Atlanta, Georgia, United States

Preparation for independent practice is essential for graduation from residency yet promoting resident autonomy without compromising faculty supervision is a challenge. In our program, senior residents gain independence during a unique inpatient consult service where attending oversight is provided via teledermatology (TD), with in-person back up available. We surveyed recent graduates of our program to compare levels of perceived autonomy between the TD-based staffing against a traditional in-person model. We also explored perceptions of potential TD-based staffing for inpatient pediatric consults. Of the 29 graduates queried, 66% responded; 7 practice in an academic setting and 12 provide inpatient consultation. Most graduates felt that both the TD-based rotation and in-person patient consult rotations overall were "extremely helpful" in preparing them for future practice (74% for each). Most graduates strongly agreed that TD-based oversight provided greater autonomy in comparison to in-person attending oversight (74%) and that being the only dermatology provider physically present on the TD-based rotation helped cultivate independence for future practice (84%). The majority agreed that more senior dermatology rotations should provide TD-based attending oversight ("strongly agree"=32%;"agree"=26%). Interestingly, 58% either disagreed (21%) or felt neutral (37%) about having a similar TD-based model for inpatient pediatric dermatology consultation. Free text comments expressed concern for the complex nature of pediatric dermatologic conditions that were felt to necessitate in-person attending oversight for every consult. Overall, we found TD-based attending oversight for inpatient dermatology consultation to be of great benefit to dermatology residents. We believe that this unique educational opportunity engenders autonomy for residents as they prepare for future practice. The discrepant comfort level with TD-based staffing for adult versus pediatric dermatology consultation warrants further investigation.

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Natural history and management of basal cell nevus syndrome: Updates from the gorlin syndrome registry
V. Eng, G. Saldanha, S. Li, I. Bailey-Healy, J. Teng, J. Tang
Dermatology, Stanford University School of Medicine, Redwood City, California, United States
Background: Patients with basal cell nevus syndrome (BCNS) are at increased risk of developing basal cell carcinomas (BCCs). Long-term data on tumor burden, co-morbidities, and management of BCNS is limited. Method: A prospective, cross-sectional study of self-reported questionnaire responses collected from BCNS patients from Feb 2012 to Oct 2016 through the national Gorlin Syndrome Registry. BCC burden was characterized based on frequency and anatomic distribution. Logistic regression analysis was performed to determine the association of BCC development with risk factors such as sex, family history, age of diagnosis/symptoms, and sun exposure. Treatment of BCCs and other co-morbid tumors are additionally characterized. Results: 87 BCNS participants (current age: 39.8 ± 20.0 years; age at diagnosis: 16.5 ± 12.4 years) reported a median of 172 BCCs (range 1 – 1715 BCCs) developing over their lifetime. The number of lifetime BCCs significantly associated with family history of BCNS ($p = 0.02$) and age (Lifetime BCCs = 5.4*Age, $p < 0.0001$). A median of 100 BCCs presented on the head, 56 BCCs on the trunk and extremities, and 10 BCCs on the breast and groin. Of the 27/87 (31%) participants with locally advanced or metastatic BCCs, roughly half (13/27) had tried a hedgehog inhibitor such as vismodegib, sonidegib, or itraconazole. Participants with BCNS are found to have an increased prevalence of tumors of the skin (KCOT, actinic keratosis, SCC, melanoma), brain (meningioma, medulloblastoma), and ovaries (fibroma, cyst). Conclusion: The results of this study demonstrate the high burden of BCCs among patients with BCNS. BCCs predominantly developed on sun-exposed area and strongly correlated with increased age. Additional interventions to prevent and treat BCCs are needed. This study establishes a clinical baseline for emerging therapies such as hedgehog inhibitors in the BCNS population.

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Treatment of actinic keratoses in veterans living with HIV
H. Yeung[1, 2], C. Adler[1, 2], C. W. Kwon[1, 2], S. C. Chen[1, 2], J. Guest[3]
[1]Dermatology, Emory University School of Medicine, Atlanta, Georgia, United States, [2]Regional Telehealth Services, Veterans Affairs, Veterans Integrated Service Network 7, Decatur, Georgia, United States, [3]Epidemiology, Emory University Rollins School of Public Health, Atlanta, Georgia, United States
Actinic keratoses (AK) are common premalignant skin lesions which may progress to become keratinocyte carcinomas. Risk factors for AK development are age, fair skin, and immunosuppression including HIV infection. While field-directed AK therapy is efficacious and may prevent keratinocyte carcinoma development, its use among patients living with HIV remains unknown. This retrospective cohort study aimed to describe AK treatment patterns in the HIV Atlanta Veterans Affairs Cohort Study (HAVACS). Data were extracted using the Veterans Affairs HIV Clinical Case Registry. AK diagnosis was defined by at least one ICD-9 or -10 code (702.0 or L57.0) during a follow-up visit. Any use of AK treatments, such as lesion-directed cryotherapy (CPT: 17000-17004) and field-directed therapies (topical 5-fluorouracil, imiquimod, diclofenac, ingenol mebutate, and photodynamic therapy) were summarized. A total of 3,480 patients in HAVACS with at least 2 infectious disease clinic visits were followed up for a mean (SD) of 8.1 (6.5) years. Among this cohort, 98 (2.8%) patients had at least 1 diagnosis of AK. For AK treatment, 63 patients (64%) underwent lesion-directed cryotherapy, 22 patients (22%) received 5-fluorouracil, 14 patients (14%) received imiquimod, 7 patients (7%) received diclofenac, 1 patient (1%) received ingenol mebutate, and 1 patient (1%) underwent photodynamic therapy. Our small data were limited to a single Veterans Affairs center, which may not be generalizable to other settings. Field-directed AK therapies were prescribed only in a minority of patients living with HIV. Optimization of field-directed AK therapy has the potential to prevent keratinocyte carcinoma development in patients living with HIV.

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Atopic dermatitis and risk of major neuropsychiatric disorders: A population-based cohort study
J. Wan[1], D. Shin[1], M. Syed[1], K. Abuabara[2], J. Gelfand[1]
[1]University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]UCSF, San Francisco, California, United States
Atopic dermatitis (AD) is a chronic skin disease now recognized to have systemic inflammatory effects which may include neuro-immunological abnormalities that are increasingly implicated in neuropsychiatric disorders. Although AD has been previously associated with anxiety, depression, and attention-deficit/hyperactivity disorder (ADHD), longitudinal studies of both children and adults are scarce. We conducted a cohort study using a U.K. population-based electronic health records database to examine the association between AD and several major psychiatric and neurodevelopmental disorders in both children and adults. A total of 644,802 adults and 434,859 children with AD were identified using a previously validated algorithm and matched on age, practice, and index date with 2,877,347 adult and 1,983,589 pediatric controls. Using Cox regression, we found that adults with AD were at greater risk for incident depression (HR 1.17, 95% CI 1.16-1.18), anxiety (1.19, 1.18-1.20), bipolar disorder (1.14, 1.06-1.23), obsessive-compulsive disorder [OCD] (1.52, 1.43-1.62), ADHD (1.38, 1.18-1.61), and autism (1.59, 1.35-1.87) after adjusting for sex, age, and socioeconomic status. In children, the effects were mostly similar but attenuated: depression (1.09, 1.07-1.11), anxiety (1.11, 1.09-1.13), bipolar disorder (1.38, 1.13-1.70), OCD (1.34, 1.25-1.45), ADHD (1.05, 1.01-1.09), autism (1.02, 0.98-1.06). AD was not significantly associated with schizophrenia in adults (1.02, 0.92-1.14) nor children (0.87, 0.68-1.11). Similar results were observed in models excluding patients with asthma or allergic rhinitis and using logistic regression to calculate prevalent odds of outcomes. Our findings from a large population-based prospective study suggest that AD is associated with several major psychiatric and neurodevelopmental disorders among both children and adults and set the stage for further research on potential mechanisms, such as AD symptoms e.g. itch and poor sleep, stigma of chronic skin disease, or shared pathophysiology.

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Antihypertensives and risk of melanoma and keratinocyte carcinoma: A systematic review and meta-analysis
O. G. Cohen[1], M. Taylor[3], S. H. Giordano[2], S. Langan[4], D. J. Margolis[1], M. R. Wehner[2, 1]
[1]Dermatology, University of Pennsylvania, Philadelphia, District of Columbia, United States, [2]Health Services Research, MD Anderson Cancer Center, Houston, Texas, United States, [3]Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania, United States, [4]Epidemiology, London School of Hygiene & Tropical Medicine, London, United Kingdom
There are many papers on the association between antihypertensive drugs and skin cancers, with conflicting results. Three recent meta-analyses on this topic have included different articles and produced different outcomes. Additionally, several new papers were not included in prior meta-analyses, which could impact the conclusions. We conducted a systematic review and random-effects meta-analysis to evaluate the most contemporary evidence on antihypertensives and basal cell carcinoma (BCC), squamous cell carcinoma (SCC), and melanoma. We identified 5793 articles. After title/abstract screening, we assessed 88 full text articles, and included 26. There were 18 drug class-skin cancer outcome combinations from 21 articles (including more than 10 million patients) that could be meta-analyzed. We found statistically significant results for ACE inhibitors (melanoma summary relative risk [sRR] 1.08, 95% CI 1.03-1.14), calcium channel blockers (melanoma sRR 1.07, 95% CI 1.01-1.13; SCC sRR 1.08, 95% CI 1.01-1.14; BCC sRR 1.17, 95% CI 1.11-1.22), thiazides (SCC sRR 1.50, 95% CI 1.00-2.25), and non-thiazide diuretics (SCC sRR 1.27, 95% CI 1.10-1.47; BCC sRR 1.06, 95% CI 1.03-1.10). In a qualitative evaluation, only three of eight articles reported a robust dose-response, though two supported our meta-analysis results: thiazides (BCC and SCC), loop diuretics (BCC), and 'photosensitizing antihypertensives' (SCC). Taken together, the results of our meta-analyses and the evaluation of dose-response reflect a need for more research to understand whether observed associations between different antihypertensives and skin cancers are causal.

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Temporal trends in the incidence of metastatic melanoma and utilization of immunotherapy in the United States

T. Bhat[1], C. Herbosa[1], W. Hodges[1], S. Kwatra[4], K. Reynolds[2], D. Chen[1], L. Cornelius[1], Y. Semenov[3]

[1]Dermatology, Washington University School of Medicine, St. Louis, Missouri, United States, [2]Oncology, Massachusetts General Hospital, Boston, Massachusetts, United States, [3]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [4]Dermatology, Johns Hopkins University, Baltimore, Maryland, United States

The aim of this study was to investigate temporal trends in the incidence of metastatic melanoma (MM) and immunotherapy utilization as well as the impact of geography on these trends. We performed a retrospective cohort study of 12,544 patients with MM extracted from Truven MarketScan®, a national private insurance claims database, from 2011-2015. Immunotherapy use was documented for claims for ipilimumab, nivolumab, or pembrolizumab within 180 days of MM diagnosis. Geographic regions were defined by standard US census regions, with the Northeast set as reference. The incidence rate of MM remained stable from 67.6 to 67.1 per million person-years from 2011-2015 (p=0.94; annual percentage change 0.3%, 95% CI -7.2%, 8.4%). Immunotherapy utilization among eligible patients increased from 4.0% in 2011 to 21.4% in 2015. Time to immunotherapy after MM diagnosis decreased from 77.9 to 71.7 days from 2011-2015. Multivariable logistic regression models revealed that female gender (OR 0.70, 95% CI 0.59-0.82), residence in the South (OR 0.78, 95% CI 0.62-0.98), and higher aggregate Charlson Comorbidity score (OR 0.85, 95% CI 0.83-0.88) significantly decreased the odds of immunotherapy use. Though immunotherapy utilization increased over time, it was still lower than expected given the role of immunotherapy as standard of care for MM. Residence in the South decreased the odds of immunotherapy use. Though the incidence of melanoma continues to rise, MM incidence has remained stable, potentially reflecting variation in diagnosis of borderline lesions. Nonetheless, our data over 5 years shows that the delay between MM diagnosis and initiation of immunotherapy has declined, though it remained greater than 2 months in 2015. Future research is necessary to investigate why immunotherapy, a life-saving therapy, has been under-prescribed.

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Survival and glycemic control in patients with squamous cell carcinoma

S. A. Ederaine[1], J. Dominguez[1], J. Harvey[2], A. Mangold[2], C. Cook[4], H. Kosiorek[3], M. Buras[3], K. Coppola[5], P. Verona[6], N. Karlin[7]

[1]Mayo Clinic Alix School of Medicine , Scottsdale, Arizona, United States, [2]Dermatology, Mayo Clinic , Scottsdale, Arizona, United States, [3]Health Sciences Research, Mayo Clinic, Scottsdale, Arizona, United States, [4]Endocrinology, Mayo Clinic, Scottdale, Arizona, United States, [5]Cancer Quality Program, Mayo Clinic, Scottsdale, Arizona, United States, [6]Information Technology, Mayo Clinic, Phoenix, Arizona, United States, [7]Hematology & Medical Oncology, Mayo Clinic Cancer Center, Phoenix, Arizona, United States

This study examined the impact of diabetes mellitus (DM) on survival in head and neck and cutaneous squamous cell cancer (SCC) patients, and the impact of SCC on glycemic control in DM. Patients with newly diagnosed SCC (n=95) were identified from the institutional cancer registry between the years 2007-2017 and matched to 95 patients with SCC without DM based on age, gender, and year of SCC diagnosis. Data on DM, cancer therapies and laboratory results were culled from the electronic medical record. Overall survival (OS) and recurrence-free survival (RFS) were estimated using the Kaplan-Meier method and compared by Cox regression analysis using stratification for matched pairs. Hemoglobin A1c (HbA1c) and glucose level during the year following cancer diagnosis were compared using mixed models. The median follow-up time was 47 months in alive patients (range 2.1 -124.3 months). For glucose, DM group status was significant (p<0.001) as DM patients had higher glucose overall compared to non-DM. In mixed model analyses, HbA1c decreased over time in DM patients (p=0.04). In patients with DM, the 5-year OS was 61%, compared to 78% in patients without DM (p=0.004). The hazard ratio (HR) was 2.60, 95% CI 1.25-5.39, p=0.01). The 5-year RFS was 55% for patients with DM compared to 78% for patients without DM (p<0.001) (HR 3.33, 95% CI 1.58,-7.02, p=0.001). The presence of coexisting DM adversely impacted OS and RFS in patients with SCC. SCC cancer did not affect glycemic control. How DM interacts with SCC to worsen outcomes requires further study.

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Top 50 dermatology influencers on Twitter

T. M. Wells[1, 2], M. Maymone[4], C. W. Rundle[3], R. Dellavalle[4]

[1]Meharry Medical College , Nashville, Tennessee, United States, [2]Vanderbilt University, Nashville, Tennessee, United States, [3]Medicine, Loma Linda University, Redlands, California, United States, [4]Dermatology, University of Colorado Denver, Aurora, Colorado, United States

Social media has reshaped the interactions between patients and health care professionals. The increased visibility and accessibility of practitioners on social media has led to emergence “influencers” in all fields of medicine, including dermatology. The growing presence of dermatology influencers on social media prompts questioning of the identities of those influencers. In this study we sought to identify the top 50 dermatology influencers on Twitter, characterize who they are, and compare their average academic impact with their social media influence. The Insight API from Right Relevance was used to generate Twitter Influence scores for the topic search “dermatology” on January 5, 2020. The accounts associated with the highest influencer scores were ranked and recorded in a database. Each account was linked to an individual name. Practitioner classification, board certification, location, and academic h-index were also recorded in the database for each account. H-index scores were generated using the Publish or Perish software on January 6, 2020. Dermatologists comprised 84% of the top 50 Dermatology Twitter influencers. Internationally trained dermatologist made up 42% of influencers. Other medical doctors made up another 10% of influencers. Of the influencers, only 6% of influencers were non-physicians, and 58% of influencers were located in the U.S. The top five geographical locations were Spain (22%), New York (16%), California (14%), Texas (8%), and the UK (6%). Academic h-index of each physician social media influencer (n=47) ranged from 0 to 53 (mean, 11.55 ± 11.13). Limitations included the inability to verify board certification for internationally trained providers. This study shows that the top dermatology social media influencers on Twitter are predominantly board-certified dermatologists and physically based in the U.S. Further studies should be conducted to identify dermatology influencers across other social media platforms.

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Burden of malignant skin melanoma in Worldwide, 1990-2017: An analysis of the Global Burden of Disease Study 2017

W. Zhang, W. Zeng, Z. Liu, X. Dong, H. Luo, Z. Zheng, Z. He, T. Ye, H. Lu

Dermatology, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, China

Numerous population-based studies have documented high morbidity and mortality of malignant skin melanoma (MSM) in light-skinned people. To update on the global epidemiology of melanoma, we provide an estimate of the burden of melanoma using data from the Global Burden of Disease (GBD) Study 2017. We analysed detailed data on melanoma epidemiology (case number and age-standardized rate (ASR)) including incidence, mortality and the disability-adjusted life-years (DALYs) metric between 1990-2017, derived from the GBD study in 2017. Estimated annual percentage changes (EAPCs) in melanoma age standardized incidence, mortality and DALYs rate (ASIR, ASMR and ASDR), by sex and region, were calculated to quantify the temporal trends in melanoma ASR. Globally, in 2017, crude number of MSM incidence was 0.3 million (95% UI 0.2 to 0.4) with an increase in ASIR of MSM between 1990 and 2017. In 2017, it was estimated that 61,665 (95% UI 47910 to 70323) deaths of MSM occurred in worldwide. There was a slight decrease in ASMR of MSM between 1990 and 2017, from 0.85% (95% UI 0.71 to 1.07) to 0.78% (95% UI 0.61 to 0.89) in worldwide. The corresponding EAPCs of ASIR and ASMR were 1.18 (95%CI 1 to 1.35) and -0.35 (95%CI -0.41 to -0.3), respectively. There were the highest EAPC of ASIR of MSM both High-income Asia Pacific (3.06; 95%CI 2.78 to 3.33) and Central Europe (3.03; 95%CI 2.93 to 3.14) worldwide. The EAPC of ASMR of MSM in both sexes is highest from 1990 to 2017 was the Guatemala (2.64; 95%CI 2.01 to 3.28) of Central Latin America, followed by Belarus (1.98; 95%CI 1.59 to 2.38) of Eastern Europe. Despite of the decreasing mortality of MSM in worldwide, it increased in many regions such as Guatemala and Belarus, which suggesting that current reducing mortality strategies should be reoriented, and specific strategies should be established in high mortality countries to forestall the increase of mortality in MSM.

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The global burden of nonmelanoma skin cancers from 1990 to 2017

W. Zhang, X. Shen, Y. Wang, Y. Lan, X. Yang, Y. Gu, J. Duan, H. Lu

Dermatology, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, China

Nonmelanoma skin cancers (NMSCs) consist of two major subtypes, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Information about the NMSCs epidemiology limited, here we provide an estimate of the global burden of NMSCs. Based on the GBD study 2017, we analysed detailed data on NMSCs of incidence, mortality and the disability-adjusted life-years (DALYs) metric between 1990-2017. Estimated annual percentage changes (EAPCs) in NMSCs age standardized incidence, mortality and DALYs rate (ASIR, ASMR and ASDR), were calculated to quantify the temporal trends in ASR. Global crude number of NMSCs incidence in both sexes were 3.3 million (95% UI 2.0 to 4.9) of BCC and 1.8 million (95% UI 1.1 to 2.6) of SCC in 2017. Although the number of cases among NMSCs had an increase from 1990 to 2017, a decrease in ASIR of BCC occured. In 2017, it was estimated that 65,097 (95% UI 63091 to 66459) deaths of SCC occurred in worldwide. The ASMR remained nearly unchanged for SCC between 1990 and 2017 in the world. For BCC of ASIR, the most significant decrease was in Australasia, while the most significant increase in Tropical Latin America. Although the SCC ASIR of Southern Sub-Saharan Africa not is the highest, there was the highest SCC ASMR and ASDR in this region. The region with greatest ASDR of BCC was America in the world. The EAPC of ASIR of SCC was high in the high Socio-demographic Index region (4.03; 95%CI 3.48 to 4.57), while Central Latin America (-1.04; 95%CI -1.49 to -0.59)) is at its minimum. Moreover, we observed an unexpected trend of the EAPC in BCC ASIR in High-income North America (-0.16; 95%CI -0.72 to 0.4) decreased from 1990 to 1997, compared to a dramatical increase in East Asia (2.86; 95%CI 2.39 to 3.32). The EAPC of ASMR of SCC is highest from 1990 to 2017 was Central Asia (2.6; 95%CI 2.23 to 2.96). Significant disparities exist among countries in NMSCs incidence, deaths, and associated disability, NMSCs remain a significant public health concern globally.

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Long-term effectiveness of spironolactone treatment for women with acne

V. Garg[2], J. Choi[1], W. James[1], J. Barbieri[1]

[1]Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Sidney Kimmel Medical College, Philadelphia, Pennsylvania, United States

Background: Use of oral spironolactone for acne has grown over the past decade. However, data on the effectiveness and safety of spironolactone is limited to small randomized trials and retrospective studies with limited follow-up. Objective: To characterize the long-term use of spironolactone for women with acne. Methods: We conducted a retrospective chart review of adult women with acne who were treated with spironolactone at an academic medical center from 2008 to 2018. We evaluated the proportion of patients whose acne had cleared at 3, 6, 12, and 24 months. To be conservative in our estimates, those who were lost to follow-up were considered to be “not clear.” In addition, the drug survival of spironolactone was calculated. Factors that may influence outcomes such as use of concomitant topicals or combined oral contraceptives, spironolactone dosing, and patient comorbidities were explored. Sankey diagrams were also used to examine dosing changes over time. Reasons for discontinuation and side-effects were examined. The impact of spironolactone on blood pressure was also evaluated. Results: Among 403 women treated with spironolactone, the most common initial dose was 100mg/day and the mean drug survival until first discontinuation was 470.7 (471.6) days. 18.1%, 31.0%, 46.9%, and 53.8% had cleared at 3, 6, 12, and 24 months respectively. The most common reason for spironolactone discontinuation was acne clearance (n=41, 44%). 23% (n=21) of discontinuations were due to side effects (15 non-menstrual, 6 menstrual). Adjusting for age and dose, menstrual side effects were significantly less common among those using oral contraceptives (OR 0.23; 95% CI 0.11-0.50). There were not significant changes in blood pressure with spironolactone treatment. Conclusion: In this large cohort of patients treated with spironolactone for acne, spironolactone appears to have good long-term effectiveness as evaluated by clearance rates at 12 and 24 months and by drug-survival. Spironolactone was well-tolerated with few patients discontinuing due to side-effects.

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Incidence and predictors of acne among transgender patients treated with masculinizing hormone therapy

N. Thoreson[1], C. Grasso[2], D. King[2], C. Shen[3, 4], E. Dommasch[5, 4]

[1]Boston University School of Medicine, Boston, Massachusetts, United States, [2]Fenway Institute, Fenway Health, Boston, Massachusetts, United States, [3]Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States, [4]Harvard Medical School, Boston, Massachusetts, United States, [5]Dermatology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States

Transgender patients treated with masculinizing hormone therapy (MHT) for gender affirmation may be at higher risk of developing acne following hormone initiation. We sought to examine the incidence and severity of acne in patients treated with MHT and factors which may predict development of acne. We conducted a retrospective cohort study using electronic medical records from a community health center which provides care to the LGBTQIA+ population for patients who started MHT between 2014 and 2017 (n=1,054). Acne severity was categorized as severe if treated with isotretinoin, moderate if treated with oral antibiotics, and mild if treated with topicals and/or no prescription treatments. Clinical and demographic factors including BMI, age, smoking status, testosterone levels, race, sexual orientation, employment, and comorbid disorders were tested for an effect on acne diagnosis and severity. Chi-square analysis, Fischer’s exact test, student’s T-test, and ANOVA were used as appropriate, and a backwards stepwise logistic regression was performed for all factors $p<0.05$ to identify independent predictors of acne. 1,054 patients were included in the analysis. Overall prevalence of acne was 345/1054 (32.7%), including 280 patients (26.6%) who developed acne after MHT initiation, with an incidence of 12.4% within the first 6 months of MHT, 20.9% within the first year, and 26.0% within the first two years. Patients who developed post-MHT acne were younger (mean age 23 years, $p<0.0005$) and had lower BMIs (mean 24.8, $p=0.006$) than patients who did not (mean age 26 and mean BMI 28.6). Patients with acne prior to MHT initiation were more likely to develop moderate or severe acne ($p=0.013$) No other clinical or demographic characteristics were found to be independent predictors of acne diagnosis or severity.

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Risk of hospitalization due to infection in patients with psoriasis: A population-based cohort study using the UK Clinical Practice Research Datalink

Z. Z. Yiu[1], R. Parisi[1], M. Lunt[1], R. Warren[1], C. Griffiths[1], S. Langan[2], D. Ashcroft[1]

[1]University of Manchester, Manchester, United Kingdom, [2]London School of Hygiene and Tropical Medicine, London, United Kingdom

Psoriasis is associated with multiple comorbidities and treated with systemic therapies that may increase the risk of serious infections. Our objective was to determine whether patients with psoriasis have a higher risk of hospitalization due to infection. We performed a cohort study of adult patients (≥18 years of age) with psoriasis delineated from the UK Clinical Practice Research Datalink (CPRD GOLD) and linked to Hospital Episode Statistics (HES) and national mortality records between 01/04/2003 and 31/12/2016. Each patient with psoriasis was matched to up to 6 individuals without psoriasis on age, sex, and primary care practice. Hospitalization due to infection was ascertained in the linked HES records. Unadjusted and adjusted stratified Cox proportional hazard models were estimated, with the adjusted model inclusive of potential confounders such as lifestyle factors and comorbid conditions. 69,312 patients with psoriasis and 338,598 matched individuals without psoriasis were followed up for a median of 4.9 years (IQR 5.9) and 5.1 (IQR 6.3) years respectively. Patients with psoriasis had a higher incidence rate of serious infection (20.5/1000 person-years, 95% CI 20.0-21.0, n=7629) compared with those without psoriasis (16.1/1000 person-years, 95% CI 15.9-16.3, n=30756). The unadjusted hazard ratio for serious infection in patients with psoriasis was 1.46 (95%CI 1.42-1.50), and the adjusted hazard ratio was 1.36 (95%CI 1.31-1.40). Psoriasis is associated with an increase in the risk of serious infection. Further research is needed to understand the mechanism by which psoriasis predisposes to a higher risk of infection.

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Rates of BCC relative to SCC are higher in younger patients, especially females

T. M. Lukowiak, L. Aizman, A. Perz, J. Etkzorn, C. Miller, T. Shin, J. Sobanko, C. Giordano, W. Higgins, M. P. Lee

Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States

In the United States (U.S.) data on keratinocyte carcinoma (KC) epidemiology has been derived from the Medicare population[1] or regional data[2]. In Medicare patients, the ratio of BCC:SCC in 2012 was approximately 1:1 in patients ≥ 65 years old[1], but in a regional study of Mayo clinic patients, the ratio of BCC:SCC was higher in patients less than 40 years old[2]. Defining sex and age-specific variations in KC epidemiology could inform targeted public health messaging. The aim of this study was to assess BCC:SCC ratios from adults (≥18 years old) between 2012-2016. This study utilized the Optum Cliniformatics Datamart, a de-identified insurance claims database of patients that broadly represent the U.S. population[3]. Patients with diagnoses of BCC or SCC (invasive or in situ) and a corresponding CPT code for destruction, Mohs micrographic surgery, or excision were included for analysis. Mean age was 68.72, and 56.63% of patients were male. 537,658 patients had paid insurance claims for 1,307,214 KCs, including 845,164 (64.65%) BCCs and 462,050 (35.35%) SCCs. For patients aged 18-39, 40-64, and greater than 65 years old, the respective BCC to SCC ratios averaged 9.98, 3.12, and 1.47. The BCC:SCC ratio was similar between the sexes in patients ≥ 65 years old (1.46 for males versus 1.49 for females), but women in younger age groups were more likely to have BCCs. For every 10-year decrement in age, the odds of having a BCC increased by 1.49 times for women ($p<0.001$) and 1.37 times for men ($p<0.001$). Holding all else constant, the odds of having a BCC were 1.89 times higher for women than men ($p<0.001$, 95% CI: 1.78 to 1.96). These data underscore that younger women in the U.S. are more likely to get BCC than SCC and may benefit from targeted detection and prevention strategies for patients.

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Health care expenditures of psoriatic patients with and without comorbid depression

N. L. Tamashunas, D. DeMeo, J. Scott

School of Medicine, Case Western Reserve University, Cleveland, Ohio, United States

While the association between psoriasis and depression is well-established, studies investigating the impact of depression on health care expenditures are limited to adult psoriasis patients with commercial insurance or Medicare. We hypothesize that psoriatic patients with depression have higher health care expenditures than psoriatic patients without depression. This retrospective cross-sectional study pooled data from the Medical Expenditure Panel Survey (MEPS), a nationally representative sample of the non-institutionalized United States population, from 2007 to 2015. Patients with one or more psoriasis conditions were identified by a 3-digit ICD code (696). Demographics and health care expenditures were compared between psoriatic patients with and without comorbid depression using Rao-Scott χ^2 and design-based Kruskal Wallis, respectively, using a weighted-subject, stratified design. A total of 1,053 unweighted psoriatic patients were identified from 2007 to 2015, of whom 259 had depression (24.6%). This amounted to 1,479,018 yearly weighted psoriatic patients (95% CI 1,321,254-1,636,781), of whom 365,091 had depression (24.7%, 95% CI 294,040-436,143). Compared to psoriatic patients without comorbid depression, psoriatic patients with depression were more likely to be female ($p<0.001$), with poor/fair perceived health status ($p<0.001$), covered by Medicaid/Medicare ($p=0.013$), divorced/separated/widowed ($p=0.008$), <100% of federal poverty level ($p=0.013$) or 200-399% of federal poverty level ($p=0.013$), and have a Charlson comorbidity index > 0 ($p=0.019$). Median yearly total health care expenditures of psoriatic patients with depression ($6,707, 95% CI $5,164-$8,249) was significantly higher than psoriatic patients without depression ($3,184, 95% CI $2,778-$3,591; $p<0.001$). As comorbid depression in adults with psoriasis is associated with higher health care expenditures, identification and management of depression in psoriasis-related visits may improve treatment and reduce cost.

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Framing application site discomfort as an efficacy signal improves willingness to continue use of topical medications

A. M. Bashyam[1], E. J. Masicampo[2], S. R. Feldman[1]

[1]Department of Dermatology, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States, [2]Department of Psychology, Wake Forest University, Winston-Salem, North Carolina, United States

One obstacle to topical therapy adherence is patient perception of treatment safety and efficacy. Medications for atopic dermatitis (AD) can cause application site discomfort (burning or stinging), leading patients to discontinue therapy. However, counseling patients can frame the sensation as a positive signal of efficacy. This study assessed differences in patient willingness to tolerate application site discomfort when application site sensation is forewarned and framed as an efficacy signal. 352 adult participants recruited through the Amazon Mechanical Turk platform were provided a hypothetical scenario about their physician prescribing a medicated cream for a skin rash and that when applied, the medication caused a burning and stinging sensation. Participants were randomized to one of three counseling groups: control/no counseling (A), counseling of potential sensation (B), and sensation is a sign the medication is working (C). Willingness to continue use of the medication was assessed using a 9-point Likert scale with 1 as “strongly not willing” and 9 as “strongly willing.” The sample represented a wide range of ages, sexes, races, and education, with no statistically significant differences between groups. When there is an unpleasant sensation, counseling patients to expect a sensation improves their willingness to continue use of a medication (B vs. A; 5.3 vs. 4.4; $p<0.001$; $d=0.46$). However, when these patients are further counseled that this sensation is an efficacy signal, their willingness is greatly increased (C vs. A; 6.9 vs. 4.4; $p<0.001$; $d=1.32$). Counseling to anticipate application site discomfort and framing such discomfort as an efficacy signal may enhance patients’ adherence to treatment and could potentially be a simple method of improving adherence and patients’ treatment outcomes.

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Physicians’ ability to determine culprit drug in SJS/TEN and areas for improvement

D. Li[1], G. Romar[1], R. Foreman[2], S. J. Divito[1]

[1]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States, [2]Pathology, Massachusetts General Hospital, Boston, Massachusetts, United States

Stevens-Johnson syndrome (SJS)/Toxic Epidermal Necrolysis (TEN) is a severe drug reaction causing mucocutaneous desquamation with high morbidity and mortality. Central to management is identifying the culprit drug to stop disease progression and recurrence. Little is known about physicians’ approach and ability to identify the culprit drug and consequences of misidentification, especially given better understanding of disease pathogenesis and tools like the algorithm of drug causality for epidermal necrolysis (ALDEN). We retrospectively analyzed 49 cases of SJS/TEN overlap and TEN from 2001-2018 at 2 tertiary care centers to determine how often physicians identified a clear drug culprit, and the clinical data and reasoning used. We then applied ALDEN to suspected drugs to re-evaluate physician assessments. Physicians identified a clear drug culprit in 9 cases (18%), relying mostly on the notoriety of common drugs. HLA association was mentioned in only 1 case, and no physicians referenced patient ethnicity, drug half-life, drug interactions, drug metabolism or a drug-scoring system. Yet in 45 cases (92%), at least 1 drug was listed under “allergy” in the medical record, and in 25 cases (51%), >1 drug was listed. ALDEN identified a clear drug culprit in 32 cases (65%), a rate significantly higher than that by physicians’ approach ($p < 0.001$). It identified culprit drugs physicians missed in 5 cases (10%) and exonerated drugs physicians listed as “allergy” in 21 cases (43%). In 16 cases (33%), ≥1 drugs listed as “allergy” had known associated HLA alleles. We find that physicians have difficulty identifying a clear culprit drug in SJS/TEN, and liberally list drugs as causing “allergy” without strong evidence of causality. Incorporation of more clinical data, an algorithm like ALDEN, or possibly HLA testing, may improve drug culprit identification and avoid potentially harmful drug avoidance recommendations.

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Association between cutaneous squamous cell carcinoma primary tumor anatomic site, laterality, and odds of invasion in the United States

S. Ghanian[1], T. Li[1], J. Han[2], A. Qureshi[1], J. Walker[1], E. Cho[1, 3, 4]

[1]Department of Dermatology, Brown University, Providence, Rhode Island, United States, [2]Department of Epidemiology, Indiana University, Indianapolis, Indiana, United States, [3]Department of Epidemiology, Brown University, Providence, Rhode Island, United States, [4]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, United States

Cutaneous squamous cell carcinoma (cSCC) is the second most common skin cancer that is associated with ultraviolet radiation exposure and immunosuppression. This study aims to examine the association between cSCC primary tumor anatomic site, laterality, and odds of tumor invasion in females in the Nurses' Health Study (NHS) and Nurses' Health Study II (NHS2) and in males in the Health Professionals Follow-Up Study (HPFS). A pathology review of cSCC cases from the NHS (n= 4,867), NHS2 (n=1,165), and HPFS (n=2,779) was conducted to investigate the association between primary tumor anatomic site and odds of tumor invasion. Logistic regression models were applied to estimate odds ratios (OR) and 95% confidence intervals (95% CI). Additionally, a subset in the NHS with information on tumor laterality (n=700) was analyzed to investigate the association between primary tumor laterality and odds of tumor invasion. The majority of cSCCs in each cohort was invasive (NHS 59%, NHS2 54%, HPFS 73%). Head and neck tumors were associated with increased odds of invasion in the NHS (OR=1.20, 95% CI=1.06,1.35), NHS2 (OR=1.52, 95% CI=1.18,1.92), and HPFS (OR=1.19, 95% CI=1.00, 1.43) compared to trunk and extremity tumors when adjusting for age at diagnosis. For the subset of NHS, the majority of tumors were left-sided (53%). Right-sided tumors were associated with a non-significant increased odds of invasion (OR=1.30, 95% CI=0.98,1.62) compared to left-sided tumors. This study demonstrates an association between primary tumor anatomic site, laterality, and odds of invasion in cSCC, which may have clinically applicable implications in cSCC diagnosis, risk stratification, and prognostication.

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High burden of patient-reported ocular disorders and symptoms in adults with moderate-to-severe atopic dermatitis

J. Weyne[1], A. Blauvelt[2], M. de Bruin-Weller[3], E. Prens[4], P. Asbell[5], D. Sierka[6], Z. Chen[1], B. Shumel[1]

[1]Regeneron Pharmaceuticals, Inc., Tarrytown, New York, United States, [2]Oregon Medical Research Center, Portland, Oregon, United States, [3]University Medical Center, Utrecht, Netherlands, [4]Erasmus University Medical Center, Rotterdam, Netherlands, [5]Hamilton Eye Institute, University of Tennessee Health Science Center, Memphis, Tennessee, United States, [6]Sanofi Genzyme, Cambridge, Massachusetts, United States

Patients with atopic dermatitis (AD) have a higher risk of conjunctivitis and other ocular surface disorders than the general population, and the incidence of ocular complications increases with AD severity. Here, we report the burden of ocular disorders and symptoms prior to treatment initiation in adults with moderate-to-severe AD with inadequate response to topical corticosteroids enrolled in LIBERTY AD CHRONOS (NCT02260986), a randomized, placebo-controlled, phase 3 trial of dupilumab. CHRONOS enrolled 740 patients after a 35-day screening period. Patients completed a survey of ocular disorders in the past year at screening, and ocular symptoms (itching, tearing, redness, and discomfort) in the past month at baseline. Among the 712 patients who responded to the survey at screening, the number (%) of patients who reported having diagnoses of ocular disorders in the previous year were as follows: atopic keratoconjunctivitis, 87 (12.2); keratoconus, 15 (2.1); perennial allergic conjunctivitis, 107 (15.0); dry eye, 146 (20.5); herpes simplex virus infection of the eye, 30 (4.2); and rosacea of the eye, 19 (2.7). At baseline, approximately 46–63% of all patients reported having ≥ 1 ocular symptom (itching, tearing, redness, or discomfort) from some of the time to all of the time in the past month; approximately 25–32% reported these symptoms as being mild, 12–22% reported them as being moderate, and 4–7% reported them as being severe. These data demonstrate a high burden of ocular disorders and symptoms in a population of adults with moderate-to-severe AD.

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Long-term treatment with dupilumab minimizes use of systemic immunosuppressants as rescue medications in adults with moderate-to-severe atopic dermatitis

L. A. Beck[1], A. Blauvelt[2], L. Sher[3], I. Hussain[4], A. B. Rossi[5], Z. Chen[6], B. Shumel[6]

[1]University of Rochester Medical Center, Rochester, New York, United States, [2]Oregon Medical Research Center, Portland, Oregon, United States, [3]Peninsula Research Associates, Rolling Hills Estate, California, United States, [4]Vital Prospects Clinical Research Institute, Tulsa, Oklahoma, United States, [5]Sanofi Genzyme, Cambridge, Massachusetts, United States, [6]Regeneron Pharmaceuticals, Inc., Tarrytown, New York, United States

LIBERTY AD OLE (NCT01949311) is a phase 3, open-label extension study that evaluates the long-term safety and efficacy of dupilumab in adults with moderate-to-severe atopic dermatitis (AD) who were previously enrolled in dupilumab clinical trials. We report the proportion of patients (pts) who required systemic rescue medications. All pts who received ≥ 1 dose of dupilumab and required ≥ 1 concomitant rescue medication during the study were included in this analysis. Rescue treatments included systemic corticosteroids, nonsteroidal systemic immunosuppressants (ISS), and phototherapy, administered at the investigator's discretion to treat intolerable AD symptoms or to manage serious intercurrent conditions. At the data cutoff date, 2,677 pts received dupilumab for up to 148 weeks. Most patients (82.4%) had completed the study through the Week 52 visit, and 13% had completed up to the Week 148 visit. Previous use of nonsteroidal systemic ISS for AD was reported in 39.3% (1,051 pts); 34.2% (915 pts) previously received cyclosporine, 10.6% (284 pts) methotrexate, and 6.4% (172 pts) azathioprine. Dupilumab treatment markedly reduced the need for systemic ISS therapies in adults with moderate-to-severe AD. During the OLE study, systemic rescue medications were required in 7.5% (200 pts); the majority (93.5% [187 pts]) received systemic corticosteroids, and 10.5% (21 pts) received systemic nonsteroidal ISS. The long-term safety profile of dupilumab remained consistent with the safety profile previously observed in controlled studies in adults with moderate-to-severe AD.

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Dupilumab prevents flares in adults with moderate-to-severe atopic dermatitis in a 52-week, randomized, controlled, phase 3 trial

J. F. Merola[1], R. Sidbury[2], A. Wollenberg[3], Z. Chen[4], A. Zhang[5], B. Shumel[4], A. B. Rossi[5]

[1]Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, United States, [2]Seattle Children's Hospital, Seattle, Washington, United States, [3]Ludwig-Maximilian University, Munich, Germany, [4]Regeneron Pharmaceuticals, Inc., Tarrytown, New York, United States, [5]Sanofi Genzyme, Cambridge, Massachusetts, United States

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by flares, defined by US and EU guidelines as acute, clinically significant worsening of signs and symptoms of AD requiring therapeutic intervention. Flare prevention is a hallmark of long-term disease control in AD. Here, we report the effect of dupilumab treatment for 52 weeks on flare prevention in adults with moderate-to-severe AD from the LIBERTY AD CHRONOS trial (NCT02260986). In this trial, 740 patients (pts) with moderate-to-severe AD were randomized 3:1:3 to subcutaneous dupilumab 300 mg once weekly, dupilumab 300 mg every 2 weeks (q2w), or placebo (PBO); all pts also received a standardized regimen of medium potency topical corticosteroids (TCS). This analysis includes pts who received dupilumab q2w (approved dose)+TCS or PBO+TCS for 52 weeks. During the 52-week treatment period, the annualized flare rate (AFR) was significantly higher in pts who received PBO+TCS (AFR = 0.77, 95% confidence interval [CI] 0.63–0.93) than in those who received dupilumab q2w+TCS (AFR = 0.17, 95% CI 0.10–0.29), representing a 78% relative reduction in annual flares in pts who were treated with dupilumab q2w (relative risk = 0.22, 95% CI 0.13–0.39). *P* values for all comparisons on event rate between dupilumab q2w and PBO were < 0.05 based on a parametric Poisson model. Based on patient self-report, in the 12 months before enrollment, 89/106 (84%) and 243/315 (77%) pts receiving dupilumab q2w+TCS and PBO+TCS, respectively, experienced flares. During the 52-week treatment period, only 14% of pts receiving dupilumab q2w+TCS experienced a flare vs 43% of pts receiving PBO+TCS. These results demonstrate that dupilumab prevents flares in adults with moderate-to-severe AD, providing continuous, long-term disease control.

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Frequency and influence of “Not Relevant” responses on the Dermatology Life Quality Index among adults with atopic dermatitis in the United States
J. Barbieri, Z. Chiesa Fuxench, J. Takeshita
University of Pennsylvania, Philadelphia, Pennsylvania, United States
Introduction: Recent data among adults with psoriasis raise concerns about frequent “not relevant” responses on the widely used Dermatology Life Quality Index (DLQI), which may result in underestimation of disease burden. To examine whether these concerns extend to other skin diseases, we evaluated the frequency and influence of “not relevant” responses on the DLQI among adults with atopic dermatitis in the United States Methods: A cross-sectional analysis was conducted using data from the Atopic Dermatitis in America survey. Adults who met the UK Working Party criteria were included (with modified age of onset criteria of <18 years). The frequency of “not relevant” responses on the DLQI was evaluated for each DLQI item. To examine whether “not relevant” responses are associated with underestimation of disease burden, Patient-Oriented Eczema Measure (POEM) and Patient-Oriented SCORAD (PO-SCORAD) scores were compared between those who responded “not relevant” and those who responded “not at all”. Differences were evaluated using Wilcoxon rank-sum tests. Results: Among 764 patients, 56.1% had at least one “not relevant” response. The median number of “not relevant” responses was 1 (IQR 0-3). Not relevant responses were most common for item 6 (“sport”, 36%), item 3 (“daily routines”, 32%), and item 9 (“sexual relationships”, 30%). Although there were some statistically significant differences in POEM and PO-SCORAD scores between those who responded “not relevant” and “not at all”, the differences were generally small and in different directions depending on the item. Conclusions: “Not relevant” responses on the DLQI are common among adult patients with atopic dermatitis suggesting issues with content validity in this population. Among several items of the DLQI, “not relevant” responses may also be associated with numerically higher or lower patient-reported disease severity, although the clinical significance of these differences is unclear.

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Implementation of a consultative teledermatology mobile application in Veterans Affairs
S. Peracca[1], A. Fonseca[2], A. Grenga[2], G. Jackson[3, 5], H. King[3, 5], J. Chapman[3], J. Whited[3, 5], M. Weinstock[2], D. H. Oh[1, 4]
[1]Dermatology Service, San Francisco VA Health Care System, San Francisco, California, United States, [2]Dermatology, Brown University/Providence VA Medical Center, Providence, Rhode Island, United States, [3]Durham VA Medical Center, Durham, North Carolina, United States, [4]Department of Dermatology, University of California, San Francisco, San Francisco, California, United States, [5]Duke University School of Medicine, Durham, North Carolina, United States
To facilitate consultative asynchronous teledermatology, Veterans Affairs (VA) introduced a mobile application at 3 sites. We evaluated the initial implementation process using a mixed methods multiple case study approach to assess readiness to implement the intervention in each facility, as well as the facilitators, barriers and contextual factors. Data sources included: 1) Interviews and an organizational readiness to change (ORC) survey conducted at one site; 2) Interviews at all sites with groups of physicians, nurses, administrators, and information technology (IT) professionals; and 3) Implementation documentation from the operational partner. Initial stakeholder support at all sites, corroborated by survey data, indicated a readiness for change. Support from both leadership and staff was a positive organizational characteristic. Each site had telehealth meetings to foster communication but only one was active with members from all departments with roles in telehealth. Each site had staff to ensure that imagers—primary users of the new app—were trained, but one had an additional staff member devoted to education of new telehealth projects; access to one-on-one guidance facilitated greater understanding of the process. At all 3 sites, staff experienced technical difficulties that negatively affected adoption, particularly due to the inability to address these problems quickly. Additional organizational characteristics that adversely affected ORC included users’ perceptions of additional workload and suboptimal IT infrastructure/support. These findings may be useful for informing rollout of future mobile telehealth endeavors in VA and other health care organizations.

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Comparisons of oral corticosteroid treatment patterns for toxicodendron dermatitis
M. Butt, S. Maczuga, A. Flamm
Dermatology, Penn State Milton S Hershey Medical Center, Hershey, Pennsylvania, United States
Toxicodendron Dermatitis (TD) is a common form of allergic contact dermatitis that affects millions of Americans every year. While some cases remain mild and can be managed with over-the-counter remedies, many patients experience significant pruritus from the eruptions and can require systemic medical intervention to ease the symptoms. The objective of this study is to evaluate medical claims associated with TD outpatient visits and compare healthcare utilization outcomes depending on the days’ supply of the initial oral corticosteroid prescription. For this study, we used Truven MarketScan© claims from 2017 with a diagnosis of TD (ICD-10: L23.7) for outpatient and pharmaceutical claims. During this time, 70,120 individuals incurred 151,672 claims for TD. Only 37.9% (n=26,571) patients received a prescription for oral corticosteroids on their initial visit. Of those that received a prescription for an oral corticosteroid, 83.9% (n=22,287) were given a 1 to 13 day supply, 13.8% (n=3,659) were given a 14 to 20 day supply, and 2.4% (n=625) were given a 21 day supply or more. Of those who had a second visit for TD (n=3,138), 38.3% (n=1,203) were not given an oral corticosteroid at their initial visit, 53.6% (n=1,682) were given an initial oral corticosteroid prescription with a day supply of 1 to 13 days, and the remaining 8.1% (n=253) having an oral corticosteroid prescribed for 14 days or more at their initial visit. The results of this study found that oral corticosteroids are relatively under-prescribed for TD as the majority of literature regarding the treatment of TD suggests a minimum course of 14 days. Also, those with TD who receive no prescription or a prescription with a shorter duration (i.e. 1 to 13 days) at an initial office or ED visit make up the majority of those who have a return visit for TD as well, which could in turn impact the cost and quality of care that these patients receive. Future studies are needed to determine how prescribing practices impact the cost of care for patients and identify means of reducing that cost burden.

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Wildfire-associated air pollution impacts clinic visits for itch and atopic dermatitis
R. Fadadu[1, 2, 3], B. Grimes[1], J. Balmes[1, 2], M. Wei[1, 3]
[1]University of California, San Francisco, San Francisco, California, United States, [2]University of California, Berkeley, Berkeley, California, United States, [3]Veterans Affairs Medical Center, San Francisco, California, United States
With climate change, the frequency and intensity of wildfires are expected to increase, leading to episodes of poor air quality that could exacerbate patients’ pre-existing dermatoses. To assess the effects of exposure to wildfire-associated air pollution on skin, we investigated if the 2018 California Camp Fire led to detectable increases in clinic visits for atopic dermatitis (AD) or itch at dermatology clinics 175 miles from the origin of the fire. We collected data from 2015-2019 on particulate matter (PM2.5) concentration and smoke plume density in San Francisco and the number of outpatient dermatology visits for AD or itch symptoms at an academic medical center. Data were analyzed using Poisson regression with empirical standard errors, and the models included exposure lags and covariates: patient age and sex, temperature, and humidity. We found that the rates of weekly pediatric AD clinic visits, adult AD clinic visits, and pediatric itch symptoms during the Camp Fire were respectively 1.75 (95% CI: 1.21, 2.50), 1.28 (1.08, 1.51), and 2.10 (1.44, 3.00) times the rate for non-fire weeks for a 0-week lag, adjusted for time of year and confounders. Every 10 $\mu g/m^3$ increase in average weekly PM2.5 concentration was associated with an adjusted rate ratio of 1.08 (95% CI: 1.04, 1.12) for weekly pediatric AD clinic visits. Thus, there was increased use of dermatology services for AD associated with exposure to air pollution from the Camp Fire, particularly for the pediatric population. With an impaired skin barrier function, AD patients may be at increased risk of adverse skin reactions following pollution exposure, which can negatively impact quality of life. Understanding the effects of air pollution on patients’ skin health can inform how dermatologists counsel patients and expand comprehension of the broader health effects of climate change.

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Incidence of depression among patients with hidradenitis suppurativa
S. Wright, A. Strunk, A. Garg
Dermatology, Donald and Barbara Zucker School of Medicine at Hofstra Northwell, Lake Success, New York, United States
Information on the risk of depression among patients with hidradenitis suppurativa (HS) is limited with much of the existing data consisting of cross-sectional studies lacking a control group. We aimed to compare risk of new-onset depression in patients with HS to that of controls without HS, and to determine clinical characteristics associated with depression among HS patients. This was a retrospective cohort analysis of 49,280 adult and 3,042 pediatric HS patients and matched controls identified using the IBM Explorys analytics platform. In separate analyses of adult and pediatric patients, depression incidence was compared between those with HS and those without HS using a Cox proportional hazards regression model, controlling for relevant confounders. Crude incidence rate (IR) of new-onset depression was 4.8 per 100 person-years in adult HS patients compared to 3.0 per 100 person-years in controls. Among pediatric patients, crude IR of depression was 4.2 per 100 person-years in HS patients compared with 2.3 per 100 person-years in controls. In unadjusted analysis, adult HS patients had a 61% increased risk of depression (HR, 1.61; 95% CI, 1.57-1.65) and pediatric HS patients had an 87% increased risk of depression (HR, 1.87; 95% CI, 1.66-2.10). In adjusted analysis, adult HS patients had a 10% increased risk of depression (HR, 1.10; 95% CI, 1.07-1.13; p<0.001) and pediatric HS patients had a 26% increased risk of depression (HR 1.26; 95% CI 1.10-1.44; p<0.001) compared to controls. Factors associated with depression among adult and pediatric HS patients included female sex, Caucasian race, smoking, and BMI/obesity. Among adult HS patients, history of alcohol use disorder and substance use disorder were also associated with new-onset depression. Pediatric and adult patients with HS are at an increased risk for depression, independent of other common risk factors for depression. Periodic screening for depression among HS patients may be warranted, particularly among those who have additional risk factors.

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The epidemiology of genital warts in the United States
W. Liszewski, P. Singh
Dermatology, Northwestern University, Chicago, Illinois, United States
Dermatologists frequently diagnose and treat genital warts. Given the expanded approval of the HPV vaccine for individuals up to the age of 45, there may be an opportunity for dermatologists to recommend the vaccine in previously ineligible individuals. To better understand the burden of genital warts in the United States—and which individuals would benefit from it--we analyzed the demographics, sexual history, and HPV vaccination status of individuals with and without a history of genital warts in the 2013-2016 National Health and Nutrition Examination Survey. A history of genital warts is more common in women than men (6.3% vs 2.8%, p<0.001), individuals with a younger age of sexual debut (p<0.001), and an increasing number of lifetime sexual partners (p<0.001). The number of individuals with an initial case of genital warts after the age of 26 represented 35.6% and 32.1% of all cases in men and women, respectively. In a multivariate regression model, men with 15+ lifetime sexual partners had an increased odds of genital warts (15.07 [3.03-74.28], p=0.004) compared with 1 lifetime partner. Each year of age was associated with increased odds of genital warts (1.04 [1.03-1.06], p<0.001), while birth outside of the US was associated with decreased odds of genital warts (0.41 [0.20-0.84], p=0.027). Women had increased odds of genital warts with each additional year of age (1.04 [1.02-1.06], p<0.001) and with 15+ lifetime sexual partners (8.75 [3.44-22.22], p<0.001) in comparison with 1 partner. Compared to White women, there were decreased odds of warts among Black women (0.45 [0.26-0.79], p=0.01). Surprisingly, receiving at least one shot in the HPV vaccine series was associated with a statistically significant decreased odds of genitals warts in men (0.22 [0.07-0.71], p=0.38) but not in women (1.32 [0.86-5.28], p=0.60). Overall, nearly one-third of individuals with a history of genital warts developed them after the age of 26, the previous age cut-off for the HPV vaccine series. The individuals at the greatest risk of genital warts are White women, American-born men, and individuals with more than 15 sexual partners.

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Impact of prior authorizations on dermatology patients at a large academic center
L. Guo[1, 2], V. Nambudiri[1, 2]
[1]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States, [2]Harvard Medical School, Boston, Massachusetts, United States
Prescription drugs in dermatology have been increasingly subject to prior authorization (PA) policies, adding burdens and barriers to both patients and providers. However, few studies have formally characterized their effect on patients. Newly prescribed dermatology medications commonly requiring PAs in 2017 at a large academic institution were retrospectively reviewed (n=515). For each prescription, whether PA was required, PA decision if applicable, and whether follow-up clinic visit occurred within 6 months were assessed. Rates of clinical improvement and adherence to the original or alternative treatment at follow-up if present were also calculated. Overall, 20.4% of prescriptions required PAs, with 58.1% of PAs approved, 29.5% denied, and 12.4% not submitted or unable to be determined. Average time from PA submission to final decision was 11.3 days. Patients with prescriptions requiring PAs (n=105) had significantly higher rates of follow-up than those that did not (n=379) (71.4% vs. 52.0%, p=0.00036) but lower rates of adherence (57.1% vs. 81.7% p=0.00024). No significant difference was found in clinical improvement of patients prescribed medications requiring PAs (including both approved and denied PAs) versus those who were not (48.6% vs. 60.5%, p=0.12). Amongst prescriptions requiring PAs, approved PAs were associated with significantly higher rates of improvement on follow-up than denied PAs (60.5% vs. 33.3%, p=0.043). Adherence was also significantly higher for approved PAs (76.7% vs. 21.7%, p=0.000022). Overall, PAs delayed access to medications. PA denial was associated with decreased rates of clinical improvement. Regardless of PA decision, PA requirements appeared to have a negative effect on adherence in dermatology and were associated with increased clinic visits. Clinical impact on patients and increased resource utilization due to undertreatment should be considered when evaluating the true “cost” of prior authorizations.

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Adherence to topical therapy for atopic dermatitis: Barriers and facilitators
A. Bazen, A. Sevagamoorthy, F. K. Barg, J. Takeshita
University of Pennsylvania, Philadelphia, Pennsylvania, United States
In spite of continuing advances in therapeutic options particularly for more severe atopic dermatitis (AD), topical treatments remain a mainstay of the therapeutic regimen for all patients. However, adherence to topical therapies remains low. Increasing adherence to topical therapies is an important contributor to improving outcomes for patients with AD. In order to better understand the barriers to and facilitators of adhering to topical therapies for AD from the patient perspective, we performed semi-structured interviews of 44 adults with AD. Interview responses were independently coded by two members of the research team using a grounded theory approach and NVivo 12 software. Median (interquartile range, IQR) age was 32 (24-50) years; 73% were female. Median (IQR) duration of AD 20 (10-27) years, and 59% of patients had moderate-to-severe AD. Major barriers to topical therapy adherence that were identified included time required to apply topicals, mess of topicals (particularly ointments), daily activities or lifestyle (e.g., work requiring frequent hand washing), concern about side effects, hopelessness due to trial and failure of numerous treatments, cost, lack of trust in medical provider, and unclear medication instructions. Specific facilitators of adherence to topical therapy that were identified included AD severity or symptom impact, social support, simplicity of treatment regimen, portable treatments, and education about treatment options and effectiveness. In sum, we identified several modifiable patient-reported barriers to and facilitators of adherence to topical therapy for AD. Notably, technology-based reminders were not identified as major facilitators of topical therapy adherence. Future studies of interventions targeting these barriers and facilitators (e.g., providing patients with easy to understand instructions, improving communication between patients and medical providers, among others) will be important in developing and implementing effective methods to improve outcomes among patients with AD.

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A randomized control trial of IT prevention messages for SMM

N. E. Admassu[1, 2], L. D. Morrison[1], R. M. Chen[1], N. Shukla[1, 3], E. Linos[1]

[1]Dermatology, Stanford School of Medicine, Stanford, California, United States, [2]School of Medicine, UCSF, San Francisco, California, United States, [3]Dermatology, UCSF, San Francisco, California, United States

Despite increased risks of indoor tanning (IT) and skin cancer among sexual minority men (SMM), IT prevention messages to date have only focused on teens and young women. We created and tested seven IT prevention messages on Facebook to assess ad engagement and impact on IT knowledge and intentions among SMM. Facebook users identifying as men 18 years and older, with LGBT interests, residing in six states with high rates of IT were randomized to see prevention messages in their newsfeed. Control group users saw their regular newsfeed. A random subset of intervention and control group users (n=4779) were asked one of three questions to assess ad recall (n=975), knowledge (n=1898), and intentions to use a tanning bed (n=1906). The age distribution was: 28% 18-24, 38% 25-34, 17% 35-44, 9% 45-54, 4% 55-64, 3% 65+. Between May 5 and June 13, 2019, messages appeared on newsfeeds 12.4 million times, reaching 1.76 million individuals an average of 7.02 times each. Intervention users were significantly more likely than controls to recall seeing a prevention video [19.1% (89/466) vs. 6.9% (35/509); OR 3.20; 95% CI 2.10-4.85; p<0.0001]. We found no difference in knowledge about risks of, or intent to use, tanning beds. Users in the intervention and control groups agreed "tanning beds are bad for you" [67.3% (600/884) and 67.9% (682/1014), respectively; OR 1.08; 95% CI 0.80-1.45; p=0.63], and were unlikely to use tanning beds in the next year [88.2% (788/893) and 88.3% (894/1013), respectively; OR 0.92; 95% CI 0.68-1.26; p=0.61]. The most successful ad in terms of reach and engagement was an animated image of a peach addressing the impact of IT on skin aging. This is the first study to develop and test IT prevention messages for SMM. Our findings demonstrate social media is a feasible tool in reaching a sizable, hard-to-reach population in skin cancer prevention efforts. Future studies are needed to assess the efficacy of social media advertising on long-term behavioral outcomes for cancer prevention.

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Clinical characteristics and quality of life burden in aquagenic pruritus: A global questionnaire-based study

P. Aggarwal[1], Y. Semenov[2], E. Boozalis[1], J. Choi[1], T. Le[1], K. Williams[3], S. Kwatra[1]

[1]Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [2]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [3]Dermatology , Johns Hopkins, Baltimore , Maryland, United States

Aquagenic pruritus (AP) is a rare pruritic dermatosis characterized by intense itching following water exposure and often results in patients avoiding water contact. Limited epidemiologic studies describe the characteristics of AP and its impact on quality of life. Our objective was to characterize the demographics, itch, and quality of life of AP patients using a descriptive cross-sectional study design. An anonymous, online Qualtrics survey was developed and delivered to 1,829 patients via a Facebook AP support group in July 2019. The survey assessed symptoms associated with AP, but also utilized established quality of life measures including the Dermatology Life Quality Index (DLQI), the 5-D pruritus scale and the Pittsburgh Sleep Quality Index (PSQI). A total of 106 respondents from 16 countries completed the survey. The mean (SD) age of respondents was 41.4 (14.2) years with the majority being female (79.3%), and Caucasian (82.1%); 45.3% of respondents were Fitzpatrick skin type 3 and 31.1% were type 2. Itch was symmetrically distributed with the greatest prevalence reported on proximal ventral surfaces, including the thighs (90%, n = 99) and upper arms (80%, n=90). The top five triggers associated with itch onset included bathing, sweating, humidity, rain, and friction over the skin. Prickling was the most common sensation felt along with itch (n=80, 75.5%). AP patients had a mean (SD) DLQI of 11.1 (7.0), 5D pruritus score of 13.9 (2.8) and a PSQI of 8.4 (3.6). Compared to mean DLQI scores of psoriasis patients, the mean DLQI score in this study suggests that AP patients have a significantly decreased quality of life. With a better understanding of AP itch distribution, severity, and quality of life, physicians can provide tailored care for this subset of itch patients.

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Melanoma in pregnancy in California, 1994-2015: A population-based study

M. Kiuru[1, 2], Q. Li[3], G. Zhu[1, 4], J. Terrell[1], K. Beroukhim[1], T. Keegan[3]

[1]Department of Dermatology, University of California, Davis, Sacramento, California, United States, [2]Department of Pathology and Laboratory Medicine, University of California, Davis, Sacramento, California, United States, [3]Center for Oncology Hematology Outcomes Research and Training (COHORT) and Division of Hematology and Oncology, University of California, Davis, Sacramento, California, United States, [4]Fourth Military Medical University, Xian, Shaanxi, China

Melanoma is the most common malignancy during pregnancy. There is continued debate regarding the impact of pregnancy on the prognosis of melanoma. Prior studies have shown mixed results and the number of recent large population-based studies are limited, especially from the United States. To address this question, we used the California Cancer Registry data linked with Patient Discharge Data and Ambulatory Surgery Center Data to identify female patients, ages 15-44 years, diagnosed with melanoma in 1994-2015, including those who were pregnant. Women with pregnancy-associated melanoma (PAM) were compared with age-matched, non-pregnant women with melanoma. Multivariable logistic regression and multivariable cox proportional hazards regression models were used. We identified 13108 female patients diagnosed with melanoma, of which 1431 had PAM. PAM was associated with tumor site (more likely on lower than upper limb), tumor thickness (more likely in situ than invasive melanoma ≤1.0 mm), and histologic type (more likely superficial spreading than lentigo maligna). Lower overall survival was associated with non-Hispanic white race/ethnicity, lower neighborhood socioeconomic status, tumor site (head/neck, trunk or upper limb vs lower limb), greater tumor thickness, lymph node involvement, and more than 90 days to surgery (vs <30), but not pregnancy. In summary, we report a population-based analysis of melanoma in pregnancy in California. Although there were no differences in overall survival, pregnancy status was associated with tumor site, thickness, and histologic type, suggesting possible biological differences. Future studies are required to better understand the biological and molecular characteristics of melanoma in pregnancy.

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Does the provision of melanoma genetic risk information change preventative behavior?

J. M. Kilgour[1], X. Hu[1], A. Fogel[2], P. Jaju[1], K. Sarin[1]

[1]Department of Dermatology, Stanford University, Redwood City, California, United States, [2]Department of Dermatology, Yale University, New Haven, Connecticut, United States

With the rise of direct-to-consumer testing, personalized genetic risk information is accessible to the public. It is unclear, however, what impact the provision of this information has on preventative behaviors for melanoma. Stanford dermatology clinic patients were offered genetic sequencing. Genetic risk scores (GRS) were calculated using 15 risk SNPs identified in prior GWAS studies. Participants were randomized to receive their GRS immediately or delayed for 6 months. All participants received standard sunscreen advice. Questionnaires were administered at baseline, 6 weeks, 3 months and 6 months to investigate behavior change, emotional impact, healthcare use and comprehension. 100 participants were enrolled; 16 were found to be at increased risk (IR), whilst 45 were at normal and 26 decreased risk (DR). Results were withheld from 13 controls. Follow up at 3 months was 84% and 55% at 6 months. Sunscreen use was significantly different between groups at 6 months (p=0.026), with the IR group reporting most frequent use (mean five-point Likert score 4.18 vs 2.82 for DR group). For the IR group, sunscreen use increased over time (p=0.024 baseline vs 6 months; 3.38 at baseline, 3.77 at 3 months, 4.18 at 6 months). Frequency of wearing shoulder-covering sleeves increased from baseline to 6 months when analyzed without risk group stratification (p=0.007; 4.18 to 4.42), and frequency of hat wearing increased for the IR group from baseline to 3 months (p=0.035; 2.56 to 3.08) and 6 months (p=0.032; 3.10). Frequency of seeking shade increased overall at 6 months vs baseline (p=0.005; 3.48 vs 3.10). Frequency of intentional tanning decreased overall at 3 months vs baseline (p=0.013; 1.39 vs 1.58); this was not significant at 6 months (1.46). The incidence of sunburn decreased overall at 3 months (p=0.013; mean 0.04 vs 0.17) but reverted at 6 months (0.16). Our study is the first to demonstrate persistent lifestyle modification in a US cohort following provision of personalised melanoma genetic risk information.

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Influence of climate on pediatric alopecia areata flares

E. A. George[1], L. Castelo-Soccio[2, 4], E. Putterman[2, 4], H. Kuhn[1, 3], B. Prindaville[1, 3], A. Qureshi[1, 3], E. Cho[1, 3]

[1]Warren Alpert Medical School of Brown University, Providence, Rhode Island, United States, [2]Pediatrics, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States, [3]Dermatology, Rhode Island Hospital, Providence, Rhode Island, United States, [4]Dermatology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States

We recently found that the frequency of alopecia areata (AA) flares follows seasonal patterns, with the highest number of flares during fall and the lowest number during summer, and explored the relationship between climate aspects and AA flares. Information on date of disease onset, first flare and second flare was extracted from the medical records of 336 pediatric AA patients (ages 1 to 12), totaling 520 episodes of AA in Philadelphia, Pennsylvania. Frequency of flares in each month was calculated. Monthly values of meteorological variables including mean ambient temperature, UV index, air pressure, humidity, cloudiness, wind speed, wind gust, number of days with rain, volume of precipitation, number of days with sun, and hours of sunlight in Philadelphia were obtained from World Weather Online. Using Spearman rank correlation (r_s) analysis, there were significant correlations ($p<0.05$) between flare frequency and mean air pressure ($r_s = 0.76$), number of days with sun ($r_s = 0.71$), mean UV index ($r_s = -0.70$) and hours of sunlight per month ($r_s = -0.61$). Stratified analyses based on atopic comorbidities revealed that the correlations between AA flare frequency and the 4 meteorological variables was stronger and significant only in atopic patients compared to patients without atopy. In atopic patients, significant correlations also appeared between flare frequency and temperature ($r_s = -0.72$), precipitation ($r_s = -0.71$) and number of days with rain ($r_s = -0.68$). Our results suggest that relationships exist between climate and AA flare frequency. Furthermore, atopic AA patients are more susceptible to the impact of climate. Physicians may counsel AA patients on these relationships. Future studies should explore how these aspects of climate affect AA pathogenesis at the molecular level.

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Quality of life in patients with facial cutaneous lupus erythematosus

J. S. Concha[1, 2], D. Yan[1, 2], A. Ravishankar[1, 2], C. Bax[1, 2], R. Borucki[1, 2], V. Werth[1, 2]

[1]Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]CMCVAMC, Philadelphia, Pennsylvania, United States

Lesions on exposed areas are known to have a significant impact in the quality of life (QoL) of patients. This study aims to compare the QoL of patients with cutaneous lupus erythematosus on the face (FCLE) versus patients without facial lesions, and to determine whether lesion activity (erythema, scale) and damage (pigmentation, scarring) on the face have an impact on QoL. This is a cross-sectional study of a database of CLE patients seen at the University of Pennsylvania. Patients with a diagnosis of CLE, have available Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) assessments, and who were able to complete the Skindex-29+3 questionnaire on the initial visit were included. Patient demographics were summarized as frequencies and percentages for categorical variables, and as median for continuous variables. For comparison of QoL across patient groups, one-way ANOVA and Bonferroni correction were used. Multivariable regression analyses were used to determine the relationship of FCLE and other variables to the QoL of CLE patients. There were 366 CLE patients in this study. Among them, 255 had FCLE. Specifically, 99 patients had both activity and damage, 124 had activity only, and 32 had damage only. The mean Skindex-29 Symptoms, Emotions and Functioning scores of patients who had both activity and damage (S=47.34, E=55.06, F=43.45) as well as activity alone (S=43.55, E=53.72, F=43.91), were significantly different from those who had no facial lesions (S=35.05, E=42.50, F=31.78) ($p <0.05$). Further, FCLE with activity and damage and active FCLE alone were significantly associated with poor QoL in all SKINDEX-29+3 domains even after controlling for the female gender and current smoking status, which are known to be associated with poor QoL scores in CLE patients. Race and CLE subtype did not have a significant effect on QOL scores ($p > 0.05$). FCLE patients have worse QoL compared with patients without facial lesions. FCLE activity drives QoL more than damage.

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Characteristics associated with physician-identified melanomas vs personally-identified melanomas

D. J. Lewis, D. Shin, M. E. Ming

University of Pennsylvania, Philadelphia, Pennsylvania, United States

Melanomas identified by patients or their friends or family are thicker than those identified by physicians, indicating that they are likely found at a later point in their development. If patients were able to identify their melanomas earlier, their prognosis potentially could be improved. We sought to determine what clinical characteristics distinguish melanomas found by physicians ("physician-identified melanomas") vs those found by patients or their friends or family ("personally-identified melanomas"). We performed a cross-sectional study of 157 melanoma patients seen at our Pigmented Lesion Clinic (PLC) who had their first melanoma within 6 months of their original PLC visit. Eleven clinical characteristics were assessed as potential explanatory variables. Study patients had a mean age of 56.9 years (SD=14.7 years), with 92 (58.6%) being male. Ninety-seven (61.8%) of the patient's melanomas were personally identified. Using a multivariable logistic regression model, personally-identified melanomas were less likely to occur in patients who were older than the mean age (OR=0.44, 95% CI: 0.21, 0.89), those whose melanomas were located on the trunk (OR=0.20, 95% CI: 0.10, 0.44), and those who did not have a spouse or partner who was able to assist with future skin examinations (OR=0.41, 95% CI: 0.19, 0.90). Other characteristics such as number of nevi, sex, and history of non-melanoma skin cancer did not have a significant association in univariate analysis, nor did they have a significant association in multivariable analysis or otherwise strengthen the multivariable model described above. These results indicate that there are particular characteristics associated with a decreased likelihood of a melanoma being detected by patients or their friends or family, highlighting potential issues to consider when designing and targeting patient education efforts aimed towards earlier detection of melanoma.

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Review of acne evaluation and management published in clinical practice guidelines for gender-affirming hormone therapy

A. M. Cartron[1], J. Zampella[2], H. Yeung[3]

[1]Dermatology, University of Maryland School of Medicine, Baltimore, Maryland, United States, [2]Dermatology, New York University, New York, New York, United States, [3]Dermatology, Emory University School of Medicine, Atlanta, Georgia, United States

Acne is a known side effect for transgender patients receiving testosterone for gender affirmation. Clinical practice guidelines and policy statements for transgender patients exist to provide evidence-based recommendations. There is a knowledge gap regarding optimal treatment of acne in transgender patients receiving masculinizing hormone therapy. We conducted a narrative review of guidelines for the management of transgender patients. Clinical practice guidelines for hormone therapy in transgender patients that were published in English were identified using Google, UpToDate, and PubMed with the search terms "transgender" and "acne" and "guidelines" or "hormones" or "testosterone." Of 12 guidelines reviewed, 7 mentioned acne as a potential adverse effect from testosterone therapy. 6 guidelines discussed acne onset, most commonly at 1-6 months of testosterone therapy initiation. 6 guidelines discussed acne duration, typically for a maximum of 1-2 years. 6 articles mentioned severity of acne. 5 articles discussed acne resolution. 3 articles discussed surveillance broadly for adverse effects of hormone therapy, but none specifically noted acne surveillance. 1 article mentioned initial treatment and additional treatments for recalcitrant cases, stating that treatment algorithms in transgender patients were identical to those in cisgender patients. None discussed pathways for referral to dermatology for acne management. Future systematic reviews using more comprehensive search strategies may uncover additional published guidelines. Current clinical practice guidelines for gender-affirming hormone therapy rarely discussed acne evaluation or management for transgender patients. Best practices for the treatment of acne should be established to improve skin and quality of life outcomes for gender-affirming hormone therapy.

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Leukocytoclastic vasculitis with and without IgA deposition is associated with renal damage: A case-control study

E. M. Leland[1], Y. Semenov[2], B. Kaffenberger[3], K. Williams[1], J. Alhariri[1], S. Kwatra[1]

[1]Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [2]Dermatology, Washington University School of Medicine, St. Louis, Missouri, United States, [3]Dermatology, Ohio State University Wexner Medical Center, Columbus, Ohio, United States

Leukocytoclastic vasculitis (LCV) is a small vessel vasculitis presenting with palpable purpura. Direct immunofluorescence (DIF) is routinely performed on skin biopsy to identify IgA deposition, which is thought to contribute to greater frequency of renal comorbidity associated with IgA vasculitis. Knowledge of characteristics between LCV with and without IgA deposition is limited. This study characterized presentations of LCV with and without IgA. Charts identified in the JHH pathologic database system with biopsy confirmed LCV between 2009-2018 were reviewed. Adult patients were stratified by presence of IgA by DIF on skin biopsy to compare demographics, comorbidities, clinical features, and hospitalization characteristics. Univariate analyses were performed using χ^2 tests and t-tests in STATA. Of 162 biopsy confirmed LCV cases, DIF was performed on 118 (72.8%). IgA deposition in skin biopsy was present in 46 (39.0%) patients. Median age was 54 (IgA-) and 51 (IgA+). IgA- LCV was associated with elevated CRP (average IgA- 8.66 vs. IgA+ 3.41; p=0.015). Differences in comorbidities included pulmonary hypertension (IgA- 3.7%, IgA+ 14.0%; p=0.024), peripheral vascular disease (IgA- 22.6%, IgA+ 7.0%; p=0.025), and asthma (IgA- 14.1%, IgA+ 2.3%, p=0.035). No difference in renal involvement (hematuria, pyuria, or proteinuria) was observed (IgA- 60.7%, IgA+ 54.3%, p=0.545). There was no difference in prevalence of AKI or CKD at diagnosis (IgA- 28.4%, IgA+ 30.6%, p=0.810) or within 365 days (IgA- 31.5%, IgA+ 42.9%; p=0.230). Patients with LCV with and without IgA deposition are both at heightened risk of renal disease, suggesting all LCV cases should be followed for potential renal injury. Future studies should evaluate differences in pathogenesis and natural history and investigate the impact of DIF in the management of patients presenting with palpable purpura.

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Increased incidence of non-melanoma skin cancers among rural kidney transplant recipients

H. Maredia, J. Motter, A. Massie, D. Segev, M. Loss

Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Skin cancers are the most common malignancy in kidney transplant (KT) recipients. Current data supports an uneven distribution of dermatological care towards urbanized areas, leading to limited access in rural settings. Patients in rural areas thus may have more difficulty in obtaining routine skin screening after transplant. We hypothesized that there would be higher risk of skin cancers after transplant among rural KT recipients and sought to examine rural-urban differences in skin cancer incidence in KT recipients in the US. National transplant data was linked to US geographic data and Medicare claims to retrospectively review 122,462 Medicare-primary KT recipients from 1999-2014. Residence was categorized into rural, micropolitan, and urban areas using the Rural Urban Commuting Area codes, which classifies Zip-code level areas based on population density, urbanization, and urban commuting. Using ICD-9 codes, we categorized skin cancer diagnoses into melanoma and non-melanoma skin cancers (NMSC), which included squamous and basal cell carcinomas. We used adjusted Cox regression to quantify the risk of skin cancer based on residence. At five years after transplantation, the incidence of NMSC was 10.5%, 9.7%, and 7.6% in rural, micropolitan, and urban areas, respectively (p<0.001). This translated into a 17% higher NMSC risk in rural areas (adjusted hazard ratio [aHR]=1.17, 95% CI 1.09-1.24, p<0.001) and a 10% higher NMSC risk in micropolitan areas (aHR=1.10, 95% CI 1.03-1.18, p=0.004) compared to urban areas. Conversely, there was no evidence of difference in melanoma risk across areas of residence. Renal transplant recipients residing in rural and micropolitan areas are at increased risk of developing NMSC. Given limited access to care in these communities, further exploration of this difference through prospective studies is warranted.

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Risk of keratinocyte carcinoma among patients with hidradenitis suppurativa

S. Ashrafzadeh[1], Y. Kim[1], G. Peters[1], H. Lee[2], M. Asgari[1]

[1]Department of Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Department of Statistics, Massachusetts General Hospital, Boston, Massachusetts, United States

Recent studies have implicated the role of the Notch pathway in keratinocyte carcinomas (KCs). Notch signaling is often upregulated in basal cell carcinoma (BCC) but downregulated in squamous cell carcinoma (SCC). Hidradenitis suppurativa (HS) is an inflammatory disorder of the apocrine glands whose pathogenesis has also been associated with downregulation of Notch signaling. Given the role of Notch in the pathogenesis of HS and KCs, we hypothesized that HS patients could be at decreased risk for BCC and increased risk for SCC. To test our hypothesis, we examined the association between HS and KC risk by comparing the incidence of BCC and SCC in HS patients to controls (patients with acne vulgaris, an inflammatory disorder of pilosebaceous glands that is not known to involve the Notch pathway). Using an age, sex, and race matched 1:2 case-control study design, we derived KC outcomes and covariates through chart review of an institutional database (Partners Healthcare, 2000-2019) and determined incident BCC and SCC risk by performing multivariate logistic regression adjusted for disease duration, BMI, smoking, immunosuppression, and number of dermatology visits. The study population consisted of 13,812 patients (4,604 HS and 9,208 acne patients), of which 59% were white, 74.7% female, and mean (SD) age was 43.7 (15.4) years. Adjusted odds ratio (OR) for incident BCC risk in HS was OR 0.51 (95% CI 0.37-0.71, P<0.001) whereas adjusted OR for incident SCC risk was 0.79 (95% CI 0.54-1.17, P=0.238). There were no statistical differences in age, gender, and race among those who developed BCC or SCC between groups (all P>0.05). Our results suggest that HS patients have reduced risk of BCC, and similar risk of SCC, compared with controls (acne patients). Further studies are needed to investigate whether decreased Notch signaling in HS patients plays a protective role in BCC tumorigenesis or whether inflammation of the pilosebaceous unit in acne affects follicular bulge cells to increase risk of BCCs.

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Crohn's disease prevalence prior to and following hidradenitis suppurativa diagnosis

M. Rosales Santillan[1], K. Savage[2], M. Porter[1], R. Parker[3], M. Simon[3], A. B. Kimball[1]

[1]Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States, [2]Drexel University College of Medicine, Philadelphia, Pennsylvania, United States, [3]Arcadia.io, Burlington, Massachusetts, United States

Previous studies have shown differing results on the prevalence of Crohn’s disease in HS [1,2]. Our study used a large claims database to determine the prevalence of Crohn’s disease in HS. The Arcadia.io database includes insurance claims and visit-level data from > 34,000,000 discrete lives. The database was searched for individuals with a diagnosis of Crohn's disease within the general patient cohort. Patients with $\geq$1 coded diagnosis of HS (ICD-9:705.83 or ICD-10:L73.2) in a clinical setting at any time were identified. Within the HS group, patients with Crohn's disease were identified at time of HS diagnosis and up to 3 years after initial HS diagnosis. Prevalence was determined by rate of diagnosed individuals out of all individuals stored at the time of the search; all identities were de-duplicated. Prevalence was further stratified by reported sex and age. The prevalence of Crohn’s disease in the entire adult cohort was 0.5% (N=36,546, general cohort population= 7.8 million) which appeared to be consistent across different age groups and sex categories. The prevalence of Crohn’s disease prior to or at time of HS diagnosis was 0.85% (HS cohort= 38,841) and had an even further increase at 1.4% (HS cohort= 39,897) prevalence following HS diagnosis. Crohn’s disease appears to have increased prevalence in HS compared to the overall cohort and even greater prevalence following HS diagnosis. Further studies may provide information in regards to disease detection. References: 1.) Deckers IE, Benhadou F, Koldjik MJ et al. Inflammatory bowel disease is associated with hidradenitis suppurativa: Results from a multicenter cross-sectional study. J Am Acad Dermatol. 2017;76(1):49-53. 2.) Eppinga H, Thio HB, van der Woude CJ. Characteristics of patients with hidradenitis suppurativa and inflammatory bowel disease. Clin Gastroenterol Hepatol. 2016;14(3):482-3.

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Differences in dermatologic care for acne vulgaris patients with autism
R. Fort, J. Sciacca Kirby, S. Maczuga
Dermatology, Penn State University College of Medicine, Hershey, Pennsylvania, United States
Atypical sensory processing occurs commonly in individuals with Autism Spectrum Disorder (ASD) and may impact the care these patients receive for skin conditions like acne vulgaris. This is a retrospective cohort study of acne-specific medication and outpatient utilization and costs from MarketScan© database (2015-2017) for continuously enrolled 11 to 18-year-old patients. People with acne were identified based on at least one instance of ICD-9/ICD-10 code (706.1/L70.0), then assigned to a cohort with ASD based on at least one instance of ICD-9/ICD-10 code (299, F84) or the cohort without ASD based on random selection of age and sex at a 10:1 ratio. Medication claims were based on NDC codes recorded at acne-specific visits and were controlled for age and sex. Outpatient utilization excluded ED and urgent care claims. Overall, 4,269 patients with acne and ASD and 42,690 patients with acne alone were identified.ASD patients received topical antimicrobials more often (25.06% vs 21.89%, p<0.0001) and had a higher mean 3-year cost per patient ($588.48 vs. $460.39, p<0.0001). ASD patients were prescribed topical retinoids less frequently (32.68% vs. 34.53%, p=0.02) with a greater mean 3-year cost per person ($888.68 vs. $814.74, p<0.0001). Oral antibiotics were used less frequently for ASD patients (18.74% vs. 20.35%, p=0.02) with a higher mean cost ($257.41 vs. $176.29, p<0.0001). Both spironolactone (0.51% vs. 1.58%, p<0.0001) and isotretinoin (8.27% vs. 11.27%, p<0.0001) were prescribed less often to ASD patients. For ASD patients, the odds of claims for topical retinoids (OR= 1.088, p=0.0139), spironolactone (OR=2.659, p<0.0001), and isotretinoin (OR=1.351, p<0.001) were higher when age was controlled. Likewise, the odds of claims for topical retinoids (OR=1.089, p=0.0143), oral antibiotics (OR=1.116, p=0.0089), and isotretinoin (OR=1.697, p<0.0001) were higher when adjusted for age and sex. *Analysis of outpatient claims is underway and will investigate utilization differences.* This study demonstrates important differences in utilization and costs for acne patients with ASD.

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Ichthyosis affects mental health in adults and children: A cross-sectional study
Q. Sun[1], I. Ren[2], T. Zaki[1], K. Maciejewski[3], K. Choate[4]
[1]Dermatology, Yale School of Medicine, Eads, Tennessee, United States, [2]Icahn School of Medicine at Mount Sinai, NYC, New York, United States, [3]Yale Center for Analytical Sciences, Yale University School of Public Health, New Haven, Connecticut, United States, [4]Dermatology, Genetics, Pathology, Yale School of Medicine, New Haven, Connecticut, United States
The impact of ichthyosis on mental health is unknown. The purpose of our cross-sectional study is to determine the relationship between quality of life (QoL) and mental health in adults and children with ichthyosis. To do this, we surveyed 181 patients (128 adults and 53 children) from the National Registry for Ichthyosis and Related Skin Types using age-appropriate QoL, depression and anxiety questionnaires. Spearman's correlation and logistic regression models determined the QoL and mental health relationship. Ichthyosis patients exhibited a high prevalence of depression and anxiety. 34.4% of adults screened positive for depression and 27.3% screened positive for anxiety. 30.2% of children screened positive for depression and 37.7% screened positive for anxiety. In both adults and children, higher severity of QoL impairment predicted anxiety and depression. Difficulties with leisure and school/work were associated with depression and anxiety in adults. Negative impact on feelings and symptoms were associated with anxiety and depression in children. Male gender was associated with depression among children. Limitations of the study include a relatively small pediatric sample, volunteer bias and use of screening tools. Despite these limitations, our study is the first to examine the interplay between QoL and depression/anxiety in ichthyosis. Our results reveal that physicians should assess depression, anxiety, and QoL in ichthyosis patients to address overlooked psychosocial complications of disease.

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The practice of dermatology: Comparison of patient characteristics and healthcare delivery between dermatology and non-dermatology providers
A. Y. Chen, I. Singh, A. J. Uy, A. W. Armstrong
Dermatology, Keck School of Medicine of University of Southern California, Los Angeles, California, United States
A thorough understanding of the overall landscape of dermatological care is important for improving patient access and healthcare policies. In this study, we sought to compare patient characteristics and healthcare delivery methods between dermatology providers and non-dermatology providers. We conducted a cross-sectional study using the National Ambulatory Medical Care Survey from 2015-2016. During this period, there were 43.5 million visits (weighted) annually to dermatology providers, which accounted for 4.6% of all ambulatory visits across specialties. Compared to patients seen by other specialties, those seen by dermatology providers tend to be older (mean age 54 versus mean age 49, p<0.0001) and have more private insurance (72% versus 58%, p<0.0001). Among visits conducted with dermatology providers, acne remains the most common reason that patients seek care, accounting for 7.9% of total visits to dermatology providers. Regarding healthcare delivery, the average wait time for scheduling an appointment is approximately 2 weeks longer for a dermatology provider than a non-dermatology provider. Compared to non-dermatology providers, dermatology providers use a lower rate of telephone consults (OR 0.37, 95% CI 0.34-0.40) and internet/email consults (OR 0.86, 95% CI 0.77-0.95). This study demonstrates that access to dermatology providers remains more limited compared to non-dermatology providers.

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Association of skin response in erythema and sclerosis with survival in chronic graft-versus-host disease
L. X. Baker[1], M. Byrne[2], P. Martin[3], S. Lee[3], H. Chen[2], M. Jagasia[2], E. Tkaczyk[1]
[1]Department of Veterans Affairs and Vanderbilt Dermatology Translational Research Clinic, Nashville, Tennessee, United States, [2]Vanderbilt-Ingram Cancer Center, Nashville, Tennessee, United States, [3]Fred Hutchinson Cancer Research Center, Seattle, Washington, United States
Skin is the most commonly affected organ in chronic graft-versus-host disease (cGVHD). Though causing significant morbidity, skin is not a common direct cause of mortality. We report survival associations of complete (CR) vs partial (PR) and no cutaneous response (NR). Using the cGVHD Consortium, a prospective observational study of cGVHD patients from 2007-2017 at 9 centers, we studied patients who were enrolled <3 months (incident) or >=3 months (prevalent) after diagnosis of cutaneous cGVHD and within 3 years of transplant. We used the 2005 NIH criteria to designate CR, PR, or NR in erythema or sclerosis at the 2nd study visit. Cox regression models were used to predict subsequent overall survival (OS) and non-relapse mortality (NRM) based on skin response. Of 468 consortium patients, 185 (40%) had erythema at entry of whom 136 (74%) achieved CR at the 2nd visit, 9% PR, 18% NR, with similar response in incident and prevalent erythema. At entry, 89 (19%) patients had sclerosis. Response was higher in incident (68% CR, 2% PR, 30% NR) compared to prevalent sclerosis (28% CR, 4% PR, 68% NR). Erythema CR in prevalent cases correlated with improved OS (median OS 1858 vs 1026 days, HR 0.29, 0.09-0.89, p=0.03) and NRM (median NRM 1194 vs 1026 days, HR 0.26, 0.08-0.92, p=0.04). CR of incident erythema was weakly associated with improved OS and NRM, with a stronger effect in the subgroup with additional affected organs. Though underpowered, CR of sclerosis had similar trends of improved OS and NRM in both incident and prevalent groups. Most incident cases of cGVHD have CR in both sclerosis and erythema by the following visit. Cutaneous CR is associated with improved survival times up to and exceeding 2 years. Sclerosis present <3 months from cGVHD diagnosis is twice as likely to resolve as sclerosis present later in the disease.

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The association between tobacco smoke exposure during childhood and adolescence and atopic dermatitis activity and severity
N. A. Al-Alusi[1], F. D. Ramirez[2], S. Langan[3], K. Abuabara[4]
[1]School of Medicine, UCSF, San Francisco, California, United States, [2]Pediatrics, UCSF, San Francisco, California, United States, [3]Non-Communicable Disease Epidemiology, LSHTM, London, United Kingdom, [4]Dermatology, UCSF, Berkeley, California, United States
Atopic dermatitis (AD) is the most common chronic inflammatory skin disease worldwide, yet the triggers of disease activity and persistence are still not completely understood. Tobacco smoke exposure (TSE) is an environmental factor that deserves particular attention because of its known effects on humoral and cellular immunity. The objective of our study was to investigate the extent to which TSE during childhood and adolescence is associated with AD activity and severity. Data were obtained from the Avon Longitudinal Study of Parents and Children, a longitudinal population-based cohort from the UK with 10,518 individuals followed from birth through adolescence. AD was based on a validated measure of maternal-reported flexural dermatitis activity at 10 time points and severity at 9 time points between the ages of 2.5 and 18 years. TSE was measured using a standardized maternal-reported measure at 8 time points between the ages of 6 months and 9 years. Child serum cotinine levels (a nicotine metabolite and validated TSE biomarker) were available at 7, 15.5, and 17 years of age. After adjusting for demographic and socioeconomic confounding, there was no significant association between TSE level and the risk of ever having AD using cross-sectional multinomial regression models (RRR: 0.80-1.19; p>0.05 at all time points) and no significant association between TSE and AD severity (RRR: 0.69-1.52; p>0.05 at all time points). At 7 years old, there was no significant association between child serum cotinine level and AD severity. In contrast, at 15.5 years, a 1 ng/mL increase in serum cotinine showed a small but statistically significant association with severe AD compared to no AD (RRR: 1.007; 95% CI: 1.000, 1.013), suggesting that active rather than passive TSE may be most relevant to disease activity and severity.

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Deep analysis of autoimmune blistering disease subtype and HLA associations in Chinese population
Y. Sun, H. Liu, F. Zhang
Department of Dermatology, Shandong Provincial Institute of Dermatology and Venereology , Jinan, Shandong, China
Autoimmune blistering diseases (AIBDs) are a group of rare acquired blistering skin diseases, which are divided into five major subtypes based on the clinical appearance and pathology: pemphigus diseases, bullous pemphigoid (BP), epidermolysis bullosa acquisita (EBA), dermatitis herpetiformis (DH) and Linear IgA bullous dermatosis (LigA). Current understanding has been greatly increased by genetic investigations mainly focus on the HLA in various populations. We have conducted the HLA association studies on different subtypes of AIBDs in Chinese population by using Next-generation (NGS) based HLA typing methods. Upon these data, we aimed to investigate the generality and heterogeneity in different disease subtype and population. A total number of 369 pemphigus (210 PV and 159 PF), 575 BPs, 36 DHs, 33 LigAs, 17 EBAs and 976 healthy controls were enrolled in the study. Genome-wide association studies were performed in the pemphigus and BPs. Associations study of HLA were conducted on the results of NGS based HLA typing. We have identified different associations for subtypes of AIBDs. For pemphigus, we confirmed HLA-DQB1*05:03 to be the strongest association with PV and PF. In addition, HLA-DRB1*14 was demonstrated to be a second independent variants for PV, while HLA-DRB1*04:06 was demonstrated to be the second independent signal for PF. For pemphigoid, HLA-DQB1*03:01 were confirmed to be the strongest association, especially for the BP180-positive group. For DH, HLA-B*08:01 and HLA-DRB1*03:01 were confirmed to be independent associations. None significant HLA associations were identified for EBA and LigA. Population heterogeneity analysis revealed effect of HLA associations on different subtype of AIBDs, especially for DH. The investigation of AIBD subtypes advanced the understanding the genetics of AIBD susceptibility and offers molecular insight into the pathophysiological mechanisms.

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Risk of second primary malignancies in Kaposi Sarcoma: A U.S. population-based study
B. Nardone[1], M. Nasca[2], W. Liszewski[1], D. P. West[1], G. Micali[2]
[1]Dermatology, Northwestern University, Chicago, Illinois, United States, [2]Dermatology, University of Catania, Catania, Italy
An increased risk for second primary malignancies (SPMs) has been reported with Kaposi Sarcoma (KS), however the risk for SPMs in those who have HIV-Related Death (HIV-RD) vs those with no HIV-RD (noHIV-RD) has not been reported. The aim of this study was to determine the risk of SPMs for KS by comparing noHIV-RD vs HIV-RD. The Surveillance, Epidemiology, and End Results (SEER) database (2000-2016) was searched to detect all patients with a primary KS who survived $\geq$ 2 mo after diagnosis (histology codes 9140/3). Site recode B ICD-O-3 was used to detect SPMs. Standardized Incidence Ratios (SIRs), ratio of the observed (O) in KS survivors, to the expected (E) in the age-adjusted general US population (O:E ratios), and 95% confidence intervals (CIs) were calculated. Cause of Death was used to categorize KS patients into two groups: those with HIV-RD vs those with noHIV-RD. Of 1,405 KS patients with HIV-RD, 119 (8.46%) developed $\geq$1 SPMs, and considering all tumor types in aggregate, there was an overall significantly increased risk for SPMs (SIR 11.77, 95% CI 9.75-14.08) while of 1,029 KS patients with noHIV-RD, 159 (15%) developed $\geq$1 SPMs (SIR 1.86, 95% CI 1.58-2.18). Compared to noHIV-RD, among the HIV-RD individuals, the risk for cervical, anal, liver, and oral cancers, as well as both Hodgkin and non-Hodgkin lymphoma, was 3-6 fold greater. An unexpected finding was the significantly increased risk for subsequent bone and for urinary system cancers in the noHIV-RD individuals but not for those with HIV-RD. These findings serve to confirm that KS survivors are at significantly increased risk for SPMs and highlight the need for ongoing surveillance for SPMs in the Kaposi Sarcoma patient population.

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Characteristics of patients with palmoplantar pustulosis compared to those with psoriasis vulgaris: A claims database study
N. Kotowsky[1], S. R. Feldman[2], E. M. Garry[3], W. C. Valdecantos[1], R. Gao[1], A. K. Golembesky[4]
[1]Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, Connecticut, United States, [2]Department of Dermatology, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States, [3]Aetion, Boston, Massachusetts, United States, [4]Boehringer Ingelheim International GmbH, Ingelheim, Germany
Palmoplantar pustulosis (PPP) is a chronic, debilitating, and painful inflammatory skin disease, characterized by localized sterile pustules on the hands and feet. There is little real-world evidence characterizing patients (Pts) with PPP. This study examined demographics, comorbidities, and concurrent medication use for PPP Pts compared to those with psoriasis vulgaris (PsO) in a real-world setting. PPP or PsO Pts were identified if they had ≥1 inpatient or ≥2 outpatient ICD-10 diagnosis codes (L40.3 or L40.0, respectively) separated by 30 to 365 days. All analyses were conducted via the Aetion Evidence Platform using IBM MarketScan Research Databases. The study period was Oct 1, 2015 – Sept 30, 2018. For comorbidities, a matched (age/sex) non-psoriasis cohort comprising persons without a history of psoriasis (but allowing psoriatic arthritis) was provided for context. At baseline, 1,579 PPP and 75,494 PsO Pts were identified, with 807 PPP and 38,950 PsO Pts having ≥12 months' follow up (FU). PPP Pts tended to be female (70.8%; 51.2% for PsO) and had a mean age of 52.8 years (49.0 for PsO). Compared to PsO Pts and the non-psoriasis group, PPP Pts were more likely to have a diagnosis of hyperlipidemia (PPP: 21.3%; PsO: 16.3%; non-psoriasis: 11.4%), obesity (PPP: 5.7%; PsO: 4.5%; non-psoriasis: 3.0%), and depression (PPP: 5.3%; PsO: 4.0%; non-psoriasis: 3.1%) at baseline. During FU, 48.7% of PPP Pts were treated with a non-biologic oral systemic therapy (31.7% for PsO). Methotrexate was the most commonly used oral therapy (17.5%) followed by apremilast (14.1%) and acitretin (13.1%). Fewer PPP Pts were on a biologic (25.2.%) compared to PsO (36.9%). PPP Pts were treated with oral systemics over biologics compared to PsO Pts, suggesting differences in clinical management.

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Characteristics of patients with generalized pustular psoriasis compared to those with psoriasis vulgaris: A claims database study

N. Kotowsky[1], S. R. Feldman[2], E. M. Garry[3], W. C. Valdecantos[1], R. Gao[1], A. K. Golembesky[4]

[1]Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, Connecticut, United States, [2]Department of Dermatology, Wake Forest School of Medicine , Winston-Salem, North Carolina, United States, [3]Aetion, Boston, Massachusetts, United States, [4]Boehringer Ingelheim International GmbH, Ingelheim, Germany

Generalized pustular psoriasis (GPP) is a rare and severe systemic disease characterized by recurrent acute flares consisting of disseminated erythematous skin rash with sterile neutrophil-filled pustules. There is little real-world evidence characterizing patients (Pts) with GPP. This study examined demographics, comorbidities, and concurrent medication use for Pts with GPP compared to those with psoriasis vulgaris (PsO) in a real-world setting. GPP or PsO Pts were identified if they had ≥1 inpatient or ≥2 outpatient ICD-10 diagnosis codes (L40.1 or L40.0, respectively) separated by 30 to 365 days. All analyses were conducted via the Aetion Evidence Platform using IBM MarketScan Research Databases. The study period was from Oct 1, 2015 to Sep 30, 2018. For comorbidities, a matched (age/sex) non-psoriasis cohort comprising persons with no history of psoriasis (but allowing psoriatic arthritis) was provided for context. At baseline, 1,175 GPP and 75,494 PsO Pts were identified, with 637 GPP and 38,950 PsO Pts having ≥12 months' follow up (FU). Pts with GPP tended to be female (63.3%; 51.2% for PsO) and had a mean age of 52.4 years (49.0 years for PsO). Compared to PsO Pts and the non-psoriasis group, GPP Pts were more likely to have a diagnosis of psoriatic arthritis (GPP: 20.6%; PsO: 6.4%; non-psoriasis: <0.1%), anxiety (GPP: 9.0%; PsO: 6.0%; non-psoriasis: 5.1%), and depression (GPP: 7.0%; PsO: 4.0%; non-psoriasis: 3.1%) at baseline. During FU, 15.7% of GPP Pts were treated with a topical medication only, but 70.0% were prescribed a systemic medication, compared to 62.2% of PsO Pts. Methotrexate was the most commonly used oral therapy (19.2%), while 22.8% were treated with an anti-TNF. Pts with GPP have more comorbidities and are more commonly treated with systemic therapies than Pts with PsO.

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Penile calciphylaxis: A retrospective analysis of ten cases

C. Gabel[1], T. Chakrala[1], R. Shah[2], M. J. Danesh[3], A. C. Walls[4], S. U. Nigwekar[5], P. I. Song[6], D. Kroshinsky[1]

[1]Dermatology, Massachusetts General Hospital, Cambridge, Massachusetts, United States, [2]Rutgers Dermatology Residency, Robert Wood Johnson Medical School, New Brunswick, New Jersey, United States, [3]Harvard Combined Dermatology Residency, Harvard Medical School, Boston, Massachusetts, United States, [4]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States, [5]Nephrology, Massachusetts General Hospital, Boston, Massachusetts, United States, [6]Dermatology, Palo Alto Medical Foundation, Palo Alto, California, United States

Background: Calciphylaxis is a disorder of vascular calcium deposition leading to ischemia and skin necrosis. Rarely, there may be penile involvement, which has not been well documented. Here we describe ten cases of penile calciphylaxis to assess risk factors and mortality. *Methods:* A case series of patients with penile calciphylaxis at Massachusetts General Hospital and Beth Israel Deaconess Medical Center was generated through retrospective chart review of patients with a diagnosis of calciphylaxis between January 2001 to December 2018. Ten male patients with calciphylaxis without penile involvement were randomly selected from a site-specific historical database as control subjects. *Results:* Among patients with penile calciphylaxis, four had chronic liver disease, compared to zero of the controls. Overall mortality was 60% (median 1.3 months, interquartile range (IQR) 6.9 months). The control group had a mortality of 60% (median 15.4 months, IQR 39.7 months). Among patients with penile calciphylaxis, four deaths were due to infected calciphylaxis lesions leading to sepsis and two were due to cardiac arrest. One of the patients who had a penile biopsy died from calciphylaxis-related sepsis. In the control group, two deaths were due to cardiac arrest and two deaths were due to infected calciphylaxis lesions. *Limitations:* Retrospective nature. Small sample size. *Conclusions:* As a rare entity, this study represents one of the largest penile calciphylaxis case-series to-date and highlights the risk of penile biopsy. Early recognition of this serious disease and treatment initiation is imperative to improve outcomes.

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Frontal fibrosing alopecia: Utilization of the Lichen Planopilaris Activity Index to assess treatment outcomes

A. Nguyen[1], R. Freese[2], D. Windenburg[1], I. Wallender[1], R. Farah[1], M. K. Hordinsky[1]

[1]Dermatology, University of Minnesota, Minneapolis, Minnesota, United States, [2]Biostatistical Design and Analysis Center, Clinical and Translational Science Institute, University of Minnesota, Minneapolis, Minnesota, United States

Frontal fibrosing alopecia (FFA) is a primary cicatricial alopecia which affects the frontotemporal scalp and face. The etiology of FFA is thought to be related to environmental exposures and a genetic susceptibility. FFA is considered to be a relatively new disease; there is a paucity of knowledge surrounding its pathogenesis, treatment and prognosis. Because FFA is histologically similar to lichen planopilaris (LPP), we chose to characterize long-term outcomes of our FFA patients using the LPP Activity Index (LPPAI), a validated scoring system introduced in 2010 which combines symptoms, signs and progression. We used this tool to assess longitudinally (0 and 12 months) the efficacy of treatments in our hair disease clinics. We completed a retrospective chart review of patients with a diagnosis of FFA seen by one investigator (MH) from 7/1/09-6/30/19. Clinical data and patient demographics were extracted from the electronic medical record Epic by the University of Minnesota Best Practices Integrated Informatics Core yielding 706 patients diagnosed with lichen planus, LPP and/or FFA. A further keyword search, with the terms frontal fibrosing alopecia or FFA, was performed using a natural language processing program, identifying 142 patients. This patient population was then limited to 24 patients due to availability of LPPAI data. A paired sample t-test was performed to evaluate differences between LPPAI scores at the baseline visit and the 12-month visit. Our analyses revealed that our patient population is predominantly female, white, and postmenopausal. Additionally, FFA patients achieved a statistically significant reduction in LPPAI score after 12 months of treatment; however, no patients were found to have a complete absence of symptoms or signs. In conclusion, the LPPAI is a valuable tool which can provide a standardized method to quantify clinical progression and response to therapy.

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Lichen planopilaris and frontal fibrosing alopecia: A possible link with rosacea

L. Burns[1], B. De Souza[1], E. Flynn[1], D. Hagigeorges[1], M. Senna[1, 2]

[1]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [2]Harvard Medical School, Boston, Massachusetts, United States

Lichen planopilaris (LPP) and its clinical variant, frontal fibrosing alopecia (FFA), are primary cicatricial alopecias. The etiopathogenesis remains unknown, however the 2 conditions primarily affect post-menopausal, Caucasian women. An increased incidence of rosacea has been identified in this patient cohort, with rates of 34-62% in FFA[1,2] compared to 1.5-10% in the general European population[3]. In this cross-sectional cohort study, we evaluate the incidence, subtype, and severity of rosacea in 66 patients presenting for evaluation of LPP/FFA. 77% (51/66) of patients were found to have rosacea on exam. 42% classified as mild, 34% as moderate and 2% as severe. Among LPP patients, 72% were found to have erythematotelangiectatic (ET) subtype with 30% ocular involvement. In FFA patients, 62% were found to have ET subtype, 21% papulopustular, and 27% with ocular involvement. The prevalence of LPP and FFA has steadily increased over the last decade, with several genetic, biologic, and environmental factors proposed. However, association with rosacea may provide new insight into the pathogenesis. A shared neurogenic inflammation (NI) mechanism may explain erythema, burning, immune infiltration, and skin fibrosis. Substance P and calcitonin gene-related peptide have been identified as possible NI mediators in rosacea with more recent implications in scarring hair loss[4]. Further understanding of this increased co-occurrence may promote early intervention and improve treatment options. References: 1. Pindado-Ortega C, et al. Frontal fibrosing alopecia and cutaneous comorbidities: A potential relationship with rosacea. J Am Acad Dermatol. 2018; 2. Porrino-Bustamante M, et al. Frontal fibrosing alopecia and rosacea: Is there any link? J Am Acad Dermatol. 2017; 3. Powell FC. Rosacea. N Engl J Med. 2005; 4. Doche I, et al. Evidence for neurogenic inflammation in lichen planopilaris and frontal fibrosing alopecia pathogenic mechanism. Exp Dermatol. 2018.

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Cutaneous langerhans cell histiocytosis in adults: Clinical features, disease course, and management among patients treated at the Dana-Farber Cancer Institute between 2003-2017

A. Bui[1], A. Laga Canales[2], E. Jacobsen[3], N. R. LeBoeuf[4, 5]

[1]Harvard Medical School, Boston, Massachusetts, United States, [2]Pathology, Brigham and Women's Hospital, Boston, Massachusetts, United States, [3]Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, United States, [4]Center for Cutaneous Oncology, Dana-Farber/Brigham and Women's Cancer Center, Boston, Massachusetts, United States, [5]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States

Langerhans Cell Histiocytosis (LCH) is a rare inflammatory neoplasm originating from myeloid dendritic cells[1]. Empiric treatment for LCH in adults has been described as a "roulette wheel" as there lack guidelines on staging, surveillance, and management[1]. Patients with cutaneous LCH may develop systemic disease and secondary hematologic malignancies; however, incidence and time to progression remains to be studied[2]. This is a retrospective cohort study of 31 patients (median age 53) with cutaneous LCH at the Dana-Farber Cancer Institute between 2003 to 2017 and literature review. 20 (64.5%) had cutaneous only disease. 5 (16.1%) had cutaneous LCH and developed extra-cutaneous LCH, with average time to progression of 84.2 months (range 46-131). 3 (9.7%) had extra-cutaneous LCH and then developed cutaneous LCH, with average time to onset of 45.7 months (8-84). 3 (9.7%) presented with cutaneous and extra-cutaneous LCH. 8 (26%) developed a secondary malignancy including diffuse large B cell lymphoma (n=2), breast cancer (n=1), chronic myelomonocytic leukemia (n=1), cutaneous T-cell lymphoma (n=1), melanoma (n=1), and thyroid cancer (n=2), with average time to diagnosis of 102 months (45-262). Patients were treated with a variety of skin-directed and/or systemic therapies. Literature review of cutaneous LCH in adults revealed 47 papers with 60 total cases. Our tertiary center experience represents the largest known retrospective cohort of cutaneous LCH in adults and may guide staging, surveillance, and treatment. 1. Allen CE et al. Langerhans-Cell Histiocytosis. *N Engl J Med.* 2018; 2. Edelbroek JR et al. Langerhans cell histiocytosis first presenting in the skin in adults: frequent association with a second haematological malignancy. *Br J Dermatol.* 2012.

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Diagnostic delay in hidradenitis suppurativa not associated with severity

P. C. Morss[1, 2], A. B. Kimball[2], M. Porter[2]

[1]University of Massachusetts Medical School, Boston, Massachusetts, United States, [2]Clinical Laboratory for Epidemiology and Applied Research in Skin (CLEARS), Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States

The natural history of hidradenitis suppurativa (HS) is not well understood. An association between diagnostic delay with increased HS severity has been suggested.[1] An IRB-approved retrospective study of 193 patients seen in an HS clinic over 4 years was performed. Data was collected at initial visit using a standardized questionnaire, including patient self-reported age at disease onset and diagnosis, gender, and race/ethnicity, and physician assessment of disease severity by Hurley stage and HS-PGA. Analyses were conducted using R software, version 3.6.0. Significance was assessed using Wilcoxon tests. Mean self-reported age at HS symptom onset was 22.1 (+/- 10.7) years while mean age at diagnosis was 28.6 (+/- 13) years. The mean lag time to diagnosis for the entire cohort was 6.5 years. The cohort was grouped into three categories: diagnosed at HS symptom onset, diagnosed between 0-5 years of onset, and diagnosed ≥5 years of symptom onset. Thirty-six percent of the cohort were diagnosed at onset (n=69), 30% within 5 years (n=57), and 33.7% were diagnosed greater than 5 years after disease onset (n=64). Within each diagnosis group, approximately the same ratio of Hurley stages (I, II, III) was observed (1:2:1, p =0.539). Time to diagnosis by Hurley stage showed no significant difference (p=0.74). No difference in time to diagnosis was found for males vs females (p=0.81) or white vs nonwhite patients (p=0.52). We report no association between time to diagnosis and Hurley stage at presentation. Patients with higher Hurley stage disease do not appear to seek care more frequently or be diagnosed more rapidly. More investigation is needed to assess whether HS patients experience disease progression during their diagnostic delay. 1. Saunte DM, Boer J, Stratigos A, et al. Diagnostic delay in hidradenitis suppurativa is a global problem. *British Journal of Dermatology*. 2015;173(6):1546-1549. doi:10.1111/bjd.14038

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Characterizing a cohort of pediatric patients with hidradenitis suppurativa

K. Babbush, A. Waldman, M. Ghias, A. Nosrati, K. Pacific, D. Lee, S. Cohen

Albert Einstein College of Medicine, Bronx, New York, United States

Hidradenitis suppurativa (HS) is a chronic inflammatory skin condition characterized by recurrent, painful inflammatory nodules that form extensive draining abscesses and dermal tracts. Median age of onset in females is 19 years and in males is 23 years. A small number of cases in pediatrics have been reported, and less than 2% of patients have disease onset before 11 years. There is a paucity of knowledge about pediatric HS. An IRB-approved retrospective chart review of pediatric patients treated at the Montefiore Hidradenitis Suppurativa Treatment Center (HSTC) from January 2015 to August 2019 identified 72 individuals. Average age at initial visit was 15.5 ± 1.34 (range 12-17) years, 62 (86.1%) were female, and BMI (documented for 35 patients) was 33.0 ± 7.78 mg/kg^2. Fourteen (19.4%) identified as Black or African-American, six (8.3%) as White, one (1.4%) as Asian, one (1.4%) as American Indian or Alaskan Native, and 50 (69.4%) as other or declined. Average Hurley stage was 1.64 ± 0.77, and HS-PGA was 2.42 ± 1.35. Most common location of lesions included axilla (84.7%), groin (59.7%), upper inner thighs (33.3%), breasts (22.2%) and buttocks (20.8%). Average duration of symptoms prior to HSTC visit was 40 months, and time since HS-diagnosis was 21 months. Twenty-six (36%) reported a family history of HS, and 41 (56.9%) reported personal history of other skin conditions including acne (27.8%), eczema (26.4%) and pilonidal cyst (6.9%). Co-diagnoses included obesity, polycystic ovary syndrome, asthma and trisomy 21. Therapies initiated at the initial visit included topical antibiotics (95.8%), oral antibiotics (52.8%), hormonal therapy (38.9%), systemic biologics (19.4%), intralesional triamcinolone (15.3%), isotretinoin (9.7%) and prednisone (1.4%). This study provides demographic and diagnostic information to characterize a pediatric cohort suffering from HS. In order to effectively treat patients with early-onset HS, one must consider the demographic information, disease progression, and comorbidities reflected in this population.

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Risk of inflammatory bowel disease in patients with atopic dermatitis- a population based cohort study

M. Syed, D. Shin, J. Wan, J. Gelfand

Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States

Atopic dermatitis (AD), a chronic inflammatory skin condition, has known associations with other allergic and non-allergic comorbidities, suggesting that it may be a systemic disorder with impacts beyond the skin. Less is known about AD and its association with other chronic inflammatory conditions such as inflammatory bowel disease (IBD). We aimed to quantify the risk of IBD among patients with AD, stratified by age compared to the general population,after adjusting for traditional risk factors. A population based retrospective cohort study was performed using The Health Improvement Network (THIN), a primary care electronic medical records database from the United Kingdom. A total of 1,079,661 patients with AD were matched on age, practice and index date to 4,860,936 unexposed controls. Outcomes of interest included IBD (Crohn's disease (CD) and ulcerative colitis (UC)). A multivariate cox proportional hazards regression model was used to calculate the hazards ratio (HR), stratified by age (<18y and ≥18y). Covariates included age, sex, Townsend index, allergic rhinitis, and asthma (for both age strata) and body mass index, smoking, and drinking (for ≥18y stratum).The risk of IBD was higher in AD patients (<18y HR:1.62, 95%CI:1.49-1.77; ≥18y HR:1.40,95%CI 1.34-1.47). Looking at specific IBD subtypes, we observed a significant association between AD and both CD (<18y HR:1.99, 95% CI:1.78-2.23; ≥18y HR:1.45,95%CI:1.35-1.56) and UC (<18y HR:1.26,95%CI:1.10-1.44; ≥18y HR:1.40, 95%CI:1.32-1.48).Similar associations were observed when asthma and allergic rhinitis were excluded from the models. Our findings from a large population-based cohort indicated an increased risk of IBD in patients with AD, with a more pronounced association in adults and with CD. Further research is warranted to identify the basis of this association.

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Clinical image identification of basal cell carcinoma and pigmented nevus based on convolutional neural networks
X. He[1], Y. Wang[1], S. Zhao[2], X. Chen[2]
[1]School of Automation, Central South University, Changsha, Hunan, China, [2]Xiangya Hospital, Central South University, Changsha, Hunan, China
This paper constructs a intelligent diagnosis model for basal cell carcinoma (BCC) and pigmented nevus (PN) based on convolutional neural networks. First, a clinical image data set of skin diseases dominated by Chinese people was constructed, in which the classification performance of five mainstream CNN models (Resnet50, InceptionV3, InceptionResNetV2, DenseNet121, Xception) was evaluated. Then, the optimal CNN classification model was compared with 30 dermatologists on 100 real-world BCC and PN patient cases. The data set contains 349 BCC and 497 PN patients, the best CNN model is Xception with the classification accuracy of 93.5%. The area under the receiving operation curve (AUROC) of BCC and PN is 97.4% and 96.9%, respectively. The performance of Xception model to distinguish the clinical images of BCC and PN is comparable to that of professional dermatologists. This study suggests that CNN model has the ability to distinguish BCC and PN of Chinese people, and lays a solid foundation for the subsequent application of artificial intelligence in the diagnosis and treatment of skin tumor.

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Limited readability and accuracy of patient facing google search results for Hidradenitis Suppurativa
N. Shukla[1], J. Jia[2], N. Admassu[2], E. Linos[2], H. Naik[1]
[1]Dermatology, UCSF, San Francisco, California, United States, [2]Stanford, Stanford, California, United States
Hidradenitis Suppurativa (HS) is a chronic inflammatory disease that affects ~1% of the Western population. In the face of median diagnostic delays of over 13 years, patients often turn to online resources for information. Our aim was to characterize the readability and accuracy of HS information available online. On January 6, 2020, 3 independent investigators searched Google for 'Hidradenitis Suppurativa,' generating 1,060000 results. Advertisements, videos and materials that did not include the terms 'Hidradenitis Suppurativa' were excluded. The top 50 patient-facing search results were included. The HTML text (including the title, body text and table headings) from each webpage was run through 4 readability tools: Flesch-Kincaid Grade Level, SMOG Grade Level, Gunning Fog and Coleman-Liau Index. We compared the readability and accuracy of HS materials written by dermatologists vs non-dermatologists (including physicians and non-physicians). Medical accuracy was determined by recommendation of physician referral and by comparing information on etiology, clinical characteristics and treatments between webpages and UpToDate. Of the top 50 Google search results, 64% were written by dermatologists and of the 36% written by non-dermatologists [50% physicians, 50% non-physicians]. The average Flesch-Kincaid Grade level of the dermatologist authored HS web pages was 12.73 and physician webpages had an average Flesch-Kincaid Grade level of 9.67 vs non-physician authored web-pages written at a 12.5 grade, despite recommendations that health materials for the general population are written at a 5th grade reading level. Information written by dermatologists was more medically accurate than that written by non-dermatologists (dermatologist 81.3%, non-dermatologist physician 27.7%, non-physician 33.3% medically accurate). In conclusion, accessibility of online HS patient information is limited, due to high reading levels across sites, and accuracy is lower of webpages written by non-dermatologist physicians and non-physicians.

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Association of processed meat and sodium intake with atopic dermatitis in adults: A pooled analysis of three cross-sectional studies in China and validation in NHANES 2005-2006
M. Shen, Y. Xiao, Y. Li, X. Chen
Department of Dermatology, Xiangya Hospital, Central South University, Changsha, China
Intake of processed food that contains high content of salt and nitrite are associated with poorer health outcomes. A recent study indicated that salt could stimulate Th2 cell differentiation in vitro experiment, and might link atopic dermatitis (AD). The study aimed to investigate intakes of processed meat, pickles, and estimated salt and nitrite with adult AD in three population-based studies among rural residents, civil servants, and workers in China. Intakes of processed meat and pickles during the past year were inquired through a food frequency questionnaire. Daily intake of salt was estimated according to food nutrition content and validated by urine sodium concentration-based estimates through the Tanaka equation. AD was ascertained by dermatologists during the field survey. NHANES 2005-2006 data was used to validate the results. AD in NHANES was defined as self-reported eczema with elevated IgE levels (>333 IU/ml). A mixed model was used to deal with the intra-cluster correlation, and to estimate the effect size in terms of adjusted odds ratio (AOR). A total of 15062 Chinese participants was analyzed. Intake of pickles for at least 1 time per week was associated with AD (AOR=1.35; 95% CI: 1.06–1.70). Intake of processed meat for at least 1 time per week was associated with AD (AOR=1.44; 95% CI: 1.11–1.87). In NHANES 2005-2006, intakes of bacon (AOR=4.47; 95% CI: 1.21–16.6) and sausage (AOR=8.59; 95% CI: 2.03–36.4), but not pickles (AOR=0.55; 95% CI: 0.07–4.67) for at least 3 times per week, were significantly associated with AD. In both Chinese and US population, cubic spline showed that the estimated daily sodium intake was linearly associated with the risk of AD. Processed meat, possibly through a mechanism involving sodium, may increase the risk of AD in adults.

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Association of night shift work with chronic spontaneous urticaria and effect modification by circadian dysfunction among workers
M. Shen, Y. Xiao, Y. Huang, X. Chen
Department of Dermatology, Xiangya Hospital, Central South University, Changsha, China
Night shift work is a social and biological stress that has been identified as a risk factor for many diseases. The study aimed to investigate the association of night shift work with chronic spontaneous urticaria (CSU), and the effect modification by circadian dysfunction. A cross-sectional survey was conducted among workers in China. Exposure was measured as the history and duration of rotating night shift work. Circadian dysfunction was indicated by excessive daytime sleepiness (EDS). CSU was diagnosed by dermatologists in the field survey. A mixed model was used to estimate the effect size, as expressed in adjusted odds ratios (AORs). A total of 7411 (92%) out of 8057 participants with complete information were included in the final analysis. The prevalence rates of CSU were 0.73% and 1.28% among workers without and with a history of night shift work, respectively. Compared with workers who never worked night shifts, the risk of CSU increased with the duration of night shift work: OR=1.55 (95% confidence interval [CI]: 0.78–3.06) for duration <5 years and OR=1.91 (95%CI: 1.12–3.26) for duration ≥5 years. EDS showed a modification effect on this association. Among workers without EDS, there was a lack of association of night shift with CSU (OR=0.94; 95% CI: 0.49–1.79). Whereas in participants with EDS, the association was significant (OR=3.58; 95% CI: 1.14–11.20). However, the effect modification by sleep disturbance was not observed. Cumulative night shift work is associated with the risk of CSU in a dose-response manner. This association may be modified by circadian dysfunction.

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Dupilumab monotherapy improves signs, symptoms and quality of life in adult and adolescent patients with erythrodermic atopic dermatitis

A. Shabbir[1], A. S. Paller[2], B. Lockshin[3], M. J. Cork[4], M. Morren[5], Y. Kataoka[6], Z. Chen[1], A. B. Rossi[7], Y. Lu[1]

[1]Regeneron Pharmaceuticals, Inc., Tarrytown, New York, United States, [2]Northwestern Uni Feinberg School of Medicine, Chicago, Illinois, United States, [3]Georgetown Uni, Rockville, Maryland, United States, [4]Sheffield Dermatology Research, Uni of Sheffield, Sheffield, United Kingdom, [5]Lausanne Uni Hospital, Lausanne, Switzerland, [6]Osaka Habikino Medical Care Center, Osaka, Japan, [7]Sanofi Genzyme, Cambridge, Massachusetts, United States

Erythrodermic atopic dermatitis (E-AD; ≥90% body surface area [BSA] affected and baseline [BL] erythema score ≥1 from Global Individual Signs Score) is often difficult to treat and highly disturbs patient (pt) physical/mental health. We report pooled efficacy data from dupilumab (DPL) monotherapy trials in adult and adolescent pts with E-AD. In 16-week DPL trials, 136 adults and adolescents with E-AD received DPL 300mg weekly (qw; n=38); DPL 200mg (adolescents: BL weight <60kg) or 300mg (adolescents: BL weight ≥60kg; and adults) every 2 weeks (q2w; n=48); or placebo (PBO; n=50). DPL monotherapy significantly improved Eczema Area and Severity Index (EASI) at Week (Wk) 16; LS mean percent changes from BL at Wk16 were −58.5%/−58.3%/−22.3% for qw/q2w/PBO (*P*=0.004/*P*=0.003 vs PBO); mean EASI (qw/q2w/PBO) decreased from 58.7/55.0/59.3 at BL to 24.4/24.3/46.4 at Wk16 (*P*=0.003/*P*=0.003 vs PBO). DPL also improved mean BSA affected (BL [qw/q2w/PBO]: 94.8%/94.6%/95.5%; Wk16: 51.7%/55.6%/81.9%; *P*=0.028/*P*=0.026); global erythema score (BL: 2.7/2.7/2.8; Wk16: 1.8/1.7/2.8; *P*=0.008/*P*=0.002) and weekly averaged Peak Pruritus Numerical Rating Scale (PP-NRS) score (BL: 7.4/7.8/8.0; Wk16: 4.2/4.8/7.4; *P*=0.0002/*P*=0.0003). More DPL- vs PBO-treated patients at Wk16 achieved ≥50% improvement in EASI (EASI-50; 44.7%/41.7%/6.0%; *P*<0.0001); EASI-75 (26.3%/25.0%/4.0%; *P*=0.002/*P*=0.011); PP-NRS ≥3-point improvement (43.2%/36.2%/0; *P*<0.0001) and ≥4-point improvement for adults/≥6-point improvement for adolescents in Dermatology Life Quality Index (48.6%/60.9%/12.0%; *P*=0.0003/*P*<0.0001). DPL had an acceptable safety profile in E-AD pts overall. DPL monotherapy significantly improves signs, symptoms and quality of life in E-AD pts.

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Sleep disturbances in chronic pruritic dermatoses are associated with increased C-reactive protein levels

S. P. Patel, R. Khanna, J. Choi, K. Williams, S. Kwatra

Dermatology , Johns Hopkins School of Medicine, Baltimore , Maryland, United States

Pruritus is a common symptom that can significantly reduce quality of life often through sleep disruption. We sought to investigate the features of disturbed sleep in patients with chronic pruritic dermatoses and test the hypothesis that increased systemic inflammation may serve as a biomarker for impaired sleep in these patients. We conducted a cross-sectional analysis of the National Health and Nutrition Examination Survey data. Odds ratios (OR), 95% confidence intervals (95%CI), and p-values using logistic regression were calculated weighted to the US Census data. Model 1 was adjusted for demographic factors (sex, age, race, education, marital status, income) while model 2 was adjusted for medical comorbidities (age, sex, allergy history, heart disease, body mass index). Chronic pruritic dermatoses were associated with waking up during the night (Model 1: OR=1.646, 95%CI [1.031-2.627], p=0.038; Model 2: OR=1.329, 95%CI [0.888-1.989], p=0.031), waking up too early in the morning (Model 1: OR=1.669, 95%CI [1.118-2.493], p=0.016 ; Model 2: OR=1.582, 95%CI [1.008-2.481], p=0.046), feeling overly sleepy during the day (Model 1: OR=1.786, 95%CI [1.144-2.789], p=0.014; Model 2: OR=1.664 , 95%CI [1.073-2.581], p=0.026), and not getting enough sleep (Model 1: OR=1.603 , 95%CI [1.049-2.450], p=0.032; Model 2: OR=1.598, 95%CI [1.064-2.399], p=0.027). Mean C-reactive protein (CRP) levels were 52.8% higher among pruritic dermatoses patients reporting trouble sleeping compared to those who did not (0.663 vs 0.434 mg/dL; p=0.034). Pruritic dermatoses were also positively correlated with CRP levels with a β coefficient of 0.142 (p=0.025). In addition to confirming sleep disturbances with pruritic dermatoses, we found that sleep disturbances are more likely to present with elevated CRP levels. Thus, clinicians should consider the potential risk for sleep-related and cardiac comorbidities in patients diagnosed with itchy skin conditions.

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Teledermatology service quality improvement at the Southeast Veterans' Integrated Service Network

T. M. DeGrazia, S. Chisolm, S. De La Feld, H. Yeung, S. C. Chen

Regional Telehealth Services, VISN7, Decatur, Georgia, United States

The Southeast Veterans' Integrated Service Network (VISN7) has a robust teledermatology program providing services to community outpatient centers in Georgia, South Carolina and Alabama. Despite rapid growth of teledermatology across the Veterans Health Administration, there has not been standardization in teledermatology practices. To provide optimal, standardized consult readings among dermatologists at the VISN7, we examined adherence to 5 mutually agreed upon best practices. Quarterly from July 2018-August 2019, each physician was assigned a month to randomly review teledermatology consult cases and to evaluate if they adhered to the best practices. Evaluators commented on at least 3 of the 5 practices for each physician, marking "yes" or "no" for adherence. Overall, adherence to best practices was high among 7 dermatologists with an average adherence of 92.0% (Q1), 88.8% (Q2), and 93.0% (Q3). Between the first and last evaluation, 2 physicians improved adherence, 2 worsened adherence and 3 were consistently 100% adherent to all practices. Average adherence to each individual practice throughout the year was as follows: 1) Attendings should sign notes within 10 business days (100%); 2) Patients with cysts and keloids should be referred to general dermatology clinic initially (91%); 3) Primary care provider biopsy clinics should be utilized when available (80%); 4) The teledermatology nurse should be instructed to call patients prescribed oral corticosteroids and/or mid-to-high potency topical corticosteroids for follow-up, and to call patients prescribed topical fluorouracil or retinoids for education (91%); 5) Patients with cosmetic diagnoses should be aware of the VA non-cosmetic policy (64%). This study was limited to a 13-month review at a single facility and only examined 5 specific practices. We found high level of adherence to pre-specified practices and identified areas of improvement for dermatologists at the VISN7. Further studies are needed to examine whether adherence to these 5 practices improve patient outcomes.

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Effects of text message reminders on data collection in a pragmatic study

B. Hefele, D. Shin, J. Gelfand, S. Baez Vanderbeek

Dermatology, University of Pennsylvania , Philadelphia, Pennsylvania, United States

Missing data is a large problem facing the clinical research community. Identifying factors that lead to increased response rate can lead to more complete data collection, robust analyses and subsequently highly impactful evidence-based and patient centered recommendations. The LITE Study is a pragmatic trial comparing the effectiveness of home versus office based phototherapy for the treatment of psoriasis. Patients complete quality of life surveys within a 7-day window every 4 weeks for 24-weeks on their mobile phones and are compensated $20 per submission. Patients enrolled during the initial seven months received app notifications within each survey window. After seven months, a text-based patient engagement service, Luma Health (Luma), was added where patients received a text message twice per survey window (a voice call transcription of the text message was sent to patients who only provided a land line). Using data from the current patient population in the LITE Study, mixed effects logistic regression was used to assess factors associated with completion of surveys. Factors included Luma-implementation of text messages, survey sequence, and patient demographics. Patients who received an additional text message reminder had higher odds for completing a survey compared to those who only received an app notification [odds ratio 3.52 (95%CI 1.6-7.5)]. Pre-Luma, 62.8% of the submitted responses were completed within the first 3 days of the 7-day survey window; post-Luma, 85.6% were submitted within the first 3 days, an absolute increase of 22.8% (p<0.001). In both pre- and post-Luma implementation periods, responses were completed between 12pm and 5:59pm more so than any other timeframe. After implementing a text message patient engagement service, response rate for patient reported outcomes increased. This approach has reduced missing primary data for the LITE Study and could be effective for other clinical research where patients are asked to self-report data.

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Understanding the impact of psoriatic disease on mental health: Results from the National Psoriasis Foundation Annual Survey
G. Gondo[1], J. Domire[1], J. F. Merola[2], A. Gottlieb[3], S. Bell[1]
[1]National Psoriasis Foundation, Portland, Oregon, United States, [2]Harvard Medical School, Boston, Massachusetts, United States, [3]Mount Sinai Hospital, New York, New York, United States
An estimated 8.3 million individuals in the United States live with psoriasis. The NPF has conducted a national survey of the psoriatic disease community for nearly 20 years. One objective of the survey is to understand the impact of psoriatic disease on the mental health of individuals living with psoriasis and psoriatic arthritis. Methods: A nationwide survey of a random sample of individuals with psoriatic disease was conducted online and by telephone. The participant pool was stratified by gender, disease type, and balanced by the estimated population of individuals with psoriatic disease by geographic region. A total of 1,570 individuals completed the survey. Results: Results from the PHQ-9 suggest that 20.8% of individuals with psoriatic disease are depression. The depression rate within the U.S. population is estimated to be 8.1%. Chi-square analysis suggests a relationship between severity of psoriasis and severity of depression as measured by the PHQ-9 ($p<.001$). Individuals with severe psoriasis reported higher rates of moderate (10.3%) and moderately severe depressive (13.3.%) symptoms compared to individuals with moderate (6.8%; 5.6%) to mild (5.4%; 4.9%) psoriasis ($p<.001$). Overall, 33% of respondents whose PHQ-9 scores indicate that they should seek treatment for their depressive symptoms live with undiagnosed depression. Conclusions: Results from a national survey of individuals with psoriasis suggest that individuals with psoriatic disease experience high rates of depression and that disease severity may impact the mental health of individuals with psoriatic disease. Survey results further suggest that the mental health needs of individuals with psoriatic disease are not being sufficiently met and underscore the importance of recent treatment guidelines issued by AAD and the NPF.

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Clinicopathological features of primary melanoma with TERT promoter and BRAF mutations in Chinese Uygur and Han nationality: A retrospective study of 63 cases
X. Kang[1, 2], Q. Li[1]
[1]Dermatology and Venereology, Xinjiang Uyghur People's Hospital, Urumqi, Xinjiang, China, [2]Xinjiang Uyghur People's Hospital, Xinjiang Uyghur, China
Objective: To detect TERT promoter and BRAF mutation of 63 primary melanoma patients, and explore the association between these mutations and clinicopathological features. Methods: A total of 63 fixed paraffin-embedded formalin tumor sections were analyzed for TERT promoter and BRAF mutation by PCR amplification and DNA sequencing. Results: Among 63 cases,26(92.9%)cases of Han was ALM predominantly, 22 (64.7%)cases of Uygur was NCSD.25 cases of TERT promoter mutations (40.32%), higher than study of Xue Bai. They found that TERT promoter mutations was only 5.9%.Our results show that TERT promoter mutations is basically balanced among Han and Uygur. The Han people were all ALM. The Uighurs are dominated by NCSD. Of these cases, 13 BRAF mutations, 12 V600E mutation and 1 L597Q mutation. 25 TERT promoter mutations, 11 of which were C228T mutation and 14 were C250T mutation.The C250T mutation was more frequent. Of the 25 melanoma patients with TERT promoter mutation, 10 combined with BRAF V600E mutation. Compared with the TERT wild-type group, the mutation group strongly associated with tumor thickness and ulceration ($P < 0.05$). Compared with the BRAF V600E wild-type group, the mutation group was significantly associated with tumor thickness and sentinel lymph node biopsy ($P < 0.05$). The combined mutation of TERT promoter and BRAF V600E was significantly associated with high-risk clinicopathologic features,including tumor thickness, sentinel lymph node biopsy, and ulceration ($P < 0.05$). Conclusions: In Xinjiang, China, the difference in ethnicity may be due to the different frequency of melanoma TERT promoter mutations and the distribution ratio of subtypes. TERT promoter mutation associated with BRAF V600E mutations.The combined mutation was significantly associated with more invasive clinicalpathology of melanoma compared to the single mutation, indicating a worse prognosis outcome.

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Emojis in dermatology: Changing the faces in medicine
C. Barber[1], R. Dellavalle[2], T. Coolman[3], V. Ranpariya[4]
[1]Michigan State University College of Human Medicine, Grand Rapids, Michigan, United States, [2]Dermatology, Colorado University, Aurora, Colorado, United States, [3]A.T. Still University- Kirksville College of Osteopathic Medicine, Kirksville, Missouri, United States, [4]Rutgers Robert Wood Johnson Medical School, Newark, New Jersey, United States
Today's modern world is focused around incorporating advancing technologies into day-to-day activities to streamline tasks, and the medical field is no different. The surge of emoticon (emoji) use has flourished in the past decade, with more platforms available for delivery, and more emoticons being created constantly. Emojis have already found purpose in medicine, as proxies for emotions expression, and understanding between providers. Studies thus far involving emojis have integrated them into depression screens and medication instruction handouts. As studies and use increase, different areas of medicine will also explore their use for emojis in practice. Although there is no reported use of emojis in the world of dermatology, we extrapolated ideas from past studies as well as proposed new uses in the field of dermatology. We offer many new and exciting uses for emojis in dermatology, with the goal to improve communication, understanding, and patient-provider interactions. Our communication and methods of information delivery are constantly evolving, as well as the population we address. This is what makes use of emojis a rising topic in the medical field, and therefore relevant in dermatology.

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Examination of current staging systems in cutaneous squamous cell carcinoma
K. Severson, S. A. Ederaine, J. Montoya, R. Butterfield, N. Zhang, J. Besch-Stokes, A. Hughes, S. Ochoa, S. Nelson, D. J. DiCaudo, A. Sekulic, M. R. Pittelkow, A. Mangold
Mayo Clinic, Scottsdale, Arizona, United States
Introduction: Cutaneous squamous cell carcinoma (cSCC) is the second most prevalent skin cancer with an estimated incidence of 700,000 cases in the United States. Our research examines the AJCC 7th, AJCC 8th and BWH staging systems. Methods: 949 retrospective cases of cSCC were randomly sampled between 2009 to 2016 from the Mayo Clinic Enterprise and stratified to mirror the US population. Chart review was performed to collect patient demographics, tumor characteristics, and outcomes. Results: The mean age was 74.5 (SD 11.8), 65.2% were male, 34.8% were female, and 95.0% were Caucasians. 14.7% of cases were immunosuppressed. 95.8% of tumors were sun exposed. The mean clinical tumor dimension was 1.3 cm (SD 1.0). 78.7% of cases were well-differentiated, 16.5% moderately differentiated, and 4.8% poorly differentiated. AJCC 7th/8th staging was 89.4%/89.5% T1, 9.9%/6.2% T2, 0.3%/3.8% T3, and 0.4%/0.5% T4/T4a+T4b, respectively. BWH staging was 85.7% T1, 10.5% T2a, 2.8% T2b, and 0.9% T3. Poor outcomes occurred with: local recurrence (3.6%), nodal metastasis (2.4%), in-transit metastasis (0.6%) and distant metastasis (0.5%). Metastatic or recurrent disease occurred in BWH, AJCC 7th and AJCC 8th staging, respectively: T1: 2.5%, 2.7% and 2.7%; T2a/T2/T2: 11.0%/27.7%/15.3%; T2b/T3/T3: 63.0%/33.3%/50.0%; T3/T4/T4a,T4b: 55.6%/75.0%/50.0%, 100%. Median disease-free survival (DFS) in months: BWH T1/T2a/T2b/T3: 96.1/39.2/14.5/13.1 ($p<0.0001$) with T2a/T2b/T3 hazard ratios (HR) of 2.62/5.13/8.59 ($p<0.0001$); AJCC 7th T1/T2/T3/T4: 95.1/32.5/NE/6.7 ($p <0.0001$) with T2/T3/T4 HR of 3.15/2.90/15.62 ($p <0.0001/0.29/<0.0001$); AJCC 8th T1/T2/T3/T4a/T4b: 95.1/33.1/10.9/NE/13.1 ($p <0.0001$) with T2/T3/T4a/T4b HR of 2.37/5.81/10.23/12.23 ($p <0.0001/<0.0001/0.001/0.01$). Conclusion: Current staging systems predict poor outcomes and DFS. However, BWH staging may offer better stratification in predicting poor outcomes whereas AJCC 8th may offer better DFS risk stratification in intermediate to high risk cSCC.

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Plasma levels of IL-6 and CRP predict risk of developing psoriasis in US women

M. Tajalli[1], T. Li[1], E. Cho[1, 3], A. Qureshi[1, 2, 3], T. Vance[1]

[1]Dermatology, Brown University, Providence, Rhode Island, United States, [2]Epidemiology, Brown University, Providence, Rhode Island, United States, [3]Medicine, Channing Division of Network Medicine, Boston, Massachusetts, United States

There are no specific biological markers that predict psoriasis. We conducted a prospective case-control study to explore whether certain inflammatory and hormonal biomarkers were associated with risk of psoriasis. In the Nurses' Health Study, 32,826 women provided blood samples from 1989 to 1990. We included incident cases of psoriasis among women free of diabetes, cardiovascular disease, and cancer at the time of blood collection. The diagnosis of psoriasis was made from1998 to 2008. Controls consisted of women who had specific biomarker data and did not have psoriasis diagnosis. Associations between biomarkers and psoriasis were estimated using logistic regression adjusted for age, BMI, exercise, smoking status and alcohol consumption. The following biomarkers were assessed: total adiponectin, HMW adiponectin, IGFBP-1, IGFBP-3, C-peptide, CRP, estrone, estradiol, IGF-1, IL-6, leptin, soluble leptin receptor, sex hormone binding globulin, testosterone and TNF R2. Biomarker levels were dichotomized using the median as the cut-off. High plasma levels of CRP (odds ratio (OR): 1.63, 95% confidence interval (CI):1.01-2.63) and IL-6 (OR: 2.30, 95% CI: 1.31-4.06) were associated with an increased risk of psoriasis. No other biomarkers were associated with psoriasis. Further analyses were performed by categorizing biomarker levels into tertiles and using the lowest tertile as the reference group. The ORs for the highest tertile were 2.32 (95% CI: 1.23-4.40, P for trend: 0.01) for CRP and 2.01 (95% CI: 1.02-3.94, P for trend: 0.04) for IL-6, which were consistent with the previous results for these two biomarkers. The results remained similar after excluding women who developed diabetes, cardiovascular diseases or cancer during follow-up. In conclusion, psoriasis risk was associated with plasma levels of CRP and IL-6 that were measured several years prior to psoriasis diagnosis. These biomarkers may be useful in predicting the risk of psoriasis.

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Treatment patterns of psoriasis by medical providers and disease severity in US women

M. Tajalli[1], T. Li[1], M. Hasan[1], A. M. Drucker[2], A. Qureshi[1, 3], E. Cho[1]

[1]Dermatology, Brown University, Providence, Rhode Island, United States, [2]University of Toronto, Toronto, Ontario, Canada, [3]Epidemiology, Brown University, Providence, Rhode Island, United States

Studies on treatment patterns of psoriasis are valuable to evaluate how efficiently psoriasis patients are treated and facilitate improved outcomes for these patients. In the Nurses' Health Study II which includes data for 116,430 female nurses, 2107 women reported to have a diagnosis of psoriasis made by a medical provider. Psoriasis Screening Tool-2 (PST-2) was sent to all of these women. PST-2 is a validated diagnostic tool for psoriasis, which also queries participants for age at disease diagnosis, treatments, type of psoriasis lesions, body surface area involved, and the provider who made the diagnosis. Among 2107 participants, 1338 completed and retuned the survey, with 1243 of them validated for having psoriasis. The average age at diagnosis was 47 years (standard deviation:15 years). 79% of the patients reported mild, 17% moderate and 4% severe disease. 41% of the patients reported plaque lesions, 22% nail, 49% scalp, 15% palmoplantar and 27% inverse psoriasis. In people with mild psoriasis, 67% were diagnosed by dermatologists and 33% by non-dermatologist providers. In those with moderate-to-severe psoriasis, 87% were diagnosed by dermatologists. In people with mild psoriasis, 58% received only topical medication, 5% only systemic, 5% systemic and topical, 4% topical and phototherapy, and 26% received no treatment. In people with moderate-to-severe psoriasis, 42% received only topical medication, 17% systemic and topical, 12% systemic, topical and phototherapy, 11% topical and phototherapy, and 11% received no treatment. Among traditional systemic therapies, methotrexate and among targeted systemic therapies adalimumab were the most commonly used medications. In conclusion, high percentage of psoriasis patients are being diagnosed and managed by non-dermatologist providers which demonstrates the importance of engaging them in educational initiative on disease treatment. Significant numbers of people with moderate-to-severe disease are undertreated.

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Real-world risk of new onset inflammatory bowel disease among psoriasis patients exposed to interleukin 17 inhibitors

S. Wright, A. Alloo, A. Strunk, A. Garg

Dermatology, Donald and Barbara Zucker School of Medicine at Hofstra Northwell, Lake Success, New York, United States

Information on real-world risk of inflammatory bowel disease (IBD) among interleukin-17 inhibitor (IL-17i) exposed psoriasis patients is limited. We aimed to compare the risk of new-onset IBD in IL-17i exposed and unexposed psoriasis patients. This was a retrospective cohort analysis using the IBM Explorys analytics platform. We calculated and compared the incidence rates (IR) of IBD in 1,821 psoriasis patients with IL-17i exposure and 213,060 psoriasis patients without IL-17i exposure. Crude 6-month IBD incidence was 0.16% (3/1,821) among psoriasis patients exposed to any IL-17i, 0.24% (3/1,246) among those exposed to secukinumab alone, and 0.11% (239/213,060) among those unexposed. Crude 1-year IBD incidence was 0.27% (5/1,821) among IL-17i exposed psoriasis patients, 0.32% (4/1,246) among those exposed to secukinumab alone, and 0.19% (412/213,060) among unexposed. After controlling for age, sex, and race, there was no significant difference in odds of developing IBD at 6-months (OR, 1.42; 95% CI, 0.45-4.43) and 1-year (OR, 1.37; 95% CI, 0.57-3.33) between exposed and unexposed psoriasis patients. Similarly, there was no significant difference in odds of developing IBD at 6-months and 1-year between secukinumab-exposed and unexposed psoriasis patients. Incidence of IBD among psoriasis patients exposed to IL-17i is low and the risk appears similar to unexposed psoriasis patients.

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The relationship between atopic dermatitis and childhood symptoms of attention deficit/hyperactivity disorder: A longitudinal cohort study

Y. Lee[3], N. Tomaszewski[1], S. Langan[2], K. Abuabara[1]

[1]Department of Dermatology, UCSF, San Francisco, California, United States, [2]Faculty of Epidemiology and Population Health, LSHTM, London, United Kingdom, [3]Tulane University School of Medicine, New Orleans, Louisiana, United States

The rise in atopic dermatitis (AD) has been paralleled by rising prevalence of attention deficit/hyperactivity disorder (ADHD) in the past few decades, especially in developed and Western countries. Previous research shows a possible association between AD and ADHD, but data have been mixed, likely due to poorly defined phenotypes and heterogeneity in measuring AD and ADHD. Furthermore, most studies have been limited by cross-sectional designs. To test our hypothesis that active and more severe early childhood AD is associated with clinician-diagnosed ADHD at age 7, and hyperactive symptoms as measured by the Strengths and Difficulties Questionnaire (SDQ) at ages 7, 10, 13, and 16 years, we analyzed data from the Avon Longitudinal Study of Parents and Children (ALSPAC), a large population-based birth cohort from the UK. Using logistic regression models, we found that AD (defined as at least two reports of flexural dermatitis from a standardized and validated questionnaire) was not associated with clinician-diagnosed ADHD at age 7 based on the Development and Wellbeing Assessment (Odds Ratio 1.07, 95% CI 0.64-1.78, adjusted for potential confounders including maternal stress during pregnancy, maternal age at delivery, maternal smoking during pregnancy, socioeconomic status, and maternal smoking). We found a trend toward higher effect estimates in younger ages, though this was not statistically significant, and we found no evidence of an interaction with parental-reported sleep quantity. Additionally, we did not find any significant cross-sectional associations between active AD and symptoms of hyperactivity based on the SDQ at four subsequent assessments between ages 7 and 16. Our results do not provide evidence to support an association between AD and ADHD in a large cohort of children followed from birth. Our research challenges the current paradigm that AD is strongly associated with ADHD.

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Neurotic excoriations: A retrospective cohort study of disease presentation, comorbidities, and treatment in 250 patients at a tertiary care center

C. Kwon[1], R. Khanna[1], K. Williams[1], Y. Semenov[2], N. Kim[1], S. Kwatra[1]

[1]Dermatology, Johns Hopkins School of Medicine, Baltimore, Maryland, United States, [2]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

There is a lack of epidemiologic data on patients with neurotic excoriations (NE). We thus conducted a retrospective chart review of patients with NE from 2007 to 2019 at a tertiary care center. Patients with ICD-9/10-CM code L98.1 from January 1, 2007 to May 1, 2019 were identified (n=315). Patients (n=250) with the diagnosis of “neurotic excoriations,” “skin-picking,” “excoriation disorder,” or equivalent synonym were identified. Patient demographics, systemic and psychiatric comorbidities, clinical course, psychiatric medications, duration of symptoms, and outcome were collected. Average age at diagnosis was 49 years, the majority of patients were female (76%), Caucasian (82%) and unmarried (61%). The most common systemic comorbidities were Type II Diabetes (16.4%), thyroid dysfunction (11.6%) and hepatitis C (6.4%). A majority (74%) had at least one psychiatric comorbidity, the most common being depression (42%), anxiety (30%) and substance use disorder (16%). Only a minority of patients (45%) were given referrals to psychiatry and most patients (64%) did not follow-up with psychiatric care. Fifty-three patients (21%) experienced improvement of symptoms. The most common treatments consisted of topical/oral antibiotics (57%), topical corticosteroids (52%), antihistamines (23%) and doxepin (17%). This is one of the first studies characterizing the population of patients affected by NE. The majority of our cohort were middle-aged, unmarried, Caucasian females with a high burden of psychiatric comorbidities Type II Diabetes. We demonstrate that despite a diagnosis of NE, only a minority of patients were seen by psychiatry. Further, treatment outcomes were generally unfavorable, highlighting the need for further research into the underlying pathophysiology and a multidisciplinary treatment approach.

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Clinical characteristics, etiology, and treatment of erythema multiforme at a tertiary care center

G. Shi[1], B. Kaffenberger[2], Y. Semenov[3], J. Choi[1], K. Williams[1], J. Alhariri[1], S. Kwatra[1]

[1]Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [2]Dermatology, Ohio State University Wexner Medical Center, Columbus, Ohio, United States, [3]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

Erythema multiforme (EM) is an acute, immune-mediated condition characterized by distinctive skin lesions and occasional mucocutaneous involvement. EM is understudied, with limited epidemiologic studies. This study sought to describe etiologies, clinical characteristics, and treatment of EM patients seen at Johns Hopkins Hospital between 2009-2019. Data was retrospectively collected for all patients with ICD-9CM/ICD-10 codes for a primary diagnosis of EM. A total of 155 patients were included, of whom 84 (54%) were female and 71 (46%) were male with a mean age at time of onset of 34.5 years. 48% of patients were white, 38% were black, and 14% were of other races. There were 45 patients (29%) hospitalized with a mean length of stay (SD) of 5.4 days (±4.7). According to current clinical guidelines, 43 (28%) patients were classified as EM major and 112 (72%) EM were classified as EM minor. 98 (63%) were isolated episodes, 52 (36%) were recurrent, and 5 (3%) were persistent. 72 (46%) cases were precipitated by viral infection (most commonly HSV, 58%), 22 (14%) were precipitated by drugs (most commonly Bactrim, 32%), and in 59 (38%) cases a precipitating cause was not found. Clinically, 121 (79%) patients presented with characteristic targetoid lesions and 90 (58%) patients presented with mucous membrane involvement. 113 (76%) of patients were treated with oral, topical, or intramuscular corticosteroids, 64 (41%) were treated with antivirals, 18 (12%) were treated with antibiotics, 20 (13%) were given supportive care, and 9 (6%) were treated with chronic immunosuppression. Greater than one third of patients did not have an identifiable cause, infections were found less frequently as etiologies, and drugs were found more commonly as inciting factors of EM than in previous studies. This study demonstrates an updated view of the epidemiology of EM from a tertiary care center in the US.

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Evaluating melanoma incidence and survival in the US veterans population

J. Remer, A. Fonseca, M. A. Weinstock

Dermatology, Providence VA Medical Center/Brown University, Providence, Rhode Island, United States

Introduction: Melanoma incidence and mortality in the US military and veteran population is complicated. While limited, prior results suggest that melanoma incidence is increased in air force members and mortality is increased in former prisoners of war. As the 5th most common cancer in the veteran population who receive care at the VA, melanoma incidence and survival is still poorly understood. Purpose: To compare incidence and survival for malignant melanoma in the US veterans who received care at the VA to the general population. Methods: Histologically confirmed melanomas between 2002-2016 for both Connecticut (CT) and general US population were identified using the VA Corporate Data Warehouse (CDW) and the Surveillance, Epidemiology and End Results (SEER) 18 database. Sex, race, cancer biology, histologic subtype, cancer stage and tumor location were obtained for each participant in both SEER and VA cohorts. Survival analysis was performed and deaths due directly to malignant melanoma were compared using the Log Rank test. Results For CT and the US, age adjusted incidence for malignant melanoma was decreased by 44.4% and 57.5% and for melanoma in situ by 52.8% and 60.5% in the veteran population when compared to the SEER cohort ($p < 0.001$). Ten year survival for malignant melanoma was significantly increased in both the CT veteran cohort at 88.8% vs 86.3% and US veteran cohort at 87.6% vs 85.8% when compared to the SEER cohort ($p < 0.001$). Discussion/Conclusion This study suggests that melanoma incidence in the veteran population who received care at the VA is decreased; while, 10 year survival is increased when compared to the SEER CT and national cohort. Reasons for improved survival in veterans who receive care at the VA could be earlier detection or better treatment for malignant melanoma. It is possible that there are melanoma cases amongst US veterans that are not registered in the VA CDW or that the VA population is not representative of the entire veteran population.

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Development of a psoriasis severity score for clinical measures in a claims database

S. A. Siegel[1], J. McGregor[1], A. Blauvelt[2], J. R. Curtis[3], K. L. Winthrop[1]

[1]Oregon Health & Science University, Portland, Oregon, United States, [2]Oregon Medical Research Center, Portland, Oregon, United States, [3]University of Alabama at Birmingham, Birmingham, Alabama, United States

Greater psoriasis disease severity is associated with higher prevalence of co-morbidities and differences in prescribing patterns. To understand effects caused by different therapies, researchers need to accurately account for disease severity. To address this gap in knowledge, using a gold standard for psoriasis severity, we developed and validated a score to predict severity in a large administrative database. Two registries, the Center for Excellence in Psoriasis and Psoriatic Arthritis (CEPPA) at Oregon Health & Science University (OHSU) and the Corrona national psoriasis registry, were linked with Medicare data for 2006-2017. Both direct linkage using social security number and probabilistic linkage utilizing date of birth, sex, dermatology provider name, and most recent dermatology encounter date were used to link data with Medicare. Registries were combined, and analyses limited to patients with ≥12 months of continuous Medicare coverage and ≥1 dermatologist-assigned psoriasis diagnostic code. Validated claims-based algorithms were used to identify potential covariates. Outcome was body surface area (BSA) dichotomized as mild (<3%) vs. moderate-to-severe (≥3%). LASSO regression was used for variable selection with 0.15 cut-off. Model fit was assessed by classification error. Sixty-four CEPPA and 172 Corrona patients met eligibility criteria (Table 1). We developed an indirect score for psoriasis severity using diagnoses of anxiety, depression, diabetes, lower back pain, and psoriatic arthritis as well as adalimumab and phototherapy use, joint surgery, lipid testing, dermatology and outpatient visits, and gender. Our model correctly predicted moderate-to-severe BSA 74.6% of the time. Misclassification was non-directional with 6 false negatives and 9 false positives. Our psoriasis disease severity score using indirect measures of BSA may be a potential method for accurately controlling for disease severity in the analysis of administrative databases.

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Risk of psoriatic arthritis in psoriasis patients on biologics and methotrexate
E. N. Lininger[1], S. A. Siegel[1], K. L. Winthrop[1, 2], A. Deodhar[2], S. Kiwalkar[2], A. Ortega-Loayza[2]
[1]School of Public Health, Oregon Health & Science University, Portland, Oregon, United States, [2]School of Medicine, Oregon Health & Science University, Portland, Oregon, United States
Psoriatic arthritis (PsA) is a chronic inflammatory arthritis characterized by joint and entheseal inflammation seen in 30% patients with psoriasis (PsO). Inhibitors of tumor necrosis factor and interleukin 23 and 17 ('biologics') are efficacious treatment options for both. We aimed to determine if the use of either systemic non-biologic or biologic therapy decreases the risk of incident PsA in patients with PsO. Records on all PsO patients seen at dermatology clinic from January 2006 - June 2019 were reviewed. Patients were considered to have PsA if they were diagnosed by a rheumatologist. Continuous and categorical covariates were compared by Student's t-test and Pearson's chi-squared test, respectively, and were included as confounders in multivariate model. We used Cox proportional hazards models to compare the risk of incident PsA diagnosis for those who initiated biologics compared to those who did not initiate biologics, and similarly for those who initiated methotrexate (MTX) compared to those who did not initiate MTX. We used backwards stepwise variable selection to build the model. Out of 617 patients with PsO, 86 (14%) had PsA at enrollment, 108 (18%) developed incident PsA, and 423 (69%) did not have incident PsA during the study period. Biologics were prescribed to 291 (47%) patients and 322 (52%) received MTX. Among those who were PsA negative at baseline and initiated biologic therapy there was no increased risk of PsA diagnosis (HR: 1.08 [95% CI: 0.74, 1.59]). The results did not change after adjustment for confounders (HR: 0.82 [95% CI: 0.54, 1.23]). There was an increased risk of incident PsA among those starting MTX (unadjusted HR: 1.68 [95% CI: 1.15, 1.60]), and this association persisted after adjustment (HR: 1.57 [95% CI: 1.07, 2.31]). These findings may indicate new evidence for prescribing patterns and need to be confirmed in a prospective study.

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Visit complexity reflects billed level of service and documentation burden
J. Ryan Wolf[1], Y. Xie[3], I. Kim[2], A. P. Pentland[1], B. T. Pentland[2]
[1]Dermatology, University of Rochester , Rochester, New York, United States, [2]College of Business, Michigan State University, East Lansing, Michigan, United States, [3]Public Health Sciences, University of Rochester, Rochester, New York, United States
The electronic medical record (EMR) promised healthcare efficiency, but has increased documentation burden for providers without yielding insight into patient outcomes. It is unclear how the EMR documentation workflow relates to the billed level of service (LOS) for clinic visits. We asked if the complexity of EMR documentation during a clinic visit correlates with the visit LOS. ThreadNet is a novel, R coded, graph-theoretic methodology that converts threads of sequence data into event networks to calculate visit complexity based on actions, roles, and workstations. It does not measure note length or content. We applied ThreadNet to time-stamped EPIC EMR audit data for 55,059 visits from dermatology clinics at University of Rochester. Each clinic visit had a billed LOS as a new patient (NPV) or follow-up patient (FUV) visit. Visit complexity was calculated for in-clinic documentation (i.e., check-in to checkout). Overall, mean visit complexities significantly differed between clinic visit LOS (mean±SD: LOS 1: 3.53±1.87; LOS 2: 3.92±1.59; LOS 3: 4.41±1.88; LOS 4: 5.34±2.28; LOS 5: 6.75±3.40, $p<0.0001$). FUV had lower overall visit complexity compared to NPV (mean±SD: 4.40±1.96 vs. 4.60±1.87, $p<0.0001$). The variation patterns of visit complexity may be a useful proxy for LOS, which could permit clinicians to focus on important content and simplify visit documentation. Thorough evaluation of visit audit trails will identify key components that influence complexity and differ between LOS. Further analyses will determine if after clinic documentation influences visit complexity. Overall, visit complexity can be used to indicate visit LOS, simplify visit documentation, and reduce EMR burden. This work is supported by the National Science Foundation (SES-1734237).

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Quality of life impact from skin diseases among persons living with HIV in Atlanta, Georgia
T. Rogers[1], B. C. Ly[1], E. Anderson[2], H. Yeung[1, 2]
[1]Department of Dermatology, Emory University School of Medicine, Atlanta, Georgia, United States, [2]Ponce De Leon Center, Grady Memorial Hospital, Atlanta, Georgia, United States
HIV remains a public health crisis in the Southern United States and disproportionately affects sexual, gender, and racial/ethnic minority persons. With advances in antiretroviral therapy, the burden of HIV-related skin diseases is often assumed to fall accordingly. The quality of life impact of these skin diseases has not been recently quantified. Medical records of 165 new patients seen at the HIV Dermatology Clinic at Grady Memorial Hospital Ponce De Leon Center in Atlanta, Georgia were reviewed from January to August 2019 in a cross-sectional study. Skindex-16 was collected as part of ongoing efforts to incorporate patient-reported outcomes into routine clinical care; domain scores were compared by demographic variables and HIV biomarkers using t-test or ANOVA. Mean age was 49 years (SD 12). 83% were male. 67% were Black, 26% were White, and 4% were Hispanic. 15% had CD4 count <200 and 20% had detectable viral load. 157 (95%) completed the Skindex-16. Diagnoses with the highest mean overall Skindex-16 scores included drug eruption, HIV-associated lipoatrophy, pruritus, fungal infection, and dermatitis. Mean (SD) Skindex-16 Symptoms, Emotions, and Functioning scores were 41.7 (33.0), 64.8 (31.1), and 43.2 (35.7). Age, gender, race/ethnicity, and CD4 count were not associated with mean Skindex-16 domain scores. Detectable viral load was associated with higher mean Skindex-16 Emotions (76.7 vs. 61.6, P = 0.02) and Functioning (58.0 vs. 40.2, P = 0.02) but not Symptoms scores. Limitations include cross-sectional design and generalizability to areas with lower HIV infection burden. Skin diseases continue to impose substantial quality of life impact in US patients living with HIV. Adhering to antiretroviral therapy and sustaining viral suppression are crucial to reduce quality of life impact from skin diseases. Multidisciplinary collaboration between HIV care providers and dermatologists can optimize skin health in persons living with HIV.

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Single-center retrospective review of the use of checkpoint inhibitors in merkel cell carcinoma patients
M. Babadzhanov[1, 3], N. Doudican[2], C. Ovits[2], T. Canavan[2], M. Stevenson[2], A. Pavlick[2], J. Carucci[2]
[1]Dermatology, NYU, New York, New York, United States, [2]Dermatology, NYU Langone Med Cntr, New York, New York, United States, [3]CUNY Brooklyn College, Brooklyn, New York, United States
Background: Given the role of the immune system in merkel cell carcinoma (MCC), immune-based therapies, including avelumab, pembrolizumab, and nivolumab, are currently FDA-approved as first-line treatment options for metastatic MCC patients. We sought to retrospectively review the efficacy of the use of checkpoint inhibitors (CPI) in MCC patients at our institution. Methods: We performed a single center retrospective review of MCC patients seen at NYU Hematology and Oncology departments between 2012-2018. Results: A total of 15 patients were identified, including 10 males and 5 females. Seven patients (46.7%) had nodal or distant disease at the time of presentation. Nine patients (60%) experienced relapse during follow-up. Of the 5 patients who received initial systemic therapy, 4 patients were administered a CPI, including a PD1 inhibitor (n=3) or CTLA4 inhibitor (n=1). Of the 9 patients who experienced disease progression, 7 patients (77.8%) were administered a CPI. We observed a trend toward increased PFS in patients whose initial treatment included a CPI (p=0.24). Conclusion: Checkpoint inhibitors offer the possibility of meaningful and durable responses for MCC patients with advanced disease at our institution. We are currently working to characterize the molecular features of MCC in this patient cohort using Nanostring analysis.

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A retrospective study of myocardial abnormalities detected on cardiac magnetic resonance imaging among patients with psoriasis compared to inflammatory skin disease controls

M. Goldenberg[1], M. Reynolds[1], S. Smart[3], J. Kaffenberger[2], S. Raman[3], B. Kaffenberger[2]

[1]The Ohio State University College of Medicine, Columbus, Ohio, United States, [2]Department of Internal Medicine, Division of Dermatology, The Ohio State University Wexner Medical Center, Columbus, Ohio, United States, [3]Department of Internal Medicine, Division of Cardiology, The Ohio State University Wexner Medical Center, Columbus, Ohio, United States

Psoriasis is an inflammatory dermatological disorder that has been associated with various cardiac manifestations. The goal of this study was to evaluate the rates of cardiac inflammation and edema on cardiac MRI in psoriasis patients compared to atopic dermatitis and rosacea control patients so that cardiac magnetic resonance imaging (CMR) can be used as a potential biomarker for psoriasis patients in the future. This was a retrospective chart review that looked at CMR records for markers of cardiac inflammation in adult patients with psoriasis, atopic dermatitis, or rosacea who were patients at The Ohio State University Wexner Medical Center between 2011 and 2018. There were 47 patients with psoriasis and 159 control patients identified. When controlling for BMI and sex, psoriasis patients had higher rates of abnormal T2 septal values (normal = 52.18 ± 3.4) compared to controls (P = 0.0483). This study's findings suggest that psoriasis patients have higher rates of edema and inflammation based on higher rates of abnormal T2 septal values on CMR compared to atopic dermatitis and rosacea controls. The study population was limited by size, as well as by the fact that the psoriasis population used had more males with a higher BMI. More research and prospective CMR mapping in greater numbers of psoriasis patients is necessary to determine the prognostic significance of these abnormalities.

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Utilization and impact of immunotherapy in stage IV melanoma using the National Cancer Database

R. Conic[1, 2], R. Knackstedt[2], G. Damiani[2], B. Gastman[2]

[1]University of Maryland, Baltimore, Maryland, United States, [2]Cleveland Clinic, Cleveland, Ohio, United States

The majority of data regarding immunotherapy for melanoma stems from trials that exclude important populations. Thus, while trials may demonstrate favorable results, not all populations may be represented. To evaluate factors affecting the utilization of immunotherapy and to stratify results based on the approval of ipilimumab in 2011 and PD-1 inhibitors in 2014, an analysis of available data from the National Cancer Database (NCDB) was performed. Stage IV melanoma patients were identified. Effects of immunotherapy on overall survival (OS) were assessed using Kaplan Meier curves and Cox proportional hazards model. 19,233 patients were analyzed, of whom, 1,998 received immunotherapy. Between 2011-2013, and in 2014, 18.6% and 28.9% of patients received immunotherapy, respectively. Younger patients and those with fewer co-morbidities or receiving care in an academic institution were more likely to receive immunotherapy. Patients without insurance were less likely to receive immunotherapy. Patients who received immunotherapy from 2011-2013 had a 33% (95% CI 30-35%) 3-year OS compared to 23% (95% CI 21-24%) for those who did not. In 2014, 3-year OS was 37% (95% CI 32-43%) for those who received immunotherapy compared to 22% (95% CI 18-26%) for those who did not (log-rank p<0.0001). This is the first analysis of a large cancer database for patients with any cancer with stratification of results based on utilization and availability of immunotherapy. Immunotherapy utilization increased yearly and improved OS. With combination immunotherapy now more widely employed, it is expected these results will continue to improve.

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Pilot study examining blood cell count ratios as a predictor of subsequent non-melanoma skin cancers

S. O'Neal[1], A. Ravishankar[1], B. Lindgren[1], N. Goldfarb[2]

[1]University of Minnesota, Minneapolis, Minnesota, United States, [2]Dermatology, University of Minnesota, Minneapolis, Minnesota, United States

Blood cell count ratios, including neutrophil-lymphocyte ratio (NLR), eosinophil-to-lymphocyte ratio (ELR), and monocyte-to-lymphocyte ratio (MLR) have prognostic implications for various malignancies. Typically, elevated ratios portend a worse prognosis, but a decreased NLR has been found in patients with NMSC. We examined the association between blood count ratios with risk of subsequent NMSC, and total number of NMSC, within 3-years of initial NMSC diagnosis. We performed a nested, retrospective study of patients in the Minneapolis VA Health Care System who had their first NMSC in 2003, and a CBC with differential within 30 days prior to or 60 days after the date of NMSC diagnosis. In the initial study, patients were included based on diabetes mellitus type II (DMII) status (3 DMII:1 non-DMII). 175 of 740 screened were enrolled in the initial study, and 50 had a CBC with differential in the window specified. Demographics, blood cell counts, and date, location and type of initial and subsequent NMSCs were collected. Statistical analysis was performed with logistic regression, p< 0.05 indicating statistical significance. Mean NLR (2.88 vs 4.24, p = 0.033) and MLR (0.33 vs 0.50, p = 0.004) were significantly lower among patients with subsequent NMSC within 3 years, after adjusting for age, NSAID use and DMII. Other covariates examined were not related to NLR or subsequent NMSC and were not analyzed. Lower NLR was negatively correlated with number of NMSCs (r = -0.33; p = 0.019) over 3 years. ELR had no significant association with risk of subsequent NMSC or number of NMSCs. Lower NLR and MLR were significantly associated with developing subsequent NMSCs, and lower NLR was associated with developing a greater number of NMSCs, within 3 years. These findings suggest a lower NLR, and possibly MLR, may help risk-stratify patients at higher risk for subsequent NMSCs. Larger studies are currently underway.

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Increased circulating eosinophil count and reduced pyridoxine levels in patients with chronic pruritic dermatoses

S. Gopinath[1], T. Pritchard[1], J. Choi[1], S. P. Patel[1], K. Williams[1], Y. Semenov[2], S. Kwatra[1]

[1]Dermatology, Johns Hopkins School of Medicine, Baltimore, Maryland, United States, [2]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

Little is known about the pathogenesis of chronic pruritic dermatoses (CPD) in adults compared to other dermatological conditions like eczema or psoriasis. In this study, we analyzed circulating eosinophil levels and nutritional deficiencies in CPD patients compared to matched controls and patients with eczema and psoriasis. We conducted a population-based study from the National Health and Nutrition Examination Survey from 2005-2006. The main outcomes were laboratory data on granulocyte counts and serum vitamin levels in participants who answered affirmatively to the questionnaires on CPD, eczema and psoriasis as well as matched healthy controls. We identified 877 (8.9%) cases of CPD, 899 (9.2%) cases of eczema and 85 (2.5%) cases of psoriasis among 23,053 adults in the US aged 20 to 59 years. These findings revealed a slightly higher percentage of females and non-Hispanic white population with CPD, eczema and psoriasis. Circulating eosinophil levels in CPD patients were higher (p <0.001; 95% CI: 0.02 to 0.06) compared to healthy controls. Interestingly, CPD patients without eosinophilia (<1.5x10^3/uL) had increased incidence of spinal fracture (p = 0.018; 95% CI: -0.07 to -0.007) as compared to CPD patients with eosinophilia (>1.5x10^3/uL). Vitamin B6 levels were also reduced in CPD by 10.84 nmol/L (p=0.011; 95% CI: -18.87 to -2.82) and Vitamin D levels were reduced by 2.22 nmol/L (p=0.035; 95% CI: -4.26 to -0.17) compared to healthy controls. Our study suggests that low levels of Vitamin B6 and Vitamin D inversely correlates with the presence of chronic pruritic dermatoses. These vitamin deficiencies suggest supplementation may reduce itch intensity in CPD. These results also show that CPD can be differentiated into distinct subsets based on circulating eosinophilic count, suggesting these patients may be amenable to therapy with immunomodulating agents.

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A retrospective review of 23 cases of pyoderma gangrenosum: Effects of comorbid conditions on treatment response

H. Nymeyer, H. K. Wong

Dermatology, UAMS, Little Rock, Arkansas, United States

Pyoderma gangrenosum (PG) is frequently associated with comorbid conditions such as inflammatory bowel disease, rheumatoid arthritis, and malignancy, but there is a paucity of data on how these comorbid conditions impact the response of PG to treatment. We retrospectively review 23 cases of PG treated at one medical institution to understand the effect that comorbid conditions have on the time to resolution of the PG. Of the 23 cases examined, 10 (43%) are associated with an inflammatory bowel disease or autoimmune hepatobiliary disease (PG with IBD). 13 (57%) are associated with other comorbid conditions or with no known comorbid condition (PG without IBD). We observe a clinically and statistically significant variation in treatment outcomes between these two groups. The median time for lesions to heal in PG with IBD was 15 months versus 54 months in PG without IBD ($p < 0.001$). 7 (70%) of the PG with IBD lesions healed by the end of the study versus 2 (16.7%) of the PG without IBD lesions. No patients in the PG with IBD group died during before the end of the study, but 2 (15%) of the PG without IBD group died before the end of the study. We speculate as to possible reasons for the disparate outcomes in the PG with IBD and PG without IBD groups.

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Health-related quality of life and economic burden of chronic pruritus

K. Whang[1], Y. Semenov[2], R. Khanna[1], K. Williams[1], V. Mahadevan[1], A. Huang[1], S. Kwatra[1]

[1]Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [2]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

Chronic pruritus is a common dermatologic condition with a significant effect on quality of life (QoL). However, its impact on health-related QoL and economic burden has yet to be fully characterized. Our study aimed to measure the health-related QoL and societal economic implications of chronic pruritus. A cross-sectional survey of 95 patients with chronic pruritus was administered using the Ontario Health Utilities Index Mark 3 (HUI3) questionnaire. Normative population data (n=4,187) from healthy US adults were obtained from the 2002-2003 Joint Canada/United States Survey of Health. Based on HUI3 scores of chronic pruritus patients compared to the control, quality-adjusted life-year (QALY) loss and economic costs were estimated. Patients with chronic pruritus had a significantly lower overall health utility score (HUI) compared to the general population (0.53±0.03 vs. 0.86±0.003, $p<0.001$). Following propensity score matching on age and adjusting for demographics and comorbid conditions in multivariable modeling, chronic pruritus was associated with worse overall health performance (coefficient -0.40, 95% CI [-0.51 to -0.30]. This effect was most accentuated in the domains of pain (coefficient -0.25, [-0.35 to -0.14]) and emotion (coefficient -0.13, [-0.20 to -0.06]). The decreased health utility score due to chronic pruritus correlated to an average loss of 6.81 lifetime QALYs lost per patient. Using conservative estimates for QALY willingness-to-pay threshold, the average QALY loss translated to an individual lifetime economic burden of $340,593. These results demonstrate that chronic pruritus is associated with significant health-related QoL impairment. There is significant individual economic burden of chronic pruritus, which highlights the necessity of further research into effective management options.

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Reliability of self-reported data on social media vs. National Residency Match Program Charting Outcomes for dermatology applicants

S. Hu[1, 2], M. Laughter[3, 2, 1], R. Dellavalle[2, 4, 5]

[1]University of Colorado School of Medicine, Aurora, Colorado, United States, [2]Dermatology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States, [3]Bioengineering, University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States, [4]Dermatology Service, U.S. Department of Veterans Affairs, Eastern Colorado Health Care System, Aurora, Colorado, United States, [5]Epidemiology, Colorado School of Public Health, University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States

Social media has risen to the forefront of online resources that dermatology residency applicants use to solicit advice and gauge the competitiveness of their application. However, there is a need to examine National Residency Match Program (NRMP) charting outcomes and self-reported academic metrics on SDN and Reddit, the two most popular social media forums used by health professional students. In this study, we compared combined match data from both SDN and Reddit to NRMP Charting Outcomes (2014, 2016, 2018). There were a total of 478 applicants between 2014-2019 who reported data on SDN and Reddit. The major categories reported include Step 1 & 2 scores, Alpha Omega Alpha (AOA) membership, medical school ranking, advanced degrees (e.g. PhD), and research productivity. This study shows a comparison of self-reported data on SDN and Reddit (2014-2019) with NRMP charting outcomes as well as the academic metrics of matched vs. unmatched applicants on social media. There was no significant difference between self-reported and NRMP data for step 1 scores, step 2 scores, and number of publications/presentations/posters (p-values ranging from 0.15 to 0.45). Overall, preliminary data suggests that social media data may correlate with NRMP charting outcomes. The study is however, limited by small sample sizes, selection bias towards reporting by successfully-matched applicants, and variability in self-reporting, making it difficult to infer the accuracy and reliability of the self-reported data. For this reason, we advise applicants to take the information they find on social media with a grain of salt.

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Longitudinal cohort study of the association between atopic dermatitis and depression throughout childhood

C. Kern[2], J. Wan[1], K. Lewinn[2], S. Langan[4], K. Abuabara[3]

[1]UPenn, Philadelphia, Pennsylvania, United States, [2]UCSF, San Francisco, California, United States, [3]UCSF, Berkeley, California, United States, [4]LSHTM, London, United Kingdom

Atopic dermatitis (AD) is one of the most common inflammatory diseases of childhood and has been associated with comorbid mental health outcomes, but there are limited data on the development of depression during childhood and adolescence, which may be a critical period for intervention. To understand origins of the relationship between AD and depression, accounting for heterogeneity in AD disease activity and severity over time, we analyzed data from an existing longitudinal cohort study representative of the UK population including 13,979 children followed from birth into adolescence. AD annual period prevalence and severity were measured using a standardized and validated question about flexural dermatitis; depressive symptoms were measured using the Short Moods and Feelings Questionnaire (SMFQ) at multiple ages. Period prevalence of AD ranged from 12.8%-18.6%, and clinically significant depressive symptoms ranged from 6.0% -17.9%. Using cross-sectional multivariable logistic regression, we tested the hypothesis that active AD in the past year was associated with SMFQ scores at 5 time points between ages 10 and 16. After controlling for potential confounders including sex, ethnicity, asthma/rhinitis, socioeconomic status and maternal mental health, we found that any active AD was associated with a 13-46% increase in the odds of depression, but the association was only significant at age 16 (OR:1.46, 95%CI 1.04-1.90), and that participants with moderate/severe disease consistently had higher odds of depression. Including a history of asthma or allergies as potential mediators did not substantially change the effect sizes. When inflammatory markers linked to depression (IL-6 and CRP) were additionally included in the models, effect sizes did not change, suggesting that other markers or mechanisms are likely to explain the association. Our results highlight the importance of interventions to address depression among children with AD, especially those with more severe disease and active disease during adolescence.

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Hidradenitis suppurativa in a cohort of sixty years and older
K. Pacific[2], K. Babbush[1], S. Cohen[1]
[1]Albert Einstein College of Medicine, Bronx, New York, United States, [2]Dermatology, Albert Einstein College of Medicine, Bronx, New York, United States
Hidradenitis suppurativa (HS) is a chronic, recurrent skin disorder characterized by painful nodules, pustules, purulent abscesses, and sinus tracts ("tunnels") leading to progressive disability. While the epidemiology of HS has been reported, this has not been explored in patients 60 years and older. Our study examines this cohort. We identified 51 patients aged 60 and older with HS who were seen Montefiore Medical Center between 2015 and 2019. There were 39 females (76.5%); 26 (50.9%) self-identified as black or African-American, 7 (13.7%) as white, 11 (21.6%) as other, and 7 (13.7%) declined to respond or were unsure. Ethnic analysis revealed 7 (13.7%) were Spanish/Latino/Hispanic, and 33 (64.7%) were non-Spanish/Latino/Hispanic , while 11 (21.6%) declined to respond or did not know. Body mass index (BMI) was available for 37 patients: 7(18.9%) were normal BMI, 6 (16.2%) were overweight, 12 (32.4%) were obese, and 12 (32.4%) were very obese. Of 49 patients who reported smoking history: 16 (32.6%) were current smokers, 18 (36.7%) were former smokers, 15 (30.6%) were never smokers. Forty-one patients reported information about temporal landmarks: the average age of symptom onset was 45.3 years (±19.7) and the average age at diagnosis was 65.2 (±5.0). Family history was documented for 32 patients: 17 (53.1%) reported a family history of HS and 15 (46.9%) did not. HS Physician Global Assessment (HS-PGA) and Hurley Scores were recorded at the initial visit for 26 patients and 37 patients, respectively. The average HS-PGA was 2.5 (±1.1), while the average Hurley stage was 2.1 (±0.92). Hidradenitis suppurativa occurs frequently among patients aged 60 and older. Our findings suggest there may be tertiary peak later in life. It appears that smoking status, BMI, and family history parallel the findings in the general HS population; however these findings indicate a need for further investigation of this cohort. Naik HB, Paul M, Cohen SR, Alavi A, Suàrez-Farñas M, Lowes MA. Distribution of self-reported hidradenitis suppurativa age at onset. JAMA Dermatol. 2019.

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FDA cleared devices produce inconsistent platelet-rich plasma product
R. Farah[3], J. A. Shaik[1], M. Inyang[1], N. Sadick[4], N. Hussain[3], R. Freese[2], G. Gregorich[1], J. Asaila[1], M. K. Hordinsky[3]
[1]Blood Bank Quality, University of Minnesota, Minneapolis, Minnesota, United States, [2]Biostatistics, University of Minnesota, Minneapolis, Minnesota, United States, [3]Dermatology, University of Minnesota, Minneapolis, Minnesota, United States, [4]Sadick Dermatology, New York, New York, United States
Platelet-rich plasma (PRP) has emerged as a novel option to treat androgenetic alopecia. However, data focused on quality of product from FDA cleared devices is lacking. Herein, we assessed and compared cellular PRP content produced from FDA cleared devices. Within one academic institution, white blood cell (WBC) counts and platelet counts from whole blood and resultant PRP were retrospectively reviewed. Institutional quality control (QC) procedures were utilized to obtain counts. Of the fifty-two PRP samples assessed, only 2 met the Insitutions QC standard (defined a 3-fold increase in platelet concentration). Paired analysis demonstrated similar platelet counts in whole blood and PRP. Platelet counts from individual devices were significantly different. Within the devices, platelet counts from PRP samples were inconsistent, failing the Shapiro-Wilk test. The Emcyte device demonstrated the highest capacity to concentrate, even though mean enrichment was just 2.9-fold and capture efficiency was found to be 34%. WBC numbers were negligible in Eclipse and Selphyl final PRP samples. However, the Emcyte device enriched PRP with WBCs similar to or greater than whole blood. These results demonstrate inconsistent PRP end product with variable platelet counts among multiple devices and within devices. QC testing standards were not passed by most samples. Efficiency of platelet capture was lower than that claimed by device manufacturers. WBC content varied by device used for preparation. Of note, no patients worsened while receiving these treatments. This study presents an additional variable in PRP preparation – inconsistent PRP product from FDA-cleared devices. Additionally, clinical improvement despite QC failure suggests platelet numbers may not be vital for clinical improvement.

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The global burden of pressure ulcers: Findings of the GBD 2017 study
A. Grada[1], C. K. Aksut[2], R. Dellavalle[2], M. Naghavi[3]
[1]Department of Dermatology, Boston University School of Medicine, Boston, Massachusetts, United States, [2]Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States, [3]Institute for Health Metrics and Evaluation, University of Washington, Seattle, Colorado, United States
Introduction: Pressure ("decubitus") ulcers present a challenge to health care professionals worldwide. The Global Burden of Disease (GBD) 2017 study is a systematic analysis to quantify the comparative magnitude of health loss at the global level. This study presents GBD 2017 results for pressure ulcers. Design and Setting: Pressure ulcer epidemiologic data sources were derived from an extensive literature search, hospital insurance data, and vital registration records. Data sources were analyzed with a Bayesian meta-regression modeling tool, DisMod-MR 2.1, to yield prevalence estimates. These were combined with disability weights to produce years lived with disability (YLDs). Deaths by age group were multiplied with reference life expectancy to generate years of life lost (YLLs). YLDs and YLLs were summed to yield disability-adjusted life years (DALYs) for 195 countries divided into 21 world regions, both sexes, 20 age groups, and years 1990–2017. Main Outcomes and Measures: Disability-adjusted life years (DALYs) in 195 countries. Results: The world regions of the Caribbean, Southeast Asia, Southern sub-Saharan Africa, Southern Latin America, Tropical Latin America, and Oceania had the greatest burden and deaths from pressure ulcers. The five individual countries with the greatest pressure ulcer burden were Barbados, American Samoa, Dominica, the Bahamas, and Suriname. Conclusion and Relevance: Our findings are at least partially explained by a convergence of high obesity prevalence and inadequate healthcare infrastructure. The substantial health and economic burden of pressure ulcers worldwide highlight the need for effective prevention measures. GBD results can help shape research and public policy.

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The global burden of acne vulgaris: Results from the GBD study 2017
A. Grada[1], C. K. Aksut[2], R. Dellavalle[2], M. Naghavi[3]
[1]Department of Dermatology, Boston University School of Medicine, Boston, Massachusetts, United States, [2]Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States, [3]Institute for Health Metrics and Evaluation, University of Washington, Seattle, Washington, United States
Background: Acne vulgaris is a common inflammatory disease that can be associated with significant psychological comorbidities. Updated estimates of acne disease burden are necessary in order to evaluate the impact of past healthcare policies and orient new healthcare strategies to address unmet needs. Methods: Data was extracted from scientific literature, national surveys, claims data, and primary care sources on the prevalence of acne. Prevalence data were combined with a disability weight to yield years lived with disability. Disability-adjusted life years (DALYs) are a sum of the years lived with disability (YLDs) and years of life lost (YLLs) – assumed to be zero. Measures of burden at global, regional, and national levels were generated for incidence, prevalence, YLDs, and DALYs due to acne. All measures are reported as absolute numbers, percentages, and crude and age-adjusted rates per 100,000 persons. In addition, acne burden was assessed by socio-demographic index (SDI). Findings: Acne was responsible for 0.1% of DALYs from all conditions studied by GBD 2017 globally. Global age-standardized DALY rate per 100,000 persons from acne was 33.93 (20.19 - 54.08) for both sexes, 30.03 (17.74 - 47.93) for males, and 37.93 (22.71-60.53) for females. Acne burden was greatest in Western Europe and North America. Age-standardized DALY burden was greater in higher SDI countries. The greatest change in Age-standardized DALY (1990-2017) was in North Africa and the Middle East. Conclusion: The disease burden of acne is disproportionately greater in high-income and high socio-demographic index countries of North America and Western Europe.

Patient-Targeted Research

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Smoking is associated with the severity of rhododendrol-induced leukoderma and with the occurrence of leukomelanoderma

Y. Fukunaga[1], K. Fukai[2], A. Umekoji-Hayashi[3], T. Ishihara[4], A. Shintani[5], D. Tsuruta[1]

[1]Dermatology, Osaka City University Graduate School of Medicine, Osaka-city, Osaka, Japan, [2]Dermatology, Osaka City General Hospital, Osaka, Japan, [3]Dermatology, Baba Memorial Hospital, Sakai, Osaka, Japan, [4]Innovative and Clinical Research Promotion Center, Gifu University Hospital, Gifu, Japan, [5]Medical Statistics, Osaka City University Graduate School of Medicine, Osaka, Japan

Rhododendrol (RD) is a skin whitening ingredient that was developed in Japan. Among the 800,000 users of RD-containing cosmetics, 20,000 patients developed localized leukoderma (RD-induced leukoderma). Forty-two % of those users showed perilesional hyperpigmentation (leukomelanoderma), and 14% of them were associated with vitiligo vulgaris afterwards. The aim of this study is to investigate the risk factors affecting the severity of RD-induced leukoderma, the occurrence of leukomelanoderma, and the association with vitiligo vulgaris. For this retrospective cohort study, we abstracted data from our dermatology medical records of 101 patients who developed leukoderma after using the cosmetics containing RD from July 2013 to December 2014. Age, BMI, the number of RD-containing products they used, smoking history and depigmentation scores at their baseline visit as well as blood test data for anti-nuclear and/or anti-thyroid antibodies were analyzed. Multivariable logistic regression and linear regression were used for analyses of leukomelanoderma, vitiligo vulgaris and characteristics at the baseline visit. Age, the number of RD-containing products used, BMI, anti-nuclear and anti-thyroid antibodies were not significantly correlated with the presence of leukomelanoderma, but it appeared that leukomelanoderma was more likely to occur in patients who had a smoking history (p=0.006). In addition, smokers showed a significant increase in their depigmentation score at the baseline visit (p=0.03). Our study demonstrates that smoking is associated with the severity of RD-induced leukoderma and the occurrence of leukomelanoderma.

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Intravenous ertapenem therapy for advanced hidradenitis suppurativa

K. Babbush, M. Ghias, A. Nosrati, K. Pacific, H. Hosgood, S. Cohen

Albert Einstein College of Medicine, Bronx, New York, United States

Ertapenem, a broad-spectrum antibiotic, has been used as an off-label treatment option for refractory hidradenitis suppurativa (HS). Previous studies have reported a significant improvement in quality of life, pain, and amount of drainage for HS patients treated with intravenous (IV) ertapenem. Additionally, key inflammatory markers, including erythrocyte sedimentation rate [ESR], c-reactive protein [CRP], interleukin-6 [IL-6], and tumor necrosis factor-alpha [TNF-a]), may be associated with disease severity in HS patients. Although clinical improvement with IV ertapenem is well recognized, changes in serum inflammatory markers have not been explored. We conducted a retrospective chart review of all patients receiving care at the Montefiore HS Treatment Center who initiated and completed at least six weeks of IV ertapenem therapy in 2019. All patients (n = 15) received daily IV ertapenem at a dose of 1 gram (500 mg renally dosed). Disease severity (HS-Physician Global Assessment [HS-PGA]; Numerical Rating Scale [NRS] pain scores) and serum inflammatory markers (ESR, CRP, IL-6, TNF-a) were quantified at baseline (week 0) and at follow-up visits (weeks 4-8) for HS patients receiving IV ertapenem. The average age of patients was 37.4 ± 12.2, and 73% were female. All patients were Hurley Stage III. Medications prior to initiating ertapenem included topical antibiotics (93%), oral antibiotics (100%), anti-androgen therapy (87%), oral corticosteroids (6.7%), and biologic immunotherapy (93%). Six patients (40%) had received surgery for HS. At follow-up visits, there were significant decreases in NRS pain scores (p=0.0083), HS-PGA scores (p=0.0005), and levels of inflammation, quantified by ESR (p=0.0012), CRP (p=0.0166) and IL-6 (p=0.0039). All patients reported decreased drainage. The dramatic improvement seen in both clinical and laboratory markers of HS disease severity supports the utility of IV ertapenem as an effective option for refractory HS.

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Words matter: A randomized controlled study evaluating the impact of decision framing on treatment preferences in adults with psoriasis and psoriatic arthritis

A. Kassardjian[1], V. S. Chat[2], L. Archuleta[1], J. Hekmatjah[3], T. Sierro[1], C. Read[1], A. Y. Chen[1], I. Singh[1], A. W. Armstrong[1]

[1]Keck School of Medicine, Los Angeles, California, United States, [2]Medical College of Georgia at Augusta University, Augusta, Georgia, United States, [3]Western Michigan University, Homer Stryker M.D. School of Medicine, Kalamazoo, Michigan, United States

It is unknown how clinicians' wording of a treatment influences patients preferences. Decision framing is the way that a choice is worded. A choice can be worded either positively (gain-framed) to explain the benefits of a therapy, or negatively (loss-framed) to explain the risks of not taking a therapy. We conducted a randomized controlled study to evaluate the effect of gain versus loss framing on patients' treatment preference. Ninety adults with psoriasis ± psoriatic arthritis were randomized 1:1 to receive a questionnaire that contains either (1) a gain-framed message that explains the benefits of receiving an injectable medication for psoriasis and psoriatic arthritis, or (2) a loss-framed message that explains the harms associated with not taking the medication. Both arms received the same information regarding possible side effects. The average age is 49.6; 64% male; 53% white; 28% with psoriatic arthritis. Each patient scored their likelihood to take the medication (0=definitely will not use the medication; 10=definitely will use the medication). Patients who received a gain-framed message had a mean score of 7.11 (SD2.20), whereas patients receiving a loss-frame message had a mean score of 8.84 (SD1.59). The difference between the groups was 1.73 (95% CI -2.54 to -0.93, p<0.0001). This study found that loss framing is more effective in influencing patients' treatment preference than gain framing. These findings suggest that patients are more likely to agree to a treatment if the clinician frames the treatment using a loss-frame approach.

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Feasibility of examining fluorescence of follicular sebum and *C. acnes* after pool water immersion

R. A. Kimball[1], M. Porter[2, 1], A. B. Kimball[2, 1]

[1]Beth Israel Deaconess Medical Center, Boston, Massachusetts, United States, [2]Harvard Medical School, Boston, Massachusetts, United States

Sebum is an oily waterproof semi liquid composed of triglycerides, fatty acids, wax esters, squalene, and cholesterol. Swimming pool water has been proposed to reduce water content of swimmers' skin due to a reduction and possible rebound of sebum. Pool water is generally treated with hypochlorite and other chemicals which would not be anticipated to emulsify sebum, as cleansers do, but as oxidizers, could lead to some dissolution. Additionally, water immersion alone has been reported to increase trans epidermal water loss and the PH of volar skin. To test the feasibility of examining follicular sebum and *C. acnes* (previously known as *P. acnes)* levels in swimmers, two experiments were conducted. In the first, 5 sources of UV light, one medical grade Woods lamp (usually 320-400nm) and four hand-held "black light" LED devices with wavelengths ranging from 375-400nm were evaluated. All fluoresced white and yellow/orange colors consistent with porphyrin and protopophyrin associated with sebum and *C. acnes* respectively. Photography was tested using both a Nikon D5200 and android smart phone; both produced viable images. Photography through the magnified Wood's lamp viewer and using flashlight with a broad 4-inch LED base (UV Beast) appeared to be most effective. Photoshop (Adobe) allowed for accentuation of contrast in images that would allow for quantification. Follicles on the back before and after a swimming session qualitatively fluoresced white (sebum) at similar levels and yellow/orange (*C.acnes*) at lower levels after swimming, suggesting that pool water was not entirely disrupting follicular sebum. Lastly, sebum was applied to glass slides and immersed in pool water for 1 and 2 hours. Minimal changes of the sebum tracings could be identified. In conclusion, sebum and *C. acnes* levels could be detected using fluorescent photography and inexpensive hand-held light sources. With some engineering modifications these readily available tools may be a useful way to monitor changes in follicular sebum and *C. acnes* fluorescence levels.

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Interim results from a comparative method validation study evaluating the use of digital photographs versus in-person assessment of rosacea

R. Hamidi[5], H. Aralis[1], K. W. Bruhn[5], I. J. Deluca[2], K. Nguyen[5], M. Tran[5], M. Patel[3], V. A. Patel[2], J. Cotliar[5], B. H. Tan[3], H. L. Sofen[4], C. V. David[5]

[1]Biostatistics, UCLA, Los Angeles, California, United States, [2]Dermatology, George Washington School of Medicine & Health Sciences , Washington, District of Columbia, United States, [3]Medicine/Dermatology, Harbor-UCLA Medical Ctr, Torrance, California, United States, [4]Medicine/Dermatology, David Geffen UCLA School of Medicine, Los Angeles, California, United States, [5]Science 37, Inc., Los Angeles, California, United States

Photography-based teledermatology has the potential to increase patient access to clinical trials, in part by removing geographic barriers that limit participation to those who reside near a trial site. Here we present interim results from a study evaluating digital photography as a method of assessing rosacea severity, in comparison with in-person methods. Eight dermatology raters performed in-person assessments of rosacea patients at a clinic in Los Angeles, California. Rosacea severity was graded using the Clinician's Erythema Assessment (CEA), Investigator's Global Assessment (IGA), and inflammatory lesion count. During the same visit, study participants were trained to take six self-photographs of their face using an iPhone SE. These photographs were housed in our cloud-based clinical trial software platform. Following a period of 28 days, raters performed rosacea severity assessments on the same patients they assessed in clinic, utilizing the digital photographs described above. To date, 34 patients (of a planned sample size of 70) have completed the study activities as outlined. Using the intraclass correlation coefficient (ICC), we compared agreement between photographic assessments of rosacea severity with the same assessments performed in-person. The ICC for CEA was 0.75 (95% CI 0.66-0.84), for IGA 0.83 (95% CI 0.74-0.89), and for total inflammatory lesion count 0.91 (95% CI 0.84-0.95), indicating agreement ranging from good to excellent. Interim results from this study indicate that patient self-photographs taken on an iPhone can be used to reliably assess rosacea severity, supporting the integration of photography-based teledermatology into future clinical trials for rosacea.

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The usefulness of an inexpensive, battery-powered, handheld microscope in low-resource dermatologic practices

N. D. Flint[1], S. Simister[1], I. Flint[2], T. Dickerson[1], A. M. Secrest[3]

[1]School of Medicine, University of Utah, Mapleton, Utah, United States, [2]Ivan D Flint Dermatology, Salt Lake City, Utah, United States, [3]Dermatology and Population Health Sciences, University of Utah, Salt Lake City, Utah, United States

Previous studies in low- and middle-income countries have shown that skin disease is very common, especially dermatophyte and scabies infections. Point-of-care microscopic evaluation of skin scraping samples improves the diagnostic accuracy of these skin pathologies. Resource limitations, however, often preclude the use of microscopy for point-of-care evaluations in low- and middle-income countries. An inexpensive (<USD$20), hand-held, battery-powered LED microscope was field-tested for potassium hydroxide (KOH) and mineral oil examinations of skin scrapings from participants who complained of a rash. The study was conducted in collaboration between the University of Utah School of Medicine and the Kwame Nkrumah University of Science & Technology School of Medical Sciences in the Atwima Nwabiagya North District in the Ashanti region of Ghana, West Africa. A board-certified dermatologist was able to microscopically confirm dermatophyte and scabies infections, which comprised 16.4% (35/213) of all evaluated skin conditions. An inexpensive, hand-held, battery-powered LED microscope can be used to effectively diagnose certain skin diseases in remote locations without the need for electricity. This point-of-care testing is readily learnable by any clinician, staff member or pharmacist. Using microscopy improves the diagnostic accuracy of skin conditions and choice of therapy and minimizes unnecessary side effects of commonly-used combination treatments.

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A deep neural network for the early image diagnosis of Stevens-Johnson syndrome/toxic epidermal necrolysis

A. Fujimoto[1], Y. Iwai[1], S. Muramatsu[3], T. Ishikawa[2], S. Shinkuma[1], R. Abe[1]

[1]Division of Dermatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan, [2]Department of Medical Informatics, Niigata University Medical and Dental Hospital, Niigata, Japan, [3]Department of Electrical and Information Engineering, Niigata University Graduate School of Science and Technology, Niigata, Japan

Background: Stevens-Johnson syndrome (SJS)/toxic epidermal necrolysis (TEN) are life-threatening cutaneous adverse drug reactions (cADRs). However, distinguishing SJS/TEN from non-severe cADRS is often difficult, especially in the early stages of disease. To overcome this limitation, we developed a computer-aided diagnosis system for the early diagnosis of SJS/TEN powered by a deep convolutional neural network (DCNN). Methods: We trained a DCNN using a dataset of 25,054 individual lesion images obtained from 113 patients diagnosed with SJS/TEN or non-severe cADRs. The DCNN's classification accuracy was compared to that of 10 board-certified dermatologists and 24 trainee dermatologists. Results: The DCNN achieved 88.8% (95% CI, 84.2–93.5) sensitivity, whereas the sensitivities of the board-certified dermatologists and the trainee dermatologists were 31.0% (95% CI, 18.8–43.2; P<0.001) and 27.5% (95% CI, 22.6–32.5; P<0.001), respectively. The negative predictive value was 96.0% (95% CI, 94.7–97.3) for DCNN, 68.1% (95% CI, 65.9–70.5; P<0.001) for the board-certified dermatologists, and 67.4% (95% CI, 65.9–70.5; P<0.001) for the trainee dermatologists. The AUC of the DCNN for SJS/TEN diagnosis was 0.872 and was significantly higher than all the board-certified dermatologists and trainee dermatologists. Conclusions: We successfully developed a DCNN to classify SJS/TEN and non-severe cADRs based on individual lesion images of erythema. The DCNN demonstrated superior performance in screening for SJS/TEN compared to dermatologists. The use of AI as a diagnostic assistance may progress diagnostic accuracy, leading improvement of prognosis for SJS/TEN patients.

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Association of granuloma annulare with type II diabetes, cigarette smoking, and liver disease: A case-control study

E. Almazan[1], M. Belzberg[2], C. X. Qin[1], K. Williams[1], J. Choi[1], B. Kaffenberger[3], Y. Semenov[4], J. Alhariri[2], S. Kwatra[2]

[1]The Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [2]Department of Dermatology, Johns Hopkins Hospital, Baltimore, Maryland, United States, [3]Division of Dermatology, Ohio State University Comprehensive Cancer Center , Columbus, Ohio, United States, [4]Department of Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

Granuloma annulare (GA) is a cutaneous granulomatous disorder of unknown etiology. There are conflicting reports of associations between GA and a range of systemic diseases. We conducted a retrospective case-control study of GA patients to clarify the relationship of GA with multiple systemic conditions. The electronic medical records of patients age 18 or older who presented to the Johns Hopkins Hospital System between January 1, 2009 and June 1, 2019 were reviewed under IRB approval. Inclusion criteria required a clinical and histopathological diagnosis of GA by a dermatologist. GA patients (n=82) were age, race, and sex matched to controls in a 1:2 ratio. Controls (n=164) presented as outpatients for benign, localized chief complaints. Continuous variables were assessed with a Student's t-test, and categorical variables with Pearson Chi-squared or Fisher's exact tests. On average, GA patients were 58 ± 16 years old. They were primarily non-Hispanic whites (85%) and women (73%). Patients with GA had a higher incidence of type II diabetes (*P*=0.0007), liver disease (*P*=0.0429), non-migraine headache disorders (*P*=0.0180), and a positive smoking history (*P*=0.0257) compared to controls. Conflicting with prior reports, no associations were found between GA and dyslipidemia, thyroid disease, or solid organ malignancy. Among GA patients, women were more likely than men to present with ophthalmic conditions (*P*=0.04), while men were more likely to have concurrent cardiovascular disease (*P*<0.0001) and type II diabetes (*P*=0.05). There were no significant differences found in the comorbid conditions of GA patients when separated by race. These results further clarify the comorbidities of GA and suggest potential areas of investigation into its pathogenesis.

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The impact of psoriasis and its associated comorbidities on quality of life: Results from the National Psoriasis Foundation Annual Survey

S. Bell[1], J. Domire[1], J. F. Merola[2], A. Gottlieb[3], G. Gondo[1]

[1]National Psoriasis Foundation, Portland, Oregon, United States, [2]Harvard Medical School, Boston, Massachusetts, United States, [3]Mount Sinai Hospital, New York, New York, United States

In 2019, the American Academy of Dermatology and the National Psoriasis Foundation issued joint care guidelines for the treatment of psoriasis that highlighted the need to address comorbidities related to psoriatic disease. For nearly 20 years, the NPF has conducted a national survey of the psoriatic disease community. The survey seeks to enhance the understanding of the impact of psoriatic disease and its associated comorbidities on the health outcomes of individuals living with psoriasis and psoriatic arthritis. Methods: A nationwide survey of a random sample of individuals with psoriatic disease was conducted online and by telephone. Participants were stratified by gender, disease type, and balanced by the estimated population of individuals with psoriatic disease by geographic region. A total of 1,570 individuals completed the survey. Results: More than 85% of individuals reported having one or more relevant comorbidities. On average, individuals reported having three comorbidities. Results from analysis of variance (ANOVA) examining the impact of the number of comorbidities on global quality of life ($p<.001$) suggest that the two are inversely related. Independent samples t-test suggests that the presence of even one co-morbidity negatively impacts quality of life ($p<.05$), as measured by DLQI. Results from independent t-tests suggest that individuals with moderate to severe psoriasis experience impaired mental health status ($p<.001$), global quality of life ($p<.001$), and disease specific quality of life ($p<.001$). Conclusions: Results from the NPF survey suggest that comorbidities are highly prevalent among individuals with psoriatic disease negatively impact their quality of life. These results underscore the importance of treating psoriatic disease systemically and holistically in order to optimize patient outcomes.

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Evaluation of extracts containing red clover using dermal papilla cells in the development of a scalp treatment system targeting hair loss and hair damage

B. Cook, J. Namkoong, M. Riggs, K. C. Holley, D. Kern, H. Knaggs

R&D, NuSkin Enterprises, Provo, Utah, United States

Hair loss and hair disorders are common conditions affecting millions around the globe. The causes are complex and not fully understood but links to genetics, lifestyle, intrinsic aging, and environment impact are all suggested. Prevalence is known to increase with age and response to treatment is notoriously variable. Treatments have a global market value of $2.5 billion (USD) driven by increasingly long and healthy lifespans, emphasis on aesthetic appearance, and the considerable emotional distress experienced by patients. In-vitro models such as Dermal Papilla Cells (DPC) from hair follicles are powerful tools for screening ingredients for hair growth promoting activities and inhibitory effects while also providing insight into possible mechanistic actions at the cellular level. In this work, we screened several ingredient blends containing either panthenol or licorice extract using the DPC model with three different sources of red clover extract. Comparison of the gene expression datasets with statistically significant Fold Change values ≥ 1.5 show unique influences by each extract blend. Using the DPC models to guide ingredient selection, a treatment serum was formulated and tested in 8-week (n=33) and 6-month (n=52) in-vivo human clinical studies using subjects with self-described thinning or damaged hair. Qualitative effects to the subject's hair and scalp were evaluated by self-perception and independent clinical grading vs baseline. Results showed statistically significant ($p\leq0.05$) improvements in several categories including volume and scalp coverage. Analysis of broken and intact hair counts from Brush Friction Hair Count Method and Macro Photography of a 2x2 cm area also show statistically significant improvement of 71.7%, 80.8% and 31.5% respectively after 6-months. Of note, in the 6-month study the percentage of positive responders reported by an independent clinical grader and self-perception was >80% and >90% respectively.

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Efficacy of immunotherapy in Merkel cell carcinoma patients with chronic immunosuppression

L. Zawacki, K. Lachance, T. Akaike, P. Nghiem

Dermatology, University of Washington, Seattle, Washington, United States

Merkel cell carcinoma (MCC) has a high propensity for recurrence and distant metastasis. Persons with chronic immunosuppression have a higher risk of developing MCC and a more aggressive disease course. Immune-checkpoint inhibitors (e.g. anti-PD-(L)1) are associated with improved disease-specific survival. However, the effectiveness and side-effect profile of these agents in immunosuppressed MCC patients is not well categorized in part because they were ineligible for prior clinical trials. This study assesses the risk-benefit profile of immunotherapy in this setting, and explores differences between forms of immunosuppression. Data were abstracted from a Seattle-based prospective registry of 1,491 MCC patients from which 29 were identified to have been treated with immunotherapy despite having chronic immunosuppression. Five types of chronic immunosuppression were represented: chronic lymphocytic leukemia (CLL, n=11), solid organ transplant (SOT, n=4), autoimmune disorders (AD, n=6), other hematologic malignancies (OHM, n=7), and HIV/AIDs (n=3). Fourteen of 29 patients (48%) had a complete response (CR), 2 patients (7%) had a partial response (PR), and 13 patients (45%) had progressive disease (PD; 11 of whom died of MCC). Progression of disease and survival status varied greatly among different types of immunosuppression. For example, all patients with concurrent OHM had CR to immunotherapy (7/7, 100%) while patients with concurrent CLL had the lowest response rate (2/11,18% had PR). In comparison, in a separate study of immune competent patients treated with anti-PD-1 agents, 28 of 50 (56%) had objective responses (CR or PR). Despite chronic immunosuppression being associated with a more aggressive disease course, the efficacy of immunotherapy in patients with chronic immunosuppression appears to be dependent on the type of immunosuppression. While there is reason for optimism for patients with certain types of immunosuppression (OHM), treatment of MCC patients with CLL in particular remains a major challenge as only 18% achieved even a PR.

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Patient safety alert: Medical image manipulation as a safety hazard for wrong-site procedures

J. S. Taylor[1], P. L. Bailin[1], A. T. Vidimos[1], J. Lucas[1], A. Vij[1], A. C. Halpern[3], D. K. Dennison[2]

[1]Dermatology, Cleveland Clinic Lerner College of Medicine, Cleveland, Ohio, United States, [2]Don K Dennison Solutions, Inc, Waterloo, Ontario, Canada, [3]Dermatology, Memorial Sloan Kettering Cancer Center, New York, New York, United States

While some medical images are manipulated for patient benefit- radiograph rotation to aid during surgery and visible image enhancement to demonstrate plastic surgical outcomes, others have been manipulated causing patient harm- inadvertent flipping of x-rays resulting in wrong- side brain surgery, and an absence of photographs has caused wrong-site surgery in dermatology. We surveyed our department staff as well as the landscape of basic medical imaging devices, software, practices and current standards to ascertain actual and potential errors. Office- generated photographs may be stored natively in an electronic medical record (EMR) or in a separate medical imaging system which in most cases may allow flipping of images. Patient generated smart phone photographs, e.g. submitted in surgical follow-up through an EMR patient portal, have been noted to be wrong -sided, likely through image editing software. Selfies, which take a mirror image are usually but not always auto- flipped. Accurate dermatologic photographs are key to preventing wrong-site procedures but there is a need for education of providers and patients on the vagaries of the multiple cameras and software. There is also a current lack of agreement on standards in dermatologic imaging which often rely on consumer-based image formats which may not allow for efficient presentation of salient image metadata, such as laterality and anatomy. Current Digital Imaging and Communication in Medicine (DICOM) standards, initially used by radiology, have been adopted for dermatology images by the US Department of Veterans Affairs and may allow leveraging of DICOM network and workflow, interoperability of images and metadata, and existing enterprise imaging infrastructure, and better compliance with required image retention and improved patient safety.

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Long-term efficacy of secukinumab in Korean patients with moderate-to-severe plaque psoriasis in a real-world setting; A single-center, retrospective study

J. Park, S. Lee

Department of Dermatology, Chonnam National University Medical School, Gwanju, Korea (the Republic of)

This study was to evaluate the long-term efficacy of secukinumab in real-world practice in Korean patients with moderate-to-severe plaque psoriasis. A total of 19 patients with moderate-to-severe plaque psoriasis, who had been treated with 300mg secukinumab were analyzed by classifying them into naïve and bio-switched groups. As a primary end-point, therapeutic efficacy of secukinumab to reach PASI 75, PASI 90, and PASI 100 response was analyzed at week 52. For long-term efficacy and drug survival of secukinumab, 11 patients were analyzed up to week 104, including three patients who were followed-up until week 280-345. At week 52, PASI 75, PASI 90, and PASI 100 responses were 95%, 89%, and 58%, respectively (n=19, all enrolled patients), with better response in naïve group (n=7). For 11 patients, who were treated up to week 104, PASI 75, PASI 90, and PASI 100 responses were 82%, 82%, and 64%, respectively, with better response in naïve group (n=2). Of Interest, three patients (1: naïve, 2: bio-switched) reached to PASI 100 response at their first treatment could maintain the same drug efficacy at their second treatment up to week 280-345, even after transient discontinuation of injection between two treatments. Our real-world data in Korean patients with plaque psoriasis showed better efficacy, especially naïve group, compared with that of previous studied phase-III clinical trials (ERASURE and FIXTURE studies). It might be related with the allowance of combination therapy, including topical agents or oral retinoic acid or methotrexate, in real-world practice. The drug survival of secukinumab is considered to be good for good-response patients at their first treatment.

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Ixekizumab achieves more rapid reduction of circulating interleukin-19 compared to guselkumab in a psoriasis head-to-head study

R. J. Konrad[1], B. J. Nickoloff[1], R. E. Higgs[1], L. K. Ferris[2], K. Reich[3], H. Elmaraghy[1], G. Gallo[1], L. Renda[1], S. Park[1], J. G. Krueger[4]

[1]Eli Lilly and Company, Indianapolis, Indiana, United States, [2]University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, United States, [3]Skinflammation® Center, Hamburg, Germany, [4]Rockefeller University Hospital, New York City, New York, United States

Previous results of a randomized, head-to-head trial between the interleukin (IL)-17A inhibitor ixekizumab (IXE) and the IL-23 inhibitor guselkumab (GUS) showed that significantly more patients treated with IXE than GUS had fully clear skin (PASI 100) after 4, 8, and 12 weeks (wks) of treatment.[1] The circulating level of the pro-inflammatory cytokine IL-19, produced by activated keratinocytes and macrophages, is a prognostic marker for skin disease activity which is elevated ~7-fold in psoriasis.[2] IL-19 levels were measured using a novel immunoassay in serum from a subpopulation of patients with moderate-to-severe plaque psoriasis after 0, 2, 4, and 8 wks of treatment with IXE or GUS (N=21 and 19, respectively). Comparisons between IXE and GUS were made using a mixed model for repeated measures. The normal range of IL-19 in a separate cohort of healthy patients (N=107) was 4-26 pg/ml (range containing 95% of values). Baseline IL-19 values for all 40 patients with psoriasis were >26 pg/ml. While IXE reduced IL-19 to <26 pg/ml (least squares mean) as early as Wk 2, GUS did not reduce IL-19 to <26 pg/ml even after 8 wks of therapy. IL-19 changes from baseline were significantly greater in IXE vs GUS patients at Wk 2 (7.1-fold vs 2.3-fold, p=0.001) and remained numerically greater at Wk 4 (7.9-fold vs 4.1-fold, p=0.06). The majority of patients who achieved PASI 100 at Wk 12 had normal IL-19 levels at Wk 2 (60%, 6/10) and at Wk 4 (82%, 9/11). In conclusion, IXE reduces IL-19 more rapidly than GUS. IL-19 may be a more objective indicator of systemic inflammation than what can be observed by examining the skin and could have prognostic use in predicting complete skin clearance. [1]Blauvelt, A et al (2019). *Br J Dermatol*. doi:10.1111/bjd.18851. [2]Konrad RJ et al (2019). *Sci Rep* 26;9(1):5211.

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Trial in progress: VALO study evaluating PTX-022 in adults with moderate-to-severe pachyonychia congenita, a rare, chronically debilitating disease that makes walking difficult or impossible

J. Teng[1], B. Shroot[3], H. Evans[4], J. Schwartz[4], J. Connor[6], J. Valentine[5], C. Hansen[2]

[1]Stanford University, Palo Alto, California, United States, [2]University of Utah, Salt Lake City, Utah, United States, [3]Palvella Therapeutics, Wayne, Pennsylvania, United States, [4]Pachyonychia Congenita Project, Salt Lake City, Utah, United States, [5]Hyman, Phelps & McNamara, Washington DC, District of Columbia, United States, [6]ConfluenceStat, LLC, Orlando, Florida, United States

INTRODUCTION: In pachyonychia congenita (PC), mutations in keratin genes cause skin fragility and impaired skin barrier function, resulting in severe plantar pain, ultimately making walking difficult or impossible. Currently, there are no approved therapies to treat PC. The keratins involved in PC are regulated by the mammalian target of rapamycin (mTOR) pathway. mTOR inhibitors therefore may repress the mutant keratin genes at the root of PC. In studies, oral mTOR inhibitors were shown to provide clinical benefit to patients. Palvella Therapeutics developed PTX-022 (QTORIN™ 3.9% rapamycin anhydrous gel) as a targeted treatment addressing the root cause of PC. PTX-022 is a topical formulation that employs a composition of excipients to enable distribution of rapamycin into the basal keratinocytes and minimize the adverse effects associated with oral mTOR inhibitors. STUDY DESIGN: The VALO Study is a Phase 2/3 randomized, placebo-controlled study of PTX-022 consisting of four periods: run-in, open-label treatment enrichment, randomized double-blind treatment withdrawal (RDBTW) and post-treatment follow-up. Participants who qualify for RDBTW are randomly assigned 4:3:3 to one of three treatment groups: vehicle gel, QD PTX-022 or BID PTX-022. KEY INCLUSION CRITERIA: Participants must have an established diagnosis of PC with genotyped keratin mutations KRT6A, KRT6B or KRT16. OBJECTIVES: The primary efficacy objective of the study is to compare the change in activity difficulty (measured by fit-for-purpose PGA-AD) in patients who have responded to PTX-022 and remain on therapy vs. responders who have been switched to placebo. STATUS: Palvella conducted the VALO Study in collaboration with PC Project, and with their assistance, has concluded trial recruitment.

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Atopic dermatitis patient focused drug development and the MoreThanSkinDeep Survey

W. Smith Begolka[1], K. Capozza[2]

[1]National Eczema Association, Novato, California, United States, [2]Global Parents for Eczema Research, Santa Barbara, California, United States

Atopic dermatitis (AD) is an inflammatory skin disease with significant physical, psychological, and social impacts, yet limited effective treatments. As part of the September 2019 MoreThanSkinDeep Patient Focused Drug Development (PFDD) meeting on AD, an online survey collected insights on disease and current treatment burdens, and unmet therapeutic needs from 1,508 AD patients and caregivers. Most respondents reported current moderate (44%) or severe (30%) AD, yet 80% noted severe AD at its worst, and 60% had overall worsening or unchanged AD since initial onset. Sixty-four percent indicated more/different body areas affected by AD over time; 67% reported consistent or increased flare frequency. The most problematic symptoms across all respondents were itch (79%), red/inflamed skin (47%), and sleep disturbance (29%). Some respondents reported anxiety (26%) or depression (22%) diagnoses, yet 74% indicated current presence of mood disturbance, which rose to 90% when AD was at its most severe. Past/current use of prescription topicals/phototherapy ranged from 28-41%, with exception of topical antimicrobials (63%), and topical steroids (97%). Most respondents (74-80%) have not used systemic therapies/biologics, yet usage was higher for oral (58%) and injectable (24%) steroids, and oral antimicrobials (51%). Numerous barriers to seeking/maintaining AD treatments were indicated; nearly half noted concern over real/potential side effects and long-term therapy. Notably, the overall impact of AD was comparable between adult patients and adult caregivers; nearly 80% reported moderate/significant quality of life impacts. Only 13% of respondents indicated their AD was very well controlled; 12% were very satisfied with available treatment options. The most important result a future treatment could provide was immediate and sustained reduction in itch (51%), followed by reduction in disease flares (25%). Future research and AD therapeutic strategies should address the underappreciated patient burden, needs and risk/benefit considerations highlighted in this survey.

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Outcomes of patients with cutaneous T-cell lymphoma (CTCL) treated with extracorporeal photopheresis (ECP): A single institution experience
A. G. Johnson[2], Q. Zhao[2], C. Chung[1], B. M. William[2]
[1]Dermatology, The Ohio State University, Columbus, Ohio, United States, [2]Hematology, The Ohio State University, Columbus, Ohio, United States
ECP is an established therapy for CTCL yet patterns of utilization, complication rates, and outcomes vary widely among treatment centers. We hereby report our experience with 13 patients (pts) treated with ECP from 2013-2019; median age was 65 (range 47-87) years. All pts had advanced stage CTCL; 8 (61.5%) with mycosis fungoides (MF) and 5 (38.5%) with Sezary syndrome (SS). Median time from diagnosis to pheresis start was 2.8 months with a median of 2 lines of prior therapy. All pts initially started on a ECP regimen of 2 consecutive days per week with 8 (61.5%) pts concurrently using oral bexarotene (Bex) and subcutaneous interferon alpha (IFN), 2 pts on ECP alone (15.4%), 2 (15.4%) with Bex only, and 1 (7.7%) with IFN only. Out of 13 pts, 9 (69.2%) had ECP via peripheral venous access (PVA) and 4 (30.8%) through central access (2 through tunneled catheters and 2 via double Vortex ports). Both pts with tunneled catheters had central line-associated infections. Out of the 9 pts who underwent ECP via PVA, 3 (33.3%) had interruptions due to inability to achieve access. Overall, 3 pts (23.1%) completed ECP without any interruptions. Eleven pts responded to ECP (Complete response; CR: 2 [18.2%], partial response; PR: 1 [9.1%], stable disease; SD: 8 [72.7%]) with a median of 1.3 months from start of pheresis to first documented response. The median number of total treatments was 15. Nine (69.2%) pts discontinued ECP after median of 4.2 months to start different therapies, 1 died suddenly of unrelated reason, and 1 discontinued ECP to pursue hospice. The median progression free and overall survival was 3.6 and 27.6 months from start of treatment. Our experience is consistent with the literature. ECP remains a highly effective treatment for CTCL. The rate of treatment discontinuation due to vascular complications (50%) is alarmingly high and warrants further investigation.

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Deep learning empowered computer-assisted diagnosis on smartphone skin images
J. Wu[1], J. Han[1], Y. Zhang[2], J. Hu[1], Q. Li[1]
[1]VoxelCloud, Los Angeles, California, United States, [2]The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States
Nowadays deep learning has been widely applied in identifying skin disease using clinical skin images. Along with the development of smartphone and communication technology, telemedicine becomes more accessible for patients to consult with medical experts. However, rare research was done to prove the feasibility of using deep learning to predict skin conditions based on smartphone captured skin images, especially taken by non-professional patients. We collected 46946 skin images from the online patient-doctor telemedicine platform in mainland China. Each image was annotated by at least 2 dermatologists mutual blindly. We trained an assembly 6 different deep neural networks to provide predictions. The model is able to predict the probability among 30 common skin conditions, including infectious conditions, inflammatory conditions, pigmentary conditions, vascular conditions, and even tumors. The model achieves the top 1 and top 3 accuracies of 0.7708 and 0.9194. The coverage error of our model is 1.4171, which indicates the model has a short depth of ranked predictions to cover all the true labels. And label ranking average precision is 0.9392, which means the model has a high fraction of higher-ranked predictions mapping with ground truth labels. Furthermore, the results show the ability to differentiate some skin conditions which have similar morphology by using a deep learning approach on patient captured skin images. In conclusion, a deep learning approach would potentially provide not only initial evaluation of skin lesions to patients but also clues for differential diagnosis to general doctors through smartphone skin photography.

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Immunostimulatory herbal supplements in patients with autoimmune skin diseases
A. Ravishankar[2, 1], D. Yan[2, 1], C. Bax[2, 1], J. S. Concha[2, 1], B. E. Shields[1], L. Pappas-Taffer[1], R. Feng[3], J. Okawa[2, 1], V. Werth[2, 1]
[1]Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Corporal Michael J. Crescenz VAMC, Philadelphia, Pennsylvania, United States, [3]Biostatistics, University of Pennsylvania, Philadelphia, Pennsylvania, United States
Complementary and alternative medicine (CAM) use is prevalent in dermatology. Certain CAMs, including Spirulina, Alfalfa, Chlorella, and Echinacea have been reported to be immunostimulatory or induce dermatomyositis (DM), cutaneous lupus erythematosus (CLE) or autoimmune blistering diseases (AIBD). As such, there is a need to characterize CAM usage in patients. We performed a retrospective chart review at UPenn to characterize CAM use among patients with DM, CLE, AIBD, and non-autoimmune controls. Information gathered included demographics, disease history, and CAM usage (Spirulina, Chlorella, Alfalfa, Green Algae, Echinacea, or other). Statistical analysis was performed using the fisher exact test. 345 patients were enrolled, including 158 DM (45.8%), 122 CLE (35.4%), 31 AIBD (9.0%), and 34 controls (9.9%). DM had the greatest proportion of Caucasians (81.6%), followed by controls (79.4%), AIBD (61.3%), and lupus (52.5%). Given the proportion of Caucasians, race was accounted for in the analysis. CAM use was reported in 13.9% of all patients (21.5% of DM, 6.6% of CLE, 9.7% of AIBD, and 8.8% of controls). CAM use was significantly greater in DM for both Caucasians ($p = 0.019$, OR 2.51) and non-Caucasians ($p = 0.003$, OR 6.79). CAM use was not associated with CLE for Caucasians ($p = 0.067$), but was significantly less for non-Caucasians ($p = 0.034$, OR 0.23). Spirulina was the most common CAM, used in 14.6% of DM, 4.1% of CLE, and 5.9% of controls. Spirulina use was significantly greater in the DM group for Caucasians ($p = 0.015$, OR 3.39) and non-Caucasians ($p = 0.016$, OR 7.63), but was not associated with CLE ($p > 0.05$). No significant relationship was observed for AIBD, controls, or other CAMs ($p > 0.05$). This study demonstrates that patients with DM should be educated regarding the risk of onset or flare from using immunostimulatory CAM such as Spirulina.

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Predicting the long-term outcomes of biologics in psoriasis patients using machine learning
S. Emam[1], A. Du[2], P. Surmanowicz[2], S. Thomsen[1], R. Griener[1], R. Gniadecki[2]
[1]University of Alberta, Edmonton, Alberta, Canada, [2]Medicine, University of Alberta, Edmonton, Alberta, Canada
Background: Real-world data demonstrate that approximately 50% of psoriasis patients treated with a biologic agent will discontinue the drug because of a loss in efficacy. History of previous therapy with another biologic, female sex, and obesity were identified as predictors of drug discontinuations, but their individual predictive value is low. Objectives: To determine whether machine learning algorithms can produce models that can accurately predict outcomes of biologic therapy in psoriasis on an individual patient level. Results: All tested machine learning algorithms could accurately predict the risk of drug discontinuation and its cause (e.g. lack of efficacy vs adverse event). The learned generalized linear model achieved a diagnostic accuracy of 82%, requiring under 2 seconds per patient using the psoriasis patients dataset. Input optimization analysis established a profile of a patient who has the best chances of long-term treatment success: biologic-naive patient under 49 years, early-onset plaque psoriasis without psoriatic arthritis, weight < 100 kg, and moderate-to-severe psoriasis activity (DLQI ≥ 16; PASI ≥ 10). Moreover, a different generalized linear model is used to predict the length of treatment for each patient with mean absolute error (MAE) of 4.5 months. However, Pearson Correlation Coefficient indicates 0.935 linear dependencies between the actual treatment lengths and predicted ones. Conclusions: Machine learning algorithms predict the risk of drug discontinuation and treatment duration with accuracy exceeding 80%, based on a small set of predictive variables. This approach can be used as a decision-making tool, communicating expected outcomes to the patient, and in the development of evidence-based guidelines.

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Alternative data presentation methods: Exploring waterfall and bubble plots in psoriasis biologic clinical trials

P. Surmanowicz[1], S. Emam[2], R. Kelm[2], A. Du[1], R. Gniadecki[1]

[1]Medicine, University of Alberta, Edmonton, Alberta, Canada, [2]University of Alberta, Edmonton, Alberta, Canada

Background: Modern patient care is built upon evidence-based medicine and relies on clinical trials to provide the empiric data to be interpreted and transformed into guidelines utilized by clinicians. Current psoriasis biologic clinical trials utilize a frequentist model (i.e. reporting statistical differences in the proportion of responders) and the PASI (psoriasis activity and severity index) score as the primary outcome measure. Although convenient and simple, these traditional methods lack reporting on individual patient data and cohort response distributions, and are therefore limiting in the conclusions they present to readers. Objective: We aim to explore the usage of waterfall and bubble plots for graphically presenting psoriasis biologic clinical trial data, and compare these alternative methods to traditional approaches to demonstrate their utility. Methods: Access to raw de-identified patient-level data was obtained through the Yale Open Data Access Project and the Clinical Study Data Request for four published psoriasis phase three clinical trials. Raw data for inter- and intra-biologic analyses were prepared into traditional line plots and the novel waterfall and bubble plots for comparison. Results: The alternative data presentation approaches provided additional information on individual patient and overall cohort distribution responses. Novel conclusions on moderate, weak, and adverse responders, as well as predictive patient and biologic factors for favorable response, were available only with the waterfall and bubble plots. Conclusions: Ultimately, the alternative data presentation graphs provided additional and more thorough data on individual and cohort distribution responses and presented additional conclusions unavailable with traditional methodologies.

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PIQ-C, a new PROMIS® tool, measures intensity and impact of itch on children with atopic dermatitis

A. S. Paller[1], J. Lai[2], S. Rangel[1], D. Ramachandran[1], N. Puar[1], V. Patel[1], K. Jackson[2], J. Silverberg[1], D. Cella[2]

[1]Dermatology, Northwestern Univ., Chicago, Illinois, United States, [2]Medical Social Sciences, Northwestern Univ., Chicago, Illinois, United States

Itch in atopic dermatitis (AD) compromises quality of life. Most itch assessments focus only on itch intensity. Our goal was to develop and validate a comprehensive PROMIS (Patient Reported Outcomes Measurement Information System®) Itch Questionnaire - Child (PIQ-C). Concept elicitation to generate the PIQ-C item pool was based on: a) literature review; b) 15 semi-structured interviews with children aged 8-17 and parents of children aged 5-17 with itch; and c) theme assessment by independent reviewers. Cognitive interviews were done (20 children; 30 parents) to ensure accurate item comprehension. Item calibration statistics were based on data from 603 children and parents (AD, n=523; epidermolysis bullosa, n=34; psoriasis, n=25; and ichthyosis, n=21). Validation of the PIQ-C was assessed using data from 251 parents and 181 8-17 year olds (self-report). Participant characteristics were: age 10.2±3.5 years (mean±SD); Eczema Area and Severity Index/EASI 17.4±12.6, Body surface area/BSA 28±19.4%. Participants completed Itch Numerical Rating Scale/NRS (mean 5.5±2.5) and several Pediatric PROMIS questionnaires. Results: A single comprehensive bank of 45 items was calibrated, enabling flexible, brief assessment options using fixed-length short forms or computer adaptive tests. ANOVA showed that PIQ-C significantly differentiated among severity levels based on Investigator Global Assessment/IGA, EASI, Patient-Oriented Eczema Measure/POEM, Children's Dermatology Life Quality Index/CDLQI. PIQ-C was positively correlated with pain (r=0.64), sleep disturbance (0.63) and sleep impairment (0.62), fatigue (0.58), , psychological stress (0.57), depressive symptoms (0.54), and anxiety (0.44), and negatively correlated with mobility (-0.46), global health (-0.40), and peer relationships (-0.36) (all p<0.001). PIQ-C discriminates children with different AD and itch severity levels and correlates well with several clinical anchors and other PROMIS measures.

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Successful treatment of vitiligo with cold atmospheric plasma-activated hydrogel

S. Zhai, Y. Xia

Department of Dermatology, The Second Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an, China, Xi'an, Shaanxi, China

Nowadays, vitiligo shows insufficient response to current therapies, partially due to excessive oxidative stress and T lymphocyte dysfunction in lesions. We have known that cold atmospheric plasma (CAP) is capable of modulating immune responses. In this study, we sought to explore the therapeutic effect of CAP on patients with active focal vitiligo. A retrospective case series of 15 patients received topical application of CAP-activated hydrogel (some lesions treated with vehicle control), followed by scoring of lesional areas, and gp100-positive cells or $CD8^+$ T lymphocytes were measured in biopsies. A 80.0% partial response rate and a 20.0% complete response rate with long-term follow-up (average of 3.3 months) were seen in patients treated with CAP-activated hydrogel. Biopsy specimens of treated sites confirmed reduction of $CD8^+$ T lymphocytes and recovery of melanocytes in CAP hydrogel-administered patients, but the controls showed no response. We observed neither hyperpigmentation at surrounding areas nor treatment-related adverse events. Therefore, we hypothesize that topical application of CAP-activated hydrogel concomitantly with single betamethasone injection is a promising therapeutic option for managing active focal vitiligo. This clinical trial registration number was ChiCTR-OPB-17013944. The protocol was approved by the Institutional Review Board of Second Affiliated Hospital of Xi'an Jiaotong University (No.2016105), and written informed consent was obtained from all patients.

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TWEAK is a critical cytokine linking vulgaris, pustular, and erythrodermic psoriasis

S. Wang, Y. Liu, H. Wang

Dermatology, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

Psoriasis is a common chronic inflammatory skin disease where various cytokines play a detrimental role. Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) has been implicated in the pathogenesis of a variety of inflammatory disorders and autoimmune diseases. However, studies conducted on the relationship between TWEAK and various psoriasis patients are limited. In this study, we aimed to explore the serum and lesional levels of TWEAK in psoriasis patients and investigated whether TWEAK is associated with clinical variables and expression of other well-known psoriasis-related cytokines including IL-17a, IFN-γ, IL-22, IL-36γ. Twenty patients with psoriasis vulgaris (PV), eight patients with pustular psoriasis (PP), eight patients with erythematous psoriasis (EP), and 20 healthy controls (HC) were enrolled in this study. TWEAK in serum and lesion was measured by commercial enzyme-linked immunosorbent assay (ELISA) and immunohistochemistry, showing obvious upregulation in three kinds of psoriasis (TWEAK in serum: F = 51.32, p <0.0001; TWEAK in lesion: F=4.536, p=0.0067). Moreover, we found that the TWEAK level was much higher in inflammatory lesions of PV and EP than pustular lesion of PP. Serum levels of cytokines were also measured using ELISA kits. The mean IL-17a, IFN-γ, IL-22, and IL-36γ levels were significantly higher in psoriasis patients than in control subjects (IL-17a: F=5.677, P <0.0001; IFN-γ: F=7.192, P = 0.0003; IL-22: F= 20.94, P <0.0001; IL-36γ: F= 7.605, P = 0.0002). Besides, serum TWEAK levels showed significant positive correlations with IL-17a (r = 0.5679, *P* = 0.0217) and IFN-γ (r = 0.6495, *P* = 0.0065) in PV, weak negative correlation with IFN-γ (r = -0.5341, *P* = 0.0331) and obvious positive correlation with IL-36γ (r = 0.8412, *P* < 0.0001) in EP, but no correlation between TWEAK and IL-22 in any psoriasis (PV: *P* = 0.3743; PP: *P* = 0.6154; EP: *P* = 0.7895). This study shows that TWEAK may be associated with the pathogenesis of PV and PP and EP via different inflammatory molecules.

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Patient and disease characteristic predictors of systemic exposure to crisaborole

V. Purohit[1], J. L. Werth[2], D. Graham[1], T. Nicholas[1]

[1]Pfizer Inc., Groton, Connecticut, United States, [2]Pfizer Inc., Collegeville, Pennsylvania, United States

Crisaborole ointment, 2%, is a nonsteroidal phosphodiesterase 4 inhibitor for the treatment of mild-to-moderate atopic dermatitis (AD). Initial pharmacokinetic (PK) studies of crisaborole showed absorption with measurable systemic levels of crisaborole. A nonlinear regression analysis using ointment dose and noncompartmental PK parameters at steady state (area under the curve [AUC_{ss}] and maximum concentration [$Cmax_{ss}$]) was conducted to correlate systemic exposure parameters with ointment dose and clearance difference between patient subpopulations. Data from 8 clinical studies were included: 3 phase 1 studies in healthy adults, 2 phase 1b studies in patients aged 12-17 years with mild-to-moderate AD, 1 phase 1 study in Japanese patients with mild-to-moderate AD, 1 phase 4 study in children aged 3-24 months with mild-to-moderate AD, and 1 phase 1b study in adults with mild-to-very severe psoriasis. The base model describing the relationship between systemic exposure and ointment dose was defined with a slope and intercept fixed to 0, with weight as covariate included as an allometric function to account for clearance differences. Race, sex, disease condition (healthy volunteer, AD, or psoriasis), baseline disease severity, and age were tested as covariates. PK data were available from 271 participants (AUC_{ss}, N=264; $Cmax_{ss}$, N=267). Mean participant age was 28.6 years, and median ointment dose was 15,800 mg (range, 4700-47,100 mg). Disease condition had the greatest impact on slope in both models, corresponding to ~2.5-fold higher AUC_{ss} and $Cmax_{ss}$ values at a given ointment dose in patients with AD or psoriasis relative to healthy volunteers. Disease severity, race, and sex had marginal effects on AUC_{ss} and $Cmax_{ss}$. Model predictions indicated that, at similar treated percentage of body surface area, crisaborole systemic exposures across age groups are expected to be in a similar range, and systemic exposures in children (>3 months of age) at maximum possible dose are unlikely to exceed the systemic exposures at the maximum possible dose in adults.

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Crisaborole in patients ≥3 months of age with mild-to-moderate atopic dermatitis (AD)

J. C. Su[1], L. J. Spelman[2], L. F. Eichenfield[3], L. F. Stein Gold[4], A. Cha[5], D. Graham[6], L. Takiya[7], J. L. Werth[7], C. Zang[7], B. Vlahos[7]

[1]Murdoch Children's Research Institute, University of Melbourne, Melbourne, Victoria, Australia, [2]Veracity Clinical Research, Woolloongabba, Queensland, Australia, [3]Rady Children's Hospital-San Diego and UC San Diego, San Diego, California, United States, [4]Henry Ford Health System, Detroit, Michigan, United States, [5]Pfizer Inc., New York, New York, United States, [6]Pfizer Inc., Groton, Connecticut, United States, [7]Pfizer Inc., Collegeville, Pennsylvania, United States

Crisaborole ointment, 2%, is a nonsteroidal phosphodiesterase 4 inhibitor for the treatment of mild-to-moderate AD. We report the efficacy and safety of crisaborole across age groups in the phase 3 studies AD-301 (NCT02118766) and AD-302 (NCT02118792) and the phase 4 study CrisADe CARE 1 (NCT03356977). Patients aged 3 to <24 months (CARE 1) or ≥2 years (AD-301/AD-302) with mild-to-moderate AD received twice-daily crisaborole (or vehicle in AD-301/AD-302) for 28 days. Safety was the primary endpoint in CARE 1. ISGA success (clear [0] or almost clear [1] with a ≥2-grade improvement from baseline) at day 29 was an endpoint in CARE 1 (exploratory) and AD-301/AD-302 (primary). CARE 1 included 137 infants, all treated with crisaborole (mean age, 13.6 months [SD, 6.42]; 64.2% male). In AD-301/AD-302, 1016 patients were treated with crisaborole (46.7% male): 335 were aged 2-6 years; 292, aged 7-11 years; 247, aged 12-17 years; and 142, aged ≥18 years. Rates of treatment-related application site pain (3.6%) and application site discomfort (2.9%) reported in CARE 1 were consistent with the rate of application site pain reported for crisaborole-treated patients in AD-301/AD-302 (4.4%; 2-6 years, 3.6%; 7-11 years, 5.5%; 12-17 years, 4.1%; ≥18 years, 5.0%); most events were mild or moderate. In CARE 1, 30.2% of patients achieved ISGA success at day 29, consistent with that observed for crisaborole-treated patients in AD-301/AD-302 (32.5%; 2-6 years, 30.5%; 7-11 years, 36.6%; 12-17 years, 30.3%; ≥18 years, 29.7%). Based on these studies, crisaborole was well tolerated and effective in patients ≥3 months of age with mild-to-moderate AD.

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Using design thinking to develop a decision aid for patients with psoriatic disease

M. T. Wan, M. Almonte, J. Gelfand, A. Ogdie

UPenn, Philadelphia, Pennsylvania, United States

Starting or switching therapy in patients (pts) with psoriatic disease can be challenging given the available therapies and complexities of therapy selection and education. True shared decision-making is challenging during short visits, but pt decision aids (DAs) can assist this. This study aimed to understand pt and clinician needs in the process of starting/switching therapy and use design thinking to develop a pt-centered DA that addresses these needs. Design thinking, a method adopted from engineering, first seeks to understand user needs to develop a prototype which is then iteratively revised based on user input. Semi-structured interviews of 10 pts and 2 focus groups with rheumatologists (N=7), dermatologists (including a nurse practitioner) (N=5) and specialty pharmacists (N=2) were conducted to inform the first prototype. Follow-up focus groups with the same clinicians and semi-structured interviews with an additional 8 pts were conducted to revise the prototype. Interview transcripts were independently coded by MTW and MA using NVivo12 to elicit pertinent themes. Clinicians felt that a DA was most useful for pts initiating/switching therapies and should include basic information about the disease, managing flares, and individual therapies. Clinicians suggested that treatment options should be identified using an algorithm that considers patient factors (e.g., comorbidities) and then select 2-3 therapies for pts to read. Pts wanted a DA that would give them access to all information about therapies including efficacy, side effects, insurance coverage, personal testimonials from other pts, and lifestyle alterations from medications. Pts and clinicians noted readability and easy navigation as necessary and were therefore refined in the final prototype. Design thinking was an effective method of creating a DA; including pts, clinicians and pharmacists significantly changed the DA developed from one that would have been designed by physicians alone. Pts desired more detailed data than anticipated by clinicians or would typically be provided in a short encounter.

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Validation of nomogram incorporating clinopathologic factors and 31GEP test for predication of cutaneous melanoma patient recurrence risk

R. Thorpe[1], H. G. Caruso[2], K. R. Covington[2], D. Brodland[3], J. A. Zitelli[3]

[1]Ada West Dermatology, Meridian, Idaho, United States, [2]Castle Biosciences, Inc, Friendswood, Texas, United States, [3]Zitelli & Brodland, PC, Pittsburgh, Pennsylvania, United States

Patients diagnosed with cutaneous melanoma (CM) have important decisions to make early on with help of their physicians. We endeavored to develop a prognostication tool to facilitate this process. The 31gene expression profile (31GEP) is a prognostic test for CM that accurately predicts patients' 5yr risk of recurrence, distant metastasis, and melanoma-related death. The 31GEP test stratifies patients as having lowest risk (Class 1A), intermediate risk (Class 1B/2A) or highest risk (Class 2B) and is an independent predictor of metastatic risk, as demonstrated in prospective and retrospective studies. Novel nomogram for predicting CM recurrence was developed from a prospectively-tested cohort of 685 patients from 9 dermatology centers with a minimum of 1yr follow-up or a recurrence event at any time (11 SLN positive). The logistic regression model was fitted on clinical and pathological data to determine relative predictive value for recurrence risk. Covariate inclusion for the model was selected by lowest Bayesian information criteria value with fewest clinical features. The final nomogram included two factors: 31GEP result and Tstage. This model was validated on an archival cohort of 901 Stage I-III CM patients from 22 centers with >5yrs follow-up or a recurrence event, of which 608 were Stage I-II. For Stage I-II patients, the 5yr distant metastasis-free survival (DMFS) and recurrence-free survival (RFS) in the validation cohort for Class 1A vs 2B were 97% vs 65% and 95% vs 51%, respectively. The performance of the nomogram was evaluated by linear regression using binomial output, which demonstrated a significant correlation between actual and predicted recurrence. The prognostic accuracy of the CM nomogram that included 31GEP test and Tstage was validated in a new and independent patient population. Incorporation of this nomogram into early decision-making process may augment the planning process for both the physician and the patient.

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Leveraging CRISPR-Cas12a for the detection of human T-cell leukemia virus type 1

C. Baker[1], Y. Chen[1], M. S. Hayden[1, 2]

[1]Geisel School of Medicine at Dartmouth, West Lebanon, New Hampshire, United States, [2]Dermatology, Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire, United States

Human T-cell Leukemia Virus type 1 (HTLV-1) is a potent carcinogenic oncovirus that, while asymptomatic in the majority, has the potential to cause devastating complications such as adult T cell leukemia/lymphoma (ATLL) or HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Despite its global burden, poor access to current testing modalities limits HTLV-1 screening in endemic regions. Here we perform pre-clinical experiments that demonstrate the potential for a sensitive, specific, and low-cost CRISPR-based screening test for HTLV-1. We took advantage of recent reports describing the ability of Cas12a enzymes and guide RNAs (gRNAs) to act as molecular sensors in field-deployable viral diagnostic assays. Upon specific recognition of the gRNA-complementary DNA sequence, e.g. HTLV-1 proviral DNA, Cas12a/gRNA complexes can catalyze nonspecific trans-cleavage of a single-stranded DNA (ssDNA) reporter, providing an amplified signal of the target recognition event. We designed and synthesized multiple HTLV-1-specific Cas12a gRNAs targeted to conserved regions of the HTLV-1 genome. We also designed custom 5'FAM-ssDNA-3'Biotin reporter molecules to allow for visualization of target-induced Cas12a trans-cleavage via differential binding of cleaved versus intact labeled ssDNA molecules to a lateral flow strip. The assay is completed in three simple steps. First, conserved regions of HTLV-1 in human genomic DNA samples are amplified isothermally using recombinase polymerase amplification. Next, HTLV-1-specific Cas12/gRNA complexes are added to the DNA sample in the presence of labeled ssDNA reporter molecules. After one hour of incubation, the HTLV-1 detection signal is read out visually on a lateral flow strip, similar to a routine home pregnancy test. Our assay is able to detect attomolar levels of HTLV-1 target DNA in infected T-cell lines within one hour and thirty minutes and lays the foundation for a robust, point-of-care HTLV-1 diagnostic test that could potentially transform the detection of HTLV-1 infection.

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Reported outcome measures in published Mohs micrographic surgery techniques: A systematic review

B. Gordon[1], P. Govas[1], B. Carroll[1, 3], A. Hurst[2], C. Wessel[1]

[1]University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, United States, [2]Atlantic Skin Cancer Surgery, Virginia Beach, Virginia, United States, [3]Case Western Reserve University School of Medicine, Cleveland, Ohio, United States

Mohs micrographic surgery (MMS) is a surgical excision technique that utilizes oriented microscopic evaluation to achieve higher cure rates for skin cancer than traditional surgical excision. In theory, the cure rate for MMS should be 100% due to its methodology, yet recurrences exist at a rate of 1%-41%. In clinical practice, recurrence rates of MMS may be impacted by considerable variability in the histologic techniques employed by MMS clinics. There is no gold standard for comparing MMS histology protocols. The most commonly reported outcomes for published MMS histology techniques are time-saving, cost-saving, and/or tissue-conservation during the procedure. While tissue-conservation is proposed as a surrogate for measuring cure rates, very few studies directly measure the outcome of cure. We hypothesized that less than 10% of technical descriptions of tissue manipulation during grossing and embedding of MMS en-face processing for cutaneous cancers are assessed for impact on cure, but instead focus on time-saving, cost-saving or tissue conservation. A systematic review was performed of published literature in MEDLINE, PubMed, EMBASE, and Cochrane library that included a description of the tissue manipulation during the grossing and embedding steps of MMS. Two reviewers performed study title/abstract review and data abstraction. Overall, 67 papers met inclusion criteria and were sufficient for meta-analysis. Only 1 paper assessed for cure/recurrence rate (1.5%), confirming our hypothesis. Additionally, 39 assessed for time-saving (39%), 11 for cost-saving (11%), and 38 for tissue-conservation (38%). In conclusion, the majority of published studies regarding MMS processing and embedding techniques make assumptions of non-inferiority of recurrence and do not formally interrogate recurrence/cure rates for skin cancers. Future studies/reports of MMS processing techniques may benefit from including cure as a reported outcome measure.

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Evaluating world health organization essential medicines list for dermatologic disease

C. W. Rundle[1], A. Fortugno[2], O. Chosidow[3], L. Naldi[4], R. Hay[5], R. Dellavalle[1]

[1]Dermatology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States, [2]College of Medicine, University of Tennessee Health Science Center, Memphis, Tennessee, United States, [3]Dermatology, AP-HP, Hôpital Henri Mondor UPEC, Créteil, France, [4]Dermatology, Azienda Sanitaria Berica, Ospedale San Bortolo, Vicenza, Italy, [5]St Johns Institute of Dermatology, Kings College, London, United Kingdom

The World Health Organization (WHO) Model List of Essential Medicines (MLEM) provides lists of medicines needed for basic healthcare systems. This study aims to appraise the dermatologic section of the WHO MLEM with respect to top Global Burden of Disease (GBD) skin disease entities. Skin burden data was extracted using the GBD results tool. Based on the top 13 GBD skin disease entities, dermatologic essential medicines were appraised with respect to *Treatment of Skin Disease, 5th edition* and *Evidence-Based Dermatology, 3rd edition.* Dermatologic essential medicines; disability-adjusted life years; first, second, and third line dermatologic therapies with grade A – E evidence; secondary appraisal with an ancillary clinical resource were utilized. The WHO MLEM provides 18 and 15 dermatologic medicines for adults and children, respectively. When compared to standard of care resources, 24.3% of total first line dermatologic options were included by the Essential Medicines list. Alternatively, 49.3% of dermatologic first line therapies were not on the dermatologic Essential Medicines List. The WHO MLEM is essential for conducting global public health efforts While the current dermatologic list of EMs covers many relevant diseases with a significant health burden, treatment of some dermatologic conditions may benefit from an expanded list of medications. The practicality and cost-effectiveness for these alternatives may be debated; however, global dermatologic burden may be better addressed with a revised list.

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Establishing the analytical and clinical validity of sonographic biomarkers in hidradenitis suppurativa
D. Grand, J. W. Frew, K. Navrazhina, J. Krueger
Laboratory of Investigative Dermatology, Rockefeller University, New York, New York, United States
Hidradenitis suppurativa (HS) is a chronic inflammatory dermatosis which currently lacks valid and reliable disease activity biomarkers. Non-invasive imaging modalities, such ultrasound, MRI, and medical infrared tomography, have demonstrated utility in detecting and characterizing subclinical HS lesions, however the analytical validity (correlating imaging with known histological and molecular markers of disease) and clinical validity (correlating imaging with validated clinical outcomes) have not been established. We present the first validity analysis of candidate sonographic biomarkers in HS in order to identify the most valid candidate biomarker for future clinical and investigational use. Ethics approval for this study was granted by the institutional review board of Rockefeller University. 22 patients with moderate to severe HS were enrolled. 20 MHz (GE Logic Q) ultrasonography was performed with matched skin biopsies as per published guidelines as well as age and sex matched healthy controls. Sonographic epidermal thickness is significantly greater in HS lesions compared to site-matched controls ($p = 0.003$) and correlates with histologic epidermal thickness ($R=0.94$). Only moderate correlation was seen between epidermal thickness and IL-17 isoforms by RT-PCR ($R=0.43$). Dermal doppler vascularity correlates broadly with inflammatory activity of clinical lesions, pain score ($R=0.85$) as well as the degree of dermal CD3+ cell ($R=0.736$) and CD11c+ ($R=0.96$) cell infiltration. Non-invasive sonographic candidate biomarkers correlate with clinical and molecular disease activity markers in HS. Whilst epidermal thickness has high analytical validity it correlated poorly with clinical disease activity. Dermal doppler vascularity illustrates very good to high analytical and clinical validity suggesting the best utility as an imaging-based disease activity biomarker in HS.

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An intelligent assistant diagnosis study of erythema and scaly skin diseases based on deep learning
Y. Wang[1], D. Yuan[2], S. Zhao[3], X. Chen[3]
[1]School of Automation, Central South University, Changsha, Hunan, China, [2]School of Computer Science and Engineering, Central South University, Changsha, Hunan, China, [3]Xiangya Hospital, Central South University, Changsha, Hunan, China
This paper proposes an intelligent assistant diagnosis study of erythema and scaly skin diseases based on clinical images, including lichen planus, parapsoriatic, lupus erythematosus, eczema, and psoriasis. First of all, we collected a skin disease data set, which contains 4,377 clinical images. In this data set, each image contains the information of the lesion area and the patient's medical history. Then, four types of convolutional neural networks were used to classify erythema and scaly skin diseases, including InceptionV3, InceptionResNetV2, DenseNet121, and Xception. The experimental results suggest that the classification accuracy of Xception model based on weights pretrained over ImageNet can reach 81.60%. In addition, we also compared the classification performance of the CNN model with 30 professional dermatologists, which shows that CNN's overall accuracy is slightly better than that of dermatologists. This paper also conducted the detection of erythema and scaly skin disease lesions based on multi-task learning, which includes two tasks: localization of disease areas and classification of diseases. We proposed an improved strategy for multi-scale feature fusion based on Faster R-CNN. For different feature fusion methods, we designed 9 sets of comparative experiments. The experimental results show that the optimal feature fusion combination is Conv1 + Conv3 + Conv5, with an average accuracy of 73.60%, which shows that the fusion of convolutional features using a wider range of convolutional layers can improve the performance more effectively. This paper will be a great help for dermatologists in remote areas to diagnose erythematous scaly disease, thus reducing the misdiagnosis and missed diagnosis of these diseases.

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Evaluation of the impact and safety of DHA-containing camouflage on the repigmentation of vitiligo: An open-label self-controlled study
M. Li, F. Wang, X. Ding, J. Du
Dermatology, Peking University People's Hospital, Beijing, China
Background: Camouflage for vitiligo patients improves their quality of life. self-tanning products contained dihydroxyacetone(DHA) have been used for the camouflage of vitiligo lesions. So far, the use of DHA-containing camouflage is not being investigated. Objective:To evaluate the impact and safety of DHA-containing camouflage on the repigmentation of vitiligo. Methods:Thirty patients with vitiligo were enrolled in this study. 2 white patches were divided into 2 groups (group A, group B). Group A was treated with camouflage as needed , group B was a blank control. In each patient, the same treatment (topical corticosteroids with or without NB-UVB phototherapy) was applied on both groups. The leukoderma area, melanin index (MI), transepidermal water loss (TEWL), extent of repigmentation and adverse events were measured and recorded at different time points (0, 4, 8, 12 weeks) in the follow-up period. The lesion improvement was assessed according to the reduction of leukoderma area, change of relative MI (RMI) and extent of repigmentation. The function of skin barrier was evaluated by the change of relative TEWL (rTEWL). Trial registration: NCT03973073 Results: 28 patients completed the study. After 12 weeks' therapy, there was no statistically difference in the reduction of vitiligo area, extent of repigmentation and the change of rTEWL in patients with combination therapy($p>0.05$). In patients with topical corticosteroids, the level of rTEWL was significantly lower in group A ($p<0.05$), and no difference in the extent of repigmentation($p>0.05$). Adverse event: there was only one patient suffered from temporary skin irritation (ithing and tingling) in group A after phototherapy at 8-12 weeks' treatment. Conclusions: Camouflage is a relatively safe way to cover up the vitiligo. It has little impact on the efficiency of vitiligo treatment and function of skin barrier.

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Evaluating the utility of post-surgical radiation for high-risk cutaneous squamous cell carcinoma in immunosuppressed patients
L. Chang[1], B. A. Nguyen[2], R. Kazi[1], P. Govas[1], M. Pugliano-Mauro[1], B. T. Carroll[3, 4]
[1]Dermatology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, United States, [2]University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [3]University Hospitals of Cleveland, Cleveland, Ohio, United States, [4]Case Western Reserve University, Cleveland, Ohio, United States
Cutaneous squamous cell carcinoma (cSCC) that presents with high-risk features such as perineural invasion is associated with a poor outcome. The National Comprehensive Cancer Network recommends consideration of post-surgical radiation for these aggressive tumors. However, the supporting evidence for this recommendation is limited for immunosuppressed patients. Recent studies demonstrated inferior outcomes of cSCC in immunosuppressed patients treated with adjuvant radiation compared with immunocompetent patients that also received radiation, highlighting the uncertain benefit of post-surgical radiation therapy in the immunosuppressed patient population. To address this gap, we performed a single-institution, retrospective chart review study to identify all high-risk cSCC cases diagnosed in immunosuppressed patients between 2009 and 2018. A total of 81 immunosuppressed patients, including 39 treated with radiation following surgery (S+XRT group) and 42 treated with surgery alone (S-only group), were included in the study. No statistically significant difference in the distribution of age ($p=1.0$), sex ($p=1.0$), AJCC8 stage ($p=0.29$), BWS stage ($p=0.57$), or type of immunosuppression ($p=0.43$) was observed between the S+XRT and the S-only groups. This single-institutional retrospective chart review did not demonstrate significant difference in the overall survival (Kaplan-Meier $p=0.8$) or local recurrence-free survival ($p=0.8$) between high-risk cSCC treated with radiation plus surgery and those treated with surgical monotherapy. The limitations of the study include the retrospective study design and the clinical complexity of multiple primary lesions in this patient population. Future randomized control trials are needed to better understand the benefit of adjuvant radiation for cSCC in immunosuppressed patients.

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Worsening CLASI damage scores in patients with cutaneous lupus erythematosus may predict development of systemic lupus erythematosus

S. M. Black[1], F. Walocko[1], S. Anderson[1], X. Li[2], B. Adams-Huet,[2], B. F. Chong[1]

[1]Dermatology, University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Population and Data Sciences, University of Texas Southwestern Medical Center, Dallas, Texas, United States

Up to 20% of cutaneous lupus erythematosus (CLE) patients develop systemic lupus erythematosus (SLE). Baseline characteristics associated with progression to SLE, including generalized DLE, have been previously identified. However, factors that change over time have not been well studied. The objective of our retrospective cohort study was to identify fixed and variable risk factors that predispose CLE patients to develop SLE. Our cohort consisted of 69 CLE patients without SLE at baseline followed for at least six months. Of these, 12 progressed from CLE to SLE (17.4%), while 57 (82.6%) remained CLE. Demographic and clinical data were compared using Fisher's exact test or Wilcoxon Rank Sum test. At baseline, CLE to SLE patients had greater American College of Rheumatology (ACR) SLE diagnostic criteria than CLE patients (median 3 (IQR: 2.5-3) vs 2 (1-3); p=0.004)) and lower (worse) Physician Global Assessment (PGA) overall skin scores (7 (5.5-7) vs 8 (7-9); p=0.01)). Generalized DLE (n=14) was significantly associated with progression to SLE vs. localized DLE (n=29) (p=0.03). Longitudinally, Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) activity and damage scores were evaluated using absolute change scores, or mean change from baseline to each follow up visit. 41.7% of CLE to SLE patients had more frequent worsening of CLASI damage scores than 15.8% CLE only (p=0.04). CLE to SLE patients had worse PGA overall skin scores at 6 months (6 (5.3-8) vs 9 (7-9); p=0.001)) and 3 years (6 (5.5-6.5) vs 9 (8-10); p=0.01)). Thus, patients who progress from CLE to SLE are more likely to have worsening CLASI damage scores and skin severity. CLE patients with increasing CLASI damage scores may need close monitoring for SLE progression.

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Computational modeling of two methods of histologic embedding during Mohs micrographic surgery

B. Carroll[2], S. Chu[1], E. Terrell[3], P. Govas[3], J. Oosten[3], L. Davidson[3], B. Ermentrout[3], D. Swigon[3]

[1]Case Western Reserve University, Cleveland, Ohio, United States, [2]University Hospitals Cleveland Medical Center, Cleveland, Ohio, United States, [3]University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Skin cancer is the most common malignancy in the United States. Mohs micrographic surgery (MMS) combines surgical excision with oriented microscopic evaluation to provide the highest cure rate for non-melanoma skin cancers. Despite a theoretical 100% margin assessment, the recurrence rate for large skin cancers is as high as 41%. One potential reason for high recurrence rates is unanticipated tissue deformation during histologic processing. Two methods of embedding are the glass-slide and the heat sink embedding method. In the glass-slide method, tissue is manipulated onto a two-dimensional surface and then immobilized by freezing. In the heat sink method, tissue is applied to a frozen two-dimensional surface that immobilizes the tissue on contact. Despite new techniques for studying tissue biomechanics, few biomechanical studies have been applied to dermatology nor MMS. We propose computational modeling of excised skin tissue to describe deformations that arise during MMS tissue processing. We hypothesize that the model of skin tissue embedded with the glass-slide will reveal distinct strain patterns relative to the heat sink embedded tissue. An anisotropic Prandtl-Reuss elastoplastic material model was applied to assess Young's modulus, Poisson's ratio, and yield stress. The three-dimensional shape of a Mohs layer of skin tissue was created with a mesh of hexahedral elements as a three-dimensional solid. Force parameters were collected with a microscopic cantilever force indenter on fresh skin tissue to define the strain on the elements. Different types of strain, including normal and von Mises effective strain were assessed. The models support our hypothesis of distinct strain patterns for the two embedding methods. Further modeling is needed to define the clinical scenarios that would be negatively impacted by these differences.

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Validation of the Skindex-mini in patients with atopic dermatitis

M. P. Dizon[1], C. Topham[1, 2], D. Haynes[1], M. Brazil[1], M. Chren[3], E. Simpson[1]

[1]Dermatology, Oregon Health and Science University, Portland, Oregon, United States, [2]Dermatology, University of Utah, Salt Lake City, Utah, United States, [3]Dermatology, Vanderbilt University Medical Center, Nashville, Tennessee, United States

Quality of life (QOL) measurements may be used to better understand the patient experience of disease and judge the effectiveness of care, and numerous instruments have been developed within the field of dermatology. Validation of QOL instruments is critical in allowing clinicians and researchers to interpret and compare findings across studies. We sought to examine the measurement properties of the Skindex-mini (SDM), a brief three-item questionnaire created specifically for routine clinical practice. A retrospective chart review was performed, including a cohort of 200 patients with atopic dermatitis (AD) in a single academic center. At the time of initial assessment, SDM responses demonstrated good construct validity with both clinician- and patient-reported global assessments of AD severity based on Spearman rank correlations (r=0.59-0.71, P<0.001) and receiver operating curve (ROC) analysis [area under curve (AUC) 0.83-0.92, P<0.001]. Changes in SDM over time demonstrated responsiveness in predicting improvement patient-reported AD severity (AUC 0.77-0.90, P<0.001). In conclusion, the present study provides support for the validity of SDM as a QOL instrument in routine clinical practice.

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Profiling of phenotypes and plasma proteins identifies biomarkers for psoriasis severity and psoriatic arthritis

J. Walsh, M. Milliken, C. Carroll, S. Belman, K. Callis Duffin, G. Krueger, B. Feng

University of Utah, Salt Lake City, Utah, United States

Biomarkers for psoriasis (Ps) and psoriatic arthritis (PsA) may elucidate the biological mechanisms of psoriatic diseases (PsD). We performed clinical phenotyping and plasma protein analysis on 234 Ps patients, including 44 with PsA, to identify protein profiles that associate with PsD. A physician-administered questionnaire was performed to determine Ps and PsA phenotypic features, including erythema, induration, scale, physician global assessment (PGA), body surface area (BSA), product of PGA and BSA (PGAxBSA), fingernail involvement, pustulosis, palmoplantar Ps, Koebner phenomenon, plaque size, plaque thickness, guttate Ps, inverse location, hyperlinearity of palms, erythrodermic disease, PsA, and duration of Ps and PsA at enrollment. We collected plasma at enrollment and, using Proximity Extension Assay, assessed concentration of 273 proteins related to inflammatory, cardiometabolic, or cardiovascular diseases. Association of each protein to a phenotype was tested by regression adjusted for age, sex, and body mass index. We used a permutation test to correct for the number of proteins and phenotypes analyzed that also considers protein-protein and phenotype-phenotype correlations. We identified significant (corrected p<0.05) associations of 1) peptidase inhibitor 3 (PI3 or elafin) with induration, erythema, and PGA, and 2) PI3, IL-17C, KLK6, IL-17A, IL-20 and CCL20 with both BSA and PGAxBSA. Suggested associations (p<0.00018, significant by Bonferroni correction for the number of proteins but not phenotypes) include PI3 and selectin-E (SELE) with plaque thickness, CD5 with scale, TNF with PsA duration and BSA, and MCP-3, TNC, CHI3L1, and OPG with both BSA and PGAxBSA. Among these proteins, PI3 and MCP-3 also associate with PsA duration with nominal significance (p<0.05, uncorrected for the number of proteins or phenotypes). This study identified potential biomarkers that associate with Ps severity and PsA duration. These biomarkers may have clinical utility for PsD.

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A clinical comparison of photobiomodulation devices for the treatment of alopecia in all skin types

S. Pratapaneni[1], M. K. Hordinsky[2], R. Farah[2], L. Voller[1], M. Winter[1]

[1]Department of Dermatology , University of Minnesota - Twin Cities, Minneapolis, Minnesota, United States, [2]Dermatology, University of Minnesota, Minneapolis, Minnesota, United States

Photobiomodulation (PBM) therapy is an emerging treatment for androgenetic alopecia (AGA). Although various devices are FDA-cleared for treatment (skin types I-IV only); no known trials to date have directly compared their outcomes against one another. Furthermore, these devices have not been studied in skin types of V-VI. We aim to clinically compare and evaluate the use of 4 FDA-cleared PBM devices in treating subjects of all skin types diagnosed with AGA. Subjects of skin types I-IV with AGA were randomized to PBM devices and received treatments per manufacturer's recommendations. Photographs of the scalp were obtained at baseline and then each subsequent month using Canfield Capture system. Self-assessment questionnaires were administered monthly throughout the study. This study first enrolled 14 pilot subjects, 11 were randomized among 6 devices for 3 months of treatment, had sufficient data to analyze their progress. 62.50% of subjects had improvements as assessed by physician scalp exams. However, at the end of the study, 45% of subjects remained dissatisfied with treatment. Based on this pilot group, the study was lengthened to 4 months of treatment and subjects randomized to 4 devices (the 2 devices most challenging to use were not used in the second part of this study). In this second part of this study, patients with skin types V and VI were also enrolled to investigate the efficacy of PBM devices on darker skin types. To date, 30 subjects have been enrolled in this study, of which 11 are skin types V and VI. 8 of skin types I-IV and 1 of skin type V successfully completed the study so far. Based on the scalp exams and photographs, improvements varied across all devices and skin types. Data obtained from the first part of this study demonstrate variable improvement in hair grading scales from scalp exams as well as variable responses on self-assessments. Therefore, greater patient numbers and continued enrollment is needed of all skin types.

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Impact of concomitant steroids on mogamulizumab efficacy in MAVORIC

O. E. Akilov[1], L. Geskin[1], M. Leoni[2], T. Ito[2], K. Dwyer[2], F. Herr[3], A. Musiek[4]

[1]University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [2]Kyowa Kirin Pharmaceutical Development, Inc., Princeton, New Jersey, United States, [3]Kyowa Kirin, Inc., Bedminster, New Jersey, United States, [4]Washington University School of Medicine, St. Louis, Missouri, United States

Topical steroids are a mainstay of treatment for patients with mycosis fungoides (MF) and Sézary syndrome (SS). Therefore, patients undergoing treatment with mogamulizumab are likely to also receive steroid therapy and an understanding of the safety and efficacy of concomitant use is beneficial for physician decision-making. MAVORIC (NCT01728805) was an open-label, phase 3 trial in which patients with previously treated MF/SS were randomized 1:1 to mogamulizumab (1.0 mg/kg weekly for the first 28-day cycle, then Days 1 and 15 of subsequent cycles) or vorinostat (400 mg daily). Objectives of this posthoc analysis were to determine how many patients were treated with steroids and the impact of steroids on the safety and efficacy of mogamulizumab therapy. Steroid use occurred in the majority of patients (ITT: 67%, 249/372; mogamulizumab arm: 68%, 127/186; vorinostat arm: 66%, 122/186). Similar numbers of patients were treated with low/intermediate potency and high potency steroids in each arm (mogamulizumab: 24% and 44%; vorinostat: 23% and 43%). No unexpected differences were reported in the AE profiles of patients with steroid use, patients without steroid use, and the ITT population. Patients receiving mogamulizumab with concomitant steroids had longer median progression-free survival (PFS) versus ITT (9.37 vs 7.70 months); PFS was similar with steroids in the vorinostat group versus ITT (3.07 vs 3.10 months). Mogamulizumab also resulted in a higher overall response rate (ORR) in patients receiving concomitant steroids compared to ITT (37% vs 28%); ORR was similar in both groups with vorinostat treatment (3% vs 5%). In the mogamulizumab arm, ORR was better for most disease stages in patients receiving concomitant steroids compared to ITT. Concomitant steroid therapy may be associated with increased patient benefit for mogamulizumab-treated but not for vorinostat-treated patients.

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Moderate to severe atopic dermatitis is associated with poor cognitive function

J. Silverberg[1, 2], D. Lei[2], M. Yousaf[2]

[1]Dermatology, George Washington University School of Medicine, Washington, District of Columbia, United States, [2]Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Atopic dermatitis (AD) is associated with itch, pain, sleep disturbance and systemic inflammation, all of which may contribute toward cognitive dysfunction. We sought to characterize the patient-burden of cognitive dysfunction in adult AD. We performed a prospective dermatology practice-based study using questionnaires and evaluation by a dermatologist (n=203). AD severity was assessed using patient-reported global AD severity, Patient-Oriented Eczema Measure (POEM), Numeric Rating Scale (NRS) for worst-itch and skin-pain, Dermatology Life Quality Index (DLQI), ItchyQOL, Eczema Area and Severity Index (EASI), objective and sleep components of Scoring AD (SCORAD). Cognitive function was assessed using Patient-Reported Outcomes Measurement Information System (PROMIS®) Cognitive Function 8-item short-form. At baseline, 118 (58.1%) patients reported at least one symptom of cognitive dysfunction in the past week, with 29 (14.3%) having mild, 11 (5.4%) having moderate and 4 (2.0%) having severe PROMIS Cognitive Function T-scores. In propensity score weighted regression models, PROMIS Cognitive Function T-scores were inversely associated with patient-reported global AD severity, POEM, NRS worst-itch and skin-pain, SCORAD-sleep, EASI and objective-SCORAD, with stepwise decreases of cognitive function with worsening AD severity. Similar results were found with all 8 individual items from PROMIS Cognitive Function. At all AD severity levels, cognitive dysfunction was associated with even higher DLQI and ItchyQOL scores compared to those without cognitive dysfunction. At follow-up, changes from baseline in PROMIS Cognitive Function T-scores were inversely correlated with changes from baseline in self-reported global AD severity, POEM, NRS skin-pain, DLQI, EASI, objective-SCORAD measures, but not NRS worst-itch or SCORAD-itch. In conclusion, cognitive dysfunction is a common and burdensome symptom in AD. Cognitive function may be an important endpoint for monitoring treatment response in AD.

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Physician assessment of alopecia areata disease severity: Results from a real-world study

R. Burge[1, 2], P. Anderson[3], C. Atkinson[3], D. Bell[3], J. Piercy[3, 4], J. McCollam[5]

[1]Global Health Outcomes, Eli Lilly and Company, Indianapolis, Indiana, United States, [2]Pharmaceutical Sciences, University of Cincinnati , Cincinnati, Ohio, United States, [3]Adelphi Real World, Adelphi Group, Cheshire, Macclesfield, United Kingdom, [4]Manchester Metropolitan University, Manchester, United Kingdom, [5]Lilly Medical, Eli Lilly and Company, Indianapolis, Indiana, United States

Information on how severity of Alopecia areata (AA) is assessed is limited. The objective was to understand how physicians determine severity in AA patients in the real world. Data were drawn from the 2019 AA Disease Specific Programme, a cross-sectional survey of US dermatologists. Physicians completed an attitudinal survey covering perceptions of severity, then completed record forms for the next 5 patients consulting with AA capturing diagnosis, physician-subjective AA severity, % scalp hair loss, and other areas affected. The results revealed 72% of physicians (n=93) surveyed indicated that amount of scalp involvement was the most important factor in determining severity; 9.5% for mild patients, 23.1% moderate, 40.7% severe. 17% indicated it was patient distress over hair loss. For actual patients (n=452), excluding AA totalis or universalis patients, mean scalp hair loss for patients assessed as mild was 8.2%, as moderate 24.2%, as severe 55.6%. Including AA totalis/universalis (n=32), the mean percentage hair loss for moderate patients increased to 24.6% and for severe to 66.4%. In conclusion, scalp hair loss is the most important factor in AA severity, though facial and body hair loss are also particularly associated with increased severity. Patient distress is also important, suggesting the burden of AA may be more than visual.

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Association of itch triggers with atopic dermatitis severity, persistence, flares and seasonality in adults

J. Silverberg[1, 2], D. Lei[2], M. Yousaf[2], S. Janmohamed[2], P. Vakharia[2], R. Chopra[2], R. Chavda[3], S. Gabriel[3], K. Patel[2], V. Singam[2], R. Kantor[2], D. Hsu[2], D. Cella[2]

[1]Dermatology, George Washington University School of Medicine, Washington, District of Columbia, United States, [2]Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [3]Galderma, La Tour-de-Peliz, Switzerland

Atopic dermatitis (AD) is associated with heterogeneous triggers of itch, which may impact AD course and severity. We sought to characterize the triggers of itch in adult AD. We performed a prospective dermatology practice-based study using questionnaires and evaluation by a dermatologist (n=334). Thirteen itch triggers were assessed using Patient-Reported Outcomes Measurement Information System® (PROMIS®) Itch-Triggers. Overall, 209 (62.6%) patients reported ≥1 itch trigger in the past week, and 114 (34.1%) having ≥3 itch triggers. The most commonly reported triggers were stress (31.7%), sweat (28.4%), dry air (26.1%), and heat (23.1%). In multivariable linear regression models, number of itch triggers was associated with more severe patient-reported global AD severity, NRS worst-itch, POEM, NRS skin-pain and objective-SCORAD, but not EASI or SCORAD-sleep. Seasonality of AD was associated with distinct itch triggers. In multivariable logistic regression models, number of itch triggers was associated ≤3 months of AD remission during the year, ≥2 AD flares and AD being worse during some seasons. Four patterns of itch triggers were identified using latent-class analysis, each was associated with different clinical characteristics. In conclusion, itch triggers are common and impact the course of AD. Itch triggers are an important endpoint to assess in AD patients.

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Comparing the Performance of Interferon Gamma Release Assays in Autoimmune Skin Diseases

D. Yan[2, 1], R. Gaffney[2, 1], R. Krain[2, 1], E. Keyes[2, 1], J. S. Concha[1], R. Feng[3], S. Schulz[3], L. Pappas-Taffer[1], R. Borucki[2, 1], A. Ravishankar[2, 1], J. Okawa[2, 1], V. Werth[2, 1]

[1]Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Corporal Michael J.Crescenz VA Medical Center, Philadelphia, Pennsylvania, United States, [3]University of Pennsylvania, Philadelphia, Pennsylvania, United States

Autoimmune skin disease patients are screened for tuberculosis (TB) before initiation of immunosuppressive drugs via interferon gamma release assays (IGRAs). The two available tests, the QuantiFERON-TB Gold Plus (QFT-Plus, Qiagen, Hilden, Germany) and the T-SPOT.TB test (T-SPOT, Oxford Immunotec, Abingdon, UK) measure IFN-γ levels produced in response to T-cell stimulation with TB antigens. The T-SPOT test uses an enzyme-linked immunospot method rather than the enzyme-linked immunosorbent assay, the basis of the QFT-Plus test. The T-SPOT results are reported as positive, negative, borderline, or invalid; QFT-Plus results are reported as positive, negative, or indeterminate. This is clinically important as an invalid or indeterminate result may lead to a delay in initiating treatment. Our aim is to compare IGRA performances in the autoimmune skin disease population. 104 patients were recruited and 3 patients excluded; 1 due to lymphopenia, 1 due to screening criteria failure, and 1 due to venous access failure. Venous samples underwent TB screening with both IGRAs. Statistical analysis was performed with Fisher's test and Mantel-Haenszel test. There were 16 indeterminate results with the QFT-Plus and 1 invalid result with the T-SPOT. One patient was positive for both tests. The frequency of indeterminate QFT-Plus results was significantly higher than the number of invalid T-SPOT results (p=0.02). Controlling for immunosuppressive medication use, hydroxychloroquine (HCQ) use was not associated with indeterminate or invalid results (OR=0.76, 95% CI (0.20,3.66)). Our study shows that in autoimmune skin population, the frequency of QFT-Plus indeterminates was significantly higher than the frequency of T-SPOT invalid tests. As indeterminate results can contribute to delays in patient care, we propose the T-SPOT test for TB screening in autoimmune skin disease patients.

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Itch-dominant atopic dermatitis: A distinct phenotype of atopic dermatitis

R. Chovatiya[1], J. Silverberg[2, 1]

[1]Northwestern University, Chicago, Illinois, United States, [2]Dermatology, George Washington University School of Medicine, Washington, District of Columbia, United States

Atopic dermatitis (AD) is a heterogenous disorder associated with multiple clinical phenotypes. In particular, AD patients may have different combinations of lesional and symptom severity, e.g. mild-moderate lesions with mild-moderate itch (mild-moderate lesions), mild-moderate lesions with severe itch (itch-dominant), or severe lesions and severe itch (severe lesions). Yet, little is known about how commonly these different AD subsets occur and their clinical characteristics. We sought to determine the characteristics and burden of itch-dominant AD. We performed a prospective dermatology-practice based study using self-administered questionnaires and skin-examination in 121 adults with AD. Overall, there was only fair concordance of EASI and objective-SCORAD with NRS worst-itch and SCORAD-itch (weighted kappa 0.20-0.42). Itch-dominant AD occurred in 18.2% (mild-moderate objective-SCORAD, severe SCORAD-itch) to 24.6% (mild-moderate EASI, severe NRS worst-itch) of adults with AD. Patients with itch-dominant AD had the highest proportions of asthma (68.2%; Chi-square, P=0.04) and food allergy (72.7%, P=0.004) and youngest age of AD-onset (median age=0 years; Mann-Whitney U test, P=0.01) compared to those with mild-moderate or severe lesions, but similar distributions of gender, race/ethnicity, hay fever, anxiety and depression. In propensity score weighted multivariable regression models, itch-dominant AD was associated with significantly higher patient-reported global AD severity, dermatology life quality index (DLQI), patient-reported eczema measure (POEM), sleep disturbance, skin-pain compared to those with mild-moderate lesions. Severe lesions were associated with significantly higher DLQI, sleep-disturbance, but lower POEM scores compared to itch-dominant AD. In conclusion, itch-dominant AD appears to be a distinct phenotype of AD, which is both common and burdensome. Further studies are needed to confirm these findings and understand the clinical course and treatment response of itch-dominant AD.

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Validation of the PROMIS Itch Questionnaire – itch severity assessments in adults with atopic dermatitis

J. Silverberg[1, 2], J. Lai[2], K. Patel[2], V. Singam[2], P. Vakharia[2], R. Chopra[2], R. Sacotte[2], R. Kantor[2], D. Cella[2]

[1]Dermatology, George Washington University School of Medicine, Washington, District of Columbia, United States, [2]Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Little is known about the validity of numeric and verbal rating scales (NRS and VRS) for itch and itch frequency for assessing itch severity in atopic dermatitis (AD). We evaluated the Patient-Reported Outcomes Information System (PROMIS®) Itch Questionnaire (PIQ) – itch severity assessment, including multiple NRS, VRS and frequency of itch assessments assessing a 7-day recall period, in adults with AD and compared their performance. Self-administered questionnaires and skin-examination were performed in 410 AD patients (age 18-90 years) in a dermatology practice setting. PIQ NRS, VRS and frequency of itch had good content validity; strong correlations with each other (Spearman correlations, P<0.0001) and weak-moderate correlations with POEM, EASI, objective-SCORAD, and DLQI (P<0.0001); and very good discriminant validity. Changes from baseline in NRS, VRS and frequency of itch were moderately to strongly correlated with each other, weakly to moderately correlated with other patient-reported (POEM, SCORAD-itch, DLQI) and clinical-reported outcomes (EASI, objective-SCORAD).There were no floor or ceiling effects for NRS or VRS itch, but there were ceiling effects for itch frequency. Each assessments was completed in <1 minute by all patients. In conclusion, NRS, VRS and frequency of itch from PIQ – itch severity showed good content and construct validity, and/or responsiveness in adults with AD, and were feasible for use in clinical trials and practice.

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Characterizing morphea subsets using a multi-center, prospective, cross-sectional analysis of morphea in adults and children
S. Prasad[1], J. L. Zhu[1], K. Schollaert-Fitch[2], R. Haley[1], K. Torok[2], H. T. Jacobe[1]
[1]University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]University of Pittsburgh Scleroderma Center, Pittsburgh, Pennsylvania, United States
Morphea is an inflammatory condition of the skin and soft tissue that results in excess collagen deposition, often producing functional and cosmetic impairment. To date, few large prospective studies have examined the clinical features of morphea. Furthermore, traditional subtype classifications have relied on expert opinion rather than unbiased analysis of clinical and demographic features of morphea patients. Using two combined prospective databases (n=944), we conducted a cross-sectional analysis using traditional univariate statistics and principal component analysis (PCA) to further characterize and identify clinically relevant subsets. Caucasian, female, and linear morphea patients comprised the majority of our cohort. New to this study, we found that adults with generalized morphea had higher activity as measured by the activity component of the Localized Scleroderma Assessment Tool ($p<0.001$) while children with linear morphea had higher Physician Global Assessment of Disease Damage scores ($p<0.001$). Univariate analysis showed features of morphea described previously including 52% linear, 25% generalized, and 10% circumscribed. Linear morphea predominated in children, while generalized and circumscribed were predominant in adults. PCA analysis revealed five phenotypes (CF1-CF5) distinguished by patient demographics and clinical characteristics. CF2 was of particular interest as it identified a novel subset predominated by patients with fatigue, pain, and depression. These patients had lower levels of disease activity and were comprised of primarily linear patients (50%). Our results suggest clinical measures of activity are closely related to subtype, indicating that values for minimal clinically important differences and responses need to be modified. We also found novel disease subsets that are not typically associated with morphea. This has important implications for practice.

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Anchoring the CLASI-A, a clinical outcome assessment (COA), to the patients' perspective of their disease
N. Williams[1], K. Williams[2], V. Werth[3, 4]
[1]Math, UTexas, Dallas, Texas, United States, [2]Temple University, Phil, Pennsylvania, United States, [3]Derm, UPenn, Phil, Pennsylvania, United States, [4]CMCVAMC, Phil, Pennsylvania, United States
The CLASI-A is a validated tool for physicians to quantify skin disease activity in cutaneous lupus and has been successfully used in several phase 1-3 clinical trials. Here, we evaluated the CLASI-A based on recent guidance from the FDA (USA) to focus on "meaningful within-patient change (i.e., improvement or deterioration from the patients' perspective) in the concepts assessed by COAs." We performed a prospective study on 250 patients with cutaneous lupus (1-25 clinic visits each). At nearly every visit, we recorded both a CLASI-A score by the physician (0-70; 70 is worst, 0 is best) and a visual analogue scale by the patient (ptVAS) to indicate his or her perception of cutaneous disease activity (0-10; 0 is worst, 10 is best), i.e., a patient global impression of severity (PGIS). At the initial visit, CLASI-A and ptVAS strongly correlated (Spearman's rho=-0.226, p=0.0003). To assess within-patient change for each patient with >1 visit (n=229), we selected the pairs of visits with the largest and the smallest differences in ptVAS scores (range of Δs = -10 to +10, in a bell-shaped distribution, two data points per patient). We grouped these data points into five categories according to ΔptVAS: [-10,-6] (markedly worse from the patient's perspective), [-5,-2] (moderately worse), [-1,1] (minimal or no change), [2,5] (moderately better), and [6,10] (markedly better). Changes in CLASI-A scores between the same two visits differed amongst the five ΔptVAS categories ($p<0.0001$ by ANOVA on ranks). Empirical cumulative distribution function (eCDF) curves and probability density function (PDF) curves for the changes in CLASI scores showed progressive improvements in CLASI-A as the patients' perception improved across the five ΔptVAS categories. Moderate worsening or improvement from the patients' perspectives corresponded to median changes in the CLASI-A score of +16.7% and -32.1%. Our data indicate that the CLASI-A can be anchored to the ptVAS, to assess meaningful within-patient changes in cutaneous disease from the patients' perspective.

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The importance of IL-36 in palmoplantar pustulosis (PPP): An immunohistochemical analysis
R. E. Schopf, N. Assy
Dermatol, Johannes Gutenberg Univ., Mainz, Germany
PPP is a rare and chronic disease that is characterized by sterile pustules, often with erythema, on the palms and soles. PPP may occur alone or in association with plaque-type psoriasis. The cause of PPP is not well understood; triggers include infections, smoking and genetic factors. At this time, treatment with cyclosporine is favorable, however, limited due to toxicity. To further elucidate pathogenesis we performed an immunohistochemical study of in 24 patients with PPP and in corresponding skin of 6 healthy control individuals. Skin biopsies were stained with antibodies against HLA-DR, IL-8, IL-17A, IL-17F, IL-23, IL-36 gamma, the chemokine CXCR3 (CD183) in IFN-induced inflammation, phospho-diesterase (PDE) 4B, and the transcription factor FOX-P3 in T-cells. The stained slides were photographed in 20x enlargement with the Leica DFC 295 microscopic system and digitally analyzed with the ImageJ Plugin-IHC Profiler scanning 82364 square micrometers. Stained cells ranged from 50 to 365 per visual field. The two-sided t-test served for statistical analysis. In PPP compared to healthy controls, we found the following significance of staining in decreasing order: IL-36 gamma ($p<0.000001$), FOX-P3 ($p<0.00001$), IL-17A ($p<0.0001$), CXCR3 ($p<0.00001$), IL-17F ($p<0.003$), IL-8 ($p<0.03$), PDE 4B ($p=0.057$), IL-23 ($p=0.10$), HLA-DR ($p=0.91$). These results clearly indicate IL-36 gamma to be the dominant cytokine in PPP. Moreover, the cytokines IL-17A and IL-17F also exhibited a significantly higher staining compared to healthy controls. Our results may provide a basis for successful new treatment in palmoplantar pustulosis.

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Clinical and demographic features of morphea patients with mucocutaneous involvement: A cross sectional study from The Morphea of Adults and Children (MAC Cohort)
H. T. Jacobe, S. Prasad, S. M. Black, S. Sharma
Dermatology, University of Texas Southwestern Medical Center, Dallas, Texas, United States
While clinical findings of morphea have been described by our group and others, genital and oral lesions have not been well characterized. To address this knowledge gap, a cross-sectional analysis was performed of patients enrolled in The Morphea in Adults and Children Registry from 2007 to 2018. Of 737 patients analyzed, 48% (n=353) had linear morphea, 31% (n=232) had generalized morphea, 12% (n=87) had plaque morphea, and 6% (n=45) had mixed. Oral lesions were present in 23 patients (3%), of which 20 (87%) had linear morphea, nine had En Coup de Sabre (39%) and 12 had Parry Romberg Syndrome (PRS) (52%). Genital lesions were present in 28 patients, the majority of which (86%) had generalized morphea. Patients with oral morphea involvement had a younger age of onset compared to genital involvement (12 and 58 years old, respectively; $p < 0.001$). Genital morphea patients had extra-genital lichen sclerosus et atrophicus (LsA) in 79% (n=22) as compared to 17% (n= 4) with oral involvement ($p < 0.001$). Morphea profunda, or deep involvement, was seen in 83% (n = 19) patients with oral involvement versus 14% (n=4) of patients with genital involvement ($p < 0.001$). Median mLoSSI and PGA-A scores for patients with oral involvement (0, IQR 0-4 and 0, IQR 0-24, respectively) was lower than patients with genital involvement (10, IQR 6-27 and 23, IQR 15-40, respectively) ($p < 0.001$ and $p = 0.002$, respectively). PGA-D scores were higher in patients with oral involvement (50, IQR 30-60) than in patients with genital involvement (20, IQR 10-25) ($p < 0.001$). Our results show that while mucocutaneous lesions are rare in morphea, oral involvement is predominant in patients with facial linear lesions, particularly PRS, while genital lesions predominate in post-menopausal women with overlying extra-genital LsA. Given the function limiting nature of these lesions, practitioners should know clinical signs that put patients at risk mucocutaneous morphea.

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Opioid use in all-cause chronic pruritus
T. T. Daugherty[1], E. M. Cole[1], R. A. Swerlick[1], S. C. Chen[1, 2]
[1]Dermatology, Emory University School of Medicine, Atlanta, Georgia, United States, [2]VISN 7, Regional TeleHealth Service, Atlanta, Georgia, United States
Pruritus occurs frequently following administration of opioids, owing to its effect on μ opioid receptors and peripheral mast cell degranulation. However, the effect of opioids on pruritus in other primary skin conditions is unknown. We performed a retrospective chart review of patients with chronic pruritus, defined as itch lasting >6 weeks, to assess the association of opioid exposure with patient-reported itch severity, using a pruritus visual analogue scale (VAS). Opioid exposure was assessed using the Georgia Prescription Drug Monitoring Program (PDMP) records for opioid medication prescriptions filled within the 90 days prior to the appointment. Our study included 154 patients (40.9% male). Diagnoses included dermatitis (39.1%), urticaria (15.4%), psoriasis (9%), prurigo nodularis/neurodermatitis (4.5%), chronic idiopathic pruritus (13.5%), and other diagnoses (18.6%). Twenty-four patients (15.6%) met criteria for opioid exposure. Opioid-exposed participants reported greater mean severity of itch, indicated by higher pruritus VAS scores (5.58 vs. 4.17, p=0.034). In addition, increased total morphine equivalents trended towards greater itch severity, although this did not reach statistical significance (4.17 vs. 5.23 vs. 6.00 for total morphine equivalents of 0, <1,000 mg, and ≥1,000 mg, respectively, p=0.087). This study highlights that opioid use may enhance pruritus in many opioid-unrelated primary skin conditions. In the setting of the current opioid epidemic, dermatologists are uniquely poised to recognize and potentially address an uncomfortable opioid side effect: pruritus. Future studies are needed to explore causative nature of opioids in the population. This knowledge may be able to provide physicians with a deeper understanding with which to fight the opioid epidemic and may motivate patients in finding non-opiate pain management strategies.

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A variant subtype of psoriasis with a unique clinical and immunological characteristics
L. Zhang[2], L. Chen[2], F. Xue[1], J. Zheng[2]
[1]Laboratory of Dermatoimmunology, Rui Jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [2]Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China
Objective We sought to characterize a variant subtype of psoriasis--eczematous psoriasis and its differences from psoriasis vulgaris. Method 5 patients diagnosed on out-patient and in-patient were selected as case group, meanwhile 11 psoriasis vulgaris and 11 eczema were selected as control group. We Assessed biopsy specimens using immunohistochemistry and quantitative real-time PCR, and cytometric bead array was used to detect inflammation-related cytokines in the serum. Result Except of basic pathological features of psoriasis, in lesion skin, eczematous psoriasis showed partial sponge edema and dermal vascular inflammation infiltration changes. Despite the characteristic upregulation in psoriasis, *IL-17A* mRNA expression was higher than psoriasis vulgaris . Interestingly, not Th17 axes but higher activation of Th2 axes was detected in serum of patients with eczematous psoriasis, and positive correlations between TNF-alpha and PASI scores ($r = 0.75$, $P < 0.05$) was found in patients with eczematous psoriasis, whereas patients with psoriasis vulgaris showed positive correlations between PASI scores and IL-17A($r = 0.87$, $P < 0.05$). Conclusion Although Eczematous psoriasis shares basic pathological features of psoriasis, it has a unique clinical, histological and immunological characteristics . we showed features of higher TH2 activation, and serum TNF-alpha levels might link TH2 and TH17 activation. The balance is transformed from Th17 to Th2, which may also be one of the main causes of the rash-like change in the rash.

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Holistic dermatology: An evidence-based review of modifiable lifestyle factors associations with dermatologic disorders
S. Hu, P. Anand, M. Laughter, M. Maymone
University of Colorado School of Medicine, Aurora, Colorado, United States
Holistic dermatology focuses on treating the human body as a whole and implementing life-style changes to enhance the treatment and prognosis of skin disease. Understanding the interplay between modifiable life factors and patients' dermatologic health will help physicians better inform patients on self-care methods to mitigate the burden of their skin disease(s). Our study reviews the current scientific literature on the relationship between modifiable life factors and dermatological outcomes of skin disorders. A systematic literature search on PubMed, Cochrane, and Web of Science was conducted to identify research articles examining the relationship between dermatology and six major categories of modifiable life factors. The search terms diet, sleep, exercise, stress, alcohol, illicit drugs, and smoking were input to identify all relevant articles with publications dates up to January 2020. Non-English language articles were excluded. A total of 128 studies were included. A substantial amount of evidence supports the relationship between modifiable life factors and dermatologic outcomes. There were the most studies on diet, stress, alcohol, illicit drugs, and smoking but all life factors were supported by some degree of scientific evidence. All modifiable life factors explored in this review play a critical role in modulating the onset and progression of skin disease. We anticipate more research studies in the future and an increasing integration of holistic dermatology into patient care.

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Measurement properties of four different patient-reported outcomes to assess sleep disturbance in adults with atopic dermatitis
D. K. Lei[1], M. Yousaf[1], S. Janmohamed[1], P. Vakharia[1], R. Chopra[1], R. Chavda[1], S. Gabriel[1], R. Sacotte[1], K. Patel[1], V. Singam[1], S. Immaneni[1], R. Kantor[1], D. Hsu[1], D. Cella[1], J. Silverberg[2, 1]
[1]Dermatology, Feinberg School of Medicine at Northwestern University, Chicago, Illinois, United States, [2]Dermatology, George Washington University School of Medicine, Washington, District of Columbia, United States
Sleep is commonly impacted in atopic dermatitis (AD). However, the ideal patient-reported outcome measures to assess sleep in AD patients has not been determined. We sought to determine the measurement properties of the Patient-Reported Outcomes Measurement Information System (PROMIS) Itch Questionnaire (PIQ)-Mood and Sleep, Sleep Disturbance (SD), Sleep-Related Impairment (SRI), and Epworth Sleepiness Scale (ESS) in adults with AD. We performed a prospective dermatology practice-based study using questionnaires and evaluation by a dermatologist (n=491). PIQ Mood and Sleep, PROMIS SD, SRI, and ESS had good convergent validity with intensity and frequency of sleep disturbance, Patient-Oriented Eczema Measure, Eczema Area and Severity Index, total and objective-Scoring AD, Numerical Rating Scale of worst-itch and average-itch, and Dermatology Life Quality Index. PIQ Mood and Sleep had significantly better correlations with other severity measures than the other sleep measures (Fisher z-scores, $P \leq 0.04$ for all). PIQ Mood and Sleep had fair ability to distinguish between levels of severity of sleep disturbance, AD or itch, i.e. criterion validity. PROMIS SD, PROMIS SRI and ESS had poor to fair ability to distinguish between different levels of sleep, AD and/or itch severity. All four sleep assessments showed fair responsiveness to change of severity of sleep-disturbance, AD and itch, had good internal consistency, with no floor or ceiling effects, and were feasible for use in clinical practice. In conclusion, PIQ Mood and Sleep, PROMIS SD, PROMIS SRI and ESS showed good construct validity, internal consistency and responsiveness in adult AD. PIQ Mood and Sleep, followed by PROMIS SD, had the best measurement properties in adult AD.

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Mercaptopurine-induced Sweet syndrome in Crohn's disease
J. Wat[1], M. Wat[1, 2], K. Honda[1]
[1]Dermatology, Case Western Reserve University School of Medicine/University Hospitals Cleveland Medical Center, Cleveland, Ohio, United States, [2]Dermatology, Temple University Lewis Katz School of Medicine, Philadelphia, Pennsylvania, United States

Azathioprine-induced Sweet syndrome (AISS) usually occurs within a month of initiation of azathioprine. We describe a 19-year-old man with Crohn's disease, which was well-controlled and chronically managed on 6-mercaptopurine (6-MP) and mesalamine, who presented with painful, hemorrhagic, centrally necrotic pustules on the bilateral dorsal hands and neutropenia. Pathology of a cutaneous lesion revealed a neutrophilic infiltrate, with a negative infectious source. Bone marrow evaluation showed hypocellular marrow consistent with 6-MP toxicity but no evidence of malignancy. Drug-induced Sweet syndrome in thirteen previously described patients co-occurred with fever, painful skin lesions most commonly on the upper extremities, and biopsy-confirmed neutrophilic dermatosis--features all present in this patient. While classically drug-induced Sweet syndrome is temporally associated with drug administration, we propose that this entity can also occur even after months of medication use. We also analyzed previous cases of AISS and azathioprine-hypersensitivity syndrome (AHSS). Unlike other cases of AISS and AHSS, peripheral neutrophilia was not seen in our index case; on the contrary, neutropenia was present. As seen in at least two other cases of AISS, lesions favored the dorsal hands and face, but lesions in this index case did not involve the trunk. Most cases involved patients with underlying inflammatory bowel disease. Therefore, we propose that azathioprine-induced Sweet syndrome favors the extremities and can still occur even after chronic use of azathioprine or 6-MP and in the absence of neutrophilia. Recognizing these atypical features in this entity and differentiating it from classical inflammatory bowel disease (IBD)-associated Sweet syndrome is important to prevent mortality from azathioprine toxicity or hypersensitivity.

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Mid-level providers and the dermatology literature: A bibliometric analysis of trends 1973-2018

M. Maymone[1], M. Laughter[1], J. D. Jones[2], P. Anand[1], R. Dellavalle[1], J. Hugh[1], C. Dunnick[1]

[1]Department of Dermatology,, University of Colorado Denver School of Medicine, Denver, Colorado, United States, [2]Strauss Health Sciences Library, University of Colorado Anschutz Medical Campus, Denver, Colorado, United States

The number of mid-level providers (MLPs) in dermatology has grown tremendously over the last five decades. This increase may be due to the imbalance between the demand for dermatology services and dermatologists. Little research has been done to examine the evolving roles of mid-level providers and the state of scientific publications on this topic. To analyze the trends in publications and key topics related to MLPs in dermatology, PubMed was screened for all articles related to MLPs in dermatology published from 1973 to 2018. The number of publications, citation count, and key topics were assessed. The general concept of the article was graded by 3 independent evaluators and scored as "pro", "con" or "neutral" toward mid-level providers. The number of publications related to MLPs in dermatology has increased over the past four decades while the proportion of papers with favorable views towards MLPs has declined. The most-cited publications highlight the ongoing workforce shortages in dermatology with an increasing prominence of mid-level providers and evaluate the efficacy between provider types. Limitations of our analysis are that we included a single database, publications written in English only, and a restricted time period from 1973 to the end of 2018.

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Patient-centered development of a digital implementation tool for integrated knowledge translation with adult atopic dermatitis patients

C. Bouchard, S. Ghazal, N. Merati, G. Isola, M. Ehteshami, C. Jack

Dermatology, McGill, Montreal, Quebec, Canada

Targeted immuno-therapy for adult atopic dermatitis has left an important gap in understanding both by patients and physicians alike. International guidelines involve complex regimens of skin care, topical therapies, trigger avoidance, and behavioral adaptation first line. Engaging patients is key to communicating these fundamentals; however, resources are often limited at the point of care. Here we describe the development of an implementation tool for integrated KT in order to address this gap. A patient-engagement framework was used in order to identify the adult atopic dermatitis patient 'end-user' greatest needs. Patient partners were formally engaged and collaboratively an objective was created to survey gaps in open-source online resources. Existing educational information and online communities, as well as patient usage patterns were explored using narrative juxtaposition. A key theme was the wide breadth of unvalidated information found to dominate patient consumption. We then applied the French et al. (2012) four-step systematic framework for developing complex implementation interventions. We found that the theoretical domain of patient-knowledge was most relevant to behavioral change. Relevant barriers identified include limited expertise at point of care, absence of face-to-face KT with clinicians, fear of negative health consequences, and self-sought information overload (world wide web, social media). A validated mobile health application was determined to be the most desirable and efficient method for implementing integrated KT. We applied mixed methods to design a mobile health application with local and national patient partners. The '*Eczema.app: virtual nurse* ' is a bilingual tool that features patient-driven topics, validated information, and multi-media content. Evidence-based education in the mobile application is tailored to the clinical context and can be personalized to the patient. We hypothesize that intervention with the '*Eczema.app: virtual nurse*' will improve patient-reported outcomes.

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Clinical characteristics of acute graft-versus-host disease in small bowel and multi-visceral transplant recipients

C. M. Brumfiel[1], P. K. Dekker[1], K. M. Saardi[2], A. H. Kroemer[3], S. S. Kaufman[3], H. B. Pasieka[2, 1]

[1]Georgetown University School of Medicine, Washington, District of Columbia, United States, [2]Dermatology, MedStar Washington Hospital Center, Washington, District of Columbia, United States, [3]MedStar Georgetown Transplant Institute, Washington, District of Columbia, United States

Acute graft-versus-host disease (aGVHD) following stem cell transplantation is characterized by rash, liver dysfunction, and diarrhea. Although aGVHD is uncommon following solid organ transplantation, we find higher rates of aGVHD after intestinal (ITx) and multi-visceral transplantation (MVT). In these cases we find a unique presentation as the donor liver (if present) and intestine are spared, leaving skin as the main target. Between 2013 and 2019, we diagnosed 17 cases of aGVHD following ITx (n=9) and MVT including both intestine and liver (n=8). Median time to onset of aGVHD was 37 days. Erythema on the trunk, palms, or both was the most common presenting skin finding (15/17 patients). All patients had skin biopsies at the time of diagnosis. Patients presented with grade 1 (7/17), grade 2 (8/17), grade 3 (1/17), and grade 4 (1/17) aGVHD. Peripheral blood chimerism studies obtained in 11 patients revealed donor CD3+ lymphocytes in 5 (45%). Two patients had passenger lymphocyte syndrome, and partial donor stem cell engraftment was diagnosed in a third patient. Native GI tract involvement (usually rectosigmoid), was found in 7/17 patients; other sites included bone marrow, and native liver. In all, 6/17 patients had no evidence of extra-cutaneous aGVHD. Treatment strategies included systemic and topical corticosteroids, anti-thymocyte globulin, extracorporeal photopheresis, and/or JAK inhibitors. Complications of immunosuppression including opportunistic infections (aspergillosis and toxoplasmosis) and post-transplant lymphoproliferative disorder occurred in 7/17 patients. In summary, rash is typically the first, and may be the only, manifestation of aGVHD following luminal organ transplants. Diagnosis may be aided by time of onset of cutaneous manifestations, extra-cutaneous signs, and peripheral blood chimerism.

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Practice changing landmark study- multi-institutional analysis of image guided superficial radiotherapy (IGSRT) for the treatment of non-melanoma skin cancer (NMSC)
L. Yu[1], D. Ladd[2]
[1]Radiation Oncology, Laserderm, Smithtown, New York, United States, [2]Tru-Skin Dermatology, Austin, Texas, United States
Practice changing landmark study- multi-institutional analysis of image guided superficial radiotherapy (IGSRT) for the treatment of non-melanoma skin cancer (NMSC). NMSC is generally treated in dermatology offices using surgical techniques. This study presents the largest multi-institutional retrospective modern series of Image Guided Superficial Radiotherapy (IGSRT) for Non-Melanoma Skin Cancer (NMSC). Between July 2013 and December 2019, 2424 NMSC lesions treated with IGSRT at 3 clinics in Texas and 1 clinic in NY were analysed. All lesions received 50, 70 or 100 kiloVoltage(kV) energy IGSRT 2-4 times weekly. KV energy selection was determined by ultrasound depth measurements and/or tumor characteristics. Patients returned 3-6 weeks after completion of therapy for dermoscopy/clinical evaluation and ultrasound imaging to confirm complete tumor clearance. Patients were then followed every 2-12 months thereafter. Any lesions in the treatment site suspicious for residual or recurrent tumor were biopsied. Exclusion criteria included tumors greater than 4 cm in diameter or non-movable tumors adherent to deeper structures. Follow-up ranged from 0.1-62.8months. At a mean follow up of 15.8 months, 29 patients with 77 lesions expired, all without recurrence. Kaplan-Meier (KM)Tumor control rates at 1, 2 and 5 years were 99.5%, 99.4% and 99.4% respectively. One, 2 and 5 year KM Overall-Survival (OS) for the entire group was 97.6%, 95.8% and 85.8% and unaffected by disease status or treatment. This study represents the largest study of NMSC treated with non-invasive IGSRT. The 99% cure rate is comparable to the best cure rates of other invasive modalities such as Mohs micrographic surgery. This innovative non-surgical technology with its high cure rate in the treatment of NMSC has the potential to transform the practice of dermatology with regard to treatment of NMSC.

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Safety and efficacy of leukocyte-rich platelet-rich plasma in the treatment of cicatricial alopecia
B. Khalid, M. K. Hordinsky, J. A. Shaik, R. Farah
Dermatology, University of Minnesota, Minneapolis, Minnesota, United States
Platelet-rich plasma (PRP) is defined as an autologous blood product with a concentration of platelets approximately three to five times the whole blood in a small volume of plasma. Due to the secretion of a plethora of growth factors by platelets in PRP that promote healing and tissue regeneration, its use has gained popularity to treat non-scarring alopecia, especially androgenetic alopecia, with promising results. However, there is limited data with only a few case studies reporting use and efficacy in scarring alopecia, wherein the hair follicle may be irreversibly destroyed due to inflammation and replaced by fibrous tissue. Different commercially available PRP systems yield an end product which may also be variably rich in leukocytes. We propose the use of leukocyte-rich PRP may be debatable due to risk of aggravating preexisting inflammation. Advocates of leukocyte-rich PRP believe the presence of leukocytes may increase growth factor content, propagate pluripotent stem cells, contribute to angiogenesis, matrix production and tissue remodeling. At the same time, growth factors secreted by platelets with anti-inflammatory properties may keep inflammatory leukocytes in a suppressive state. We used leukocyte-rich PRP to treat three patients with scarring alopecia; two with lichen planopilaris and one with frontal fibrosing alopecia. Each underwent monthly treatment for three months with a PRP device that enriched for platelets by 3-fold but also contained white blood cells whose concentration ranged from 0.5 to 4 times that in whole blood. Following the treatment sessions, none of the patients reported increased itching, burning, erythema or worsening of symptoms. Decreased perifollicular erythema was noticed in one patient. Overall, patients either remained stable or improved, although not significantly, with some new vellus or indeterminate hair fiber growth. Thus, leukocyte-rich PRP appeared to be safe and somewhat beneficial to one subject in our study with a small sample size. Further studies with more patients treated for longer duration need to be conducted to conclusively assess the safety and efficacy of using leukocyte-rich PRP in inflammatory alopecias.

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IL-13 production and accumulation in lesional, non-lesional and *ex vivo* activated skin of atopic dermatitis patients
G. Isola, V. Gimenez-Riviera, C. Jack
Dermatology, McGill, Montreal, Quebec, Canada
IL-13 is a key cytokine mediating pathogenesis in atopic dermatitis patient skin. The cellular source and accumulation sites of this cytokine remain elusive in human skin, largely due to methodological limitations. Here we introduce three validated methodologies for the detection of IL-13 in skin biopsies and skin-derived T cells of patients with atopic dermatitis. Using confocal microscopy and flow cytometry, we show that IL-13 protein is highly sensitive to fixation with PFA, which masks key epitopes for the optimal detection by specific antibodies; this limitation requires the use of PFA-free protocols or heat-mediated protein retrieval for accurate quantification and study of this cytokine in the skin of patients. In order to distinguish between accumulation and production sites of IL-13 in human skin, we compare the localization of this cytokine at the protein level with its mRNA expression using a novel in situ hybridization method. Furthermore, we compare the production of this cytokine and its mRNA in lesional, non-lesional and ex vivo activated skin of patients with atopic dermatitis. Our results demonstrate the production of IL-13 by cells expressing low to high levels of CD45 with lymphocytic morphology localized in the epidermal stratum granulosum and spinosum and more markedly in the papillary dermis. In line with this finding, the isolation and analysis of cutaneous T cells using our skin explant model and IL-13 secretion assay by flow cytometry revealed a population of memory Th2 and Tc2 lymphocytes present in lesional skin. The detection of IL-13 mRNA correlated with some but not all sites of IL-13 protein expression, identifying different areas of production and consumption of this cytokine in the skin. These methods pave-the-way for more precise identification of the role of IL-13 in human skin during health and disease.

Pharmacology and Drug Development

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Formulation and evaluation of topical products with *Helianthus Annuus* ozonized oil
M. Andreassi, C. Salvini, L. Furi, J. Zanardi, P. Brizzi, V. Travagli
Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Siena, Italy
This work was aimed to study the activity of topical products formulated with *Helianthus annuus* normal and ozonized oil produced in Italy, Recent studies have suggested a possible moisturizing and elasticizing activity of *Helianthus Annuus* ozonized oil. For such characteristics *Helianthus Annuus* ozonized oil appears an appropriate ingredient to be used in topical preparations for the treatment of skin hydration. The oil was incorporated into O/W emulsion in a standard formulation at percent concentration of 3%, 5% and 10 % respectively of *Helianthus Annuus* normal and ozonized oil, one O/W emulsion was prepared without active ingredient as control. The investigation was carried out on 10 healthy female volunteers, between the ages of 20 and 40, with normal or dry skin. Each product was applied to the volar surface of the forearm at a dose of 3 mg/cm^2. As control, the same cream without active ingredient was applied to the other forearm. To evaluate TEWL and skin elasticity, was used the device Aveal 220 (Sylton diagnostic systems). The skin hydration action of the emulsions was evaluated in relation to basal value, and the emulsion without active ingredient, respectively after 15 minutes and 8 days. The skin elasticity was evaluated after 15 minutes and 8 days. The results showed that the 6 emulsions with *Helianthus Annuus* normal and ozonized oil, compared to the emulsion without active, significantly increase the degree of hydration and elasticity of the skin. The 10% formulation of *Helianthus Annuus* ozonized oil has a greater power of hydration of the skin compared to other emulsions, both short and long term and is the emulsion that has produced better results.

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Saikosaponin A induces HEKa cell apoptosis via ROS generation

M. Liu, Y. Zheng

Dermatology, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, China

Background*:* Psoriasis is a common, chronic and noninfectious skin disease with the complex pathogenesis which is reported as excessive proliferation and aberrant apoptosis of keratinocytes. Saikosaponin A (SSA) is a kind of triterpenoid saponin extracted from Chinese traditional medicine *Radix bupleuri*, possessing various biological functions such as anti-inflammatory, immune regulation and anti-tumor. But the effect of SSA on keratinocytes is unclear. *Objective:* To explore the effect of SSA on keratinocytes and to analyze the corresponding mechanism. *Methods: 1.* The cell viability was detected by MTT assay and the apoptosis was examined using the annexin V-FITC/PI flow cytometry double staining method. Apoptosis-related protein were determined by Western blot. *2.* After stimulation with SSA, DCFH-DA and JC-1 were used to detect intracellular reactive oxygen species (ROS) levels and mitochondrial membrane potential. MitoSOX Red and MitoTracker Green were used to observe generation and distribution of ROS in mitochondria by confocal laser scanning microscope; ROS-related protein was detected by Western blot. *3.* Antioxidants N-acetyl-cysteine (NAC, 10mM) was added to HEKa cells 1h before SSA(20μM) treatment to identify whether SSA induced the apoptosis of HEKa cells through ROS production. *Results: 1.* Compared with the control group, SSA significantly inhibited the viability and induced the apoptosis of HEKa cells with changes in BCL-2 family protein levels. *2.* SSA promoted ROS production of mitochondria and decreased Δψ, which caused changes in ROS-related protein levels and oxidative stress damage to cells. *3.* SSA-treated HEKa cells produced more ROS, but this effect was inhibited by the antioxidants NAC. Furthermore, NAC treatment attenuated HEKa cell apoptosis and proliferation suppression of SSA. *Conclusion*: SSA can inhibit proliferation and induce apoptosis of HEKa cells through ROS generation, making SSA a potential promising candidate drug for future research.

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Development and first-in-human characterization of a potent oral CCR4 antagonist for the treatment of atopic dermatitis

L. E. Cheng, A. Jorapur, S. Jacobson, O. Talay, S. Miakicheva, D. Trujillo, N. Lee, J. Jankicevic, D. Wustrow, P. Kassner, W. Ho, D. Brockstedt

RAPT Therapeutics, Inc., South San Francisco, California, United States

Atopic dermatitis (AD) is predominantly driven by T helper type 2 cells (Th2). Accumulation of Th2 cells depends on CCR4-mediated recruitment of Th2 cells by the CCR4 ligands CCL17 and CCL22. Both are elevated in inflamed tissue, and levels correlate with disease activity and severity. Here, we describe RPT193, a novel, highly potent and specific oral CCR4 antagonist. In multiple preclinical mouse models of allergic skin inflammation, we demonstrated efficacy and reduction of Th2 cytokines with once daily dosing of RPT193 that is comparable to antibodies specific to IL-4 receptor and IL-13. The safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of escalating single and multiple oral (once daily for 7 days) doses of RPT193 are currently being evaluated in healthy adults. The study has enrolled healthy subjects into 4 single and 4 multiple dose cohorts (randomized 3:1 to receive RPT193 ranging from 50-400 mg or placebo). As of an ad hoc interim analysis, 64 healthy subjects have received single or multiple doses of RPT193 or placebo. Blinded safety review suggests RPT193 has acceptable safety and tolerability with only mild or moderate treatment-emergent adverse events after single or multiple doses. No stopping criteria were met at any dose tested. RPT193 demonstrated linear PK, a terminal half-life of ~24 hours, and accumulation upon multiple dosing. CCR4 receptor occupancy of >80% at 24 hours post-dose was attained in most subjects receiving single doses and all subjects receiving multiple doses of RPT193. In summary, preclinical data indicate that CCR4 antagonism with RPT193 inhibits allergic skin inflammation in mice akin to targeting IL-13 or the IL-4 receptor. Clinical experience thus far indicates encouraging safety, PK consistent with once daily dosing, and desired target coverage. The data warrant further investigation of RPT193 in patients with AD and potentially other allergic immune conditions.

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Biological evaluation of litchi derived products as dermatological agents

D. Huang[1], Z. Sun[1], X. Shen[1], Q. Gu[1], M. Soeberdt[2], C. Abels[2], J. Xu[1]

[1]School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou, Guangdong, China, [2]Dr August Wolff, Bielefeld, Germany

Litchi (Litchi chinensis Sonn.) is a fruit native to South China, and popular for the taste and health benefits. Litchi products, such as litchi juice and vinegar (extracted or fermented from litchi fruit) have potential dermatological benefits. In this paper, we have evaluated litchi vinegar and juice regarding protective effects on HaCaT keratinocytes following either UVB irradiation or poly (I:C) by assessing cell survival (OD, optical density; MTT assay), reactive oxygen species (ROS), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) activity. In addition, different inflammatory parameters were measured, e.g. IL-1b, TNF-a, or IL-8. As result litchi vinegar or juice (1) are not cytotoxic at therapeutic concentration; (2) protect HaCaT keratinocytes against UVB irradiation or poly(I:C); (3) reduces oxidative stress through inhibiting ROS and enhancing SOD and GSH Px activity; (4) suppressing inflammatory parameters, e.g. IL-1b, TNF-a, and IL-8. The ingredients very likely responsible for these effects are primarily epicatechin, rutin and chlorogenic acid.

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ATx201 modulates biomarkers of skin barrier function and cutaneous inflammation in patients with moderate atopic dermatitis

E. Guttman-Yassky[1], R. Bissonnette[2], A. Pavel[1], E. Saint-Cyr Proulx[2], P. Prokocimer[3], R. Toft-Kehler[3], M. Sommer[3]

[1]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [2]Innovaderm Research, Montreal, Quebec, Canada, [3]UNION Therapeutics, Hellerup, Denmark

ATx201 is a small molecule, which decolonizes *Staphylococcus aureus* and improves the diversity of the skin microbiome in patients with mild-to-severe atopic dermatitis (AD) (DECOLAD). We now report the safety and immune-modulatory effect of ATx201 in patients with moderate AD. In this randomized, double-blind, intraindividual, and vehicle-controlled Phase 2 trial, thirty-one patients received ATx201 CREAM 2% and matching vehicle (1:1) once daily for 3 weeks (NCT03304470), with a 12-day follow-up period. Analysis of the safety data revealed that ATx201 CREAM 2% was generally safe and well-tolerated in subjects with moderate AD lesions. The histological and transcriptional profiling analysis (IHC, microarray and RT-PCR) demonstrated that treatment with ATx201 CREAM 2% significantly ($p < 0.05$) increased expression of biomarkers related to skin barrier function (PNPLA3, ACOX2, DGAT2, FAXDC2, etc.), and decreases expression levels of markers related to inflammation including Th17 (S100A7, S100A9, CCL20, PI3, CXCL1, IL17C, STAT3), Th2 (IL10, IL4R, CCL26, CCL18, etc.), Th1 (CCL2, CCR1, etc.) and inflammatory cells (Langerin/CD207) compared to vehicle at Day 22. Finally, several biomarkers that were significantly modulated by ATx201 CREAM 2% were significantly correlated with improvement in TSS, TAA scores and the TSS component scores ($p < 0.05$), such as S100A8 ($\rho_{TSS} = 0.83$), KRT16 ($\rho_{TSS} = 0.77$), PI3 ($\rho_{TSS} = 0.69$), IL13 ($\rho_{TSS} = 0.68$), IL22 ($\rho_{TSS} = 0.67$) and MMP12 ($\rho_{TSS} = 0.55$). IHC analysis further revealed that 15/29 (51.7%) subjects were classified as histological responders receiving ATx201 CREAM 2% versus 9/29 (31.0%) receiving vehicle. These data suggest that topical application of ATx201 is safe and improves biomarkers of skin barrier function and suppresses biomarkers of AD-associated inflammation across multiple T helper cell pathways.

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Preclinical rationale for a first-in-human trial to evaluate the safety and preliminary efficacy of desmoglein 3 chimeric autoantibody receptor T cells (DSG3-CAART) for mucosal pemphigus vulgaris

A. S. Payne, J. Lee, D. K. Lundgren, X. Mao, S. Manfredo-Vieira, S. Nunez-Cruz, E. F. Williams, C. Assenmacher, E. Radaelli, B. Wang, C. T. Ellebrecht, J. A. Fraietta, M. C. Milone

University of Pennsylvania, Philadelphia, Pennsylvania, United States

We previously established proof-of-concept that antigen-specific B cell depletion can be achieved with gene-engineered T cells expressing chimeric autoantibody receptors (CAARs) comprising DSG3, the autoantigen in the autoimmune blistering disease mucosal pemphigus vulgaris (PV), fused to CD137-CD3ζ signaling domains. After specific lysis of anti-DSG3 B cells, DSG3-CAART cells are stimulated to proliferate and engraft, leading to potentially durable remissions of autoimmune disease. Here we present final preclinical studies supporting the DSG3-CAART investigational new drug application. DSG3-CAART specifically lysed primary human anti-DSG3 B-cells from PV patients and demonstrated dose-related activity in a PV hybridoma model, resulting in decreased hybridoma burden, decreased serum and tissue-bound autoantibodies, and increased DSG3-CAART engraftment. In an exploratory PV active immune model with physiologic anti-DSG3 IgG levels, DSG3-CAART inhibited antibody responses against pathogenic DSG3 epitopes and autoantibody binding to epithelial tissues, leading to clinical and histologic resolution of blisters. Soluble anti-DSG3 antibody stimulates DSG3-CAART cells to promote IFNγ secretion, homotypic clustering, and clonal proliferation, consistent with an activated T cell phenotype. In vitro screening of a panel of primary human cells with DSG3-CAART and high-throughput screening of a commercial membrane protein array with the soluble DSG3 CAAR ectodomain did not identify productive interactions with desmosomal or other off-target proteins. Collectively, these studies have informed the clinical design of a first-in-human trial of DSG3-CAART for mucosal PV and may facilitate the preclinical development of future CAART therapies for other antibody-mediated diseases.

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Mechanisms by which combined inhibition of BET and HDAC inhibits proliferation and induces apoptosis in CTCL

L. Zhao[1], G. Wood[2]

[1]Dermatology, University of Wisconsin-Madison, Madison, Wisconsin, United States, [2]Dermatology, U. Wisconsin, Madison, Wisconsin, United States

Previously, we showed that the combination of BET inhibitors (BETi) and histone deacetylase inhibitors (HDACi) acted synergistically to induce significant apoptosis (60-80%) in CTCL lines and leukemic CTCL cells within 96 hours without significant apoptosis of normal CD4+ T cells (<10%) (Zhao et al., Neoplasia, 2019). We showed that mediators of apoptosis (e.g. cleaved caspases 8 and 9) were increased and proliferative drivers (e.g. NFkB, cyclin D1, c-Myc) were decreased. Our current gene expression studies showed that BETi/HDACi treatment of CTCL cells at nanomolar levels suppressed several pro-survival factors early (e.g. AKT, NFkB) while simultaneously upregulating a wide variety of pro-apoptotic factors (e.g. multiple caspases, death receptors/ligands, Bcl2 family inhibitory factors), mainly at 6 and 96 hours. Some pro-survival factors also increased at 96 hours but by then, apoptosis was already well underway. BETi/HDACi reduced AKT, TP73, DR3 and TRAF2 greater than 2-fold at multiple time points. DR3 and TRAF2 can activate NFkB. TRAF2 and TP73DN isoforms can inhibit apoptosis. AKT can promote CTCL-associated proliferative drivers such as c-Myc and NFkB, and cell survival factors like Bcl2 while inhibiting pro-apoptotic factors like Bax. Using a clinically relevant AKT inhibitor (MK2206), we showed that AKT inhibition in CTCL partially mimics the effects of BETi/HDACi, with modest inhibition of proliferation and reduced metabolic activity but no induction of apoptosis. To further explore the role of differential inhibition of BET family proteins expressed in CTCL, we found that siRNA knockdown of BET2 and BET4, but not BET3, inhibited c-Myc expression. Knockdown of all 3 reduced GATA3 expression. Our results further define the mechanisms by which BETi/HDACi treatment inhibits proliferation and induces apoptosis in CTCL. Our findings support this novel combination therapy for advanced CTCL.

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Comparing molecular cutaneous improvement in atopic dermatitis with various treatment modalities facilitates personalized approaches

J. Glickman[1], C. Dubin[1], J. Han[1], D. Dahabreh[1], S. Garcet[2], J. Krueger[2], A. Pavel[1], E. Guttman-Yassky[1]

[1]Icahn School of Medicine at Mount Sinai, NY, New York, United States, [2]Laboratory for Investigative Dermatology, The Rockefeller University, NY, New York, United States

Atopic dermatitis (AD) is the most common chronic inflammatory skin disease. Recently, there is active therapeutic development in AD, with testing of targeted and broad treatments, to address various disease mechanisms. A comparison of the molecular effects of these treatments on AD skin abnormalities can facilitate a deeper understanding of the key pathogenic elements in AD, fostering further therapeutic developments, but it is still lacking. Using a meta-analysis-based approach, we are comparing immune and barrier effects of key systemic treatments used or tested in AD, including cyclosporine A, narrow-band ultraviolet B, dupilumab/anti IL-4R, fezakinumab/anti-IL-22, ASN002/JAK-SYK antagonist, ustekinumab/anti IL-12/23p40, as well as topicals (topical corticosteroids, crisaborole/anti PDE4) on lesions of AD patients. We evaluate relative improvements with various drugs on AD-related gene signatures in skin biopsies from AD patients, using clinical trial data in which Affymetrix U133Plus 2.0 gene-arrays were performed on lesional and nonlesional skin before and during treatment. While cyclosporine showed the highest overall cutaneous improvement, narrow Th2-targeting with dupilumab achieved the highest Th2 inhibition in skin with accompanying broad changes in other mechanisms beyond Th2, and greatest changes in cardiovascular/atherosclerosis markers. Our study also highlights specific advantages of targeting a particular axis. For example, Th22/IL-22 targeting with fezakinumab is particularly efficacious in improving the AD barrier dysfunction, but induces only modest changes in skin inflammation. Our approach may facilitate future development of a personalized medicine approach in AD by identifying the best treatments for individual phenotypes and patients needs, based on immune, barrier, and atherosclerosis profiles.

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Rilzabrutinib (PRN1008) shows BTK-mediated mechanisms of action supporting clinical development for immune-mediated diseases

C. Langrish, M. Francesco, Y. Xing, J. Bradshaw, T. Owens, P. Nunn

Principia Biopharma, South San Francisco, California, United States

Bruton tyrosine kinase (BTK) is a critical immune signaling enzyme expressed in B and innate immune cells and is an essential element downstream of BCR and FcR signaling. Rilzabrutinib (PRN1008) is an oral, reversible, covalent BTK inhibitor that drives durable BTK occupancy with low off-target effects shown by other BTK inhibitors. Preclinical PRN1008 activity was evaluated in biochemical studies and in vivo models of inflammation and canine pemphigus. PRN1008 showed kinase selectivity for BTK with an enzyme inhibition IC_{50} of 1.3 nM; functional BTK target occupancy of 91% (±2%) was achieved in PBMCs at 4 h. In a skin IgG antibody (FcγR)-mediated acute Arthus reaction rat model, PRN1008 10, 20, and 40 mg/kg led to significant, dose-dependent improvements in immune complex-mediated inflammation and injury (P<0.01 all doses vs vehicle). In a passive cutaneous anaphylaxis mouse model that utilizes mechanisms similar to human allergic disease, PRN1008 20 and 40 mg/kg significantly inhibited IgE antibody (FcεR)-mediated immune responses (P<0.01 both doses vs vehicle). In naturally occurring canine pemphigus foliaceus, an autoantibody-mediated autoimmune disease that dogs and human share, 4 dogs treated with PRN1008 showed a rapid clinical improvement. All animals achieved complete or substantial disease control measurable by improved canine PDAI scores and without requiring corticosteroid use. Anti-inflammatory effects in dogs were visible within 2 wk and tolerability was excellent. BTK target occupancy in PBMCs from dogs was >70% within 4 h of PRN1008 treatment. Overall, PRN1008 preclinical results show simultaneous mechanisms of rapid and sustained anti-inflammatory effects by blocking inflammatory immune cells, eliminating autoantibody destructive signaling, and preventing new autoantibody production. These results provide a strong biologic basis for rilzabrutinib (PRN1008) in B cell- and autoantibody-driven autoimmune disorders, supporting ongoing clinical studies of pemphigus (phase 3) and immune thrombocytopenia (phase 2).

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Quantitative ligand and receptor binding studies reveal IL-36 activation mode

V. Todorovic[1], L. Zhou[2], S. Kakavas[1], L. Wang[2], B. Sielaff[2], R. Sadhukhan[2], P. L. Richardson[1], E. DiGiammarino[1], C. Sun[1], V. E. Scott[1]

[1]AbbVie, North Chicago, Illinois, United States, [2]AbbVie, Worcester, Massachusetts, United States

IL-36 cytokines are pro-inflammatory members of the IL-1 family upregulated in a host of inflammatory skin disorders. They signal through the IL-36 receptor (IL-36R) and a shared subunit IL-1 receptor accessory protein (IL-1RAcP). Targeting antagonism of IL-36/IL-36R binding by biologics and small molecules is being actively pursued for several dermatological disorders. Therefore, it is critical to gain understanding of the mechanism of ligand-receptor binding. The mode of activation for the IL-36 pathway is proposed to be similar to IL-1 in that the IL-36 agonist forms a binary complex with IL-36R, which recruits IL-1RAcP. Recently, it has been shown that IL-36R also interacts with IL-1RAcP even in the absence of an agonist. To elucidate the activation mode, all possible binding events for IL-36 ligands/receptors were outlined and examined in direct binding assays. Results show that the agonists bind to the extracellular domain of IL-36R with micromolar affinity while they do not show detectable binding to IL-1RAcP. Under surface plasmon resonance (SPR) experimental conditions, IL-1RAcP does not show detectable binding to IL-36R. In the presence of IL-36α, however, IL-1RAcP binds to IL-36R strongly. These results suggest the main path to IL-36R/IL-36α/IL-1RAcP ternary complex is through the IL-36R/IL-36α binary complex, which recruits IL-1RAcP. To predict the binding affinity of IL-36R/IL-1RAcP, which could not be measured directly, we engineered an Fc linked construct to induce heterodimerization of IL-36R/IL-1RAcP. Through a complete thermodynamic cycle, the affinity is predicted to be double-digit micromolar. SPR analysis also shows that IL-36R antagonist (IL-36Ra) binds to IL-36R with higher affinity and a much slower off rate than the agonists, providing a biochemical basis for efficient antagonism. These results depict the landscape of IL-36 ligand and receptor interactions, providing a better understand IL-36 pathway activation and inhibition.

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Improved effect on two cases of different subtypes of porokeratosis with superficial X-ray radiotherapy

Y. Chen, Q. Li

Dermatology Department, Dermatology Hospital, Southern Medical University, Guangzhou, Guangdong Province, China

Porokeratosis (PK) is a heterogeneous group of skin disorders with unclear aetiology. This group includes multiple clinical variants. PK can cause cosmetic disturbances and poses the risk of malignant transformation. However, the current therapies for PK yield unsatisfactory results. To evaluate the therapeutic effects of superficial X-ray radiotherapy (SXRT) on PK, two patients with local PK who experienced failed multiple topical treatments were treated with SXRT. One patient with porokeratosis of Mibelli (PM) received one course of treatment for the lesion on their buttocks and two courses for neck lesions. The other patient with porokeratosis ptychotropica (PP) received one course of treatment. Both patients are currently being followed up. Both patients expressed high satisfaction with their therapeutic results. SXRT induced more positive responses in our patients, more positive clinical improvement in a short amount of time, more satisfying cosmetic results and fewer side effects than their previous treatment methods. SXRT is highly tolerable and should be considered a promising treatment for local PK, including PM and PP. Moreover, long-term follow-up after withdrawal and large-scale clinical studies are needed to evaluate the true effectiveness of the treatment definitively.

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To assess the antifungal activity of Jublia® under real-world conditions

W. Lee[1], C. Otto[2], K. Abdul[3], D. M. Siegel[1]

[1]Dermatology, SUNY Downstate Health Sciences Univ, Brooklyn, New York, United States, [2]Pathology, SUNY Downstate, Brooklyn, New York, United States, [3]NYU, NY, New York, United States

Onychomycosis affects up to 8% of the population and is most commonly caused by Trichophyton rubrum (T. rubrum) and Trichophyton mentagrophytes (T. mentagrophytes). Systemic antifungals provide higher rates of clearance compared to topical antifungals, but are associated with potentially serious side effects and drug-drug interactions. Efinaconazole, a triazole antifungal, is approved by the FDA as a 10% nail solution (Jublia®) for the treatment of distal and lateral subungual onychomycosis. Efinaconazole's activity has been demonstrated using agar diffusion assays (ADAs) centered on cadaveric nail clippings; however, it has not been studied under real-world conditions that may provide a more accurate approximation of its true effectiveness. Efinaconazole was applied once daily to all fingernails of 20 healthy study subjects for 21 consecutive days and nail clippings were obtained to assess antifungal activity. ADAs performed with nail clippings taken 14 days after discontinuing efinaconazole resulted in zone sizes of 70 ± 2.1 mm for T. rubrum and 50.5 ± 6.8 mm for T. mentagrophytes, indicating that efinaconazole remains active in the nail plate even after it has been discontinued. Disk diffusion assays to compare the susceptibility of T. rubrum and T. mentagrophytes to efinaconazole and terbinafine showed that 1 mg of efinaconazole resulted in complete inhibition (85 mm zone) of T. rubrum and T. mentagrophytes; by contrast, doses of terbinafine up to 20 mg had no activity (6 mm zone) against T. rubrum and 2 mg of terbinafine produced a zone of 56 ± 1 mm against T. mentagrophytes. Our findings indicate that efinaconazole remains in the nail plate for 14 days after treatment is discontinued, and has a lower minimal inhibitory concentration (MIC) than terbinafine. Additionally, our data suggest that efinaconazole may be more effective than terbinafine for T. rubrum and T. mentagrophytes. This information may assist health providers wishing to prescribe efinaconazole for onychomycosis.

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GZ17-6.02 promotes autophagy and cell death in actinic keratoses via ATM-dependent mTOR inhibition

C. West[1], L. Booth[2], D. Von Hoff[3], S. Kwatra[4], P. Dent[2]

[1]Genzada Pharmaceuticals USA, Inc, Sterling, Kansas, United States, [2]Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, Virginia, United States, [3]tgen, Phoenix, Arizona, United States, [4]Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

GZ17-6.02 (602) is a novel investigational compound composed of curcumin, harmine, and isovanillin undergoing phase I clinical trials in oncology for solid tumors and lymphoma (NCT03775525). The goal of the present study is to determine the efficacy of 602 in killing actinic keratoses (AK) and to elucidate the molecular mechanisms underlying these effects. In a trypan assay, low concentrations of 602 killed AK cells to a greater degree than 5-fluorouracil ($p<0.05$). Alterations in cell signaling caused by 602 in AK cells were evaluated via fluorescence microscopy. 602 activated ATM, AMPK, and ULK1, resulting in inactivation of AKT, mTORC1 and mTORC2, reduced expression of K-RAS, N-RAS, HSP90, HSP70, and GRP78, reduced phosphorulation of ERBB 1/2/3/4, and increased phosphorylation of PERK and eIF2α. Mechanistic roles for individual proteins during the killing process were investigated via knockdown gene expression of suspected modulators of cell death. Knock down of Beclin1, ATM, AMPK, ULK1, or eIF2α suppressed the lethality of 602. Expression of constitutively active mTOR reduced its lethality. In control cells expressing LC3-GFP-RFP, 602 enhanced the formation of GFP+ vesicles after 4h. After 8h, the numbers of GFP+ vesicles declined, while RFP+ vesicle levels increased. Activated mTOR suppresed GFP+ autophagosome formation after 4h and 8h and no increase in RFP+ autolysosomes was observed. In summary, 602 induces autophagy, increases endoplasmic reticulum stress signaling, reducing expression of K-RAS and N-RAS, and inactivating AKT and mTOR. Expression of activated mTOR reduced killing, autophagosome formation and abolished autophagic flux. Our data argue that 602 causes cell death in AK cells via a targeted signaling mechanism and indicate that 602 may represent a novel therapeutic agent in the treatment of actinic keratoses.

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Crystal structure of sarecycline bound to the 70S bacterial ribosome reveals structural differences from other tetracyclines at atomic resolution

Z. Batool[2], I. B. Lomakin[3], Y. Polikanov[2], C. G. Bunick[1, 3]

[1]Dermatology, Yale University, New Haven, Connecticut, United States, [2]Biological Sciences, University of Illinois at Chicago, Chicago, Illinois, United States, [3]Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut, United States

Sarecycline is a new tetracycline-class antibiotic approved for the treatment of acne vulgaris. Tetracyclines share a common four-ring naphthacene core and inhibit protein synthesis by interacting with the 70S bacterial ribosome. Sarecycline is distinguished chemically from other tetracyclines because it has a 7-{[methoxy(methyl)amino]methyl]} group attached at the C-7 position of ring D. It represents the longest and largest C-7 moiety for any antibiotic in its class. To investigate the functional role of this C-7 moiety, we determined the crystal structure of sarecycline bound to the Thermus thermophilus 70S ribosome complex. 70S Thermus thermophilus ribosomes were mixed with tRNA(fMet), M-UAA mRNA, and sarecycline and then crystallized using vapor diffusion. X-ray data was collected at Argonne National Laboratory, and processed using HKL-2000, Coot, and Phenix software. A 2.8 Å resolution structure revealed sarecycline binds the 70S ribosome at the small (30S) ribosomal subunit A site. The polar edge of the naphthacene core contacted helices 31 and 34 of the 16S rRNA similar to other tetracyclines. Importantly, unlike other tetracyclines, sarecycline's C-7 moiety extends into the mRNA binding channel where it may interfere with mRNA movement through the channel or disrupt mRNA interaction with the anticodon of the A-site tRNA. Sarecycline is a new therapeutic option for acne vulgaris; therefore, clinicians should be aware that molecular differences exist in how sarecycline binds to the ribosome compared to other tetracyclines. Further research is needed to evaluate whether these structural findings correlate with clinical improvement in patients.

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Plasma exosomal miR-375-3p regulates ferroptosis in keratinocytes by targeting lipid transporter GPX4 in SJS/TEN

C. Zhang, G. Wang, M. Fu

Xijing Hospital, Fourth Military Medical University, Xi'an, China

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are life-threatening, cutaneous adverse drug reactions that are accompanied by keratinocyte cell death. Ferroptosis is a recently recognized form of regulated cell death driven by lipid-based reactive oxygen species (ROS) accumulation. However, the molecular mechanisms of ferroptosis regulation are still largely unknown in SJS/TEN. Exosomes are nanometer-sized membranous vesicles in various body fluids. They contain functional proteins, mRNAs, and miRNAs. Nevertheless, the potential roles of plasma exosomes and the underlying mechanisms in SJS/TEN have yet to be explored. In our study, we uncovers that exosomes isolated from plasma of SJS/TEN patients showed the expected size between 30 and 150nm by using transmission electron microscopy and flow nanoanalyzer. Then, exosomes were further characterized by western blot, and shown to be positive for exosomal markers CD9, CD63, CD81 and TSG101. Deep sequencing analysis and qRT-PCR of plasma exosomal derived miRNAs demonstrated that the miR-375-3p level was the markedly upregulated in the exosomes of 40 SJS/TEN patients, positively correlated with disease severity. Then, plasma derived exosomes from patients were internalized by human primary keratinocytes and promoted malondialdehyde (MDA), free iron, and lipid ROS accumulation. These factors are important ferroptosis markers. Additionally, ectopic expression of miR-375-3p suppressed glutathione peroxidase 4 (GPX4), an enzyme converts lipid hydroperoxides to lipid alcohols, resulting in increased ferroptotic cell death. Overexpression of GPX4 attenuated miR-375-3p mimic-mediated ferroptosis of keratinocytes. Lastly, knockdown of miR-375-3p inhibited ferroptosis, which completely prevented SJS/TEN-like responses in a mouse model of SJS/TEN. Our results indicate that GPX4 downregulated by the overexpressed miR-375-3p mediates keratinocyte ferroptosis in SJS/TEN patients. Circulating exosomal miR-375-3p might be used as a potential disease marker for the diagnosis of SJS/TEN.

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Improved local drug delivery with bioadhesive nanoparticles in the treatment of skin cancer

J. K. Hu[1], H. Suh[2], M. Qureshi[1], J. M. Lewis[1], W. M. Saltzman[2], M. Girardi[1]

[1]Dermatology, Yale School of Medicine, New Haven, Connecticut, United States, [2]Department of Biomedical Engineering, Yale School of Engineering and Applied Science, New Haven, Connecticut, United States

Nanoparticles (NPs) have been utilized to enhance delivery of rapidly-degraded chemotherapies in multiple animal tumor models. We explored the role of NPs in the local treatment of squamous cell carcinoma (SCC). Using biodegradable poly(lactic acid)-hyperbranched polyglycerol (PLA-HPG), we encapsulated camptothecin (CPT) to create nonadhesive NPs (NNPs), which were then chemically converted to bioadhesive NPs (BNPs) that have previously exhibited augmented association with tumor microenvironments. We characterized the NPs by hydrodynamic size (270 ± 103 nm), finding an improved surface area of effect relative to free CPT. We then assessed anti-tumor efficacy *in vitro* by exposing a murine SCC cell line (PDVC57) to free drug, empty NNPs, empty BNPs, NNP-CPT, or BNP-CPT, and found that BNP-CPT conferred significant cytotoxic advantage relative to controls ($p < 0.0001$). To examine the advantages of BNP-CPT *in vivo,* we transplanted PDVC57 into syngeneic C57Bl/6 mice. PDVC57 tumors were injected with free CPT, NNP-CPT, or BNP-CPT, and harvested 0, 48, and 240 hours later, at which point remaining CPT in each tumor was quantified. At 48 and 240 hours following treatment, a substantially greater percentage of CPT was recovered from tumors injected with BNP-CPT (48 h: 72.4%; 240 h: 44.7%) when compared to free CPT (48 h: not detectable, ND; 240 h: ND) and NNP-CPT (48 h: 13.4%; 240 h: ND), indicating enhanced BNP-CPT association within SCC tumors. This improved intratumoral drug retention translated to increased survival in the SCC mouse model, and tumors treated with BNP-CPT (12.5 mg/kg, 4.8% CPT loading) with adjuvant CpG (10 μg) exhibited significant growth retardation when compared to tumors treated with free CPT and adjuvant CpG (p=0.0229). Together, our results indicate that local delivery of encapsulated agents offers improved intratumoral drug retention and bioavailability, representing a viable non-surgical alternative for cutaneous malignancy.

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Rationale and design for the Kallikrein Inhibitor in Netherton Syndrome (KINS) pivotal clinical trial

K. W. Johnson[1], A. Hovnanian[2], J. Teng[3], A. S. Paller[4], K. Choate[5], P. Elias[6], Z. Laura[1], T. Nguyen[1], D. Smith[1], A. Hsu[1], L. Alani[1], C. Lai[7], L. Hsu[1]

[1]LifeMax Labs, Redwood City, California, United States, [2]Inserm, Paris, France, [3]Stanford University, Palo Alto, California, United States, [4]Northwestern University, Chicago, Illinois, United States, [5]Yale University, New Haven, Connecticut, United States, [6]UCSF, San Francisco, California, United States, [7]Lai Consulting, Palo Alto, California, United States

Netherton Syndrome (NS) is a potentially life-threatening autosomal recessive disorder involving skin barrier breakdown, inflammation, and allergy. NS affects individuals from birth through adulthood, but standard interventions provide limited benefit and there are no FDA- or EMA-approved therapeutics. We summarize the Kallikrein Inhibitor in Netherton Syndrome (*KINS*) study, the first Phase 2/3, multicenter, randomized, double-blind, vehicle-controlled trial to evaluate the safety, efficacy and tolerability of a novel topical therapeutic (LM-030) in NS patients. NS is caused by loss of function mutations in the *SPINK5* gene which encodes the serine protease inhibitor LEKTI. Hence, the epidermal barrier is broken down due to excessive activation of skin proteases, particularly kallikrein-related peptidase 5 and 7 (KLKs 5&7) and elastase 2 (ELA2). LM-030 (previously BPR277) is a low MW cyclic peptide with selective potent inhibition of KLK7 and moderate inhibition of ELA2, and favorable skin penetration with limited systemic exposure. As listed on the EU clinical trials site, a Phase 1/2 trial in 79 subjects was completed with daily topical application of ointment (predominantly 1%) onto defined target areas in healthy volunteers for 2 weeks or atopic dermatitis and NS patients for 4 weeks. The treatment was generally safe and well-tolerated. 7 of 15 NS patients exhibited a 'treatment effect' defined as a 2-point improvement from baseline in the Total Lesional Sign Score for Netherton Syndrome (TLSS-NS) vs 1 of 15 with vehicle control. Inhibitor concentrations in skin exceeded plasma levels by 1000-fold. These data support further evaluation of LM-030 in NS. The *KINS* study, targeted for initiation in the US and EU in mid-2020, will enroll over 100 patients. Additional design details will be presented in the presentation.

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Validation of an atopic dermatitis model in mice by repeated intra-dermal challenges with ovalbumin

H. Rashid, R. Samadfam

Discovery, Charles River, Senneville, Quebec, Canada

Background: Atopic dermatitis (AD) is a chronic inflammatory skin condition that significantly affects quality of life. A number of animal models have been developed over the years to mimic pathophysiology of AD. Among the different AD models, those induced by epicutaneous application of allergens and haptens are more common. However, limited absorption of peptide and protein allergens after epicutaneous application is challenging and requires long-term application of adhesive skin patches. In this present study, we examined induction of AD-like symptoms in mice with repeated intra-dermal challenges with the protein allergen, ovalbumin. Methods: BALB/c mice were first sensitized on Days 1 and 8 by intraperitoneal injection with a mixture of alum and ovalbumin (OVA). These mice were then challenged with OVA by intra-dermal injections in the right ear on Days 15, 19, 24 and 29. Ear thickness, redness and scaling were evaluated following the challenges. On Day 29, terminal serum, spleen and ear samples were collected for immunology and histopathology analysis. Results: The study was done in two phases. Following the first phase, 20µg of OVA and 2mg of alum for the sensitization and 20µg of OVA for the intra-dermal challenges were chosen as the optimal dose levels for induction of AD-like symptoms on the ear. Induction of AD also caused an increase in spleen mass and total IgE level in the serum. In the second phase, efficacies of standard steroidal drugs dexamethasone (oral) and clobetasol (topical) as well of the non-steroidal phosphodiesterase-4 inhibitor, crisaborole (topical) were assessed. While clobetasol and dexamethasone significantly attenuated the dermatitis scores, crisaborole had no effect in this model. Some additional immunology and histopathology assessments are currently on going. Conclusion: The current results show that AD-like symptoms can be induced in mice with repeated intra-dermal challenges with ovalbumin in the ear. This could eliminate the technical challenges involved with epicutaneous application of protein allergens for induction of AD models.

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The discovery of new therapeutic combinations for Merkel cell carcinoma by small-molecule synergy screening

K. Garman[1], T. Gelb[1], D. Urban[2], M. Hall[2], I. Brownell[1]

[1]Dermatology Branch, NIH, Bethesda, Maryland, United States, [2]NCATS, NIH, Bethesda, Maryland, United States

Merkel cell carcinoma (MCC) is a rare and highly metastatic neuroendocrine skin cancer. The majority of MCC tumors are virus positive (VP-MCC) and are associated with integrated Merkel cell polyomavirus, whereas the remainder of MCC are virus-negative (VN-MCC) tumors that are characterized by ultraviolet light associated mutations. Metastatic MCC is treated by immunotherapy or chemotherapy. Conventional chemotherapy is usually unbeneficial in MCC, and although immune checkpoint inhibitor (ICI) therapy can be effective, it is contraindicated in many patients and others have disease progression despite immunotherapy. To identify new treatments for MCC, we performed high-throughput small molecule screening of ~4,000 drugs for their ability to reduce MCC viability in VP-MCC and VN-MCC cell lines relative to immortalized control cell lines. We identified navitoclax as selectively effective against VP-MCC cell lines compared to VN-MCC and controls. Navitoclax use in the clinic is limited by a dose-dependent thrombocytopenia. To identify drug combination that will lower the effective dose of navitoclax and thereby decrease the risk of thrombocytopenia, we performed a synergy screen between navitoclax and each of the 1912 clinically relevant compounds in the NCATS Mechanism Interrogation PlatE (MIPE) library. This screen identified synergistic effects between navitoclax and multiple drug categories. High priority drug combinations are now being validating *in vivo* using pre-clinical xenograft models of MCC in order to identify novel therapeutic combinations with higher efficacy and lower side effects. By focusing on repurposing approved drugs, these combinations will be immediately available to patients who do not benefit from ICI.

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The evaluation of the application of glucocorticoids

X. Zhang, M. Pan

Dermatology, Ruijin Hospital Affiliated to Medical College of Shanghai Jiaotong University, Shanghai, China, Shanghai, Shanghai, China

The administration of glucocorticoids is widely used in the treatment of bullous diseases. However, long-term use of systemic glucocorticoids will cause some side effects, such as infection, diabetes, hypertension, osteoporosis and so on. Recently, the topical steroids were applied to reduce the side effects. [1,2] In order to investigation the side effects between the topical and systemic application of the steroids, we tried to designed the experiments based on the betamethasone application on 8-week-old C57BL / 6 male mice. The experiment was divided into two groups, topical glucocorticoids group and systemic oral glucocorticoids group. The topical group was divided into four doses of 0.044mg, 0.44mg, 0.88mg and 0mg per day. The systemic oral glucocorticoids group was divided into 0.044mg and blank control group. After 2 weeks of continuous administration， the effect of topical betamethasone on mice was examined. In the aspect of systemic effect, the weight and spleen index of mice decreased, and the pathological changes of liver, such as liver edema and pyknosis necrosis, were observed. In terms of the local effects on the back skin of mice, the expression of glucocorticoid receptor increased [3] and the thickness of the epidermis became thinner. Conclusion: The side effects of systemic use of glucocorticoids can also be seen in the topical use of glucocorticoids, especially in the case of large doses, which may be related to percutaneous absorption. So we advise side effects of topical glucocorticoids should be monitored regularly. [1] Ference J. D. Last A. R. Choosing Topical Corticosteroids[J]. American family physician, 2009, 79(2):135-140. [2] Hengge U.R. Ruzicka T. Schwartz R. A. et al. Adverse effects of topical glucocorticosteroids[J]. Journal of the American Academy of Dermatology, 2006, 54(1):0-15. [3] Roumestan C. Gougat C. Jaffuel D. et al. Glucocorticoids and their receptor: mechanisms of action and clinical implications][J]. La Revue de Médecine Interne, 2004, 25(9):636

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Investigation of omega-3 mechanisms involve in psoriatic plaque healing using a psoriatic skin model produced by tissue engineering

M. Simard[1, 2], S. Morin[1, 2], G. Rioux[1, 2], J. Fradette[1, 3], R. Pouliot[1, 2]

[1]Centre de recherche en organogénèse expérimentale de l'Université Laval/LOEX, Axe Médecine Régénératrice, Centre de Recherche du CHU de Québec-Université Laval, Québec, Quebec, Canada, [2]Faculté de Pharmacie, Université Laval, Québec, Quebec, Canada, [3]Département de Chirurgie, Faculté de Médecine, Université Laval, Québec, Quebec, Canada

Psoriasis is a dermatosis characterized by keratinocyte hyperproliferation, and abnormal epidermal differentiation. Clinical studies have shown that supplementation of the diet with omega-3 fatty acids could improve the symptoms of psoriatic patients. However, the mechanisms involved are still poorly understood.The aim of this study was to investigate the effects of α-linolenic acid (ALA) on the keratinocyte proliferation and differentiation of a psoriatic skin model. Healthy (HS) and psoriatic skin substitutes (PS) were produced according to the self-assembly method of tissue engineering, using culture media supplemented with 10 µM ALA. Gas chromatography analyses showed that the added ALA was efficiently incorporated into the phospholipids of the epidermis, since levels of ALA metabolites, namely EPA (7-fold) and DPA (3-fold) were significantly higher ($p<0.001$) in phospholipids of PS^{ALA+} than in PS^{-}. The epidermis of PS^{ALA+} was more organized and was significantly thinner than the epidermis of PS^{-} ($p<0.001$). Moreover, addition of ALA decreased keratinocyte proliferation, as fewer cells were stained with Ki67 in PS^{ALA+} epidermis than in PS^{-} epidermis. Expression of filaggrin and keratin 14 were respectively increased and decreased after supplementation with ALA, thus, showing a restored differentiation in PS^{ALA+}. Finally, protein kinase arrays revealed that among the 43 kinases analyzed the most striking change in the phosphorylation status in response to the ALA supplementation was ERK1/2, whose phosphorylation level was higher in the PS^{ALA+} than in the PS^{-} (2-fold). Taken together these results confirm that omega-3 fatty acids decrease the pathologic phenotype of psoriatic skin substitutes by normalizing keratinocyte proliferation and differentiation.

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Identification of highly potent and selective Interleukin-1 receptor associated kinase 4 (IRAK4) degraders for the treatment of hidradenitis suppurativa

A. Slavin, V. Campbell, M. Mayo, H. Rong, X. Zheng, N. Ji, M. Weiss, S. Rusin, K. Sharma, J. Gollob, N. Mainolfi

Immunology, Kymera Therapeutics, Cambridge, Massachusetts, United States

Interleukin-1 receptor associated kinase 4 (IRAK4) plays a central role in myddosome signaling via kinase and scaffolding functions, making it an attractive target for the treatment of TLR- and IL-1R-driven inflammatory diseases. IL-1 family cytokines and TLRs, are central to the pathophysiology of hidradenitis suppurativa (HS), a Th1- and Th17-mediated neutrophilic, chronic inflammatory skin disease. Kymera has developed orally administered hetero-bifunctional molecules that selectively target IRAK4 for degradation and elimination by the ubiquitin proteasome pathway. These degraders have broad and potent activity *in vitro* against IL-6, TNF-a and other proinflammatory cytokines and chemokines induced by TLR agonists and IL-1 family cytokines that is superior to IRAK4 kinase inhibitors. The ability to strongly suppress inflammation and superiority over small molecule kinase inhibitors is even more pronounced after combination of TLR agonists and IL-1β. *In vivo*, orally-dosed IRAK4 degraders are well-tolerated in rodent and dog species and achieve exposures leading to >95% protein knockdown in spleen, PBMC and skin. IRAK4 degraders are highly active in the mouse imiquimod psoriasis model, with reduction of skin thickening and both Th1 and Th17 cytokines. Additionally, IRAK4 degraders block neutrophil infiltration and IL-1b production in the mouse MSU air-pouch model. The demonstrated activity against TLR- and IL-1R-driven Th1 and Th17 inflammation *in vitro* and *in vivo*, coupled with favorable drug-like properties and strong pharmacodynamic effect in both circulating immune cells and skin, supports the development of IRAK4 degraders in HS and other autoimmune diseases. Entry into the clinic is planned for the later part of 2020.

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Effects of CCR4 antagonists in cutaneous T-cell lymphoma cells

J. S. Enriquez, P. Bijani, M. Duvic, X. Ni

Dermatology, The University of Texas MD Anderson Cancer Center, Houston, Texas, United States

CC chemokine receptor 4 (CCR4) is responsible for T-cell skin homing. It is overexpressed in type 2 helper T-cells (Th2) as well as cutaneous T-cell lymphoma (CTCL) cells. Mycosis fungoides (MF) and Sézary syndrome (SS) are two most common types of CTCL. Currently, Mogamulizumab, a humanized anti-CCR4 antibody, has been FDA-approved to treat MF and SS. However, the clinical response rates for SS patients was 30% and for MF patients was 22% and severe drug eruptions following treatment were reported. Thus, a more effective and less toxic strategy is needed for CCR4-targeted therapy. CCR4 antagonists have been studied in diseases where Th2 cells participate, such as asthma and atopic dermatitis, but little has been done for CTCL. Here, we tested two CCR4 antagonists, C021 (class I antagonist) and AZD2098 (class II antagonist), in MF derived cell line (MJ) and SS derived cell line (Hut78). The effects of two compounds on cell chemotaxis, proliferation, apoptosis, and Th2 cytokine secretion were assessed. As expected, CCR4 was highly expressed on MJ and Hut78 cells. MJ and Hut78 cells showed chemotactic responses to TARC/CCL17 and MDC/CCL22. Both C021 and AZD2098 inhibited chemotactic responses to TARC/CCL17 and MDC/CCL22 in MJ and Hut78 cells. Of note, C021 inhibited chemotaxis to CCL17 at a much lower concentration (IC50: 0.186 μM) than to CCL22 (1.3 μM) in MJ cells. AZD2098 inhibited chemotaxis to CCL17 at a much lower concentration (IC50: 0. 12 μM) than to CCL22 (0.866 μM) in Hut78 cells. Interestingly, only C021 downregulated CCR4 expression on cell surfaces. Only C021 inhibited cell proliferation, at a higher concentration (IC50: 3.21 μM in MJ cells; 5.98 μM in Hut78 cells), and induced cell apoptosis. AZD2098 had no such effect at any concentrations tested. In addition, the expressions of IL-5 and IL-13 proteins were decreased after treatment with C021 but not with AZD2098. Our results suggest that both CO21 and AZD2098 have inhibitory effects on chemotaxis of CTCL cells, but only C021 exerts other effects on CTCL cells. The *in vivo* study is on the way.

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Increased psoriasis severity with obesity is associated with insufficient adiponectin-mediated regulatory T cell response

H. Liu, S. G. Yu, K. R. Kwan, M. S. Bonkowski, A. S. Paller

Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Psoriasis is a chronic inflammatory skin disease characterized by Th17 cell skewing. Obesity, the most common psoriasis co-morbidity, is associated with greater disease severity. We showed earlier onset and greater disease severity, as well as reduced adiponectin expression, in high fat diet (HFD)-fed obese mice compared to regular diet (RD)-fed controls when psoriasis-like disease is induced by topically-applied imiquimod (IMQ). Obesity is associated with a reduced Treg response, but its role in psoriasis is unknown. We postulated that insufficient lesional Treg cells may promote greater severity of psoriasis with obesity. Immunohistochemical analysis of lesional skin of RD mice treated with IMQ to induce psoriasis revealed increased resident Treg cells compared with healthy (NT) skin (IMQ 116.7cells/mm^2 vs. NT 51.1 cells/mm^2, $p<0.01$). In obese mice, IMQ fails to increase Treg cells (16.5 cells/mm^2, $p<0.001$ vs. IMQ-treated RD mice; $p=0.99$ vs. HFD mice without IMQ) and increases psoriasis severity. Intraperitoneal treatment with ADP355, an adiponectin mimetic, restores in HFD mice IMQ-induced Treg upregulation (80.15 cells/mm^2; $p=0.67$ vs RD mice) and improves psoriasis-like disease severity (reducing modified Psoriasis Areas and Severity Index/PASI by 40.9%, $p<0.01$; epidermal thickness by 39%, $p<0.01$; and epidermal hyper-proliferation by 56%, <0.001), and expression of psoriasis-associated inflammatory markers (TNFA by 98%, DEFB4 97%, PI3 89%, IL17A 73%, IL6 57%, and IL22 49%; all $p<0.001$ vs. vehicle-injected HFD mice). PBMCs exposed to 72 h of TGF-β plus IL-2 yielded no increase in $CD25^+/FOXP3^+$ cells with ADP355 vs. PBS, suggesting that adiponectin mediates Treg cell recruitment or survival, but not differentiation. Overall, our findings suggest that adiponectin deficiency may contribute to the greater severity of psoriasis in obesity through a reduced regional Treg cell response.

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Pan-caspase inhibition is a novel immunotherapeutic against MRSA skin infections in mice

M. Alphonse, J. H. Rubens, R. Ortines, N. Orlando, A. Patel, D. Dikeman, Y. Wang, I. Vuong, D. Joyce, J. Zhang, M. M. Mumtaz, Q. Liu, C. Youn, G. J. Patrick, A. Ravipati, R. J. Miller, N. Archer, L. S. Miller

Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Staphylococcus aureus causes the majority of skin infections, and the emergence of methicillin-resistant *S. aureus* (MRSA) strains has created a public health threat. There is an unmet clinical need for non-antibiotic immunotherapies to combat MRSA. Herein, the pan-caspase inhibitor Quinoline-Val-Asp-Difluorophenoxymethyl ketone (Q-VD-OPH) was investigated for efficacy against an MRSA skin infection in mice. A single intraperitoneal injection of Q-VD-OPH 4 hour's post-infection substantially decreased skin lesion sizes and rapidly reduced bacterial burden compared with vehicle or untreated wildtype (wt) mice. Q-VD-OPH inhibited the inflammasome component ASC speck formation and caspase-1-mediated IL-1β production. However, Q-VD-OPH had similar therapeutic efficacy in mice deficient in ASC, IL-1β, caspase-1, or gasdermin D (the inflammasome-activated pore-forming protein). Interestingly, caspase-11-deficient mice treated with inhibitors to caspases 1 and 8 had similar enhanced immunity as Q-VD-OPH, suggesting that the activity of Q-VD-OPH occurred via inhibition of caspases 1, 8, and 11. Furthermore, Q-VD-OPH resulted in less cell death with increased TNF and TNF-producing monocytes/macrophages in the infected skin, which was critical for immunity as Q-VD-OPH had no efficacy in mice deficient in TNF or TNF/IL-1R and anti-TNF antibody-treated wt mice. Finally, Q-VD-OPH also enhanced immunity against *Streptococcus pyogenes* and *Pseudomonas aeruginosa* skin infections. Collectively, pan-caspase inhibition represents potential host-directed immunotherapy against MRSA and other bacterial skin infections.

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Dynamic cytokine profiles combined with ELISPOT assay are useful in immunologically confirming the dapsone hypersensitivity syndrome

H. Liu, L. Sun, Q. Zhao, F. Zhang

Shandong Provincial Hospital for Skin Diseases & Shandong Provincial Institute of Dermatology and Venereology, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, Shandong, China

Background: Dapsone hypersensitivity syndrome (DHS) is induced by drug-specific T cells. The specific dynamic cytokine profiles of DHS are unknown and the diagnosis of DHS in clinical practice remains a challenge. Objective: To investigate the DHS specific dynamic cytokine profiles, and explore specific enzyme-linked immunospot (ELISPOT) assay in the diagnosis of DHS. Methods: 14 types of cytokine levels (IFN-γ, Fas Ligand, Granzyme B, IL-4, IL-5, IL-6, IL-10, IL-13, IL-15, IL-17A, IL-21, IL-22, IL-23, and TNF-α) in DHS patients and dapsone tolerant patients carrying HLA-B*13:01 were analyzed using Luminex Bioplex assay. The performance of an IFN-γ/Granzyme B/IL-5 three-color fluoroSpot ELISPOT assay and Single-color ELISPOT assays based on traditional enzyme-tagged reagents in immunologically confirming DHS was evaluated in 16 DHS patients, four dapsone tolerant patients and 12 healthy donors. Results: Dynamic cytokine profiles showed that IFN-γ, IL-5, IL-13, Granzyme B and TNF-α were specifically up-regulated expression in DHS. In 16 patients with DHS, 9 (56.25%) patients were positive on IFN-γ ELISpot, 11 (68.75%) patients were positive on Granzyme B ELISpot and 11 (68.75%) patients were positive on IL-5 ELISpot, and when combination of IFN-γ, Granzyme B and IL-5 ELISPOT, the sensitivity increased to 87.5% (14/16) with the specificity of 100%. onclusions: An IFN-γ/Granzyme B/IL-5 ELISPOT assay with sensitivity of 87.5% was established, which could be treated as a promising tool in the diagnosis of DHS.

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Differential ligand binding distinguishes therapeutic from pathologic Aryl Hydrocarbon Receptor (AhR) modulating agents: Implications for inflammatory skin disease

S. Smith[2], K. McHale[1], K. Creech[2], D. Rickard[2], C. Jayawickreme[2], D. Wu[3], F. Rastinejad[4], D. Rubenstein[1]

[1]Dermavant Sciences Inc, Durham, North Carolina, United States, [2]GlaxoSmithKline, Upper Providence, Pennsylvania, United States, [3]Shandong University, Qingdao, China, [4]University of Oxford, Oxford, United Kingdom

The aryl hydrocarbon receptor (AhR) is a transcription factor highly expressed in epithelial cells and immune cells and is emerging as a therapeutic target for inflammatory skin disease. AhR activity can be influenced by several naturally occurring and manmade agonists and antagonists. Despite the discovery of 2,3,7,8-Tetrachlorodibenzodioxin (TCDD) as an AhR agonist, many compounds exert their effects via the AhR pathway, resulting in different physiological responses and clinical manifestations. Tapinarof (DMVT-505, GSK2894512, WBI-1001) is a naturally identified hydroxylated stilbene new molecular entity that is under investigation for the topical treatment of psoriasis (PsO) and atopic dermatitis (AD). Utilizing competition binding experiments in cellular assays and with purified AhR-ARNT heterodimers, we demonstrate that tapinarof and TCDD do not compete for binding to AhR. These data demonstrate that tapinarof and TCDD interact with AhR in distinct ways, likely engaging via distinct binding pockets on the receptor and leading to distinct biological outcomes. In support of this, we demonstrate that the functional consequences of AhR agonism by tapinarof and TCDD differ. Collectively, these data differentiate distinct AhR ligand classes, therapeutic AhR modulating agents (TAMA) versus pathologic AhR modulating agents (PAMA) and provide further mechanistic explanation for the differential effects of these ligand classes.

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Complementary sun protection of two antioxidants (OTZ and DTG) in a sunscreen emulsion based on skin release

C. Jacques[1], D. Bacqueville[1], C. Jeanjean-Miquel[1], C. Genies[1], M. Noizet[1], A. Tourette[1], D. Redoules[2], S. Bessou-Touya[1], H. Duplan[1]

[1]Pierre Fabre Dermo-cosmetique, Toulouse, France, [2]Avene Laboratories, Lavaur, France

Sunscreens have been shown to be extremely effective in preventing DNA lesions due to ultraviolet (UV) radiation. Indeed, recent studies indicate that the number of UV-induced lesions decreased up to 95% depending on sunfilter combination and sun protection factor (SPF). Thus, sunblockers protect the skin from the harmful effects of UV, but they do not provide 100% protection. As a result, sunscreens are now being developed with antioxidants to provide additional protection against oxidative stress-mediated cell damage especially reactive oxygen species (ROS). We investigated the bioavailability, release and antioxidative properties of a sunscreen formulation (emulsion) containing two antioxidants, oxothiazolidine (OTZ) and delta-tocopheryl glucoside (DTG) using reconstructed human epidermis (RHE) model. OTZ reacts directly with ROS to form taurine; while DTG is metabolized into α-tocopherol to achieve antioxidative activities. Their penetration and metabolism 0.5-24 h were measured after solar-simulated irradiation as well as their antioxidative responses on RHE models. Oxidative stress markers included malondialdehyde (MDA), superoxide dismutase (SOD) and catalase activities. The two antioxidants had different penetration profiles: OTZ was rapidly and extensively absorbed whereas DTG was slowly absorbed. The yield of protection increased over time, with maximal protection 2 h post-irradiation. DTG slowly penetrated in the RHE and was present in the epidermis at all post-irradiation timepoints, thus allowing a slow but constant supply of α-tocopherol over at least 24 h. By contrast, the OTZ antioxidative protection was immediate but short-lived due to its rapid penetration. These results indicate a complementary sunlight protective action of OTZ and DTG; with immediate bioavailability of OTZ, and a prolonged skin delivery of α-tocopherol from the slower bioavailability and metabolism of DTG.

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An *Aquaphilus dolomiae* extract modulates cutaneous sensitivity on *in vitro* models of neuro-inflammation

F. Lestienne, C. Viode, I. Ceruti, S. Carrere, S. Bessou-Touya, H. Duplan, N. Castex-Rizzi

PCO, Pierre Fabre Dermocosmetic, Toulouse, France

Substance P is one of the neurotransmitters mainly involved in neurogenic inflammation on cutaneous level, a key point in the pathogenesis of inflammatory skin disorders such atopic dermatitis (AD), associated pruritus, and sensitive skin. In vitro models evaluated the effect of the original biological extract of Aquaphilus dolomiae E0 (Ad-E0) on cutaneous neurogenic inflammation. Ad-E0 significantly inhibited SP-stimulated release of IL-1β and TNF-α from normal human epidermal keratinocytes; significantly and dose-dependently inhibited SP-stimulated activation of human mast cells; significantly inhibited veratridine-stimulated release of SP from human sensory neurons; modulated expression of genes involved in lipid synthesis, innate immunity, corneocyte scaffolding and epidermal differentiation in a histamine-sensitized reconstructed human epidermis model; and, when applied topically to ex vivo human explants, inhibited IL-8 and histamine release. Topically applied Ad-E0, once formulated, may improve neuro-inflammation in patients with inflammatory skin disorders.

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Identification of a human skin commensal bacterium that selectively kills cutibacterium acnes
A. ONeill[1], T. Nakatsuji[1], M. Williams[1], R. Mills[2], A. Hayashi[3], D. Gonzalez[2], R. L. Gallo[1]
[1]Dermatology, University of California San Diego, San Diego, California, United States, [2]Pharmacology, University of California San Diego, San Diego, California, United States, [3]Medicine, Tohoku University, Sendai, Japan
Cutibacterium acnes (C. acnes) is one of the most common bacterial species on human skin and can promote acne vulgaris. Most current therapeutics for acne are antibiotics that fail to discriminate C. acnes from the other skin resident microflora, many of which have important roles in health. Such approaches have significant drawbacks due to poor antimicrobial activity on the skin surface and emergence of antibiotic resistance. The microbiome represents a vast resource for drug discovery as its members engage in constant conflict to outcompete one another by deploying diverse strategies for survival. Our aim was to identify and develop new antimicrobials from the skin microbiome, as a biotherapy for acne. To do this, we conducted a functional screen of coagulase-negative staphylococci collected from swabs of the face and arms of healthy individuals and recorded their antimicrobial activity against C. acnes. Amongst the antimicrobial hits, we found that Staphylococcus capitis (S. capitis E12) strain exhibited potent and selective activity against all acne- and health-associated C. acnes strains tested, but not against several other important commensal bacteria. HPLC and LC/MS analyses of S. capitis E12 supernatant identified the antimicrobial metabolites as four distinct amphipathic phenol soluble modulin β (PSMβ) peptides. These PSMβ peptides were found to act synergistically and were not toxic to human keratinocytes. Formulation and application of a purified S. capitis E12 extract onto ex vivo pig skin colonized with C. acnes, resulted in a 1-log decrease (p> 0.02) in CFU after 24 h. More impressively, the extract applied topically onto SKH1 mouse skin colonized with C. acnes, resulted in a 3-log decrease (p>0.01) in CFU after 48 h. Overall, these data show how some peptides from the skin microbiome can be highly selective against C. acnes and highlights their potential application as a biotherapy for acne vulgaris.

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Treatment of facial flushing and erythema by carvedilol in rosacea patients with anxiety, a prospective randomized controlled clinical study
J. LI, Z. Song
Dermatology, First Affiliated Hospital of the Army Medical University, Chongqing, China
Introduction: Treatment of erythematotelangiectatic rosacea (ETR) is extremely challenging, because of the severe facial flushing and anxiety, which form a vicious cycle of reciprocal causation in the pathogenesis. There are no known specific treatments available, some cases have reported that beta-blokers play a role in the treatment of rosacea, but there are not yet any randomized controlled prospective, with larger seriers, clinical studies to evaluate the effects of systemic betabloker therapy in treating ETR. Neither study assessed the effect of it in patients with only ETR or persistent centrofacial erythema with transient flushing. Objective: the aim of this study was to compare the efficacy and safety between the monotherapies of carvedilol and minocycline and identify which treatments is better on persistent centrofacial erythema with transient flushing and anxiety. Materials and Methods: This is a prospective, single-center, single-blind, randomized, controlled clinical study. 102 patients received carvedilol 5mg bid and 51 patients received minocycline 100mg qd for 3 months. The rosacea-specific Qol instrument (RosaQol) ,Patientself assessment(PSA), Clinicians erythema assessment(CEA), Generalized anxiety disorderGAD-7) and Patient health questionnaire (PHQ-9) were performed by questonaires and take a picture of face every 2 weeks. Results：Long-term treatment of carvedilol showed a better controlling of the most common symptoms of rosacea than minocycline；No obvious side effects were found in rosacea patients with treatment of carvedilol；Long-term treatment of carvedilol improved the anxiety status of patients with rosacea. Conclusion: Our study found that compared to minocycline, carvedilol can significantly reduce erythema with flushing and anxiety of rosacea patient, also had a maintenance effect after drug withdrawal. The novel use of this drug could be an effective systematic treatment for flushing and erythema phenotype of rosacea(or ETR) .

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Discovery of new olfactory receptors in human keratinocytes: A potential role on skin stress response
R. Duroux[1], A. Mandeau[1], Y. Quesnel[2], E. Loing[1]
[1]R&D, Lucas Meyer Cosmetics, Toulouse, France, [2]ChemCom, Brussels, Belgium
Olfactory receptors (ORs) are mainly expressed in the nasal olfactory epithelium but can also be found in different cell types throughout the body to regulate physiological cell functions beyond olfaction. Recently, ORs have been detected to be functionally expressed in the skin when activated with odorants. Olfaction is known to be disturbed under stress condition and is widely used in aromachology to afford relaxing effect. Therefore, we questioned the role of skin olfactory receptors in response to this environmental factor. For the first time, we showed the presence of OR10A6, OR2AG2 and OR11H4 in human primary keratinocytes cells and on human skin.n ex-vivo skin model stressed with high levels of epinephrine, a stress hormone, during 9 days was developed. In addition to an acceleration of the cell activity and perturbation of DNA repair with a significant increase of Loricrin, G6pDH and gH2AX of respectively +132%, +27% and +71% ($p<0.05$), we observed by qPCR and immunofluorescence a decrease of the expression of the 3 ORs ($p<0.001$) compared to control. Identification of phenylethyl alcohol (PEA) and a PEA-rich rose extract as an agonist of these receptors was realized on cloned ORs. These agonists allowed to protect the skin against the impact of stress but also to restore ORs level of expression, especially for OR2AG2. Overall, these finding suggest that OR10A6, OR2AG2 and OR11H4 are involved into stress response mechanisms and demonstrate that olfactory receptors activation may serve as a new target to fight skin stress.

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Treatment of moderate-to-severe acne with once-daily tazarotene 0.045% lotion in pediatric patients: Pooled analysis of two phase 3 studies
L. F. Eichenfield[1], E. Tanghetti[2], E. Guenin[3]
[1]Departments of Dermatology and Pediatrics, University of California, San Diego School of Medicine and Rady Children's Hospital, San Diego, California, United States, [2]Center for Dermatology and Laser Surgery, Sacramento, California, United States, [3]Ortho Dermatologics, Bridgewater, New Jersey, United States
Acne, common in adolescence, can be treated with topical retinoids, such as tazarotene (TAZ). TAZ is an anti-inflammatory and comedolytic prodrug whose active form, tazarotenic acid, selectively binds retinoic acid receptors β and γ. A lower-dose 0.045% TAZ lotion was developed using polymeric emulsion technology, as gel or cream 0.1% TAZ formulations can cause irritation and limit use. In two phase 3, double-blind, vehicle-controlled 12-week studies (N=1,614), patients with moderate-to-severe acne were randomized (1:1) to receive TAZ 0.045% lotion or vehicle once-daily. The objective of this pooled post hoc analysis was to evaluate efficacy and safety of TAZ 0.045% lotion in pediatric patients aged 10-13 years (n=136) and 14-17 years (n=548). Efficacy assessments included changes from baseline in inflammatory/ noninflammatory lesions and treatment success (≥2-grade reduction in Evaluator's Global Severity Score [EGSS] and clear/almost clear). Adverse events (AEs) were also assessed. At week 12, mean percent reductions in inflammatory and noninflammatory lesion counts were significantly greater with TAZ versus vehicle in both age groups (least-squares mean inflammatory 10-13 years: -55.6 vs -37.0%; 14-17 years: -53.3 vs -41.2%; noninflammatory 10-13 years: -47.7 vs -28.2%; 14-17 years: -52.7 vs -32.9%; $P<0.01$ all). More patients achieved treatment success with TAZ versus vehicle in both age groups ($P<0.05$, both). There were no significant differences between TAZ-treated age groups in lesion count reductions or treatment success. Most treatment-emergent AEs with TAZ or vehicle were of mild or moderate severity in both age groups. Tazarotene 0.045% lotion in this new polymeric formulation was efficacious and well tolerated in adolescent patients with moderate-to-severe acne.
Funding: Ortho Dermatologics

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Long-term management of moderate-to-severe plaque psoriasis: Maintenance of treatment success following cessation of halobetasol propionate 0.01%/tazarotene 0.045% lotion

L. F. Stein Gold[1], M. G. Lebwohl[2], N. Bhatia[3], T. Lin[4], R. Pillai[5]

[1]Henry Ford Hospital, Detroit, Michigan, United States, [2]Icahn School of Medicine at Mount Sinai, New York, New York, United States, [3]Therapeutics Clinical Research, San Diego, California, United States, [4]Ortho Dermatologics, Bridgewater, New Jersey, United States, [5]Bausch Health US, LLC, Petaluma, California, United States

Psoriasis is an immune-mediated disease that may have frequent remissions/exacerbations. Treating psoriasis by combining tazarotene (TAZ) with a topical corticosteroid, such as halobetasol propionate (HP), may enhance efficacy while reducing side effects of HP, which limit long-term use. TAZ also sustains response posttreatment and may play a role in maintenance therapy. This 1-year, open-label study assessed a fixed-combination, once-daily HP 0.01%/TAZ 0.045% lotion in participants with moderate-to-severe psoriasis. HP/TAZ was stopped for those achieving treatment success (Investigator Global Assessment [IGA] score of clear [0] or almost clear [1]) at wk 8; those without treatment success continued with once-daily HP/TAZ. At wk 12, participants demonstrating ≥1-grade IGA improvement from baseline continued and were managed in 4-wk cycles (no treatment success: continued HP/TAZ; treatment success: no treatment until next evaluation). Maximum continuous exposure was 24 wk. Of 550 participants with post-baseline safety data, 318 (57.8%) achieved treatment success; 54.4% of those within the first 8 wk. A post hoc analysis evaluated maintenance of effect in participants that were enrolled ≥8 wk and who achieved clear during the study (n=56). Of these participants: 28.6% did not require any HP/TAZ retreatment after first achievement of clear, 53.6% did not require retreatment for ≥85 days, 62.5% for ≥57 days, and 83.9% for ≥29 days. Though the study design was limited by requiring individuals to stop using HP/TAZ lotion at the time of first treatment success, many patients achieved clear skin, half of whom did not require retreatment for at least 3 months. These data indicate a longer maintenance of therapeutic effect with HP 0.01%/TAZ 0.045% lotion. Funding: Ortho Dermatologics

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Small molecule blockade of T-type calcium channels by DX416 inhibits itch and reduces corresponding inflammation in acute and chronic itch mouse models

J. Kreitinger[1], L. Walker[1], V. Gadotti[2], N. Wageling[1], G. Zamponi[2], F. Diaz[1], P. Diaz[1, 3]

[1]DermaXon, Missoula, Montana, United States, [2]The University of Calgary, Calgary, Alberta, Canada, [3]BMED, University of Montana, Missoula, Montana, United States

The processing of itch signals is regulated by many different ion channels. Among those are T-type calcium channels which are important regulators that contribute to the firing behavior of pruriceptors, or itch sensing neurons. The human genome encodes three different T-type channels: Cav3.1, Cav3.2, Cav3.3. It has been shown recently that pharmacological blockade of T-type calcium channels inhibits acute itch and that immune cells can express Cav 3 channels with roles in effector function. Thus, we hypothesized that local administration of the novel small molecule T-Type calcium channel inhibitor, DX416, would decrease cutaneous itch both through reducing pruriceptors signals triggering itch and decreasing local inflammatory immune responses potentiating itch. To test this, we employed three murine models of itch including histamine-induced acute itch, chloroquine-induced acute itch, and acetone-ether-water (AEW)- induced chronic itch. Compared to vehicle treatments, intradermal administration of 30ug DX416 significantly inhibited itch (>65%, $p<0.05$, n=6) in all three models. Skin punch biopsies from treatment sites were used for custom RT-qPCR array analysis of cytokine and chemokine expression of each itch model. We observed marked alterations to cytokine and chemokine expression fitting a TH2 subset profile as well as decreased itch-related transmembrane proteins such as histamine receptor 4 (HRH4). In vitro studies in human peripheral blood mononuclear cells and monocyte-like cells supported that 5μM DX416 markedly reduced protein secretion of itch-related cytokines including IL-31 (60pg/ml Vehicle to 20pg/mL DX416, $p<0.05$) and TNFα (1500pg/mL Vehicle to 500pg/mL DX416, $p<0.05$). Collectively, our data revealed that small molecule blockade of T-type calcium channels represents a promising therapeutic strategy for the inhibition of itch and concurrent inflammation.

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Targeting 14-3-3e-CDC25A interactions to trigger apoptotic cell death in skin cancer

T. R. Holmes[1], J. Al-Matouq[1, 2], M. Holmes[1], N. Sioda[1], J. Rudd[1], L. Nicola[1], S. Lovas[1], L. Hansen[1]

[1]Biomedical Sciences, Creighton University, Omaha, Nebraska, United States, [2]Mohammed Almana College of Health Sciences, Dammon, Saudi Arabia

Non-melanoma skin cancer is the most common form of cancer worldwide. We previously documented an anti-apoptotic role for CDC25A in cutaneous squamous cell carcinoma (SCC), an activity dependent on its association with 14-3-3 proteins. We hypothesized that targeting CDC25A-14-3-3ε interactions may be an effective strategy for inducing skin cancer cell apoptosis. Co-immunoprecipitation revealed that CDC25A associated with 14-3-3ε, 14-3-3γ and 14-3-3ζ in SCC cells but not normal keratinocytes. Additionally, in SCC cells, overexpression of CDC25A induced Akt/BAD/Survivin pro-survival signaling while knockdown of 14-3-3ε inhibited pro-survival signaling, suggesting that 14-3-3ε and CDC25A similarly activate pro-surivval signaling pathways to suppress cell death. To target the interaction of 14-3-3ε with CDC25A for cancer therapy, we developed two novel phospho-peptides, pS and pT, corresponding to each of the 14-3-3 binding sites of CDC25A, to specifically interfere with 14-3-3ε binding to CDC25A. Co-immunoprecipitation demonstrated that pS and pT treatment successfully inhibited 14-3-3ε-CDC25A interactions in SCC cells. Subsequently, we found that peptides pT (IC_{50}=22.1 mM), and pS (IC_{50}=29 mM) induced SCC cell death as indicated by both neutral red cell viability assays and elevated Caspase-3/7 activity ($p=0.0127$ and $p=0.0485$, respectively). To determine the efficacy of pS and pT treatment in-vivo, SCC xenografts were treated with peptide or vehicle daily for two days. Immunofluorescent analysis of tumors revealed increased apoptotic cell death and decreased pro-survival P-Akt (S473) and Survivin in pS and pT treated tumors, demonstrating the effectiveness of the peptides in vivo. These findings illustrate the potential utility of inhibiting 14-3-3ε-CDC25A interactions for skin cancer treatment and lay a framework for the development of more efficacious derivatives of pS and pT peptides.

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Targeting CtBP-mediated proinflammatory gene transcription to treat skin inflammation

H. Li[1], C. Zhang[1], B. Li[2], M. Fujita[1], D. Norris[1], X. Wang[2], M. Huang[1]

[1]Dermatology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States, [2]Pathology, University of Colorado Anschutz Medical Campus, Aurora, Colorado, United States

Inflammatory skin diseases (ISDs) such as psoriasis and allergic contact dermatitis affects millions of people and poses a major public health burden. Aberrant cytokine production is a prominent characteristic of ISDs, although the molecular mechanisms underlying the imbalance between pro- and anti-inflammatory gene expression remain underexplored. C-terminal-binding protein (CtBP) 1 and 2 are transcriptional coregulators that repress diverse cellular processes. Our recently studies have uncovered a previously unrecognized proinflammatory role of CtBP in skin inflammation. CtBP1 overexpression in transgenic mouse keratinocytes causes a psoriasis-like phenotype including increased epidermal proliferation, immunocyte infiltration and proinflammatory cytokine expression in skin. Expression of the CtBPs is elevated in both human psoriatic skin lesions and the inflamed skin of two mouse ISD models, the imiquimod-induced psoriasis and the DNFB-induced contact hypersensitivity. Keratinocytes stimulated by imiquimod or DNFB exhibit transactivation of CtBP2 and CtBP-controlled proinflammatory genes that is accompanied by increased recruitment of CtBPs to the target promoters. Furthermore, we demonstrate that distinct CtBP-specific inhibitors can effectively suppress the expression of the CtBP target genes by evicting CtBPs from their target promoters and relieve symptoms of skin inflammation with topical treatment in both mouse ISD models. Together, these findings indicate that the CtBPs can promote skin inflammation by transactivating a select set of proinflammatory genes and suggest new avenues for therapeutic modulation of inflammation and immune responses in ISDs.

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The topical tanning agent dihydroxyacetone induces stress response gene expression and signaling in human reconstructed epidermis and SKH1 hairless mouse skin

J. Perer, J. Jandova, J. Fimbres, E. Jennings, J. Galligan, A. Hua, G. T. Wondrak

Pharmacology and Toxicology, College of Pharmacy and UA Cancer Center, University of Arizona, Tucson, Arizona, United States

Chemical tanning is widely regarded as a safe alternative to solar UV-induced skin tanning, but the cutaneous biology impacted by chemical tanning remains largely unexplored. Chemical tanning is based on the formation of melanin-mimetic cutaneous pigments ('melanoidins') from spontaneous glycation reactions between epidermal amino acid/protein components and reactive sugars including the glycolytic ketose dihydroxyacetone (DHA). Here, we have examined cutaneous effects of acute DHA exposure on cultured human keratinocytes, epidermal reconstructs, and mouse skin employing gene epression array analysis and immunodetection. In human HaCaT keratinocytes, DHA (1-20 mM; 6 h) did not impair viability while causing a stress response with activation of phospho-protein signal transduction [p-p38, p-Hsp27, p-eIF2α] and gene expression (*HSPA6*, *HMOX1*, *CRYAB, CCL3*), not observed in response to the tanning-inactive DHA-control glycerol. Formation of DHA-specific advanced glycation endproducts resulting from posttranslational protein adduction was confirmed by mass spectrometric detection of *N*-ε-(carboxyethyl)-L-lysine and N^7-carboxyethyl-L-arginine. Skin cells with CRISPR-Cas9 elimination of the carbonyl stress response gene *GLO1* (glyoxalase 1) displayed hypersensitivity to DHA cytotoxicity. A topical DHA regimen elicited a similar stress response in human epidermal reconstructs [Epiderm™; 1-10% DHA in carrier; 6-24 h] as revealed by expression array (*HSPA1A*, *HSPA6*, *HSPD1*, *IL6*, *DDIT3, EGR1*) and IHC analysis (CEL, HO-1, p-Hsp27). In SKH1 mouse skin, gene expression analysis confirmed a topical DHA-induced stress response substantiated by IHC-detection of CEL and p-Hsp27. Given the worldwide use of chemical tanners including DHA in consumer products these prototype data deserve further molecular exploration in living human skin.

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AHR agonist RLV102 suppresses inflammation in mouse models of eczema and psoriasis

Y. Zhou[1], L. Tang[2]

[1]University of British Columbia, Vancouver, British Columbia, Canada, [2]Resolvex Pharmaceuticals, Vancouver, British Columbia, Canada

Aryl hydrocarbon receptor (AHR) signal transduction pathway is involved in barrier formation and regulation of inflammation of the skin and the digestive system. Recently tapinarof, an AHR agonist, has shown effect for treatment of psoriasis in mouse models and in early phase clinical trials, revealing therapeutic potential by modulating AHR activities. Here we describe development of RLV102, a potent next generation AHR agonist and report its effect on psoriasis and atopic dermatitis in animal models. Structural modification of tapinarof was performed to improve its transcutaneous absorption, resulting in a compound that showed 5 times higher potency in inducing expression of AHR target gene, Cyp1A1 and filaggrin in cell based assays. In imiquimod induced psoriasis model and MC903 induced atopic dermatitis model, RLV102 significantly inhibited development of skin inflammation at concentrations as low as 0.1% when applied topically. The results reported revealed that RLV102 is a novel AHR agonist with therapeutic potential for treatment of chronic inflammatory skin diseases such as psoriasis and eczema.

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A novel treatment for skin repair using a combination of a MR antagonist + Vitamin D3

D. Biyashev, U. V. Onay, S. Evans, M. Demczuk, K. Lu

Dermatology, Northwestern University, Chicago, Illinois, United States

Exposure of the skin to toxic chemotherapy agents such as nitrogen mustard (NM) triggers activation of inflammatory dermal macrophages (Macs) with high expression of iNOS and TNFa resulting in delayed wounds. We have shown that Intervention with vitamin D3 (VD) mitigates Mac-mediated inflammation resulting in skin repair. Data from our in vitro drug screen using RAW 264.7-NFkB reporter cell line shows that VD with spironolactone (SP) has additive inhibition of NFkB activity. SP is a mineralocorticoid receptor antagonist known to be protective in chronic heart failure and other clinical conditions due to its abilitiesto delay progression of tissue injury and inhibit inflammation. Therefore, we wanted to investigate in vivo whether SP alone or +/- VD has increased abilities of mediating skin repair. Using our skin injury model, we observe that the combination treatment (SP + VD) prevents tissue swelling in vivo at 24, 48, and 72 hr post injury (p=0.03, n=8-10) as measured by bi-fold thickness. SP +VD delayed the formation of skin necrosis and hemorrhagic crust development, not seen with SP alone. The combination treatment results significantly in more rapid skin repair by day 14 (35% wound reduction, p=0.016, n=9) and day 21 (25% wound reduction, p=0.01, n=9). Analysis of skin immune cells by flow cytometry shows that SP+VD results in a significant increase in skin Macs (p=0.04, n=13-14) that are of the repair M2 phenotype ($CD206^+/Ly6C^-/F4\text{-}80^+$) vs. inflammatory M1 phenotype ($CD206^-/Ly6C^+/F4\text{-}80^+$). To determine the potential mechanism of the rapid repair, whole skin lysates were analyzed for hallmark inflammatory factors. We observe significant reduction (50-60%) in MMP9, CCL2, IL-1a, IL-b, and iNOS (p=0.0069, p=0.015, p=0.04, p=0.03, and p=0.01, respectively, n=5). A priori, spironolactone is not known or predicted to play a role in acute wound repair or reduction of tissue destructive factors in the skin. Taken together, these data demonstrate a novel treatment regimen to augment inflammation following skin injury to promote wound repair.

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Inverse docking assisted identification of flavonols as c-Kit, CDK2 and mTOR inhibitors for melanoma and non-melanoma skin cancer management

T. Roy, S. Banang-Mbeumi, S. Boateng, R. N. Chamcheu, A. Walker, J. Chamcheu

College of Pharmacy, University of Louisiana at Monroe, Monroe, Louisiana, United States

Melanoma and non-melanoma skin cancers (NMSCs) are two major forms of skin cancers. Melanoma is the most aggressive form of skin cancer related deaths while basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are most prevalent forms of NMSCs with over 5.3million yearly cases in the US. Due to huddles including resistance, bioavailability and adverse effects with available treatments, there is the need for effective strategies against skin cancer progression. The cyclin dependent kinase-2(CDK2), c-kit and mammalian target of rapamycin (mTOR), are attractive targets for new anticancer drug development. We recently identified fisetin, a natural bioflavonol as an anticancer agent targeting the mTOR central signaling. Here, we synthesized 22 new flavonol derivatives alongside fisetin, and employed inverse *in-silico,* integrated *cellular and kinase-based* screening approaches for identifying novel CDK2, c-kit, and mTOR inhibitors. These agents were evaluated for *in-vitro* anticancer activity against human melanoma (A375), and two NMSC (UW-BCC1 and A431), compared to normal (HaCaT and melanocytes) cells. Treatment with (0.0-40μM) of compounds exhibited significant dose-dependent decreases in cell growth/viability with minimal effects on normal cells ($p<0.05$). Eleven identified CDK2, c-kit, and mTOR kinase inhibitors showed higher anti-cancer activity with over 3-100-folds lower IC_{50} IC_{50} (0.2, 1.50, 4.7, 5.30, 8.2, 8.3, and 8.6) μM compared to fisetin reference (*$p<0.001$*. Furthermore, the potent derivatives, markedly modulated wound closure, colony formation, induced apoptosis (evident by increased caspases-3/Bax/Bcl2 expression), and modulated the increases in deregulated targets in melanoma and NMSC including phosphorylated p70S6K, c-kit, mTOR, VEGF and ERK1/2. These data identify novel more active flavonol compounds as a promising therapeutic lead to be further developed for the control of melanoma and NMSCs.

Photobiology

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Synergistic effect between cigarette smoke and sunlight on human keratinocytes

A. Grenier[1, 3], P. J. Rochette[1, 2], R. Pouliot[1, 3]

[1]Centre de recherche en Organogénèse Expérimentale de l'Université Laval/LOEX, Axe Médecine Régénératrice, Centre de recherche du CHU de Québec-Université Laval, Québec, Quebec, Canada, [2]Département d'ophtalmologie et ORL-chirurgie cervico-faciale, Faculté de médecine, Université Laval, Québec, Quebec, Canada, [3]Faculté de Pharmacie, Université Laval, Québec, Quebec, Canada

Several environmental factors influence the skin aging process, including exposure to sun and cigarette smoke. Studied separately, these factors are known to accelerate skin aging. However, effects of a potential synergy between these two factors have been poorly characterized. It is well known that some cigarette smoke components can accumulate in the skin by contact or by systemic effect after inhalation and that solar rays can penetrate the epidermis and dermis. The aim of this study is to assess the harmful effects of this synergy on skin and more precisely on skin aging. A cigarette smoke extract (CSE) was obtained by capturing the soluble fraction of cigarette smoke using an impinger. Sun exposure was performed with a solar simulator at 14.5 and 29 kJ/m^2 of UVA, representing 15 and 30 minutes of sun exposure at its zenith. The CSE phototoxicity was determined on human keratinocytes using a test assessing the cellular metabolic activity (MTS assay). These two environmental factors alone showed no cytotoxicity up to the maximal doses tested. When irradiated, CSE was highly phototoxic. Indeed, keratinocytes exposed to 5% CSE showed a 39±1% and 2±17% cellular viability when exposed to 14.5 kJ/m^2 and 29 kJ/m^2 UVA respectively (p-value<0.001 compared to PBS control). Pre-treating keratinocytes with an antioxidant prior to exposure improved cell viability caused by the synergy between CSE and sunlight. Our results show a synergistic toxicity between cigarette smoke and solar irradiation on skin cells and that toxicity is, at least in part, caused by a photo-oxidation reaction. Our work is now focussing on deciphering and further investigating the mechanisms involved in this synergy.

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Skin squamous cell carcinoma (SCC)-derived exosomes after ALA-PDT treatment deliver microRNA-3473 to induce M1 macrophage polarization via NF-κB pathway

Q. Zeng, X. Wang

Institute of Photomedicine, Shanghai Skin Disease Hospital, Shanghai, China

Topical ALA-mediated PDT, ALA-PDT, is a novel therapeutic modality widely used to treat actinic keratosis, Bowen's Disease, superficial skin SCC, and other cancerous and precancerous skin diseases. Several studies have proved that ALA-PDT can inhibit SCC growth and reduce tumor volume. Subsequent research suggested that ALA-PDT not only directly induced tumor cells apoptosis, but also improve tumor microenvironment through regulation of immune cells. However, the anti-tumor immune function of ALA-PDT is still need to be elucidated. Exosomes are emerging as important elements that participate in intercellular communication and tumor microenvironment modulation, but the exact mechanisms by which tumor exosomes facilitate the generation of tumor microenvironment remain unclear. Here we investigated the effects of SCC-derived exosomes after ALA-PDT treatment on macrophage polarization. We also performed microRNA sequencing analysis of exosomes to identify the microRNA that mediated macrophage polarization. The microRNA-associated intracellular signaling pathway in macrophages was further investigated. Compared with no ALA-PDT treated exosomes (N-PDT-EXOs), ALA-PDT treated exosomes (PDT-EXOs) markedly induced M1 macrophage polarization, which subsequently inhibited SCC proliferation, migration and invasion in vitro and in vivo. MicroRNA sequencing analysis identified miR-3473 as the most enriched microRNA in PDT-EXOs. Further investigation confirmed that miR-3473 mediated PDT-EXOs-induced M1 macrophage polarization by activating NF-κB signaling pathway. Our study elucidated a mechanism that ALA-PDT influence M1 macrophage polarization via PDT-EXOs, which could facilitate the formation of the immune-activated microenvironment. Moreover, PDT-EXOs also promote dendritic cells (DC) maturation in vitro and in vivo. Thus, our research may contribute to in-depth molecular understanding of the ALA-PDT on anti-tumor immune function.

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UVB radiation with high photon density induces dendritic cell maturation and contributes to cutaneous immune suppression via Treg cell expansion

C. Lan, S. Huang, S. Lai

Department of Dermatology, Kaohsiung Medical University Hospital and Kaohsiung Medical University, Kaohsiung, Taiwan

Phototherapy is an important treatment modality used in dermatology practice, and UVB radiation is often used for treating inflammatory skin disorders due to its immune suppressive effects. Currently, several UVB emitting radiation with different irradiance (W/cm2) are available. Clinical reports have shown different response profiles from devices emitting similar wavelengths at different irradiance. We previously showed that at equivalent fluence (mJ/cm2), high irradiance (HI) UVB imparted more immune suppressive effect as compared to its low irradiance (LI) counterpart. The current study was launched to explore the mechanisms involved. Using animal model, we showed that at equivalent fluence, HIUVB induced significantly higher immunosuppressive effect as compared to its LIUVB counterpart. Moreover, induction of more T regulatory cells (Treg) was noted in HIUVB treated group as compared to its LIUVB treated counterpart. It was recognized that dendritic cell (DC) maturation after UVB induces Treg cell proliferation. Using cell model, we showed that at equivalent fluence, HIUVB induced significantly higher HLA-DR, C80, CD86 expression on DCs as compared to its LIUVB counterpart. Additionally, at equivalent fluence, conditioned media (CM) from keratinocytes (KC) treated with HIUVB induced significantly more DC maturation as compared to CM derived from KC treated with equivalent fluence of LIUVB. Previously, aryl hydrocarbon receptor (Ahr) activation was shown to play a part on DC induced immune suppression. HIUVB treated DC and KC showed higher activation of Ahr as compared to their respective counterparts treated with equivalent fluence UVB at LI. Taken together, our results suggest that at equivalent fluence, HIUVB is more capable of immune suppression through induction of Treg expansion as compared to ts LIUVB counterpart, and differential DC modulation due to difference in UVB irradiance contributed significantly to the observed phenomenon.

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A novel painless ALA-PDT in the treatment of skin diseases

X. Wang, Q. Zeng, L. Shi, P. Wang

Institute of Photomedicine, Shanghai Skin Disease Hospital, Tongji University School of Medicine, Shanghai, China

Pain during conventional photodynamic therapy (C-PDT) is the main limiting adverse effect in its use in dermatology. During the course of C-PDT, most patients experience sensations of burning and tingling pain that vary in intensity. In some cases, patients have to stop the treatment because of unbearable pain. It is generally believed that C-PDT pain is mediated by free radicals generated by PpIX; ROS can either stimulate nerve endings directly or mediate pain through inflammatory by-products. Pain remains the top obstacle that prevents C-PDT application to all patients. Here, we modified C-PDT into a painless PDT treatment (Modified photodynamic therapy , M-PDT, also named novel painless ALA-PDT) by decreasing ALA incubation time and increasing light dose. Our study showed that pain in M-PDT group was significantly reduced in the treatment of actinic keratosis, acne, and condyloma acuminate. Even, in some cases the pain almost vanished. And M-PDT has better efficacy than C-PDT. Further investigation confirmed that M-PDT also inhibit tumor growth on cutaneous squamous cell carcinoma (SCC). Interestingly, compared with C-PDT, M-PDT induced more ROS which should stimulate nerve endings but not. We elucidated a mechanism that the low average amount of ROS may be the reason why M-PDT is painless. Our findings indicate that M-PDT have wide clinical value in skin disease.

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Macrophage depletion preserves dermal collagen in UVB exposed mice
M. Sharma[1, 2], V. Werth[1, 2]
[1]Dermatology, CMCVAMC, Philadelphia, Pennsylvania, United States, [2]Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States
We previously reported that Etanercept blocks UVB-induced recruitment of neutrophils and macrophages into the dermis in mice, but paradoxically, accelerates loss of dermal collagen by increasing breakdown of mature collagen and decreasing synthesis of procollagen. To examine the role of macrophages in dermal collagen preservation during UVB exposure, we performed macrophage depletion in mice. Macrophages increase inflammation and tissue remodeling in many conditions by producing MMPs and TGFβ. MMPs can degrade collagen and TGFβ can induce procollagen synthesis. C57BL/6J mice were treated i.p with 2 doses (0.15 ml) of Clophosome-A-Clodronate Liposomes (CCL) of 48h interval. CCL treatment was started 5 days before UVB exposure. Mice were UVB-irradiated (100mJ/cm^2/d for 5d) and sacrificed 3h after the last exposure. Collagen fragmentation was evaluated by immunoblot for Type I collagen. Skin sections were stained by IHC for procollagen, and by picrosirius red for collagen fibers. Under circular polarized light, picrosirius red differentiates collagen fibers as red or yellow (mature) and green (thin) fibers. Red fibers were decreased in UVB-irradiated mice compared to non-irradiated controls ($p<0.01$). Macrophage-depleted UVB-treated mice showed more red fibers relative to control UVB-treated mice ($p<0.001$). UVB increased fragmentation of collagen compared to controls. CCL treatment prevented UVB-induced collagen fragmentation *vs* UVB-exposed mice. MMP13 was significantly increased in UVB-irradiated mice *vs* sham and CCL-treated controls ($p<0.001$). Macrophage-depleted UVB-treated mice showed significantly less MMP13 *vs* UVB-treated mice ($p<0.001$). CCL treatment increased the synthesis of procollagen and TGFβ in UVB-irradiated mice ($p<0.001$) more than UVB-treated mice. In conclusion macrophage depletion prevents collagen loss, associated with increased synthesis of procollagen, increased TGFβ and less degradation of mature collagen in UVB-treated mice associated with decreased MMP13. These studies suggest macrophages importantly contribute to collagen alteration in acute photodamage.

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Red light emitting diode (LED) light treatment promotes memory through up-regulation of *trpm4* in Zebrafish
S. Yu[1], M. You[2], W. Yang[2], C. Cheng[2], H. Chang[3], H. Yu[4]
[1]Department of Dermatology, Kaohsiung Medical University, Kaohsiung, Taiwan, [2]National Health Research Institutes, Miaoli County, Taiwan, [3]National Applied Research Laboratories, Hsinchu, Taiwan, [4]Kaohsiung Medical University, Kaohsiung, Taiwan
Although light from light emitting diodes (LEDs) has become a very common and important environmental factor in our daily lives, there is limited research regarding LED light's possible effects on biological functions on the skin and the brain. Herein, we investigated the long-term effects of white (WL), blue (BL), and red (RL) LED light on cognitive learning and memory recall by using a zebrafish animal model. Zebrafish that were 36 days post fertilization (dpf) were used for light exposure. Light intensity was 7.2 J/cm^2 for 120 mins per day for 30 days. A water T-maze was used to evaluate fish cognitive learning and memory. A memory recording test was performed using a T-maze but with the cue cards swapped. To examine the long-term effect, we used zebrafish at the age of 5 months, 7 months, and 11 months. The memory recording test showed that, at 11 months, the RL group had the best memory recall in finding the target zone even after the target arm was changed. By using qPCR, the expression levels of *trpm4*, *grin2aa*, and *dlg4* in the skin were increased in RL compared with the control (*trpm4*, RL = 3.43±0.29, $P<0.001$; *grin2aa*, RL = 2.49±0.19, $P<0.001$; *dlg4*, RL = 1.9±0.18, $P<0.05$). In addition, the up-regulation of *trpm4* in the brain (RL = 1.67±0.16, $P<0.01$) correlated to enhanced memory following RL treatment. The up-regulation of *trpm4* in the brain was only observed in the RL group, not in WL and BL groups. Our results identify a light-based stimulation system enhances long-lasting zebrafish memory, which has the potential to provide important insights into the relationship between LED lighting and animal behavior.

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Solar simulated light induces cutaneous SCC in inbred mouse strains: Development of a clinically relevant mouse model
A. C. Adams[4], A. M. Macy[4], H. Cui[3], J. Merida[2], S. E. Dickinson[3, 4], D. J. Glembocki[2], D. J. Roe[1, 3], K. T. Hastings[4, 3]
[1]Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, Arizona, United States, [2]US Dermatology Partners Pathology Lab, Scottsdale, Arizona, United States, [3]University of Arizona Cancer Center, Tucson, Arizona, United States, [4]University of Arizona College of Medicine, Phoenix and Tucson, Arizona, United States
To address the need for clinically relevant cutaneous squamous cell carcinoma (cSCC) mouse models, we exposed SKH-1, FVB/N, Balb/c and C57BL/6 mice to solar simulated light (SSL), the environmental etiologic factor in human disease. Outbred SKH-1 mice are highly susceptible to SSL-induced tumor formation. However, an outbred strain limits the ability to evaluate MHC-restricted, antigen-specific T cell responses and perform studies with genetic mutants. The susceptibility of inbred strains FVB/N, Balb/c and C57BL/6 is unknown. UVA-340 bulbs were used to deliver SSL, because the ultraviolet spectrum closely resembles that from the sun. Mice were exposed to SSL 3 times per week starting with 1.2 kJ/m^2 UVB and 13.9 kJ/m^2 UVA. The dose was increased by 10% weekly to a maximum dose of 10 kJ/m^2 UVB and 115.4 kJ/m^2 UVA. SKH-1 mice received 22 weeks of SSL exposure with a cumulative dose of 257 kJ/m^2 UVB and 2,967 kJ/m^2 UVA. Other strains received 42 weeks of SSL exposure with a cumulative dose of 856 kJ/m^2 UVB and 9,884 kJ/m^2 UVA. SKH-1 mice were most susceptible to SSL-induced tumor formation with a median tumor onset of 19 weeks compared with 38 and 41 weeks for FVB/N and Balb/c mice, respectively ($p < 0.0001$). Tumors were histologically confirmed as papillomas (cSCC in situ) and invasive cSCC. At 44 weeks after the initiation of SSL exposure, no tumors had developed in C57BL/6 mice. A disadvantage of the SSL-induced tumor model is the long timeframe required to generate tumors. To address this disadvantage, we generated cell lines from the SSL-induced cSCC tumors in inbred mice. Thus, Balb/c and FVB/N mice are susceptible to SSL-induced cSCC formation. Syngeneic cSCC cell lines can be developed as transplantable cSCC models. These complementary approaches will facilitate future investigation.

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UVB-generated microvesicle particles mediate systemic immunosuppression
L. Liu[1], C. M. Rapp[1], S. Zheng[3], J. B. Travers[1, 2]
[1]Pharmacology & Toxicology, Wright State University, Dayton, Ohio, United States, [2]Dayton Veterans Administration Medical Center, Dayton, Ohio, United States, [3]The Ohio State University, Columbus, Ohio, United States
Microvesicle particles (MVP) are small membrane bound particles released from cells that have been demonstrated to act as signal transporters between cells via their abilities to transport bioactive molecules. Previously our laboratory has reported that environmental injuries such as ultraviolet B radiation (UVB) and thermal burn injury can generate MVP release via a novel pathway involving the Platelet-activating factor (PAF) receptor in epithelial cell lines and human skin. Our current studies using pharmacologic and genetic approaches demonstrate that MVPs released from keratinocytes in vitro and mice in vivo in response to UVB (UVB-MVP) are dependent upon PAF receptor and acid sphingomyelinase (aSMase). We identified the calcium sensing receptor (CaSR) as a marker for keratinocyte-derived MVP. Treatment of mice and humans with UVB resulted in the formation of UVB-MVP in plasma which were CaSR-positive, indicating that UVB-MVP derived from the epidermis travel systemically. We also report that UVB-MVP express cytokines as well as high levels of PAF activity. Finally, as PAF mediates the delayed systemic immunosuppressive effects of UVB, we determined the role of UVB-MVP in UVB-mediated inhibition of contact hypersensitivity responses to the neoantigen dintrofluorobenzene using aSMase-deficient mice. These studies reveal that UVB does not generate systemic immunosuppression in mice lacking aSMase, yet PAFR agonists given systemically are immunosuppressive. These findings suggest that MVP provides a mechanism by which the metabolically labile bioactive lipid PAF leaves the epidermis in response to acute environmental stressors like UVB. Moreover, targeting UVB-MVP could provide a novel strategy to block UVB-induced immunomodulatory effects.

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Evidence that wounding of geriatric skin which upregulates IGF-1 levels protects against both abnormal carcinogenic UVB responses as well as from the development of non-melanoma skin cancer

J. B. Travers[1], M. G. Kemp[1], D. Spandau[2]

[1]Pharmacology, Wright State University, Dayton, Ohio, United States, [2]Indiana University, Indianapolis, Indiana, United States

UVB irradiation causes specific DNA damage to keratinocytes that can lead to cancer-causing mutations if they are allowed to persist in proliferating cells. Previously, we demonstrated that the activation status of the insulin-like growth factor-1 receptor (IGF-1R) regulates the cellular response of keratinocytes to UVB exposure. Briefly, geriatric skin is deficient in IGF-1 expression resulting in an aberrant IGF-1R-dependent UVB response consisting of basal keratinocytes proliferating while still harboring unrepaired DNA damage. This abnormal UVB response contributes to the development of aging-associated non-melanoma skin cancer, especially squamous cell carcinoma. As the dermal fibroblast is the source of IGF-1 in skin, and aging results in senescent fibroblasts which produce less IGF-1, we have used wounding strategies which enhance IGF-1 expression in an attempt to normalize the abnormal, pro-carcinogenic geriatric UVB response. Here we demonstrate that localized wounding of geriatric skin with fractionated laser resurfacing (FLR) results in decreased numbers of senescent fibroblasts and increased levels of IGF-1. Moreover, acute UVB treatment of previously wounded skin results in decreased numbers of basal keratinocytes co-expressing thymine dimers and the proliferative antigen Ki-67, in comparison to UVB-treated control (not wounded) skin. Long-term studies reveal that the wounding effect induced by FLR appears to be durable in geriatric subjects at one- and two-years post-treatment. Finally, a prospective interventional study treating one upper extremity (forearm & wrist) of geriatric subjects with considerable actinic damage (>5 actinic keratoses on each arm) resulted in decreased numbers of non-melanoma skin cancers developing on the FLR-treated vs untreated extremity. These studies suggest that wounding procedures could protect geriatric skin from the abnormal pro-carcinogenic UVB responses associated with aging.

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UVB induced EMT-like phenotype in keratinocytes is mediated by TLR3 activation

A. Schneider, R. Feehan, C. Garner, Z. Cong, A. Flamm, E. Billingsley, A. Nelson

Dermatology, Penn State College of Medicine, Hershey, Pennsylvania, United States

Risk factors for non-melanoma skin cancer (NMSC) include UVB exposure, genetic mutations, immunosuppression, and chronic inflammation. Activation of Toll-Like-Receptor 3 (TLR3), which recognizes dsRNA, leads to downstream activation of NF-κB and an upregulation of inflammatory cytokines. TLR3 protein expression is higher in moderately differentiated squamous cell carcinomas (SCCs) and infiltrative basal cell carcinomas (BCCs) compared to well-differentiated SCCs by immunohistochemistry ($n > 6$) suggesting TLR3 expression correlates with more aggressive NMSCs. We hypothesized that UVB induced keratinocyte damage leads to TLR3-dependent signaling promoting an epithelial-to-mesenchymal transition (EMT)-like phenotype, which may contribute to NMSC carcinogenesis. *In vitro*, normal human embryonic keratinocytes (NHEKs) exposed to UVB (10 mJ/cm^2) or a TLR3 agonist, (poly(I:C), 20 mg/ml), induced TLR3 mRNA, protein expression, and pathway activation ($n \geq 6$; $p < 0.05$). *In vivo*, GLI1, Notch1, and p53 mRNA expression is altered in sun-exposed vs non-sun-exposed human skin and *in vitro* in UVB and poly(I:C) treated NHEKs. Strikingly, both UVB and poly(I:C) treated NHEKs exhibit a sustained EMT-like morphology with ~35% of keratinocytes developing a spindle-like phenotype. Gene expression of key EMT-associated transcription factors, ZEB1 (3-fold), SNAI1 (2-fold), and TWIST1 (2-fold) are significantly elevated ($n \geq 12$; $p < 0.05$) and EMT proteins, vimentin and fibronectin, are increased more than 2-fold with TLR3 activation ($n = 2$; $p < 0.05$). *In vitro* scratch assay data suggests increased migratory capacity in TLR3 activated keratinocytes. Finally, chemical inhibition of TLR3 with competitive inhibitor CU CPT 4a prior to UVB or poly(I:C) treatment significantly ($n \geq 6$; $p < 0.05$) reduces EMT gene expression and phenotypic changes. All together, these data suggest that TLR3 activation leads to EMT-like changes in keratinocytes which likely contributes to NMSC development.

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Low-level pulsed wave red light induces type I procollagen protein and ATP production at shorter treatment times as compared to continuous wave red light

W. Li, P. Thepmankorn, R. Parsa, M. Southall

Johnson and Johnson Consumer Inc, Skillman, New Jersey, United States

Low-level light therapy uses non-thermal irradiance to modulate cellular functions via laser or light emitting diodes (LEDs). Red light has been clinically shown to stimulate wound healing, improve hair growth, relieve pain and inflammation as well as reduce skin wrinkles. Recent studies suggest that the biological responses to continuous wave light treatment may decrease or desensitize over time, and therefore continuous wave light treatment may not provide optimum efficacy. Thus, we sought to characterize the cellular effects of non-continuous "pulsed" wave (PW) vs continuous wave (CW) lights on induction of extracellular matrix gene expression in skin cells. The aim of the current study was to determine in human skin fibroblasts if PW red LED light can stimulate collagen production and ATP synthesis as well as compared to CW red light. Procollagen type I and ATP productions were evaluated by enzyme-linked immunosorbent assay (ELISA) and Adenosine triphosphate (ATP) assay, respectively. Treatment with PW red light at 0.3 J/cm^2 (8 mW/cm^2 with 500 Hz or 800 Hz for 100 seconds, duty cycle 40%) significantly increased procollagen type I production in human fibroblasts, similarly to CW red light at 0.3 J/cm^2 (0.5 mW/cm^2 for 10 minutes, duty cycle 100%). Moreover, PW red light at 0.3 J/cm^2 rapidly and significantly increased ATP production which was sustained over 30 minutes, similarly to CW red light at 0.3 J/cm^2. In conclusion, low-level pulsed wave red light induced biomodulation in human fibroblasts at a significantly shorter treatment time than continuous wave light. Thus, a non-continuous, pulsed wave light mode delivering similar benefits in a significantly shorter exposure time may have substantial advantage for consumer compliance and convenience, enhancing therapeutic value for skin rejuvenation, wound-healing and pain management.

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Photoprotective efficacy of a new sunscreen formulation SPF50$^+$ against chronic UV-induced skin damage in an *ex vivo* human skin model

D. Bacqueville[1], J. Severine[1], L. Duprat[1], M. Saint Aroman[3], S. Bessou-Touya[1], H. Duplan[2]

[1]Pharmacology, Pierre Fabre Dermo-Cosmétique, Toulouse, France, [2]R&D, Pierre Fabre Dermocosmetique, Toulouse, France, [3]Laboratoires Dermatologiques A-Derma, Lavaur, France

Introduction: The prevalence of skin cancer has increased in last decade and is mostly due to chronic sunlight exposure. Therefore, it is important to prevent sun-induced skin damage by using photoprotection strategies. Objective: To assess the photoprotective efficacy of an innovative emulsion containing specific sunfilters combination to protect the skin against UV-induced lesions. Materials and Methods: An *ex vivo* human skin model was developed to mimic chronic exposure of solar-simulated radiation (CSSR). The sunscreen formulation presented a high sun protection factor (SPF50$^+$) and was applied before each SSR. Results: The skin model showed that chronic SSR induced an increase of the expression of the tumor suppressor p53 in keratinocyte nuclei. This biomarker is commonly mutated in skin cancer where its level has been associated to an enhanced stability. CSSR treatment also revealed that the cell cycle regulator p21 was upregulated and the caspase-3 was activated in response to DNA damage in the skin explants. Thus, CSSR induced cell cycle arrest and apoptosis. The data were confirmed by the detection of sunburn cell in the epidermis of CSSR-exposed skin. Topical application of SPF50$^+$ emulsion afforded an effective photoprotection against CSSR-induced skin lesions. The CSSR-exposed explants treated with sunscreen were protected from p53 upregulation. Concomitantly, cell cycle arrest and apoptosis were almost prevented after sun formulation application. Conclusion: The SPF50$^+$ emulsion had a high photoprotective efficacy in chronic SSR-exposed skin model and protected the skin from UV-induced damage. Thus, the sunfilter formulation may help to improve photoprotection against sunlight-induced skin damage.

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Efficacy of a nomad SPF50+ sunscreen stick with broad spectrum protection in both UVB+A and NIR

M. Noizet[1], P. Lapalud[2], A. Tourette[1], A. Houcine[1], D. Bacqueville[1], T. Douki[3], C. Jean Decoster[4], S. Bessou-Touya[1], H. Coubetergues[2]

[1]Pharmacology, Pierre Fabre Dermo-Cosmétique, Toulouse, France, [2]Clinical department, Pierre Fabre Dermo-Cosmétique, Toulouse, France, [3]Commissariat energie atomique, Grenoble, France, [4]Lavoratoire Dermatologique AVENE, Lavaur, France

Several studies have shown that the ultraviolet (UVB/A) from terrestrial solar radiation are strongly implicated in the etiology of most skin cancers via the generation of DNA lesions and reactive oxygen species (ROS). Moreover, skin is also exposed to solar near infra-red (NIR) radiation which are responsible of oxidative stress generation. Therefore, it is important to protect the most sensitized skins (actinic keratosis or atopic dermatitis) in all circumstances/all day long. Thus, the aim of this project was to develop a SPF50+ sunfilter product in stick form to provide a nomad protection for sensitized skins. We demonstrated a very good genoprotection after an acute solar simulated irradiation in an *in vitro* reconstructed human epidermal (RHE) model with a very high protection of UV-induced cyclobutane pyrimidine dimers formation. Moreover, the quantification of ROS revealed that the topical application of sunscreen decreased significantly the free radical production in an *ex vivo* human skin model after acute UVA irradiation. In addition, the stick SPF50+ protection was evaluated on healthy subjects by the quantification of NIR-generated malondialdehyde (MDA), marker of lipid peroxidation. The topical application of the product decreased significantly the MDA production after NIR irradiation. Thus, the nomad sunfilter product provides an efficient protection against IR-generated oxidative stress. Finally, we showed that topical application of the sunscreen induced a physical barrier reinforcement on the RHE model by transepithelial electric resistance. Altogether, we demonstrated that the SPF50+ sunfilter stick shows a broad spectrum photoprotection not only UVB+A but also NIR, associated to a physical barrier reinforcement, providing a complete protection of the most sensitized skins.

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Narrowband UVB (311nm) phototherapy maintains its high efficacy in psoriasis throughout repetitive treatment cycles

P. Wolf, M. Wiedenbauer, T. Graier, T. Benezeder, A. Gruber-Wackernagel, F. Legat, A. Hofer

Dermatology, Medical University of Graz, Graz, Styria, Austria

In contrast to systemic agents, phototherapy is administered intermittently in the treatment of psoriasis. At least for systemic agents (such as biologics), there is a concern that after treatment interruption, restarting treatment may lead to a weaker therapeutic response compared to that of after initial treatment (e.g. due to formation of neutralizing antibodies). In this study, we analyzed the efficacy of narrowband UVB (311nm) phototherapy (NB-UVB) under daily life conditions in patients who had received one or more phototherapy cycles. The analysis included data from a cohort of 70 patients with chronic/guttate plaque psoriasis (cohort I) who had been treated with NB-UVB at our center between 2010 and 2015 and data from another cohort of 32 patients (cohort II) who had been treated with different phototherapeutic modalities including NB-UVB from 2015 onwards. The 70 patients in cohort I achieved an average reduction of the initial psoriasis area and severity index (PASI) in the first therapy cycle of 81%, with a PASI90, PASI70 and PASI50 rate of 61, 81, and 99%, respectively. A second therapy cycle given to 18 of the patients in cohort I resulted in an average PASI reduction of 72%, with PASI90, PASI75, and PASI50 of 67, 94, and 94%, respectively. In the direct comparison of the therapy cycles of the 18 patients who received two cycles, the average PASI reduction was 74% after the first cycle and 72% after the second cycle. There was no significant relationship between PASI reduction and age (p=0.626), gender (p=0.178) or initial PASI (p=0.288). Similar therapeutic results were observed in cohort II. Our ongoing work with serum samples of cohort I and II addresses whether the levels of certain proinflammatory cytokines and chemokines (such as CCL22) and microRNA's at baseline may predict therapeutic response to NB-UVB. Together this work indicates that NB-UVB is an efficient first-line therapy for the treatment of psoriasis that maintains its efficacy even after repetitive administration

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Age and insulin-like growth factor-1 (IGF-1) status impact translesion synthesis (TLS) pathway activation in human keratinocytes and skin epidermis

M. G. Kemp, R. J. Hutcherson, A. Alkawar, A. J. Castellanos, R. D. Gabbard

Pharmacology and Toxicology, Wright State University, Dayton, Ohio, United States

Several studies have indicated that UV-induced DNA photoproducts are removed from epidermal genomic DNA at a slower rate in geriatric skin than in young adult skin. Such a situation may result in a greater need for the translesion synthesis (TLS) pathway of DNA replication, in which specialized DNA polymerases are recruited to sites of DNA damage to replicate across DNA adducts in either an error-free or error-prone manner. Here we show that skin epidermis from geriatric individuals (>65 years of age) exhibits higher levels of mono-ubiquitinated PCNA, a biochemical marker of TLS pathway activation, than the skin of young adults (aged 21-29) following exposure to 700 J/m^2 of UVB light. Low IGF-1 expression and deficient IGF-1/IGF-1 receptor (IGF-1) signaling are well-characterized properties of geriatric skin, and we observe that IGF-1R inhibition is associated with higher levels of PCNA mono-ubiquitination in both keratinocytes in vitro and skin epidermis ex vivo after UVB irradiation. Interestingly, the TLS polymerase pol eta (pol η), which accurately replicates across UV-induced thymine dimers and is a transcriptional target of p53, failed to be properly induced in keratinocytes after UV exposure. Thus, our results indicate that IGF-1-deficient geriatric skin may be more dependent on other, more mutagenic TLS polymerases to synthesize DNA across UV photolesions, which may contribute to the higher incidence of non-melanoma skin cancers in geriatric populations.

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Cyp11a1 derived secosteroid, 20(OH)d3 as a novel therapeutic agent for the prevention and treatment of uvb induced skin cancer

P. Guroji[1], S. Qayyum[1], T. Kim[1], Z. Janjetovic[1], M. Athar[1], A. Slominski[1, 2]

[1]Dermatology, The University of Alabama at Birmingham, Birmingham, Alabama, United States, [2]Pathology, Birmingham VA Medical Center, Birmingham, Alabama, United States

Non-melanoma skin cancers are more prevalent in people prone to repeated solar UVB expose. Though UVB is required for Vitamin-D3 production, yet it manifests tumorigenesis. 1,25(OH)2D3 protects from several autoimmune disorders and malignancies, however, due to its calcemic effects, therapeutic uses at pharmacological doses are limited. Previously, our lab reported that CYP11A1 produces 20(OH)D3 and is non-calcemic in mice and rats at pharmacological doses (60 µg/kg). Loss of PTCH gene function activates Sonic hedgehog (SHH) signaling, which drives BCC and perhaps SCC. We decided to evaluate the therapeutic potential of 20(OH)D3 against UVB induced skin tumorigenesis in Ptch1$^{+/-}$/SKH-1 mice. Mice were treated with UVB (180mJ/cm2), twice a week and 20(OH)D3 was administered topically as well as subcutaneously. Tumor sizes were recorded on a weekly basis. Mechanistic studies of skin and tumors were conducted using immunohistochemistry, RNA-seq, ELISA and western blot analysis. Histological studies confirmed the tumor type. LC-MS studies revealed that 20(OH)D3 has excellent bioavailability in the skin/serum. Treatment of mice with 20(OH)D3 significantly protected from tumorigenesis, tumor volume and survival compared to vehicle control. Furthermore, 20(OH)D3 affords protection (> 70%) against UVB induced carcinogenesis. Mechanistic studies provided substantial evidence that 20(OH)D3 modulates the SHH pathway activation. Furthermore, *In vitro* studies complimented our *in vivo* results. Altogether, this study reveals that 20(OH)D3 has potential to block UVB induced skin carcinogenesis.

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Association of clinical and demographic factors with phototherapy outcomes in patients with atopic dermatitis
D. D. Huh, Y. S. Roh, N. Kim
Department of Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States
For many patients with Atopic Dermatitis (AD), first-line interventions fail to alleviate symptoms. UVA, PUVA, and UVB phototherapy have been utilized as a safe and cost-effective therapeutic options for a variety of inflammatory skin conditions including AD. We conducted a retrospective cohort study to examine the efficacy of phototherapy in the management of AD and to identify patient characteristics associated with treatment compliance and outcomes. We conducted a chart review of 145 AD patients treated at the Johns Hopkins Phototherapy Unit from 2009-2017 to characterize the demographics, comorbidities, concomitant medications, number of treatments, and UV irradiation dosing, as well as the post-treatment outcomes. All analyses were conducted with Stata. 43% of the cohort was male, median [IQR] age 41 [26-56], median [IQR] BMI 25.98 [22.87-31.32], 36% White, 51% Black, 0.7% Indian or Alaska Native, and 12% Asian or Pacific Islander. 53% reside within Baltimore and 70% are enrolled in private insurance. 60% were compliant to the full course of treatment, 72% experienced improvement of symptoms, and 13% experienced side effects. Patient compliance significantly correlated with improvement of symptoms (p<.0001). Insurance status, dosage of therapy, and side effects also influenced whether a patient benefited from phototherapy. Cited reasons for noncompliance included time commitment, inconvenience, and cost of treatment. Insurance status, dosage of therapy, side effects, and compliance to treatment are significant contributors to the efficacy of phototherapy. As the ability of a patient to complete 20+ phototherapy sessions is critical to the success of phototherapy, patient-clinician conversations regarding compliance may be helpful prior to prescribing treatment. Further research should aim to improve tolerability of the treatment and providers and payors need continued education to ensure that socioeconomic factors such as insurance coverage are not barriers for patients to access phototherapy.

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CrispR/Cas9 deletion of TLR4 impacts the UV-induced stress response in human keratinocytes
V. Kirschnerova[1], M. Khawam[1], B. Seligmann[2], C. Curiel[1, 3], G. T. Wondrak[1, 4], S. E. Dickinson[1, 5]
[1]University of Arizona Cancer Center, Tucson, Arizona, United States, [2]BioSpyder Technologies, Carlsbad, California, United States, [3]College of Medicine, University of Arizona, Tucson, Arizona, United States, [4]Pharmacology and Toxicology, University of Arizona, Tucson, Arizona, United States, [5]Pharmacology, University of Arizona, Tucson, Arizona, United States
Damage incurred by exposure to ultraviolet (UV) radiation stimulates multiple stress-responsive signaling pathways in the skin. There is mounting evidence that Toll-like receptor 4 (TLR4) mediates key inflammatory responses in skin cells, including keratinocytes. Pharmacological blockade of TLR4 using resatorvid (TAK-242) inhibits not only acute UV-induced signaling *in vitro* and *in vivo*, but blocks UV-induced skin carcinogenesis in mouse models. In order to better characterize the UV-induced responses linked to TLR4, we have recently utilized CRISPR/Cas9 techniques to delete TLR4 from the genome of human HaCaT keratinocyte cells in culture. Characterization of TLR4 KO cells reveal a unique UV sensitive phenotype as compared to wildtype controls. TLR4 KO cells demonstrate increased sensitivity to UV-induced apoptosis as detected by flow cytometry of annexin V/propidium iodide stained cells, and by immunodetection of increased PARP cleavage. In contrast, TLR4 KO cells display markedly reduced intracellular levels of reactive oxygen species (ROS) detectable by DCF fluorescence in response to UV stimulation. Moreover, TempO-Seq®-based transcriptomic profiling revealed attenuated expression of UV-induced stress response genes (e.g., TP53, HMGB1, JUND, FRA1, DUSP4) in TLR4 KO cells. Consistent with our earlier findings that resatorvid blocks UV-induced AP-1 inflammatory signaling *in vitro* and in reporter mice, TLR4 KO status causes attenuation of UV-induced cfos expression that is unresponsive to resatorvid treatment, a finding consistent with an emerging role for the TLR4-AP-1-inflammation axis in skin photodamage. Future experiments will employ this novel TLR4 KO keratinocyte cell line as a mechanistic tool to dissect the role of TLR4 in skin inflammatory signaling and oxidative stress in the context of skin photodamage and carcinogenesis.

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The cutaneous response to solar UV radiation is attenuated by chlorination stress originating from swimming pool disinfectants
J. Jandova[1], A. Hua[1], S. E. Dickinson[2], A. Shakya[1], G. T. Wondrak[1]
[1]Pharmacology and Toxicology, College of Pharmacy and UA Cancer Center, University of Arizona, Tucson, Arizona, United States, [2]Pharmacology & UA Cancer Center, University of Arizona, Tucson, Arizona, United States
Hypochlorous acid (HOCl) is the active oxidizing principle released by standard swimming pool disinfectants used on a global scale, but the cutaneous consequences of human exposure to HOCl remain largely unknown, posing a major public health concern relevant to populations around the world. Here, for the first time, we have profiled the HOCl-induced cutaneous stress response in reconstructed human epidermis and SKH1 hairless mouse skin. In addition, we have investigated the molecular consequences of co-exposure to solar simulated ultraviolet (UV) radiation and HOCl, a procedure mimicking environmental exposure experienced by recreational swimmers. Performing gene expression array analysis (Oxidative Stress Plus RT^2 Profiler™ PCR Array) in organotypic human reconstructed epidermis (Epiderm™) exposed to topical HOCl (10-100 µM; 30 min-6 h), we have identified *TXNRD2*, a gene encoding the mitochondrial antioxidant enzyme thioredoxin reductase 2, as a novel key response factor and mechanistic determinant of chlorination stress sensitivity. Importantly, in SKH1 hairless mouse skin, UV/HOCl co-exposure studies revealed that the HOCl-induced cutaneous antioxidant response blocks inflammatory gene expression (*COX2*, *NOS2*) elicited by acute UV exposure, a finding consistent with emerging clinical evidence in support of a therapeutic role of topical hypochlorite preparations for the suppression of inflammatory skin conditions (atopic dermatitis, psoriasis). Taken together, these data indicate that HOCl co-exposure may block solar UV-induced skin inflammatory signaling. Given the public health relevance of human exposure to chlorination stress in the context of drinking water disinfection and recreational swimming pool use, an urgent need exists for further detailed molecular investigations.

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XPC dissociation from damaged DNA and efficient global nucleotide excision repair depend on vitamin D receptor
C. Wong[1], D. H. Oh[1, 2]
[1]Dermatology Research Unit, San Francisco VA Health Care System, San Francisco, California, United States, [2]Department of Dermatology, University of California, San Francisco, San Francisco, California, United States
Vitamin D and its receptor, VDR, together and independently, have been associated with DNA repair, though the mechanism by which they act is unclear. Upon ultraviolet irradiation through 3 mm pores in otherwise opaque filters to create focal spots of DNA damage, epidermal keratinocytes from both VDR-null mice and human keratinocytes depleted of VDR with siRNA exhibited slower removal of 6-4 photoproducts than normal control cells over 90 minutes. Co-staining with antibodies to XPC, the initial UV-induced DNA damage recognition sensor, revealed that XPC rapidly accumulated at DNA damage foci and gradually faded over 90 minutes as nucleotide excision repair proceeded in control human keratinocytes. In VDR-depleted keratinocytes, XPC associated with DNA damage with comparable efficiency, but the dissociation of XPC were delayed so that more XPC was retained at 30 minutes and more XPC was bound to DNA damage than in control cells. A simple model of XPC dynamics fit to the data confirmed reduced XPC off rates in the absence of VDR. The XPF endonuclease which acts later in the nucleotide excision repair process bound with comparable kinetics in control and VDR-depleted cells, but the magnitude XPF binding was reduced in the latter. These results suggest that VDR is important for normal dissociation of XPC from damaged DNA, and that VDR's absence can lead to non-productive binding of XPC, reduced completion of the nucleotide excision repair complex, and less efficient removal of DNA damage.

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5-(3', 4'-Dihydroxyphenyl-valerolactone) regulates DNA methylation in UVB-irradiated keratinocytes
A. Kim[1], B. Mock[1], H. Lee[2], Y. Kang[1], D. Kim[1, 2], J. Shin[1, 2]
[1]Skin Biology Research Center, Department of Biochemistry, CHA University School of Medicine, Seongnam-si, Gyunggi, Korea (the Republic of), [2]Dermatology, Bundang Medical Center, Seongnam-si, Gyunggi, Korea (the Republic of)
5-(3',4'-Dihydroxyphenyl)-γ-valerolactone (DHPV) is a colonic microbial metabolite derived from cacao. In the previous study, we found that cacao powder modulates DNA methylation and inhibits UVB-induced AP-1 activation and MMP-1 expression. To further investigate whether major metabolite of cacao, DHPV, has UV protective effect as cacao, we evaluated the regulatory role of DHPV on DNA methylation in keratinocytes after UVB irradiation. In this study, we found that UVB irradiation altered methylation profiles in HaCaT cells and these changes were ameliorated by DHPV. As well as methylation changes, DHPV inhibited UVB-induced accumulation of DNA methyltransferase 1 (DNMT1). Inhibition of DNMT1 accumulation by DHPV could be explained by the down-regulation of AKT1 phosphorylation with DHPV treatment. Supporting the AKT1-DNMT-DNA methylation pathway, a PI3K inhibitor, LY-294002, inhibited UVB-induced phosphorylation of AKT1 resulting in the reduction of DNMT1. Overall, our results indicate that DHPV regulates DNA methylation by suppression of AKT1 phosphorylation and DNMT1 stabilization, and suggest the protective role of DHPV on keratinocytes from UVB-induced epigenetic changes. Considering that DNA methylation changes are frequently observed in skin cancers, we suggest that DHPV could be a potential substance that can protect our skin from UVB-cancer initiation.

Pigmentation and Melanoma

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Late-stage melanoma diagnosis in New York State (NYS)
P. Shah, S. Bajaj, D. Polsky
The Ronald O. Perelman Department of Dermatology, New York University Langone Health, New York City, New York, United States
Early-stage (ES) melanoma at diagnosis is independently prognostic for improved survival compared to late-stage (LS) melanoma, defined as having regional or advanced spread. Additionally, decreased physician supply and lower socioeconomic status are associated with diagnosis at more advanced stages. We investigated population-level and county-level associations with LS diagnosis in NYS, a region of lower ambient sun exposure than areas frequently the subject of melanoma epidemiology studies in the US. We conducted a retrospective review of the NYS Cancer Registry data from 1995 to 2016, identifying 72,349 melanoma cases. Incidence rates were age-adjusted and reported per 100,000 persons. From the 1990s to the 2010-2016 period, overall incidence of melanoma increased from 10.4 to 18.9, ES incidence increased from 6.8 to 13.3, and LS incidence increased from 1.6 to 2.7. Disproportionately greater associations with LS were demonstrated in the elderly (80+ years, OR 1.80; 95% CI 1.64-1.97), male (OR 1.80; 1.64-1.97), Black (OR 3.62; 3.13-4.19) or other non-White (OR 2.61; 2.14-3.18), and Hispanic (OR 2.11; 1.88-2.37) patient populations. Counties with highest proportions of LS diagnoses were not those of greatest population or those with greatest melanoma frequency. Of the ten counties with the highest proportion of LS diagnoses (18% - 22%), only two had populations greater than 2.5 million people. Designation as a rural county (p=0.48) or healthcare professional shortage area for primary care (p=0.21) determined by the Health Resources and Services Administration did not increase the risk for LS diagnosis. In conclusion, the incidence of LS melanoma in NYS continues to increase, and specific population characteristics and geographic regions are disproportionately associated with LS diagnosis. The data also suggests that proximity to primary care physicians alone may not be sufficient for early diagnosis of melanoma. Additional investigation for LS diagnosis incidence and access to dermatology care is underway.

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Association between the frequency of indoor tanning and pain among women in the United States
B. Wang, J. Han, X. Li
Epidemiology, Indiana University , Indianapolis, Indiana, United States
Some people may use indoor tanning as a treatment for chronic pain. We tested the association of indoor tanning frequency with pain feeling and pain medication usage in the Nurses' Health Study II, a large well-characterized cohort in the United States. The study population consists of 75,957 female Caucasian nurses. We used the linear regression model and logistic regression model for the association of indoor tanning frequency with pain feeling and pain medication usage, respectively. The frequency of indoor tanning is significantly association with pain after controlling for various confounding factors. Compared with never indoor tanners, the multivariable-adjusted odds ratio (OR) (95% confidence interval [CI]) of pain killer use was 1.23 (95% CI, 1.18 to 1.29) for women who used indoor tanning 1-2 times/year, 1.55 (95% CI, 1.46 to 1.65) for those who used 3-11 times per year, and 1.83 (95% CI, 1.65 to 2.04) for those who tanned 12+ times per year. In linear regression, there was a trend towards more frequent indoor tanning use with higher pain scores ($p<0.001$). Participants were exclusively female nurses in the U.S., preventing generalization of results to other occupational groups or to men. Other limitations include self-reported indoor tanning frequency and pain and potential recall bias. Frequency of indoor tanning is positively associated with pain feeling and pain medication use. Those who use indoor tanning for pain relief should be aware of its harmful effect on skin and addictive features.

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A retrospective multicenter study of melanoma in children and adolescents
S. Consortium
Pediatric Dermatology Research Alliance, Indianapolis, Indiana, United States
Background: Melanoma in the pediatric population is rare, with distinct clinical features compared to adult disease. Risk factors are not well understood in children and adolescents. This study aims to characterize melanoma in the pediatric population and explore potential risk factors and negative patient outcomes. Methods: Multicenter retrospective study of patients <20 years of age diagnosed with melanoma between 1/1/1995 – 6/30/2015 from 11 academic medical centers. Information was compared to control patients, matched to each study patient by gender and age group, from healthy patients seen in the Dermatology Program at Boston Children's Hospital from 2000-2015. Results: Melanoma was diagnosed in 321 children and adolescents. The majority were diagnosed in adolescence (72.6% at age 11 or greater). The most common subtypes included Spitz (41%) and superficial spreading (34%), and 11% of cases were found to arise from a congenital nevus. Sentinel lymph node biopsy was performed in 30% of cases, and was positive in 47% of these cases, and 20 patients died from disease (7%). Patients diagnosed with melanoma were more likely to have other cutaneous malignancy (p=0.006) or family history of melanoma (p=0.04) compared to controls. There was no significant association between overall survival and risk factors of family melanoma history, sunburn history, or iatrogenic factors of prolonged immunosuppression, radiation therapy, or chemotherapy. Discussion: Pediatric melanoma has diverse clinical presentations. Better understanding of these cases and their outcomes may allow for improved risk stratification of children and adolescents diagnosed with melanoma.

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Imiquimod spares disfiguring surgery for large facial lentigo maligna

K. O'Hern[1, 2], M. Chambers[1], M. Chapman[2]

[1]Dartmouth Medical School, Lebanon, New Hampshire, United States, [2]Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire, United States

Surgical excision of lentigo maligna (LM) in anatomically constrained sites such as the face can result in adverse functional and cosmetic consequences. Patient age, comorbidities, lesion size, or location can make adequate margins impossible and/or disfiguring. The off-label use of topical imiquimod has been considered as an alternative. This case represents clearance of LM in an 86-year-old female with a 10 cm facial LM involving the eyelid treated with topical imiquimod. An 86-year-old Caucasian female with no personal/family history of skin cancer presented with a 24-month history of an enlarging, 10 cm, irregularly brown, pigmented, macule, without nodules, ulcerations, or erosions on the left zygomatic cheek extending to the lateral superior and inferior eyelids. Diagnostic biopsy demonstrated LM. Definitive removal via surgical excision was discussed, but given the patient's age, large size of the lesion, and difficult repair with eyelid involvement, a less invasive option was chosen by the patient and treating team. Topical tretinoin 0.1% cream was applied daily for two weeks followed by imiquimod 5% five times per week for 12 weeks. Given a minimal response at 2 weeks, imiquimod was increased to daily. Imiquimod was halted for 2 weeks due to a lip infection after 7 weeks of treatment and then resumed for a total of 70 applications over 12 weeks. During imiquimod therapy, the patient experienced appropriate pruritus and erythema that resolved after treatment cessation. A post-treatment biopsy showed no residual LM. She was scheduled for annual skin exams with no evidence of recurrence at 4 months post-treatment, but was lost to follow-up and died without evidence of disease 3 years later.

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Risk factors for recurrence with topical imiquimod cream for lentigo maligna: Survival analysis with 17 years follow-up

M. Chambers[1, 2], K. O'Hern[2], C. Baker[1], D. Badin[1], E. Saunders[2], M. Chapman[2]

[1]Dartmouth Medical School, Lebanon, New Hampshire, United States, [2]Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire, United States

Topical imiquimod 5% cream has been investigated as an off-label primary or adjuvant treatment for melanoma in situ lentigo maligna (LM) type, although long-term follow-up data are lacking. In this single-institution retrospective analysis we evaluated treatment response and recurrence from 2002-2019 for LM treated with primary or adjuvant topical imiquimod (5%) with or without tazarotene gel 0.1% as pretreatment. 98 patients were identified with a total of 103 lesions analyzed. Mean follow-up time was 5.9 years (SD: 5.2), with 29.1% of cases having >10 years follow-up. The overall response rate was 97.1%. There were 3 treatment non-responders and 8 local recurrences (8/100 lesions, 8.0%) developing at an average of 2.9 years after treatment (SD: 2.7 years). Risk factors for recurrence were analyzed using survival analysis (recurrence-free survival with Kaplan-Meier Curves). Significant variables included history of a failed excision prior to imiquimod therapy (p= 0.03) and clinical clearance (p= 0.01). Variables which tended towards significance included <60 applications (p= 0.08) and an insufficient overall treatment (a combination of # of applications, # of weeks, # applications/week, and tazarotene pretreatment: p= 0.10). Non-significant variables included age (>65), size (>1.0 cm), and histologic clearance. Our analysis supports previously published literature, while overcoming barriers in sample size and follow-up time to present a novel survival analysis from this large cohort. Our observed low recurrence in a large case series with long-term follow-up suggests the efficacy of topical 5% imiquimod for LM and emphasizes the need for randomized control trials comparing imiquimod to conventional/staged surgery.

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The relationship between genetic mutations and clinicopathological manifestations in Korean cutaneous melanoma: A study based on next-generation sequencing

J. Byun, J. Heo, G. Choi

Dermatology, Inha University School of Medicine, Incheon, Korea (the Republic of)

Identifying individual mutated genes have been proposed for effective biomarkers and therapeutic targets in cutaneous melanoma. Next-generation sequencing (NGS) shows comprehensive genomic mutations with high sensitivity. We assessed the genetic alteration in patients with melanoma and their prognosis according to clinicopathological manifestations. We analyzed clinicopathological features of 11 patients with melanoma who obtained surgical resection and examined genetic mutations using NGS. Among 11 cases of melanoma, gene mutations were identified in five. Four, except one on the neck, occurred on the acral sites. Case #1 presented lentigo maligna melanoma with vertical growth pattern on the neck. NGS showed CCND1 amplification and TP53 mutation. There was no recurrence under follow-up without adjuvant treatment for 2 years, but the patient diagnosed rectal cancer 12months after surgery. Case #2 showed acral lentiginous melanoma (ALM) on the foot. NRAS mutation was identified, and metastasis to lymph nodes and lung occurred 8 months after surgery. After eighteenth anti-PD1 therapy, the treatment was changed to dacarbazine due to disease progression. Case #3 presented ALM on the great toe and case #4 presented nodular melanoma on the heel. NGS confirmed RAF1 amplification and KRAS mutation, and FGFR1 amplification and PTEN deletion, respectively. Both of them occurred metastasis to lung; 14months after amputation in case #3 and 8 months after wide excision in case #4. Case #5 confirmed tumorigenic phase of ALM on the sole and KIT gain was found. There is no recurrence after surgery for 10 months. The study of various genetic mutations identified in melanoma is an emerging issue. To analyze the factors affecting therapeutic outcome including genetic mutations and their associated target therapy, further studies of each genetic mutation and review of related mutations in melanoma are needed.

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α-MSH induces melanogenesis via up-regulation of Opsin1 in cultured human skin melanocytes *in vitro*

W. Zeng, J. Mou, H. Lu

Dermatology, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, China

Background: Physiological skin color change is regulated by environmental cues such as sun light and also modulated through the neurological and/or endocrine control of chromatophores. Opsin1 found in bird and fish pineal gland and hypothalamus was suggested to have roles in regulating skin color change. Our previous studies have shown that Opsin1 is expressed in human skin melanocytes. α-MSH is a well known endocrine hormone that induces pigmentation in human skin. However, the question whether this hormone interacts with Opsin1 in modulating human skin melanogenesis has not been fully addressed. Objectives: To investigate whether α-MSH induces Opsin1 expression and affects melanogenesis. Method: Melanocytes were obtained from human foreskin with two-step enzyme-digestion and then cultured in M254 medium supplied with human melanocyte growth supplement for three passages. Cells were pretreated with α-MSH (1 μM) for 30 minutes and the cells were collected 3 hours after the medium was changed. Cellular sediment from medium was solubilized in 400μl of 1M NaOH in 70 degrees centigrade for 3h to dissolve melanin and the absorbance was measured by spectrophotometer at 405nm. Melanin products were calculated by normalizing the total melanin values with protein content. RT-qPCR was used to detect Opsin1, Opsin2, Opsin3, Opsin4, Opsin5 mRNA level. Western blot was applied to measure Opsin1, Opsin2, Opsin3, Opsin4, Opsin5, TYR, TRP1, TRP2, MITF and p-MITF expression. Result: Compared with control group, α-MSH treatment enhanced melanin production. While Opsin1, Opsin2, Opsin3, Opsin4, Opsin5 mRNA level showed marginal difference, Only Opsin1 mRNA level was significantly increased. At protein level, compared with the control group, Opsin 1, TRP 1, TRP 2, MITF and p-MITF in α-MSH group were increased, but Opsin2, Opsin3, Opsin4, and Opsin5 did not show remarkable change. Conclusion: α-MSH up-regulates Opsin1 and enhances melanogenesis in cultured human epidermal skin melanocytes in vitro.

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GDP/GTP exchange inhibitor, FR900359, synergizes with chloroquine in GNAQ/11-mutant melanoma

A. Truong[1, 4], M. Onken[2], K. Blumer[2], M. McMahon[3, 4]

[1]Oncological Sciences, University of Utah, Salt Lake City, Utah, United States, [2]Washington University School of Medicine, St. Louis, Missouri, United States, [3]Dermatology, University of Utah, Salt Lake City, Utah, United States, [4]Huntsman Cancer Institute, Salt Lake City, Utah, United States

GNAQ and GNA11 (GNAQ/11) encode for G-alpha proteins, and mutations in these genes are found in approximately 2% of melanoma, including 80% of uveal melanoma. Constitutive activation of G-alpha proteins leads to downstream activation of multiple oncogenic pathways, such as MAPK signaling. Unfortunately, metastatic uveal melanoma is generally refractory to all available systemic therapies, including MAPK targeted therapies, indicating a critical need for novel therapies. Recently, the GDP/GTP nucleotide exchange inhibitor, FR900359, was characterized to inhibit growth of GNAQ-mutant uveal melanoma cells *in vitro*, but has been limited in translatability due to a narrow therapeutic window. We show that FR900359 is a potent inducer of autophagy in GNAQ- and GNA11-mutant cell lines. These findings were specific to GNAQ/11, as treatment of a BRAF-mutated cell line with FR900359 did not affect autophagy levels. The addition of an FDA-approved and widely available autophagy/lysosome inhibitor, chloroquine, resulted in synergistic cytotoxicity (Loewe model) in combination with FR900359 at concentrations as low as 30 nM. These results were also seen when FR900359 was combined with uveal melanoma cells that expressed a dominant-negative mutant of ATG4B autophagy protein, as well as lysosome inhibitor, bafilomycin A1. This not only demonstrates the cytoprotective role of autophagy as a result of G-alpha signaling inhibitor treatment, but also lowers the effective dose of FR900359 by more than three-fold when an autophagy inhibitor is added. Our results may be informative for future first-in-human studies of FR900359 by demonstrating that the addition of chloroquine may widen the therapeutic window of FR900359. These findings provide a novel and potentially effective combination treatment approach for GNAQ/11-mutant melanomas.

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TGF-β2 upregulates OPN3 in human epidermal melanocytes independent of TGF-β2R *in vitro*

Y. Wang, H. Lu, Y. Lan, X. Yang, Y. Gu

Dermatology, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, China

Background: Previous studies have shown that OPN3 belongs to the photosensitive G Protein-Coupled Receptors families and is involved in the growth and melanogenesis of human epidermal melanocytes. However, the question whether OPN3 is regulated by TGF-β2 is yet to be answered. Objective: To explore the relationship between TGF-β2 and OPN3 in human epidermis melanocytes. Methods: Human epidermal melanocytes were stimulated with various concentrations of TGF-β2 *in vitro*. The expressions of OPN3 gene was monitored after the treatment of 10ng/ml TGF-β2, 10μM LY2109761 and 9 mM U73122. Western blot was used to detect the expression of OPN3, TGF-β2R, Smad2, p-smad2, Smad3 and p-smad3 at the level of protein. Results: At 24h, 48h and 72h after 10ng/ml TGF-β2 treatment, the expression of OPN3 increased at the protein level, and peaked at 48h *in vitro*. After incubation simultaneously with 10 ng/ml TGF-β2 and 9 mM U73122 for 48 hours, the expression of OPN3 protein was elevated significantly, compared with the control group. However, there was no significant difference in the expression of OPN3 between the experimental group and 10 ng/ml TGF-β2 stimulation group. At 48h after adding 10μM LY2109761, there was no significant change in the expression of OPN3 and TGF-β2R between the experimental group and the control group,but the expression of Smad2, p-smad2, Smad3, p-smad3 decreased at the protein level *in vitro*. After incubation simultaneously with 10 ng/ml TGF-β2 and 10μM LY2109761 for 48 hours, the expression of OPN3 and TGF-β2R at the protein level increased compared with the control group, while the decreased expression of Smad2 and p-smad2 were also found at the protein level. There was no significant difference in the expression of Smad3 and p-smad3 between the experimental group and the control group. Conclusions: Taken together, our findings demonstrate that TGF-β2 up-regulates the expression of OPN3 in human epidermal melanocytes independent of TGF-β2R *in vitro*.

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Inactivating p53 is essential for nerve growth factor receptor to promote melanoma initiating cells-stemmed tumorigenesis

L. Jiang[1], S. Huang[2], J. Wang[2], H. Lu[3], S. Zeng[2], H. Lu[2]

[1]Dermatology, Guizhou Provincial People's Hospital, Guiyang, Guizhou, China, [2]Department of Biochemistry & Molecular Biology and Tulane Cancer Center, Tulane University School of Medicine, New Orleans, Louisiana, United States, [3]Dermatoloy, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, China

Nerve growth factor receptor (NGFR, CD271 or p75NTR) is highly expressed in melanoma initiating cells (MICs) and critical for their proliferation and tumorigenesis, and yet the underlying mechanism(s) remain largely elusive. We previously showed that NGFR inhibits p53 activity in a negative feedback fashion in other cancer cells. Here we report that this feedback inhibition of p53 by NGFR plays an essential role in maintaining the sphere formation (stem-like phenotype) and proliferation of MICs and in promoting MICs-derived melanoma growth in vivo. Knockdown of NGFR markedly reduced the size and number of spheroid formation of melanoma cells, which can be rescued by ectopically expressed NGFR. This reduction was also reversed by depleting p53. Consistently, knockdown of NGFR led to the suppression of MICs-derived xenograft tumor growth by inducing the p53 pathway. These results demonstrate that the NGFR-p53 feedback loop is essential for maintaining MICs stem-like phenotype and MICs-derived tumorigenesis, and further validate NGFR as a potential target for developing a molecule-based therapy against melanoma.

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Dual HDAC and LSD1 inhibition as a novel strategy to overcome BRAF inhibitor resistance

F. Gibson[1], A. Hanly[1], K. Kuang[1], J. Kalin[2], M. Wu[1], P. Cole[2], R. Alani[1]

[1]Dermatology, Boston University School of Medicine, Boston, Massachusetts, United States, [2]Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts, United States

Despite recent advances in the development of targeted and immunotherapies for metastatic melanoma, the majority of tumors demonstrate intrinsic or acquired therapeutic resistance. While the mechanisms of therapeutic resistance in melanoma are varied and often undefined, epigenetic regulation of gene expression and transcriptional programming may play a pivotal role. The potential role of epigenetic changes in the development of resistance to therapies in melanoma and other cancers has led to the development of numerous epigenetic agents as potential anti-cancer therapies; however, the lack of target selectivity of such agents results in a narrow therapeutic window. We have developed a novel, dual-warhead hybrid agent, Corin, that potently and specifically targets the CoREST chromatin-modifying complex by simultaneously inhibiting HDACs1/2 and LSD1. Derived from a class I HDAC inhibitor (entinostat) and an LSD1 inhibitor (tranylcypromine analog), Corin has been shown to preferentially inhibit the growth of melanoma cells compared to other cancer cell lines and consistently outperforms the anti-proliferative effects of its parent HDAC and LSD1 inhibitors when used in combination. Preliminary studies suggest that Corin is able to resensitize BRAF inhibitor (BRAFi)-resistant melanoma to BRAFi therapies. Combinational treatment of Corin and a BRAFi was effective in slowing BRAFi-resistant melanoma tumor growth in a mouse xenograft model. Gene expression analysis revealed that Corin is a potent inducer of tumor suppressor genes, many of which have been observed to be epigenetically silenced in cancer. This novel, dual action inhibition presents a specific and potent approach to targeting epigenetic mechanisms to overcome acquired BRAFi resistance in melanoma and may serve as a model for overcoming intrinsic and acquired therapeutic resistance in other cancers as well.

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A20 determines the therapeutic effect of anti-PD-1 immunotherapy in melanoma

W. Guo, J. Ma, X. Yi, T. Gao, C. Li

Department of Dermatology, Xijing Hospital, Xi'an, Shaanxi, China

The therapeutic effect of immune checkpoint blockade (ICB) therapy, especially the inhibition of programmed cell death protein 1 (PD-1) and its ligand PD-L1, has been verified in melanoma treatment. However, the dissatisfied response rate and therapeutic efficacy of anti-PD-1/PD-L1 therapy remains a major challenge for melanoma treatment. Here, we reported A20 as a critical regulator that determines the therapeutic effect of anti-PD-1 immunotherapy in melanoma. Through the un-targeted MS-based proteomic analysis, we first found that high expression of A20 was significantly associated with therapeutic resistance to anti-PD-1 immunotherapy in melanoma patients. We then proved that the suppression of tumoral A20 expression potentiated the anti-tumor activity of infiltrated $CD8^+$T cells and increased the efficacy of anti-PD-1 antibody treatment in pre-clinical mice model. A20 promoted PD-L1 expression in tumor cell to impair infiltrated $CD8^+$T cell cytotoxicity and contributed to melanoma immune escape and therapeutic resistance to anti-PD-1 immunotherapy. Moreover, up-regulated A20 expression facilitated PD-L1 transcription via the ubiquitination and degradation of PHB and thereby ameliorating its inhibition of STAT3. Our findings reveal a previously un-recognized regulatory role of A20 in anti-tumor immunity, and demonstrate that A20 can be exploited as a promising target to overcome immune escape and improve the effect of anti-PD-1 immunotherapy in melanoma.

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Targeting the α9-nAChR/PD-L1 axis in antiproliferative effects of *Allium cepa* L. var. *proliferum* Regel extract on melanoma cells

H. Nguyen[1], Y. Ho[2]

[1]International Master Program in Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan, [2]School of Medical Laboratory Science and Biotechnology, College of Medical Science and Technology, Taipei Medical University, Taipei, Taiwan

Programmed cell death ligand-1 (PD-L1) is critical for melanoma development, progression and treatment. The α9 nicotinic acetylcholine receptor (nAChR) is shown to affect melanoma cell proliferation. *Allium cepa* L. var. *proliferum* Regel extract possesses anticancer properties. However, the mechanism of *Allium cepa* L. var. *proliferum* Regel extract in the antiproliferative effects of melanoma cells remains unclear. Using UPLC-ESI-MS/MS method, a total of 42 compounds were identified in *Allium cepa* L. var. *proliferum* Regel extract classified into five categories, including organosulfur compounds (962.20 ± 34.55 μg/g), polyphenols (100.40 ± 12.55 μg/g), flavonoids (58.36 ± 10.75 μg/g), organic acids (54.04 ± 2.69 μg/g), and alkaloids (15.08 ± 3.10 μg/g) where we found various bioactive components targeted to nAChRs. We analyzed the genetic dependency of α1-10 nAChRs from Achilles_CRISPR dataset (n=38) using CRISPR-Cas9 technology. α9-nAChR contributed significantly to the survival of melanoma cells (dependency scores < 0). We evaluated α1-10 nAChRs and PD-L1 mRNA expression from CCLE_Expression dataset (n=50). α9-nAChR was the only gene that strongly correlated to PD-L1 expression (r=0.6, p<0.001). There was also a strong correlation between α9-nAChR and PD-L1 expression (r=0.7, p<0.001) in the tissue microarray (n=192) using the IHC staining. Knockdown or overexpression of α9-nAChR significantly down- or upregulated the expression of PD-L1 via the transcription factor STAT3 binding to the PD-L1 promoter, respectively, and affected melanoma cell proliferation. *Allium cepa* L. var. *proliferum* Regel extract inhibited melanoma cell proliferation through inactivation of α9-nAChR/PD-L1 axis. Our results suggest that α9-nAChR/ PD-L1 axis regulates the cell proliferation of melanoma and *Allium cepa* L. var. *proliferum* Regel extract is considered as a potential candidate to prevent melanoma.

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Oncogenic melanocyte stem cells, driven by regenerative niche signals, give rise to heterogeneous melanoma resembling human melanoma

Q. Sun[1], I. Katehis[1], W. Lee[1], Y. Mohri[2], M. Takeo[1], C. Lim[1], X. Xu[3], P. S. Myung[4], R. Atit[5], M. Taketo[6], R. Moubarak[7], M. Schober[1], I. Osman[1], D. Gay[8], D. Saur[9], E. K. Nishimura[2], M. Ito[1]

[1]Dermatology and Cell Biology, New York University School of Medicine, New York, New York, United States, [2]Tokyo Medical and Dental University, Tokyo, Japan, [3]University of Pennsylvania, Philadelphia, Pennsylvania, United States, [4]Yale University, New Haven, Connecticut, United States, [5]Case Western Reserve University, Cleveland, Ohio, United States, [6]Kyoto University, Kyoto, Japan, [7]Pathology, New York University School of Medicine, New York, New York, United States, [8]CEA/DRF/IBFJ/iRCM/LRTS, Fontenay-aux-Roses cedex, France, [9]Technische Universität München, München, Germany

Melanoma, the most lethal form of skin cancer, is rarely curable at its advanced stages. The early events of this disease, during which treatment would be beneficial, remain poorly elucidated. Melanocyte stem cells (McSCs) residing in the hair follicle niche were proposed to be cells-of-origin for melanoma. To understand the cellular and molecular mechanisms regulating the initiation and progression of McSC-derived melanoma, we established a novel c-Kit-CreER-driven melanoma mouse model that enabled us to specifically target McSCs and trace their oncogenic behaviors. Coupling this model with an advanced imaging technology, we now demonstrate that oncogenic McSCs first expand in the niche and then migrate to the epidermis to form epidermal melanoma that later invade into the underlying dermis and undergo metastasis. Furthermore, normal Wnt and Endothelin signals, secreted by epithelial niche cells during hair anagen onset, can be hijacked to promote the malignant transformation of McSCs. Finally, transcriptional profiling revealed a strong resemblance between murine McSC-derived melanoma and human melanoma in heterogeneity and gene signatures. These results suggest that follicular McSCs can be an ultimate origin of melanoma and that follicular niche can control McSC oncogenic transformation. The similarities of McSC derived melanoma with human melanoma in epidermal to dermal progression, heterogeneity and gene expression suggest the potential utilization of this mouse model as a pre-clinical model for human melanoma.

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Associations of *BIRC2/3/5* copy number gains with clinicopathological features of acral melanomas in Taiwan

C. Chu, Y. Sheen

Department of Dermatology, National Taiwan University Hospital, Taipei, Taiwan

Anti-apoptotic molecules can protect melanoma cells from apoptosis, thus enhancing their aggression. One of the factors responsible for the difficulties in engaging the apoptotic cascade efficiently in melanoma is the up-regulation of conserved inhibitor of apoptosis proteins (IAPs). All IAPs share up to three conserved zinc-binding baculoviral IAP repeats domains. The best described members are cIAP1, cIAP2, X-linked IAP, and surviving, which are encoded by Baculovirus inhibitor of apoptosis repeat containing genes (*BIRC2, BIRC3, BIRC4, and BIRC5*). These proteins are associated with chemoresistance and poor outcome in many cancer types. Acral melanoma is the most common subtype of melanoma in Asians. Our previous report found that frequencies of *NRAS/KRAS* mutations, altered cell cycle regulation, *BIRC2/3/5* copy number gain, and amplification of receptor tyrosine kinase genes were significantly enriched in acral melanomas. In addition, *BIRC2/3* copy number gain was only detected in the acral melanomas, and acral melanomas had significantly more *BIRC2/3/5* copy number gain than the cutaneous melanomas. Most of *BIRC2/3/5* copy number gains were detected in acral melanomas developed in stress-bearing areas. Moreover, the melanomas with *BIRC2/3/5* gain were associated with ulceration and greater Breslow thickness. Our further analysis found that cell cycle alteration, *BIRC2/3/5* copy number gain, and lymph node status were significant prognostic factors in 45 acral melanoma in a univariate analysis. In a multivariate analysis, altered cell cycle and lymph node status remained the most crucial independent prognostic factors in acral melanomas. We identified *BIRC2/3/5* might be a critical determinant of survival in the acral melanoma patients.

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β3-tubulin knockdown interferes with microtubule dynamics, cell-cycle regulation, and microvesicle release in human melanoma cells

M. Altonsy[2], P. Surmanowicz[1], A. Ganguly[2], G. Lauzon[2], R. Mydlarski[2]

[1]Medicine, University of Alberta, Edmonton, Alberta, Canada, [2]University of Calgary, Calgary, Alberta, Canada

Introduction: Microvesicles (MV), ranging in size from 100 nm to 1000 nm, play an important role in carcinogenesis by promoting angiogenesis and tumor metastasis, interfering with anti-tumor immunity, and inducing multidrug resistance. The release of MVs requires structural changes in microfilaments, intermediate filaments, and microtubules. Class III β-tubulin (β3-tubulin), one of seven β-tubulin isotypes, is a microtubule component involved in malignant transformation and cancer development. Herein, we characterize the effects of β3-tubulin knockdown on microtubule dynamics, cell cycle regulation, and microvesicle formation in human melanoma cells. Methods & Results: Human malignant melanoma cells were cultured and transfected with either β3-tubulin siRNA or non-targeting siRNA. Western blot analysis, RNA isolation, immunofluorescent microscopy, and MV purification were performed 48 hours after transfection. Cell cycle analysis was conducted 24 and 48 hours post-transfection. The A375 cells were found to constitutively express β3-tubulin mRNA and protein. Knockdown of β3-tubulin in A375 cells impaired microtubule dynamicity, induced cell cycle arrest, activated apoptosis signaling pathways, and inhibited microvesicle release. Conclusions: Taken together, the data suggest that β3-tubulin knockdown interferes with microtubule dynamics, cell-cycle regulation, and MV release in human melanoma cells. By understanding the significance of β3-tubulin in carcinogenesis, the dermatologist will gain diagnostic, prognostic and therapeutic insight essential for the management of melanoma patients.

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Ulcerated melanomas exhibit epigenetic changes in epidermal and immune response genes

G. Micevic, M. Bosenberg

Dermatology, Yale School of Medicine, New Haven, Connecticut, United States

The purpose of this study is to identify the genetic and epigenetic changes associated with melanoma ulceration. Clinically, ulceration is consistently associated with shorter disease-free and shorter overall melanoma-specific survival. Ulcerated melanomas have also been associated with a higher risk of melanoma recurrence. However, the molecular changes associated with ulceration are largely unknown. In this study, we examined the differences in DNA methylation patterns between ulcerated and nonulcerated melanoma samples. We uncovered that ulcerated melanomas exhibit significantly altered DNA methylation of genes with epidermal functions, including desmogleins (*Dsg1, Dsg4*), desmocollin 1, filaggrin 2, keratin and keratin associated proteins with functions in the intermediate filament cytoskeleton ($p < 0.001$). Ulcerated melanomas exhibited signification methylation changes in chemokine signaling (CCL4,14,15,16,20,23; $p<0.001$), interferon signaling (IFNG, IRF4, GZMB, UBD, GBP2, $p<0.001$) and immune response signaling (FCRL3, CD200, SLAMF7, IL32, TRAT1, $p<0.001$). Uncovered changes in DNA methylation were associated with significant transcriptional changes including keratin family members, (KRT16, KRT17, KRT6, KRT75, KRT78, KRT9, KRT 6C), E-cadherin, desmogleins and desmocollin ($p<0.0001$, Student's t-test). Ulcerated melanomas exhibited a lower median number of somatic mutations compared to nonulcerated tumors (242 vs. 296, $p<0.05$, Kruskal Wallis). Ulcerated melanomas showed a significantly higher mitotic rate ($p<0.001$, Kruskal Wallis Test), Breslow depth (50 vs. 16 mm, $p<0.001$, Kruskal Wallis), and occurred at a later median age (63 vs. 57 years, $p<0.001$). Ulceration was associated with significantly shorter melanoma overall and disease-free survival ($p<0.0001$, Log-rank test). Collectively, these findings shed light on molecular alterations exhibited by ulcerated melanomas, and suggest that ulceration is associated with specific epigenetic and transcriptional changes in epidermal function and immune response signaling, whose functional roles should be further investigated.

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CD147 promotes tumorigenesis in squamous cutaneous cancer through recruiting MDSC into epidermis through CXCL1/2

Y. Guo, X. Zhang, X. Chen, C. Peng

Dermatology, Xiangya Hospital, Central South University, Changsha, Hunan, China

CD147, a transmembrane glycoprotein belongs to the immunoglobulin superfamily, was highly expressed in various cancers. Recent studies demonstrated that CD147 promoted tumor progression by regulating inflammatory factors and chemokines. Our previous studies found CD147 promoted cutaneous squamous-cell carcinoma (cSCC) through inducing EGFR endocytosis. However, whether CD147 could regulate cSCC by influencing immune cells remains unclear. In this study, we found spontaneous tumors in CD147 epidermal over-expressing transgenic mice ($Tg^{CD147\text{-}Epi}$). RNA sequencing was then performed, and overexpression of CD147 significantly up-regulated CXCL1/2, which could induce MDSCs. The immunofluorescence staing showed that MDSC infiltration was significantly increased in tumor tissues. We then generated DNBA/TPA mouse model. Interestingly, the tumorgenesis of $Tg^{CD147\text{-}Epi}$ mice started notably earlier than Tg^{wt}, so were the tumor volume and tumor number. Meanwhile, the overexpression of CD147 in JB6, a murine keratinocyte, enhanced cell proliferation and migration. Considering the expression of CD147 was elevated in cSCC tissues, we knocked down CD147 in A431, a human cSCC cell line, resulting in inhibition in cell growth and migration. We then treated the $Tg^{CD147\text{-}Epi}$ mice with TPA for 12 hrs. The rt-PCR showed an elevation of CXCL1/2 in TPA-treated epidermis, and flow cytometry proved the reduced MDSC in $Tg^{CD147\text{-}Epi}$ compared to Tg^{wt}. Next, we used CD147 epidermal knock-out transgenic mice ($Tg^{CD147\text{-}KO}$) for TPA treatment. Consistent with previous results, CXCL1/2 decreased remarkably in $Tg^{CD147\text{-}KO}$ mice. Those results indicated that CD147 promoted cSCC through regulating MDSC. The proteome profile found p-PLCγ1 was significantly up-regulated when over-expressing CD147 in JB6 cells. It was then confirmed that CD147 up-regulated p-PLCγ1 under the stimulation of TPA, thereby activating AP-1 and increasing the expression of CXCL1/2. In conclusion, our study revealed a novel mechanism of CD147 in promoting cSCC through recruiting MDSC into epidermis.

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Targeting lncRNAs AC and BX reveals their critical role in melanoma and allows to identify novel phosphor-catalytic vulnerabilities

V. Feichtenschlager, C. Emily, M. Vujic, I. Vujic, C. Chen, A. Lee, S. Ortiz-Urda

Dermatology, University of California, San Francisco, San Francisco, California, United States

More than 80% percent of melanomas harbor mutations in either NRAS or BRAF. Our goal was to identify targets that are key in the process of melanocyte tumorigenesis and molecules that can be used to prevent this event. We have identified two long non-coding RNA (lncRNA) transcripts AC and BX key in the process of melanocyte tumorigenesis and melanoma progression. Tested in a variety of BRAF and NRAS mutated, drug resistant melanoma cell lines and other NRAS cancers such as lung ca and neuroblastoma, RNAi and antisense oligonucleotide (ASO) mediated knockdown of AC and BX lead to a vast reduction of tumor growth in vivo. High-throughput Kinase Activity Mapping (HT-KAM), a method for mapping phospho-catalytic dependencies of cells, unveiled AC and BX dependent kinases. Their inhibition not only mimic the knockdown-effects but also shows synergistic effect with ASO treatment. In addition, by using HT KAM technology, we were able to identify phospho-catalytic vulnerabilities of melanoma cells resistant to current targeted therapeutics. Moreover, knockdown of our transcripts causes a significant induction of PDL1 receptor ligand (key for immunotherapy response). Our studies are innovative because we offer new therapeutics and a diagnostic test to identify new drug combinations to restore therapeutic response for immune and targeted therapy.

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Semi-quantitative 5-hmC expression by immunohistochemistry is a useful adjunctive technique to help distinguish spitz nevi from spitzoid malignant melanomas

K. Bell[1], M. Tahir[4], R. Lazova[2], N. Rodic[3]

[1]Georgetown University School of Medicine , Washington DC, District of Columbia, United States, [2]California Skin Institute, San Jose, California, United States, [3]MedStar Washington Hospital Center, Washington, D.C., District of Columbia, United States, [4]Lancaster General Hospital, Lancaster, Pennsylvania, United States

The differentiation between Spitz nevi (SN), Atypical Spitzoid neoplasms (ASN) and Spitzoid malignant melanomas (SMM) represents a challenge to dermatopathologists. ASN are lesions in the gray zone for which a definitive histopathologic diagnosis of benign or malignant cannot be made with absolute certainty. We sought to investigate whether the major epigenetic biomarker, 5-hydroxymethylcytosine (5-hmC), is differentially expressed and can aid in the diagnosis of benign and malignant Spitzoid neoplasms. Nine cases of SN, four cases of ASN, and seven cases of SMM from the Yale Spitzoid Neoplasm Repository were immunolabeled with the 5-hmC biomarker. Each case was scored by examining 50 lesional nuclei for staining on a scale from 0-3: (i) 0, no immunostaining; (ii) +1, weak immunostaining (qualitatively less than 5-hmC level in maturing keratinocytes in the upper stratum spinosum); (iii) +2, moderate immunostaining (similar to 5-hmC level in maturing keratinocytes); and (iv) +3, strong immunostaining (stronger than 5-hmC level in maturing keratinocytes). A product score was then calculated by multiplying the number of nuclei by the intensity of staining. For instance, if 25 of 50 lesional nuclei immunolabeled weakly with 5-hmC, the final product score would be [(25/50)*(1/3)]*100 = 15. Although 5-hmC was present in both SN and SMM, it was expressed much stronger and more diffusely in SN with a product score of 63.41 whereas the expression was notably diminished in SMM with a product score of 38.57 ($p<0.05$). Contrary to our hypothesis, the 5-hmC biomarker did not discriminate between SN and ASN nor did it differentiate ASN from SMM. Given the difference in biomarker expression seen in SN and SMM, 5-hmC could be used as an ancillary study to help in the differential diagnosis and distinguish SN from SMM.

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The effective killing of the difficult-to-treat melanomas with the combinations of MCL1 inhibitors S63845/MIK665 plus Navitoclax

N. Mukherjee[1], J. Skees[1], K. Todd[1], D. West[1], K. Lambert[1], W. Robinson[1], C. Amato[1], K. Couts[1], R. Van Gullick[1], M. MacBeth[1], K. Nassar[1], A. Tan[1, 2], Z. Zhai[1], M. Fujita[1], S. Bagby[1], D. Norris[1, 3], Y. Shellman[1]

[1]Dermatology, University of Colorado, Aurora, Colorado, United States, [2]Moffitt Cancer Center, Tampa, Florida, United States, [3]Veterans Affairs Medical Center, Denver, Colorado, United States

Despite the advancement of melanoma care, a subset of melanoma patients do not respond or relapse with current targeted or immuno-therapies. Therefore, it is necessary to explore other biological processes can be therapeutic. The BCL-2 family of proteins contributes to a cancer cell's resistance to treatment, and BH3 mimetics drugs that target BCL-2 are effective in hematological cancers. We used genetic knockdown and BH3 mimetics therapeutics to target BCL-2's anti-apoptotic defenses, and identified MCL1 and BCLXL as crucial melanoma pro-survival members. We then examined the effects of combining BH3 mimetics that target MCL1 and BCLXL *in vivo* and *in vitro*. We prioritized clinical-trial-ready compounds, such as ABT263 (Navitoclax) and S63845/S64315 (MIK655). We used commercial cell lines, as well as cells derived from patients with difficult-to-treat melanomas. The melanoma panel included the subtypes acral, mucosal, superficial spreading, and nodular, as well as diverse mutations, such as wild type for BRAF, NRAS and NF1. *In vitro*, the combined inhibition of MCL1 and BCLXL resulted in greater killing than either single agent ($p < 0.05$) in multiple assays. Genetic knock-down and knock-out studies showed that the combination-induced cell death was not dependent on pro-apoptotic BCL2 family members BID, BIM, or NOXA. Moreover, in mouse xenograft model, the combination inhibited tumor growth of multiple melanomas ($p <0.01$), had tolerable toxicity, and reduced sphere forming capacity ($p < 0.05$) in a downstream *in vitro* assay. In summary, this study suggests that dual targeting of MCL1 and BCLXL can be an alternative treatment for melanoma patients who have exhausted current treatment options.

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The phosphorylation of CD147 by Fyn plays a critical role in melanoma cell growth and metastasis

X. Zhang, Y. Guo, X. Chen, C. Peng

Dermatology, Xiangya Hospital, Central South University, Changsha, Hunan, China

CD147, also known as extracellular matrix metalloproteinase inducer (EMMPRIN), is a transmembrane glycoprotein that is highly expressed in tumor cells, particularly melanoma cells, and plays critical roles in tumor cell metastasis through the regulation of matrix metalloprotease (MMP) expression. Studies have demonstrated that CD147 could promote melanoma progression through post-translational modification, such as glycosylation. However, the role of phosphorylation of CD147 in melanoma remains unclear. In this study, we identified Fyn, a member of Src family that regulates diverse physiological processes, as a novel interacting protein of CD147. Our findings demonstrated that Fyn directly phosphorylates CD147 at Y140 and Y183. Two phosphospecific antibodies against Y140 and Y183 were developed to validate the phosphorylation of CD147 by Fyn. Moreover, the CD147-FF (Y140F/Y183F) mutation impaired the interaction between CD147 and GnT-V, leading to decreased CD147 glycosylation and membrane recruitment. In addition, CD147-FF significantly blocked MMP-9 expression as well as cell migration. Moreover, we found that Fyn is overexpressed in melanoma clinical tissues as well as in melanoma cell lines. Knockdown of Fyn expression markedly attenuated the malignant phenotype of melanoma cells in vitro and in vivo through downregulation of CD147 phosphorylation, indicating that Fyn/CD147 is a potential target molecule in melanoma treatment. Finally, through virtual screening, we identified amodiaquine as a potential inhibitor targeting the Fyn/CD147 axis. amodiaquine treatment dramatically inhibited the phosphorylation of CD147 by Fyn, thus attenuating melanoma cell growth and invasion in vitro and in vivo, suggesting that amodiaquine is a promising inhibitor for melanoma treatment. In conclusion, our findings showed that the Fyn/CD147 axis plays an essential role in melanoma cell metastasis and growth and that amodiaquine, which targets this axis, is a promising inhibitor for melanoma treatment.

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Relationship between the physical properties of skin with pigmented spots and amount of desmoglein 1 in the stratum corneum

R. Maruyama, A. Kajiwara, A. Tada

POLA CHEMICAL INDUSTRIES,INC., Yokohama, Japan

The stratum corneum (SC) of the skin is thicker in areas with pigmented spots than in areas without pigmented spots. Despite this difference in SC characteristics, the physical properties of skin with pigmented spots have not yet been studied. We compared skin hardness and measured the amount of desmoglein 1 (Dsg1) in the SC at sites with and without pigmented spots. Since cell adhesion proteins can increase SC thickness, we assumed that the physical properties of the SC are also associated with cell-cell adhesion. We analyzed 105 sites with pigmented spots and 93 sites without pigmented spots on the faces of 20 Japanese women (aged 40-52 years) using an IDM 800 Indentometer®. In addition, we tape-stripped these sites to analyze Dsg1 levels in the SC using a method we developed. We also searched for botanical extracts capable of reducing the amount of Dsg1 in the SC. Compared to sites without pigmented spots, sites with pigmented spots had L* and b* value that were significantly lower and higher, respectively. The amount of melanin in the SC at sites with pigmented spots was higher than without pigmented spots. Sites with and without pigmented spots could be distinguished based on L* value, b* value and the amount of melanin. We found that the indentometer®'s value was significantly smaller, and that sites were harder in the skin with than without pigmented spots. Significantly more Dsg1 was found in the SC at sites with pigmented spots than without pigmented spots. We found that an extract of wild thyme (*Thymus serpyllum*) reduced the amount of Dsg1 on the SC. In conclusion, the finding that skin with pigmented spots was harder compared to skin without pigmented spots suggests that the amount of Dsg1 in the SC affects skin hardness. We consider that the hardness of skin with pigmented spots could be improved to levels that are equivalent to those of skin without pigmented spots by reducing the amount of Dsg1 in the SC using an extract of wild thyme.

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Sestrin2 contributes to vemurafenib resistance in braf mutant metastatic melanoma cells by detoxifying intracellular reactive oxygen species

G. Zhu, S. Wang, T. Gao, C. Li

Department of Dermatology, Xijing Hospital, Fourth Military Medical University, Xi'an, China

Melanoma is the most life-threatening skin cancer, accounting for 75% of skin cancer-associated death. Vemurafenib was approved by FDA for the treatment of late-stage melanoma patients with BRAF mutation. However, clinically, most cases developed resistance to it. Hereby we conduct a study to explore the possible role of a stress responsive protein, Sestrin2, in vemurafenib resistance. Previously, we have demonstrated Sestrin2 has a protective role in melanoma cells in response to anoikis via detoxifying intracellular reactive oxygen species (ROS). In the current study, we first found Sestrin2 was up-regulated in melanoma cells and tissues acquired vemurafenib resistance. Overexpression of Sestrin2 in BRAF mutant melanoma cells significantly shifted the IC50 curve while knockdown of Sestrin2 impaired the viability of vemurafenib-resistant cells in exposure to vemurafenib. Furthermore, we found the increased apoptosis induced by Sestrin2 knockdown was associated with elevated intracellular ROS. Microarray assay revealed mTOR pathway might be responsible for the vemurafenib resistant effect of Sestrin2. To sum up, in this study, we found Sestrin2 can be induced by the survival stress in BRAF mutant melanoma cells by vemurafenib treatment. By detoxifying intracellular ROS, Sestrin2 exerts a prospective role against vemurafenib.

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IL6/stat3 pathway drives MMP9 to promote invasion in melanoma

L. Lu, X. Chen, J. Su

Department of Dermatology, Xiangya Hospital, Central South University, Changsha, Hunan, China

Interleukin-6 particularly plays a critical role in number of biological events. Different cancer cells, cancer-associated cells and resistant cancer cells overexpress and secrete IL-6 in tumor microenvironment. While, IL6 which was originally identified as a B-cell differentiation factor, is a multifunctional cytokine that regulates the immune response, the acute phase response and inflammation. There are increasing evidences showed that IL-6 can initial promoting anti-tumor immunity to inhibit cancer progress. Those contradictory view urge us to explore the function of IL6 in melanoma. We report that IL6 was overexpression in melanoma patients and melanoma cell lines. Patients with high IL6 expression in melanoma species conferred a worse prognosis. Knock down IL6 markedly attenuated melanoma cell proliferation, invasion and metastasis in vitro and in vivo. Anti-IL6 antibody siltuximab can treat subcutaneous tumorigenic nude mice effectively. To further investigate the mechanism of IL6 promote invasion in melanoma. We downregulated IL6 and found the cell cycle were mainly prolonged in G1 phase and shortened in S phase, DNA damage was increase. Human phosphor-kinase array showed a significant decrease in stat3 and p-stat3, MMP9. Taken together, above findings indicated that IL6 stimulates tumor invasion and metastasis by adjust cell cycle, apoptosis, and STAT3/MMP9 expression and these actions can be inhibited by a neutralising anti-IL6 antibody siltuximab, which serves as a new understanding of the function of IL6 in melanoma, and enhanced the basis of IL6 as a melanoma target.

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$PM_{2.5}$ is an AhR agonist that upregulates melanogenesis in human melanoma cells A375

F. Peng[3], Z. Chen[1], M. Furue[2], J. Zhang[1]

[1]Peking University People's Hospital, Peking, China, [2]Kyushu University Hospital, Fukuoka, Japan, [3]Dermatology, Peking University People's Hospital, Peking, China

To investigate the effects of $PM_{2.5}$ on A375 human melanoma cells and the possible mechanism. The $PM_{2.5}$of winter haze was collected in Beijing. A375 was treated with different concentrations of $PM_{2.5}$ suspensions. The morphology of A375 cells was observed by microscopy. The number of viable A375 decreased and the cells lost their normal shape gradually with the treatment of $PM_{2.5}$. $PM_{2.5}$ also inhibits the A375 cells viability and induces blockage of S phase.The protein expression of CDk1 and CyclinE1 were significantly decreased after $PM_{2.5}$ treatment. $PM_{2.5}$ induces IL-1α, IL-6, IL-8 production by Elisa. In contrast, it has no impact on TNF-α. ROS was effectively induced by $PM_{2.5}$.We examined melanin in A375 using transmission electron microscopy, and observed more melanin following $PM_{2.5}$treatment. Furthermore, $PM_{2.5}$significantly induced a dose-dependent increase of melanin content in A375.To elucidate the mechanism of melanin synthesis, we examined the effect of $PM_{2.5}$on human tyrosinase activity. $PM_{2.5}$ markedly induced tyrosinase activity and induced tyrosinase mRNA and protein upregulation.To determine whether $PM_{2.5}$activates AhR signaling, we examined AhR, CYP1A1 and CYP1B1 expression by qRT-PCR and western blotting analysis. It confirmed that $PM_{2.5}$induced the upregulation of CYP1A1 mRNA and protein in A375s treated with $PM_{2.5}$. PM2.5 can exert toxicological effects on human melanoma cells A375. $PM_{2.5}$-induced reactive oxidative species (ROS) and melanogenesis maybe dependent on AhR.

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A systems biology approach for skin brightening, including autophagy as a critical mechanism to control pigmentation

K. Dong[1], D. Layman[1], N. Pernodet[1, 2]

[1]Research & Development, The Estee Lauder Companies, Melville, New York, United States, [2]Estee Lauder Research Laboratories, Melville, New York, United States

We use a lens of systems biology to develop our high-performance skincare products. When targeting brightening, the importance of addressing the pigmentation machinery (tyrosinase inhibition, melanin transfer) is well established. We have, however, identified autophagy, which has recently been shown as another pathway critical not only to pigmentation, but also to inflammation as an important target as well. Autophagy, a cellular degradative and recycling pathway, is essential for cell efficiency, homeostasis, and longevity. This pathway is also a critical player in cellular aging. As a highly conserved mechanism, it is responsible for the recycling and renewal of intracellular organelles, lipids, and proteins and is also a vital source of energy for cells. A decrease in autophagic activity has been associated with age. And as skin ages, age spots and other pigmentation problems increase. We have determined that a reduction in autophagic activity translates to an increase in intracellular damage, oxidative stress, and inflammation, which in turn contributes to the accumulation of pigmentation when melanin is not properly turned over. In this study we specifically addressed the role of autophagy activity in helping to address hyperpigmentation. Using a specific yeast extract, we were able to support autophagy activity and show a decrease in inflammatory mediator expression as well as in melanin production. These results were compared to the activity of vitamin C, an inhibitor of melanin production. Finally, the two actives were combined to target different pathways reflecting our overall systems biology approach to address hyperpigmentation.

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Targeted degradation of CD147 proteins in melanoma

Z. Zhou, J. Long, X. Chen, C. Peng

Dermatology, Xiangya Hospital, Central South University, Changsha, Hunan, China

CD147, a transmembrane glycoprotein highly expressed on melanoma cells, plays a vital role in tumor proliferation, invasion, metastasis, thus may be a potential drug target for melanoma. Theoretically, degradation of a target oncogenic protein in cancer cells is superior to inhibition of the same protein in efficacy. PROTACs (proteolysis targeting chimeras) are chimeras connected via a linker which could simultaneously bind to a target protein and an E3 ligase, thereby leading to ubiquitination and subsequent degradation of the target by the ubiquitination system. Herein we reported the discovery of the first CD147 PROTACs derived from nature product PAB (pseudolaric acid b). We choose carboxylic acid group as the modification site for the linker, lenalidomide-type ligands for the E3 ligase CRBN. Various carbon linkers were synthesized and assayed. In these PROTACs, 81b showed the most degradation ability on CD147. Immunoblotting data showed that after 6 hours of treatment, 81b effectively decreased the level of CD147 in a dose-dependent manner, the DC50 was 3.1 μM. To confirm the degradation of CD147 is through PROTAC, rather than PAB or lenalidomide. we applied the above ligands and ubiquitin enzyme inhibitor MG132 to the cells in the same way. The data showed that the degradation induced by 81b could be effectively blocked by pretreatment with CD147 ligand, CRBN ligand (lenalidomide) and proteasome inhibitor (MG132), and the degradation effect of CRBN ligand was not achieved by using CD147 ligand alone. By immunoblot then we found that the downstream signal pathway MMP9,p-STAT3 of CD147 weakened with the degradation of CD147, which was consistent with the data we obtained in the cell phenotype. The degradation of CD147 inhibited the growth and invasion of cells. Moreover, in vivo experiments indicate that 81b has a greater inhibitory effect on tumorigenesis and development in mouse xenografts than PAB. In conclusion, our data show that 81b is a very strong CD147 degradation agent, and further optimizer structure may produce new drugs for the treatment of melanoma.

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Immunoprevention of chemical melanomagenesis through early recognition of oncogene mutations

K. Yang[1], M. Sherwani[1], B. N. McDaniel[1], A. Stephens[1], N. Yusuf[1, 2], C. Elmets[1, 2]

[1]UAB, Birmingham, Alabama, United States, [2]BVAMC, Birmingham, Alabama, United States

Melanoma, an aggressive malignancy of melanocytes, is responsible for more deaths than any other skin cancer. We have developed an *in vivo* murine model of melanomagenesis in which benign pigmented lesions progress to become invasive melanomas. The polyaromatic hydrocarbon DMBA (7,12-dimethylbenz(a)anthracene) was applied to the skin of C3H/HeN mice followed by repeated exposure to phorbol 12-myristate 13-acetate (TPA). This treatment produces a specific activating mutation in the H-ras oncogene, resulting in a single amino acid substitution (leucine for glutamine) at the 61st residue. Vaccinating mice with the mutant H-ras peptide fragment (AGLEEYSAM) elicited a protective response against melanomagenesis. To determine the effect of IL-12 and IL-23 on the efficacy of immunization, cohorts of IL-12p35 knockout (KO), IL-23p19 KO, and wild type (WT) C3H/HeN were immunized subcutaneously with mutant H-ras or control peptide (100μg/100μl in PBS). The first immunization was followed with two booster doses of the peptides. Mice were rested for a week, after which DMBA (0.1%) was topically applied once on their skin followed by biweekly application of TPA for 15 weeks. Mice that were immunized with mutant H-ras peptide showed a significant decrease (p<0.001) in development of melanocytic nevi in WT mice in comparison to WT mice that were treated with control peptide. The protective effect of mutant H-ras peptide was abolished in IL-12p35 KO mice. Interestingly, vaccination with mutant H-ras peptide offered initial protection against melanocytic nevi in IL-23p19 KO, which was abolished at later stages of melanomagenesis. These findings indicate that IL-12 is necessary for success of mutant H-ras peptide vaccination against melanoma and that IL-23 helps with short-term but not long-term efficacy.

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Keratinocyte behaviour in normal appearing vitiligo skin

D. Kovacs, E. Bastonini, P. Iacovelli, A. Pacifico, C. Cota, M. Picardo

San Gallicano Dermatological Institute, Rome, Italy

Cytoarchitectonic and functional interactions among epidermal and dermal compartments are known to regulate melanocyte behaviour and survival. The presence of a deregulated microenvironment able to affect melanocyte homeostasis has been described in both hyper- and hypo-pigmentary disorders. In vitiligo, several data indicate a structural and functional impairment not only of melanocytes, but also of the other cutaneous cells, which appears broadly extended, even in apparently normal skin. In line with these remarks, our group has recently reported some modifications in non lesional fibroblasts, which show a myofibroblast phenotype associated to signs of premature senescence. This fibroblast phenotype, in turn, is able to perturb melanocyte adhesion, furthering their detachment and loss. Here we broaden the *in vitro* and *ex-vivo* analyses also to the epidermal compartment, focusing on keratinocyte features possibly responsible for compromising the well-being of melanocytes still present in regularly pigmented skin areas. Microscopical evaluation of non lesional vitiligo keratinocytes in culture revealed an overall enlargement of their size. These cells display also a slow proliferation rate, as well as a deregulation/delay of differentiation and stratification processes, as assessed by the reduction of early and late differentiation markers at mRNA and protein levels. An inappropriate cytoskeletal actin rearrangement, associated to lower expression and discontinuous distribution of the adhesion molecule E-cadherin in comparison to control cells are also detectable. These structural and functional alterations highlighted in non lesional epidermal cells may perturb, over time, the whole skin integrity and the appropriate communication between keratinocytes and melanocytes, rendering the latter cells more susceptible to cell-cell adhesion defects and disappearance.

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Anti-tumor effects and mechanism of 4'-bromo-resveratrol in a $BRAF^{V600E}$/ $PTEN^{NULL}$ melanoma mouse model

G. Chhabra, C. K. Singh, M. A. Ndiaye, N. Ahmad

Dermatology, University of Wisconsin-Madison, Madison, Wisconsin, United States

New therapeutic modalities are required for an effective and recurrence-free lasting treatment of metastatic melanoma, which is one of the deadliest forms of cutaneous malignancy. 4'-Bromo-resveratrol (4BR), is a dual small molecule inhibitor of Sirtuin-1 and -3, both of which play tumor-promoting roles in melanoma. Earlier, we showed that 4BR imparted anti-proliferative effects against human melanoma cells *in vitro*. Here, we determined the *in vivo* efficacy and mechanism of 4BR against melanoma in a genetically engineered $BRAF^{V600E}$/$PTEN^{Null}$ mouse model that recapitulates human disease with lymph nodes and lung metastases. Tumors were induced by topical application of 4-hydroxytamoxifen on shaved backs of 10-week-old mice, and the effects of 4-BR (30 mg/kg b.wt.; *intraperitoneally*; 3d/week for 5 weeks) treatment were assessed on pigmented melanoma tumors. 4BR treatment significantly reduced the size and volume of primary melanoma tumors, as well as the metastatic burden in lungs with no adverse effects. Further, mechanistic studies with skin/tumor samples demonstrated downregulation of cell proliferation markers (Ki67, PCNA, Survivin), melanoma promoting growth factor IGF1, as well as upregulation of the tumor suppressor protein IGFBP5. As Sirtuins-1 and -3 are linked to immunomodulation, to explore the mechanism of 4BR treatment, we performed differential gene expression analysis using the nCounter PanCancer Immune panel (770 genes) and nSolver software. Results indicated that 4BR significantly downregulated (≥2-fold) expression of multiple genes promoting melanoma metastasis (Fn1, S1008a, Fap, Col3a1, Angpt2, Itga1, and Ptgs2), associated with chemokine/cytokines and their receptors (Ccr1, Ccl6, Il1b, Ccl24, Ccl9, Il1rl1 and Ccr5), and innate/adaptive immunity (Itgam, Nlrp3, Colec12 and Irf7). Overall, sirtuin inhibition by 4BR (or other means) appears to be a promising anti-cancer therapy with anti-metastatic and immunomodulatory effects, warranting further studies, including clinical investigations.

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Re-evaluation of analogues of a potent tyrosinase inhibitor
J. Jerome, T. Mammone
Research & Development, The Estee Lauder Companies, Melville, New York, United States
Human skin color is partly determined by the amount and type of melanin, a light-absorbing molecule produced by melanocytes, specialized pigment producing cells in the skin. A key step in the synthesis of melanin in skin is the conversion of tyrosine to DOPAquinone by the enzyme tyrosinase. Multiple approaches exist for inhibiting melanin biosynthesis, many of which target tyrosinase. Tyrosinase is the rate limiting enzyme in the reactions leading to the production of the two melanin pigments, eumelanin and pheomelanin. Activation of this enzyme increases the production of melanin, resulting in an increase in pigmentation. Conversely, tyrosinase inhibition results in a decrease in melanin production and diminishes pigment. Historically, UP302 (1-(2,4-dihydroxyphenyl)-3-(2,4-dimethoxy-3-methylphenyl)propane), a synthetic derivative of the plant extract *Dianella ensifolia*, has been shown to effectively inhibit tyrosinase in vitro. Additional derivatives have been tested, with UP274M (UP274) (4-(3,5-dimethoxyphenethyl) benzene-1,3-diol) showing the most promise. Here, we compare the effects of UP274 to UP302 on melanin formation in cell lines and in tissue culture. To evaluate UP274 efficacy as compared to UP302, we assessed (1) melanin production, spectrophotometrically, in the B16-F10 melanoma cell line and (2) changes in pigmentation in melanocyte-containing reconstructed skin models via spectrophotometric analysis and macroscopic images. A significant decrease in melanin content was observed after treatment with 5.94 and 11.88 μM of UP274, as compared to UP302 at 49.96 μM, in B16-F10 cells after 48 hours. In addition, after 5 days of apical treatment in skin models, UP274 yielded a dose-dependent decrease in pigmentation which showed parity or a slight decrease in pigmentation compared to UP302. These data suggest that UP274 is more efficacious than UP302 *in vitro* and is active at lower levels.

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Identification of RNA biomarker candidates in melanocytic tumors using digital spatial profiling
M. Kiuru[1, 2], M. Kriner[3], G. Zhu[1, 4], J. Terrell[1], M. Hoang[3], J. Beechem[3], J. McPherson[5]
[1]Department of Dermatology, University of California, Davis, Sacramento, California, United States, [2]Department of Pathology and Laboratory Medicine, University of California, Davis, Sacramento, California, United States, [3]NanoString Technologies, Inc., Seattle, Washington, United States, [4]Fourth Military Medical University, Xian, Shaanxi, China, [5]Department of Biochemistry & Molecular Medicine, University of California, Davis, Sacramento, California, United States
Early diagnosis of melanoma is imperative for improved prognosis, but current diagnostic methods are based on histological examination and can be inaccurate for a subset of tumors. Additionally, biomarkers of early melanomagenesis and the role of the microenvironment are poorly defined. To identify novel biomarkers, we measured RNA expression of >1,000 cancer- and immune-related genes with spatial resolution in 200μm-diameter regions of interest (ROIs) from patient-derived formalin-fixed, paraffin-embedded (FFPE) tissue sections of common melanocytic nevi, dysplastic nevi, melanoma in situ, and melanoma using the NanoString GeoMX™ Digital Spatial Profiler (DSP). ROI clustering analyses confirmed distinct expression patterns across cell types and tumor types. We coupled linear regression of ROI groups with dimensionality reduction analysis to identify genes enriched specifically in melanocytes, keratinocytes or immune cells in melanoma. These genes include known biomarkers as well as novel candidates, and enrichment of these genes in melanoma was verified by an orthogonal bulk RNA-seq cohort and protein expression by immunohistochemistry. Lastly, spatial analysis of melanoma revealed striking intra-tumoral heterogeneity, thus providing a snapshot of cancer development within a single specimen. In summary, our results demonstrate the value of a spatially resolved assay performed in situ on FFPE tumor material for precise molecular profiling of the tumor and its microenvironment and provide a framework for discovery of cell type-specific biomarkers with utility for diagnosis, prognosis, and prediction of response to therapy. GeoMx™ DSP is for research use only and not for use in diagnostic procedures.

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Using a novel p300 HAT inhibitor as epigenetic therapy to treat BRAF inhibitor-resistant melanoma cells
A. Hanly[1], F. Gibson[1], K. Kuang[1], E. Kim[2], B. E. Zucconi[3, 4], M. Wu[1], P. Cole[3, 4], R. Alani[1]
[1]Dermatology, Boston University School of Medicine, Boston, Massachusetts, United States, [2]Surgery, University of Massachusetts Medical School, Worcester, Massachusetts, United States, [3]Medicine, Division of Genetics, Brigham and Women's Hospital, Boston, Massachusetts, United States, [4]Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts, United States
Despite initial response to targeted therapy in many patients with advanced melanoma, innate and acquired resistance to BRAF inhibitor treatment limits the long-term success of monotherapy with these agents. Reactivation of the mitogen activated protein kinase (MAPK) pathway in melanoma cells is a crucial mechanism in the development of resistance to targeted therapy and usually does not occur through new genetic mutations. Therefore, epigenetic alterations such as histone modifications have emerged as key mediators of the ability of melanoma cells to achieve resistance. We showed that A485, a novel p300 histone acetyltransferase (HAT) inhibitor, led to decreased proliferation and morphological changes in BRAF inhibitor-resistant melanoma cells, particularly in MITF-high cell lines. A485 acts through the MITF-FOXM1 transcriptional axis, whereby inhibition of p300/CBP downregulates MITF, which in turn downregulates FOXM1, a pro-proliferative and pro-survival MEK-target gene. Combinational treatment with A485 and a BRAF inhibitor showed superior growth inhibition in BRAF inhibitor-resistant cells compared to treatment with a BRAF inhibitor alone. Given that MITF is known to play a role in the ability of melanoma cells to acquire resistance to BRAF inhibitors, p300/CBP inhibition demonstrates a promising potential mechanism in overcoming BRAF inhibitor resistance. Future work aims to determine the effects of A485 and BRAF inhibitor treatment on downstream targets in both sensitive and BRAF inhibitor-resistant melanoma cell lines to identify novel differentially-regulated genes and signaling pathways in response to combination treatment.

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p38 signaling regulates human cutaneous metastatic melanoma (MM) invasion and MM-dependent disruption of keratinocyte differentiation
A. Kiss[1], C. Wei[2], Z. Aligabi[1], M. Barlas[1], E. Murphy[2], F. Glass[2], A. Friedman[2], T. Efimova[1, 2]
[1]Anatomy & Cell Biology, GWU, Washington, District of Columbia, United States, [2]Dermatology, GWU, Washington, District of Columbia, United States
Advancing our understanding of MM invasion mechanisms is vital for developing new mechanism-based therapies and improving MM outcomes. Here we describe an optimized organotypic human skin equivalent co-culture system of primary epidermal keratinocytes, MM cells, and stromal fibroblasts recently developed in our laboratory in order to reliably model early invasive behavior of MM as well as melanoma-keratinocyte crosstalk in the tissue microenvironment that more accurately reflects human disease pathology. p38 kinases p38α/δ are the predominant p38 isoforms in keratinocytes, while p38α/β are the most abundant p38s in MM cells. However, the roles of p38 kinases in regulation of human cutaneous MM invasion or in control of melanoma-keratinocyte communication remain to be elucidated. Our data showed that in human skin equivalents harboring human A375 MM cells, pharmacologic inhibition of p38α/β isoforms with specific inhibitor SB203580 led to increased invasion of A375 cells into the dermis, as manifest by significantly increased size of the dermal nests of A375 cells relative to size of those in control vehicle-treated cultures. The hyper-invasive MM phenotype observed in skin equivalents treated with SB203580 was reversed by treatment with potent pan-p38 inhibitor Compound 62 back to the levels displayed by the control cultures. These data suggest that p38α/β function to restrict MM invasion, and support a role for keratinocyte p38δ in promoting MM invasion in this model system. Furthermore, reflecting effect of melanoma on keratinocyte differentiation as observed in human disease, skin equivalents harboring A375 MM cells displayed a marked disruption of keratinocyte differentiation program as evidenced by reduced cornification, absence of granular layer, and severely diminished expression of differentiation markers. Pan-p38 inhibition partially restored keratinocyte differentiation, supporting a role for p38 signaling in MM-dependent loss of the latter in this system.

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An epidermal model containing melanocytes for skin pigmentation and lightening studies

M. Klausner, B. Breyfogle, A. Armento, J. Oldach, S. Ayehunie

MatTek Corporation, Ashland, Massachusetts, United States

Considerable interest exists in evaluating raw materials and/or skin care formulations which cause lightening of the skin. These products are utilized to modulate one's natural skin color or to combat skin pigmentation disorders such as melasma, dark spots, solar lentigo, and other hyperpigmentation lesions. To aid in the development and testing of such products, we have developed a skin whitening protocol using the epidermal skin model, MelanoDerm™, to evaluate both raw materials and skin lightening formulations. MelanoDerm is a highly differentiated, three-dimensional tissue culture model of human epidermis that contains normal human melanocytes (NHM) and keratinocytes (NHK). Epidermal tissues have been produced containing NHM of varying skin phototypes which follow the pigmentation level of the donor tissue, i.e. black > Asian > Caucasian. For lightening studies, tissues were treated topically three times a week over a two to three week period to mimic consumer application. Several over-the-counter skin lightening products were evaluated in cultures containing NHM from black and Asian donors. Over the treatment period, negative control cultures became increasingly pigmented with retention of normal epithelial morphology. In contrast, tissues treated topically with cosmetic skin lightening agents containing tyrosinase inhibitors such as kojic acid and magnesium ascorbyl phosphate remained lighter than the control cultures. The skin lightening effect on treated tissues was quantitatively evaluated for melanin content using a Solvable melanin assay and for skin brightness (L* value) using a hand-held spectrometer. Treated tissues showed significant changes in overall melanin content and brightness compared to control tissues. These results suggest that this model can provide valuable *in vitro* data for screening raw materials prior to the commencement of costly clinical trials and that it will be useful to study melanogenesis, skin lightening, and other pigmentation phenomena of the skin.

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MAPK inhibition synergizes with TNF-alpha to promote the expression of STAT1, IRF-1 and MHC class I

B. P. Pollack[1], Y. Chang[2]

[1]Dermatology, Atlanta VA Health System/Emory University, Decatur, Georgia, United States, [2]Immunology and Microbiology, Emory University, Atlanta, Georgia, United States

Molecular events that activate the MAPK signaling pathway are key events in melanoma pathogenesis and MAPK inhibitors (MAPKIs) such as those targeting BRAFV600E and MEK1/2 are currently in use to treat melanoma patients. In addition to their effects on tumor cell proliferation and survival, MAPKIs can increase T cell infiltration into tumors and MHC expression and clinical trials combining MAPKIs with immune-based therapies are currently being performed. TNF-alpha is a pleiotropic cytokine that plays a complex role in melanoma and has been shown to block apoptosis induced by MAPKIs. In contrast, the interplay between TNF-alpha and MAPKI-mediated immune effects are largely undefined. To further interrogate interactions between TNF-alpha and MAPKIs, we treated human melanoma cell lines with the BRAFV600E inhibitor vemurafenib or the MEK1/2 inhibitor trametinib alone or in the presence of TNF-alpha. Using A375 and HT-144 cells as models for BRAF mutant melanoma, we found that MAPKIs synergized with TNF-alpha to increasing MHC class I surface levels > 20 fold. In addition, cell surface levels of programmed death ligand 2 (PD-L2) were also increased by the combination of TNF-alpha and a MAPKI. Additional studies determined that these changes were associated with increases in steady state mRNA levels and total protein levels of MHC class I genes HLA-A, B and C . To define the mechanisms involved in this synergistic response, we examined levels of STAT1 and found that the combination of TNF-alpha and a MAPKI increased levels of STAT1 mRNA by roughly 10 fold and that of STAT1 protein by 4-5 fold. Increases in phospho-STAT1-Y701 and S727 were also observed. Similar increases were seen in levels of IRF-1 mRNA and protein. These studies suggest that MAPKIs can influence the cellular responses to TNF-alpha in a manner that promotes the expression of genes typical of an interferon signature.

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Gene profiling of acral melanomas

P. Valencia Pena, J. Li

Dermatology, University of Miami, Miami, Florida, United States

Acral melanoma is a subtype of melanoma that occurs most often in dark skinned individuals. Different from the melanoma subtypes occurring in sun-exposed areas in white patients, dark skinned patients most often present lesions in palms, soles, nails and heels and in advanced stages. Even though advances in genomics and molecular technologies are refining our understanding of biological mechanism of melanoma, most of the studies have been done in non-acral melanomas in white individuals. Those studies have led to identifying common mutations or alterations in genes of BRAF, NRAS and KIT. However, in case of acral melanoma, these alterations represent only about 40~45% of the cases in total. Thus the gene alterations that give rise to the majority of acral melanomas are currently unknown. In this preliminary study, we conducted research of gene profiling with a panel of 50 cancer-related genes using Illumina Next Generation Sequencing technology. 13 acral melanoma tissue DNA samples were analysis. From 13 tumors, we identified 2 with cKIT mutation, 2 with NRAS mutation, and 1 with both cKIT and NRAS mutations. In contrast to the frequent mutations identified in common forms of superficial spreading melanoma and nodular melanoma, this low occurrence of BRAF, NRAS and cKIT mutations in acral melanoma tested is consistent with the reported data (less than 45%), supporting our hypothesis that potential novel driving force of gene mutations are involved in the development of the majority of acral melanomas.

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Dysplastic nevi (DN) patients have high DC-HIL-expressing myeloid-derived suppressor cells (MDSC) that may confer increased risk for melanoma

J. Chung[1], V. Ramani[2], P. Cruz[2], S. Savory[2], K. Ariizumi[2]

[1]Dermatology, UT Southwestern Medical Center at Dallas, Dallas, Texas, United States, [2]Dermatology, UT Southwestern Medical Center, Dallas, Texas, United States

MDSC are the most potent suppressors of T-cell function due to expression of the DC-HIL receptor. We showed degree of DC-HIL$^+$MDSC proliferation in blood of melanoma patients to correlate with worsening cancer stage but also found DN patients to display a wide range of this index. In FACS analysis, DC-HIL$^+$MDSC/PBMC in DN cases ranged from 0.2 to 4.6% DC-HIL$^+$MDSC/PBMC with a median of 2%. This was significantly higher than DN-free and age-matched healthy donors (HD) whose median index was 0.1 ± 0.1% and of melanoma in-situ (MIS) at 1.1 ± 0.6%, but lower than stage 3 melanoma at 2.2 ± 0.9%. Moreover in DN, the T cell-inhibitory activity of MDSC (40-70% suppressed IFNγ response) was markedly higher than for HD (~10%), but lower than stage 3 MDSC (70-95%). We examined DC-HIL regulation by UVB using monocytes (Mn) because MDSC are a minuscule fraction. UVB-irradiated *in vitro* and measured DC-HIL mRNA by qRT-PCR. UVB irradiated (100J/m^2) Mn from DN showed 2.5-fold higher DC-HIL mRNA than non-irradiated Mn, lower than for HD Mn (10-fold). Since DC-HIL is a target gene for UVB-responsive MITF transcription factor, we examined its role in UVB-upregulation. MITF expression was restricted to Mn among blood leukocytes and upregulated by UVB, 3.2-fold rise in DN and 6.3-fold in HD. UVB-inducibility considerably varied among DN cases (1.5-7-fold). UVB-irradiated Mn from DN were more potent T cell-suppressors than non-irradiated Mn. These effects were reversed by pretreatment of Mn with MITF-specific siRNA or anti-DC-HIL mAb. We also found DC-HIL$^+$ myeloid cells in lesional DN skin. We thus conclude that DC-HIL expression by MDSC is upregulated by the UVR/MITF axis and that DN cases with high DC-HIL-MDSC may be at greater risk for melanoma.

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Malassezin: A proof of concept study documenting the efficacy of a novel microbiome-based ingredient for facial hyperpigmentation

P. Grimes[1], E. T. McCraw[2], A. Einziger[3], M. Einziger[3]

[1]Vitiligo and Pigmentation Institute of Southern California, Los Angeles, California, United States, [2]Skin Science Advisors, LLC, Peachtree Corners, Georgia, United States, [3]Versicolor Technologies, Santa Monica, California, United States

Malassezin is a natural indole compound produced by the fungus Malassezia furfur which causes Tinea Versicolor characterized by patches of hypo and/or hyperpigmentation. Our preliminary in-vitro and ex-vivo experiments documented the ability of Malassezin to decrease skin pigmentation. The objective of this randomized, double blind, controlled study was to investigate the skin lightening effects of novel formulations of Malassezin for facial hyperpigmentation. This 22-week study enrolled subjects with melasma(n=8) and dyschromia(n=12) caused by photodamage. Subjects had mild, moderate, or severe hyperpigmentation. They were randomized to 1 of 4 groups including vehicle, 0.1%, 0.5%, and 1.0%. Subjects were evaluated at baseline, 2, 4, 8, 14, 18 and 22 weeks. Twenty subjects were enrolled and 16 completed the study. As early as 2 weeks, colorimetry assessments (Mexameter MX18) showed improvement. The 1.0% formulation group showed a significant percent reduction in the melanin index compared to vehicle for involved skin (2.93% vs 0.27% respectively). At 14 weeks, clinical assessments and photography using the Visia-CR (Canfield Scientific) showed that there was a decrease in facial hyperpigmentation in 69% of subjects. The lightening effects were sustained during the 8-week regression period from week 14 to week 22. Histopathological assessments(Fontana Masson staining) showed a reduction in melanin. There were no clinically significant adverse events observed during the study. This proof of concept study documents the very novel efficacy and safety of malassezin for skin lightening.

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Laser Capture Microdissection and genetic analysis identify dysplastic nevi as a subgroup of common nevi rather than a progressive state towards melanoma

K. Navrazhina[1], J. W. Frew[1], N. Gulati[1, 2], D. Grand[1], S. Garcet[1], H. Mitsui[1], J. G. Krueger[1]

[1]Laboratory for Investigative Dermatology, The Rockefeller University, New York, New York, United States, [2]Department of Dermatology, NYU Grossman School of Medicine, New York, New York, United States

Whether the dysplastic nevus (DN) is a premalignant lesion serving as a precursor to malignant melanoma (MM), or a subset of the common melanocytic nevus (CN) has remained a source of controversy. We employed Laser Capture Microdissection (LCM) in combination with microarray analysis to identify melanocyte gene expression signatures specific to DNs. Excisional biopsies of CNs, DNs and primary MM were obtained. LCM was performed on frozen sections to isolate melanocytes followed by cDNA microarray analysis. Principal component analysis and phylogenetic tree clustering demonstrated that all pigmented lesions have a distinct melanocytic profile compared to normal skin (FCH>1.5, p<0.05). DNs and CNs clustered together separate from MM and normal skin. We identified over 17,000 differentially expressed genes between the melanocyte population of DNs and normal skin. Upregulated genes unique to DNs included follicular and adnexal associated genes (KRT15, HIPK2, S100A2). The LCM method was able to identify targetable kinases including those involved in cell cycle progression, growth factors, cytokine receptors and epigenetic markers, with known genes associated with neoplastic melanocyte function absent from DNs. We then compared the LCM analysis to a bulk microarray analysis. The top upregulated genes detected by bulk analysis were of the immune and stromal background. The data demonstrate that the histologically diagnosed DNs have no significant melanocytic enriched genes compared to CNs. Alterations in the surrounding immunological and stromal milieu may account for the differences between DNs and CNs rather than melanocyte specific markers and profiles. Taken together, our data suggest that the DN is an aberrant tissue state rather than a portion of a linear progression towards MM.

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Bioluminescent identification of a novel EMT-directed experimental therapeutic blocking invasion and metastasis in human malignant melanoma

J. Jandova, G. T. Wondrak

Pharmacology and Toxicology, College of Pharmacy and UA Cancer Center, University of Arizona, Tucson, Arizona, United States

Epithelial-mesenchymal transition (EMT) is a key molecular pathway and promising therapeutic target in human melanomagenesis. Employing a phenotypic screen that examines therapeutic suppression of EMT in A375 malignant melanoma cells, we have now identified a drug-like chemical entity, 4-[(7-chloro-2-methoxy-1,5-dihydrobenzo[b] [1,5]naphthyridin-10-yl)imino]-2,6-bis(pyrrolidin-ylmethyl)cyclohexa-2,5-dien 1-one (PYD), as a potent EMT inhibitor. In a SCID mouse metastasis model employing time-resolved bioluminescent detection of lung tumorigenesis after tail-vain injection of stable A375-luciferase transfectants (monitored over three weeks post-injection), lung tumor burden imposed by A375-luc2 cells was attenuated by PYD-based systemic intervention (100 mg/kg; p.o., q.d., 3 d regimen), a curative outcome achievable without causation of any organ toxicity or negative impact on body weight. Differential transcriptomic analysis futher substantiated PYD-induced inhibition of EMT-related gene expression [upregulated: *ECAD*, *IL1RN NUDT13, SNAI3*; downregulated: *BMP1*, *CTNNB1, FN1, FZD7*, *MMP2, MMP9*, *MYC, NCAD*, *SNAI2*, *TFRC, TWIST1*, *VIM*, *WNT5A, ZEB1*, *ZEB2* (up to tenfold; $p < 0.05$)]. Phenotypic transwell migration and Matrigel™ 3D-invasion assays, confirmed by PCR- and ELISA-based detection of MMP9 downregulation, revealed PYD as a micromolar inhibitor of melanoma cell invasiveness. Moreover, prolonged PYD exposure (24-48 h) caused melanoma cell apoptosis with complete rescue by pan-caspase inhibiton (zVAD-fmk). Taken together, these data provide strong preclinical evidence in support of the heretofore unexplored therapeutic efficacy of PYD blocking melanoma cell invasiveness and metastasis *in vitro* and *in vivo*.

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Facility type and location impact survival of spindle cell melanoma

S. Behbahani[1], K. M. Karanfilian[1], W. C. Lambert[1, 2, 3]

[1]Rutgers New Jersey Medical School, Jersey City, New Jersey, United States, [2]Department of Pathology, Immunology, and Laboratory Medicine, Rutgers University, Newark, New Jersey, United States, [3]Dermatology, Rutgers University, Newark, New Jersey, United States

Spindle cell melanoma (SCM) is a rare histological subtype of malignant melanoma composed of spindled neoplastic cells. Due to its rarity, population-based clinical and epidemiological characterization is necessary. We searched the National Cancer Database (NCDB) for all cases of SCM confirmed histologically diagnosed from 2004-2016 and excluded those with missing survival information (n=3906). Kaplan-Meier (KM) and Cox proportional-hazards models were used to analyze the epidemiology and overall survival (OS) of SCM. The median age of diagnosis was 66. Five-year and 10-year OS were 65.0% and 49.6%, respectively. Males represented 64.3% of patients. Head and neck (39.6%) was the most common primary site. Surgery alone (80.2%) was the most common treatment. The mean Breslow thickness was 3.47 mm (SD ±3.00 mm) with 31.1% with ulceration. 79.4% of staged cases presented with American Joint Commission on Cancer (AJCC) Stage I or Stage II disease. 44.4% of cases were treated at academic/research institutions followed by community programs (36.1%) and integrated network cancer programs (INCP) (12.7%). Age (HR=1.05; CI 95%[1.04-1.06]), black race (HR=3.0; CI 95%[1.60-5.62]), Breslow thickness greater than 9.8 mm (HR=1.38; CI 95%[1.04-1.82]), ulceration (HR=1.51; CI 95%[1.30-1.75]) CDCC score greater than 2 (HR=2.28; CI 95%[1.72-3.02]), and facility locations in the East South Central (HR=1.60; CI 95%[1.19-2.15]) and West South Central United States (HR=1.47; CI 95%[1.06-2.05]) were independently associated with decreased OS. Females (HR=0.85; CI 95%[0.72-0.99]), primary tumors on the trunk (HR=0.735; CI 95%[0.61-0.89]) and upper extremities (HR=0.71; CI 95%[0.55-0.92]), Stage I disease (HR=0.36; CI 95%[0.13-0.99]), and treatment at INCP (HR=0.76; CI 95%[0.68-1.42]) are independently associated with increased OS. Our results suggest that when accounting for patient and tumor characteristics, facility type and location significantly impact OS of patients with SCM.

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MITF-mediated changes of tumor architecture, tensile stress and extracellular matrix control intratumor heterogeneity in melanoma

L. Spoerri[1], C. A. Tonnessen-Murray[1], K. A. Beaumont[2], D. Hill[2], R. Jurek[3], G. Gunasingh[1], W. Weninger[2], N. K. Haass[1, 2]

[1]The University of Queensland, Brisbane, Queensland, Australia, [2]Centenary Institute, Sydney, New South Wales, Australia, [3]CSIRO Astronomy & Space Sciences, Sydney, New South Wales, Australia

Dynamic heterogeneity is a prime source for drug resistance, and understanding its underlying mechanism is crucial to design effective therapies. Using real-time cell cycle imaging (FUCCI) and single-plane illumination microscopy (SPIM), we demonstrate dynamic heterogeneity in melanoma spheroids and xenografts. This heterogeneity was characterized by the presence of clusters of proliferating cells and clusters of G1-arrested cells in the same spheroid or xenograft. The location of the quiescent zones suggested oxygen/nutrient deprivation as the cause of cell cycle arrest, and the G1-arrested cells reversed to cycling when re-cultured under normoxia in 2D culture. Increased levels of MITF, a transcription factor strongly associated with melanoma development, progression and therapy response, consistently decreased this heterogeneity in vitro and in vivo. While this phenomenon was not associated with a reduced hypoxic core, high MITF expression allowed proliferation under hypoxia. Importantly, modulation of MITF expression lead to changes of spheroid architecture, tensile stress and expression of extracellular matrix (ECM) and cell-ECM adhesion and crosstalk proteins. Atomic force microscopy (AFM) of spheroids revealed that these changes are accompanied by stiffness modulation of cells and ECM. In addition, we incorporate fluorescent stress beads into spheroids to assess forces that cells undergo at different locations within these structures. Mechanistically, inhibition of the Rho/ROCK signaling pathway mimics both morphology and cell cycle effects of high MITF expression. These findings support a novel role of MITF in controlling intratumor melanoma heterogeneity through changes in cell-ECM crosstalk and mechanotransduction and may therefore open avenues for new therapeutic approaches.

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Malassezin: A preclinical assessment of a novel microbiome-based skin lightener

E. T. McCraw[1], E. Coryell[1], M. Duvvuri[3], P. Grimes[5], M. Einziger[2], A. Einziger[2], T. Macdonald[4], J. Sackier[2]

[1]Skin Science Advisors, LLC, Peachtree Corners, Georgia, United States, [2]Versicolor Technologies, Malibu, California, United States, [3]PharmaDirections, Inc, Cary, North Carolina, United States, [4]University of Virginia, Charlottesville, Virginia, United States, [5]Vitiligo and Pigmentation Institute of Southern California, Los Angeles, California, United States

The Malassezia species are commensal to the skin microbiome and implicated in skin and scalp diseases, including tinea versicolor and seborrheic dermatitis. In cases of tinea versicolor, Malassezia overgrowth often results in skin pigmentation changes (including both hypo- and hyper-pigmentation). The purpose of this study was to link the benign pigmentation change to malassezin, an indole metabolite secreted by *Malassezia furfur,* and demonstrate the safety and ability of malassezin to decrease melanin in in-vitro models. Malassezin, was chemically synthesized and tested negative in the Computational Genotoxicity Assessment (Leadscope, Columbus, OH) and confirmed by Mini-Ames Test in *Salmonella typhimurium* and *Escherichia coli* (Pharmaron, Beijing, PRC). Phototoxicity assessment using an in vitro 3D skin tissue (Epiderm™, MatTek Corpotation) was negative. Studies of malassezin in Melanoderm models (Institute for In Vitro Sciences) demonstrated melanin reduction and compared favorably to Kojic Acid. *Ex vivo* studies (Laboratoire BIO-EC, Longjumeau, France) using differential gene expression (Affymetrix Human Clariom S chip, ThermoFisher Scientific, Waltham, MA; Genemarkers, LLC, Kalamazoo, MI) did not show changes expected from typical pigmentation modifying mechanisms. In addition, malassezin was not found to be a tyrosinase inhibitor (Sunny BioDiscovery, Santa Paula, CA). Malassezin, an indole metabolite of *M. furfur*, has been shown to be a safe and effective pigment lightening agent in-vitro, with a potentially new mechanism of action.

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Vitiligo clinical and lesional molecular features associated with favorable response to NBUVB combined with topical tacrolimus

Q. Yang[1], J. Xu[2], M. Su[1], G. Zhang[1], X. Zhang[2], H. Lui[1], P. Zhou[3], Y. Zhou[1]

[1]University of British Columbia, Vancouver, British Columbia, Canada, [2]Huashan Hospital, Shanghai, China, [3]Shanghai Skin Hospital, Shanghai, China

Vitiligo is an autoimmune disease resulting in death of melanocytes, causing disfiguring loss of skin pigmentation. It is known that numerous differentially expressed genes (DEGs) are present in vitiligo lesional skin. However, it is unknown if vitiligo DEGs are correlated with meaningful clinical characteristics, such as disease severity, duration and therapeutic response. The purpose of this study is to evaluate the clinical correlates of vitiligo DEGs. Thirty seven vitiligo patients were recruited for this study, each consented to skin biopsies from lesional as well as non-lesional skin. RNA sequencing was performed to determine DEGs of lesional skin, and statistical analysis was performed to detect DEGS correlated with lesional duration and favorable therapeutic response to NBUVB-combined with topical tacrolimus. The results showed revealed significant overlap in DEGs between short lesional vitiligo duration and faborable response to NBUVB+Tacrolimus therapy, in that both contained DEG signatures of more active immune response compared with cases with long disease duration and less-favorable response. In conclusion, short lesional disease duration and favorable response to phototherapy are characterized by specific sets of differentially expressed genes in vitiligo lesional skin, which may be useful management decision making for vitiligo in the future..

Skin of Color

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Using scanning electron microscopy to elucidate the role of hair shaft malformation in the pathogenesis of Central Centrifugal Cicatricial Alopecia

E. F. Araoye[1], N. Dlova[2], C. Aguh[1]

[1]Dermatology, Johns Hopkins School of Medicine , Baltimore , Maryland, United States, [2]Department of Dermatology, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, Durban, KwaZulu-Natal, South Africa

Central Centrifugal Cicatricial Alopecia (CCCA) is a scarring alopecia most common in black women. Its presentation follows a seemingly genetic pattern affecting clusters of women within a family. A study that aimed to elucidate the genetic basis for CCCA found an increased incidence of mutations and decreased expression of PID3, a gene that encodes for peptidyl arginine deiminase type 3, essential for the formation of a normal hair shaft, in scalp samples of patients with CCCA (1). Of note, PID3 is one of three genes implicated in uncombable hair syndrome, a disorder where the hair shaft appears triangular or heart shaped in cross section when viewed under scanning electron microscopy (SEM) (2). It is unclear if CCCA represents a hair shaft disorder and there have been no studies assessing the hair shafts of patients with CCCA. Our study aimed to investigate differences in features of the hair shaft in regard to cross-sectional shape, area, aspect ratio and circularity. The measure of circularity gives numerical information on the shape in cross section with a measure of 1.0 representing a perfect circle and 0.8 representing an oval shape. Hair samples were obtained from the vertex and occipital scalp of CCCA and age matched controls using a gentle pull technique targeting telogen hairs. Images of the hair samples were taken under SEM, and analysis and measurements were obtained using ImageJ software. This study will help elucidate the role hair shaft differences play in the pathogenesis of CCCA. References: 1. Malki L, Sarig O, Romano M-T, et al. Variant PADI3 in Central Centrifugal Cicatricial Alopecia. *New England Journal of Medicine*. 2019 2. Ü. Basmanav FB, Cau L, Tafazzoli A, et al. Mutations in Three Genes Encoding Proteins Involved in Hair Shaft Formation Cause Uncombable Hair Syndrome. *Am J Hum Genet*. 2016

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Do race and ethnicity impact healthcare utilization and costs? A population study among U.S. non-melanoma skin cancer patients
T. Sierro[1], L. Blumenthal[1], J. Hekmatjah[3], V. S. Chat[2], C. Read[1], A. Kassardjian[1], A. W. Armstrong[1]
[1]USC Keck School of Medicine, Los Angeles, California, United States, [2]Medical College of Georgia at Augusta University, Los Angeles, California, United States, [3]Western Michigan University, Homer Stryker M.D. School of Medicine, Sherman Oaks, California, United States
Racial and ethnic differences in healthcare utilization and expenditures for non-melanoma skin cancer (NMSC) patients are unknown. Compared to white patients, NMSC is less prevalent in other racial groups. However, it is important to evaluate healthcare use and costs among racial and ethnic minorities with NMSC to identify gaps in care. This study aims to identify and compare healthcare expenditures and utilization among non-Hispanic white, non-Hispanic black, and Hispanic white patients with NMSC. We performed a nationwide cross-sectional study using the Medical Expenditure Panel Survey (MEPS) from 1996 to 2015. Among 50,895,706 NMSC patients (weighted) from the 20-year period, 49,653,877 (97%) were non-Hispanic white, 155,980 (0.3%) were non-Hispanic black, and 701,682 (1.4%) were Hispanic white patients. After adjustment for socio-demographic characteristics and comorbidities, compared to non-Hispanic whites, Hispanic whites had significantly more ambulatory visits (4.79 vs 3.28, p=0.01). Compared to non-Hispanic whites, non-Hispanic blacks had significantly more ambulatory visits (11.66 vs 3.28, p=0.037), inpatient visits (.32 vs .01, p=.04), and prescription medications (1.28 vs 0.31, p=0.05), as well as higher prescription medication costs ($244.83 vs $42.95, p=0.03). In conclusion, racial and ethnic minority patients with NMSC utilized more healthcare resources. Additionally, non-Hispanic black patients also incurred greater prescription medication costs.

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Expectations of care among African-American Women with hair loss: A cross-sectional study
B. De Souza, L. Burns, D. Hagigeorges, E. Flynn, M. Senna
Dermatology, Harvard Medical School-Massachusetts General Hospital, Boston, Massachusetts, United States
Despite being the fourth most common diagnosis among African-Americans presenting to the dermatologist, alopecia is often self-managed by African-American women (AAW) with only 23% seeking medical care.[1,2]In this study, we aim to capture patient-reported healthcare utilization for alopecia, related treatments, and barriers to alopecia care among AAW with hair disorders. This cross-sectional study utilized REDCap to distribute a survey to AAW hair groups and profiles on social media in winter 2019. Respondents were required to be at least 18 years old, identify as an AAW with a hair disorder, and live in the United States. Of over 100 respondents, most patients sought medical information from online platforms.[2]Confirming previous studies, less than half of participants sought medical care for alopecia, thus highlighting the poor utilization of healthcare resources by AAW.[2]Subjects reported difficulty accessing a medical provider and the providers' lack of understanding of Afro-textured hair as the most common barriers to medical treatment. These findings suggest that medical providers are rarely the source of medical information among this patient population. Online resources, particularly social media, play a significant role in influencing health-seeking behavior among AAW and should be utilized to educate this population on the symptomatology, diagnosis, and therapeutic options for hair loss disorders.

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Supporting diversity in skin science: Development of a summer curriculum for underrepresented minority medical students
M. Paul, J. Lester, R. Ricardo-Gonzalez, H. Naik
Dermatology, University of California, San Francisco, San Francisco, California, United States
Diversity in the scientific workforce fosters innovation, improves research quality, increases the likelihood of generalizable findings, and builds public trust. Despite a recent commitment to diversity in dermatology, gender, racial and ethnic diversity in skin science is still lacking. We developed a 6-week summer curriculum for underrepresented minority (URM) medical students conducting research with a faculty mentor in our department. The curriculum goals were to: create a supportive environment for URM students to develop research mentorship relationships, expose URM students to the breadth of research in dermatology, and facilitate acquisition of skills needed for academic success. The curriculum consisted of a weekly lunch seminar series led by faculty; peer mentoring with residents; and an informational career panel. Students attended resident didactics, and networking events were held to facilitate mentoring. All students completed program evaluations. Seven URM students (6 rising MS2s, and 1 rising MS4) from 5 medical schools participated (5 female, 5 black, 2 Hispanic, 1 Native American, 3 bi-racial). Two students received Dermatology Foundation Diversity Supplement awards and 2 received departmental funding designated for URM students. 5/7 reported having an academic mentor and 4/7 reported having a dermatology mentor at their home institutions. Upon program completion, students reported improved understanding of dermatology research and careers in dermatology, and feeling better prepared to read an article, give a presentation and write an academic paper. 4/7 reported financial constraints and affordable housing as the primary barrier to program participation. All were extremely likely to continue their mentoring relationship with their assigned mentors. We report an initiative aimed at supporting and cultivating racial and ethnic diversity in investigative dermatology. Longitudinal data are needed to understand the impact and long-term outcomes of this initiative.

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Enhanced molecular signatures in cutaneous lupus erythematosus patients support distinct pathogenic pathways in African American patients
J. L. Zhu[1], L. Tran[2], F. Zheng[2], J. James[2], J. Guthridge[2], B. F. Chong[1]
[1]Dermatology, University of Texas Southwestern Medical Center, Dallas, Texas, United States, [2]Arthritis & Clinical Immunology, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, United States
Cutaneous lupus erythematosus (CLE) is an autoimmune disease with clinical sequelae such as skin damage that disproportionately affects African Americans (AAs). Recently, a novel approach in gene expression analyses was devised that groups genes into transcriptional modules (i.e. apoptosis, protein synthesis, inflammation) to identify relevant gene signatures. This technique helped identify a unique interferon signature in systemic lupus patients. Thus, we applied this modular analysis approach to identify molecular signatures unique to AA CLE patients. We conducted RNA sequencing of whole blood transcriptomes from 66 CLE patients (52% (N=34) AAs) and subsequently performed modular analyses. Modules were associated with patient subgroups distinguished by demographic, clinical, and laboratory features. An unsupervised cluster analysis identified eight distinct clusters of CLE patients. Statistical analyses comparing module scores of these clusters were performed using Kruskal-Wallis tests with Bonferroni's correction. We observed that two groups with mostly AA CLE patients (n=12 (35.3% of AAs)) had a predominant T cell signature (M4.1, M4.15 (both $p<0.0001$)). Seven inflammation module scores (M3.2, M4.2, M4.6, M4.13, M5.1, M5.7, M7.1 (all $p<0.0001$)) were markedly elevated in two clusters of mainly non-AA patients (N=16 (51.6% of non-AAs)). Additionally, neutrophil (M5.15) and cell death (M6.13, M6.6 (all $p\leq0.0001$)) molecular signatures were up-regulated in these two patient clusters suggesting that neutrophils and cell death processes could be driving the inflammatory response in non-AA CLE patients. Thus, our data suggests that unique cell populations and processes may be driving disease pathophysiology in AA and non-AA CLE patients, potentially affecting their disease course and treatment selection.

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A new free teledermatology platform effectively delivers specialist recommendations for uninsured Latino patients

N. Rodriguez, C. L. Simpson

Univ. of Pennsylvania, Philadelphia, Pennsylvania, United States

Access to specialist medical care in the U.S. is limited for economically disadvantaged patients and this worsens healthcare disparities for minority populations. To address this issue, the American Academy of Dermatology launched a new free teledermatology platform to deliver specialist advice to primary care providers (PCPs) in under-resourced clinics. Using the smartphone-based program, we offered consultations to PCPs in an urban community health clinic serving uninsured patients with a focus on Latino immigrants, who face various barriers to care. PCPs submitted patient histories and photographs, which were reviewed by remote dermatologists, who provided a diagnosis and plan of care or referred the patient to be seen in-person. To assess the effectiveness of the new platform, we retrospectively reviewed all consults (N=130) and found a completion rate of 98% with 3 incomplete cases due to failure to receive photographs; the mean time to the dermatologist reply was 7.6 hours. Importantly, we found in 63% (80/127) of completed cases, no in-person appointment was deemed necessary and a definitive plan of care was provided. Among the 37% (47/127) of consults referred for evaluation in clinic, 33 needed in-person examination due to diagnostic uncertainty while 14 required a procedure. For 24 deferred cases, teledermatologists gave an interim care plan while awaiting an appointment, thus accelerating delivery of actionable specialist recommendations in 82% of cases overall. We also assessed if the number of photographs was correlated with consult outcome, but found no significant difference between the mean number of uploaded images for diagnosed versus deferred cases (2.6 vs 2.1, p=0.10). In summary, we found the new free teledermatology platform provided rapid and reliable delivery of specialist-recommended advice to PCPs and obviated the need for in-person evaluation in the majority of cases. Moreover, our results demonstrate the potential of expanding teledermatology outreach to aid PCPs serving disadvantaged patients and to help mitigate healthcare disparities in dermatology.

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Abnormal autophagosome formation increased melanocyte sensitivity to H2O2-induced oxidative stress in vitiligo

T. Cui, S. Li, K. Li, Z. Jian, C. Li

Dermatology, Xijing Hospital, Xi'an, Shannxi, China

Autophagy is a controlled self-digestion process which can protect cells against oxidative damage. Dysregulated autophagy has been demonstrated to increase melanocyte sensitivity to oxidative stress in vitiligo. However, the exact mechanism is still not clear. This study aimed to determine the implications of autophagy for melanocyte survival in response to oxidative stress. Our results demonstrated that the autophagic flux in PIG1 exposure to H2O2 was significantly enhanced compared with that in PIG3V, which were accompanied by high level of ROS accumulation, membrane potential changes, and increased apoptosis. It indicates that vitiligo melanocytes exhibited hypersensitivity to H2O2-induced oxidative injury due to dysregulated autophagy. To further explore the mechanism, we performed RNA sequencing to compare the RNA expression in PIG1 and PIG3V cells exposed to H2O2, the bioinformatic analysis indicate that autophagosome formation was impaired in vitiligo melanocytes, our in vitro study also showed that inhibition of autolysosome degradation can not lead to autophagosome accumulation in vitiligo melanocytes, confirming that the impairment of autophagosome formation is responsible for the defects of autophagy in vitiligo melanocytes, the further study also showed that overexpression of HSF1, the main transcription factor for Atg5 and Atg12, could reduce H2O2-induced oxidative damage of vitiligo melanocytes. Our data demonstrated that owing to the deficiency of HSF1 expression, the autophagosome formation was blocked, which resulted in autophagy impairment and further increase the sensitivity of vitiligo melanocytes to oxidative stress. Our results indicating that targeting autophagy may be a potential therapy option.

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Racial differences in the health-related quality of life of chronic pruritus patients

K. Whang[1], Y. Semenov[2], R. Khanna[1], K. Williams[1], V. Mahadevan[1], S. Kwatra[1]

[1]Department of Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [2]Department of Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

Chronic pruritus is a condition with profound impact on quality of life (QoL), which has been shown to vary with race. The goal of our study was to characterize the racial differences in the health-related QoL of patients diagnosed with chronic pruritus. We administered a cross-sectional survey of 95 patients with chronic pruritus utilizing the Ontario Health Utilities Index Mark 3 (HUI3) questionnaire. We obtained normal population data from healthy US adults (n=4,187) from the 2002-2003 Joint Canada/United States Survey of Health. HUI3 scores, representing overall health performance and health in specific domains, were compared between the groups and stratified by race. Chronic pruritus patients were significantly more likely to be black compared to the general population (OR 6.67, 95% CI [4.26-10.48], p<0.001). Among the subset of chronic pruritus patients diagnosed with prurigo nodularis, black race was associated with decreased overall health performance in multivariate regression adjusting for demographics and itch severity (coefficient -0.49, 95% CI [-0.98 to -0.01]). This association was not observed in other diagnosis classes for chronic pruritus. Black chronic pruritus patients had a significantly higher average quality-adjusted life year (QALY) loss, calculated based on HUI3 scores, than white chronic pruritus patients (7.66 vs. 6.18 years, p=0.003). The QALY loss by black chronic pruritus patients translates to an increased individual lifetime financial burden of $383,036 compared to $309,011 for white chronic pruritus patients. This study demonstrates racial differences impacting health-related QoL and economic burden of chronic pruritus. Further research must be performed to study etiologic factors responsible for the observed racial disparities.

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Patient race affects dermatologists' assessments and treatment of psoriasis

A. Sevagamoorthy, A. Bazen, D. Shin, F. K. Barg, J. Takeshita

University of Pennsylvania, Philadelphia, Pennsylvania, United States

Racial disparities in psoriasis treatment have been suggested. Little is known about how physicians' assessments and treatment patterns may contribute to these disparities. We aimed to evaluate whether patient race, gender, or socioeconomic status affect dermatologists' assessment and treatment of psoriasis. We conducted a cross-sectional survey study of a random sample of dermatologists (N=3,352) who are members of the American Academy of Dermatology. Each dermatologist was randomly assigned one of eight identical survey options which differed only by either patient race (white vs black), gender (male vs female), or socio-economic status (high vs low). Each vignette described and visually depicted a 50-year old with severe psoriasis followed by questions assessing the dermatologist's confidence in the diagnosis of psoriasis and their first line treatment recommendation. We performed multivariable logistic regression to evaluate the associations among patient characteristics and the dermatologist's confidence in psoriasis diagnosis and treatment recommendation. In total, 668 dermatologists returned the survey yielding a response rate of 19.9%. Most dermatologists were between the ages of 35 and 54 years (54%), white (74%), and practiced in a single specialty private setting (49%). Dermatologists were less likely to be confident in the diagnosis of psoriasis among black patients compared to white patients (odds ratio [OR] 0.15, 95% confidence Interval [CI] 0.08-0.29). Lack of confidence in the diagnosis of psoriasis was also associated with a lower likelihood of recommending appropriate treatment for severe psoriasis with phototherapy, oral systemics or biologics (OR 0.35, 95% CI 0.17-0.71), independent of disease severity assessment and other patient and dermatologist characteristics. Our findings identify differences in the confidence of psoriasis diagnosis among dermatologists by patient race, which likely drive treatment recommendations whereby black patients are more likely to be underdiagnosed and undertreated for their psoriasis.

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Keloids are associated with Th2, JAK3, and CCR9/CCL25 inflammation

J. Wu[1], E. Del Duca[1], M. Espino[1], A. Diaz[1], N. Zhang[1], A. Gontzes[1], Y. Estrada[1], J. G. Krueger[2], A. Pavel[1], E. Guttman-Yassky[1]

[1]Dermatology, Icahn School of Medicine at Mount Sinai, New York, New York, United States, [2]Laboratory for Investigative Dermatology, Rockefeller University, New York, New York, United States

Keloids occur due to disturbed wound healing and abnormal collagen production, often affecting African American (AA) and Asian populations. An increased prevalence of atopic conditions, particularly asthma, has been described in keloid patients. However, its immune pathomechanism has not been studied, inhibiting therapeutic development. To evaluate the inflammatory signature of keloids in skin we obtained lesional and nonlesional biopsies from 3 AA patients with new onset keloids and 5 AA healthy controls. We profiled the cellular and molecular phenotype of keloids using immunohistochemistry, RNA-seq, and RT-PCR. Significant increases in cellular infiltrates were found in keloids including $OX40^+$ T-cells and $OX40L^+$ dendritic cells, $tryptase^+$ mast cells, and $periostin^+$ cells. Expressions of CCR9 and its ligand CCL25, that regulate cellular recruitment in early asthma inflammation, were significantly upregulated in keloid lesions ($p<0.05$). Lesional skin also showed upregulation of immune markers related to Th2 (IL13, IL4R, OX40L, CCL25), Th17 (CCL20, PI3), Th1 (CXCL9/10/11), and JAK3 signaling ($p<0.05$). T-cell migration (CCR7, CCL19) and cytotoxic (granzyme B) markers were also highly upregulated in keloid lesions ($p<0.05$). Similar trends of upregulations were found in nonlesional compared to normal skin. Among significantly down-regulated markers in both lesional and nonlesional skin, was the negative regulator IL-37 ($p<0.05$). We also identified increased fibrosis/bone/cartilage-differentiation products, consistent with prior studies, ($p<0.05$). Overall, our data show a strong Th2/JAK3 inflammatory milieu, indicating the potential use for Th2 targeting agents in keloids, similar to other atopic indications, such as atopic dermatitis and asthma.

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Doctor-level multi-classification of skin diseases and a dataset for the Yellow Race

X. Chen, S. Zhao, Y. Kuang, M. Chen, W. Zhu

Department of Dermatology, Xiangya Hospital, Central South University, Changsha, Hunan, China

Convolutional Neural Networks has superior performance in image recognition. But it relies on a large amount of data. However, there has not been public skin disease dataset for a specific race. This paper builds such a dataset for the yellow race. It includes 108,248 images from 474 different skin diseases, and part of these images are also annotated with location for individual skin lesions. All these annotations are validated by at least 3 dermatologists and matched with corresponding pathology information as golden standard. Moreover, each image in this dataset are matched with clinical history. Based on this dataset, a framework for skin disease diagnosis was proposed. This framework was designed based on the properties of skin lesions, such as scattering and irregular shape. For an input images, this framework first detects individual skin lesions to generate local results, and then these local results combine to come up with the final result which indicate the disease category for the input image. We conducted a competition between our framework and 31 professional dermatologists. And in the competition, some indistinguishable images from six common skin diseases are used as the testing data, including skin benign tumors (Seborrheic Keratosis), skin malignant tumors (Basal Cell Carcinoma), connective tissue disease (Lupus Erythematosus), allergic skin disease (Eczema), and bullous skin disease (Pemphigus), erythematous papule scaly skin disease (Psoriasis). Comparing the performance on the same testing data, our framework achieved average precision of 64.75% (top1) and 84.77% (top3), and for dermatologists there are 62.13% (top1) and 78.15% (top3). Which shows that, in our dataset for the yellow race, the framework proposed in this paper reached the average level of dermatologists. It laid the foundation to the building of intelligent diagnosis and treatment platform for skin diseases.

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Understanding the intersectional stigma of HIV-related dermatologic disorders in Kenya

D. McMahon[1], L. Butler[2], N. Busakhala[3], L. Chemtai[4], A. Semeere[5], H. Byakwaga[5], M. Laker-Oketta[5], J. Martin[6], I. Bassett[7], E. Freeman[7]

[1]Harvard Medical School, Boston, Massachusetts, United States, [2]University of Connecticut, Storrs, Connecticut, United States, [3]Moi University, Eldoret, Kenya, [4]AMPATH, Eldoret, Kenya, [5]Infectious Disease Institute, Kampala, Uganda, [6]UCSF, San Francisco, California, United States, [7]Massachusetts General Hospital, Boston, Massachusetts, United States

Stigma is an independent determinant of health inequities for people with HIV and skin disease, yet few studies have examined how the intersection of these conditions influences overall stigma. This intersectional stigma may be particularly prominent for people with Kaposi's Sarcoma (KS), a common HIV-related dermatologic condition in sub-Saharan Africa. In this study we used qualitative interviews with KS patients in Kenya to assess HIV stigma, dermatologic stigma, and intersectional stigma for both conditions. All patients $\geq$18 with newly diagnosed Kaposi's sarcoma within the AMPATH clinic network in Western Kenya from 2016-2019 were enrolled in the parent study. Of these, 88 were purposively selected to participate in a semi-structured interview. Coded transcripts were analyzed with framework analysis using the Health Stigma and Discrimination Framework. Our findings highlight six themes demonstrating how the intersection of HIV and dermatologic stigma produce health inequities: (1) Multiplied fears of contagion and (2) Physical disability and disfiguration resulted in (3) Loss of relationships and employment, as well as produced (4) External fears of witchcraft, ultimately leading to (5) Social isolation and (6) Decreased motivation to travel to and engage with the health system. To improve healthcare engagement for people with HIV-related skin disorders, the intersection between dermatologic conditions and HIV should be considered. Potential interventions to reduce stigma include incorporation of dermatologic screening and treatment at existing HIV centers, support groups for patients with shared identities, as well as continued efforts to reduce community-level and structural drivers of stigma.

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Salt and pepper dyspigmentation in dermatomyositis with TIF1-γ autoantibodies

K. Svigos, D. Gutierrez, A. Femia, N. K. Brinster, K. Lo Sicco

The Ronald O. Perelman Department of Dermatology, New York University School of Medicine, New York, New York, United States

A 48-year-old man with dermatomyositis (DM), significant muscle involvement, and transcription intermediary factor 1-γ (TIF1-γ) autoantibodies was referred to dermatology for widespread areas of dyspigmentation over six months. His rheumatologist treated him with tapering oral glucocorticoids and six infusions of rituximab 1000mg. With this therapy, his pruritic dyspigmentation progressed with only slight improvement of proximal muscle weakness. On physical examination, there were hypopigmented patches with erythema on the face, trunk, and back, notably sparing follicular ostia. Mucosal examination was unremarkable. Sclerodactyly, digital tuft pits, and matted telangiectasias were absent however nailfold capillary dilatation was noted. The resemblance of this dyspigmentation to "salt and pepper" changes typical of systemic sclerosis was concerning for a sclerodermoid-overlap syndrome, prompting additional serologic and histopathologic evaluation. An antinuclear autoantibody test by immunofixation was positive with a titer of 1:320 in a diffuse, speckled pattern. A test for TIF1-γ autoantibodies was positive. Serologic tests for U1RNP, SSA-52/60, Jo1, Mi-2, Pl-7, Pl-12, EJ, KU, U2-snRNP, U3RNP, OJ, SAE1, XP-2, MDA5, dsDNA, Scl-70, RNA-Polymerase 3, centromere, SRP, and p- and c-antineutrophil cytoplasmic autoantibodies were negative. A punch biopsy of the left arm showed superficial perivascular lymphocytic infiltrate, vacuolar interface dermatitis, a thickened basement membrane, and increased dermal mucin. Given the lack of scleroderma-specific autoantibodies and histopathology supporting DM, the patient's speckled dyspigmentation was attributed to poorly-controlled cutaneous DM. He was subsequently prescribed oral methotrexate with significant repigmentation over three months. This case demonstrates that in darker skin types, interface dermatitis can create prominent dyspigmentation which may be a presenting cutaneous finding in TIF1-γ DM.

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Risk of C. Difficile infection among hidradenitis suppurativa patients prescribed prolonged clindamycin course

R. Kwak[1, 2], A. P. Charrow[1]

[1]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States, [2]Harvard Medical School, Boston, Massachusetts, United States

Hidradenitis Suppurativa (HS) is a chronic disease with a higher incidence among African American (AA) and Hispanic population in the United States[1]. Prolonged antibiotics (e.g. clindamycin +/- rifampin for 10-12 weeks) are recommended[2], yet the risk of CDI among HS patients receiving the prolonged course is unknown. We performed a retrospective chart review of all patients at Brigham and Women's and Massachusetts General Hospitals between 1999 to 2019. We identified 503 patients who were diagnosed with HS, seen by a dermatologist, and treated with clindamycin, of whom 209 were confirmed to have received oral (n=190, 90.9%) and/or intravenous (IV) (n=25, 12.0%) clindamycin for HS. Of these patients, only 1 patient (0.48%, 61-year-old African American, non-Hispanic male) developed CDI (toxin confirmed), which occurred 4 days after a 1-time dose of IV clindamycin prescribed for an I&D of an HS-associated abscess. No patient developed CDI following a prolonged oral clindamycin course (45 patients, 22.2%). Dermatologists were more likely to prescribe the recommended prolonged course (e.g. 10-12 weeks) ($p<0.01$) and to prescribe clindamycin with rifampin ($p<0.01$) than other providers. Only 26.6% of oral clindamycin courses were prescribed with rifampin despite recommended guidelines.[2] More AA patients received a prolonged course of oral clindamycin (>10 weeks) and rifampin compared to Caucasian patients ($p<0.01$). There was no statistical difference in the clindamycin course length or rifampin co-prescription between Hispanic and non-Hispanic patients. Alikhan A, Lynch PJ, Eisen DB. Hidradenitis suppurativa: a comprehensive review. J Am Acad Dermatol. 2009 Apr;60(4):539-61. Alikhan A, Sayed C, Alavi A, et al. North American clinical management guidelines for hidradenitis suppurativa: A publication from the United States and Canadian Hidradenitis Suppurativa Foundations: Part II: Topical, intralesional, and systemic medical management. J Am Acad Dermatol. 2019 Jul;81(1):91-101.

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Clinical efficacy analysis of narrow spectrum intense pulsed light in the treatment of acne-related erythema in Chinese patients

Y. Yang, Y. Zheng

The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

Objective: To evaluate the efficacy and safety of Dye narrow-band pulsed light (DPL) in the treatment of Acne Vulgaris in Chinese patients. Methods: The clinical data of 50 patients with erythema after facial acne who had received treatment in the Hospital between January 2019 to June 2019 were analyzed.50 patients with acne-related erythema, aged 15 to 36 years, with a course of 2 to 6 years, with an average of 2.6 years. The following conditions are excluded: (1) a viral infection on the face or the wound incision has not healed; (2) a history of photosensitivity or taking photosensitivity drugs within 4 weeks; (3) patients who have taken oral retinoic acid in the past six months; (4) pregnancy and actating women; (5) patients with heart, kidney, liver insufficiency or other serious underlying diseases; (6) patients with mental illness. Using Harmony XL and Dye-PL hand tools at wavelengths of 500 to 600 nm (Alma Lasers, Caesarea, Israel), 50 patients with post-acne Erythema were treated 3 times at 4-week intervals. The degree of facial erythema, skin brightness, the degree of pain during treatment, and the adverse reactions after treatment were measured and recorded before each treatment and at 2 weeks after treatment. Basic Cure: improvement ≥90%; marked effect: improvement ≥60%; progress: improvement ≥30%; invalid: improvement <30%. Results: 50 patients completed treatment and follow-up. The effective rate of DPL for treating acne-related erythema was 100%, and the erythema and pigments were improved to varying degrees without serious adverse reactions. Conclusion: Narrow spectrum intense pulsed light is effective in the treatment of post-acne Erythema with few side effects, short recovery time and good tolerance. DPL has a good clinical application prospect.

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SFRP2-expressing, *COL11A1*-expressing fibroblasts are the major fibroblast population within keloids

I. Dougherty, V. Cantu, D. Glass

UT Southwestern Medical Center, Dallas, Texas, United States

Fibroblasts perform a multitude of processes within skin: extracellular matrix (ECM) production, regulation of inflammation, facilitate cutaneous repair after injury, and serve as pluripotent mesenchymal cells. Recent evidence suggests that there are two main major fibroblast populations in human skin: *SFRP2*-expressing fibroblasts and *FMO1*-expressing fibroblasts, and five minor populations, each expressing discrete genes: *CRABP1*, *COL11A1*, *FMO2*, *PRG4*, or *C2ORF40*. Keloids are benign fibroproliferative tumors that result from an exaggerated response to cutaneous wound healing. Keloids have an increased number of fibroblasts that produce increased levels of ECM; however, little is known about which fibroblast subpopulation is responsible for the keloid phenotype. To better characterize keloids, we performed whole transcriptional profiling of biopsies from keloids and adjacent non-lesional skin (n=3). We identified 218 upregulated and 126 downregulated differentially expressed genes (DEGs) between keloid and NL skin across all three sample pairings. Within our DEGs we found that *SFRP2* and *COL11A1* were upregulated in keloid tissue versus normal skin. Real-time PCR confirmed significant upregulation ($p<0.05$) of *SFRP2* and *COL11A1* in keloid tissue versus adjacent non-lesional skin. Our data suggest that keloid fibroblasts are derived from an expansion of *SFRP2*-expressing, *COL11A1*-expressing fibroblasts. As *SFRP2*-expresing and *COL11A1*-expressing fibroblasts are thought to be involved in matrix deposition and connective tissue cell differentiation, respectively, our findings help explain the aberrant ECM production and connective tissue dysplastic gene expression found within keloids.

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Dermatologists' assessments and treatment of atopic dermatitis differ by patient race

J. Takeshita, A. Sevagamoorthy, A. Bazen, D. Shin, F. K. Barg

University of Pennsylvania, Philadelphia, Pennsylvania, United States

Racial/ethnic, gender, and socioeconomic disparities in dermatologic care have been suggested for various skin diseases. The contribution of dermatologists' practices to such disparities remains unknown. We aimed to evaluate whether dermatologists' assessments and treatment of atopic dermatitis (AD) differ by patient race, gender, or socioeconomic status (SES). We conducted a cross-sectional survey study of a random sample of dermatologists (N=3,351) who are members of the American Academy of Dermatology. Each dermatologist received one of eight identical survey options that included a clinical vignette that differed only by either patient race (white vs. black), gender (male vs. female), or SES (high vs. low). Each vignette described and visually depicted an 18 year-old with severe AD followed by questions about disease severity, quality-of-life impact, and first-line treatment recommendation. We performed multivariable logistic regression to evaluate the associations among patient characteristics and the ratings of AD severity, quality-of-life impact, and recommended treatment. In total, 596 dermatologists returned surveys yielding a response rate of 17.8%. Most dermatologists were between 35 and 44 (27%) or 55 and 64 (23%) years old, white (72%), and practiced in a single specialty private setting (39%). Dermatologists were less likely to rate black patients as having severe AD (odds ratio 0.41; 95% confidence interval 0.29-0.59) and large impact on their quality-of-life due to AD (0.20; 0.05-0.76) compared to white patients. Black patients were less likely than whites to receive recommendations for treatment with phototherapy, oral systemic, or biologic (0.67; 0.45-0.99), especially among white dermatologists; this may be due to differences in dermatologists' assessments of AD severity between black and white patients. Our findings highlight differences in dermatologists' determination of AD disease burden by patient race which appear to drive treatment decisions and may lead to undertreatment of black patients with AD.

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Racial disparities in biologics utilization for psoriasis

W. Hodges[1], T. Bhat[1], C. Herbosa[1], S. Kwatra[2], A. Musiek[1], C. M. Mann[1], Y. Semenov[3]

[1]Division of Dermatology, Washington University, St. Louis, Missouri, United States, [2]Department of Dermatology, Johns Hopkins University, Baltimore, Maryland, United States, [3]Department of Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

Biologic therapies are an effective and increasingly common treatment option for moderate to severe psoriasis. However, little is known about biologics utilization in patients of different races. We sought to determine if treatment disparities exist in biologic medication use for psoriasis between different races by analyzing patient records at a large academic medical center. Data was collected using SlicerDicer in the Epic electronic medical record and statistical analyses were performed using SAS Studio version 3.8. Biologics utilization rates were calculated as a percent of all psoriasis patients within each race. We identified 13,084 psoriatic patients treated between 1/1/2010 and 8/31/2019, of whom 88.1% (n=11,524) were Caucasian and 7.2% (n=949) were Black. Compared to Caucasians, Blacks with psoriasis were more likely to be younger, female, and unmarried ($p<0.01$). They also had significantly lower rates of biologics use for psoriasis compared to Caucasians (14.5% vs 20.1%; OR 0.67, 95% CI 0.56-0.81). The majority of this disparity was driven by differences in the use of TNF-alpha inhibitors (10.2% vs 15.6%, OR 0.62 95% CI 0.50-0.76). Blacks were also significantly less likely to use IL-17 inhibitors (2.8% vs 4.2%, OR 0.67 95% CI 0.45-0.99), but no significant differences existed in use of IL-12/23 or IL-23 inhibitors. This treatment gap persisted from as early as 2012 (2.0% vs 6.5%, p=0.030) until the end of the follow-up period in 2019 (16.7% vs 21.6%, p=0.003), despite increasing rates of biologics use in both races. There were no significant differences in the use of non-biologic systemic psoriasis therapies between Blacks and Caucasians (21.8% vs 23.6%, p=0.196). Future studies are needed to further understand why biologics use is lower in Black patients with psoriasis. Awareness of racial disparities in the use of biologic therapies may assist clinicians in meeting the needs of vulnerable patient populations.

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Friend or foe: Elevated sera levels of IgM autoantibodies targeting hair follicle components detected in patients with Hidradenitis Suppurativa

M. L. Kerns[1], C. Carmona-Rivera[2], C. Zhu[3], Q. Li[3], W. D. Shipman[4], L. A. Barnes[5], M. J. Kaplan[2], G. A. Okoye[6, 1], A. S. Byrd[6, 1]

[1]Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [2]NIAMS, NIH, Bethesda, Maryland, United States, [3]Immunology and Microarray Core Facility, UTSW Medical Center, Dallas, Texas, United States, [4]Weill Cornell/Rockefeller/Sloan-Kettering Tri-Institutional MD-PhD Program, New York, New York, United States, [5]Dermatology, Stanford University School of Medicine, Stanford, California, United States, [6]Dermatology, Howard University College of Medicine, Washington, District of Columbia, United States

Through their recognition of self-antigen, a repertoire of germline encoded IgM antibodies perform the vital housekeeping task of phagocytic clearance of dying cells by the innate immune system. However, pathogenic levels of IgM autoantibodies (AAbs) can recognize neo-epitopes exposed in damaged tissue and initiate uncontrolled inflammation. Hidradenitis suppurativa (HS) is a chronic debilitating disease characterized by recurrent draining nodules and scarring in hair bearing areas of the skin. Hair follicle rupture, due to an unknown stimulus, is postulated to cause an abnormal innate immune response culminating in sterile neutrophil inflammation. Herein, we sought to characterize the sera levels of IgM AAbs in patients with HS using an array-based high-throughput autoantibody screening. Given that HS disproportionately affects skin of color, we obtained sera samples from 21 patients, of which 17 were African Americans. The binding of IgM AAbs with antigens was detected using cy5-labeled anti-human IgM, and the antibody score of each AAb was calculated based on the signal intensity and signal to noise ratio. Increased autoreactivity was detected for IgM against galectin and cytokeratin 19 and positively correlated with HS clinical severity. Notably, both galectin and cytokeratin 19 are expressed in the hair follicle, the site of early cellular damage in HS. Further experimentation is required to determine whether IgM AAbs are playing a protective or pathogenic role, but the positive correlation with increased clinical severity suggests the latter.

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IgG autoantibodies correlate with Hidradenitis Suppurativa clinical severity

W. D. Shipman[1], M. L. Kerns[2], C. Carmona-Rivera[3], C. Zhu[4], Q. Li[4], L. A. Barnes[5], M. J. Kaplan[3], G. A. Okoye[6, 2], A. S. Byrd[6, 2]

[1]Weill Cornell/Rockefeller/Sloan-Kettering Tri-Institutional MD-PhD Program, New York, New York, United States, [2]Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [3]NIAMS, NIH, Bethesda, Maryland, United States, [4]Immunology and Microarray Core Facility, UTSW Medical Center, Dallas, Texas, United States, [5]Dermatology, Stanford University School of Medicine, Stanford, California, United States, [6]Dermatology, Howard University College of Medicine, Washington, District of Columbia, United States

Hidradenitis suppurativa (HS) is a chronic skin disorder with debilitating draining abscesses and sinus tracts in intertriginous areas. Although classically viewed as an autoinflammatory disorder, in part mediated by hyperactive neutrophils, HS is associated with several autoimmune conditions, including inflammatory bowel disease (IBD) and spondyloarthritis (SpA). Key mediators of autoimmunity, high-affinity, somatically mutated IgG autoantibodies (AAbs) reflect a disruption of homeostatic pathways related to cell clearance and antigen-receptor signaling. We previously reported an increase in total IgG in HS sera and tissues. Thus, we sought to characterize IgG autoreactivity in patients with HS using an array-based high-throughput AAb screening. Sera samples collected from 21 HS patients (without any known autoimmune disorder) were used to detect the binding of IgG AAbs with antigens using cy3-labeled anti-human IgG, and the antibody score of each AAb was calculated based on the signal intensity and signal to noise ratio. Compared with controls, sera from HS patients had increased autoreactivity to collagen- I, II, V, and VI, which is a known mechanism in SpA; immune regulatory proteins PD-L1 and PD1; nuclear proteins nucleoporin 62, nucleosome, and nucleolin; calreticulin, a target in IBD; and azurocidin, a target of antineutrophil cytoplasmic antibodies. All of the aforementioned autoantigens had a positive correlation with disease severity. Taken together, the novel identification of potential pathogenic IgG AAbs in HS patient sera expands the understanding of HS pathogenesis to likely include an autoimmune mechanism and uncovers myriad therapeutic targets.

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Midkine and pleiotrophin are expressed in keloids

V. Cantu, A. Tran, I. Dougherty, D. Glass

UT Southwestern Medical Center, Dallas, Texas, United States

Midkine (MDK) is a 15.5kDa heparin-binding growth factor induced by retinoids and inhibited by corticosteroids. It is closely related in structure to another low molecular weight protein, Pleiotrophin (PTN, 18.9kDa). Both MDK and PTN are involved in many important biological pathways including embryogenesis, tumorigenesis, and wound healing. They bind to a number of different receptors, including Protein-Tyrosine Phosphatase, Receptor-Type Zeta-1 (PTZPRZ1), which is known to play a key role in regulating cell growth, differentiation and transformation. MDK has been reported to stimulate collagen production (both Type I and Type III) and glycosaminoglycan synthesis in primary dermal fibroblasts. However, the roles of MDK, PTN and PTPRZ1 in the development of keloids have been largely unexplored. To further investigate whether MDK, PTN and PTPRZ1 play a significant role in keloid formation we have studied their expression in keloid and normal whole tissue. Results from both whole transcriptome sequencing and real-time PCR indicate that *MDK*, *PTN* and *PTPRZ1* are upregulated in keloid tissue. Western blots have further identified their expression in keloid tissue. Our results suggest that MDK, PTN and PTPRZ1 could play an important role in the pathogenesis of keloids.

Skin, Appendages, and Stem Cell Biology

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A study of skin-age analysis method using five parameters and skin characteristics of subjects using First Care Activation Serum for long-term period

S. Jang, G. Cho, S. Choi, Y. Jung, J. Han, E. Kim

Amorepacific R&D Center, Yongin-si, Gyeonggi-do, Korea (the Republic of)

Introduction : Recently, many researches have been actively conducted to evaluate age by artificial intelligence using various parameters. Prediction of skin age is important because biological age and skin age differ due to various environmental factors, beauty habits, and genetic factors. This study predicted skin age by using multiple regression analysis by five skin parameters that affect aging, and analyzed the predicted age of the group using the First Care Activation Serum and the group without it. Method : 290 women between the ages of 22 and 80 have been recruited in the study. Ninety-five people used First Care Activation Serum for at least three years (Test group), while 195 others did not use (Control group). Subjects were measured by ten parameters (The skin hydration, skin color, gloss, wrinkles, Trans-epidermal water loss, Transparency, Sebum, pH, Melanin and Erythema). Result : Five parameters (The skin hydration, wrinkles, skin color, transparency, and gloss) were changed according to age among ten parameters. Thus, these five parameters were used to perform multiple regression analysis to derive a regression formula for predicting skin age. The regression formula for predicting age using the five skin parameters was suitable for predicting age by having adjusted R-square of 0.685 and showing significant results. Test group was used First Care Activation Serum for an average of 12.05 years, and the predicted skin age was 17.91 years younger than those that did not use product. Discussion : In this study, we developed a formula for predicting skin age using five simple skin measurements. Furthermore, this regression formula is suitable for predicting age because it has 68.5% of the expression's explanatory power. The study also confirmed that skin age may vary depending on beauty habits.

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Study of clinical and pathological features in 729 cases of nevus sebaceous

T. Wang, Q. Li, L. Zhao, S. Geng

Dermatology, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China

Objective: To observe the manifestations and pathological features of NS and analyze the composition of concomitant or secondary diseases in NS. Methods: Collect baseline data of patients with NS and classify their clinical manifestations and review the pathological diagnosis of NS, then analyze the statistical results with SPSS21.0. Results: 1. There were 729 cases of NS, 434 (59.53%) for males and 295 (40.47%) for females, with a male to female ratio of 1.47:1. The average age of surgical excision was 16.16 years. Divided into groups in three different ages, there were 254 infants (34.84%), 270 (37.04%) adolescents, and 205 (28.12%) adults. Among 729 cases of NS, 695 (95.33%) of them had a single type lesion, and 34 of them (4.67%) had multiple lesions. The involved parts were prone to the head and face, and other parts included the buttocks and extremities were rare. 2. There were 673(92.3%) cases of NS without other concomitant diseases. Among the 673 subjects, the epidermis without significant changes were mainly in infants and children ($P<0.05$), papillary hyperplasia and verrucous hyperplasia occurred mainly in puberty ($P<0.05$); Cases of sebaceous glands directly open to the epidermis mainly existed in puberty ($P<0.05$); the proportion of immature hair follicles in puberty was higher than that in adult ($P<0.05$). 3. There were 56(7.68%) cases of NS with other concomitant diseases, including 9(1.23%) cases of TB and 5(0.68%) cases of BCC. There were 12(1.65%) cases of SCAP, 1(0.13%) case of sebaceous epithelioma, 2(0.27%) cases of compound nevus, 1(0.13%) case of navus lipomatosus superficialis and so on. Conclusion: Clinical and pathological manifestations of NS are relevant to the ages. But they are not completely matched, for there are individual differences. Among the tumors associated with NS, the proportion of TB is the largest, BCC is not as much as previously thought, suggesting a low percentage of BCC in the third stage of NS. Prophylactic excision may be over-treatment, and conservative treatment may be a better choice, but cosmetic resection remains reasonable.

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The study on the role of FOXE1 in the pathogenesis of psoriasis

M. Liu, Y. Zheng

Dermatology, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, China

Background: FOXE1 is a DNA-binding protein involved in the regulation of cell growth and differentiation. It has been found that FOXE1 is involved in the pathogenesis of several kinds of cancer. But its specific role and mechanism in psoriasis remain unclear. *Objectives:* The aim of this study is to investigate the role of FOXE1 in the pathogenesis of psoriasis. *Methods:* 1. Immunohistochemistry was used to detect the expression of FOXE1 in normal skin tissues and psoriatic lesions; qRT-PCR and Western Blot (WB) were used to determine the expression of FOXE1 in healthy controls and psoriatic lesions, as well as the imiquimod mice models. 3. After stimulated with M5, the expression of FOXE1 in HaCaT and HEKa cells was detected. 4. After transfected with siRNA, the cell proliferation was examined by CCK-8 assays. Flow cytometry was used to detected cell cycle and apoptosis; qRT-PCR was used to detected inflammatory cytokines. The cell cycle and apoptosis-related protein were detected by WB. 5. Cells were transfected with lentivirus to overexpress FOXE1, and the same methods were used to detect cell proliferation, cell cycle, cell apoptosis and inflammatory cytokines. 6. WB was used to detect ERK/p-ERK after knockdown and overexpression of FOXE1. *Results:* 1. The expression of FOXE1 in psoriatic lesions and imiquimod mice was higher than that of normal skin and control mice. 2.FOXE1 was upregulated in HaCaT and HEKa cells after stimulated with M5. 3. Knockdown of FOXE1 inhibited human keratinocyte proliferation, arrested cell cycle in G1/S phase, and inhibited the expression of cell cycle-associated protein and inflammatory cytokines; 4. Overexpression of FOXE1 promoted the expression of cell cycle proteins, promoted cell proliferation, increased the number of cells in S phase, but had no obvious effect on inflammatory response; knockdown or overexpression of FOXE1 had no obvious effect on keratinocytes apoptosis; 5. FOXE1 activated the ERK1/2 pathway. *Conclusion:* FOXE1 is involved in the pathogenesis of psoriasis by regulating keratinocytes proliferation and inflammatory response through the ERK activation.

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A study of novel skin elasticity index using by CutiScan®

S. Kim, M. Kim, J. Han, E. Kim

AMOREPACIFIC, Yongin-si, Gyeonggi-do, Korea (the Republic of)

Skin is a complex with viscoelastic characteristic, composed of the subcutaneous tissue, the epidermis and dermal matrix. The skin undergoes degenerative changes with aging; reduction of elasticity, epidermal thickness and collagen, elastic fiber, increment of wrinkle, dryness and anisotropy. The CutiScan® provides information not only the viscoelastic properties but also on anisotropy of the skin. There are total 17,640 displacement raw data including time (4seconds) and angle (360 degrees) information and existing elasticity parameter(V3) of this system appears 360 values after measurement. These overwhelming data are hard to understand the skin elasticity change. Therefore, we calculated the average of the displacements over all angle ranges, indicating the skin's pliability (overall displacement parameter), and after calculating the mean and standard deviation of the angular displacement, the anisotropy of the skin was expressed through the difference of the calculated values of each corresponding position (asymmetric factor parameter). The purpose of this study is to evaluate the three-dimensional skin elasticity change on human skin using novel index. For this study, 22 female subjects (11 Young group; 30-39years, average of 36.545±2.697years, 11 Old group; 50-60years, average of 54.182±3.488years) were recruited and measured on thigh elasticity. As compared to Young group, Overall displacement parameter (pliability) were significantly decreased at Old group ($p<0.05$). Also, Asymmetric factor parameter (anisotropy) was significantly increased at Old group ($p<0.05$). In addition, we applied this novel index to the anti-aging product and confirmed improvement in skin elasticity. In conclusion, the new elasticity index is able to confirm the three-dimensional skin elasticity changes.

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Diet and the dermal white adipose tissue: Analyzing shifts in the cutaneous lipid landscape in response to dietary change

W. Gao[1], M. Edwards[1], B. Thompson[2], J. McDonald[2], T. A. Harris-Tryon[1, 3]

[1]Dermatology, UT Southwestern Medical Center, Dallas, Texas, United States, [2]Molecular Genetics, Center for Human Nutrition, UT Southwestern Medical Center, Dallas, Texas, United States, [3]Immunology, UT Southwestern Medical Center, Dallas, Texas, United States

The increased prevalence of diet-induced obesity and its associated skin conditions continues to plague modern populations. Though it has been established that diet affects subcutaneous and visceral white adipose, less is known about the impact of the diet on the dermal white adipose tissue — a superficial layer of cutaneous adipose with a variety of metabolic and immune functions. This study sought to investigate the impact of diet on the dermal white adipose tissue (dWAT). Four groups of mice were fed either: 1) a standard diet higher in proteins, 2) a ketogenic diet higher in fat, 3) a western diet higher in fat and carbohydrates, or 4) a tailored steatohepatitis (NASH) diet high in fat, fructose, and cholesterol. Skin samples were fractionated and analyzed by LC/MS to determine lipid composition. Compared to the standard group, the dWAT of mice on the experimental diets showed an increase of monounsaturated TAGs and a decrease of polyunsaturated TAGs. Interestingly, though mice on the ketogenic and western diets showed an increase in saturated TAGs in the dWAT, mice on the NASH diet showed a decrease of saturated TAGs when compared to the standard group. Of note, mice from all three experimental diet groups showed significant decreases in the levels of omega-3 fatty acids in their skin, specifically alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). These essential fatty acids have been shown to play a role in maintaining skin moisture, decreasing inflammation, improving wound healing, and promoting hair growth. Additional investigation will be needed to determine how these changes in dWAT lipid composition affect our skin's complex function and contribute to cutaneous disease pathophysiology.

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Protecting skin matrisome to prevent skin aging progress

P. Warnault[1], L. Duriot[1], A. Salwinski[1], H. Chajra[2], M. Frechet[2]

[1]Plant Advanced Technologies PAT, Vandœuvre-lès-Nancy, France, [2]CLARIANT, Toulouse, France

Extracellular matrix (ECM) proteins are major components of human body and precisely human skin. In the skin the ECM is the most abundant in the dermal compartment. The human matrisome has been characterized recently, to clarify the definition of an ECM protein. Matrisome genes were divided in two main groups: the core matrisome and the matrisome-associated genes. While, the core matrisome group is divided in three categories: collagens, proteoglycans and glycoproteins; the matrisome-associated genes were classified as encoding ECM-affiliated proteins, ECM regulators or secreted factors. Skin matrisome is essential for maintaining dermal mechanical properties and homeostasis, therefore safeguarding its structure and physiology is critical. As aged and UV-related alterations of the skin matrisome components have been described (decline of collagens and decorin or stimulation of MMP-1 and CCN-1 (Cysteine-rich angiogenic inducer 61)), it appears clear that protecting skin matrisome from these alterations whatever the origin (oxidative stress, pollution …) is a way to limit skin declines leading to premature skin aging. The use of an innovative process called plant milking technology based on aeroponic culture conditions allows the development of innovative and sustainable cosmetic active ingredients highly enriched in specific compounds. We developed from Morus Alba tree a root extract (MAE) containing high levels of active prenylated compounds. MAE modulates the gene expressions of CCN1, collagen type III, decorin and MMP-1 which belong respectively to the following matrisome categories: ECM affiliated proteins, collagens, proteoglycans and ECM regulators. CCN-1 controls the transcription level of MMP-1, a protease involved in the degradation of dermis and dermal epidermal junction. MAE prenylated molecules bind collagenase with a high affinity and are strong collagenase inhibitors. MAE demonstrates effective anti-aging effect, as confirmed by a smoother and plumped skin with less visible wrinkles.

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Untangling G-protein-coupled receptor signaling and Creb in hair follicle homeostasis

M. Miranda[1], I. P. Avila[2], J. Esparza[2], W. E. Lowry[2]

[1]Molecular Biology, University of California, Los Angeles, Los Angeles, California, United States, [2]Molecular, Cell, and Developmental Biology, University of California, Los Angeles, Los Angeles, California, United States

Hair follicle stem cells (HFSCs) located in the stem cell compartment called the bulge have been shown to be the cells responsible for growing hair shafts *de novo* during each hair cycle, influenced by intrinsic and extrinsic signals among epithelial, mesenchymal, and nervous tissues. The interaction between the sympathetic nervous system and HFSC behavior is largely understudied. Sympathetic nerve innervation of the bulge has been previously shown to be hair-cycle dependent, and adrenoreceptor agonists have been demonstrated to modulate hair growth. Clinical drugs that reduce adrenergic signaling (beta blockers) report hair loss as a common side effect. Thus, several lines of evidence point to G-protein-coupled receptor (GPCR) signaling in regulating the hair cycle. GPCR typically leads to activation of adenyl cyclase which regulates levels of cAMP, ultimately leading to activation of the Creb transcription factor. We hypothesized that GPCR/cAMP/Creb signaling may regulate HFSC quiescence and activation. We treated telogen-stage murine dorsal skin with beta-2-adrenergic receptor agonists and observed an acceleration of the hair cycle, in addition to robust phospho-Creb immunostaining in the bulge -- consistent with Creb activation across the normal hair cycle, where increased phospho-Creb was present in the bulge upon anagen entry. We have taken advantage of small molecules that act on certain steps in GPCR/Creb signaling to further elucidate direct downstream molecular networks in the HFSC niche. Interestingly, we found that Creb stimulation effectively increased glycolysis in HFSCs, priming them for activation. Going forward, we will identify the impact of GCPR/cAMP/Creb signaling on HFSC metabolic state. Extensive characterization of GPCR/Creb signaling in HFSC homeostasis will therefore allow for novel methods to regulate the hair cycle and also provide a molecular mechanism by which direct nerve-stem cell interactions can modulate hair growth.

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Clinical efficacy of topical autophagy activator on acne-prone skin

K. Shin[1], K. Shin[2], S. Yoon[1], J. Jung[1], E. Hwang[1], H. Chung[1], S. Lee[3], S. Jeong[1]

[1]Research Division, Incospharm Corp., Daejeon, Korea (the Republic of), [2]Department of Food Science and Nutrition, Hallym University, Chuncheon, Korea (the Republic of), [3]Department of Dermatology, Yonsei University College of Medicine, Seoul, Korea (the Republic of)

Hyperkeratosis in follicular infundibulum, increased sebum formation, and inflammation are major factors in acne pathogenesis. Recent studies about the important roles of autophagy signaling in sebogenesis and epidermal differentiation suggest potential benefits of autophagy activator on acne. In this study, *in vitro* and clinical studies were performed to investigate the effects of autophagy activator on acne-prone skin. Autophagy signaling in sebocytes SZ95 and keratinocytes was observed and effects of autophagy activating peptide on sebum generation in sebocytes was also investigated. Clinical efficacy of autophagy activator in acne-prone skin was evaluated through 8 week, double-blind, randomized, vehicle-controlled study and changes in skin surface lipid components were further analyzed. As results, in both cultured sebocytes and keratinocytes, increased expression of LC3-II protein by tested peptide was observed, which suggests a stimulation of autophagy signaling. Sebogenesis induced by testosterone and linoleic acid treatment was also inhibited by autophagy activator treatment, while expression of epidermal differentiation marker proteins in cultured keratinocytes also observed. In consistent with *in vitro* data, reduction of whitehead and skin surface lipid quantity, as well as trans-epidermal water loss (TEWL) was observed in test peptide containing formulation applied skin. Reduction of squalene, as a marker lipid of sebum, and increase of cholesterol, as a marker lipid of keratinocyte-derived lipid, was also observed after 8 weeks of usage. These results suggest that topical application of autophagy activator can down-regulate the sebum formation and improve the skin barrier function. Considering the important roles of sebum and skin barrier function in acne pathogenesis, autophagy activator can be a new preventive or therapeutic option for acne.

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Impairment of ceramide biosynthesis pathway in bioengineered human skin models influences epidermal differentiation

C. Capallere, L. Mouret, C. Plaza, C. Meyrignac, C. Coquet-Morel, J. Botto

Ashland, Global Skin Research Center, Sophia Antipolis, France

Among the various functions of the skin, the epidermal barrier plays a key role in protecting the body from excessive water loss and the entry of exogenous substances. One of the essential components of the permeability barrier is the extracellular lipid matrix of the stratum corneum. The lipid matrix is mainly composed of ceramides, cholesterol and fatty acids. Among these lipids, ceramides are critical to maintain the water permeability barrier function of the skin. The main enzyme responsible for the conversion of glucosylceramides into ceramides is the β-glucocerebrosidase (GBA). With the goal to study the impact of a decreased expression of the skin GBA on the integrity of the epidermal barrier, 3D bioengineered skin models with down-regulated GBA expression were developed. The consequences of this decrease were investigated particularly at the level of barrier function proteins and lipid composition, detected by LC/MS. The innovative skin bioengineered model obtained in this study combined with chemical analysis of the lipids represent a promising tool to identify biofunctional ingredients or chemical substances with modulatory potential *in vitro.*

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Exosomes derived from human umbilical cord mesenchymal stem cells relieve psoriasis-like skin inflammation

Y. Zhang, J. Yan, Q. Sun

Dermatology, Shandong University, Qilu hospital, Jinan, Shandong, China

Exosomes are microvesicles secreted from the endosomal membrane and have been shown to act as regulators of cell-cell communication.The immunomodulatory effect of mesenchymal stromal cells (MSCs) is partially mediated by MSC-derived exosomes (MSC-exo).MSC-exo are a very attractive candidate for cell therapy applications in several inflammatory diseases.Psoriasis is a chronic immune-mediated inflammatory skin disease.In recent years,studies have proved that the IL-23/IL-17 axis is closely related to the immunological pathogenesis of psoriasis,and plays a key role in the occurrence and development of psoriasis.Because of the immunomodulatory properties of umbilical cord mesenchymal stem cell-derived exosomes (hucMSCs-Exo),we investigated whether hucMSCs-Exo can ameliorate psoriasis inflammation,and explored the underlying mechanisms. We found that 1.Subcutaneous injection of hucMSCs-Exo significantly relieved symptoms of psoriasis in IMQ-induced mice;in addition,the treatment with hucMSCs-Exo decreased the expression of STAT3/p-STAT3,IL-17,IL-23 and CCL20;2.HucMSCs-Exo co-cultures with DCs suppressed the maturation and activation of DCs,and inhibited the secretion of IL-23;3HucMSCs-Exo co-cultures with hacat cells and it also reduced levels of STAT3/p-STAT3,IL-17,IL-23,CCL20. Our findings suggest tha1.Subcutaneous injection of allogeneic hucMSCs-Exo significantly prevented psoriasis development in IMQ-induced mice,likely through suppression of immune cells and cytokines associated with IL-23/IL-17 axis through multiple pathways;2.Our data offers a novel therapeutic approach to chronic inflammatory skin diseases such as psoriasis by leveraging immunomodulatory effects of hucMSCs-Exo.

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DNA dioxygenases Tet1/2/3 control hair matrix keratinocyte differentiation and hair shaft shape via regulation of hair keratin gene expression

G. Chen[1], Q. Xu[2], M. Fessing[3], A. Mardaryev[3], A. Sharov[1], G. Xu[2], V. A. Botchkarev[1]

[1]Dermatology, Boston University, Boston, Massachusetts, United States, [2]Shanghai Institute of Biological Sciences, Shanghai, China, [3]Centre for Skin Sciences, University of Bradford, Bradford, United Kingdom

DNA methylation and subsequent oxidation of 5-methylcytosine into 5-hydroxymethylcytosine (5hmC), catalyzed by the TET1/2/3 family enzymes, are key epigenetic events regulating development, stem cell differentiation and cellular reprogramming in mammals, while genetic ablation of all three *Tet* genes is lethal. Here, we show that 5hmC modified DNA and Tet1/2/3 proteins show dynamic changes in their abundance in the developing hair follicles (HFs), as well as during the hair cycle. High levels of 5hmC, Tet2 and Tet3 were seen in differentiating hair matrix keratinocytes (KCs), outer and inner root sheaths, hair shaft and dermal papilla. To uncover the roles for Tet1/2/3 in the control of HF development and cycling, we used genetically modified mice with *Krt14-Cre* mediated ablation of *Tet* genes (*Krt14-Cre/ Tet2*$^{fl/fl}$*/Tet3*$^{fl/fl}$ or DKO, *Krt14-Cre/ Tet1*$^{fl/fl}$*/Tet2*$^{fl/fl}$*/Tet3*$^{fl/fl}$ or TKO). Consistently with the expression patterns for *Krt14* gene, the level of 5hmC was markedly decreased in the epidermis and hair follicle, as well as in hair matrix keratinocytes, which are the progenitors of the hair shaft. *Krt14-Cre* mediated *Tet2/3* or *Tet1/2/3* ablation resulted in waivy hairs and hair loss compared to WT and *Tet2/3* single knockout controls. Smaller hair bulbs and altered hair shape were observed in the DKO and TKO mouse skin. Furthermore, RNA-seq analysis of the RNA isolated from primary keratinocytes revealed a significant decrease in expression of the inner root sheath keratin genes *(Krt25, Krt26, Krt27, Krt28, Krt71, Krt72)* upon *Tet2/Tet3* ablation. In summary, our data demonstrate the role of Tet-mediated 5hmC DNA oxidation in the control of hair shaft-specific keratinocyte differentiation and hair shaft shape, as well as in regulation of hair keratin gene transcription.

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Keratinocyte differentiation is coupled to mechanical cues through the LINC complex

A. G. Zieman[1], R. Stewart[3], A. E. Zubek[1, 2], E. Carley[3], I. Jalilian[3], M. King[3], V. Horsley[1, 2]

[1]Molecular, Cellular and Developmental Biology, Yale University, New Haven, Connecticut, United States, [2]Dermatology, Yale University, New Haven, Connecticut, United States, [3]Cell Biology, Yale University, New Haven, Connecticut, United States

Skin tissue is resilient to mechanical forces, but also can respond to prolonged mechanical forces. For example, tissue expanders are used to generate more skin for breast reconstruction surgery. At the cellular level, multiple mechanical forces are exerted on epidermal keratinocytes. Progenitor keratinocytes, unlike differentiated keratinocytes, have both cell-cell adhesions and cell-extracellular matrix (ECM) adhesions that keep progenitor keratinocytes attached to the basement membrane. During keratinocyte differentiation, progenitor keratinocytes lose cell-ECM adhesions and detach from the basement membrane, but the exact mechanism of how this occurs is not fully understood. External forces must be communicated from the plasma membrane at the outside of the cell to the nucleus, and the Linker of Nucleoskeletal and Cytoskeletal (LINC) complex is poised on the nuclear membrane to sense these mechanical forces. We were interested in determining if there is tension on the LINC complex in keratinocytes and if this tension influences the function of epidermal keratinocytes. Tension on the LINC complex was measured using a FRET-based Nesprin tension sensor, which showed that keratinocytes undergoing differentiation have less tension on the LINC complex compared to progenitor keratinocytes. To investigate the functional consequences of tension on the LINC complex, mice lacking LINC complex components SUN1 and SUN2 proteins were generated. Multiple lines of evidence indicate that *Sun1/2* null keratinocytes undergo precocious differentiation both in culture and *in vivo*. In conclusion, this work demonstrates that mechanical tension on the LINC complex maintains keratinocytes in a progenitor state and identifies tension on the LINC complex as a new regulator of keratinocyte differentiation.

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Discovering the signaling pathways underlying mouse Merkel cell development using FACS-based single cell RNA-seq

L. Miao[1], M. Kelly[2], S. Barkdull[1], L. Collado[1], M. Kelley[2], I. Brownell[1]

[1]Dermatology Branch, NIAMS, NIH, Bethesda, Maryland, United States, [2]Laboratory of Cochlear Development, NIDCD, NIH, Bethesda, Maryland, United States

Merkel cells (MC) are innervated light touch sensors that comprise less than 0.3% of the mouse epidermis. Terminally differentiated MC are derived from epithelial progenitors and possess both epidermal and neuronal features. Study of MC development provides a model of lineage-specific epithelial differentiation and gives insight into the tumorigenesis of Merkel cell carcinoma (MCC). Using fluorescence-activated cell sorting (FACS)-based single-cell RNA sequencing (scRNAseq) and MC-specific GFP reporter mice, we captured the transcriptome of single MC from embryonic, neonatal, and postnatal skin. Single GFP+ cells were isolated, lysed, and cDNA library for individual cells were generated using Smart-seq2 method. We determined the transcriptome of 1152 single cells including GFP+ MC at all states of differentiation and GFP- control cells. Sequencing yielded ~3 million filtered reads per cell. Full-length mRNAs were mapped, and splice variants were identified with an average of 6000 genes per cell. Cell transcriptomes clustering identified MC at different differentiation stages, from epithelial precursor to terminally differentiated MC. Trajectory analysis traced the transcriptional changes occurring during MC differentiation. MC-specific genes and transcriptional regulators that define MC differentiation were identified. Transcriptional signatures associated with active cell signaling pathways were identified for each stage of MC differentiation. Comparison of MC progenitors to keratinocytes identified Shh, Egf, Bmp4, and Fgf signaling as being active in early MC specification. Taken together, our FACS-seq approach captured high quality scRNA-seq data to analyze the differentiation of MC from developing mouse skin. This analysis identified novel markers for the MC lineage, characterized the stages of MC differentiation, and defined transcriptional networks and signaling pathways underlying MC differentiation.

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Single cell transcriptomics reveals dermal fibroblast heterogeneity and a progenitor population that shapes fibroblast heterogeneity

X. Zhang[1], W. Liu[1], L. Sun[1], M. Yin[1], S. Wu[1], L. Zhang[1, 2]

[1]School of Pharmaceutical Sciences, Xiamen University, XiaMen, FuJian, China, [2]Department of Dermatology, University of California, San Diego, La Jolla, California, United States

Dermal fibroblasts (dFBs) are an essential component of skin; they not only produce and organize the extracellular matrix of the dermis, but also are essential for wound healing, hair growth, fibrosis and defense against infection. Fibroblast heterogeneity has long been recognized in mouse and human skin, but the cellular hierarchy and mechanisms governing fibroblast heterogeneity are incompletely understood. Here, we used single-cell RNA-sequencing to study how cellular heterogeneity of murine skin is turned at the transcriptional level during post-natal periods using single cells isolated from the skin of new born (P1), young (3 weeks) and adult (2 month) mice. Unbiased clustering of >10,000 single-cell transcriptomes revealed 29 distinct population of the skin. Within these clusters, Pdgfra clearly marked 13 dFB clusters, which were then re-clustered into 23 dFB clusters. Pseudotime analyses of these dFB clusters identified a $Pdgfra^{+}CD24^{hi}Thy1^{lo}Sca1^{lo}$ progenitor population that was highly abundant in neonatal skin early in life but declined in adulthood. Pseudotime analyses revealed that this progenitor population gave rise to several FB subtypes, including dermal papillary FBs, reticular FBs that produce high levels of type1 collagen, adipocytes that produce antimicrobial peptide *Camp* as well as the interstitial reticular FB that are enriched with inflammatory gene signature during post-natal development. The ability of this progenitor population to commit to collagen 1 producing reticular dFB and to differentiate into adipocytes was confirmed by primary dFB culture in vitro. Together, our study allows the reconstruction of gene expression programs during fibroblast development and provides insights into how fibroblasts develop heterogeneity from progenitors during adulthood.

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Molecular analysis of atopic dermatitis pathogenesis in NC/NgaTnd mice

C. K. Singh, C. A. Mintie, M. A. Ndiaye, G. Chhabra, N. Ahmad

Dermatology, University of Wisconsin, Madison, Wisconsin, United States

Atopic dermatitis (AD), a chronic inflammatory disease marked by skin itching and lesions, affects >26 million people in the US. A detailed understanding of the molecular pathogenesis of AD may provide novel means for the management of this debilitating disease. To this end, here we used 2,4-dinitrofluorobenzene (DNFB)-induced NC/NgaTnd mouse model that closely mimics AD in humans. After hair removal, AD was induced in 5-week-old female mice by weekly topical application of 0.15% DNFB to dorsal skin. Skin lesions appeared at week 5, and by week 10, severe AD-like skin lesions were present. The mice were found to have increased erythema/haemorrhage, dryness/scaling, and inflamed ears. Further, AD mice had increased epidermal thickness, mast cell infiltration, serum IgE, spleen weight, and larger lymph nodes. Using a mouse cytokine array, we identified significant increases in tumor necrosis factor (Tnf), interleukins (Il6, Il22, Il23, Il28), and chemokines (Cxcl1, Cxcl10, Ccl2, Ccl4, Ccl5, Ccl7). To further explore the molecular pathogenesis of AD, we employed global quantitative proteomics. A total of 714 proteins were identified, of which 68 were significantly modulated ≥2-fold in AD mice. Using Ingenuity Pathway Analysis software (IPA), we identified acute phase response (APR) signaling as an important canonical pathway, with the top 4 upregulated proteins (Hp, Fgb, Fgg, Hpx) being positive APR proteins related to inflammation. Functional annotation of the 68 modulated proteins included inhibited microtubule dynamics and cytoskeleton organization and increased secretion of molecules. Upstream analysis of modulated proteins predicted increased Il6, Il1β, Osm, Stat3, and Tnf signaling and decreased Ins1 and Nfe2l2 signaling. Importantly, APR signaling and several others identified in our proteomics analysis (A1bg, Myh13, Mocs3, Pcdh15, Lrg1; all >5-fold change) have not been linked to AD thus far. Further studies are needed to validate how interactions of these modulated proteins are involved in AD development and progression.

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Terminal arborizations of itch-sensing neurons exhibit large receptive field in the skin and regionally specific organization in the spinal cord

L. Han, Y. Xing, H. Steele, H. Hilley, K. Lawson

Biological Sciences, Georgia Institute of Technology, Atlanta, Georgia, United States

Itch sensation is initiated by the activation of a specific subset of sensory neurons that project their peripheral and central axons to the skin and spinal cord respectively. Different subtypes of sensory neurons exhibit distinct terminal arborization subserving their functions. Sensory arborization of itch-sensing neurons has never been observed due to the lack of proper molecular genetic tool. Here we have genetically labeled a small subset of DRG sensory neurons using a newly generated $MrgprC11^{CreER}$ mouse line. Sparse Cre combination labels about 1% of all DRG neurons. Majority of them are expressing multiple itch receptors including MrgprC11, MrgprA3, histamine receptor H1 (H1R) and IL31ra, demonstrating that these cells represent a small subset of itch-sensing neurons. Using whole mount skin PLAP histochemistry staining, we revealed the morphology of itch-sensing arborizations in both the skin and spinal cord. We found that itch-sensing skin arbors are characterized by free endings with extensive axonal branching in the superficial epidermis and large receptive field. The average area of itch arbors is triple the size of the non-itch-sensing nociceptive skin arbors. This is consistent with the large innervation territories of itch-sensing nerves that have been observed in human skin. We also found that the central arbors of itch-sensing neurons exhibit regionally specific organization in the spinal cord. The central arbors of distal limbs innervating neurons show round morphology whereas the arbors of the trunk innervating neurons exhibit long and thin morphology, although the two types of arbors are of the same size. This regionally specific terminal arborizations suggest sensory acuity for itch in the distal limb. These findings improved our understanding of the basic mechanisms of itch and provide novel insights into the topographical organization of the neural circuits mediating itch.

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Generation of human hair follicle organoids *in vitro* and *ex vivo* by co-culture of primary human hair matrix keratinocytes and dermal papilla fibroblasts

I. Piccini[1], K. Bakkar[2], C. Collin-Djangoné[2], J. Gherardini[1], R. Paus[1, 3, 4], M. Bertolini[1]

[1]Monasterium Laboratory, Muenster, Germany, [2]L'Oréal Research and Innovation, Aulnay-sous-Bois, France, [3]University of Miami Miller School of Medicine, Miami, Florida, United States, [4]University of Manchester, Manchester, United Kingdom

Hair transplantation for the management of androgenetic alopecia is often limited by the number of donor HFs available for transplantation. Therefore, HF neogenesis technologies that enlarge the number of donor HFs are needed. Here, we assessed the potential of human primary adult hair matrix keratinocytes (HMx), i.e. the cells interacting with dermal papilla fibroblasts (DPs) *in vivo,* in generating HF organoids when co-cultured with DP spheroids *in vitro* and in human skin organ culture, compared to primary human adult epidermal keratinocytes (NHEK). Both types of keratinocytes effectively generated HF organoids *in vitro*, yet NHEK with higher success rate than HMx. DP inductivity declined over time in both types of HF organoids (i.e., gradual decrease in *VERSICAN, NOGGIN, LEF1 mRNA*, VERSICAN protein expression, and alkaline phosphatase activity), despite an increase in *IGF1, HGF*, and *TGFß2* transcripts. HMx-DP organoids revealed higher expression of the pre-cortical hair matrix keratin, K85, which precedes hair shaft formation, as compared to ORS-associated keratins, i.e. K6, K14 and K5, while NHEK-DP organoids showed high expression of all types of keratins. Upon intradermal injection into organ-cultured human skin, fluorescently labelled human HMx and DP cells proliferated until day 3 but did not undergo apoptosis later on possibly reflecting commitment to differentiation. When HMx and DP spheroids were co-cultured shortly *in vitro* and then placed into human scalp skin, small HF organoids positive for VERSICAN, K85, and K6 formed during 10 days of organ culture. Thus, the morphogenic potential of isolated human HMx may be used for both, medical HF neogenesis and as a preclinical screening system for testing candidate hair growth-promoting agents *in vitro* and *ex vivo*.

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Extreme organization of supra-basal cells allows the building of modular feather architectures for adaptable flight

C. Chuong[1], W. Chang[2], H. Wu[2], M. Lei[2], W. Juan[2]

[1]Pathology, University of Southern California, Los Angeles, California, United States, [2]Integrative Stem Cell Center, China Medical University, Taichung, Taiwan

The skin forms the interface between an organism and its environment. To venture into diverse eco-spaces, animals evolve different integumentary organs to adapt. Here we study how feathers are made from a flat epidermis. A feather is made of two modules: a central shaft (rachis) and the bilateral vane which is made of barb branches. The feather has to be light and strong. In the follicle, feather epidermis first forms an epithelial cylinder with basal cells facing the pulp dermis inside. Then supra-basal cells are partitioned to form the rachis and barbs. In the rachis region, supra-basal keratinocytes are further partitioned into stiff cortex and vacuolated medulla with different keratin types, a process controlled by *Bmp* and *TGF*β signaling. In the barb region, supra-basal cells are organized into periodic ridges, and each ridge is further partitioned into two barbules. The barbule cell shape can be cylindrical, plate-like or hooklet-shaped. Hooklet is formed by asymmetric cell junction distribution and keratin expression, and allows barbules to be weaved into the feather vane in a velcro-like mechanism. Transcriptome analyses and functional studies show anterior-posterior *Wnt2b* signaling within the dermal papilla controls barbule cell fates with spatio-temporal collinearity of within-feather barb differences. Flight feathers of birds with different flight characteristics, ranging from ostrich, chicken, duck, eagle, finch, humming bird and penguin are examined and each show optimal bio-architectural design that adapt them to the need of their distinct eco-spaces. Analyses of one-billion year old amber feather fossil provide 3D perspective on the evolutionary process. Some results are published in "The making of a flight feather: Bio-architectural principles and adaptation, CELL, 2019, 179:1409-". More molecular mechanisms on the control of the extreme complex arrangement of the differentiated supra-basal cells will be discussed.

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Hyperactivation of sympathetic nerves drives melanocyte stem cell depletion

B. Zhang[1], S. Ma[1, 2, 3], I. Rachman[4], M. He[1, 5], P. Baral[6], S. Choi[1], W. A. Gonçalves[10], Y. Shwartz[1], E. M. Fast[1, 7], Y. Su[4], L. I. Zon[1, 7, 8], A. Regev[2, 8, 3], J. D. Buenrostro[1], T. M. Cunha[6, 9], I. M. Chiu[6], D. Fisher[4], Y. Hsu[1]

[1]Stem Cell and Regenerative Biology, Harvard University, Cambridge, Massachusetts, United States, [2]Klarman Cell Observatory, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, United States, [3]Department of Biology and Koch Institute, Massachusetts Institute of Technology, Cambridge, Massachusetts, United States, [4]Cutaneous Biology Research Center, Department of Dermatology, Massachusetts General Hospital, Charlestown, Massachusetts, United States, [5]Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts, United States, [6]Department of Immunology, Harvard Medical School, Boston, Massachusetts, United States, [7]Division of Hematology/Oncology, Boston Children's Hospital/Dana-Farber Cancer Institute, Boston, Massachusetts, United States, [8]Howard Hughes Medical Institute, Chevy Chase, Maryland, United States, [9]Department of Pharmacology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil, [10]Graduate Program in Cellular Biology, Institute of Biological Science, Federal University of Minas Gerais, Belo Horizonte, Brazil

Empirical and anecdotal evidence have associated stress with accelerated hair greying (formation of unpigmented hairs), but the scientific evidence linking the two is scant. Here, we report that acute stress leads to hair greying through fast depletion of melanocyte stem cells (MeSCs). Combining adrenalectomy, denervation, chemogenetics, cell ablation, and MeSC-specific adrenergic receptor knockout, we found that stress-induced MeSC loss is independent of immune attack or adrenal stress hormones. Rather, hair greying results from activation of the sympathetic nerves that innervate the MeSC niche. Upon stress, sympathetic nerve activation leads to burst release of the neurotransmitter norepinephrine, which drives quiescent MeSCs into rapid proliferation, followed by differentiation, migration, and permanent depletion from the niche. Transient suppression of MeSC proliferation prevents stress-induced hair greying. Our studies demonstrate that acute stress-induced neuronal activity can drive rapid and permanent loss of somatic stem cells, and illustrate an example in which somatic stem cell maintenance is directly influenced by the overall physiological state of the organism.

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Optimized Vitamin C prodrug for controlled release and antioxidant activity

C. Jacques, C. Genies, D. Bacqueville, N. Borotra, M. Noizet, A. Tourette, S. Bessou-Touya, H. Duplan

Pierre Fabre Dermo-cosmetique, Toulouse, France

Vitamin C (vitC) plays key roles in many biological processes and it is a powerful anti-oxidant which protect the skin against UV exposure. Nevertheless, vitC is unstable under oxidative conditions. A pro-vitamin C, Ascorbic acid 2-glucoside (AA2G) was developed to stabilize vitC. The purpose of the study was to assess skin delivery, metabolism and antioxidant effect of AA2G compared to two concurrent products with 5% of encapsulated vitC or with 15% of free vitC. Skin delivery and metabolism studies were performed on human skin explants by UHPLC/UV. For anti-oxidative protection, three parameters were evaluated by measuring superoxide dismutase, catalase following UV exposure and malondialdehyde by GC/MS. We demonstrated prodrug concept with a good stability of AA2G and release of vitC. A better bioavailability was observed with prodrug compare to encapsulated vitC and equivalent to free vitC. A reservoir effect of AA2G in the SC was also observed which allows a controlled release of vitC according to the needs of the skin. These results are in accordance with a better anti-oxidant activity of the prodrug compare to the encapsulated vitC and equivalent to the free vitC with a lower percentage in the formulation 1,8% of prodrug versus 15% of free vitC.

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IL1α, IL6, and GMCSF are Downstream Mediators of IL17A that Promote Asymmetric Stem Cell Self-Renewal in Psoriasis

H. Li[1, 2], A. Charruyer[1, 2], T. Weisenberger[1, 2], A. Khalifa[1, 2, 4], R. Nguyen[1, 2, 3], R. Ghadially[1, 2]

[1]Dermatology, UCSF, San Francisco, California, United States, [2]Dermatology, VA Medical Center, San Francisco, California, United States, [3]Keck School of Medicine, Los Angeles, California, United States, [4]Faculty of Science, University of Zagazig, Zagazig, Egypt

Interleukin 17A (IL17A) is a key cytokine in psoriasis and IL17A antibody therapy is an effective treatment for psoriasis. We previously showed that IL17A increases asymmetric stem cell self-renewal divisions in human keratinocytes and plays a role in the hyperproliferation of the epidermis observed in psoriasis. We hypothesized that downstream IL17A-induced keratinocyte cytokines were mediators of the increased asymmetric stem cell self-renewal divisions of psoriasis. We studied cytokines produced by keratinocytes after IL17A treatment and determined their effects on asymmetric stem cell self-renewal divisions in human keratinocytes in vitro, using sister pair analysis. Using ELISA, we showed that IL17A significantly increased the production of IL1α, IL6, IL8 and GMCSF by keratinocytes. IL1α, IL6, and GMCSF (but not TNFα and IL8) increased asymmetric stem cell self-renewal divisions in keratinocytes versus vehicle-treated keratinocytes (48.4±4.0% versus 36.4±3.8%, 51.2±1.1% versus 37.3±0.8%, and 47.8±2.7% versus 34.2±1.0%, $P < 0.05$, n=3, respectively). Increasing doses of IL1α antibody, IL6 antibody, and GMCSF antibody significantly inhibited IL17A-induced asymmetric stem cell self-renewal in a dose-dependent fashion. In summary, this study has identified key mediators involved in the increased asymmetric stem cell self-renewal divisions in psoriasis and may provide new avenues to finding treatments for the cutaneous manifestations of psoriasis. This study also confirms the important role of asymmetric stem cell self-renewal in inflammatory diseases and underscores the potential of manipulation of stem cell self-renewal as a therapeutic strategy.

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Phenotypic plasticity of cutaneous squamous cell carcinoma mediated by cyclooxygenase-2

H. Moon[2], D. Kim[1], L. Donahue[1], A. White[1]

[1]Cornell University, Ithaca, New York, United States, [2]University of California, Davis, Davis, California, United States

Murine hair follicle stem cells containing the KrasG12D mutation and p53 loss of function are able to form tumors that represent mesenchymal-like spindle cell morphology. This spindle form of squamous cell carcinomas exhibit hallmarks of epithelial to mesenchymal transition and grow rapidly with invasive characteristics. This mouse model thus provides a method for testing molecular candidates that are critical for a mesenchymal-like phenotype in cutaneous tumors. Cox-2 has been shown to have a role in progression of cutaneous squamous cell carcinomas composed of epithelial-like cells. Cox-2 is significantly expressed in hair follicle stem cell-arising tumors, but the role of Cox-2 in these mesenchymal-like tumors remains unclear. Here, we show that loss of function in Cox-2 in hair follicle stem cells that also express KrasG12D and loss of p53 generate tumors with a change in phenotype from mostly mesenchymal-like cells to mostly epithelial-like. Loss of Cox-2 demonstrates upregulation in epithelial markers and downregulation in the mesenchymal markers found in tumors harboring fully functioning Cox-2 *in vivo*. This study demonstrates that Cox-2 expression at the origin of hair follicle-originating tumors predisposes to a mesenchymal-like morphological and molecular phenotype.

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A master regulator function of hairless in skin homeostasis and immune regulation

W. Ha[1], M. Mihlan[2], A. Hinde[1], K. Krotova[1], G. Aslanidi[1], L. Xie[1], R. Ricci[2], A. Christiano[3], L. Liu[1, 3]

[1]The Hormel Institute, University of Minnesota, Austin, Minnesota, United States, [2]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Université de Strasbourg, Strasbourg, France, [3]Dermatology, Columbia University, New York, New York, United States

The hairless (Hr) gene plays an essential role in hair follicle development and skin homeostasis. Both humans and mice lacking hairless activity suffer from complete hair loss and abnormal epidermal proliferation and differentiation, although the mechanisms underlying such developmental defects remain largely unknown. We recently demonstrated that Hr encodes a histone demethylase and acts as an epigenetic regulator of the expression of its target genes. Gene expression profiling studies identified pro-inflammatory IL-1 family cytokines, including IL-36 and IL-1β, among the major targets of Hr. In addition to elevated levels of IL-1 family cytokines in Hr-deficient mouse skin, we found increases in CD3+ T cells in both the epidermis and dermis from Hr-knockout (Hr-/-) mouse. We confirmed that the epidermal T cells were predominantly dendritic epidermal γδ T cells. While there was little change in CD8+ T cells between Hr-/- and Hr+/- skin, there were significant increases in CD4+ (22% vs 15%) T cells and CD4+/CD25+ regulatory T cells (7.43% vs 1.97%) in Hr-/- skin. Despite the increased amount of regulatory T cells, Hr-/- mice are prone to developing dermatitis that is absent in Hr+/- control mice. Furthermore, we found a significant reduction in epidermal stem cell population in Hr-/- mice based on the expression of Cd34+, Cd49f+, Lgr6 and Tfrc markers by both immunofluorescence and FACS analysis. Skin wound healing in Hr-/- mice, however, was not affected by the decrease in epidermal stem cell population as compared to age-matched Hr+/- mice. Together, these findings demonstrate a master regulator role of hairless in skin immune homeostasis and epidermal stem cell development that is linked to the abnormal hair and skin phenotypes in Hr-deficient mice. The molecular pathways underlying such pivotal regulatory functions are under investigation.

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Evidence for epithelial cells in blood and bone marrow of untreated murine and human subjects

S. Holtorf[1], J. Monts[1], T. Schuster[1], R. Morris[2]

[1]The Hormel Institute, Austin, Minnesota, United States, [2]The Hormel Institute/University of Minnesota, Austin, Minnesota, United States

Cytokeratin positive cells are frequently found in the blood and bone marrow of patients with epithelial cancers and are attributed to metastasis. Interestingly, we observed in a number of publications that unaffected human controls expressed traces of cytokeratin mRNAs. To determine the presence of epithelial cells in normal blood and bone marrow, we used immunofluorescence microscopy (IF), Krt1-14;mTmG transgenic mice, qRT-PCR, and fluorescence activated cell sorting (FACS). We have made several interesting findings. First, we discovered, rare but reproducible cytokeratin immunoreactive cells the size of small lymphocytes in blood and bone marrow of untreated adult mice. We found that Epithelial Cell Adhesion Molecule (EpCAM) positive cells in whole human blood and bone marrow constituted 0.083% and 1.94% respectively (average of at least 4 experiental and 2 biological replicates) regardless of the number of cells counted. Virtually 100% of the EpCAM+ cells were immunoreactive to pan-cytokeratin as determined by IF microscopy. Second, using Krt1-14;mTmG transgenic mice that express GFP in epidermis under the Krt1-14 promoter, we found low (8.6 native GFP+ cells per 10^6 cells analyzed), but significant numbers ($p<0.0005$) of GFP+ cells in bone marrow of normal adult mice when compared with negative controls. Third, qRT-PCR demonstrated very low but reproducibly detectable expression of cytokeratin mRNAs in blood and bone marrow, that when compared with purified epidermal keratinocytes, was 1000x and 100,000x less, respectively. Moreover, FACS analysis of fresh bone marrow disclosed multiple subpopulations of EpCAM+ cells when compared with hematopoietic and mesenchymal lineage markers. We conclude from these observations that cytokeratin proteins and mRNAs are expressed at low, but reproducibly detectable levels in blood and bone marrow cells thus setting the stage for determining epithelial stem cell functions in general, and epidermal stem cell functions in particular, of these interesting cells.

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Evolution of an Engrailed 1 enhancer underlies expanded sweat gland density of humans
D. Aldea[1], Y. Atsuta[2], B. Kokalari[1], S. Schaffner[3], Y. Kamberov[1]
[1]Genetics, Perelman School of Medicine/University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Harvard Medical School, Boston, Massachusetts, United States, [3]Broad Institute of MIT and Harvard, Cambridge, Massachusetts, United States
In humans, heat dissipation is mainly achieved by the evaporation of sweat. Key to the effectiveness of this thermoregulatory mechanism is the human-specific evolution of a dramatic increase in eccrine sweat gland density. The genetic changes underlying the evolution of this adaptive trait are unknown. In humans, expression of the Engrailed 1 (En1) transcription factor correlates with the onset of sweat gland formation. In mice, regulation of ecotdermal En1 expression is a major determinant of natural variation in gland density between strains and increased En1 promotes the specification of more eccrine glands. In light of these findings, we hypothesized that modulation of En1 expression could be an underlying mechanism for the adaptive changes in human sweat gland density. To test the hypothesis that changes in En1 expression impacted human sweat gland density, we investigated the regulation of En1 expression in humans and other species. Using comparative genomics combined with functional validation of enhancer activity in mice, we identified a suite of En1 enhancers active during sweat gland development. We then compared the relative activity of the mouse, human, and non-human primate sequences of these elements in human and mouse keratinocytes. We find that among these elements, the activity of one enhancer- previously identified as a region of positive evolution in humans-is strikingly higher than that of all other species. Using a humanized mouse knock-in model in which we replaced the endogenous mouse enhancer with its human ortholog, we show that the human enhancer upregulates En1 transcription in cis and that this is sufficient to increase eccrine sweat gland density in mouse skin. Our data indicate that the rapid evolution of an En1 enhancer led to increased expression of ectodermal En1 on the human lineage and drove the expansion of eccrine sweat gland density that is a signature trait of our species.

Tissue Regeneration and Wound Healing

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Promoting cutaneous wound healing with nutraceutical porcine type I collagen peptides
K. Mistry[1], B. Van Der Steen[2], B. Vanhoecke[2], F. van Holthoon[3], A. Kleinnijenhuis[3], T. Clifford[1], M. Labus[1], P. Lovat[1], A. McConnell[1]
[1]Newcastle University , Newcastle upon Tyne , United Kingdom, [2]Rousselot, Ghent, Belgium, [3]Triskelion, Utrechtseweg, Netherlands
Type I collagen, the most abundant protein in human skin is integral to cutaneous wound healing, promoting fibroblast proliferation whilst increasing wound tensile strength. Ageing leads to decreased type I collagen synthesis and greater disorganisation within the extracellular matrix resulting in impaired wound healing, an increasing burden in an ageing population. Synthetic type I collagen peptides have been shown to enhance cell migration and proliferation leading to the current hypothesis that nutraceutical porcine type I collagen peptides may enhance dermal collagen levels in aged individuals and promote cutaneous wound healing. To test this hypothesis, donor matched primary young (18-35 yrs) or aged (60+ yrs) fibroblasts were seeded onto clinically achievable concentrations of type I collagen porcine peptides prior to assessment using *in vitro* assays of wound closure, proliferation or ki67 expression. Results demonstrated porcine collagen peptides significantly promoted wound closure of both young and aged fibroblasts. Inhibition of cell proliferation with mitomycin C decreased peptide-induced migration while MTS cell viability assays and immunofluorescence for Ki67 expression demonstrated porcine collagen peptides induced fibroblast proliferation. *In vivo* studies of porcine peptide ingestion additionally demonstrated increased hydroxyproline concentrations (a biomarker for collagen I peptide absorption) 2 hours after ingestion in both young and aged individuals to a level equivalent to peptide concentrations used *in vitro* assays and suggesting age does not impact collagen peptide absorption. Collectively this data suggests type I porcine collagen peptides may offer a viable therapeutic strategy to promote cutaneous wound healing in both young and elderly individuals.

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Biphasic modulated pulsed microcurrent treatment of skin
D. Kern, M. Riggs, H. E. Knaggs
R&D, Nu Skin Enterprises, Inc, Provo, Utah, United States
Application of low level AC or DC current to the skin to assist drug delivery, reduce pain, affect muscle contraction, accelerate bone and chronic wound healing have been used medically since 1830 when Carlo Matteucci reported that injured tissue generated an electrical current. In some therapeutic modalities, such as the treatment of Bell's palsy, a reduction of fine lines and wrinkles, improved skin tone and plumpness have been reported. In the early 1970s skin aestheticians incorporated microcurrent treatment into their product offerings claiming improvement in the appearance of the skin. To investigate possible cosmetic benefits from microcurrent therapy applied to the skin, a home use device delivering 40 uA/cm^2 in the form of a biphasic modulated pulsed waveform was created and used with an inert electrically conductive gel in an IRB-approved 8 week clinical study. Forty female subjects, 25 – 40 years of age, Fitzpatrick I-IV were enrolled and evaluated instrumentally, by clinician and self-assessment. Using a clinician scoring range of 1 (low) to 9 (high), comparing a conductive gel with microcurrent application to conductive gel only, improvement in numerous skin attributes were seen immediately and progressively over time. Depending on the specific attribute, generally one or more full grade changes were seen for hyperpigmentation, skin tone evenness, radiance, wrinkles, fine lines, plumpness, sagging, texture, pores and overall skin appearance quality. The biphasic modulated pulsed microcurrent treatment may provide skin benefit by changing the resistivity of the skin due to structural changes, increasing circulation within the skin, enhancing the regeneration of collagen and connective tissue or by an increased rate of ATP generation. Microcurrent treatment of skin can improve the appearance of the skin.

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RNase L is a regeneration repressor gene
N. Islam[1], E. M. Wier[1], M. Alphonse[1], H. Liu[1], D. Kim[1], A. Li[1], S. Reddy[2], L. S. Miller[1], H. Weiliang[3], S. Lee[1], S. Kim[1], G. Wang[1], M. Kane[3], R. Silverman[4], L. Garza[1]
[1]Dermatology, Johns Hopkins School of Medicine, Baltimore, Maryland, United States, [2]Plastic Surgery, Johns Hopkins School of Medicine, Baltimore, Maryland, United States, [3]Pharmaceutical Sciences, University of Maryland, Baltimore, Maryland, United States, [4]Cancer, Lerner Research Institute, Cleveland, Ohio, United States
When compared to animals across other phyla, mammals have restricted regeneration and more fibrosis. This limited regenerative capacity may reflect a loss of pro-regeneration programs or active suppression by genes functioning akin to tumor suppressors. To uncover the programs governing regeneration in mammals, we investigated Wound Induced Hair Neogenesis (WIHN), a rare example of regeneration in adult mammals. Through comprehensive screening of transcripts associated with WIHN—as well as after rejuvenation lasers in human subjects--, we found that the endoribonuclease RNase L associates with regeneration/rejuvenation, but actually functions as a powerful suppressor of regeneration. *Rnasel*$^{-/-}$ mice exhibit remarkable regenerative capacity, with increased WIHN (n=10, p<0.0001) and accelerated wound healing following injury (n=3, p<0.05). This is mediated through the production of IL-36α, which is increased in *Rnasel*$^{-/-}$ mice, enhances WIHN when added exogenously (n=3, p<0.01), and is required for WIHN given its absence in IL36R-/- mice (n=3, p<0.01). Consistent with the known role of RNase L to stimulate caspase-1 signaling, we find that in wild type mice, pharmacologic inhibition of caspases promotes regeneration in an IL-36-dependent manner. These responses are not limited to skin, but occur following intestinal injury as well, suggesting that suppression of regeneration is a general attribute of mammalian wound healing. Taken together, this work suggests a therapeutic strategy to uncover latent regenerative capacity and promote functional response to injury.

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Bacteria induce skin regeneration

G. Wang, H. Liu, E. M. Wier, R. Ortines, N. Islam, A. Li, Y. Xue, Y. Wang, N. Archer, L. S. Miller, L. Garza

Dermatology, Johns Hopkins School of Medicine, Baltimore, Maryland, United States

Wound Induced Hair follicle Neogenesis (WIHN) is a rare adult organogenesis model where stem cells form *de novo* hair follicles following full-thickness wounding. As wounds inevitably contact the skin microbiota, it is important to understand the role of the skin microbiome in WIHN. To do so, we modified bacterial burdens and tested WIHN. We used 3 levels of microbial burden to measure WIHN: For minimal bacteria loads, we used germ-free (GF) mice, applied antibiotic ointment (Neosporin) or frequent cage changes of standard specific pathogen free (SPF) mice housing. For intermediate bacterial loads and as a baseline comparator, we used standard SPF mice housing. For maximal bacteria loads, we injected each of the top three strains of SPF mice skin commensal bacteria to the wound bed early during wounding. We found that GF mice (fold= -14.4, n=5, $p=3.5X10^{-5}$), mice treated with antibiotic (fold= -7.9, n=5, $p=8.4X10^{-5}$) or mice in clean cages (fold= -2.8, n=4, p=0.015) have lower regeneration capacity respectively and lower stem cell marker expression. Increase commensal bacteria loads can enhance SPF mice regeneration capacity (fold=3.3, n=6, $p=7.5X10^{-5}$) and also promote stem cell marker expression. Mice deficient in Myd88 (fold=-29.6, n=7, $p=8.3X10^{-7}$) and IL1R deficient mice (fold=-9.4, n=5, $p=0.4X10^{-4}$) have poor regeneration capacity, and are resistant to the ability of exogenous bacterial to enhance WIHN. Keratinocytes and Myeloid cell-specific Myd88 deficient mice have similar regeneration capacity to wild type mice. Taken together, these results demonstrate that commensal and exogenous bacterial burden enhance regeneration. This is mediated through IL1R- Myd88 signaling, but not in keratinocytes or myeloid cells. Future studies will define the cell type and cytokine responsible for activation of Myd88 and WIHN during commensal exposure.

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Effect of novel disaccharide for construction of living skin equivalents

J. Muto[1], S. Fukuda[2], Y. Shirakata[1], T. Tsuda[1], E. Tan[1], X. Dai[1], K. Shiraishi[1], H. Mori[1], M. Murakami[1], S. Higashiyama[2], K. Sayama[1]

[1]Department of Dermatology, Ehime University Graduate School of Medicine, Toon, Japan, [2]Division of Cell Growth and Tumor Regulation, Proteo-Science Center, Ehime University, Toon, Japan

Living skin equivalents (LSEs) containing well-differentiated keratinocytes cultivated on fibroblast-populated dermal substitutes have been used to treat burn wounds, skin defects of epidermolysis bullosa. The dermal matrix and the fibroblasts in LSEs modulate epidermal growth and differentiation through dynamic interactions. Various biomaterials have been used as dermal matrix substitutes, but the search has continued for an ideal matrix that is readily available and has minimal toxicity. The non-reducing disaccharide trehalose is a singular molecule, which has been conserved throughout evolution in prokaryotes, plants, invertebrates, but is absent in vertebrates including mammals. In this study, we investigated the effect of trehalose mixed in the fibroblast-rich type 1 collagen gel and tested if it could affect the construction of LSEs. When trehalose was added in the fibroblast-populated collagen gel, the epidermal sheets showed the significantly rapid and extensive spread yet were morphologically and histologically normal. Histological and flow cytometric evaluation showed significantly more Ki67 positive and G2 phase fibroblasts in trehalose-containing LSEs. We also pre-treated 2D-cultured fibroblasts with treahalose, and embedded them in the collagen-gel without trehalose. Even in this condition, the epidermal sheets showed significantly rapid and extensive spread. To examine the dynamic nature of the response, human dermal fibroblasts were treated with trehalose for 24 hours. The trehalose treatment increased the ERK1/2 and Akt phosphorylation, and FGF2 mRNA levels assessed by real-time quantitative PCR significantly compared with control. Taken together, our data show that fibroblasts treated with trehalose accelerates the proliferation of the epidermal sheets and suggests targeting this pathway may be therapeutically useful.

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Acute IL-17A production by a 3D immunocompetent psoriatic skin substitute to study the interaction between epithelial and immune cells

G. Rioux[1, 2], S. Morin[1, 2], M. Simard[1, 2], S. Guérin[1, 3], R. Pouliot[1, 2]

[1]Centre de recherche en Organogénèse Expérimentale de l'Université Laval/LOEX, CUO-Recherche, Axe Médecine Régénératrice, Centre de Recherche du CHU de Québec- Université Laval, Québec, Quebec, Canada, [2]Faculté de Pharmacie, Université Laval, Quebec, Quebec, Canada, [3]Département d'Ophtalmologie, Faculté de Médecine, Université Laval, Québec, Quebec, Canada

Psoriasis is a chronic inflammatory dermatosis mediated by T cells through the IL-23/IL-17 axis. The hallmark cytokine of the Th17 cells, IL-17A, activates keratinocytes leading to the secretion of various chemokines that also account for leukocytes infiltration, thus producing an activation loop leading to the chronicization of the psoriatic lesions. The lack of suitable preclinical models reflecting the complex phenotype of this skin disease is a major obstacle to the further study of psoriasis pathogenesis. In this study, we aimed to optimize T cell culture methods and their incorporation into a 3D models in order to obtain long-term IL-17A production. To this end, both healthy (HS) and psoriatic (PS) skin substitutes have been produced according to the self-assembly method. T cells were isolated from whole blood by negative selection with the *EasySep Direct Human T cell* isolation kit and activated with phorbol 12-myristate 13-acetate (PMA, 25ng/ml) and ionomycin (1µg/ml). Activated T cells were then seeded into the 3D skin models. The location of $CD3^+$ T cells within the skin substitutes was demonstrated by immunofluorescence staining. PS displayed a more pronounced leucocyte infiltration than HS. Compared to the previous activation method, the activation with PMA and ionomycin allowed the production of IL-17A. Moreover, a higher production of IL-17A was obtained with the psoriatic skin model than with the healthy one ($p<0.001$). Overall, these results suggest that the 3D immunocompetent psoriatic skin model is a promising tool for the study of the psoriasis pathogenesis as it allows to further mimic the physiopathological context found in native psoriatic skin.

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The role of YAP/TAZ in the pathogenesis of skin fibrosis

L. Lu[1], N. Gao[1], X. Ma[1], Z. Liu[1], X. Wang[2], S. Yang[3], G. Han[1]

[1]Department of Dermatology, Peking University International Hospital, Beijing, China, [2]Department of Pathology, University of Colorado Denver Anschutz Medical Campus, Aurora, Colorado, United States, [3]Department of Dermatology, Peking University First Hospital, Beijing, China

YAP/TAZ are key molecules in the Hippo signaling pathway that regulate organ size and promote the development and progression of proliferative diseases such as tumors through the induction of cell growth and inhibition of apoptosis. Skin fibrosis is characterized by persistent proliferation of fibroblasts and excessive production of extracellular matrix. Given the key role of the Hippo signaling pathway in other cells, we speculate that YAP/TAZ may also be involved in the pathogenesis of fibrosis. Using immunofluorescence/immunohistochemistry, we found increased nuclear staining of YAP/TAZ in tissue samples of keloid and early stage scleroderma, both of which are typical fibrotic skin diseases. Compared to normal human fibroblasts, primary cultured fibroblasts derived from keloid had increased levels of YAP/TAZ proteins and faster migration in cell scratch migration assays. Inhibition of YAP/TAZ by both siRNA interference assay and treatment with verteporfin, an inhibitor of YAP/TAZ, significantly inhibited cell proliferation, reduced cell migration and induced cell apoptosis. Our study provides evidence that YAP/TAZ may play crucial roles in the pathogenesis of fibrosis and are potentially new targets for anti-fibrotic drugs.

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Effects of cholera toxin and isoproterenol as cAMP stimulators for a psoriatic reconstructed skin model

S. Morin[1, 2], M. Simard[1, 2], G. Rioux[1, 2], R. Pouliot[1, 2]

[1]Axe Médecine Regénératrice, Centre de recherche en Organogénèse Expérimentale de l'Université Laval/LOEX, Quebec, Quebec, Canada, [2]Pharmacie, Université Laval, Québec, Quebec, Canada

Psoriasis is a pathology mainly characterized by the hyperproliferation of epidermal cells, which leads to abnormal cell differentiation. A change in cyclic adenosine monophosphate (cAMP) levels could be causing this altered proliferation, as cAMP plays a major role in epidermis cellular growth. However, whether levels of cAMP in psoriatic skin are enhanced or decreased is a matter of much controversy. The aim of this study was therefore to quantify the levels of cAMP in psoriatic skin substitutes produced with two different cAMP enhancers, cholera toxin and isoproterenol, and to evaluate what impacts these levels have on the main characteristics of the pathology. Psoriatic skin substitutes were produced according to the self-assembly method, using culture media supplemented with cholera toxin (10^{-10} M) or isoproterenol (10^{-6} M). Histological aspects of skin substitutes showed that the living epidermis of those produced with isoproterenol was thinner than those with cholera toxin, suggesting that the hyperproliferation of keratinocytes was decreased. The expression of involucrine, filaggrin and keratin 10 of substitutes supplemented with cholera toxin was altered as in native psoriatic skin, whereas with isoproterenol, the expression of those proteins was more representative of healthy skin. In addition, in psoriatic skin substitutes, the substitution of cholera toxin for isoproterenol leads to an important decrease in epidermis cAMP concentrations, as found in healthy skin substitutes. In fact, the presence of isoproterenol in the culture media seemed to diminish the psoriatic phenotype, mainly by decreasing the cAMP levels. Therefore, our study revealed that cholera toxin is the only cAMP stimulator that can correctly reproduce psoriatic features, leading to a rise in cAMP amount.

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A20 and its repressor DREAM expression govern susceptibility to fibrosis in systemic sclerosis

W. Wang[1], B. Claudia Schock[4], H. Abdala-Valencia[1], B. Jeong[1], S. Bale[1], R. B. Christmann[2], R. Marangoni[1], S. Berdnikovs Ph.D.[1], E. Herzog[3], J. Varga[1], S. Bhattacharyya[5]

[1]Medicine, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [2]Biogen, London, United Kingdom, [3]Yale University, New Haven, Connecticut, United States, [4]Queen's University, Belfast, United Kingdom, [5]Feinberg School of Medicine, Northwestern University, Chicago, Illinois, United States

Recent GWAS have uncovered consistent genetic linkage of SSc fibrotic phenotypes with TNFAIP3, encoding the ubiquitin-editing enzyme A20. A20 has been previously implicated in negative regulation of innate immunity, and hypomorphic A20 variants are associated with autoimmunity in SLE, RA,and others.The transcription factor DREAM binds to the A20 promoter to repress expression. Unbiased transcriptome analysis of skin and lung biopsies from SSc patients showed significantly decreased A20 levels and robust anti-correlation with fibrotic TGF-beta signaling. In contrast, the negative regulator of A20 DREAM was significantly elevated in SSc biopsies and anti-correlated with A20. In human skin and lung fibroblasts and ADSC, and mouse preadipocytes, A20 potently inhibited both profibrotic gene expression and myofibroblast transition via blocking multiple SSc-relevant pathways including canonical and non-canonical TGF-beta. We generated A20 haploinsufficient mice that, comparable to humans harboring A20 risk alleles, showed exaggerated organ fibrosis, and altered transcriptome profiles including enhanced fibrotic and inflammatory gene signatures. Furthermore,fibroblasts-specific deletion of A20 showed exaggerated fibrosis in mice. Conversely, DREAM-null mice were protected from organ fibrosis. Adiponectin elicited a sustained increase in A20 in fibroblasts whereas the anti-diabetic drug repaglinide, which blocks DREAM, enhanced A20 expression. Together, these studies uncover a novel role for A20 and DREAM as the primary checkpoints in modulating fibroblast activity in SSc. Implicating DREAM as a novel pathogenic factor in SSc could stimulate the discovery of drugs to block DREAM and enhance A20 function for the treatment of multiple organ fibrosis.

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Overcoming wound healing complications of radiotherapy in breast dermal fibroblasts through the influence of pre-adipocytes

L. V. Trevor[1, 2], K. Riches-Suman[2], A. Mahajan[1], J. M. Thornton[2]

[1]Plastic Surgery and Burns Research Unit, University of Bradford, Bradford, England, United Kingdom, [2]Centre for Skin Sciences, University of Bradford, Bradford, England, United Kingdom

Impaired wound healing in irradiated tissue is a significant clinical problem which may be improved by stromal vascular fraction (SVF) containing pre-adipocytes. We studied the characteristics of dermal fibroblasts (DF)s derived from human breast skin exposed to radiotherapy to investigate challenges presented by irradiation prior to reconstruction, and whether the pre-adipocyte secretome has potential therapeutic benefit. Tissue collected from breast reduction (Ctrl), or reconstruction following 40 Gy hypofractionated radiotherapy (IR) was processed for histology, and isolation of primary DF and SVF cultures. IR skin had a flattened epidermal-dermal junction and extensive disorganisation of the reticular dermis. IR DFs took substantially longer to explant and culture. IR DFs had an increased perimeter ($p=0.06$), reduced circularity ($p<0.01$) but similar mean cell area to Ctrl DFs. IR DFs exhibited impaired proliferation (CyQuant) and migration in a scratch wound assay, but had a higher metabolic activity (AlamarBlue) than Ctrl. Immunocytochemistry demonstrated the presence pre-adipocyte markers CD10, CD105 and CD73, and absence of CD45 in the cultured SVF cells. The effect of conditioned medium (CM) collected from pre-adipocytes was compared with 1ng/ml fibroblast growth factor(FGF)-2 (n=2 Ctrl, n=4 IR donors). Pre-adipocyte CM and FGF-2 increased proliferation and metabolism of Ctrl DFs; but had no effect on IR DFs. In contrast, pre-adipocyte CM stimulated migration of IR DFs in a scratch wound assay. In summary, IR DFs have a stellate morphology, higher metabolism, reduced proliferation and slower migration during in vitro wound closure; characteristics which may contribute to poorer wound healing following radiotherapy. Human pre-adipocytes secrete soluble factors that stimulate the migration of IR DFs. Thus, the SVF may present an opportunity for future therapies to address the negative consequences of skin irradiation following breast reconstruction.

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TEAD1 and TEAD3 play redundant roles in the regulation of human epidermal proliferation

J. Li, G. L. Sen

Dermatology, University of California at San Diego, La Jolla, California, United States

Hippo signaling pathway is important for regulating cell growth, proliferation and organ development. It functions through the Yes-associated protein 1 (YAP1), which acts with the TEAD family transcription factors to regulate essential genes for proliferation and cell growth. However, it is not known whether TEAD (1-4) genes play redundant or non-redundant role in the human epidermal tissue development. We knocked down each individual TEAD and in combinations in human keratinocytes and found that only double knockdown of TEAD1 and TEAD3 resulted in a significant decrease of CTGF and AXL mRNAs as well as other critical self-renewal and proliferation genes (BIRC5, FGFBP1, CCNA2, MYC, CDK1, CYR61). We also observed a dramatic decrease of KI67 staining in regenerated human epidermis. To explore the mechanisms, we performed TEAD1 ChIP-seq and found that 67% of TEAD1 peaks overlapped with enhancers regions marked by H3K27ac and H3K4me1. The co-localization of TEAD1 peaks and enhancers was found at genes such as FGFBP1, CYR61, CTGF and AXL. In summary, we demonstrated that TEAD1 and TEAD3 function redundantly to maintain human epidermal growth through binding enhancers to regulate self-renewal related genes.

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Bioprinted skin integrates and forms epidermal rete ridges in full-thickness wounds

A. M. Jorgensen, A. Gorkun, M. Varkey, C. Clouse, S. Lee, J. J. Yoo, S. Soker, A. Atala

Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States

Current tissue engineered skin fails to meet the need for skin replacement in full-thickness wounds. Bioprinting technology allows for fabrication of full-thickness skin with multiple cell types organized into biomimetic layers in vitro. The purpose of this study is to determine if bioprinted skin will integrate, form an epidermal barrier, and vascularize when implanted into full-thickness wounds on mice. Cells were isolated from human skin, expanded in vitro, suspended in a fibrinogen bioink, bioprinted to form a biomimetic tri-layer skin construct, and implanted onto 2.5 x 2.5cm full-thickness excisional wounds on mice. A significant difference in total wound closure was found between bioprinted skin and non-treated wounds, as well as an accelerated time to wound closure (14.8 vs. 24.5 days, $p<0.001$). By day 90, H&E and Masson's trichrome staining revealed rete ridge formation in the bioprinted skin comparable to native human epidermis, compared with a flat epidermis in controls. Picrosirius red stained samples demonstrated a normal basket weave collagen orientation in bioprinted skin-treated wounds, and hypertrophic scar-like, parallel collagen alignment in controls (41.3% vs. 80.0% alignment, $p<0.001$). Immunohistochemical staining confirmed the presence of human cells in the regenerated skin, the formation of epidermal rete ridges, and incorporation of human endothelial cells in infiltrating host blood vessels. Measurement of blood vessels demonstrated an increase in small diameter blood vessels in bioprinted skin treated wounds compared to control (8.4 vs. 4.3 blood vessels, $p<0.01$). In conclusion, bioprinted skin accelerates full-thickness wound closure, with implanted cells laying down a healthy, basket-weave collagen matrix. The remodeled skin forms epidermal rete ridges and a vascular network composed of both graft and infiltrating host vessels. Ultimately, this technology could translate into a new treatment for full-thickness wounds in human patients.

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Topical type VII collagen increased elastic fiber formation, accelerated wound closure and reduced scarring of pigskin wounds

D. Woodley, J. Cogan, Y. Hou, M. Hao, A. Kwong, W. Li, M. Chen

Dermatology, Univ. of Southern California, Los Angeles, California, United States

Non-healing skin wounds are common and represent major medical, economic and social problems worldwide. Currently, there is a paucity of effective therapy for skin wounds. We have shown previously in several murine skin wound models that recombinant human type VII collagen (rhC7) dramatically accelerates wound closure and inhibits scarring. Murine skin wounds, however, heal much differently than human skin wounds. Therefore, in this study, we evaluated the effect of topical rhC7 on pigskin wounds. Pigskin is the most similar to human skin of all known species. We found that pigskin wounds treated topically with rhC7 exhibited marked acceleration of wound closure compared with wounds treated with vehicle alone (VE), platelet-derived growth factor (PDGF-BB, the only FDA-approved wound healing agent) or Dermacol (commercially available collagen) by increasing re-epithelialization. We assessed healed wounds for elements of scarring by immunohistochemistry and histology. Topical rhC7 decreased fibrogenic transforming growth factor β1 (TGF-β1) and increased anti-fibrogenic TGF-β3. In addition, topical rhC7 reduced collagen deposition, α-smooth muscle actin positive myofibroblasts, connective tissue growth factor, and periostin in the healed neodermis – all consistent with less scar formation. We also evaluated inflammation and angiogenesis. Compared with VE, rhC7-treated wounds had reduced expression of factor VIII (endothelial cells) and inflammation markers including Ly6G (granulocytes) and IL17 (Th17 cells). The lack of a dermal elastic fiber network is characteristic of human skin scars. Most interestingly, rhC7-treated wounds exhibited robust, increased elastic fiber formation compared with VE-treated wounds. Lastly, despite decreased collagen deposition, rhC7 increased the tensile strength of healed pigskin wounds. We conclude that topical rhC7 increases elastic fiber generation, accelerates wound closure, reduces inflammation and inhibits scarring. RhC7 may be a novel therapeutic agent for treating skin wounds.

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Recombinant *T. pallidum* protein TP0136 promotes the migration of fibroblasts by inducing MCP-1/CCR2 expression through signalling involving the TLR4, ERK, JNK, PI3K and NF-κB signalling pathways

W. Ke

STD, Dermatology Hospital, Southern Medical University, Guangzhou, Guangdong, China

Background: Wound healing involve the migration of fibroblasts. The microenvironment of fibroblast migration is not beneficial for the survival of most pathogens due to lack of oxygen. However, for *T. pallidum*, the hypoxic environment is relatively suitable for survival. Ulceration lesion chancre, the early stage of syphilis, can heal spontaneously within a few weeks without treatment. The specific mechanism underlying this process is still unknown. Objective: Tp0136 is one of a few proteins produced by *T. pallidum* that bind and adhere to fibronectin, and the transcription level of Tp0136 is significantly elevated during the healing period in a rabbit model of syphilis. Therefore, we aimed to analyse the role of Tp0136 in the migration of fibroblasts and the related mechanism. Methods: The migration ability of fibroblasts was stimulated with Tp0136 and detected by a wound-healing assay. RT-PCR and ELISA detected the expression of migration-associated cytokines MCP-1, IL-6 and MMP-9. TLR 4 expression was detected by RT-PCR. The protein levels of CCR2 and relevant signalling pathway molecules were measured by western blotting. Results: Tp0136 significantly promoted fibroblast migration. Subsequently, the levels of MCP-1 and its receptor CCR2 were increased in this process. The migration of fibroblasts was significantly inhibited by an anti-MCP-1 neutralizing antibody or CCR2 inhibitors. Furthermore, studies demonstrated that Tp0136 could activate the ERK/JNK/PI3K/NF-κB signalling pathways through TLR4 activity and that signalling pathways inhibitors could weaken MCP-1 secretion and fibroblast migration. Conclusion: Tp0136 promotes fibroblast migration by MCP-1/CCR2 expression through the TLR4, ERK, JNK, PI3K and NF-κB signalling pathways. Understanding the important role of Tp0136 in fibroblast migration and the relevant mechanisms could contribute to an improved understanding of the pathogenesis of *T. pallidum*, especially chancre self-healing in the early stages of syphilis infection.

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Acne-associated strains of *Cutibacterium acnes* induces significantly higher MMP-1 expression, a potent mediator of extracellular matrix degradation

A. Awad[1, 2], M. Qin[1], T. To[1], J. Kim[1]

[1]Dermatology, University of California, Los Angeles, Los Angeles, California, United States, [2]Burrell College of Osteopathic Medicine, Las Cruces, New Mexico, United States

Scarring is a common sequelae of inflammatory acne vulgaris caused by the gram-positive bacterium, *Cutibacterium acnes* (*C. acnes*). Aberrant extracellular matrix remodeling seen in scarring may be due to upregulated activity of matrix metalloproteinases (MMPs) in the inflammatory response, resulting in loss of collagen deposition, increased tissue destruction, and imperfect repair during the wound healing process. *C. acnes* was shown previously to induce MMP-1 (collagenase) and MMP-9 (gelatinase) expression. Past studies have also shown that different *C. acnes* phylotypes induce distinct immune responses. In this study, we evaluated whether stimulation with acne (C_A) versus healthy (C_H) skin-associated *C. acnes* strains induces differential MMP expression levels. Through *in vitro* stimulation of peripheral blood mononuclear cells (PBMCs) with 3 C_A strains and 3 C_H strains, we observed an increased expression of MMP-1 and MMP-9, with little to no expression of MMP-3, MMP-13, and MMP-14. Notably, C_A strains induced a 4-fold higher level of expression of MMP-1 than C_H strains. Studies of various inflammatory diseases have also shown that COX-2 regulates inflammation and MMP activity. We found that treatment of PBMC's with a COX-2 inhibitor, followed by stimulation of C_A and C_H strains, significantly decreased baseline MMP-1 expression, but had no effect on MMP-9 expression. This data suggests that while various MMPs are upregulated in the presence of C_A and C_H, C_A may be differentially inducing more activity of MMP-1, a COX-2 dependent potent mediator of extracellular matrix degradation. In turn, this may contribute to the overall inflammatory response seen in acne vulgaris, causing increased tissue destruction and further leading to scar formation. This knowledge regarding the molecular mechanism involved in acne associated scarring allows new preventive therapeutic strategies to be developed and implemented.

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Nipple fibroblasts express low levels of fibrotic inducer DPP4 and facilitate wound healing with minimal scarring

S. Vargas[2], A. Filipowicz[2], M. Keel[2], H. Zhou[1], D. Spandau[1], M. J. Turner[1], J. G. Foley[1, 2]

[1]Dermatology, Indiana University, Indianapolis, Indiana, United States, [2]Med Sci, Indiana University School of Medicine, Bloomington, Indiana, United States

Sites of specialized skin in humans, including the nipple-areola complex exhibit minimal scaring after wounding. The distinct epidermal and dermal features of these skin regions are induced and maintained by signaling from local fibroblasts. In contrast, trunk fibroblasts induce scarring after wounding and this is mediated by a subpopulation of that express high levels of dipeptidyl peptidase IV (DPP4/CD26). Profiling of transcripts of adult fibroblasts from a murine model of ectopic nipple development based on keratin 14 promoter driven parathyroid-related protein (KrP) transgene, identified increased levels of ovarian hormone receptors and matrix production, but decreased *DPP4* and *TGFB1* levels. Using FACS, fibroblasts from ventral skin of the KrP mouse and wild type littermates were evaluated for CD26 and PDGFRa. High levels of CD26 was expressed on ~70% ventral dermal fibroblasts, whereas ~90% of KrP were low or null for the marker. To investigate the fibrotic response of the nipple-like skin, female KrP and wild type littermates were subjected to ventral skin wounds. Closure rate of splinted and un-splinted wounds did not differ between transgenics and littermates. However, at 21 days after wounding, histomorphometric measures of epidermis and collagen bundles indicated scarring was minimal in KrP wounds. Immunohistochemical analysis failed to find significant differences in numbers M1 macrophages, neutrophils, and myofibroblasts at 1 to 7 days after wounding. Four days after wounding there were increased M2 macrophage counts in KrP mice, but no changes in *MCP-1* or *TGFa* transcript levels. At 9 and 11 days, higher levels of *Col1α1, Col3α* and *fibronectin* transcripts were present in KrP wounds. These findings suggest the fibroblasts that induce the specialized skin of the nipple lack the marker CD26 and do not produce fibrotic matrix associated with scaring.

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Multi-view light sheet florescence microscopy (LSFM) for imaging cellular self-assembly in spheroids of human hair follicle dermal papilla cells and keratinocytes

S. Li[1], F. W. Lischka[2], P. Klover[1], J. Wang[1], I. M. Herman[3], T. Darling[1]

[1]Dermatology, Uniformed Services University of the Health Sciences, Bethesda, Maryland, United States, [2]Biomedical Instrumentation Center, Uniformed Services University of the Health Sciences, Bethesda, Maryland, United States, [3]Developmental, Molecular and Chemical Biology, Tufts University School of Medicine, Boston, Massachusetts, United States

Heterotypic spheroids are used to study mesenchymal-epithelial interactions important for skin development and regeneration. In order to investigate the behavior and interaction of human hair follicle dermal papilla cells (DPC) and keratinocytes during in vitro three-dimensional (3D) co-culture, we labeled DPC and keratinocytes with two different fluorescent cell tracking dyes and prepared spheroids in DPC medium by hanging-drop technique. After incubation for 1 or 2 days, spheroids were live embedded in an agarose cylinder and image stacks were generated in multi-view mode from several angles using dual-sided LSFM. In presence of 100 µg/ml rat tail collagen I, cells aggregated into a single 300-500 µm spheroid, whereas multiple spheroids of various sizes formed in absence of collagen. Cells appeared randomly distributed after 1 day, but after 2 days the vast majority of DPC were located in the core with most keratinocytes distributed in the periphery, with or without collagen. LSFM can be used to study self-assembly of DPC and keratinocytes in spheroids in order to gain insights into factors regulating organogenesis during skin regeneration and hair follicle neogenesis. The sub-micrometer resolution of the LSFM microscope in x-y dimension in conjunction with multi-view imaging allows for detailed quantification of distribution of cell populations in 3D space.

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Metabolic products of *Cutibacterium acnes* protect against cutaneous biofilm formation by *Staphylococci*

K. Nakamura, A. ONeill, M. Williams, L. Cau, T. Nakatsuji, R. L. Gallo

Dermatology, University of California San Diego, San Diego, California, United States

Bacterial biofilms are a major factor in delayed wound healing. Many staphylococcal species can form biofilms that have the potential to harm the host. DNA sequencing and qPCR of 16S RNA from bacteria isolated from human hair follicles by laser capture microdissection has shown that *Staphylococci* are highly abundant in the normal human follicles and co-exist with *C. acnes*. However, despite the high density of *Staphylococci* in follicles, biofilms seldom occur on healthy skin. We hypothesized that interactions between species in the commensal skin microbiome may regulate biofilm formation. To test this, two different strains of *S. epidermidis* and *S. aureus* were exposed to the conditioned media from several species of *Cutibacteria* including 2 ATCC defined strains of *C. acnes*. Biofilm formation was observed by crystal violet staining of *S. epidermidis* and *S. aureus* grown on plastic, and all *Cutibacteria* species and strains tested inhibited biofilm formation by 80% ($P<0.002$) at concentrations that were not bacteriostatic. To identify the mechanism of action, conditioned media from *C. acnes* was subjected to biochemical analysis. The active product was stable at 100°C, resistant to proteinase K and lysozyme, and volatile after lyophilization. These properties suggested that the active agents could be short chain fatty acids (SCFAs). *C. acnes* produces several SCFAs including acetate, propionate, isobutyrate, and isovalerate. Exposure of *S. epidermis* and *S. aureus* to pure SCFAs at the concentrations made by *C. acnes* confirmed that acetate, propionate, isobutyrate, and isovalerate inhibited *Staphylococci* biofilm formation. Similar to the *C. acnes* conditioned media, these concentrations of SCFAs were not antibacterial. Our data suggest that a beneficial consequence of diversity in the skin microbiome is that SCFAs from *Cutibacteria* inhibit biofilm formation by *Staphylococci*. Recognition of this function can be useful to wound care where low microbial diversity may permit biofilm development and delayed wound healing.

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Circadian factors BMAL1 and CLOCK control transcriptional innate antiviral immunity programs in response to skin wounding

S. Kirchner[1, 4], V. Lei[1], M. Coates[1], C. Handfield[1], D. Corcoran[3], X. Ling[1, 5], J. Shannon[1, 2], P. Rosa Coutinho Goulart Borges Mariottoni[1], D. Hughes[6], D. Waters[6], K. Dzirasa[6, 7, 8], A. S. MacLeod[1, 2, 4]

[1]Dermatology, Duke University, Durham, North Carolina, United States, [2]Immunology, Duke University, Durham, North Carolina, United States, [3]Center for Genomic and Computational Biology, Duke University, Durham, North Carolina, United States, [4]Molecular Genetics and Microbiology, Duke University, Durham, North Carolina, United States, [5]Suzhou Ninth Peoples Hospital, Suzhou, China, [6]Neurobiology, Duke University, Durham, North Carolina, United States, [7]Psychiatry and Behavioral Sciences, Duke University, Durham, North Carolina, United States, [8]Neurosurgery, Duke University, Durham, North Carolina, United States

Antiviral proteins (AVPs) including the oligoadenylate-synthase (OAS) and Interferon induced transmembrane protein (IFITM) families have protective roles within the innate immune system. However, little is known about their regulation in skin. BMAL1 and CLOCK, regulators of the circadian rhythm, have known importance in a number of immune functions. We hypothesized that the circadian clock may regulate cutaneous AVP expression. We demonstrate that murine skin displays homeostatic oscillations of AVP expression through the day, and that AVPs exhibit modest rhythmic expression in primary human keratinocytes post-circadian synchronization using serum starvation or dexamethasone shock with a periodicity of 20 to 24 hours. siRNA knockdown of CLOCK also decreased AVP expression *in vitro*. Further *in silico* analysis revealed that murine and non-human primate skin display circadian expression of AVPs. Notably, we have found that skin wounding at different times of day induces variable AVP expression. Wound-induced transcription of AVPs also was attenuated in ClockΔ19 circadian mutant mice. These findings support a paradigm where circadian rhythm may control time-of-day anticipatory AVP transcriptional production in order to prioritize cutaneous defenses when the host is more likely to be wounded and encounter pathogens. Further work is needed to establish the mechanistic links of wounding, circadian rhythm, and the AVP response to ultimately permit a better understanding of our skin's homeostatic and wound-induced viral defense mechanisms.

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Targeting GM3 synthesis improves wound healing in human diabetic skin equivalents

T. R. Holmes, K. Lewandowski, K. R. Kwan, M. S. Bonkowski, A. S. Paller

Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States

Chronic foot ulcers are a major cause of morbidity and mortality in 25% of individuals with type 2 diabetes (T2D). Our laboratory has previously shown increased expression of sphingolipid GM3 and GM3 synthase (GM3S) in human diabetic foot skin. Preventing increases in GM3 in a diet-induced mouse diabetic model or in high glucose-treated 2D keratinocyte cultures improved wound healing and scratch wound closure, respectively. To further test the role of increases in GM3/GM3S on wound healing in human T2D, a diabetic 3D human skin equivalent (HSE) model using diabetic foot ulcer fibroblasts (DFUFs) and normal keratinocytes was generated. When a wound was created in the 3D HSE model, closure was reduced by 70% compared with wounded 3D skin with normal foot fibroblasts (NFFs). We hypothesized that the DFUFs express high levels of GM3 and that inhibiting GM3 production would reverse the human 3D diabetic wound healing impairment. Based on lipidomic analyses, we have found that treatment with a glucosylceramide synthase inhibitor (GCSI) reduces all species of GM3 in human keratinocytes by 77-89% because of substrate reduction. Indeed, cultured DFUFs had a 2-fold increase in GM3S expression compared to NFFs and treatment of DFUFs with a GCSI (500 nM) accelerated 2D scratch wound closure within 24 h compared to vehicle-treated DFUFs ($p<0.02$). In wounded 3D diabetic HSEs, treatment with GCSI improved wound closure, with 67% closed (based on epidermal gap) by 3 days with topical GCSI treatment versus 17% with vehicle treatment ($p<0.03$). BrdU incorporation in wounded DFUF HSEs revealed improved proliferative capacity in rafts treated with GCSI (16.1 $BrdU^+$ cells/linear mm) compared to vehicle-treated rafts (12.4 $BrdU^+$ cells/linear mm) ($p<0.03$). These studies further attest to the importance of GM3 in human diabetic wound healing impairment and suggest a central role for fibroblast responses to GM3 depletion in reversing the impaired keratinocyte migration of T2D.

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Dual LSD1/HDAC inhibition accelerates skin wound healing

M. Kida[1], M. Wu[1], P. Cole[2], V. Falanga[1], A. Sharov[1], R. Alani[1]

[1]Dermatology, Boston University School of Medicine, Boston, Massachusetts, United States, [2]Division of Genetics, Departments of Medicine and Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts, United States

The epigenetic machinery regulates epidermal homeostasis and keratinocyte differentiation is normal skin, while epigenetic control of wound healing remains relatively underexplored. The HDAC1/2 and LSD1 enzymatic activities within the CoREST complex are commonly associated with silencing of gene expression. Corin is a synthetic hybrid agent derived from the HDAC inhibitor (MS-275) and LSD1 inhibitor. In this study, using in vivo mouse tail wound healing model, we found that HDAC1 and LSD1 expression levels in epidermal keratinocytes show dynamic changes after injury compared to uninjured skin. Corin treatment resulted in accelerated wound closure in a dose-dependent manner. Histological analysis revealed that 100nM corin significantly promoted regeneration of the epithelium 10 days after wounding. In epidermal keratinocytes treated with corin, the expression of H3K9Ac was increased compared to controls, while the expression of H3K4Me2 was not changed. In addition, corin treatment significantly stimulated migration of human keratinocytes in vitro, while their proliferation was reduced. qRT-PCR gene expression analysis of corin-treated keratinocytes showed significant upregulation of the genes associated with cell migration, such as *CD24*, *EPHB2*, *ITGAX*, *PTGS*, *SCT1*, *SERPINB2*, *SERPINE1*, and *SLPI*. In summary, our study demonstrates that dual HDAC1 and LSD1 inhibition by corin promotes acetylation of H3K9 and skin wound healing via stimulation of migratory activities of epidermal keratinocytes.

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Involvement of papillary and reticular fibroblasts in dermal angiogenesis

E. Aymard[1], A. Mauroux[2], C. Monnot[2], S. Germain[2], F. Ruggiero[3], L. Muller[2], B. Closs[1]

[1]R&D Department, SILAB, Brive la Gaillarde, France, [2]College de France, CNRS, INSERM, PSL Research University, Center for Interdisciplinary Research in Biology (CIRB), Paris, France, [3]UMR 52422 CNRS – ENS – UCBL, IGFL, Institut de Génomique Fonctionnelle de Lyon, Lyon, France

Wound healing is a multi-step complex mechanism during which dermal angiogenesis is a critical factor. Human dermis has a complex architecture, divided into papillary and reticular dermis which are characterized by distinct extracellular matrix (ECM) and vasculature. Nowadays, the contribution of each subtype of fibroblasts in the generation of specific ECM and vasculature is poorly documented. The aim was to determine if papillary and reticular fibroblasts generate specific 3D microenvironments and then evaluate their impact on angiogenesis. RNAseq transcriptomic analysis of papillary and reticular cell sheets revealed that genes related to angiogenesis and ECM were the most regulated in both subtypes. Interestingly, an angiogenic gene signature was identified with an enrichment in paracrine pro-angiogenic genes in the papillary fibroblasts. In agreement, conditioned medium from papillary fibroblasts culture displayed a higher angiogenic potential compared to reticular fibroblasts. Papillary and reticular fibroblasts also showed specific matrisomes *(i.e* ensemble of genes encoding ECM and ECM-associated proteins) linked to biological functions suggesting the deposition of distinct ECM. Differences in composition / ultrastructure of ECM were also checked at the protein level. Finally, when cultivated with endothelial cells, papillary microenvironment induced a denser network of thinner capillaries compared to the reticular one, thus reproducing some features of dermis vasculature. These results indicate that papillary and reticular fibroblasts generate specific microenvironments that differentially impact angiogenesis. Dermal fibroblast heterogeneity should be taken into consideration for generation of vascularized 3D skin models, as well as for wound healing and regenerative medicine applications.

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Filling Injection of Platelet-rich Plasma Gel as A New Method to Treat En Coup de Sabre Scleroderma

T. Huang, G. Hu, B. Yang

Guangdong Provincial Dermatology Hospital, Guangzhou, Guangdong, China

Platelet-rich Plasma (PRP) plays an important role on the tissue regeneration process. A new preparatioin method of PRP provides an filling injection product which was named Platelet-rich Plasma Gel (PRPG) and could provied synergic effects on skin elasticity, dermal remodeling and re-vascularization. The aim of this pilot study was to assess the clinical benefit of PRPG filling injection on the treatment of en coup de sabre scleroderma. Five patients who were diagnosed under the confirmation of histopathology, and assessed by ultrasonography. Treatment was administered with PRP at a concentration 3.5 times above baseline. And under sterile condition, pre-seperated PRP was aspirated into several needle tubes, gradually heated in a dry bath machine, and prepare PRPG under the terminal temperature of 70 centigrade for 14 minutes. Under swelling anesthesia, subcutaneous tissue gaps were formed. Then PRPG was safely filled in these gaps without the risk of injection into the blood vessels. Therefore, a kind of structure mimic subcutaneous soft tissue was performed to slowly release cell and growth factors which enhance the regeneration effects on the remodeling of dermal tissue and reconstruction of vascular network. The subjective symptoms of pain and tightness were significantly relieved after at least one time of injecton. And after completion of 3 cycles of treatments at monthly intervals, objective lesions showed the improvement of skin elatisity, dermal thickness, subcutaneous tissue thickness and subcutaneous vascular network density. Even hair growth can be observed in those atrophy scalp filed. The main limitations of this study were the low number of cases, lack of a comparison group and the difficulty of identifying the exact role of each factor which would be studied in next clinical trial. However, PRPG is a good alternative of tissue filler substitution instead of hyaluronic acid and collagen, and represents a new and potentially regenerative treatment option for en coup de sabre scleroderma with minimal associated side effects.

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Aquaphilus dolomiae S0, a thermal spring water active compound, showing broad repairing properties of on *in vitro* models of injured skin

M. Noizet[1], P. Bianchi[1], M. Galliano[1], A. Caruana[1], J. Brandner[3], S. Bessou-Touya[1], H. Duplan[2]
[1]R&D, Pierre Fabre Dermocosmetique, Toulouse, France, [2]R&D, Pierre Fabre Dermocosmetique, Toulouse, France, [3]University Hospital Hamburg-Eppendorf, Hamburg, Germany

The clinical efficacy of Avène thermal spring water in atopic dermatitis, psoriasis or in reducing erythema post laser resurfacing have been shown by several studies. In addition to these soothing and immunomodulatory properties, *in-vitro* experiments have also demonstrated effects of Avène thermal spring water on stimulation of keratinocyte differentiation and improvement of membrane fluidity, suggesting a potential effect on skin barrier and repair. An investigation of the deep aquifer of the Avène thermal spring water pointed out a new microorganism as a potential source of these unique properties. Based on its distinctive phenotypic and genotypic characteristics, this newly identified strain was assigned to a new genus, as a representative of a novel species called *Aquaphilus dolomiae*. It is a chemoorganotrophic non-spore-forming bacterium of the b-Proteobacteria class. In the present study, the activity of S0, an original biological extract of *A. dolomiae*, was evaluated on *in-vitro* models of injured skin. The compound showed positive properties on primary fibroblast proliferation and keratinocyte migration. When formulated, it favored skin re-epithelialization on a 3D model of wounded skin explants. Moreover, we showed that S0 could prevent wound infection by up-regulating numerous antimicrobial peptide genes and inducing hBD2 peptide release. All together, these results show broad repairing properties of the *A. dolomiae* extract S0, helping skin repair and preventing complicated wounds.

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Ultra-low profile, soft pressure sensors with wireless communication for wound healing applications

Y. Park[4, 1], K. Kwon[1], J. Kwak[1], S. Kwak[1], D. Yang[1], D. Pontes[2, 1], M. Zhang[2, 1], S. Xu[4, 2, 1], J. Rogers[4, 1, 3]
[1]Northwestern Univ CBIE, Chicago, Illinois, United States, [2]Dermatology, Northwestern Univ School of Med, Chicago, Illinois, United States, [3]Materials Science and Engineering, Northwestern Univ, Chicago, Illinois, United States, [4]Northwestern Univ Simpson Querrey Institute, Chicago, Illinois, United States

Compression garments (CGs) such as bandages and stockings are critical in the management of a wide range of lower extremity conditions, particularly venous leg ulcers (VLUs). In order for CGs to deliver therapeutic benefit, the interface pressure between the fabric and the skin much typically reach at least 30 mmHg. However, this threshold value is highly sensitive to the type of CG material used, correct placement by a trained healthcare provider, body position, and leg volume changes. Currently, PicoPress® (Microlabitalia, Padua, Italy) is the gold-standard tool to measure interface pressure. Unfortunately, the system is expensive, and requires an air-bladder wired to a large base unit, limiting the system to only point measurements. Using advanced materials science techniques, we report the development of a soft, wearable, flexible and wireless pressure sensor that consists of a gold thin wire (50 nm) integrated on a 3D polyimide structure and elastomer (Ecoflex-30, 2.4 mm in diameter, 1 mm in thickness). Once packaged, this low cost sensor is 15 mm x 45 mm can be comfortably placed between CGs and the skin without the risk of skin injury. Data is transmitted wirelessly to any standard smartphone. Testing in healthy normal subjects, the 3D sensor measured an interface pressure within 10% agreement with the PicoPress® across the full range of pressures (0 to 120 mmHg) by detecting the deformation of the elastomer (R^2>0.98). A wearable interface pressure sensor will enable more effective compression therapy with CGs at the point of application and in the home setting. Future directions include evaluating the performance and tolerability of the device on patients with an active history of VLUs.

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Src-family kinases enhance corneal wound healing and compensate for neurotrophic keratopathy

V. Lee[2], R. T. Kweon[1], L. T. Wushanely[1], W. T. Foos[1], C. T. Lin[1], J. T. Seykora[1]
[1]Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]University of Pennsylvania, Philadelphia, Pennsylvania, United States

Corneal wounds are a leading cause of blindness and therapeutic approaches that target early epithelial wound healing can minimize the risk of blindness. Preliminary studies of corneal wounds showed increased SFK activity at the wound edge. Therefore, we hypothesized that increased SFK activity may promote corneal epithelial wound healing in vitro and in vivo. Our data show that in vitro wound healing in primary human corneal epithelial cells (HCEC) is enhanced when SFK levels are increased by sodium orthovanadate ($NaVO_4$). Scratch assays of HCECs were performed with increasing doses of $NaVO_4$ or controls and were imaged every hour for 24 hours. In parallel, cells lysates were collected after wounding and subjected to western blot analysis for activated SFKs. In vivo wound experiments were performed on the following genetic models: 1) wildtype (WT); 2) $Fyn^{-/-}$, 3) and K14-FynY528 transgenic mice. Transection of the ciliary branch of the trigeminal nerve was performed unilaterally in one set of mice. For corneal epithelial wounding, a diamond burr was used to debride both corneas. Mice were serially imaged after wounding. $NaVO_4$ activated SFKs and Akt and enhanced wound healing. In addition, wound healing was faster in FynY528 mice compared to WT, while $Fyn^{-/-}$ mice exhibit slower wound healing compared to WT. Wound healing rates were slower in eyes with trigeminal nerve resection compared to controls. Our data demonstrate that SFK and Akt activation enhances wound healing in HCEC. Loss of trigeminal nerve innervation slows wound healing, suggesting a critical role for nerve function in epithelial wound healing. Corneal epithelial wound healing may be augmented despite loss of corneal innervation with increasing SFK activity pharmacologically or genetically. These data suggest that delayed corneal epithelial wound healing from loss of corneal innervation can be compensated by increasing SFK activity. Further studies to delineate the downstream effectors of SFK in regards to wound healing should be conducted.

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Single-cell approaches to uncover adipocyte precursor heterogeneity and differentiation mechanisms in the skin

G. Rivera[1], K. Kamimoto[1], E. Butka[1, 2], W. Kong[2, 1], S. Morris[1, 2]
[1]Developmental Biology, Washington University in St. Louis, St. Louis, Missouri, United States, [2]Genetics, Washington University in St. Louis, St. Louis, Missouri, United States

Dermal white adipose tissue (dWAT) is key for multiple processes in the skin, ranging from immune response to hair growth. The generation of mature adipocytes in the skin relies on the proliferation and differentiation of adipocyte precursors (APCs). APCs can be identified by the expression of surface markers such as CD29, CD34, Sca1, and CD24, however the true extent of APC heterogeneity remains largely unknown. Using single-cell RNA sequencing (scRNA-seq) we reveal an unprecedented degree of heterogeneity in skin APCs. Using bioinformatics tools we predict the segregation of APCs in at least two broad populations of APCs with different differentiation capabilities; a CD26+/CD9- population which displays reduced in vitro adipogenic differentiation and a CD9+ population with increased propensity to differentiate into mature adipocytes. To test the relationship between these two APC populations we have developed a cell-tracing assay that is compatible with scRNA-seq approaches. Using these powerful tools, we will be able to establish a skin APC hierarchy and unveil the mechanisms that allow the different APC populations to transit throughout the different stages of differentiation toward becoming mature adipocytes. These findings will have a significant impact on our understanding of adipocyte development and maintenance in the skin.

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ARHGAP29 regulates keratinocyte migration *in vitro* but not *in vivo*
T. Reeb[1], A. Stein[1], L. Rhea[1], D. Wessels[2], D. Soll[2], M. Dunnwald[1]
[1]Anatomy and Cell Biology, The University of Iowa, Iowa City, Iowa, United States, [2]Biology, The University of Iowa, Iowa City, Iowa, United States
ARHGAP29 is a Rho GTPase Activating Protein with a high affinity for the small GTPase RhoA. In endothelial cells, ARHGAP29 regulates tubulogenesis and endothelial barrier function. However, very little is know about the role of ARHGAP29 in keratinocytes. We previously demonstrated that ARHGAP29 was reduced in *Irf6*-deficient skin and keratinocytes. Beause IRF6 is required for keratinocyte migration, we hypothesize that ARHGAP29 also regulates keratinocyte migration. To test this hypothesis, we used CRISPR-Cas9 technology to generate keratinocyte cell lines possessing two mutant alleles for *ARHGAP29*. *ARHGAP29* mutant keratinocytes had more rounded cell morphology, irregular cellular edges and a disorganized actin cytoskeleton. We also observed an increased incidence of cellular blebbing, a phenomenon previously associated with increased RhoA activity. Using these cells, we performed *in vitro* scratch assays and observed that *ARHGAP29* mutant keratinocytes were significantly delayed in their ability to close the scratch. Live imaging analysis of individual cells at the scratch edge showed that *ARHGAP29* mutant keratinocytes migrated with significantly reduced migration speed, directionality, and persistence. To test a similar role for ARHGAP29 *in vivo*, we generated a keratinocyte-specific knockout of *Arhgap29*. These animals were viable and did not exhibit macroscopic cutaneous defects. Following excisional wounds, keratinocyte migration was not altered 7 days post-injury. Together, these data demonstrate that ARHGAP29 is required for proper keratinocyte morphology and migration *in vitro*, and additional wound healing time points will be needed to fully investigate the function of ARHGAP29 in *in vivo* keratinocyte migration.

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Variability in secretion of growth factors by platelets is intrinsic to alopecia patients undergoing platelet-rich plasma therapy
J. A. Shaik, N. Estharabadi, R. Farah, M. K. Hordinsky
Dermatology, University of Minnesota, Minneapolis, Minnesota, United States
Platelet α-granules release growth factors (GFs) that promote healing and tissue regeneration by stimulating cell proliferation and differentiation. Platelet-rich plasma (PRP) prepared from autologous blood has shown to be beneficial in treating alopecia, however, clinical response can be inconsistent. Due to several fold enrichment of platelets secreting large quantities of GFs following PRP injections, variable GF secretion by platelets between patients may contribute to inconsistent clinical response. Herein, we analyzed variation in expression and secretion of GFs by platelets across patients undergoing PRP therapy for hair loss. Aliquots of de-identified PRP samples prepared by a single commercially available device from subjects undergoing therapy for alopecia during a series of sessions over a nine-month period were used. Platelet expression and secretion of transforming growth factor beta1 (TGFβ1), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF2) were analyzed. A Shapiro-Wilk test for normal distribution indicated that expression and secretion of all GFs by platelets were highly variable between patients. Since several FDA-cleared devices are currently being used to prepare PRP, we further analyzed whether platelet quality could vary based on the type of device used. We compared platelet GF secretion in PRP samples prepared from two distinct commercial devices and found no significant difference between them. We also found no significant differences in platelet GF secretion when comparing PRP samples from non-cicatricial and cicatricial alopecia patients. Finally, we provide evidence that platelets from each patient secrete inconsistent levels of GFs. Therefore, comparing clinical response to therapy with platelet GF secretion profile in individual patients could potentially result in not only predicting clinical outcome but also understanding the basic mechanism by which PRP promotes hair growth in alopecia.

Translational Studies

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No detectable systemic absorption of topically applied 0.02% and 0.04% chlormethine (CL) gel in patients with mycosis-fungoides cutaneous T-cell lymphoma (MF-CTCL)
C. Querfeld[1], J. Scarisbrick[2], P. Quaglino[3], E. Papadavid[4], P. Ortiz Romero[5]
[1]City of Hope Cancer Center, Duarte, California, United States, [2]University of Birmingham, Birmingham, United Kingdom, [3]University of Turin, Turin, Italy, [4]Attikon University Hospital, Athens, Greece, [5]Universidad Complutense, University Hospital 12 de Octubre, Madrid, Spain
CL (also known as mechlorethamine) 0.016% w/w gel (equivalent to 0.02% CL HCl) is an approved skin-directed therapy for treatment of MF-CTCL. The 201 study evaluated efficacy and safety of CL 0.02% gel vs equal strength CL ointment. The 202 study evaluated response and tolerability in patients (pts) from the 201 study who did not achieve a CR after 0.02% topical treatment and used 0.04% gel formulation. Bioanalysis of blood samples from pts treated with 0.02% or 0.04% CL gel was used to assess if systemic absorption occurred. For 201 gel-treated pts (n=16), blood was taken prior to, 1, 3 and 6 hrs after CL application, and after 1 month's treatment; quantitation limit was 41.5 ng/mL. For 202 gel-treated pts (n=15), blood was drawn at 0 and 1 hr of month 0 (or month 2/4 if pt was on study), and a third sample taken at the next visit. Bioanalysis was performed using a sensitive, validated LC-MS/MS method, with a quantitation limit of 5 ng/mL. Blood samples were taken from pts with a range of baseline demographics and clinical characteristics such as disease stage, % BSA affected, dose frequency, and localized treatment vs whole body application. Most pts received daily CL gel treatment with blood samples taken after the first dose and subsequent samples taken after 1–6 months of treatment. Neither first analysis of pts using 0.02% CL gel nor second analysis of pts using 0.04% CL gel have found detectable CL in any blood sample. The lack of detectable serum levels of CL would suggest that there is no systemic absorption of topical CL gel at these concentrations, and that systemic drug interactions are therefore unlikely. These results also correlated with the local activity of CL gel and the observation that there were no hematologic toxicities or other systemic adverse events in the studies.

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Calibration performance of deep neural networks for image classification declines on real-world, versus curated, test sets
A. T. Young[1, 2], J. Pfau[1, 2], M. J. Keiser[1], M. Wei[1, 2]
[1]University of California, San Francisco, San Francisco, California, United States, [2]Veterans Affairs Medical Center, San Francisco, California, United States
Deep convolutional neural networks (CNNs) perform on par with or better than dermatologists for melanoma image classification. Rather than yes-no predictions, CNNs output a probability distribution over a set of predefined disease classes, whereby prediction confidence is the max probability over the disease classes. A well-calibrated CNN, such that confidence forecasts observed prediction accuracy, is critical for clinical application. However, CNNs tend to be overconfident and therefore must first be calibrated, a topic not yet studied for image-based skin lesion diagnosis. There is a need for metrics to assess whether calibration performance varies depending on the characteristics of the test dataset. We trained 80 CNNs on images from our institution and several international public datasets, comprising 3,563 melanomas and 10,094 nevi. We calibrated the CNNs on validation sets non-overlapping with the training and test sets, using the temperature scaling method to adjust for overconfidence. We then measured calibration performance using area under the response rate-accuracy curve (AURRA) and expected calibration error. Our top dermatologist-level CNN model was reasonably well-calibrated on high-quality, curated dermoscopic and non-dermoscopic image test sets (AURRA 0.96 and 0.93, respectively). However, on a real-world teledermatology test set, calibration performance was considerably lower (AURRA 0.66). Additionally, we show that the validation set used for calibration has a significant impact on calibration performance. In conclusion, CNN calibration performance can drop significantly on real-world vs curated research datasets, revealing a need for reporting of standardized calibration metrics before clinical use.

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Micro-regional transcriptome reveals local dermal-epidermal intercorrelation in atopic dermatitis

T. Miyai[1, 2, 3], H. Kawasaki[1, 3, 4], M. Hosokawa[5], H. Matsunaga[5], R. Satoh[1, 2], A. Sekita[1, 2], H. Takeyama[5, 6], M. Amagai[3, 4], H. Koseki[1, 2, 7]

[1]Disease Biology, RIKEN MIH, Yokohama, Japan, [2]Dev. Genetics, RIKEN IMS, Yokohama, Japan, [3]Dermatology, Keio Univ. School of Medicine, Tokyo, Japan, [4]Skin Homeostasis, RIKEN IMS, Yokohama, Japan, [5]Nano & Life Innovation, Waseda Univ., Tokyo, Japan, [6]Life Sci. and Med. Biosci., Waseda Univ., Tokyo, Japan, [7]Cell. Mol. Med., Chiba Univ. School of Medicine, Chiba, Japan

Skin consists of a coordinated structure and multiple appendages, all of which could be affected under specific disease conditions such as atopic dermatitis (AD). Therefore, spatial information of gene expression should be essential for understanding the pathogenesis of skin inflammation. To obtain spatial profiles of the skin transcriptome, we have performed site-specific RNA-seq of 100 μm-diameter regions obtained by punch microdissection in wild-type (WT) and AD model mice. We collected 120 spots (epidermis, dermis, and hair follicle) in total from 14 proximal areas (WT: n=4, non-lesion (NL): n=6, and lesion (LS): n=4) on the frozen tissue sections. We found that some NL areas showed weak expression of inflammatory cytokine genes in the dermis, and the same group of genes exhibited differences in dermal expression even among histologically similar areas in LS. These results indicate that disease status, that is “hidden” in conventional skin gene expression analysis, could be revealed by our novel method. Because we speculated that inflammatory state could also be detectable by epidermal gene profiles, we performed correlation analysis in adjacent dermis-epidermis pairs. Interestingly, dozens of epidermal genes strongly correlated with dermal inflammatory cytokine levels; some (e.g., stefins) showed positive, and others (e.g., apolipoproteins) exhibited negative correlations. Further investigation using human tissues from healthy volunteers and AD patients confirmed the epidermal genes identified here were indicative of the inflammatory condition. Therefore, our study demonstrates the concept of dermal-epidermal intercorrelation in inflammation, and the micro-regional transcriptomics provides a better resolution for the skin pathology.

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A guideline improves interrater reliability of assessing leukocyte motion *in vivo* in human skin

Z. Zhao[1, 2], J. R. Patrinely[1, 2], I. Saknite[2], M. Byrne[3], M. Jagasia[3], E. Tkaczyk[1, 2]

[1]Department of Veterans Affairs, Nashville, Tennessee, United States, [2]Vanderbilt Dermatology Translational Research Clinic, Vanderbilt University Medical Center, Nashville, Tennessee, United States, [3]Vanderbilt-Ingram Cancer Center, Nashville, Tennessee, United States

Reflectance confocal microscopy (RCM) enables *in vivo*, real-time visualization of adherent and rolling leukocytes in human skin microvasculature. We found that adherent and rolling leukocytes are increased in cutaneous graft-versus-host disease (GVHD) [1]; however, the interrater reliability of these manual counts is unknown. We acquired 1164 RCM videos from 45 subjects in a cross-sectional study: 10 healthy, 15 GVHD patients, and 20 post-hematopoietic cell transplant controls. Based on standard definitions (adherent: paused >= 30 seconds; rolling: rotation on its axis along the vessel wall), two trained raters blinded to diagnosis independently counted adherent and rolling leukocytes in an initial set of 88 videos. They subsequently discussed discrepancies and created a guideline, with which they independently scored the remaining 1076 videos. The interrater intraclass correlation coefficient (ICC) of adherent leukocytes improved from poor (ICC: 0.056; 95% Confidence Interval: -0.117, 0.236; n=88) to good (0.830; 0.810, 0.847; n=1076), while that of rolling leukocytes improved from poor (0.385; 0.191, 0.550) to moderate (0.619; 0.580, 0.655). A key point of disagreement that the guideline addressed was to distinguish mimicking scenarios from adherent (e.g. basal keratinocytes) and rolling (e.g. leukocyte tumbling within a blood flow) leukocytes, which contributed to the increase in ICCs. This guideline will enable reproducible future studies of human cutaneous leukocyte trafficking. 1. Saknite I, Byrne MT, Jagasia M, Tkaczyk ER. Noninvasive Microscopic Imaging Reveals Increased Leukocyte Adhesion and Rolling in Skin of Acute Graft-Versus-Host Disease Patients Compared to Post-Transplant Controls. Blood. 2019;134(Supplement_1):4533. doi:10.1182/blood-2019-123795

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Spirulina stimulates inflammatory cytokine production in dermatomyositis *in vitro*

C. Bax[1, 2], Y. Li[1, 2], A. Ravishankar[1, 2], S. Maddukuri[1, 2], J. Patel[1, 2], D. Yan[1, 2], V. Werth[1, 2]

[1]Department of Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]CMCVAMC, Philadelphia, Pennsylvania, United States

Spirulina, a popular herbal supplement, stimulates the immune system, as determined by in vitro and in vivo studies. Our recent epidemiologic data suggest that Spirulina is associated with the onset or exacerbation of pre-existing autoimmune skin diseases, such as Dermatomyositis (DM). The purpose of this study was to 1) investigate the immunostimulatory effects of Spirulina and 2) characterize the role of the Stimulator of interferon genes (STING) and TLR4 pathways. PBMCs were isolated from DM and normal controls and stimulated with increasing concentrations of Spirulina supernatant (0, 0.3, and 1 mg/ml) or with Spirulina supernatant and STING antagonist, H-151, or TLR4 antagonist for 18 hours. Spirulina significantly increased PBMC production of IFNβ and TNFα in both controls and DM patients. With stimulation at 0, 0.3, and 1 mg/ml Spirulina concentrations, DM PBMCs secreted mean (standard error of mean) IFNβ of 14.18 pg/ml (4.18), 38.26 (4.69), and 97.42 (23.76), respectively ($p<0.05$)(n=7), and TNFα of 444.15 pg/ml (223.48),1699.74 (427.14), and 1539.18 (187.26) (n=6). STING antagonist H-151 suppressed secretion of IFNβ in Spirulina-treated PBMCs of all tested DM patients (n=5). "Good Responder," defined as patients with >20% decrease in IFNβ levels with STING antagonist, at Spirulina 0.3 mg/ml had mean (SEM) IFNβ levels of 33.78 pg/ml (1.51) pre and 13.19 (7.11) post-antagonist, and at 1 mg/ml had IFNβ levels of 59.47 pg/ml (7.04) pre and 33.81 (8.17) post-antagonist ($p<0.05$) (n=3). TLR4 inhibitor decreased IFNβ levels from mean (SEM) 38.29 pg/ml (5.40) pre-antagonism to 26.2 pg/ml (8.60) post in 0.3 mg/ml Spirulina-treated PBMCs (n=4). Spirulina increases production of key inflammatory cytokines TNFα and IFNβ and can activate both the TLR4 and STING pathway in vitro in DM patients, thus providing a potential mechanism by which Spirulina use may lead to disease onset or flare in susceptible patients.

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Machine learning (ML) predictive algorithm for childhood-onset chronic pruritic dermatoses (CPD) identifies acrylamide and glycidamide as itch modulators

S. Wongvibulsin[1], Y. Semenov[2], R. Khanna[1], S. Zeger[3], S. Kwatra[1]

[1]Dermatology, Johns Hopkins Medicine, Baltimore, Maryland, United States, [2]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States, [3]Biostatistics, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, United States

The factors responsible for childhood-onset CPD are not fully known. We hypothesize that environmental factors play a role and ML algorithms can increase understanding of childhood-onset CPD. We employ the classification random forest (RF) algorithm to systematically evaluate the association between CPD and environmental exposures in children (ages 16 and younger) in the 2005-2006 NHANES. We define incident CPD as presence of an itchy rash for at least 6 months with first rash occurrence within the past year. Variables in the analysis include demographics and environmental exposures, as described in NHANES demographics and laboratory data sections. Analyses were conducted using nhanesA and randomForestSRC R packages. We identified 69 individuals aged 16 years or younger with CPD developing within the past year. With random sampling of pediatric controls, the RF training data (80% of overall dataset) included 110 individuals (mean age 7.17 +/- 5.47) with 55 CPD cases and the test data (remaining 20% of dataset) included 28 individuals (mean age 5.50 +/- 5.10) with 14 CPD cases. Using RF for feature selection, we identified the top predictors as acrylamide, glycidamide, and age. Afterwards, we constructed the RF model using only these predictors. The performance of the final RF model is as follows: accuracy: 0.89, sensitivity: 0.86, specificity: 0.93, positive predictive value: 0.92, and negative predictive value: 0.86. Our results demonstrate the ability to predict CPD in the NHANES pediatric population and to identify top predictors of potential clinical importance. Extensions can further increase understanding of how interactions between environmental exposures, genetics, and lifestyle factors contribute to CPD. Overall, our approach has implications for improving prediction and prevention of CPD.

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Increased leukocyte pausing in the upper dermal microvasculature of cutaneous acute graft-versus-host disease patients by noninvasive reflectance confocal video microscopy

J. R. Patrinely[1, 2], I. Saknite[2], Z. Zhao[1, 2], M. Byrne[3], M. Jagasia[3], E. Tkaczyk[2]

[1]Vanderbilt University School of Medicine, Nashville, Tennessee, United States, [2]Department of Veterans Affairs and Vanderbilt Dermatology Translational Research Clinic, Nashville, Tennessee, United States, [3]Vanderbilt-Ingram Cancer Center, Nashville, Tennessee, United States

Acute graft versus host disease (aGVHD), one of the most common and potentially deadly complications of hematopoietic cell transplantation (HCT), is often difficult to diagnose with traditional clinical and histopathologic examination. Through five quantitative parameters extracted from noninvasive reflectance confocal microscopy videos, we compared the differences in upper dermal microvasculature of patients post-HCT with skin aGVHD (N=10) and with no organ aGVHD (post-HCT controls, N=10). We used a clinical reflectance confocal microscope, the Vivascope 1500 (Caliber I.D., Rochester, NY), to image volar forearm and upper chest blood vessels. Patients were similarly distributed in terms of gender, age, days post-HCT, and underlying disease. We found an increased number of “paused” or temporarily stopping leukocytes (median of 2 vs. 1) in aGVHD patients compared to post-HCT controls. Although the size of paused leukocytes was similar (median of 8 µm), the time of leukocytes being paused (median of 4 vs. 2 seconds) was higher in aGVHD compared to post-HCT controls. Interestingly, we found no difference in the blood vessel size (median of 9 µm) or density (median of 3 blood vessels) among both groups. In a limited number of patients, we found increased leukocyte pausing in similar-sized upper dermal blood vessels of aGVHD patients, compared to post-HCT controls.

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Seeing water in the skin: Hyperspectral imaging in the short-wave infrared

L. Shmuylovich[1], D. K. Mishra[2], H. Hurbon[2], A. Yu[2], T. Du[2], T. Wang[2], M. Berezin[2]

[1]Dermatology, Washington University School of Medicine, St Louis, Missouri, United States, [2]Radiology, Washington University School of Medicine, St. Louis, Missouri, United States

Noninvasive skin imaging typically relies on light absorption and scatter of chromophores like melanin, hemoglobin, and collagen in the visible and near-infrared. Low absorption makes water essentially invisible in this spectral range. Because fluid accumulation is a key pathophysiologic component of many skin diseases, an imaging modality where water has high absorption would be useful. The short-wave infrared (SWIR) range (900-1700 nm) has high lipid and water absorption and relatively low melanin and hemoglobin absorption. We built a SWIR hyperspectral imaging (SWIR-HSI) system, where 510 reflectance spectra from 881-1710 nm are acquired at each pixel, and custom software maps any combination of these 510 pixel-based spectra to grayscale or pseudo-RGB images. We hypothesized that SWIR-HSI could directly assess the acute-spongiosis-associated increase in epidermal fluid seen in allergic contact dermatitis (ACD), which is a common and burdensome rash. Visible photography and SWIR-HSI was obtained daily from one subject with poison ivy induced ACD over 19 days. Wavelengths with reflectance spectra that maximized a metric of image contrast between affected and unaffected skin were determined and used to generate pseudo-RGB images. SWIR-HSI revealed fluid accumulation in ACD lesional skin with a spectral signature matching the spectral signature of a control normal saline intradermal injection. Reflectance spectra from 1070 nm, 1340 nm, and 1605 nm provided the highest contrast pseudo-RGB images. Normalizing lesional reflectance intensity to unaffected skin defined a quantitative measure of tissue fluid accumulation. SWIR-HSI may have novel applications in diagnosis and monitoring of a variety of skin disorders where tissue fluid balance is altered. Furthermore, owing to the low absorption of melanin in the SWIR range, SWIR-HSI imaging should be relatively pigmentation-independent, thereby making it easier to visualize cutaneous eruptions in skin of color patients.

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Increased urine phthalate metabolite levels associated with eczema diagnosis

S. P. Patel[1], R. Khanna[1], K. Williams[1], J. Choi[1], Y. Semenov[2], S. Kwatra[1]

[1]Dermatology , Johns Hopkins School of Medicine , Baltimore , Maryland, United States, [2]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

The skin is exposed to unprecedented levels of pollution which can activate inflammatory pathways, affect the immune system, and induce oxidative stress. In particular, the use of phthalates as plasticizer in consumer products has created health concerns since the pollutant is believed to cause hormonal and immune dysregulation. We thus investigated the association between phthalate exposure and eczema. Using the National Health and Nutrition Examination Survey, we conducted a cross-sectional analysis to investigate the association between urinary phthalate metabolite levels and eczema. Patients with a previous eczema diagnosis and active disease within the past year were included in our study cohort. We calculated odds ratios (OR), 95% confidence intervals (95%CI), and p-values using logistic adjusting for gender, age, race, allergy history, poverty, body mass index, smoking status, and urine creatinine. Logistic regression revealed that eczema was associated with increased levels of the following urinary phthalate metabolites: Mono(carboxynonyl) phthalate (MCNP) (OR=1.328; 95%CI [1.002, 1.760], p=0.049), mono(carboxyoctyl) phthalate (mCOP) (OR=1.606, 95%CI [1.070, 2.410], p=0.025), and mono-isononyl phthalate (mNP) (OR=1.811, 95%CI [1.027, 3.104], p=0.041). Mean analysis showed that the geometric means of mCOP (8.53±2.54 vs 5.03±0.18, p=0.039) and mNP (1.53±0.23 vs 1.05±0.02, p=0.011) were higher for eczema patients as comparted to the control group with no significant difference in the geometric mean of MCNP (3.50±0.90 vs 2.57±0.08, p=0.235). Inflammatory skin diseases can be exacerbated by pollution exposure, and our data suggest that phthalates may be contributing to eczema symptoms. Public health efforts to reduce pollution and screening eczema patients for pollution exposure can be beneficial given the association of elevated urinary phthalate metabolites in eczema patients.

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Management of pyoderma gangrenosum in the perioperative period

A. Ortega-Loayza[1], C. Haag[1], L. Bacik[2], E. Latour[3], D. Morse[4], N. Fett[1]

[1]Dermatology, Oregon Health & Science University, Portland, Oregon, United States, [2]Dermatology, Penn State Hershey Medical Center, Hershey, Pennsylvania, United States, [3]Knight Cancer Institute, Portland, Oregon, United States, [4]Dermatology, UT Houston, Houston, Texas, United States

Pyoderma gangrenosum (PG) classically presents with the rapid evolution of painful skin ulcerations in an acute inflammatory stage, which is typically treated with immunosuppression. A subset of PG patients treated with immunosuppression can develop refractory, non-inflammatory ulcers, of which there are reports describing successful surgical outcomes (e.g. healing). However, the potential for pathergy can complicate disease management, as surgical procedures are generally contraindicated in patients with PG. There is no current consensus regarding optimal perioperative treatment for patients with PG undergoing surgery, PG-related or otherwise. Therefore, we sought to describe perioperative management practices and risk factors that may predict response to surgical intervention by conducting a comprehensive literature review in medical literature databases. We identified 126 published cases, from 81 publications, of surgical intervention in patients with active PG, which was defined as patients with current PG ulcers or currently taking immunosuppressive medication for the treatment of these ulcers. Among these, 16.7% experienced post-operative disease progression, defined as the development of new PG ulcers or clinical deterioration of existing PG ulcers. There were not any specific perioperative treatment regimens or clinical risk factors (the presence of active PG, location of PG, or duration of PG lesions before surgical procedure) which were identified as statistically significant predictors of disease recurrence. Although limited by case series design and publication bias, this study is a valuable means of hypothesis-generation for this rare condition. Multicenter prospective studies addressing the approach to non-healing ulcer and to the risk of surgery at non-ulcer sites of PG patients should be considered to answer this important clinical question.

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Redness has higher interrater reproducibility than body surface area in measuring extent of photographed cutaneous graft-versus-host disease

K. Parks[1], Z. Khera[1], X. Liu[2], T. Reasat[2], L. X. Baker[1], M. Jagasia[3], B. Dawant[2], E. Tkaczyk[1]

[1]Department of Veterans Affairs and Vanderbilt Dermatology Translational Research Clinic, Nashville, Tennessee, United States, [2]Department of Electrical Engineering and Computer Science, Vanderbilt University, Nashville, Tennessee, United States, [3]Vanderbilt-Ingram Cancer Center, Nashville, Tennessee, United States

Reliability for estimating disease body surface area (BSA) has interested investigators but is not known for photos. We evaluated accuracy and agreement of marking active disease from 3D photos of cutaneous chronic graft-versus-host disease (cGVHD). Prior to the study, three non-physician trainees (medical student KP, post-doc XL and PhD student TR) read background cGVHD literature. The Canfield Vectra H1 camera was used to capture a set of 15 3D photos from 8 patients, including 7 body sites. Then before and after 6 months of instruction by a board-certified dermatologist (ERT), each trainee used Vectra analysis software to demarcate the active areas of cGVHD in the set. For each photo, we calculated the error of each trainee in marked BSA (cm2) and total redness (CIELAB a* coordinate) relative to ERT. Interrater intraclass correlation coefficient (ICC) of trainees went from 0.70 (95% confidence interval 0.44-0.87) before to 0.87 (0.69-0.95) after training for BSA and from 0.92 (0.83-0.97) to 0.98 (0.95-0.99) for total redness. The overall error (average relative error across all 15 photo) in BSA improved from 11% pre- to 8% post-training for KP; 18% to 16% for XL; and 38% to 11% for TR. The overall error in total redness changed from 14% pre- to 8% post-training for KP; 17% to 15% for XL and 10% to 11% for TR. Trainees reduced their relative error in marked BSA from a mean of 22% to 12% with training, while improving their interrater reliability ICC metric from 0.70 to 0.87. Corresponding figures for redness went from 14% to 11% and 0.92 to 0.98. While training improved BSA assessments from “moderate” to “good,” BSA never reached the pre-training “excellent” reliability of the total redness metric.

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Clinical and immunological profiles of BP-specific IgE autoantibodies in bullous pemphigoid

Y. Shih, H. Yuan, J. Zheng, M. Pan

Dermatology, Ruijin Hospital, Shanghai Jiao Tong University, China, Shanghai, China

A subset of bullous pemphigoid (BP) patients show anti-BP180 and anti-BP230 IgE in the serum, yet the data about the diagnostic value of anti-BP230 IgE and the clinical presentation of BP remains inconclusive.

Following our clinical observation, a branch of BP patients presenting with topical-steroid therapy resistant, especially when anti-BP230 IgE autoantibodies were detected by concurrent ELISA testing. We hypothesized that anti-BP230 IgE plays an important role in BP. We investigated IgE autoantibodies in BP patients by ELISA, indirect immunofluorescence (IIF) and western blot. 54 BP patients were divided into two groups- topical-steroid-sensitive group and topical-steroid-resistant group. Clinical and immunological features were statistically analyzed among the two groups. In 156 BP patients, 96(62%) BP patients with elevated level of total IgE. IgE autoantibodies to BP180 and BP230 were found in 25 and 36 of 96 BP sera, respectively. The level of BP230 IgE was significantly higher than level of BP180 IgE ($P<0.05$, $N=96$). BPDAI scores($p<0.0001$), total IgE($p<0.05$), blood eosinophil counts($p<0.001$) and anti-BP230 level($p<0.001$) were significantly higher in topical-steroid-resistant group than in topical-steroid-sensitive group. There are 27 (64%) of 42 topical-steroid-resistant patients with blister/erythematous(BE) phenotype and mostly presented with anti-BP230 IgE. Anti-BP230 IgE levels and IgE deposition on BMZ increased after IgG depletion in topical-steroid-resistant group, indicating a positive deposition for IgE along the BMZ would be possible with high anti-BP230 IgE and concomitantly low IgG levels. In BP patients, the positivity rate of anti-BP230 IgE is higher than anti-BP180 IgE. It implied that IgE preferentially bound to BP230 rather than BP180. Anti-BP230 IgE as a marker implies resistant to the topical steroid therapy and BE phenotype in BP. Combine with clinical phenotypes and performing anti-BP230 IgE detection, they can be as implication whether systemic steroid therapy could be initiated.

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Development of an *ex vivo* rabbit eye model for bacterial conjunctivitis

A. Caserta[1], J. E. Neil[1], J. Lenn[2], M. Brown[2]

[1]Tissue Culture, MedPharm , Durham, North Carolina, United States, [2]MedPharm, Durham, North Carolina, United States

This ex vivo rabbit ocular model was designed to mimic the common eye disease bacterial conjunctivitis and evaluate the response of ocular therapeutics. Fresh rabbit eyes were extracted, either whole globe or dissected into cornea, surrounding sclera and lens, and mounted into agar/media substrate. The eyes were then cultured at 37°C for up to 3 days with LPS/PGN stimulation to determine the optimal method to mimic bacterial conjunctivitis inflammation and to evaluate the associated biomarkers by RT-qPCR. A 72 hour time-dependent profile evaluating different biomarkers by RT-qPCR resulted in the ability to upregulate bacterial conjunctivitis-associated biomarkers differentially in the cornea and sclera of both dissected and intact rabbit globe. Initial assessment in the dissected cornea and sclera, upregulation of gene expression of S100A9 (ca. 34 fold), IFNγ (ca. 5 fold), MMP9 (ca. 20 fold), IL1β (ca. 8.5 fold), IL6 (ca. 60 fold), IL8 (ca. 11 fold), and TNFα (ca. 80 fold) is apparent. In the whole globe looking at cornea and sclera, upregulation of gene expression of S100A9 (ca. 12 fold), IFNγ (ca. 10 fold), MMP9 (ca. 35 fold), IL1β (ca. 4 fold), IL6 (ca. 15 fold), IL8 (ca. 4 fold), and TNFα (ca. 9 fold) is apparent. This gene expression exhibits a clear differential induction of conjunctivitis-associated inflammation in response to LPS/PGN stimulation in culture in the ex-vivo model of both dissected and whole globe rabbit eye. This unique model can be used for the non-clinical ex vivo testing of ocular therapeutics for the treatment of bacterial conjunctivitis by mimicking the conditions and evaluating the biomarkers associated with this disease. Further characterization as well as challenge with topical steroid treatments will further determine the translational capability of this model to clinical pathology.

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Acetyl zingerone opposes deleterious effects of skin aging by bolstering matrisome synthesis, neutralizing oxidative stress and inhibiting DNA damage

W. R. Swindell[1], T. Meyer[2], S. Premi[3], K. Bojanowski[4], R. Chaudhuri[2]

[1]Internal Medicine, The Jewish Hospital, Cincinnati, Ohio, United States, [2]Sytheon Ltd, Boonton, New Jersey, United States, [3]Moffitt Cancer Center, Tampa, Florida, United States, [4]Sunny Biodiscovery Inc, Santa Paula, California, United States

Acetyl zingerone (AZ) is a new molecule structurally related to Zingerone (Z), a naturally occurring phenolic alkanone found in ginger, but designed for improved stability and antioxidant function. In microarray studies performed using reconstituted human epidermis, both Z and AZ increased Notch pathway gene expression (NOTCH1, MAML3) and decreased expression of genes linked to ECM disassembly (MMP3, CTSV, NOXO1) and reactive oxygen species metabolism (PMAIP1, ARG2). Although Z and AZ each inhibited in vitro MMP-1, MMP-3 and MMP-12 activity, inhibition of MMP-3 and MMP-12 was greater with AZ. Moreover, AZ led to more consistent increases in the expression of genes encoding collagens (COL11A2, COL6A3, COL4A1), proteoglycans (VCAN, OGN, PODN), ECM regulators (TIMP1, OGFOD1), and ECM glycoproteins (SPARC, GLDN, FBLN2), while concomitantly opposing gene expression patterns associated with fibroblast senescence, keratinocyte differentiation, and IL-17A stimulation. Immediate treatment of melanocytes with AZ after UVA irradiation significantly inhibited ongoing formation of delayed cyclobutane dimers, while incubation with AZ in keratinocytes before UVA exposure significantly decreased ROS formation, confirming that AZ does not behave as a photosensitizer. Compared with α-tocopherol and Z, AZ exhibited significantly better photostability and efficacy to neutralize free radicals, physically quench singlet oxygen, and scavenge peroxynitrite. These results suggest mechanisms by which AZ improves upon the core molecular structure of Z to effectively bolster the synthesis of ECM components. We have further demonstrated that AZ limits genotoxic stress secondary to ROS formation or UV light exposure. AZ thus provides a translational step forward towards development of a more efficacious molecule to treat or prevent the consequences of intrinsic and extrinsic skin aging.

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Natural killer cell deficiency reveals a novel immunotherapy strategy for atopic dermatitis

M. R. Mack[3], J. R. Brestoff[1], M. M. Berrien-Elliott[2], T. B. Yang[3], A. M. Trier[3], P. L. Collins[1], H. Niu[4], N. D. Bodet[3], J. A. Wagner[2], E. Park[5], A. Z. Xu[3], F. Wang[3], M. McCullen[1], R. Chibnall[3], M. L. Council[3], D. J. Margolis[6, 7], D. Sheinbein[3], E. Vivier[8, 9], P. Lovato[10], M. Cella[1], M. Colonna[1], W. M. Yokoyama[5], E. M. Oltz[11], T. A. Fehniger[2], B. Kim[3, 1]

[1]Pathology and Immunology, Washington University School of Medicine, Saint Louis, Missouri, United States, [2]Oncology, Washington University School of Medicine, Saint Louis, Missouri, United States, [3]Dermatology, Washington University School of Medicine, Saint Louis, Missouri, United States, [4]Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, United States, [5]Rheumatology, Washington University School of Medicine, Saint Louis, Missouri, United States, [6]Dermatology, Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [7]Biostatistics, Epidemiology and Informatics, Perelman School of Medicine, Philadelphia, Pennsylvania, United States, [8]Aix Marseille University, Marseille, France, [9]INSERM, Marseille, France, [10]Skin Research, LEO Pharma, Ballerup, Denmark, [11]Microbial Infection and Immunity, Ohio State University, Wexner School of Medicine, Columbus, Ohio, United States

Atopic dermatitis (AD) is a widespread, chronic skin disease associated with aberrant allergic inflammation. While current treatments involve either broad or targeted immunosuppression strategies, enhancing aspects of the immune system to control disease remains largely untested. We demonstrate that patients with AD harbor a blood natural killer (NK) cell deficiency that has both diagnostic value and improves with therapy. Multidimensional CyTOF analysis and RNA-seq profiling of patients' peripheral blood NK cells revealed subset-level changes associated with activation-induced cell death. Both pharmacologic and genetic NK cell depletion in mice resulted in enhanced type 2 inflammation in the skin, characterized by increased group 2 innate lymphoid cells (ILC2s) and eosinophils, suggesting that NK cells play a critical immunoregulatory role. Based on these findings, we employed an NK cell-boosting IL-15 superagonist and observed marked improvement in AD-like disease in mice. These findings reveal a previously unrecognized role for NK cells and IL-15 superagonism, currently in development for cancer immunotherapy, as a novel immunotherapeutic strategy for AD.

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Functional drug screening identifies candidate synergistic combinations for CTCL therapy

S. Yumeen[1], F. N. Mirza[1], J. M. Lewis[1], A. O. King[1], S. Kim[1], K. R. Carlson[1], S. Umlauf[2], Y. Surovtseva[2], F. M. Foss[1], M. Girardi[1]

[1]Yale School of Medicine, New Haven, Connecticut, United States, [2]Yale Center for Molecular Discovery, West Haven, Connecticut, United States

Cutaneous T cell lymphoma (CTCL) is a malignancy of T lymphocytes that may involve the peripheral blood in advanced stages. Reported gene copy number alterations and single nucleotide variants in CTCL have suggested therapeutic targets in altered and aberrantly activated pathways. Combination targeted agent treatment strategies are emerging as an approach to increase efficacy and reduce risk via drug synergy. We aimed to investigate the potential roles of single and combination approaches utilizing agents targeting JAK, BCL2, BET, and HDAC in CTCL cells. Peripheral blood malignant CTCL cells were isolated from 16 patient samples and exposed in vitro to 8 therapies alone and in combination, and comparisons made to healthy lymphocytes and five CTCL cell lines (HH, MyLa, Hut78, SeAx, and Sez4). CTCL patient samples showed differential response to JAK inhibition, with JAK2 expression levels negatively correlated to 50% inhibitory concentration (IC50) values. CTCL patient samples also expressed significantly greater JAK2 (36-fold, p=0.0004), STAT5B (65-fold, p=0.0002) and BCL2L2 (11-fold, p=0.02) than control lymphocytes. Under parallel conditions, patient samples were found resistant to bexarotene, methotrexate, and talazoparib monotherapy, but these agents potentiated malignant cell kill when used in combination with BCL2, BET, HDAC, or proteasome inhibition. Combination inhibition of JAK and BCL2 showed the strongest potentiation of CTCL kill, driven by both intrinsic and extrinsic apoptosis pathways. These data help elucidate specific therapeutic combination strategies that may potentiate cytotoxic effects against the malignant cells across a diverse set of CTCL patients.

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Dietary grape intake protects against UV damage in humans by augmenting DNA repair

A. S. Oak, R. Shafi, M. Elsayed, B. Mishra, L. Saag, A. T. Slominski, S. Bae, M. Athar, C. Elmets

Dermatology, University of Alabama at Birmingham, Birmingham, Alabama, United States

Compounds found in grapes, such as ellagic acid, resveratrol and polyphenols, protect against photodamage in animal models. The purpose of this single-group, open-label clinical study was to determine whether grapes had a similar effect in humans. The minimal erythema dose (MED) was determined in 19 healthy volunteers, (Fitzpatrick skin types I-III), and biopsies were taken from sun-exposed and non-sun-exposed skin. They were instructed to consume 25 g of freeze-dried grape powder 3x/day for 2 weeks. The MED was measured and posttreatment biopsies were taken. The posttreatment MED increased significantly compared to pretreatment values by 74.80% (p=0.03, 95% CI [8.01, 141.60]). Subjects reported no significant adverse effects. Significantly lower levels of cyclobutane pyrimidine dimers, gamma-H2AX, and TUNEL-positive cells were observed by immunohistochemistry. Gene expression analysis was then conducted using NanoString. Higher expression of *ATM,* which encodes a key protein kinase involved in recognition and repair of double-strand DNA breaks, was observed after treatment and was more pronounced in subjects whose MED increased after grape treatment. Upregulation of genes associated with damage recognition in both transcription-coupled repair and global genome repair (e.g., *XPCC4*, *XPA*, and *ERCC6*) were observed after treatment. Upregulation of *MLLT3*, which increases the catalytic rate of RNA polymerase II transcription, was observed as well. Because UV exposure increases the inflammatory response and because some of those mediators augment DNA repair, NanoString was also examined for inflammatory mediators. Significant downregulation of UV-induced proinflammatory genes (e.g., *IL1B, IL8, TNFRSF10A* and *IL12RB2*) was observed after grape treatment. We conclude that oral consumption of table grapes has photoprotective effects in healthy human volunteers and significantly lowers markers of DNA damage. The protective effects are associated with upregulation of genes involved in DNA damage recognition and repair.

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Arthritis-associated pyoderma gangrenosum: A systematic review

E. Sawka[1], M. Friedman[2], A. Ortega-Loayza[3]

[1]School of Medicine, Oregon Health & Science University, Portland, Oregon, United States, [2]Medicine, Oregon Health & Science University, Portland, Oregon, United States, [3]Dermatology, Oregon Health & Science University, Portland, Oregon, United States

Pyoderma gangrenosum (PG) is a rapidly-progressive neutrophilic dermatosis commonly associated with systemic inflammatory diseases. Our understanding of the relationship between PG and arthritis is lacking and the literature is currently dominated by case series and reports. We performed a systematic review in PubMed from 1950 to 2019 to determine the incidence of inflammatory arthritis among patients presenting with PG, the most common types of arthritis present in PG patients, and trends in treatment and outcomes. Seventeen studies that reported incidence of arthritis in PG patients and 84 case reports of PG and arthritis were identified. The median incidence rate of arthritis in patients presenting with PG was 17.4%. The most common types of arthritis were rheumatoid (54%), unspecified inflammatory (26%), psoriatic (8%), and spondyloarthritis (5%). In almost every case, joint symptoms preceded PG, by a median of 10 years. Almost half of ulcers healed within 6 months. Of the cases with complete resolution, the median healing time was 3 months. In the majority of cases, the initial treatment failed with the most common being antibiotics and high dose steroids. Of the successful treatments, medium to high dose steroids were the most frequently used (47%), followed by biologics (41%). Of the biologics, infliximab, etanercept, and anakinra were notably effective. This study shows that PG is frequently preceded by arthritis, most commonly rheumatoid arthritis. Clinicians used a wide variety of treatment regimens with variable outcomes. While larger studies are needed to standardize treatment of arthritis-associated PG, this study suggests that high dose steroids and biologic medications are often effective for these patients.

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An *in vitro* assay of inflammatory monocyte-keratinocyte activation predicts *in vivo* activity of BET inhibitors in a preclinical model of psoriasis

X. Wu, Z. Shi, D. Hsu, M. Huynh, D. Yamada, J. Chong, L. Mendoza, S. Hwang
Dermatology, University of California Davis, Sacramento, California, United States

Bromodomain and extra-terminal (BET) proteins perform a key role in epigenetic control of gene expression. Targeting BET bromodomains has been proposed in Th17-mediated autoimmune diseases such as psoriasis, which involves interplay between monocyte-macrophage cells and keratinocytes. To determine if we could create an effective in vitro model to screen BET inhibitors that would have activity in blocking psoriasiform dermatitis (PsD) in vivo, we co-cultured HaCaT keratinocytes with U937, a myeloid monocyte cell line which expresses TNF-α. Upon coculturing with U937, HaCaT cells exhibited a marked increase in secretion of a characteristic set of Th17-associated chemokines and cytokines, including CCL2, IL-8, IL23 and CCL20. Among ten small molecules known to target BET proteins, OTX015 and ABBV075 were found to inhibit inflammatory Th17 cytokine gene expression by >70% at concentrations of 0.1 μM and 0.02 μM, respectively. By contrast, another BET-targeting small molecule, ARV825, showed relatively less inhibitory activity. Next, the three agents above were tested in a mouse model of topical imiquimod (IMQ)-induced PsD to determine if in vitro efficacy correlated with in vivo therapeutic effects. Daily oral dosing at 25mg(~50μmol)/kg OTX015 or 2.5mg(~5μmol)/kg ABBV075 in IMQ-induced mice were able to reduce disease severity (>50% by PSI score), transepidermal water loss (>50%), numbers of infiltrated neutrophils and IL-17-producing-γδ low-T cells (~70% and ~50% respectively) compared to vehicle treatment without obvious toxicity. Of note, ARV825 showed no improvement at 50mg (~50μmol)/kg in respect to therapeutic reduction of inflammatory markers at a clinical and molecular levels. In summary, our data show that several, but not all, BET inhibitors ameliorate PsD at non-toxic dosing levels. Our in vitro system and in vivo animal model can serve as pre-clinical screening tool for selection of small molecules that may be clinical helpful in psoriasis.

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A point-of-care, real time artificial intelligence system to support clinician diagnosis of a wide range of skin diseases

M. Zhang[1], B. Dulmage[2], K. Tegtmeyer[1], M. Colavincenzo[1], S. Xu[1]
[1]Dermatology, Northwestern Univ School of Med, Chicago, Illinois, United States, [2]Dermatology, The Ohio State Univ, Columbus, Ohio, United States

Diagnosing skin disease is challenging for non-dermatologists. Artificial intelligence (AI) clinical decision tools offer the potential to improve diagnostic accuracy. While previous AI tools have been used to classify skin cancers, there are limited technologies able to provide diagnostic support for both neoplastic and inflammatory skin conditions. Thus, we present the development and early validation of a point-of-care, real time AI tool that provides morphological classification of a wide range of skin diseases. The AI tool was built via an ensemble of 5 distinct convolution neural network (CNN) models and trained on more than 77,000 dermatologist-labeled images from the VisualDx (Rochester, NY) library. We tested the performance of this AI tool compared to 15 board-certified internal medicine physicians with and without a visual aid of dermatological morphologies on a set of 16 new, de-identified clinical images of 13 distinct morphologies. This cohort of IM physicians achieved a baseline 36% accuracy in describing the morphologies without any aids, which subsequently improved to 68% with the use of a visual key of correct morphologies ($p<0.001$). The AI's single top prediction also achieved 68% accuracy in the same set of images—and when broadened to top 3 predictions, accuracy improved to 80%. The AI was later applied to a larger set of 222 heterogeneous images taken from a wide range of smartphones, digital cameras, and lighting, achieving an overall accuracy of 69% when limited to its single top prediction and 92% when broadened to its top 3 predictions. Sub-stratification into Fitzpatrick I-III skin types (n=169 images) and Fitzpatrick IV-VI skin types (n=53 images) demonstrated no statistical significance in accuracy ($p=0.79$). Selection of the correct morphology as the first step in building a dermatological differential diagnosis, as suggested by the AI tool, is a key step in allowing clinicians to maintain decision autonomy while mitigating the risk of deskilling.

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Molecular characterization of mucosal lichen planus

A. N. Privalle[1], A. Meves[1], J. Lehman[1, 2], R. Guo[1, 2], J. Sartori-Valinotti[1]
[1]Dermatology, Mayo Clinic, Rochester, Minnesota, United States, [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, United States

Lichen planus (LP) is an inflammatory mucocutaneous disease. The precise pathogenesis remains elusive. A critical event in the initiation of immune responses in LP lesions is for memory T cells to migrate from the circulation into the skin leading to cytokine/chemokine release (i.e. IFN-γ, IL-1β, IL-6, IL-8, IL-17, JAK3 and TNF-α). We sought to quantitatively examine the differential expression of various molecular mediators in idiopathic mucosal lichen planus and to test if the expression pattern is anatomic site-specific. Using an institutional database, at least 10 LP cases per anatomic site were identified. The histopathological diagnosis was reviewed by the principal investigator (JSV). The tissue was processed for Fluidigm quantitative PCR. Expression patterns of 36 markers based on postulated involvement, including JAK1, JAK2, JAK3, PDE4B, PDE4D, CD271, STAT1, and STAT3, were analyzed for each tissue sample. A pairwise Dunn's test was used to detect any statistically significant differences in gene expression between anatomic sites. 62/74 (84%) samples (15 lip, 14 gingiva, 13 penis, 5 buccal, 5 anorectal, 4 vulva, 4 tongue) demonstrated quantifiable gene expression. The gene expression of TNNT3 (troponin T type 3; $p = 0.003$), HSPB6 (heat shock protein, alpha-crystalline-related, B6; $p = 0.006$), and TNNC2 (troponin C type 2; $p = 0.02$) was statistically significant between the tongue and gingiva, with the tongue demonstrating higher expression in all three. No other statistically significant anatomic site differences resulted. This is the first study to show differential expression of genetic markers in LP based on anatomic site. Such data may help to further elucidate the pathogenesis of LP and guide future pharmacologic discoveries. Limited literature has suggested PDE4 and JAK inhibition as viable treatments for LP. In our study, the molecular mediators within these pathways demonstrated similar expression among anatomic sites, suggesting treatments may have similar efficacy regardless of disease location.

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Microbiome therapy of atopic dermatitis by application of rationally selected human commensal skin bacteria

T. Nakatsuji[1], T. Hata[1], L. Tong[1], J. Cheng[1], F. Shafiq[1], A. Butcher[1], A. Spergel[4], K. Johnson[3], B. Jepson[3], A. Calatroni[3], P. Taylor[2], D. Leung[2], R. L. Gallo[1]
[1]Dermatology, UCSD, La Jolla, California, United States, [2]National Jewish Health, Denver, Colorado, United States, [3]RhoFed Inc, Chapel Hill, North Carolina, United States, [4]NIAID, NIH, Bethesda, Maryland, United States

Disease severity in atopic dermatitis (AD) is negatively influenced by *S. aureus* (SA). SA can be inhibited by antimicrobial molecules made by some strains of coagulase-negative Staphylococcus (AM+CoNS). A lack of AM+CoNS correlates with SA abundance and disease severity in AD. A previous clinical trial showed autologous AM+CoNS could reduce SA on AD skin within 24 hrs. Recently, some CoNS were also found to inhibit expression of SA toxins by blocking quorum sensing through production of autoinducing peptides (AIPs). For this reason, a single strain of *S. hominis*(SHA9) that produces both AMs and an AIPwas selected for further testing of safety and potential therapeutic efficacy in mice and humans. Application ofSHA9 to OVA-sensitized Balb/c $FLG^{ft/ft}$mice reduced SA survival by >99% ($P<.05$). In these mice, SHA9 was also a potent anti-inflammatory agent and suppressedIL-4 by 89.6%, IL-13 by 84.6% and TSLP by 87.3% ($P<.05$). SHA9 also increased skin cathelicidin expression by 9.2 fold ($P<.01$) to provide enhanced host defense. Efficacy was in part due to the action of AIP; genetic deletion of AMs from SHA9 did not completely eliminate the anti-inflammatory activity but did diminish the capacity to eliminate SA. SHA9 was also evaluated in a double-blind, vehicle-controlled phase 1 clinical trial on 54 moderate to severe SA-positive AD adult subjects. SHA9 or vehicle was applied BID to upper extremities for 7 days and skin swabs were obtained for up to 11 days. No difference in adverse events was observed between active and vehicle groups. Compared to vehicle, SHA9 reduced SA abundance on lesional skin by 99% at day-7 ($P<.001$). This reduction persisted up to day-11 (99%, $P<.001$) and correlated with a decrease in local EASI at day-9 ($P=.001$) and day-11 ($P=.007$). These data show the safety and efficacy of a highly-defined and rationally selected member of the skin microbiome to manage AD.

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Intravenous gentamicin therapy for junctional epidermolysis bullosa patients harboring nonsense mutations

M. Hao[1], R. Antaya[2], J. Cogan[1], C. Hamilton[2], Y. Hou[1], A. Kwong[1], D. Woodley[1], M. Chen[1]

[1]University of Southern California, Los Angeles, California, United States, [2]Yale School of Medicine, New Haven, Connecticut, United States

Generalized severe junctional epidermolysis bullosa (GS-JEB) is an incurable and fatal inherited blistering skin disease most commonly caused by nonsense mutations in *LAMA3*, *LAMB3*, or *LAMC2* genes. These mutations impair the ability to produce functional laminin 332, needed for epidermal-dermal adherence. Previously, we showed that topical gentamicin therapy generated new, functional laminin 332 and improved wound healing in GS-JEB patients. Although effective, topical administration of gentamicin to the entire skin surface is cumbersome and would not treat mucosal sites, including the upper respiratory tract. In this study, we administered intravenous (IV) gentamicin to three GS-JEB patients with nonsense mutations in either *LAMB3* or *LAMA3*. At day 0, multiple Test Sites from open wounds and intact skin were selected for measuring wound closure and new blister formation. Three patients received daily infusions of 7.5 mg/kg gentamicin for 14 days and one patient received daily infusions of 10 mg/kg gentamicin for 24 days. Skin biopsies were examined for the expression of laminin 332, and wounds were evaluated using standardized photographs before and at one and three months after treatment. We also evaluated the patients' overall clinical improvement using EB disease activity scores. After IV gentamicin, Test Sites exhibited newly created laminin 332 at the dermal-epidermal junction of the patients' skin. In addition, IV gentamicin promoted wound closure and improved the patients' clinical scores. Most interestingly, we also observed improvement of airway symptoms in GS-JEB patients. Lastly, increasing the dosage and duration of infusions resulted in more laminin 332 expression and greater clinical improvement. No adverse effects or auto-antibodies against new laminin 332 were observed. IV gentamicin may offer JEB patients a readily available, safe and effective treatment which improves wound healing and quality of life.

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Thioredoxin regenerates elastic fibers in the dermis

T. Tohgasaki, S. Kondo, S. Nishizawa, X. Yu, S. Ishiwatari, T. Sakurai

Beauty Science Research Center, FANCL Corporation, Yokohama, Kanagawa, Japan

Wrinkles and sagging occurring with aging affect the human physical appearance, resulting in poor quality of life. Therefore, care methods for these need to be improved. Wrinkles and sagging are thought to be caused by alterations in the internal skin structure; however, the specific morphological alterations and molecular mechanisms remain unclear. Previously, we observed the three-dimensional (3D) structure of the elastic fibers in the dermis by using tissue decolorization technology, and established a novel computational method to analyze its structural characteristics (volume, surface area, blanch numbers, length, diameter, and straightness). We then found that the fibers become thicker, shorter, and curved with aging, by using this method. In this study, thioredoxin (TRX), which promotes antioxidant function and tropo-elastin molecule production in fibroblasts, was applied to human abdominal extirpated skin, which was then cultured for 5 days, to verify the potential of TRX to improve these structural alterations. We then evaluated the elastic fiber structure using 3D computational analysis with imaging. Our results showed that the elastic fibers in the extirpated skin significantly regenerated, with increased length and straightness, after culturing for 5 days with TRX application. Elastic fibers are known to be degenerated and lost by ultraviolet radiation and inflammation, and the regeneration rate of elastic fibers in the dermis of adults is extremely slow. Therefore, their repair is difficult. However, we found elastic fibers in the extirpated skin to be regenerated with characteristics similar to those in youngsters, by application of TRX on the skin surface. This regeneration may be caused by TRX's functions as an antioxidant and inducer of elastin production in fibroblasts. Although the molecular mechanisms underlying the elastic fiber regeneration by TRX need to be elucidated, TRX has the potential to improve the dermal structure of the skin.

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Role of STAT6 in advanced-stage cutaneous T-cell lymphoma

A. Gaydosik[1], D. Queen[2], O. E. Akilov[1], L. Geskin[2], P. Fuschiotti[3]

[1]University of Pittsburgh, Pittsburgh, Pennsylvania, United States, [2]Columbia University College of Physicians and Surgeons, New Yok, New York, United States, [3]Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, United States

Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of lymphoproliferative disorders derived from skin-homing memory T cells. Mycosis fungoides (MF) and Sezary syndrome (SS) are the most common subtypes. Aberrant cytokine expression in the MF/SS tumor microenvironment (TME) is a major factor in disease pathogenesis and progression. We have previously shown that MF/SS malignant lymphocytes produce high levels of IL-13, which acts as an autocrine factor for tumor cells and suppresses tumor-cell immunosurveillance. Furthermore, our studies indicate that IL-13 synergizes with IL-4 in inducing SS cell growth and implicate IL-13 signaling via the Signal Transducer and Activator of Transcription-6 (STAT-6), an up-stream mediator common to both IL-4 and IL-13 signaling. Significantly, we found high numbers of activated STAT-6^{+} cells in the affected skin of MF patients, particularly in advanced stages, implicating STAT-6 as a critical signaling mediator in MF/SS lymphocytes. To investigate the underlying molecular mechanism, we combined genome-wide transcriptional profiling with STAT-6 inhibition to identify the STAT-6-regulated genes in advanced-stage MF/SS tumors. In malignant lymphocytes, we found that STAT-6 regulates the expression of genes associated with control of cell cycle progression and genomic stability, and its inhibition decreased proliferation. Furthermore, we showed that STAT-6 enhances expression of Th2 cytokines in malignant and reactive T cells by up-regulating the expression of GATA-3. Finally, we demonstrated that STAT-6 contributes to the pro-tumoral M2-like phenotype of macrophages in the TME of advanced-stage MF by up-regulating the expression of genes associated with immunosuppression, chemotaxis, and tumor matrix remodeling. Thus, STAT-6 contributes to MF/SS malignancy by several mechanisms including enhancing proliferation of tumor lymphocytes and promoting an immunosuppressive and pro-tumoral microenvironment that favors tumor growth and invasion.

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Lesional immune cells in cutaneous acute graft-versus-host disease: A prospective cohort study

R. Sennett[1], B. Jama[2], B. Hinds[1], D. Tzachanis[3], G. Morris[2], A. Marsch[1]

[1]Dermatology, University of California, San Diego, San Diego, California, United States, [2]Pathology, University of California, San Diego, San Diego, California, United States, [3]Medicine, University of California, San Diego, San Diego, California, United States

Hematopoietic stem cell transplant (HSCT) is an intricate process that carries a risk of similarly convoluted complications, including graft-versus-host-disease (GVHD). Acute and chronic GVHD are distinct entities, defined by a combination of historical, clinical and pathologic data, but both are generally thought to stem from self-propagating aberrantly activated immune cells inflicting end organ damage, with the potential to cause significant morbidity and mortality. Survival rates after HSCT have improved significantly over the past few decades, but GVHD remains a major hurdle in improving the efficacy and safety of transplant. A deeper understanding of the cellular and molecular mechanisms driving the progression of this reaction is necessary to better prevent and treat GVHD. Towards this end, skin biopsy samples were collected from lesional and unaffected skin in five patients with acute cutaneous GHVD. Fresh tissue was processed for fluorescence-activated cell sorting (FACS) and analysis of macrophages and lymphocytes, demonstrating a predominance of CD8+ T-cells. The percent of lymphocytes and macrophages as a representation of total cells varied between patients, and was not always consistent between lesional and unaffected sites. The heterogeneity in immune cell profiling that was observed between patients in this study could reflect the diverse demographics, conditioning and transplant conditions of each individual. This initial study only gives a glimpse into the underlying molecular mechanisms of cutaneous GVHD progression but paves the way for additional studies to examine the cellular and molecular landscape in greater detail.

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Immunotherapy in advanced Merkel cell carcinoma: Frequent responses but a potentially concerning recurrence rate after therapy discontinuation

Y. Moshiri[1], N. Alexander[1], T. Akaike[1], C. Doolittle-Amieva[1], T. Pulliam[1], L. Zaba[2], P. Nghiem[1, 3]

[1]Dermatology, University of Washington School of Medicine, Seattle, Washington, United States, [2]Department of Dermatology, Stanford University, San Jose, California, United States, [3]Skin Oncology, Fred Hutchinson Cancer Research Center, Seattle, Washington, United States

Merkel cell carcinoma (MCC) is an aggressive skin cancer that often recurs. MCC tumors can respond to PD-1 pathway inhibitors rapidly, however, it is unclear how often these responses persist after discontinuation of therapy. We retrospectively assessed 159 persons with advanced MCC treated with first-line anti-PD-(L)1 agents. Non-responders were defined as those with progressive disease (PD) or stable disease (SD), while responders had partial response (PR), or complete response (CR), based on clinician assessment. Of the 159 patients, 106 (66%) responded to anti-PD-(L)1 therapy and 53 (33%) did not. Among responders, 66 (42% of cohort) had CR and 40 (25%) had PR. Among 61 responders whose therapy was discontinued electively or due to treatment complications, 22 (36%) have had a recurrence, while 39 (64%) have not. Median duration of therapy in responders without recurrence was 356 days, and in those with recurrence was 303 days. Time-to-recurrence data were available for 15 of the 22 patients whose tumors recurred and the median interval was 198 days after drug discontinuation. Median follow-up time for responders who have not recurred was 725 days. Responders and non-responders did not differ significantly in the following characteristics that were examined: age, sex, immune status, Merkel cell polyomavirus serostatus, primary site of disease, and prevalence of immune-related adverse events. The observed 66% overall response rate in this MCC cohort is high relative to most solid tumors. However, the 36% recurrence rate among responders, following immunotherapy discontinuation, raises questions about how to best manage patients in the longer term, including a possible role for low dose-intensity maintenance therapy.

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Baseline differences in circulation between early vs late responder patients with vitiligo treated with ruxolitinib cream

M. Howell, Y. Hu, Z. Zhu, K. Sun, F. Kuo, K. Butler, S. Owens

Incyte Corporation, Wilmington, Delaware, United States

Vitiligo is a chronic, inflammatory skin disease characterized by increased interferon-gamma signaling through Janus kinase (JAK) 1 and JAK2 and subsequent activation of CD8+ T cells, which target melanocytes resulting in areas of depigmentation. Ruxolitinib cream, a JAK1/JAK2 inhibitor, is under investigation for vitiligo treatment in a 52-week, randomized, double-blind, phase 2 study (NCT03099304). Significantly more patients treated with ruxolitinib cream vs vehicle achieved ≥50% improvement in facial Vitiligo Area Scoring Index (F-VASI50) at Week 24 (primary endpoint); at Week 52, patients treated with ruxolitinib cream 1.5% twice daily (BID) attained the highest F-VASI50 response (57.6%). This analysis investigated the differences between early and late responders (ER and LR, respectively) following treatment with ruxolitinib cream. Patients were classified as ER if they achieved F-VASI50 or greater at Week 24; all other patients were classified as LR. In ER, F-VASI improved by 79.9%±4.0% and 91.9%±1.5% at Weeks 24 and 52, respectively. In contrast, F-VASI improved by 1.1%±7.3% and 25.1%±13.4% in LR at Weeks 24 and 52. Broad serum proteomics of 54 patients identified 76 out of 1104 proteins were differentially expressed between ER and LR at baseline at $p < 0.05$. Eleven proteins were up-regulated in ER while the rest were down-regulated in ER. Overall, this analysis identified significant differences between ER and LR that require deeper scientific interrogation and may be important in stratifying the therapeutic benefit for patients with vitiligo.

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Direct mechanical measurements of skin to quantify evolution of sclerotic disease

L. X. Baker[1], F. Chen[1], Y. Ssempijja[1], L. Dellalana[1], A. Vain[2], M. Jagasia[3], E. Tkaczyk[1, 3]

[1]Department of Veterans Affairs and Vanderbilt Dermatology Translational Research Clinic, Nashville, Tennessee, United States, [2]University of Tartu Institute of Physics, Tartu, Estonia, [3]Vanderbilt-Ingram Cancer Center, Nashville, Tennessee, United States

There is an unmet need for objective cutaneous measurements to track the progression of sclerosing diseases, such as chronic graft-versus-host disease (cGVHD). The handheld Myoton device extracts biomechanical parameters of stiffness, frequency, and relaxation time characterizing soft tissue through its damped oscillation response to a mechanical micro-impulse. Cutaneous measurements using Myoton have differentiated sclerotic cGVHD patients from post-transplant controls. In a prospective longitudinal pilot study, we assessed Myoton's ability to numerically monitor sclerosis over time. Between Apr 2017 and Dec 2019, we measured the skin of sclerotic cGVHD patients (n=9) with the Myoton at each clinic visit. Each subject had a personalized set of anatomic sites that were measured at each visit, yielding an average stiffness, frequency, and relaxation time for each visit. Simple linear regression was performed on the series of parameter averages for each subject. Visit notes were reviewed to assess clinical correlation. Two patients had cGVHD progression, corresponding to the only positive outlier slopes in stiffness (36 N/m/month; 31N/m/month) and frequency (1.3 Hz/month; 0.5 Hz/month). Three patients had clinical improvement, two of whom had negative outlier slopes in stiffness (-162 N/m/month; -40 N/m/month) and frequency (-2.3 Hz/month; -1.0Hz/month), and positive outlier slopes in relaxation time (2.1 ms/month; 1.2 ms/month). The remaining four patients were clinically stable and had regression slopes that did not differ significantly from 0 in any parameter. Changes in skin stiffness, frequency, and relaxation time measurements over time reflect clinical perception of skin response in sclerotic cGVHD patients. A larger study is needed to develop a predictive model for clinical response based on Myoton measurements.

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Pathogenesis based therapy improves cutaneous abnormalities in porokeratosis- A pilot study

L. Azmony[1, 2], Q. Sun[3], C. Hamilton[3], Y. H. Lim[3], J. S. Leventhal[3], A. S. Paller[4], K. Choate[3]

[1]Dermatology, Rabin Medical Center, Petach Tikva, Israel, [2]Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel, [3]Dermatology, Yale School of Medicine, New Haven, Connecticut, United States, [4]Dermatology and Pediatrics, Northwestern Univ. Med School, Chicago, Illinois, United States

Porokeratosis is a heterogenous group of keratinization disorders associated with mevalonate pathway gene mutations. Treatments options for the disease are few and often ineffective. On the basis of the possible pathogenic role of pathway end-product deficiency (cholesterol) and accumulation of toxic precursors, we studied the efficacy of topical lovastatin/cholesterol in different variants of porokeratosis. We recruited 1 patient with disseminated superficial actinic porokeratosis (DSAP), 2 patients with porokeratosis palmaris et plantaris disseminate (PPPD) and 2 patients with linear porokeratosis (LP). Patients were genotyped prior to initiation of therapy and applied topical lovastatin/cholesterol twice daily for 6 months with the rest of the untreated affected skin serving as a control. Response was evaluated using clinical photographs every visit. Three patients had germline *MVD* mutations and 2 patients had germline *PMVK* mutations. Topical lovastatin/cholesterol (but not cholesterol alone) almost cleared lesions after 4 weeks of therapy in DSAP and partially improved lesions in PPPD and LP. There were no adverse events. In conclusion, topical cholesterol/lovastatin is an effective and safe therapy for porokeratosis that validates the utility of pathogenesis-based therapy that replaces deficient end products and prevents accumulation of potentially toxic metabolites.

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Establishment of a model of ras oncogene induced senescence in endothelial cells

A. Costa[1], M. Teichmann[1, 2], M. Bonner[1], J. Mackelfresh[1], L. Gilbert[1, 2], S. Rao[1], J. Arbiser[1, 2]

[1]Dermatology, Emory University, Atlanta, Georgia, United States, [2]Atlanta VAMC, Decatur, Georgia, United States

Oncogene induced senescence underlies many processes in dermatology, such as driver mutation induced vascular malformations, nevi, and seborrheic keratoses. These processes are difficult to study given that cells with driver oncogenes rapidly senescence in culture, whether the cells are transduced with a driver oncogene or cultured from a primary lesion. In order to provide a model easily accessible to the research community, we have established a model of oncogene induced senescence through the sequential introduction of a temperature sensitive large T oncogene followed by oncogenic H-ras into microvascular endothelial cells. Shift to the nonpermissive temperature of 39oC results in shutting off large T oncogene. We studied the signaling and metabolic changes that underlie oncogene induced senescence in our model system. The transcription factor IRF7 was downregulated, which might enable these lesions to become immune priveleged. P38 signaling was also downregulated, suggesting that agents that induce p38 signaling might cause regression of benign lesions with driver mutations.Temperature shift also resulted in an irreversible induction of p16ink4a and downregulation of lamin B, hallmarks of oncogene induced senescence. Metabolic changes induced by the termperature shift that are specific to ras include downregulation of succinate dehydrogenase alpha, p53 S15, and pBAD S112. This model will be generalizably useful for researchers studying oncogene induced senescence and help elucidate novel therapies for unmet needs like driver induced vascular malformations.

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Clinical and histopathologic characteristics of metastatic and locally aggressive basal cell carcinomas

L. X. Baker[1], E. Grilletta[2], J. P. Zwerner[2], A. S. Boyd[2], L. Wheless[2]

[1]Vanderbilt University School of Medicine, Hermitage, Tennessee, United States, [2]Dermatology, Vanderbilt University Medical Center, Nashville, Tennessee, United States

Due to the rarity of metastatic or locally advanced basal cell carcinoma (mBCC, laBCC), most of the data regarding their characteristics come from single case reports and small series. None have performed a thorough evaluation of the histologic parameters of mBCC. To address this gap, we searched the entire medical record at Vanderbilt University Medical Center to identify potential cases of locally advanced or metastatic BCC occurring between 1984 and January 2019. A retrospective chart review was performed of all identified patients to determine case status. Where available, we reviewed pathology slides of biopsies or resections of the primary tumors. Forty patients with either laBCC (n=17) or mBCC (n=23) were identified. Most cases occurred in males (80%), arising most commonly on the head and neck (73%), the upper extremities (10%), and the trunk (7.5%). At diagnosis of mBCC cases, 7 (30%) were localized, 4 (17%) were locally aggressive, and 5 (22%) had involved regional lymph nodes. Pathology slides were available for 13 mBCC and 10 laBCC cases. Of 13 mBCC cases, the histologic subtypes include infiltrative (n= 9), nodular (n=7), superficial (n=2), morpheaform (n=2), and sclerotic (n=2), accounting for multiple patterns present in specimens. Perineural invasion was identified in 2 laBCC and 6 mBCC cases, with 3 mBCC invading nerves >0.1mm. Ulceration was present in 7 laBCC and 8 mBCC cases. Tumor infiltrating lymphocytes were noted in 2 laBCC and 1 mBCC cases. 21/23 were Clark Level IV or V, with a mean depth of invasion of >8mm for both types. The mean mitotic rate was 3.8 mitoses/mm^2 for both types. 60% of locally aggressive and 77% of BCCs that ultimately metastasized were reported as having previously recurred locally. In this large series of locally advanced and metastatic BCC, we observed that most of the mBCC had infiltrative morphology and deep invasion. An elevated mitotic rate, perineural invasion and local recurrences are all common findings in laBCC and mBCC.

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Continuous hair density differentiation to score extent of alopecia areas automatically

T. Mane[1], A. Bayramova[1], L. Castelo-Soccio[1, 2, 3], E. Bernardis[1]

[1]Department of Dermatology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [3]Division of Pediatrics, Section of Dermatology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States

Commonly used alopecia severity scales usually rely on having clinicians individuate areas of hair loss and compare their locations and/or sizes in order to map the hair loss appearance to a score, such as overall percent alopecia (*e.g.,* Severity of Alopecia Tool, SALT, score) or hair loss top extent score (*e.g.,* Central Scalp Alopecia Photographic, CSAP, scale). To automate this process, we developed a computational tool that takes standardized photographs of patients' scalps and returns an overall percent hair loss as well as a top extent alopecia shape score as output without any user input. Scores are computed from hair density information extracted automatically at every point of the patient's scalp. This is achieved by combining two complementary neural network architectures that mimic the way clinicians infer hair loss information upon a visual inspection. One network encodes the overall hair density appearance and the other captures local hair texture details. When compared to manually annotated scores, our results showed a less than 6% difference with the SALT score and over 70% exact match on the CSAP scale with the remaining 30% being off by only one grade.

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IL-1β is a potential central mediator to Papulopustular Rosacea pathology as determined by paired transcriptomic and proteomic analysis

J. Harden[1], Y. Shih[2, 3], D. Rajendran[1], J. Xu[3], R. Li[3], H. Hofland[1], A. L. Chang[3]

[1]Research - Immunology, Dermira, Inc, Menlo Park, California, United States, [2]Taipei Medical University-Shuang Ho Hospital, New Taipei City, Taiwan, [3]Department of Dermatology, Stanford University, Redwood City, California, United States

Papulopustular rosacea (PPR) is a chronic inflammatory skin disease characterized by redness, sensitive skin, and inflammatory lesions. Multiple inflammatory pathways have been described to be upregulated in PPR; however, a complete mechanistic understanding of the central mediators of PPR lesions requires further study. To this end, we quantitatively evaluated both the transcriptomic (RNAseq) and proteomic (OLINK® and MSD®) signature of paired non-lesional (NLS) and lesional (LS) PPR biopsy explants (n=5 patients). We identified 92 differentially expressed genes (DEGs) and 20 differently expressed proteins (DEPs) between paired PPR LS and NLS explants. In confirmation of multiple previous studies, we identified a complex inflammatory milieu in PPR lesional explants, with upregulation of many chemokines, cytokines, and tissue remodeling genes and proteins. MAPK and TNF signaling pathways were the most significantly upregulated pathways in LS tissue, and both signaling pathways highlighted IL-1β as a potential central mediator to PPR pathogenesis. To mechanistically evaluate the role of IL-1β in PPR lesions, we exogenously stimulated NLS explants with IL-1β and performed the same paired transcriptomic and proteomic assessment as described above. IL-1β stimulation of NLS biopsy explants resulted in a transcriptomic and proteomic profile similar to LS PPR. This suggests that even in the absence of de novo influx of immune cells, IL-1β is sufficient to drive a PPR-like profile from NLS tissue-resident cells. Although multiple pathways may play a role in PPR pathology, our paired quantitative transcriptomic and quantitative analysis highlights a potential role of IL-1β in PPR pathogenesis and suggests that targeting this cytokine may be a useful approach for treatment of this disease.

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A computational method to automate acne lesion counting

E. Bernardis

Department of Dermatology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, United States

Counting primary acne lesions such as comedones, pustules/papules, or nodules is a first necessary step to measuring acne severity both for patient monitoring and for clinical trials. While clinicians quickly estimate the number of lesions upon a visual inspection, a precise count of all lesions present can be time consuming, and, additionally, for clinical trials, many hours of training are often needed to decrease inter-rater variability. The task becomes even more difficult when working with photographs, as in addition to the multifaceted appearance of acne lesions, challenges are posed by varying illumination and skin color. We present a computational method to automate extraction of all acne lesions from photographs of affected skin via an unsupervised image segmentation algorithm. This is achieved by encoding local geometry cues within a global segmentation framework that allows to simultaneously extract all acne lesions as 'foreground spots' separating them from their common 'skin background'. The resulting segmentation shows over 86% accuracy when compared to lesions outlined manually. By analyzing local shape and color, each area is then classified into one of the primary acne lesions to provide a lesion count which can then be fed as input to an acne severity scale to obtain a severity score.

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Understanding the triggers and consequences of atopic dermatitis

A. Snyder[1, 2], V. Taliercio[1], L. Webber[1], A. Langner[3], B. Rich[3], A. Beshay[1], D. Ose[2], R. Hess[2, 4], J. Rhoads[1], A. M. Secrest[1, 2]

[1]Dermatology, University of Utah, Salt Lake City, Utah, United States, [2]Population Health Sciences and Dermatology, University of Utah, Murray, Utah, United States, [3]School of Medicine, University of Utah, Salt Lake City, Utah, United States, [4]Internal Medicine,, University of Utah, Salt Lake City, UT, Salt Lake City, Utah, United States

Atopic dermatitis (AD) is a common skin condition that can affect many aspects of a patient's health-related quality of life (HRQoL). However, during the time-constrained clinical visit, clinicians rarely capture a complete picture of the impact of AD on the daily home lives of our patients. This study aims to understand triggers and consequences of AD to help clinicians better grasp the full implications of living with AD on an individual and their family members. For this qualitative study, 24 patients with AD and 12 family members participated in focus groups and interviews with questions on how AD affects all aspects of their lives and relationships. Transcripts from the focus groups and interviews were coded by two researchers who then agreed on common themes; these researchers conducted thematic analysis using grounded theory approach. Common themes between triggers and consequences included *clothing*, *fragrance*, *emotions and stress*, *exercise and sweating*. Avoiding these triggers led to negative consequences that affected daily life. These triggers changed how patients made decisions as to which activities or products they would allow for themselves. Common themes in consequences included impacts on *daily activities*, *emotional and mental health*, and *social interactions*. Family members also faced consequences in how AD affected their loved ones' *emotions* and *sleep*. Further, *negative and upsetting* remarks by family members and their *understanding* (or the lack thereof) weighed heavily on the quality of life and relationships for those with AD. AD affects many aspects of patients' lives, both in lifestyle changes for trigger avoidance and in the consequences that stem from managing symptoms and living with a visible and misunderstood skin condition.

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Insights into the far-reaching effects of the itchiness of atopic dermatitis

V. Taliercio[1], A. Snyder[2, 1], L. Webber[1], A. Langner[4], B. Rich[4], A. Beshay[1], D. Ose[2], R. Hess[2, 3], J. Rhoads[1], A. M. Secrest[1, 2]

[1]Dermatology, University of Utah, Salt Lake City, Utah, United States, [2]Population Health Sciences and Dermatology, University of Utah, Murray, Utah, United States, [3]Internal Medicine, University of Utah, Salt Lake City, Utah, United States, [4]School of Medicine, University of Utah, Salt Lake City, Utah, United States

Atopic dermatitis (AD) is a chronic inflammatory skin condition characterized by xerotic and eczematous lesions. Severe itchiness is the cardinal symptom of this disease and significantly impacts individuals' health-related quality of life. This study aimed to qualitatively explore the experiences of participants with AD and their family members who live with the itchiness of AD. Twenty-four participants with AD and 12 of their family members participated in focus groups and interviews. After two researchers independently coded the narratives, we conducted a thematic analysis using a grounded theory approach to arrive at a consensus on the major themes. Five themes emerged: miserable experiences, physical damage from itching, daily activity limitations, limitations on social activities and relationships, and emotional impact. Participants described the itchiness as a *miserable experience* that is very hard to control or cease, often causing continuous disruptive sensations day and night. Itchiness impacts everything participants do, including limitations on *daily activities* such dressing, wearing makeup, and sleeping. The discomfort and embarrassment from scratching in public and the resultant reactions of others hinder *social activities and relationships* and affect the *emotional state* of those with AD. Various emotional reactions were reported, including irritation, embarrassment, and depression. This study, including direct quotes of those with AD and their loved ones, provides valuable insight into the relatively unexplored experience of living daily with AD. Patients with AD face many hurdles in dealing with itchiness, and the wide-ranging effects of itchiness need to be better understood by clinicians who treat those with AD.

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When itchiness is not enough: Understanding the impact of pain on experiences with atopic dermatitis

A. Snyder[2, 1], V. Taliercio[2], L. Webber[2], A. Langner[3], B. Rich[3], A. Beshay[2], D. Ose[1], R. Hess[4, 1], J. Rhoads[2], A. M. Secrest[2, 1]

[1]Population Health Sciences and Dermatology, University of Utah, Murray, Utah, United States, [2]Dermatology, University of Utah, Salt Lake City, Utah, United States, [3]School of Medicine, University of Utah, Salt Lake City, Utah, United States, [4]Internal Medicine, University of Utah, Salt Lake City, Utah, United States

Pain, separate from pruritus, is a relatively unappreciated, and poorly understood, symptom of atopic dermatitis (AD) that can affect patients' health-related quality of life (HRQoL). This qualitative study involved 24 patients with AD and 12 family members who participated in focus groups and interviews aimed at understanding the impact of pain on AD patients and their family members to capture how this symptom affects HRQoL. Thematic analysis was conducted using grounded theory approach. Two researchers independently coded the transcripts and reached a consensus on major themes. One theme was that itchiness and pain can merge together; pain was often caused by or otherwise associated with itchiness and may result from open sores and denuded skin from scratching. A second theme that emerged was that pain was most often referred to as a burning sensation, though other sensations were reported, including mild persistent discomfort, stinging, and stabbing. A third theme was that pain affected daily life, including impacting what clothes those with AD could wear and daily activities like sleep, social activities, and relationships. The location of the painful areas also limited activities, such as sex. Finally, a fourth theme that emerged was that some with pain from AD tried to control their pain with over-the-counter and prescription treatments, some of whom felt these were necessary to maintain their daily routines. Pain can significantly impact the HRQoL of AD patients and their loved ones and should be discussed in clinic visits when caring for AD patients.

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Quality of life among family members of patients with atopic dermatitis and psoriasis

V. Taliercio[1], A. Snyder[2], L. Webber[1], A. Langner[3], B. Rich[3], A. Beshay[1], D. Ose[2], R. Hess[2, 4], J. Rhoads[1], A. M. Secrest[1, 2]

[1]Dermatology, University of Utah, Salt Lake City, Utah, United States, [2]Population Health Sciences and Dermatology, University of Utah, Murray, Utah, United States, [3]School of Medicine, University of Utah, Salt Lake City, Utah, United States, [4]Internal Medicine, University of Utah, Salt Lake City, Utah, United States

Chronic inflammatory skin diseases like psoriasis (PsO) and atopic dermatitis (AD) severely impact the quality of life (QoL) of patients. However, the effect of these diseases can extend beyond the affected patients and diminish their families' QoL as well. We held focus groups and interviews with 23 family members–most commonly significant others–twelve had a family member with AD and 11 had a family member with psoriasis to understand these impacts. After two researchers independently coded the transcripts, we conducted a thematic analysis using a grounded-theory approach to arrive at a consensus on the major themes. Five themes emerged: *Physical health*–some family members reported waking up throughout the night due to loved ones' itchiness, leading to fatigue and poor concentration. *Psychological health*–family members frequently feel sad, frustrated, irritated, worried, or embarrassed about their loved ones' AD or PsO. *Dependence*–some patients require extra attention for their skin condition, needing family members to help apply treatments, give medication, and take them to appointments. *Social relationships*–family members may do fewer activities with the patient or need to be selective about activities. Personal relationships, as well as sexual relations, can be affected. *Shared environment*–since patients and their family members share the same home environment, AD and PsO can affect frequency of household chores and alter the home environment to reduce patients' exposure to possible triggers and irritants. Chronic skin conditions affect many aspects of the lives of loved ones, and dermatologists should be aware of these impacts when making treatment decisions.

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Investigating T cell phenotype and function in delayed-type drug hypersensitivity reactions

G. Romar[1], P. Hsieh[1], M. Liang[2], B. Schmidt[3], R. Foreman[4], S. J. Divito[5]

[1]Dermatology, Brigham & Women's Hospital, Boston, Massachusetts, United States, [2]Dermatology, Boston Child. Hospital, Boston, Massachusetts, United States, [3]Pathology, Boston Child. Hospital, Boston, Massachusetts, United States, [4]Pathology, Ma. Gen. Hospital, Boston, Massachusetts, United States, [5]Dermatology, Brigham & Women's Hospital, Boston, Massachusetts, United States

Delayed-type drug hypersensitivity reactions (DHR) range in severity from mild rash to severe sloughing of skin and mucosal surfaces, with or without internal organ involvement. Pathobiology is poorly understood including the phenotype and function of T cells mediating DHR. We performed transcript analysis of 186 genes using Nanostring on FFPE skin samples from adult and pediatric patients with morbilliform drug eruption (MDE) (n=7), Drug Rash with Eosinophilia and Systemic Symptoms (DRESS) (n=6), and Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN) (n=14). The diagnosis in all cases was clinically and pathologically confirmed. Only SJS/TEN > 10% TBSA blistered skin were included to reduce diagnostic error. Healthy skin served as controls (n=11). Preliminary results revealed significantly increased CD3 and CD8, and central memory T cell markers CD45RO, CD62L, and CCR7 in SJS/TEN and DRESS compared to healthy controls, but not in MDE samples. Skin resident memory T cell markers CD69 and CD103 were not elevated in any DHR group. All three DHR demonstrated a Th1/Tc1 skewing. Microscopy confirmed that the majority of CD3+ T cells were CD45RO in all three DHR yet a minority of T cells were CD103+. CD3+ T cells consisted of both CD4+ and CD8+ subsets, were largely CLA+, and were ab type, not gd type T cells. A group of patients with MDE were identified that were profoundly lymphopenic, indicating that they had (near) absent circulating T cells. These lymphopenic skin samples contained CD4+ and CD8+ T cell subsets that were predominantly CD45RO+ and CLA+, and were of equivalent numbers as healthy controls. These data suggest that skin resident memory T cells can mediate MDE, but that central memory T cells are recruited to skin in SJS/TEN and DRESS. These findings may explain why MDE is skin limited while SJS/TEN and DRESS involve multiple tissues/organs.

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Novel injectable coolant for reducing cutaneous nerve fiber density

S. Moradi Tuchayi, Y. Wang, I. Pence, C. Evans, R. Anderson, L. Garibyan

Wellman Center for Photomedicine, Massachusetts General Hospital; Department of Dermatology, Harvard Medical School, Boston, Massachusetts, United States

Postherpetic neuralgia (PHN) is a dermatologic disorder with no established cure. The distinct dermatomal distribution of PHN makes the peripheral nerve supplying the dermatome and its distal cutaneous branches the ideal therapeutic target; however, the short acting nature of current medications limits the use of this localized approach. Herein we demonstrate the effects of a drug-free, injectable coolant with long-lasting pain suppression capabilities that could potentially be used for treatment of PHN. We have previously reported reduced cutaneous nerve fiber density following topical cooling with cryolipolysis treatment. From here we went on to develop an injectable coolant to target nerves at any anatomic location accessible by a needle. We showed that injection of coolant around the rat sciatic nerve leads to local disruption of myelin. This in turn leads to decrease in nocifensive function for up to 60 days. In this study, using the rat sciatic nerve, we examined the mechanism of reduced nociceptive response after coolant injection. We investigated the effects of the injectable coolant on sciatic nerve branches distal to the injection site and on cutaneous nerve fibers using Coherent anti-Stokes Raman scattering (CARS) microscopy and immunofluorescence (IF) staining. CARS showed myelin degradation in branches distal to the injection site. Corrected correlation parameter index, an indicator for organization of myelin structure, decreased in fibular branch from 0.86±0.08 at baseline to 0.40±0.22 (P<0.0001) at day 14 post injection. Myelinated dermal nerve fiber density was quantified in skin of the hind paw innervated by the sciatic nerve. IF staining showed decreased density of myelinated dermal nerve fibers from baseline value of 56±3/mm^2 to 22±3/mm^2 (P<0.0001) at day 14. Thus, coolant injection reduces pain by decreasing cutaneous nerve fiber density. Although more work needs to be done, we hope this could potentially be used to treat PHN associated pain.

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Targeted IL-17RA antagonism ameliorates histological and transcriptomic features of hidradenitis suppurativa: A proof of concept study

J. W. Frew, K. Navrazhina, S. Garcet, J. Krueger

Laboratory of Investigative Dermatology, Rockefeller University, New York, New York, United States

Hidradenitis Suppurativa is an autoinflammatory disorder of keratinization with a major inflammatory contribution from the Th17 axis. We have previously shown that in addition to previously described IL-17A & IL-17F; IL-17C is found at higher levels by both IHC and rt-PCR in lesional HS skin. We asked whether the IL-17 axis mediates disease activity by using an IL-17RA antagonist Brodalumab to examine histological and transcriptomic features of disease in 10 individuals with HS. Biopsies as per previously published consensus criteria were taken at Baseline and at Weeks 4 and 12 after treatment with an IL-17RA antagonist. Elevated levels keratinocyte derived genes (S100A9: logFCH =10.19; CXCL1: logFCH=5.46; CXCL8: logFCH=5.2), B-cell associated genes (IGHG3: logFCH=14.49) and cell trafficking chemokines (CXCl13: logFCH=7.62) were significantly elevated compared to normal site matched controls. IL-17RA antagonism resulted in statistically significant reductions in CXCL1, CXCL8, IL-36a to the level of unaffected HS patient tissue as confirmed by rt-PCR by Week 4. Histology demonstrates a significant reduction in CD11+ and CD3+ dermal cells (measured by quantitative IHC) by week 4 (p<0.01) continuing on to Week 12. Significant reductions in transepithelial neutrophil trafficking (measured by quantitative IHC) into dermal epithelialized tunnels was also noted. Morphological changes in epidermal psoriasiform hyperplasia were seen by Week 12 of therapy. Epidermal psoriasiform hyperplasia and trans-epithelial neutrophil migration in HS epidermis and epithelialized tunnels have parallels with Psoriasis Vulgaris and Generalized Pustular Psoriasis. IL17RA antagonism demonstrates rapid (by week 4) reduction in expression of B and T cell trafficking chemokines as measured by RT-PCR and significant alterations in epidermal morphology by Week 12. This highlights the role of IL-17 mediated feed-forward inflammation as in psoriasis and transepithelial neutrophil trafficking in dermal tunnels in HS.

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Antisynthetase syndrome and dermatomyositis immunophenotyping

J. Patel[1, 2], A. Ravishankar[1, 2], S. Maddukuri[1, 2], M. Bashir[1, 2], C. Bax[1, 2], V. Werth[1, 2]
[1]CMCVAMC, Philadelphia, Pennsylvania, United States, [2]Derm, U Penn, Philadelphia, Pennsylvania, United States

Antisynthetase syndrome (AS) and Dermatomyositis (DM) are rare inflammatory autoimmune diseases that some consider as separate entities. AS is poorly defined and some patients have skin lesions consistent with DM. To investigate differences between AS and DM relative to control (HC), we identified immunophenotypes. 3 AS, 3 DM, and 3 HC formalin-fixed, paraffin-embedded (FFPE) samples obtained from back, arm, or leg were stained via immunofluorescence for MxA and IFNβ. Three 20x regions were acquired on Nikon Eclipse. A panel of 35 metal conjugated antibodies separately was stained and areas of 500x800 µm (ROI) were ablated at 200Hz on the Hyperion Imaging System(Fluidigm). Cell segmentation was performed in Visiopharm. Mean pixel intensities (MPI) per cell were analyzed using histoCAT and ImageJ. One-way ANOVA and Dunn's Multiple comparison test was performed with all values reported as AS/DM/HC mean± SEM. Skin lesions of AS and DM patients MPI did not differ significantly in MxA (15.6±1.0/12.7±2.7/1.4±0.5; $p<0.05$ with AS vs DM $p>0.05$) or IFNβ expression (18.4± 2.4/21,20.9± 1.2/1.3±0.2; $p<0.05$ with AS vs DM $p>0.05$). AS and DM lesions did not differ in the following cells/ROI except CD4: CD4 (70± 42/178± 28/11±2; AS vs DM $p<0.001$), CD8 (49± 37/55±21/20±12), MAC387 (6± 3/22± 10/4±2), pDC (4± 1/14± 7/5±1), CD11C+ (25± 8/44± 11/11±6), mast (14± 5/21± 12/25±11), and FOXP3+ CD4(18±14/64± 32/5±2); all $p<0.001$ with AS vs DM $p>0.05$ except CD4). AS and DM lesions also did not differ in MPI of key inflammatory pathways: pSTING (7.1± 1.4/8.2± 1.3/1.5±0.6), IL31 (0.87± 0.2/1.0± 0.1/0.1±0.02), IFNγ (1.5± 0.38/2.8± 0.2/0.3±0.08), IL4 (4.4± 0.7/4.6± 0.5/0.8±0.1), and IL17 (1.0± 0.2/1.2± 0.2/0.2±0.01); $p<0.0001$ with AS vs DM $p>0.05$. Patients with both AS and DM lesional skin did not differ from DM alone in immunophenotyped or type I IFN protein. AS with DM skin lesions were not differentiated from DM, as the pathogenic cellular characteristics between the two were the same.

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Machine learning for measuring scratching in atopic dermatitis using a skin-mounted, soft and wireless sensor: Model selection, feature extraction, and training set performance

K. Chun[2], J. Kim[3], H. Jo[3], J. Lee[3], R. Lee[1, 5], M. Zhang[1, 5], K. Lee[3], J. Chang[3], A. Fishbein[4], A. S. Paller[1], J. Rogers[5], S. Xu[1, 5]
[1]Dermatology, Northwestern University, Chicago, Illinois, United States, [2]Electrical and Computer Engineering, The Univ of Texas at Austin, Austin, Texas, United States, [3]Sibel Health, Evanston, Illinois, United States, [4]Pediatrics Allergy and Immunology, Northwestern Univ School of Med, Chicago, Illinois, United States, [5]Northwestern Univ CBIE, Chicago, Illinois, United States

Pruritus is a hallmark of atopic dermatitis (AD). Measuring scratching activity, a natural response to itch, is one potential surrogate marker for assessing pruritus. However, current methods such as actigraphy to objectively quantify pruritus, particularly in children, are limited due to confounding from non-scratching hand motions and omitting fine finger scratching. In order to more accurately measure scratching, we present the use of a small, skin-mounted soft flexible sensor (4.5 cm x 2.1 cm, 7 g) with an embedded high frequency 3-axis (0-2000 Hz) accelerometer in direct mechanical communication with the dorsum of the hand to simultaneously capture motion and acoustic signatures of scratching. We generated 9,600 training data sets of scratching and non-scratching behavior across multiple body locations from healthy subjects (n=10). We then tested and compared the performance of three machine learning models (random forest, gradient boosting, and adaboost) and logistic regression to identify the optimal classifier. The algorithm was evaluated by applying 5-fold cross validation for 100 iterations with frame randomization in each iteration. The gradient boosting classifier demonstrated the most accurate performance (95% precision, 96% recall, and 95% specificity). Both motion (x-axis 10-80 Hz; z-axis 20-200 Hz) and acoustic (z axis >200 Hz) were represented as the 3 most important features for the model, suggesting the importance of capturing both signatures. Ongoing studies will validate the performance of this sensor and algorithm with pediatric AD patients.

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'DermAnnotation' is All You Need; methodology to transfer knowledges of dermatologists to artificial intelligence

K. Shido[2], E. Kondo[1], K. Kojima[3], K. Yamasaki[2], S. Aiba[2]
[1]Electrical, Information, and Physics engineering, Tohoku university, Sendai, Japan, [2]Dermatology, Tohoku university, Sendai, Japan, [3]Tohoku Medical Megabank Organization, Tohoku University, Sendai, Japan

Potential of artificial intelligence (AI) for image analysis is proved year by year in many fields including medical situation. Since most of skin diseases reveal their symptoms on skin surface, AI application to dermatology clinic is highly expected. However, even though lesions are on skin, anonymous image collection of skin diseases cannot develop accurate dermatology AI or lead misdiagnostic AI to make confusion in dermatology in worst scenarios, because dermatological diagnosis is a sort of state-of-the-art inspection technique. Therefore, in order to create precise diagnostic AI, a large amount of image data, which are annotated by skilled dermatologists, are required. To support dermatologists make annotation on skin disease images, we developed 'DermAnnotation', an AI application dedicating to annotate types of skin eruptions. 'DermAnnotation' have learned types of skin eruptions, and is programed to anticipate area of skin eruptions on images and to propose the possible types of skin eruptions on skin images. Dermatologists just check whether proposed types of skin eruptions are correct or not. If dermatologists click areas of the proposed types of skin eruptions are not correct, 'DermAnnotation' further proposes another possible type of skin eruption. Moreover, 'DermAnnotation' simultaneously runs machine learning on images that dermatologists suggested annotation. By using 'DermAnnotation', dermatologists can save time by not annotating every skin eruption, and dermatologists also can educate 'DermAnnotation' to more accurately annotate skin eruptions. Thus 'DermAnnotation' facilitates to transfer the dermatologists' knowledge to AI because 'DermAnnotation' support dermatologists to give initial annotations on skin disease images.

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Treatment of Netherton syndrome patients with Ixekizumab: A case series

C. Barbieux[1, 3], M. Bonnet des Claustres[1, 3], M. Bagot[2], E. Bourrat[2], A. Hovnanian[1, 3]
[1]U1163, INSERM, Paris, France, [2]Dermatology, Hospital Saint-Louis, Paris, France, [3]Institut Imagine, Paris, France

Netherton Syndrome (NS) is a rare autosomal recessive skin disease due to loss-of-function mutations in *SPINK5* encoding LEKTI. NS combines a severe skin barrier defect with superficial scaling, skin inflammation, and multiple allergies associated with high serum IgE levels. Increased IL-17 pathway has been reported in NS patients' skin and peripheral blood, identifying IL-17 as a potential therapeutic target. We report compassionate treatment of 3 adult NS patients with the humanized anti-IL-17 monoclonal antibody ixekizumab (IXE). Patients received a 160mg starting dose (W0), then 80mg 2x/month for 12 weeks (induction phase) followed by 80mg 1x/month for 12 weeks (maintenance phase) (W24). Efficacy was assessed by clinical and biological readouts. The treatment was well tolerated. DLQI, 5-D pruritus, IASI-E and IASI-S scores showed a rapid and significant decrease during the induction treatment, which were maintained until W24. During the induction phase, the 3 patients reduced their use of topical steroids, could wear sleeveless clothes and did not experience new acute flare. However, during the maintenance phase, they experienced some new attenuated flares which led to discontinuation of the drug after W24. Biological assessments showed a significant decrease in neutrophil infiltration in patient 1 and 3 skin. The IL-17-induced chemokines CCL20 and CXCL13 serum levels were increased at baseline, with CCL20 levels increasing and CXCL13 values decreasing during treatment. Pro-allergic cytokines TARC and MDC serum levels were increased and remained unchanged during treatment. Peripheral lymphocyte phenotyping showed increased and subnormal Th17 and Th2 subsets, respectively, at baseline, with a trend to increase at W24. In contrast, Tregs which were initially significantly increased, normalized at W24. Altogether, our data show that IXE treatment induced evident clinical benefits. Although attenuated flares occurred during maintenance, the patients reported persistent improvement of their quality of life.

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Major producers of IFNg and CB2 receptor distribution in dermatomyositis

S. Maddukuri[1, 2], J. Patel[1, 2], C. Bax[1, 2], M. Wysocka[1], V. Werth[1, 2]

[1]Dermatology, University of Pennsylvania, Montville, New Jersey, United States, [2]Dermatology, Corporal Michael J Crescenz Veterans Affairs Medical Center, Philadelphia, Pennsylvania, United States

Dermatomyositis (DM) is an autoimmune disease that affects skin and muscle. Type I IFNs, such as IFNb, are implicated in the pathogenesis of DM, but little is known about IFNg. Our prior studies showed a reduction of IFNg after treatment with Lenabasum, a nonpsychoactive CB2 receptor (CB2R) agonist. We aim to identify major producers of IFNg and understand the distribution of CB2R in the blood vs skin of DM patients. Flow cytometry was performed on PBMCs from 3 healthy controls (HC) and 7 DM patients. In HCs, we found no significant difference in the percentage of CD8 (31%) and CD4 cells (26%) producing IFNg ($p=0.70$). In DM patients, we found a greater percentage of CD8 cells (54%) producing IFNg compared to CD4 (15%) ($p=0.004$). There was no significant difference in lymphocyte distribution, with respect to the percentage of CD4 and CD8 cells, between HC and DM patients. CD8 cells also produced more IFNg in the skin of DM patients compared to HC, according to image mass cytometry (IMC) findings ($p=0.03$). In DM patient blood, CB2R expression is significantly greater on myeloid (mDC) and plasmacytoid (pDC) dendritic cells when compared to minimal expression on CD4 and CD8 cells ($p<0.001$). There is no significant difference in CB2R expression between HC and DM in CD4 and CD8 cells; however, there was a difference in mDCs and pDCs ($p<0.001$). Although CB2R expression on lymphocytes, mDCs, and pDCs is generally <10% in the blood, results from flow cytometry performed on cells eluted from a lesional DM skin biopsy show significant CB2R expression on CD4 cells (39%) and on mDCs (28%). Presence of CB2R on CD4, CD8, and mDCs in DM skin has also been confirmed by IMC. This suggests differential CB2R expression on lymphocytes and dendritic cells in the blood vs skin of DM patients. Increased CB2Rs in the skin could indicate increased action of Lenabasum in skin compared to blood. Increased CB2Rs on lymphocytes could indicate a preferential action of Lenabasum on lymphocytes compared to dendritic cells.

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Emulating IL-17 – CCL20 axis to identify surrogate markers of psoriasis using 3D psoriasis tissue model

S. Ayehunie, T. Landry, Z. Stevens, A. Armento, M. Klausner

MatTek Corporation, Ashland, Massachusetts, United States

Psoriasis is a chronic, immune mediated inflammatory skin disease marked by hyperproliferation, abnormal keratinocyte (KC) differentiation, and leukocyte infiltration. Interleukin (IL)-17 – CCL20 axis has been identified as a critical pathway In the Pathogenesis of psoriasis. Overexpression of IL-17A has been implicated with epidermal hyperproliferation and a robust inflammatory response. Since T cells, particularly Th17 cells (IL-17 producing cells), are implicated in inflammatory skin diseases and because targeting IL-17 has been a promising approach in clearing moderate to severe plaque psoriasis, we investigated the role of T cell cytokines such as IL-17 alone or in combination with interferon (IFN)-γ (Th1 cytokines) in exacerbating inflammatory responses using a reconstructed 3D human psoriatic tissue model. The reconstructed psoriatic organotypic human cell-based tissue model was exposed systematically to IL-17 / IFN-γ for up to 96 hr (4X exposure) to examine keratinocyte responses. The results showed that: 1) IL-17 increases the Th-17 cell chemoattractant (CCL-20), the neutrophil chemoatractant (IL-8), and the anti microbial peptides (HBD2 and elafin (PI3) by 6.9, 2.8, 2.4, and 3.4 fold, respectively. 2) IFN-γ induces overexpression of the chemoattractant for activated T cells (CXCL11), IL-8, and TNF-α by 324, 3.7, and 2.2 fold, respectively. 3). IL-17 and IFN-γ exacerbate inflammation synergistically by increasing expression levels of the neutrophil chemoattractants (CXCL5, IL-8), HBD2, PI3, and TNF-α by > 3 fold. Here, we show that a self-sustaining inflammatory feedback loop that involves the T-cell cytokines (IL-17, IFN- γ) and KC/fibroblast derived innate immune responses of CCL20, CXCL5, HBD2, PI3, and IL-8 is established. Release/expression level of CCL-20, IL-8, IL-6, and CXCL-5 can serve as surrogate markers to examine effect of anti-inflammatory drug responses. In conclusion, these surrogate markers can be used as valuable tools to screen new IL-17 inhibitors for the treatment of moderate-to-severe psoriasis.

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Metabolomics: Unlocking the blueprint of skin aging in Caucasian and Chinese women

K. Corallo[1], E. Pelle[1], M. Morse[3], N. Pernodet[1, 2]

[1]Research & Development, The Estee Lauder Companies, Melville, New York, United States, [2]The Estee Lauder Companies, Melville, New York, United States, [3]Clinical Operations, The Estee Lauder Companies, Melville, New York, United States

For more than a decade, we have demonstrated the importance of circadian rhythm for skin homeostasis and repair. To advance this research, our objective was to identify metabolite profile changes in skin over a 12-hour period, and to understand how age and treatment influence these changes in both Caucasian and Chinese women. An emerging technique for skin diagnostics is metabolomics, or the study of metabolites, which are small molecule substrates, intermediates, and end-products of biological processes. The metabolite profile reflects the influence of genetic and environmental factors (e.g., microbiome and pollution), which is critical when evaluating skin status, as the skin is in direct contact with the external environment. This precision tool gives a snapshot of skin condition relative to time and the environment. Using non-invasive tape-strips, we sampled young (20<age<25 years) and mature (60<age<70 years) female facial skin in both populations at 7AM and 7PM. Samples were subjected to untargeted metabolomic profiling via LC/MS. Results were compared to evaluate circadian-, age-, and treatment-related changes *in vivo*.. We identified hundreds of metabolites – 300 in Caucasian women and 272 in Chinese women – which showed significant changes with age, as well as in the key metabolites measured in the morning versus evening. Furthermore, metabolites associated with damage accumulation were higher in the aging population but were reduced to levels closer to those of young participants following an 8-week treatment. To our knowledge, this is the first temporal evaluation of the facial skin metabolome, and the first study to demonstrate the recovery of metabolites in aging participants through a skincare treatment.

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Rapid capture, extraction, and analysis of sweat samples for quantification of inflammation biomarkers using a novel wearable microfluidic system

M. Zhang[1, 2, 4], A. Aranyosi[2, 3], S. Lee[2, 3], J. Model[2, 3], A. Leech[2, 3], W. Li[2, 3], S. Chen[2, 3], J. Rogers[2, 3], A. S. Paller[1], R. Ghaffari[2, 3], S. Xu[1, 2]

[1]Dermatology, Northwestern Univ School of Med, Chicago, Illinois, United States, [2]Northwestern Univ CBIE, Chicago, Illinois, United States, [3]Epicore Biosystems, Cambridge, Massachusetts, United States, [4]Medical Scholars, Vanderbilt Univ School of Med, Nashville, Tennessee, United States

Eccrine sweat is a rich, accessible biofluid comprised of a mixture of electrolytes, metabolites, hormones, and cytokines. Collection of sweat typically relies on absorbent pads that are incompatible with remote monitoring and deployment in field settings due to contaminants in the pads, long-term exposure of sweat to skin, and the need for centrifuges for extraction. Recent advances in the development of wearable microfluidic sensors that capture and analyze sweat dynamics offer new possibilities for tracking sweat biomarkers. This study presents a novel, wearable microfluidic device (the 'Discovery patch') that carefully captures small sweat volumes (10-150 µL), quantifies rates of excretion, and supports extraction for external lab analysis. Discovery patches were skin-mounted on the forearms of healthy subjects (n=10) who perspired in an environment chamber (40-45°C). Sweat captured in the Discovery patch was extracted and stored in cryovials, frozen, then analyzed by immunoassay to quantify cytokines (IL-1α, IL-1RA, and IL-8) across 3 anatomic regions (upper forearm and bilateral lower forearms). IL-1α and IL-1RA were present at significant quantities (2.0±1.6 and 2.9±2.9 ng/mL, respectively), whereas IL-8 was near the noise floor (1.1±0.7 pg/mL). Moreover, IL-1α and IL-1RA concentrations were independent of sweat rate ($R^2=0.1$ and $R^2=0.02$) and anatomic region ($p=0.93$ and $p=0.99$), and demonstrated diurnal fluctuations ($p<0.01$ and $p<0.05$, respectively), a striking phenomenon reported previously in serum tests. These results show that the Discovery patch is a robust tool for non-invasive tracking of inflammation biomarkers. Future directions include assessment of sweat cytokines in atopic dermatitis patients to locally monitor flare-ups and enable individualized tracking of patient responses to drug treatment.

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Interleukin-9 promotes malignant T cell survival by inhibiting oxidative stress and lactic acidosis in cutaneous T cell lymphoma

S. Kumar[1], B. Dhamija[1], S. Marathe[1], A. Karulkar[1], N. Sharma[2], H. Jain[2], R. Purwar[1]

[1]Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai, India, [2]Tata Memorial Hospital, Mumbai, India

Cutaneous T cell lymphoma (CTCL) represents a distinct subgroup of non-Hodgkin lymphomas of mature skin tropic malignant T cells. Although the immune dysregulation is critical for the pathogenesis of lymphoma, the precise mechanism of CTCL pathogenesis remains unknown. Recently, few studies reported the defective T cell function during the onset and progression of certain types of lymphoma. However, the presence of interleukin-9 (IL-9) producing Th9 cells and more importantly, the roles of IL-9/Th9 axis in tumor cell metabolism and survival remain unexplored. With this study, we performed multidimensional blood endotyping in large cohorts of CTCL patients and revealed distinct immune hallmarks of the disease. Importantly, there was a higher frequency of “skin-homing” Th9 cells in CTCL patients. However, advanced-stage CTCL patients had severely impaired frequency of skin-homing Th1 and Th17 cells, indicating attenuated anti-tumor immunity. Interestingly, T cells of CTCL patients express IL-9 receptor (IL-9R), and there was negligible IL-9R expression on T cells of healthy donors. Functionally, IL-9/IL-9R interaction on CD3+ T cells of CTCL patients reduced oxidative stress, lactic acidosis, and apoptosis and ultimately increased their survival. In conclusion, our data suggest the critical role of the IL-9/Th9 axis in CTCL pathogenesis and strategies targeting Th9 cells might harbor great potential in developing robust CTCL therapy.

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Buddy relationships in dermatologic excisions: A novel one-to-one peer mentorship program for patients with skin cancer

S. Zullo, C. Miller, T. Shin, J. Etkzorn, J. Sobanko

Dermatology, University of Pennsylvania, Philadelphia, Pennsylvania, United States

Facial skin cancers impair psychosocial health. Peer mentorship programs provide emotional and social support for many oncologic patients but have not been studied in skin cancer. This pilot study evaluated feasibility and satisfaction with a peer mentorship program for facial skin cancer patients treated with Mohs micrographic surgery (MMS). An IRB-approved, randomized controlled trial was performed in an academic center from 2018 to 2019. Patients one year removed from MMS treatment of a facial skin cancer were enrolled and trained as mentors. Patients with a new facial skin cancer were randomized into control or mentee groups at a visit before or on the same day as their Mohs surgery. Mentees were paired with a mentor and asked to regularly communicate before and after their surgery as well as receiving standard physician counseling. Controls received only standard physician counseling. Post-study surveys with 17 feedback indices pertaining to satisfaction and mentor pairing qualities were collected 3 months following MMS. Descriptive statistics and ANOVA testing evaluated and determined statistically significant ($P<0.05$) differences in program satisfaction scores. 11 mentors, 13 pre-surgery mentees, 11 same-day surgery mentees, and 25 controls were enrolled. Overall program feedback scores were favorable (mean 5.2±1.1 on a 6-point Likert scale). Significant ($p<0.05$) differences in mean scores were observed between pre-surgery and same-day surgery mentee groups on 9 different indices. Application of this novel peer mentorship program for MMS skin cancer patients was well-received with excellent overall patient satisfaction scores. The timing of mentorship initiation significantly influenced patient perception of the program with participants who received pre-surgical intervention reporting greater program satisfaction on individual feedback indices. This low-cost, easily implemented mentorship model was favored by mentors and mentees and could prove helpful for supporting skin cancer patients receiving MMS treatment.

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Differentially expressed plasma proteins in pityriasis rubra pilaris patients treated with ixekizumab

J. L. Strunck[1, 2], D. Haynes[1], C. Topham[2], A. Ortega-Loayza[1], T. M. Greiling[1]

[1]Oregon Health and Science University, Portland, Oregon, United States, [2]University of Utah School of Medicine, Salt Lake City, Utah, United States

Pityriasis Rubra Pilaris (PRP) is a rare and debilitating cutaneous disease characterized by widespread red scaly plaques, follicular papules, and palmoplantar keratoderma. The pathogenesis of PRP is poorly understood, although overexpression of Th17 cytokines have been reported suggesting an inflammatory pathogenesis that may share features with psoriasis. In this study, we used OLINK proximity extension assay technology to quantitate 92 plasma inflammatory proteins of 11 PRP patients treated with ixekizumab (NCT03485976). Samples were obtained at baseline (week-0) and the final study visit (week-24). Comparisons of plasma protein concentrations were made between pretreatment and posttreatment samples and between responders (as defined by a ≥50% improvement in Psoriasis Area and Severity Index [PASI50]) and nonresponders. P-values were adjusted for multiple hypotheses. Of the 92 proteins analyzed, we identified a paradoxical 5.7-fold upregulation of IL-17A at week-24 compared to baseline ($p<0.000001$), in contrast to previous reports of decreased plasma IL-17A gene expression in patients treated with ixekizumab for psoriasis. When stratified by treatment response status, responders had significantly lower levels of IL-17 and TNF family cytokines, including IL-17C ($p<0.0001$) and TNF ($p=0.001$), at week-24 compared to nonresponders, suggesting that additional inhibition of the Th17 axis may be required to treat recalcitrant cases of PRP. This observation was supported clinically by a nonresponder patient who had treatment success with an increased dose of ixekizumab after trial completion. To our knowledge, this is the first quantitative protein analysis of PRP. These findings support prior studies implicating dysregulation of the Th17 axis in PRP and may help elucidate relevant pathways to target and better treat PRP. Further research is warranted to compare samples to a control population, and to compare these systemic biomarkers to local changes in skin samples.

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Next-generation sequencing and computational modeling identifies the genomic signature of isotretinoin in acne patients

Z. Nolan, A. Schneider, Z. Cong, A. Longenecker, D. Thiboutot, A. Nelson

Dermatology, Penn State College of Medicine, Hershey, Pennsylvania, United States

Despite nearly 40 years of clinical use and research, the mechanism of action of isotretinoin in the treatment of severe acne is still relatively unknown. Utilizing next-generation sequencing and computational modeling, we investigated the genomic changes induced by isotretinoin treatment in both cell lines and in patient skin samples. Sebocytes (SEB-1), keratinocytes (NHEK), and fibroblasts (NHDF) were treated with either vehicle or 0.1μM isotretinoin for 72hrs. Skin punch biopsies were collected from non-lesional back skin of acne patients prior to isotretinoin treatment, after 1, 8, or 20 weeks of treatment, and 6 months after cessation of therapy ($n \geq 6$/time point). RNA-sequencing was performed using the Illumina HiSeq 2500, quality filtered, aligned to the human reference genome GRCh38 using STAR, and quantified using RSEM. Differentially expressed genes (DEGs) were determined using a limma+voom based pipeline and pathway analyses were completed with Ingenuity Pathway Analysis (IPA). Within each cell type isotretinoin induces a specific gene signature and uniquely impacts retinoid metabolism, keratin production, and estrogen signaling. The p53 canonical pathway ($p=2.47\times10^{-6}$) and gene regulation by tretinoin ($p=4.99\times10^{-26}$) are significantly increased and unique to sebocytes. As expected, DEGs derived from patient skin demonstrated a gene expression pattern linked to duration of isotretinoin treatment. Histone modification, fatty acid oxidation, retinoid metabolism, and immune regulation pathways are significantly impacted at 1 week ($p<0.05$) but not changed at later time points. Lipid metabolism ($p=.03$) and cholesterol biosynthesis ($p=.007$) pathways are significantly decreased at 8 weeks and 20 weeks of treatment. This combination of in vitro and clinical samples allows for unprecedented insight into the genomic effects of isotretinoin bringing us closer to understanding the mechanism of action in patients.

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Molecular analysis of primary melanoma T cells identifies patients at risk for metastatic recurrence

W. Pruessmann[2], J. Rytlewski[1], J. Wilmott[3], M. C. Mihm[2], G. H. Attrill[3], B. Dyring-Andersen[2], P. Fields[1], Q. Zhan[2], A. J. Colebatch[3], P. M. Ferguson[3], J. F. Thompson[3], K. Kallenbach[4], E. Yusko[1], R. A. Clark[2], H. Robins[1], R. A. Scolyer[3], T. S. Kupper[2]

[1]Adaptive Biotechnologies, Seattle, Washington, United States, [2]Brigham Health/Harvard, Boston, Massachusetts, United States, [3]MIA, Sydney, New South Wales, Australia, [4]Zealand University, Roskilde, Denmark

Primary melanomas >1 mm thickness can be cured by resection, but may recur metastatically. We assessed the prognostic value of “T cell fraction” (TCFr) and “repertoire T cell clonality”, as measured by high-throughput sequencing of the T cell receptor beta-chain (TCRB), in primary melanomas (n=377) from patients followed five years. We included T2-T4 melanomas. All samples were scored for TIL content by histopathology. To assess T cell subsets, we further stained sections by multiplex immunohistochemistry (mIHC) for CD3, CD8, CD4, FoxP3, CD39 and CD103. TCFr accurately predicted progression-free survival (PFS) and was fully independent of melanoma thickness, ulceration, mitotic rate, or age, all of which were co-dependent predictive variables. TCFr was second only to tumor thickness in its predictive value, using a gradient-boosted model. A cut-off of 20% TCFr performed best (HR 0.39 high-vs-low TCFr, p = 1.28E-5). Only 23.4% of T3 melanomas with ≥ 20% TCFr progressed in five years, compared to 54.2% progression of those with < 20% (HR 0.3, p = 0.0076). A TCFr >20% was protective regardless of tumor ulceration or mitotic rate. Patients with resected regional nodal disease and high TCFr in the primary had a markedly decreased risk of progression (HR 0.37, p=0.0046) and had a PFS rate similar to that of patients without nodal disease and a low TCFr. TCFr high and TCFr low samples had comparable numbers of CD4+, CD8+ and “tumor-specific” CD8+CD39+CD103+/- T cells by mIHC. Finally, TCFr by HTS was more accurate than conventional histopathological TIL grading to predict 5-year PFS (AOD, p = 0.0055). Our study suggests that a successful T cell-mediated antitumor response exists in primary melanomas. Combined with Breslow thickness, this test provides the most accurate prognostic staging of primary melanomas to date.

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Oral glucoraphanin and curcumin supplements induce the key cytoprotective enzyme NAD(P)H dehydrogenase [quinone] 1 (NQO1) in the skin of healthy human subjects

A. Chien[1], S. Rachidi[1], R. Wang[1], K. Wade[2], H. Liu[2], J. Fahey[3], S. Kang[1]

[1]Dermatology, Johns Hopkins, Baltimore, Maryland, United States, [2]Pharmacology & Molecular Sciences, Johns Hopkins, Baltimore, Maryland, United States, [3]Medicine, Johns Hopkins, Baltimore, Maryland, United States

Oxidative stress plays a pivotal role in skin aging and carcinogenesis. NAD(P)H dehydrogenase [quinone] 1 (NQO1; EC:1.6.5.2) is a prototypical phase 2 enzyme involved in cytoprotection from oxidative stress and mutagenicity. Phytochemicals such as sulforaphane (SF) or curcumin (CUR) can be highly protective by inducing these enzymes in mammals. Topical SF or its precursor glucoraphanin (GR) from broccoli sprouts induce NQO1 activity in mice. Ex vivo treatment of full thickness human skin with SF induces NQO1 gene expression. CUR, from turmeric, induces NQO1 and p53 levels upon long term treatment of T cell lymphoma-bearing mice, and in vitro, it inhibits NQO1 activity and promotes p53 degradation in thymocytes and myeloid leukemia cells. We investigated the effect of glucoraphanin and of CUR, in vivo, in 18 healthy human volunteers (9 males, 9 females, age (avg, [range]) 38.9, [18-69] years. Subjects were randomized to receive daily GR, which is converted to SF upon ingestion (450 mg; 1 mmol; n=6), CUR (1000 mg; 2.7 mmol; n=6), or both (450mg GR + 1000 mg CUR; n=6), as oral supplements. Punch biopsies were obtained from the buttocks, first at baseline after 8 days of a diet low in both compounds, then after 8 days of receiving oral supplements. Compared to baseline, all treatments induced NQO1 mRNA levels: 3.1-fold with GR, 3.3-fold with CUR, and 3.6-fold with the combination of GR and CUR (all p <0.05, two-tailed student t-test). Supplements were well tolerated and compliance was excellent. Oral glucoraphanin (SF precursor) and curcumin are well tolerated and result in upregulation of NQO1 gene expression in human skin in vivo, suggesting an antioxidant and cytoprotective benefit from these supplements. Further implications on cutaneous biology such as UV-induced pyrimidine dimers and extracellular matrix homeostasis are to be elucidated and are a focus of our studies.

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Concordance of teledermatology assessment with in-person, histopathological and laboratory results

A. Tam[1], N. Bhari[2], S. Khandpur[2], M. Ramam[2], M. Wei[1, 3]

[1]University of California, San Francisco, San Francisco, California, United States, [2]All India Institute of Medical Sciences, New Delhi, New Delhi, Delhi, India, [3]Veterans Affairs Medical Center, San Francisco, San Francisco, California, United States

Almost 90% of the world’s population has mobile coverage; recent advances in mobile technology hold promise to improve access to dermatology care worldwide. However, research on the use of mobile technology for teledermatology in underserved regions remains limited. We conducted a pilot study to compare diagnostic concordance of assessment of images vs. in-person assessment, histopathological and/or laboratory results in an outpatient dermatology clinic at an academic institution in India. Of the 152 patient cases, 127 cases had both in-person assessment and diagnosis rendered by photo 1-2 months later, by the same dermatologist, with an intra-observer concordance of 91.3%. An inter-observer consensus diagnosis was assessed in 121 cases, with a top-1 and top-3 concordance of 88.4% and 95.9%, respectively. One-third (n=48) of the cases had a path/lab diagnosis with 91.7% concordance with in-person diagnosis. Some cases (n=32) had diagnoses rendered via all 3 modalities; overall concordance was 84.4%. We also assessed barriers to dermatological care and acceptability of use of mobile apps as diagnostic support tools, by conducting a pilot survey study from October 2018 to May 2019. A total of 112 patients and 103 physicians completed the survey. Patients overwhelmingly (96.3%) indicated importance of dermatological care to their well-being, and noted far distances for care (mean 235.8 ± 411.8 km) and long wait times for appointments (42.9% patients waited >2 weeks). No patients or physicians had experiences with skin-screening apps, but welcomed the prospect of using one (79.4% patients, 78.6% physicians). Significantly, patient expressed needs and physician expressed needs differed. This data provides support for the clinical utility and acceptance of image-based apps in resource-limited settings. With further validation, mobile technology could play a role in helping to meet the dermatological needs of underserved communities.

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Association of urine mono-benzyl phthalate levels with increased psoriasis severity

S. P. Patel[1], J. Choi[1], R. Khanna[1], K. Williams[1], Y. Semenov[2], S. Kwatra[1]

[1]Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [2]Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

Plastic products produced with phthalates as plasticizers have created health concerns since phthalates are believed to cause hormonal and immune dysregulation. Thus, we sought to explore the association between phthalate exposure and psoriasis. Using the 2005-2006 National Health and Nutrition Examination Survey, we conducted a cross-sectional analysis to investigate the association between urinary phthalate metabolite levels and psoriasis severity. We calculated odds ratios (OR) using logistic regression and performed a linear regression to assess for a relationship between urine phthalate levels and psoriasis severity. All models were adjusted for gender, age, race, allergy history, poverty, body mass index, smoking status, and urine creatinine. Increased levels of mono-benzyl phthalate (mBzP) (OR=2.126, 95%CI [1.233, 3.664], p=0.010) were associated with psoriasis diagnosis. The metabolite mBzP was also found to increase with increasing psoriasis severity in our model (β=0.250, 95%CI [0.025, 0.475], p=0.031). Participants without psoriasis had a mBzP geometric mean of 7.451±0.528 ng/mL which was significantly lower than patients with moderate or extensive psoriasis with their geometric mean of 29.782±15.8 ng/mL (p=0.015). Participants with a diagnosis of psoriasis reporting little or no current symptoms had the lowest geometric mean of 3.209±1.038 which was lower than those without psoriasis (p=0.024) and the cohort with moderate or extensive psoriasis (p=0.005). Our data reveals a dose-dependent relationship between a phthalate metabolite and psoriasis severity suggesting that phthalate exposure may contribute to worsened disease. Given that phthalate exposure is ubiquitous with global plastic use, physicians should consider screening psoriasis patients for pollution exposure given the association of elevated mBzP in these patients.

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Endotype analysis in mucous membrane pemphigoid

K. Messingham[1], T. Crowe[1], M. Greiner[1], J. Hellstein[1], D. VanDaele[1], S. Palmer[1], J. Fairley[2]

[1]University of Iowa, Iowa City, Iowa, United States, [2]Dermatology, University of Iowa, Iowa City, Iowa, United States

Mucous membrane pemphigoid (MMP) is an autoimmune blistering disease involving mucosae with heterogeneous disease presentation and autoantigen reactivity. The goal of this study was to determine if MMP could be resolved into endotypes, based on clinical features or antigenic target. 71 patients (37 female/ 34 male) who met clinical and histologic criteria for MMP were identified in our REDCap database; clinical information and sites of involvement were confirmed by exam and chart review. Serum autoantibody targets were evaluated via indirect immunofluorescence, ELISA (BP180, BP230, Col VII), and blotting (BP180, α6β4 integrin and Laminin 332). Oral mucosa was the most common site of involvement (98%) followed by skin (44%), ocular (35%), anogenital (27%) nasal (24%), pharyngeal (13%) and laryngeal (13%). Genital involvement was significantly higher in females (p=0.04), while ocular involvement was more common in males (p=0.03). A correlation analysis revealed close association of laryngeal and pharyngeal involvement (r = 0.689, p<0.0001), and an association between those sites and nasal involvement (r ≥ 0.304, p<0.01). Ocular and nasal involvement were also weakly associated (r = 0.267, p = 0.024). Analysis of antigenic specificity of 68 patient's autoantibodies identified 51 (75%) as BP180, 3 (4%) as laminin 332 and 4 (6%) as Col VII, 10 were indeterminant (15%). α6β4-reactivity showed extensive overlap with other antigens. BP180-reactivity was associated with skin lesions (r = 0.374, p = 0.0344). Col VII reactivity was associated with ocular involvement (r = 0.306, p = 0.046), and laminin-332 reactivity was associated with pharyngeal and laryngeal lesions (r = 0.423, p 0.007). This suggests that clinical endotypes may be related to antigenic targets. Analysis of a larger number of patients is needed to confirm these findings and determine if endotypes of MMP predict outcomes and/or response to treatment.

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Utilization of an oxygen-sensing bandage to distinguish cellulitis from clinical mimickers

C. Gabel[1], H. Marks[2], E. Roussakis[2], A. Raff[1], C. Thomas[2], E. Nguyen[1], R. Shah[1], C. Evans[2], D. Kroshinsky[1]

[1]Dermatology, Massachusetts General Hospital, Cambridge, Massachusetts, United States, [2]Massachusetts General Hospital, Boston, Massachusetts, United States

Cellulitis is a cutaneous infection of the dermis and subcutaneous tissue. There are no existing gold standard diagnostic techniques for differentiating between cellulitis and clinical mimickers ("pseudocellulitis"). New optical technologies in development could aid diagnosis, specifically: an oxygen-sensing bandage developed by this group. There is evidence that inflammation, metabolic derangement and vascular changes alter tissue oxygenation (pO2). Detection of these changes may help differentiate infectious vs. non-infectious causes of inflammation. A paintable transparent liquid bandage is used to visually display pO2.(1) This formulation quantitatively reports tissue oxygenation (pO2) via a ratiometric red/green color change fitting the Stern Volmer relation. The formulation consists of New-Skin nitrocellulose liquid bandage embedded with an oxygen-sensing metalloporphyrin exhibiting red phosphorescence, and fluorescein, a green-fluorescing reference dye. Tissue oxygenation maps are collected using a commercial Nikon DSLR camera fitted with custom UV excitation and dual bandpass emission filters. In an ongoing inpatient human clinical trial, this technology is evaluated in an inpatient patient population admitted with cellulitis or pseudocellulitis of a limb. The described bandage is applied to both the affected and unaffected limb. Thus far, the data of 7 patients has been analyzed, finding that at the end of a 20-minute observation period, the control site was more oxygenated than the infected site, regardless of its temporal path, suggesting the bandage has potential in distinguishing infection vs. healthy tissue. By understanding the effect of bacteria-induced inflammation on tissue oxygenation, a more robust model for diagnosis can be built. 1. Li, Z. et al. (2017) 'Non-invasive monitoring of skin inflammation using an oxygen-sensing paint-on bandage', Biomedical optics express, 8(10), pp. 4640–4651.

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Comparison of changes in serum metabolites before and after treatment with IL-17A monoclonal antibody and halomethasone in psoriasis

Y. Le[1], L. Zhang[1], L. Chen[1], X. Li[1], Y. Yu[2], J. Zheng[1]

[1]Dermatology, Rui Jin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [2]School of Pharmacy, Fudan University, Shanghai, China

OBJECTIVE: To analyze the changes of serum metabolites of patients with psoriasis after IL-17A monoclonal antibody (mAb) treatment and halomethasone treatment, and to compare the effects of the two treatment methods on patients' systemic metabolism. METHODS: Metabolomics techniques based on gas chromatography-mass spectrometry were used to analyze the serum metabolites before and after the treatments in 32 patients treated with IL-17A mAb and 16 patients treated with halomethasone. RESULTS: 19 serum differential metabolites were screened out before and after IL-17A mAb treatment, while 9 serum differential metabolites were screened out after treatment with halameasone. The most significant metabolic changes caused by the two treatments is the decrease in free fatty acids (FFA) in the serum, but IL-17A mAb treatment has led to more FFA changes than halameasone treatment. Associated with FFA changes, IL-17A mAb treatment also resulted in a 39.9% decrease in beta-hydroxybutyric acid (3-HB) (p = 0.0084) and a 91.4% increase in azelaic acid (p = 0.045). IL-17A mAb treatment also uniquely caused a significant increase in ammonia metabolic activity, the polyamine amino acids ornithine and lysine increased by 32.6% (p = 0.0379) and 15.1% (p = 0.0103), and urea increased by 15.0 % (P = 0.0332) and uric acid decreased by 24.4% (p = 0.0253). Halomethasone treatment uniquely caused a 20.1% increase in serum inositol (p = 0.0184). CONCLUSION: Compared with Halomethasone treatment, IL-17A mAb treatment caused changes in more types of metabolites in the patient's serum, especially the simultaneous reduction of FFA and the lipid beta oxidation product 3-HB, indicating that lipids metabolism may provide an energy source for the activation of the IL-17A inflammatory pathway. Ammonia metabolism was up-regulated after IL-17A mAb treatment, indicating that the body's ability to excrete ammonia is enhanced after treatment.

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Assessing performance of deep neural networks used for image classification by stress testing

A. T. Young[1, 2], J. Pfau[1, 2], M. J. Keiser[1], M. Wei[1, 2]

[1]University of California, San Francisco, San Francisco, California, United States, [2]Veterans Affairs Medical Center, San Francisco, California, United States

Deep convolutional neural networks (CNNs) can outperform dermatologists on image classification. However, their performance in a real-world setting, where image capture is subject to variations in lighting, zoom, focus, etc. is not well studied. Recent work in the machine learning community has reported that CNNs predictions are not stable to perturbations of the input image, e.g. a simple rotation. Standardized metrics of model robustness, to be reported in addition to accuracy, are needed to assess the readiness of dermatologist-level CNNs for clinical use. We trained a CNN model on images of 3,563 melanomas and 10,094 nevi. CNN performance was evaluated on hold-out test sets with images of different types (dermoscopic vs non-dermoscopic), collected for varying purposes (for biopsy site identification vs teledermatology vs research). For the diagnosis of melanoma vs nevus, the model outperformed dermatologists on a curated dermoscopic test set (CNN sensitivity/specificity 90.0%/77.5% vs 157 dermatologists 74.1%/60.0%); a curated non-dermoscopic test set (CNN sensitivity/specificity 95.0/75.0% vs 145 dermatologists 89.4%/64.4%); and a real-world teledermatology test set (CNN sensitivity/specificity 84.2%/67.1% vs 14 dermatologists 82.0%/55.1%). Yet, for selected lesions, the model can output different diagnoses, depending on stress testing by changing image rotation, brightness, contrast, blur, or other slight variations. When stress testing the model by testing on diagnostic classes not seen during training, the model erroneously predicts as confidently as melanoma or nevi. We show that the structural similarity index (SSIM), a measure of image similarity between the Grad-CAM saliency maps of the reference and perturbed images, can forecast both prediction accuracy and whether the model prediction is robust to image perturbations. In summary, we have developed a novel method, *stress testing*, to better forecast model accuracy by assessing CNN model robustness to perturbations of input images.

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The IgG1 isotype of anti-MDA5 antibody may dominate severity of interstitial lung disease in dermatomyositis

M. Chen, J. Zheng, M. Pan, H. Cao

Department of Dermatology, Shanghai Jiaotong University Affiliated Ruijin Hospital, Shanghai, China

Objective To identify anti-MDA5 antibody subtype (IgG, IgA, IgM) and anti-MDA5 IgG subclasses, and to investigate their association with clinical severity. Methods Clinical features, laboratory findings and serum of 36 DM/CADM patients with anti-MDA5 antibody positive were collected and analyzed. Anti-MDA5 IgG was measured by enzyme-linked immunosorbent assay and line-blot immunoassay. Anti-MDA5 subtype (IgG, IgA, IgM) and anti-MDA5 IgG subclasses were measured by enzyme-linked immunosorbent assay. All data were analyzed using SPSS 23.0 software or GraphPad 6 software. Results In 36 anti-MDA5 Ab positive DM/CADM patients, 12 died of interstitial lung disease and 24 survived till the latest visit. The incidence rate of necrotic ulceration, ulcerative Gottron rash, acute interstitial pneumonia were significantly higher in patients dead than survivors(all P<0.05). Survivors had significantly higher serum LDH and ferritin. Cut-off value of anti-MDA5 IgG was identified as 50U/ml, with 100% sensitivity and 99.42% specificity. Incidence rate of acute interstitial pneumonia, mortality rate and serum ferritin were significantly higher in MDA5 IgG1+ DM/CADM patients (n=26) than MDA5 IgG1- patients (n=10) (P=0.0027, 0.015, 0.0011, respectively). Till the latest visit, overall survival of patients with MDA5 $IgG1^+IgG4^+$ was 37.5% and median survival time was 6 months. The overall survival of patients with MDA5 $IgG1^+IgG4^-$ and MDA5 IgG1- was 61.1% and 100%, respectively. Log-rank (Mantel-Cox) test of the survival curves showed significant difference, P=0.037. Serum ferritin was significantly elevated in MDA5 $IgG1^+IgG4^+$ and MDA5 $IgG1^+IgG4^-$ patients than MDA5 IgG1- patients (both P<0.01). Conclusion Serum levels of MDA5 IgA may be a substitute for anti-MDA5 IgG. MDA5 IgG1 is the principle component of MDA5 IgG subclasses and correlates with AIP and prognosis in patients with DM/CADM, which might participates in the pathogenesis of anti-MDA5 antibody associated ILD.

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Fluorescence Imitating Brightfield Imaging (FIBI): A novel application of rapid, non-destructive and slide-free skin tissue imaging

T. Engel[1], A. Rangchi[2], M. Fung[2, 3], M. Kiuru[2, 3], F. Fereidouni[2], R. Levenson[2]

[1]University of California Davis School of Medicine, Sacramento, California, United States, [2]Pathology and Laboratory Medicine, University of California Davis, Sacramento, California, United States, [3]Dermatology, University of California Davis, Sacramento, California, United States

Purpose: To investigate the functionality of Fluorescence Imitating Brightfield Imaging (FIBI) for imaging skin tissue samples. Methods: The surfaces of three (with more to follow) skin samples from the UC Davis Dermatopathology tissue bank were deparaffinized using xylene and ethanol washes, then stained with hematoxylin, Scott's bluing reagent and eosin. A color camera captured images from 405 nm visible range (blue) excitation light illuminating the specimens. Standard hematoxylin and eosin (H&E) slides were cut from specimens prior to deparaffinization and compared to FIBI images. Results: FIBI specimen staining and imaging took on average five minutes and was non-destructive. Images were scored by two dermatopathologists based on their similarity to standard brightfield microscopy and suitability for diagnostic use. Compared to H&E glass slides, FIBI images were comparable in visualizing dermal structures such as collagen and epidermal layers. We also observed greater visualization of some components such as basement membrane, along with fewer artifacts. Conclusion: Preliminary imaging suggests FIBI may be a useful tool for dermatologic microscopy as an alternative to traditional tissue fixation and sectioning. The imaging process is quick, simple and inexpensive, lending to future applications in limited resource areas and procedures benefiting from rapid specimen processing. FIBI images can also be converted to H&E-like images for familiarity and ease of interpretation.

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Sex differences in the molecular cause of perioral skin wrinkling

I. Brown, C. Dillen, B. C. Ly, N. Shyam, S. Kang, A. Chien

Dermatology, Johns Hopkins, Baltimore, Maryland, United States

Wrinkling is the hallmark of skin aging. We have previously reported that perioral wrinkling is more severe in females; however the molecular basis for this is unknown. We enrolled 12 subjects (n=6 male/female) age 54-86 with Griffith's photoaging severity grade between 4 and 8 (0=none, 8=severe) and took biopsies from both the perioral and periocular region. Using qPCR, we assessed RNA expression levels of collagen I, collagen III, cysteine-rich angiogenic inducer 61 (CYR61), and insulin like growth factor 1 (IGF-1). CYR61 causes dermal fibroblast senescence in response to photodamage, leading to the decreased collagen production linked to skin wrinkling. IGF-1 is produced by dermal fibroblasts and plays a role in regulating the hair cycle and promoting oxidative stress. While there was no difference between females' and males' Griffith's grade (6.67 and 5.67 respectively, p=0.096) or periocular wrinkling grade (3.2 and 2.6 respectively, p=0.421), females did have a significantly more severe perioral wrinkling grade when compared with males (6.2 and 2.8 respectively, p=.0.035). Consistent with the increase in severity in perioral wrinkling, females also expressed significantly more CYR61 (p=0.018) than males, however, they also expressed more collagen III (p=0.016). In this location, there was no significant difference in collagen I (p=0.115) or IGF-1 (p=0.124) expression between males and females, although females trended towards higher expression of both, with the latter marker's role in oxidative stress potentially contributing to the increased perioral wrinkling in females. In the periocular region, there were no significant differences between males and females in the expression levels of all four markers. While both sexes had similar overall photoaging and gene expression in the periocular region, the perioral region expressed significant molecular differences between the sexes, which may contribute to the greater perioral skin wrinkling seen clinically in females. It also highlights the complex process of skin aging and the influence of specific anatomic milieu in this process.

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Early effector T-cell densities predict response in patients with advanced Merkel cell carcinoma treated with anti-PD-1

S. Lu, F. Succaria, B. Green, N. Giraldo-Castillo, J. Taube

Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Background: We previously nominated PD-1+ cell densities in pre-treatment tumor specimens as a potential biomarker for response to pembrolizumab (anti-PD-1) in patients with advanced Merkel cell carcinoma (MCC). Here, we used multiplex immunofluorescence (mIF) in a separate cohort of MCC patients treated with anti-PD-1 to determine which immune cell subset(s) contribute PD-1 to the tumor microenvironment (TME). We also characterized PD-1 expression intensity on lymphocytes, as an indication of their functional state, and tested for an association with anti-PD-1 response. Methods: We performed mIF staining on pre-treatment formalin-fixed paraffin embedded tumor specimens from 23 unique patients. The mIF panel included PD-1, CD4, CD8, CD20, FoxP3, and CD56 (tumor marker). The density of each cell type and intensity of PD-1 expression (PD-1^{lo}vs. PD-1^{hi}) were determined and correlated with response assessments by RECIST1.1. Results: Total PD-1 cell densities were higher in responders vs. non-responders, supporting our original findings. Additionally, median CD4, CD8 and CD20 cell densities were increased in those who responded to therapy. While CD8+ cells are often cited as contributing the majority of PD-1 to the TME, we found an equal proportion was contributed by CD4+ cells. Approximately 4% of the CD8+PD-1+ cells also expressed FoxP3. Of the immune cell phenotypes tested, CD8+FoxP3+PD-1^{lo}densities showed the strongest association with response to anti-PD-1 ($p=0.01$, Mann-Whitney U test). Conclusions: The next generation of tissue-based biomarkers for predicting response to immunotherapy are likely to utilize multiplex IF/IHC technologies, which can quantify complex cell phenotypes in situ. Here, we highlight CD8+FoxP3+PD-1^{lo}as a key cell type associated with anti-PD-1 response. Representing early, tumor-specific effector T-cells with strong proliferative potential and IFN-gamma secretion, CD8+FoxP3+ cells warrant additional exploration as a predictive biomarker.

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Increased eosinophils as a biomarker for therapeutic response in patients with chronic pruritus of unknown origin

R. Khanna, S. P. Patel, K. Williams, S. Kwatra

Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Chronic pruritus of unknown origin (CPUO) is pruritus lasting greater than 6 weeks that is not linked to a causal disease process. With a lack of approved therapies, CPUO is extremely difficult to manage. We thus conducted a retrospective review of 23 patients collecting demographics, medical history, laboratory data including complete blood count with differential, pruritus characteristics, and response to treatment. Of these patients, those with an absolute eosinophil count above 0.30 K/cu mm, eosinophil percent above 4%, or pronounced eosinophils on skin biopsy were identified. Treatment effect was recorded using the itch numerical rating scale (NRS). Among non-eosinophilic CPUO patients, 55% reported symptom improvement with gabapentin compared to just 14.3% of eosinophilic CPUO patients ($p=0.001$). Mean NRS dropped from 9.4 ± 0.40 to 2.75 ± 1.75 ($p=0.025$) among non-eosinophilic CPUO patients taking gabapentin. Most eosinophilic CPUO patients (86%) reported no change in symptoms with gabapentin. Unlike gabapentin, immunomodulators led to symptom improvement in all patients with increased eosinophils ($n=4$) and resulted in no change in symptoms among non-eosinophilic patients ($n=4$) ($p=0.005$). Mean NRS decreased from 8.25 ± 0.75 to 5 ± 0.25 ($p=0.024$) in eosinophilic patients treated with immunomodulators. Our results show that CPUO patients with normal eosinophil counts are more likely to have neurological disease or back pain ($p=0.030$ and 0.056, respectively) and to respond to neuromodulator therapy as compared to CPUO patients with increased eosinophils. Our results also suggest that CPUO patients with increased eosinophils likely have an immune-mediated etiology to their itch. We propose that patients with CPUO undergo complete blood count with differential with option for tissue biopsy to evaluate for eosinophil predisposition to guide the selection of targeted therapies.

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Lebrikizumab attenuates the neuronal enhancement of pruritus through IL-13 consistent with clinical anti-itch effects observed in phase 2b moderate-to-severe atopic dermatitis patients

E. Simpson[1], A. S. Paller[2], F. Cevikbas[3]

[1]Oregon Health & Science University , Portland, Oregon, United States, [2]Northwestern, Chicago, Illinois, United States, [3]Dermira Inc, Menlo Park, California, United States

Recent studies have provided a better understanding of cytokines playing a role in pruritus (itch), a cardinal feature in atopic dermatitis (AD). Among these cytokines, IL-13 is a key mediator of multiple pro-inflammatory processes in AD. Other type-2 cytokines, such as TSLP, IL-33, and IL-4 modulate neurons directly, in addition to their pro-inflammatory effects. Lebrikizumab, a novel, high-affinity, monoclonal antibody targeting IL-13, is under investigation for the treatment of moderate-to-severe AD. To understand the neuronal role of IL-13 and the directed action of Lebrikizumab, we employed a human neuronal model as a tool for mechanistic insight into the clinical phase 2b data. In a randomized, double-blind, placebo-controlled phase 2b clinical trial in adults with moderate-to-severe AD (NCT03443024), lebrikizumab resulted in dose-dependent, statistically significant improvement vs placebo on measures of AD severity (eg, Eczema Area Severity Index and Investigator's Global Assessment) and itch (pruritus numeric rating scale) at week 16. Itch improvement as early as day 2 in this trial suggested a direct effect on sensory nerves that mediate itch. The objective of these preclinical studies was to test the direct effects of IL-13 and lebrikizumab in a human dorsal root ganglion model. In the functional neuron model studies, human dorsal root ganglia were treated with IL-13 in combination with different histaminergic and non-histaminergic pruritogens in the presence or absence of lebrikizumab. Responses were directly monitored with live cell calcium imaging. IL-13 potentiated the neuronal responses to known pruritogens such as serotonin, BAM8-22 and histamine, and lebrikizumab attenuated these IL-13-driven responses. These preclinical findings suggest a direct neuromodulatory role for IL-13 in itch associated with AD and may be part of the mechanistic basis for lebrikizumab's anti-itch effects observed in the phase 2b clinical trial.

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A variant of Mohs micrographic surgery: The muffin technique

P. Surmanowicz, A. Sivanand, A. Du, R. Gniadecki

Medicine, University of Alberta, Edmonton, Alberta, Canada

Background: Moh's micrographic surgery (MMS) is the gold standard treatment for non-melanoma skin cancers (NMSC) such as basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Despite the advantages of MMS, the technique also has limitations. The procedure can be time-consuming and, due to the bulky nature of many cutaneous tumors, appropriately sectioning and flattening the specimen for cryosection without omitting tissue is difficult. A novel method has recently been proposed as an alternative to the traditional MMS approach. Due to the procedure's similarity to the removal of the paper lining encasing a muffin, it has been aptly named the "Muffin Technique". Herein, we describe the "Muffin Technique" and outline the procedure's safety and effectiveness in a real-world institution.Methods: We conducted a retrospective chart review of all patients with BCC or SCC who underwent MMS with the Muffin Technique at the University of Alberta Dermatology Centre from June 2016 until September 2019. Details on demographics, disease burden, and procedure effectiveness and complications were analyzed and compared to existing MMS data to compare this variant technique to the traditional approach. Results: A total of 64 patients were included with 64 BCCs and 5 SCCs who underwent Muffin Mohs. There were no major procedural complications, and 92.75% of the surgeries had clear margins after the first incisions, 100% after second round re-excisions. Surgical wounds were significantly smaller than with traditional approaches. All patients remain disease-free upon most current follow-up.Conclusions: The Muffin Technique represents a variation on the traditional Mohs approach and enables paraffin fixation that may be stored for pathologic evaluation for longer, allows for larger specimens to be evaluated on a single slide, and possesses advantages for smaller incisions and wound healing. We hope to introduce the field of dermatology to this alternative surgical approach to ultimately improve patient care.

Late-Breaking Abstract Table of Contents

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Abstract Booklet

Adaptive and Auto-Immunity

LB915
A targeted CRISPR screen to identify essential genes for the T_{RM} T cell response to cancer
J. B. Williams, Y. Contreras Duarte, E. Rotrosen, T. S. Kupper
Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States
Bunkered in barrier tissue, tissue-resident memory T (T_{RM}) cells are strategically positioned to react quickly to tissue perturbations, such as infection, injury, or cancer. Recent studies have highlighted a potential role for T_{RM} cells as a targetable T cell population due to their abundance in most tissues. To better understand the role T_{RM} cells play in the antitumor immune response, we developed a targeted CRISPR screen to identify the essential genes regulating the T_{RM}-response to tumors. We first developed a murine tumor model system in which OT-I cells could be adoptively transferred and re-isolated from tumors. Yumm1.7 tumor cells were transduced with OVA fused to mCherry. SIINFEKL reactivity to Yumm.OVAmCh tumors was confirmed by pentamer staining, ELISPOT, and OT-I proliferation in vivo. To promote T_{RM}-responses we used a model of skin scarification where tumors develop in the uppermost layers of the skin. In this model, approximately 1/3 tumors are spontaneously rejected. On day 7 after tumor inoculation, skin-residing T cells expressed CD103 and CD69, which was not observed in tumors engrafted subcutaneously. Further, $CD8^+$ T cells isolated from the skin of rejected tumors contained SIINFEKL-reactive T_{RM} cells. Next, we optimized T cell transduction to achieve approximately 20% efficiency, which was enriched to 50% after puromycin selection. Finally, to achieve statistically powerful guide-RNA (gRNA) coverage we chose to target select genes expressed in T_{RM} cells and $CD8^+$ tumor-infiltrating lymphocytes (TILs). To this end, we compared gene expression profiles of T_{RM} cells and TILs across several studies. From this cross-study comparison, we chose both up- and down-regulated genes (log2 fold-change > 1.5) totaling 1,469 genes. In conclusion, the epicutaneous tumor model system provides a vehicle to study T_{RM}-responses to melanomas. Future studies will apply a targeted-deletion OT-I T cell library to these tumors to reveal genes critical for the T_{RM}-response to tumors.

Carcinogenesis and Cancer Genetics

LB916
Effects of the platelet-activating factor-receptor and microRNA-149 in lung cancer growth and therapy effectiveness
S. J. Chauhan, A. Thyagarajan, R. P. Sahu
Wright State University, Dayton, Ohio, United States
Lung cancer remains the leading cause of cancer-related deaths, with low response rates to the current treatment options, indicating the need to explore potential factors, involved in lung cancer growth or impeding the efficacy of therapeutic agents. Studies, including ours, have shown the critical roles of a G-protein coupled, platelet-activating factor-receptor (PAF-R) signaling in augmenting tumor growth or limiting therapy effectiveness in various experimental cancer models. While several mechanisms of the PAF-R pathway have been proposed, its effect with microRNAs (miRs) has not been studied. In particular, while miR-149 has been shown to play oncogenic roles in other cancer types, it functions as a tumor suppressor in lung cancer. The current study aimed to determine the effects of PAF-R and miR-149 in lung cancer growth and therapy effectiveness. We first evaluated the functional significance of PAF-R and miR-149 using A549 and H1299 human non-small cell lung cancer (NSCLC) cell lines as tools. These tumor lines express endogenous PAF-R and miR-149, and PAF-R activation by PAF agonist (CPAF) significantly increased-, whereas miR-149 mimic transfection inhibited cell proliferation in a dose-dependent manner. Interestingly, miR-149 mimic significantly attenuated CPAF-mediated increased proliferation of A549 cells, as also confirmed by miR-149 expression analysis via qPCR. We then examined PAF-R and miR-149 effects on currently used targeted therapy (i.e., erlotinib and gefitinib) responses. Both these agents inhibited the survival of A549 and H1299 cell lines in a dose- and time-dependent manner. While CPAF significantly blocked erlotinib and gefitinib (at ~IC_{50} dose)-mediated decreased cell proliferation, PAF-R antagonist and miR-149 mimic did not exert any effects. While additional studies are needed, these findings indicate that miR-149 overcomes PAF-R-mediated increased cell proliferation effect, and PAF-R activation attenuates cytotoxic response of targeted therapy.

LB917
Platelet-activating factor-receptor signaling mediates targeted therapy-induced microvesicle particle release in lung cancer cells
S. J. Chauhan, A. Thyagarajan, Y. Chen, R. P. Sahu
Wright State University, Dayton, Ohio, United States
Microvesicle particles (MVP) are nano-sized extracellular vesicles, secreted in response to stimuli such as therapeutic agents by a variety of cell types including tumor cells. MVP are involved in mediating several cellular responses including modifying tumorigenicity or sensitivity of therapeutic agents. Studies, including ours, have shown that tumor cells expressing a G-protein coupled, platelet-activating factor-receptor (PAF-R) augments pro-oxidative stressors including chemotherapy-mediated MVP release. The current study determined the role of PAF-R signaling in targeted therapy (i.e., erlotinib and gefitinib)-mediated response on MVP release using PAF-R-expressing human A549 and H1299 non-small cell lung cancer (NSCLC) cell lines. Using PAF-R agonist, CPAF and phorbol myristate acetate (PMA), a PAF-R independent agonist as positive controls, our first studies observed that erlotinib and gefitinib induce MVP release from both NSCLC cell lines in a dose- and time-dependent manner. Notably, MVP secretion peaked between 4 to 8 hours' time points with similar effects, so we used a 4-hour time point with an optimal dose for MVP release. As MVP biogenesis regardless of the stimuli, involves acid sphingomyelinase enzyme (aSMase), we observed that aSMase-specific inhibitor significantly blocked erlotinib and gefitinib-mediated, and also CPAF and PMA-induced MVP release from A549 cells. To confirm the PAF-R dependency, we tested that PAF-R knockdown via specific siRNA as measured by qPCR or PAF-R antagonist significantly blocked only targeted therapy- and CPAF-mediated but not PMA-induced MVP release. Mechanistically, mitogen-activated protein kinase (MAPK, particularly, ERK and p38) pathway inhibitors significantly attenuated erlotinib and gefitinib-mediated MVP release. Overall, our studies indicate the potential role of PAF-R signaling in augmenting targeted therapy-mediated MVP release, which could have cellular and systemic effects in lung cancer.

LB918

Tirbanibulin, a novel inhibitor of tubulin polymerisation and src kinase signaling, for actinic keratosis (AK): Results of two phase-3 studies and 1-year follow-up data

A. Blauvelt[1], S. Kempers[2], S. Puig[3], G. Martin[4], E. Fumero[5], A. Grada[5], D. Cutler[6], J. Fang[6]

[1]Oregon Medical Research Center, Portland, Oregon, United States, [2]Associated Skin Care Specialists, Fridley, Minnesota, United States, [3]Hospital Clínic de Barcelona, Barcelona, Spain, [4]Dermatology Associates, Maui, Hawaii, United States, [5]Almirall, Barcelona, Barcelona, Spain, [6]Athenex, Inc, Buffalo, New York, United States

Objectives: Two pivotal Phase III randomized, double-blinded, vehicle-controlled, studies (KX01-AK-003/KX01-AK-004) evaluated the efficacy and safety of tirbanibulin ointment 1% vs. vehicle in adults with AK on the face/scalp. Methods: Eligible subjects with 4-8 clinically visible AK lesions in a 25 cm^2 area were randomized 1:1 to receive tirbanibulin or vehicle (5-day once-daily self-application). Primary endpoint was complete clearance of AK at Day 57. Safety assessments included local skin reactions (LSRs) and adverse events. Recurrence rate up to 1-year post Day 57 in subjects with complete clearance of treated AK lesions at Day 57 were evaluated. Results: 702 subjects were enrolled (n=351 in 31 sites per study); >99% completed Day 57. At Day 57, the complete clearance rate was significantly higher with tirbanibulin vs. vehicle (KX01-AK-003: 44% vs. 5%, P<0.0001; KX01-AK-004: 54% vs. 13%, P<0.0001). Most adverse reactions were transient mild-to-moderate application-site pruritus/pain, erythema, and flaking/scaling. No deaths, discontinuations, or serious AEs related to tirbanibulin occurred. In total, 202 subjects (tirbanibulin, n=173; vehicle, n=29) entered the Recurrence Follow-up Period; the estimated rate of recurrence at 1-year post Day 57 was 72 - 74%. Recurred AK lesions were mostly single lesions (58%). Baseline number of lesions (>5) and previous AK treatments in the treatment area were correlated with recurrence (OR 2.1 and 3.0, respectively). Conclusion: Tirbanibulin is a first-in-class topical treatment for AK with a short treatment duration that resulted in statistically higher complete AK clearance compared with vehicle and was very well tolerated. Recurrence rate at 1 year was 72 - 74%.

LB919

Aging does not facilitate nodular BCC development in mice expressing oncogenic *Smo*

M. Grachtchouk, D. Wilbert, A. Hoover, D. Pyrozhenko, M. Verhaegen, A. A. Dlugosz

Dermatology, University of Michigan, Ann Arbor, Michigan, United States

Aberrant Hedgehog (Hh) signaling drives the development of basal cell carcinomas (BCCs), which arise mainly in elderly patients. Loss-of-function *PTCH1* or gain-of-function *SMO* mutations are oncogenic drivers in human BCC, but neither of these genetic alterations effectively yield nodular BCCs in genetically-engineered mice. Because nearly all prior BCC modeling studies have been performed in juvenile mice, we set out to assess whether aging is permissive for BCC development using a Cre-inducible oncogenic *SmoM2* allele, activated in skin of young versus aged mice. As expected, young (4 month-old) *SmoM2*-expressing mice formed microscopic basaloid hamartomas following 9 weeks of transgene activation, with nodular BCC-like lesions never detected. Similarly, aged (22-24 month-old) mice, expressing SmoM2 for up to 8 months, also failed to develop nodular BCCs. Histology of *SmoM2*-driven hamartomas in skin of young and aged mice was indistinguishable, and nearly all epithelial cells in lesions from both groups expressed K5, K17, and Sox9. Remarkably, while Ki67+ proliferating cells were abundant in cells at the periphery of hamartomas in young mice, proliferation was markedly reduced in hamartomas in aged mice. Our findings show that oncogenic *Smo* expression yields similar histologic phenotypes in both young and old mice and an apparent reduction in proliferating cells in aged mice. The absence of BCCs both in young and elderly *SmoM2*-expressing mice argues against a critical role for aging in facilitating full-blown skin tumorigenesis in this mouse model.

Epidermal Structure and Barrier Function

LB920

IQGAP3 is an important mediator of the psoriatic phenotype of keratinocytes

A. Zolotarenko, A. Zavarukhina, S. Bruskin

Laboratory of Functional Genomics, Vavilov Institute of General Genetics RAS, Moscow, Russian Federation

Keratinocytes are immunocompetent cells important for the structural and barrier function of skin. Psoriatic keratinocytes are characterized by the enhanced proliferation and reduced differentiation rates as well as by the elevated production of proinflammatory cytokines and chemoattractants. In this research we have knocked-down a scaffold protein IQGAP3 using shRNA in HaCaT keratinocytes (HaCaT_shIQ3) in order to test the hypothesis if IQGAP3 mediates the psoriatic phenotype of keratinocytes. IQGAP interacts with cell adhesion molecules, with the cytoskeleton, with signaling molecules to regulate cell morphology, motility and kinase pathways. Earlier we have identified IQGAP3 to be overexpressed in skin of psoriatic patients and to be stimulated by the proinflammatory cytokines IL17, TNFa and IFNg. RNA-seq of the HaCaT_shIQ3 cells has shown that among the GO enriched by the downregulated genes were: positive regulation of MAPK cascade, negative regulation of inflammatory response, epidermis development, negative chemotaxis, cell adhesion, keratinization, keratinocyte differentiation, intracellular signal transduction. Among the GO enriched by the upregulated genes were positive regulation of macrophage cytokine production, extracellular matrix organization, positive regulation of I-kB/NF-kB signaling, calcium transport, cell adhesion, positive regulation of inflammatory response, lipid metabolic process, positive regulation of IL6 production, NO biosynthetic process. Almost all the onthologies mentioned above are involved in psoriatic process, highlighting the importance of the IQGAP3 for this disease. Using xCELLigence real time cell analysis system we have evaluated the growth rates of HaCaT_shIQ3. Compared to the control cells, HaCaT_shIQ3 demonstrated slower growing rates, delayed wound healing and were in a lesser degree growth-stimulated by psoriatic proinflammatory cytokines. Thus we concluded IQGAP3 to be an important mediator of the psoriatic phenotype of keratinocytes.

LB921

Relationship between functional group interaction of lipid components in stratum corneum and barrier function of skin

Y. Obata[1], Y. Ofuchu[1], H. Setoyama[1], K. Ishida[2]

[1]Hoshi University, Tokyo, Shinagawa, Japan, [2]Takasago International Corporation, Kanagawa, Hiratsuka, Japan

The stratum corneum located at the outermost layer of the skin plays a physical protective function for living body from exogenous hazards. Numerous studies have shown that disruptions in the organization of stratum corneum lipids may be associated with decreased skin barrier function. In this study, we used CER [NDS] and CER [NP] as ceramides (CER), cholesterol, and palmitic acid (PA) to understand the barrier function formed by the organization of lipids. Lipid models containing typical subclass CER prepared, its nanostructure and characteristics was examined by differential scanning calorimetry, synchrotron X-ray diffraction and infrared spectroscopy. A number of unclear phase transitions were observed in the healthy human stratum corneum with temperature scanning based on change in lipid organization, while clear phase transitions were observed in the NP model and the NDS model. In addition, the eutectic model in which two types of CERs were mixed showed thermal behavior pretty similar to that of the human stratum corneum, suggesting that many lipids may form a eutectic mixture with each other in the human stratum corneum. On the other hand, the infrared absorption spectra indicated that the absorption of the carbonyl group derived from PA disappears at high temperatures in both the NDS model and the NP model, suggesting that PA may be fallout from the rigid organization of lipids. In addition, the maximum wave number of the absorption indicating the hydrocarbon chain packing showed a slight shift in the NDS model along with the temperature scan, and a large shift in the NP model, confirming the difference in the effect of desorption of PA. This suggests that PA in the NDS model is slightly involved in the overall fluidity of lipids, whereas in the NP model, desorption of PA may increase the fluidity of hydrocarbon chains. This result indicates that the NDS model may maintain a stable structure against external stimuli. Thus, it was considered that NDS was important for skin barrier function.

LB922

The evaluation of moisturizing products on *in vitro* dry-skin mimicking 3D model

S. Hong, A. Nguyen, R. Metha, K. Kadoya

Allergan plc, Irvine, California, United States

Dry skin is a characteristic appearance of impaired skin barrier function. Since dry skin is due to excessive water loss, topical application of moisturizing products has been a main remedy to keep more moisture in skin and to ameliorate adverse symptoms. However, the effect of active ingredients on the biological structure of dry skin has not been widely tested due to a lack of methodology to properly evaluate them systematically. Previously (LB1083, SID meeting 2019), we demonstrated Tracer assay (barrier function assay), focusing on the effect of moisturizers to address water loss on the 3D cell culture model (EpiDerm, MatTek). The goal for this study was to establish a dry skin model, by disrupting only the uppermost stratum corneum (SC) with petroleum ether (PE) treatment, which was confirmed with Lucifer Yellow penetration assay. Commercially available moisturizer products were applied on the surface of the tissue and incubated for 24 hours, then Tracer assay was performed as demonstrated previously. In parallel qPCR and ELISA analyses were performed against tight junction and SC formation biomarkers. The results show that; 1) PE treated tissue simulated dry skin by partially disintegrating the top layer of SC, which was ascertained by deeper and bright signal in Lucifer Yellow penetration assay, and induced more water loss than the untreated control (80% vs. 35%). 2) Application of commercial moisturizer products improved barrier function of PE treated tissue (48%~67%) in Tracer assay. 3) Application of products upregulates gene expression of tight junction, lipid generation and cornified envelope formation selectively (1.3 ~ 2.3 folds) and increase the level of protein expressions of filaggrin (~20%) and loricrin (~10%). We successfully demonstrated and established experimental platform for assessing moisturizing products in terms of occlusive and biological effects with in vitro 3D skin model.

LB923

Reduced complexity stratified epidermal equivalent cultures from immortalized keratinocytes

B. A. Nanes[1, 2, 3], G. Danuser[2, 3]

[1]Department of Dermatology, UT Southwestern Medical Center, Dallas, Texas, United States, [2]Lyda Hill Department of Bioinformatics, UT Southwestern Medical Center, Dallas, Texas, United States, [3]Department of Cell Biology, UT Southwestern Medical Center, Dallas, Texas, United States

Three-dimensional keratinocyte culture models mimicking stratified squamous epithelium may reveal aspects of skin architecture and function unobservable in traditional two-dimensional monolayer cultures. Such skin equivalent cultures are well-established and were among the first organotypic culture systems developed. However, traditional skin equivalent cultures are relatively complex, requiring primary keratinocytes with limited replication potential and relying on a fibroblast and collagen dermis-like layer to stimulate keratinocyte differentiation. While the development of specialized media has allowed the creation of epidermal equivalent cultures without the underlying fibroblast layer, reliance on primary keratinocytes limits the use of many experimental tools, such as genome editing. Using a keratinocyte cell line immortalized with hTERT and cdk4, we have created a reduced complexity epidermal equivalent culture model more amenable to mechanistic studies based on molecular manipulation. For cells cultured at an air-liquid interface, commonly available growth medium components are sufficient to trigger differentiation. In contrast, keratinocytes in submerged culture maintain in an undifferentiated state, even when fully confluent. The reduced complexity of this immortalized keratinocyte epidermal equivalent culture compared to traditional skin equivalent cultures will significantly increase its experimental utility.

Genetic Disease, Gene Regulation, and Gene Therapy

LB924

Presentation of a novel variant of DOCK8: Heterozygous mutation with clinical findings

A. S. Weissman[1], K. Field[2], S. F. Dinetz[3], C. Mani[4], B. B. Wray[2], K. R. May[2]

[1]Medical College of Georgia at Augusta University, Augusta, Georgia, United States, [2]Division of Allergy-Immunology and Pediatric Rheumatology, Dept. of Pediatrics, Augusta University, Augusta, Georgia, United States, [3]Division of Allergy-Immunology, Dept. of Pediatrics, Nemours Children's Hospital, Orlando, Florida, United States, [4]Division of Pediatric Infectious Disease, Dept. of Pediatrics, Augusta University, Augusta, Georgia, United States

Hyper-IgE syndromes (HIES) are characterized by skin abscesses, increased serum IgE, eczema, and recurrent pneumonias. The most common autosomal recessive (AR) HIES are homozygous dedicator of cytokinesis 8 (DOCK8) loss-of-function mutations. These patients develop atopic diseases and cannot control cutaneous infections. While AR homozygous HIES DOCK8-deficiency clinical information has increased, little is known about the impact of DOCK8 heterozygous mutations. We describe a 10y male with a novel heterozygous point mutation in the DOCK8 region at c.624-12 T>A. He initially presented at 6m with eczema, acute urticaria, potential asthma, and total IgE of 190 kU/L. Around 3y, he developed specific IgE food and environmental sensitivities and dermatitis flares, later complicated by cutaneous fungal infection. He had 3 hospitalizations for severe eczema herpeticum and MRSA skin infections. At 5y, his eosinophil count was $1.6x10^3/mm^3$ with total IgE 15,828 kU/L. To avoid future hospitalization, he was prescribed antibiotic and antiviral prophylaxis and placed on an IgE sensitivity-guided elimination diet. At age 7y, he was referred to the National Institutes of Health (NIH) for further evaluation. In the past few years, his eczema has improved, he has ongoing evidence for IgE sensitivities, and possible asthma. His absolute eosinophil count and total IgE levels have decreased, possibly related to avoidance measures. The clinical significance of heterozygous mutations in the DOCK8 region has not been described previously. This case underscores the potential importance of non-homozygous mutations. Analysis of simple and complex heterozygous mutations may improve our understanding of DOCK8 genetic variants.

LB925

Non-invasive gene-expression analysis of cutaneous T-cell lymphoma

B. Jansen[1], J. A. Rock[2], D. Hansen[3], M. Hanhan[1], Z. Yao[1]

[1]DermTech, La Jolla, California, United States, [2]Clinical, DermTech, La Jolla, California, United States, [3]Pacific Pathology, San Diego, California, United States

Purpose: The purpose of this study is to non-invasively identify disease specific genes expressed in skin samples of patients with cutaneous T-cell lymphoma (CTCL). Materials and Methods: Epidermal skin samples were collected non-invasively with adhesive patches from lesional and non-lesional skin of CTCL patients and from non-CTCL skin samples. Gene expression was evaluated on cDNA reversely transcribed from isolated total RNA using magnetic bead extraction and qPCR reactions. Results: Fourteen target genes were assessed across a sample set including 12 CTCL and 11 control samples. Of these 14 genes, 4 showed significant changes in gene expression ($p<0.05$) and 4 showed a meaningful differential expression, with Student t-test p values close to 0.05 (0.054, 0.056, 0.067 and 0.076, respectively). Using algorithmic analysis of Ct values on gene expression of these genes, we were able to differentiate CTCL patient samples from controls with an AUC > 0.93 and an expected assessment accuracy of over 90%. Conclusions: A group of differentially expressed CTCL target genes was identified in non-invasively obtained skin samples. The employed adhesive patch-based sample collection platform paired with the selected CTCL target genes may help to identify CTCL patients early and without surgical biopsies

Innate Immunity, Microbiology, and Microbiome

LB926

Hyaluronic acids (HAs) molecular size-dependent biological functions on UVB-induced DAMPs-mediated keratinocyte inflammation

L. Hu[1], Y. Sato[1], K. Takagi[1], T. Ishii[1], Y. Honma[1], J. Muto[2]

[1]Rohto Pharmaceutical Company, Kyoto, Japan, [2]Department of Dermatology, Aichi Medical University, Nagakute, Aichi, Japan

Physiologic responses mediated by hyaluronic acid (HAs) depend on their molecular weights and binding receptors, TLR4 and CD44. Low molecular weight HA (LMW-HA) induces inflammation, while high molecular weight HA (HMW-HA) works as an anti-inflammation factor. UVB-induced epidermal inflammation is usually initiated by endogenous molecules released from damaged cells upon skin tissue injury, called damaged-associated molecular patterns (DAMPs). Calprotectin, a typical DAMP, usually causes severe skin inflammation through activating TLR4 pathways. Since both LMW-HA and HMW-HA have inhibitory functions on TLR-mediated macrophage inflammation, HAs are assumed to suppress UVB-induced Calprotectin-mediated skin inflammations. In this study, ultra-LMW-HA, uLMW-HA (0.8 kDa) and HMW-HA (1200kDa), are utilized in the evaluation of UVB-irradiated keratinocyte inflammation. Calprotectin and proinflammatory cytokines secretion were demonstrated on UVB-irradiated keratinocytes. Results showed increased amounts of IL-6 and IL-8 in the medium after irradiation. Under HAs treatments on UVB-irradiated keratinocytes, IL-6 secretion showed a nearly 20% reduction($p<0.05$), which indicates the anti-UVB-induced inflammatory effect of uLMW-HA and HMW-HA. Additionally, we treated the calprotectin-treated keratinocytes with HAs since it was clear that UVB up-regulated Calprotectin secretion. Results showed suppressive effects of HAs on IL-6 secretion, and HMW-HA presented more substantial inhibitory effect compared to uLMW-HA. Furthermore, TLR4 downstream protein, TRAF6 expression was down-regulated in uLMW-HA-treated conditions, which supported uLMW-HA's blocking effects on Calprotectin/TLR4 signal. It is firmly believed that understanding the detailed molecular mechanisms of natural skin response to HAs will be helpful in guiding future HA-containing formulations designs.

Patient Population Research

LB927

Predictors of post-operative wound dehiscence: An analysis of the northwestern medicine enterprise data warehouse (nmedw)

V. Harikumar, K. A. Reynolds, D. Hsu, B. Chen, J. Lazaroff, E. Gwillim, E. Poon, M. Alam

Northwestern University, Chicago, Illinois, United States

Background: Dehiscence, a post-surgical complication, is associated with suboptimal patient outcomes and higher costs. Identifying patients at high risk can facilitate interventions aimed towards prevention. Objective: To identify risk factors for dehiscence after skin closure surgery. Methods: We analyzed the Northwestern Medicine Enterprise Data Warehouse (NMEDW), a registry of EHR patient data, to identify cases of dehiscence. "Wound dehiscence" was searched in patient charts from 2014- 2015. Patients must have had a surgical procedure within 45 days of the mention. Extracted variables included demographic information, medical comorbidities, and medication use. Frequency analysis was conducted for demographic variables. Bivariate logistical regression was performed for each of the independent variables. Finally, multivariate logistical regression analysis was performed with only the variables determined to be statistically significant in bivariate analysis. Results: 447 dehiscence cases and 1625 controls by CPT code were obtained. By multivariate logistical regression, dehiscence was associated with younger age (18-39 odds ratio [OR] 1.67, 95% confidence interval [CI] 1.17-2.37), obesity (OR 1.46, 95% CI 1.04-2.06), and anticoagulant use (heparin OR 4.8, 95% CI 3.36-6.9; LMW heparin OR 4.8 95% CI 3.3-6.9; warfarin OR 7.4 95% CI 3.8-14.6; factor X OR 10.7 95% CI 2.7-42.4). Dehiscence was inversely associated with Charleston Comorbidity Index scores (OR 0.92, 95% CI 0.88-0.97). Anemia was significantly associated with dehiscence in bivariate but not multivariate analysis (OR 1.35, 95% CI 0.973-1.89, P=0.072). Limitations: Limitations include sample size, reliance upon ICD9 coding, and potential differences in patient care across services. Conclusions: Predictors of surgical dehiscence include young age, obesity, and anticoagulant use. Increasing CCI is inversely associated with dehiscence.

LB928

Association of indoor tanning frequency during early life with other addictive behaviors among US women

H. Tsibris[1], H. Nan[2, 3, 4], X. Li[3, 5]

[1]Dermatology, Brigham & Women's Hospital, Boston, Massachusetts, United States, [2]Medicine, Brigham & Women's Hospital, Harvard Medical School, Channing Laboratory, Boston, Massachusetts, United States, [3]Epidemiology, Richard M Fairbanks School of Public Health, Indiana University, Indianapolis, Indiana, United States, [4]Global Health, Richard M Fairbanks School of Public Health, Indiana University, Indianapolis, Indiana, United States, [5]Melvin & Bren Simon Cancer Ctr, Indianapolis, Indiana, United States

Frequent indoor tanning bed users may be more likely to participate in other addictive behaviors. We evaluated the association of indoor tanning usage with other addictive behaviors including smoking, alcohol use, and caffeine consumption amongst a large prospective cohort of 75,957 female registered nurses using logistic regression analyses adjusted for potential confounding factors. 24.5% of the participants reported indoor tanning. We observed dose-response relationships between the frequency of indoor tanning and the likelihood of past or current smoking (*P*-trend <0.0001 for both). Compared with participants who never tanned, frequent indoor tanners (≥12 times per year) were more likely to be past (odds ratio [OR], 1.51; 95% confidence interval [CI], 1.34-1.70) or current smokers (OR, 2.47; 95% CI, 2.18-2.80). We also found a significant association between the frequency of indoor tanning and the likelihood of elevated alcohol use or coffee intake (*P*-trend <0.0001 for both). Women who tanned frequently were more than two times as likely to consume ≥14 alcoholic drinks per week at ages 23-30 (OR, 2.21; 95% CI, 1.72-2.83) or ≥3 alcoholic drinks on any day in a typical month (OR, 2.00; 95% CI, 1.81-2.20) compared to participants who never tanned. The OR of consuming 6 or more cups of coffee daily was 2.21 (95% CI, 1.68-2.90) for frequent tanners compared with those who never tanned. In this study of US women, we found an association between indoor tanning use and other addictive behaviors. Frequent tanners were more likely to smoke, binge drink, and consume more coffee. Our findings have implications for public health strategies aimed at preventing tanning bed dependence and may help to identify individuals at higher risk of multiple addictions.

LB929

Determinants of palliative care utilization for metastatic melanoma

R. Tripathi[1], J. S. Bordeaux[1], R. I. Nijhawan[2]

[1]Dermatology, Case Western Reserve University School of Medicine/University Hospitals Cleveland Medical Center, Cleveland, Ohio, United States, [2]Dermatology, University of Texas Southwestern Medical Center, Dallas, Texas, United States

Background: Although palliative therapy for metastatic disease is associated with improved quality of life, longer overall survival, and lower end-of-life costs, little is known regarding the utilization of palliative therapy for metastatic melanoma. Our objectives were to investigate factors associated with use of palliative therapy for metastatic melanoma, compare mortality by receipt of palliative therapy, and describe trends in palliative therapy utilization over time. Methods: Cross sectional retrospective study of hospital registry data using the National Cancer Database (2004-2015) of patients with Stage IV cutaneous melanoma (n=21,670). Multivariable logistic regression was used to evaluate factors associated with receipt of palliative therapy. Univariate analyses were used to trend palliative therapy use over time and compare palliative therapy receipt by time to treatment. Kaplan-Meier curves were created to compare mortality for patients by receipt of palliative therapy. Results: 3,125 (14.4%) of patients received palliative therapy. Patients initiating treatment earlier were more likely to receive palliative therapy ($p<0.01$ for all modalities). Controlling for all covariates, increased hospital distance (aOR 0.72), location in the Pacific (aOR 0.56), and private insurance (aOR 0.62) were associated with decreased receipt of palliative therapy. Metastases to the bone (aOR 1.53), liver (aOR 1.52), and lung (aOR 1.22), as well as receipt of chemotherapy (aOR 1.25) and radiation (aOR 4.94) were associated with increased receipt of palliative therapy ($p<0.01$). Patients receiving palliative therapy lived over twice as long (19.18 months vs. 8.50 months, $p<0.01$). Use of palliative therapies is steadily increasing over time ($p<0.01$). Conclusions and Relevance: Systemic differences exist in utilization of palliative therapy. Targeted interventions to improve access to palliative therapy are critical in improving care for patients with metastatic melanoma.

LB930

Leaving against medical advice among patients hospitalized for dermatologic conditions

R. Tripathi[1], R. S. Mazmudar[1], J. S. Bordeaux[1], J. Scott[2]

[1]Dermatology, Case Western Reserve University School of Medicine/University Hospitals Cleveland Medical Center, Cleveland, Ohio, United States, [2]Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Background: Leaving against medical advice (AMA) is associated with worse outcomes for hospitalized patients and disproportionately affects vulnerable patient populations. Despite the growing burden of skin disease in hospitalized patients in the United States, little is known regarding predictors for leaving AMA among patients hospitalized for dermatologic conditions. Objective: To investigate the patient, clinical, and hospital factors associated with leaving AMA in patients hospitalized for dermatologic conditions. Methods: Retrospective cross-sectional study of the nationally representative National Inpatient Sample from 2009-2015. Weighted multivariable logistic regression models were used to determine factors associated with leaving AMA in patients hospitalized for dermatologic conditions. Results: 4,523,930 patients were hospitalized for dermatologic conditions and 66,091 (1.46%) left AMA. In multivariable analyses, young adult (age 18-39 aOR 1.41) male (aOR 1.56) patients who were either uninsured (aOR 1.91) or insured by Medicaid (aOR 1.77) were most likely to leave AMA. Patients with higher income had lower odds of leaving AMA (aOR 0.78, $p<0.001$). Patients with skin/subcutaneous infections (aOR 1.19) were most likely to leave AMA, while those with inflammatory skin conditions (aOR 0.81) were least likely to do so ($p<0.05$). Patients admitted electively (aOR 0.56) and undergoing major procedures (aOR 0.38) were less likely to leave AMA ($p<0.001$). Patients at urban teaching (aOR 1.51) and nonteaching (aOR 1.70) hospitals were more likely to leave AMA than those in rural hospitals ($p<0.001$). Conclusion: Understanding contributors to leaving AMA for patients hospitalized with dermatologic conditions is critical in developing targeted interventions to limit early termination of treatment and improve healthcare service utilization.

LB931

Regional differences in biologic treatment patterns and achievement of outcomes within the Corrona Psoriasis Registry across the US

C. Enos[1], K. O'Connell[1], R. Harrison[2], R. McLean[2], B. Dube[2], S. Bell[3], A. Van Voorhees[1]

[1]Eastern Virginia Medical School, Norfolk, Virginia, United States, [2]Corrona, LLC, Waltham, Massachusetts, United States, [3]National Psoriasis Foundation, Portland, Oregon, United States

US treatment targets for psoriasis have been proposed, yet there is little nationwide data on the use and effectiveness of biologics to achieve these targets. This study will determine the geographic variation of biologic treatment patterns and outcomes among adult US patients with psoriasis in the Corrona Psoriasis Registry. Patients were enrolled at 100 sites recruited from 32 states. Analyses included 737 biologic patient-initiations in 2018. Demographics and disease measures were recorded at initiation and 6-months. Proportions of patients achieving response and discontinuing or switching therapy at 6-months were compared across US Census Divisions. At initiation, mean age was 50 years. Proportions of patients varied across regions (e.g.7% in West-South-Central (WSC), 30% in Northeast) as did demographic distributions (women 36-62%, whites 32-97%, obese 30-60%, college graduates 21-47% Medicaid recipients 4-43%). There was a statistically significant difference among regions for the proportions of patients achieving PASI75 ($p=0.01$), with the WSC and East-South-Central (ESC) having the lowest (38 & 41% vs. 51-60% in other regions). Overall difference in achieving BSA≤1 was not statistically significant ($p=0.16$) but was achieved less frequently in the ESC and WSC vs. other regions (BSA≤1 38-39% vs. 46-55%) . Frequencies of discontinuing and switching ($p<0.001$) therapies varied; ESC had the highest proportion of discontinuations (28% vs. 6-15% in other regions) and switches (21% vs. 2-12%). Reasons for discontinuation differed across regions ($p=0.014$), with efficacy most common (54%). Although the registry is not population-based, these findings suggest further investigation into potential geographic differences in patient characteristics and biologic utilization and the potential impact on effectiveness.

LB932

Reasons for psoriasis hospitalizations with inpatient mortality

E. Edigin[1], P. O. Eseaton[2]

[1]Internal Medicine, John H Stroger Jr. Hospital of Cook County, Chicago, Illinois, United States, [2]University of Benin Teaching Hospital, Benin, Edo, Nigeria

Psoriasis is a chronic autoimmune disease with an increased risk of medical comorbidities that results in hospitalizations and occasional inpatient mortality. Data were abstracted from the National Inpatient Sample (NIS) Database (the largest collection of inpatient hospitalization data in the United States). The NIS was searched for psoriasis hospitalizations in 2017 with ICD-10 code “L04” as the principal or secondary diagnosis. The principal discharge diagnosis” for psoriasis hospitalizations with in-hospital death was divided into 19 ICD 10 code categories. There were 30 million discharges included in the 2017 NIS database. Of those, 165,215 hospitalizations had principal or secondary ICD 10 code for psoriasis. Inpatient mortality occurred in 2985 of these hospitalizations (1.81%). These hospitalizations were for patients who were mainly males (57%), whites (65%), with an average age of 69 years, average LOS of 8.7 days and mean total hospital charge of $133,081. The top 5 principal discharge ICD 10 code categories in psoriasis hospitalizations with inpatient mortality in descending order of frequency were as follows: infections 930 (31.16%), cardiovascular 605 (20.27%), respiratory 420 (14.07%), digestive 325 (10.89%), hematology/oncology 225 (7.54%). The most common principal diagnoses in psoriasis hospitalizations with in-hospital mortality were sepsis (from an unspecified organism), followed by acute and chronic hypoxic respiratory failure, non-ST segment elevation myocardial infarction, chronic kidney disease with heart failure secondary to hypertension, and sepsis from Methicillin-Resistant Staph Aureus in descending order of frequency. For psoriasis hospitalizations with in-hospital mortality, infections were the most common ICD 10 code category and sepsis was the most common specific ICD 10 code principal diagnosis. Prevention, early detection and adequate management of infections is key to reducing inpatient deaths in psoriasis hospitalizations.

LB933

Views and beliefs of people engaged in virtual discussions of vitiligo: A qualitative study of messages in online forums

M. B. Gadarowski, A. M. Bashyam, S. R. Feldman

Department of Dermatology, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States

Virtual asynchronous discussion platforms can provide firsthand insight on disease management, psychosocial impact, burden on quality of life, and anecdotal experiences between healthcare providers and the public. We examined the content exchanged by people engaged in online vitiligo forums. An interpretive research paradigm—a means to capture subjective experiences—was utilized to assess public online forum content and the beliefs of individual participants. 39 relevant forums were identified, nine of which met inclusion criteria for a total of 382 anonymous users. An inductive thematic analysis revealed major themes and subthemes including: vitiligo disease management, homeopathy/home remedies, psychosocial impact, public perceptions, and camouflage/concealment. Many users feel vitiligo is neither “life-threatening” nor a “physical ailment,” but nevertheless struggle with stress, depression, and how to manage negative public interactions. Exchanges between participants were overwhelmingly sympathetic, providing encouragement and validation. Coping mechanisms ranged from reliance on friends and family, to personal stories on how to embrace and accept vitiligo. While many discussed appropriate treatments, false reports of “cures” and success with homeopathy emerged, with endorsements of solutions ranging from herbal medicine to diet plans. Contrary to misconceptions that patients with darker skin types experience greater stress, many self-reported Caucasian users explicitly reported lowered self-confidence and high stress levels. Vitiligo forums were overall supportive to participants, with users sharing their personal accounts on how to live with the condition. Users commonly refer to other participants for advice and help on treatments, how to explain their condition, and how to navigate relationships and daily life. After visit care plans may benefit from including information on these areas.

LB934

Outcomes of psoriasis with and without joint involvement

E. Edigin, M. M. Rivera Pavon

Internal Medicine, John H Stroger Jr. Hospital of Cook County, Chicago, Illinois, United States

Psoriasis is a autoimmune disorder with occasional joint involvement. We analyzed the outcomes of psoriasis with and without joint involvement. The primary outcome was inpatient mortality, while secondary outcomes were hospital length of stay (LOS) and total hospital charge. Data were abstracted from the National Inpatient Sample (NIS) 2016 and 2017 Database. The NIS was searched for psoriasis hospitalizations with and without joint involvement as principal or secondary diagnosis using ICD-10 codes. Multivariate logistic and linear regression analysis was used to adjust for possible confounders for the primary and secondary outcomes respectively. There were over 71 million discharges included in the combined 2016 and 2017 NIS database. 323405 hospitalizations were for patients aged≥ 18 years, who had either a principal or secondary ICD 10 code for psoriasis. 77980 (24.11%) and 245425 (75.89%) of these hospitalizations were for psoriasis with joint and without joint involvement respectively. 5485 adult psoriasis hospitalizations (1.70%) resulted in inpatient mortality. 1105 (20.15%) of the deaths occurred in psoriasis with joint involvement vs 4380 (79.85%) without joint involvement (P=0.0019). The adjusted odds ratio (AOR) for inpatient mortality for psoriasis with joint compared to without joint involvement was 0.89 (95% CI 0.76-1.05, P=0.159). Psoriasis with joint involvement hospitalizations had a mean decrease in adjusted LOS of 0.15 days (95% CI 0.26-0.04, P=0.007) compared to without joint involvement (statistically, but not clinically significant). Psoriasis with joint involvement hospitalizations had an increase in adjusted mean total hospital charge of $3655 compared to without joint involvement (95% CI 2146-5164, P=0.000). Hospitalizations for psoriasis with joint involvement do not have increase inpatient mortality compared to without joint involvement. However, it increases total hospital charge, which increases the burden to the health care system. Collaboration between the dermatologist and rheumatologist is needed to optimize outcomes.

LB935

Impact of prior authorization on patient behavior to fill prescription following appointment

A. M. Bashyam, M. B. Gadarowski, A. Ali, W. Haidari, S. D. Pearce, S. R. Feldman, L. C. Strowd

Dermatology, Wake Forest School of Medicine, Winston-Salem, North Carolina, United States

This study evaluated the impact of prior authorization (PA) on patient behavior in filling prescriptions by assessing treatment delay and abandonment. Adult patients prescribed topical tacrolimus for any dermatologic condition in a six-month period were identified. Patients with electronic prescriptions at retail pharmacies were included. Patients whose insurance did not require PA were compared to those who required PA (and were approved). Eligible patients were contacted for a phone questionnaire. Regression analyses controlled for age, sex, race, ethnicity, and insurance. 133 of 231 (58%) eligible patients consented to participate; 93 (70%) of whom did not require PA and 40 (30%) had an approved PA. 7 (18%) patients abandoned treatment in the approved PA group compared to 4 (4%) patients who did not require PA (adjusted OR=5.9; p=.02). In addition to a median 5-day delay in prescription approval for PA, an extra 4 days passed before patients obtained medication from approval date. This delay from approval to pick-up was larger than the delay from prescription to pick-up in the group not requiring PA (4 vs. 1 day; p<.001). In patients who did not require PA, 54 (61%) filled their prescription the same day as their appointment, whereas 7 (21%) patients who required a PA filled their prescription the same day that it was approved (adjusted OR=.17; p<.001). PA led to high rates of treatment abandonment and resulted in an unexpected discrepancy between the time from PA approval to pick-up compared with the time from appointment to pick-up in the group not requiring PA. The difference between groups in filling prescriptions may indicate a disruption of behavioral momentum, where patients typically go straight to the pharmacy after an appointment. PA’s impact may be greater than simply its delay in treatment approval.

LB936

Tanning bed use and the risk of anxiety and depression

E. X. Wei[1], H. Nan[2], X. Li[2, 3]

[1]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States, [2]Epidemiology and Global Health, Richard M. Fairbanks School of Public Health, Indiana University, Indianapolis, Indiana, United States, [3]Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, Indiana, United States

This study investigates whether early life indoor tanning frequency early in life is associated with risk of incident depression and anxiety later in life. The Nurses’ Health Study II (NHSII) is a prospective cohort study, which started in 1989 with 116,434 US female nurses (ages 25–42 at enrollment). Depression was defined as clinician-diagnosed depression and/or antidepressant use. Anxiety is defined as having an Crown-crisp anxiety scale items greater than or equal to 6 or Generalized Anxiety Disorder score of greater than or equal to 15. We used Cox proportional hazards models to estimate hazard ratios (HR) and 95% confidence intervals (CIs) for the risk of incident depression in relation to indoor tanning frequency. Logistic regression analysis was performed to estimate odds ratios (OR) and 95% CIs for association between early tanning frequency and risk of anxiety. In multivariate analysis, higher risk of later in life depression was associated with early life indoor tanning frequency in a dose-dependent manner (p trend <0.0001). Participants who reported tanning bed use 1-2, 3-11 and 12 or more times per year had 12% [HR=1.12, 95% CI (1.05-1.19)], 22% [1.22 (1.13-1.32)] and 45% [1.45 (1.27-1.67)] increased risk for depression later in life, respectively, compared to those who never used indoor tanning. Individuals who reported indoor tanning early in life also had a dose-dependent increased in risk for anxiety later in life (p trend <0.0001). After adjusting for potential confounders, the risk for anxiety was significant in those who reported 3-11or 12 or more times use per year, with 11% [OR=1.11, 95%CI (1.03-1.19)] and 25% [1.25 (1.11-1.41)] increased in risk, respectively, compared to those who had never used the tanning bed. Early life indoor tanning usage is associated with risk of depression and anxiety among US women in a dose dependent manner.

LB937

Post-operative radiation therapy for low-risk Merkel cell carcinoma is associated with reduced local recurrences for primary tumors on the head/neck, but not other sites

M. Bierma[1], P. Goff[2], K. Lachance[1], D. S. Hippe[3], S. Schaub[2], Y. Tseng[2], S. Apisarnthanarax[2], J. Liao[2], P. Nghiem[1], U. Parvathaneni[2]

[1]Dermatology, Univ of WA, Seattle, Washington, United States, [2]Radiation Oncology, Univ of WA, Seattle, Washington, United States, [3]Radiology, Univ of WA, Seattle, Washington, United States

Merkel cell carcinoma (MCC) is a rare, aggressive skin cancer with a recurrence risk of ~40%. Primary treatment typically includes surgery with adjuvant post-operative radiation therapy (PORT) to the primary site to reduce local recurrence (LR) risk. Here, we assess the impact of PORT on LR rates in MCC patients with stage IA head and neck (HN) vs. non-head and neck (N-HN) primary tumors. We conducted a retrospective analysis of 118 low-risk MCC patients treated between 2006 and 2019. Inclusion criteria were study enrollment <180 days from diagnosis, primary tumor ≤2 cm, negative pathological margins, negative sentinel lymph node biopsy, no immunosuppression, and no lymphovascular space invasion. No patient received systemic therapy as part of initial management. LR was defined as tumor recurrence within 2 cm of the primary surgical bed and estimated with the Kaplan-Meier method. Sixty-six patients received PORT (26 HN, 40 N-HN) while 52 patients were treated with surgery alone (28 HN, 24 N-HN). There were 7 LRs (6 HN, 1 N-HN), with a significantly higher risk of LR among patients treated with surgery alone compared to surgery plus PORT (12% vs. 1%, p=0.043). While the addition of PORT significantly reduced LR rates among HN patients (22% vs. 0%, p=0.024), there was no significant difference in LR rates among N-HN patients treated with surgery alone vs. surgery plus PORT (0% vs. 1%, p=1.000). MCC-specific survival among patients receiving PORT vs. surgical monotherapy was not significant (p=1.000). These findings confirm prior reports that PORT is associated with lower LR rates in patients with primary HN MCC, even among patients with the lowest risk disease such as this cohort. In contrast, for low-risk MCC primary tumors on the limbs and trunk, excellent outcomes were achieved in the absence of PORT.

Patient-Targeted Research

LB938

Efficacy of FMX101 4% topical minocycline foam for the treatment of moderate-to-severe acne vulgaris: Integrated summary from three phase 3 studies

J. Del Rosso[1], L. F. Stein Gold[2], J. Weiss[3], L. Green[4], V. Stakias[5], I. Stuart[5]

[1]JDR Dermatology Research/Thomas Dermatology, Las Vegas, Nevada, United States, [2]Henry Ford Health System, Detroit, Michigan, United States, [3]Gwinnett Clinical Research Center, Snellville, Georgia, United States, [4]George Washington University School of Medicine, Rockville, Maryland, United States, [5]Foamix Pharmaceuticals, Bridgewater, New Jersey, United States

Background: FMX101 4% is a novel FDA-approved minocycline-containing topical foam for the treatment for acne vulgaris. Objective: Compare the efficacy of FMX101 4% vs vehicle foam for acne based on the integrated analysis of 3 Phase 3 studies. Methods: In 3 multicenter, randomized, double-blind, Phase 3 studies (FX2014-04, N=466; FX2014-05, N=495; FX2017-22, N=1488), FMX101 4% was compared to vehicle in subjects with moderate-to-severe acne. Subjects applied the study drug once daily for 12 weeks. The co-primary efficacy endpoints were the absolute change from baseline at week 12 in inflammatory lesions and the proportion of subjects with Investigator Global Assessment (IGA) treatment success. Percent change from baseline at week 12 in noninflammatory lesions was a secondary endpoint. Results: The integrated population included 2449 subjects (FMX101 4%, n=1378; vehicle, n=1071). FMX101 4% demonstrated statistically significant benefit vs vehicle for both co-primary endpoints. There was a significantly greater reduction in inflammatory lesions from baseline at week 12 in the FMX101 4% group compared to vehicle (*P*<.0001), and a significantly greater percent of FMX101 4% subjects who achieved IGA treatment success at week 12 compared to vehicle (*P*<.0001). The effect of FMX101 4% was observed as early as week 3, and was maintained throughout the 12-week trial. There was a significantly (*P*=.0019) greater percent reduction in noninflammatory lesions at week 12 in the FMX101 4% group vs vehicle. Conclusions: FMX101 4% demonstrated statistically significant benefits compared to vehicle in treating moderate-to-severe acne vulgaris in the pooled population of 3 Phase 3 studies.

LB939

Pruritic and dyskeratotic dermatosis: An unusual cutaneous manifestation

K. R. Kwan[1], S. Sears[1], J. Malone[1], P. Rady[2], S. Tyring[2], S. Bahrami[1], M. Huelsman[1], J. Callen[1]

[1]Dermatology, University of Louisville School of Medicine, Louisville, Kentucky, United States, [2]Dermatology, UTHSC Houston, Houston, Texas, United States

Immunocompromised patients are at increased risk for opportunistic infections. Pruritic and dyskeratotic dermatosis (PDD) is a rare, recently described clinical disease in immunocompromised individuals caused by reactivation of Human Polyomavirus (HPyV) 6 or 7,detectable predominantly in the epidermis. Histologically, specimens lack viropathic effect and may demonstrate “Peacock plumage” characterized by irregular columns of parakeratosis and spotty intraepidermal dyskeratosis. The condition manifests as diffuse, pruritic, gray-brown, lichenified plaques. We present a patient with PDD with an unusual clinical presentation of keratotic spines, not characteristic of HPyV 6 or 7 infection, but more commonly seen in trichodysplasia spinulosa (TS), a cutaneous eruption caused by TS-associated human polyomavirus (TSPyV). The patient is a 44 year-old African-American man with a history of kidney and pancreas transplant who presented for evaluation of a 2 year history of a diffuse pruritic, burning rash associated with dyspigmentation and lichenification in addition to keratotic spines involving the dorsal hands and elbows and pits involving the wrists and palms. Two biopsies from the wrist and upper extremity showed mild acanthosis, spongiosis, hyperkeratosis, and prominent intraepidermal dyskeratosis. Human Polyoma Virus qPCR testing was performed and returned positive for HPyV7 and negative for TSPyV. Treatment with oral acitretin 25 mg daily and topical cidofovir cream has begun. PDD should be included in the differential diagnosis of a diffuse pruritic rash with keratotic spines and pits in an immunosuppressed patient. While keratotic spines reflect the similar viral class of origin of PDD and TS, the histologic features of the two conditions are disparate. Despite the histologic lack of viral cytopathic effect, the presence of columnar parakeratosis or spotty intraepidermal dyskeratosis should alert the pathologist to the diagnosis of PDD when there is history of immunosuppression.

LB940

Ixekizumab versus guselkumab: 24-week clinical responses and 4-week gene expression data

A. Blauvelt[1], A. Pinter[2], Y. Tada[3], H. Elmaraghy[4], G. Gallo[4], B. J. Nickoloff[4], L. Renda[4], S. Park[4], L. Guenther[5]

[1]Oregon Medical Research Center, Portland, Oregon, United States, [2]University Hospital, Frankfurt am Main, Germany, [3]Teikyo University School of Medicine, Tokyo, Japan, [4]Eli Lilly and Company, Indianapolis, Indiana, United States, [5]Guenther Research Inc., London, Ontario, Canada

The objective of IXORA-R was to compare the efficacy of the interleukin (IL)-17A antagonist ixekizumab (IXE) and the IL-23p19 inhibitor guselkumab (GUS) over 24 weeks of treatment. Adults with moderate-to-severe plaque psoriasis (sPGA ≥3, PASI ≥12, BSA ≥10%) were randomized to IXE or GUS (1:1; dosing per label). Significantly more patients treated with IXE than GUS had clear skin (PASI 100) from Weeks 4 to 12.[1] Here, we report 24-week results for efficacy and safety as well as gene expression data through Week 4. Efficacy comparisons (PASI 100 and Physician's Global Assessment of Fingernail psoriasis [PGA-F]=0) were made using Cochran-Mantel-Haenszel test adjusted by pooled site using nonresponder imputation for missing data. A prespecified non-inferiority test of IXE versus GUS was performed for PASI 100 at Week 24 (pre-set non-inferiority margin: -11.4%). IXE was non-inferior to GUS for PASI 100 at Week 24 (IXE 50%, GUS 52%, difference: -2.3% [95% CI −8.4% to 3.8%]), with no statistically significant difference (p=0.414). Of those with baseline PGA-F >0, more patients receiving IXE compared with GUS showed completely clear nails at Week 24 (63% vs. 44%; p<0.001). Using 8 public datasets, we identified 286 overexpressed genes in psoriasis. Gene expression at Weeks 1, 2, and 4 versus baseline was compared for lesional skin biopsies taken from a subset of patients (IXE, n=32; GUS, n=21). A greater number of overexpressed genes were reduced by IXE than GUS at Weeks 1-4 (Week 1: 156 vs. 5; Week 2: 191 vs. 41; Week 4: 260 vs. 113). In conclusion, IXE was non-inferior to GUS in skin clearance but superior in clearing nails at Week 24, with no new safety signals, and IXE more rapidly reduced overexpressed psoriasis genes than GUS as early as Week 1. [1]Blauvelt, A et al. (2019). *Br J Dermatol*. doi:10.1111/bjd.18851.

LB941

Dupilumab for atopic dermatitis in children aged ≥6 to <12 years in phase 3 LIBERTY AD PEDS trial: Analysis by baseline weight

A. S. Paller[2], E. C. Siegfried[3], L. A. Beck[4], J. T. O'Malley[5], B. Shumel[1]

[1]Regeneron Pharmaceuticals Inc., Tarrytown, New York, United States, [2]Northwestern University Feinberg School of Medicine, Chicago, Illinois, United States, [3]Saint Louis University, Saint Louis, Missouri, United States, [4]University of Rochester Medical Center, Rochester, New York, United States, [5]Sanofi, Boston, Massachusetts, United States

We evaluate dupilumab efficacy and safety by pre-specified baseline weight stratification in children aged ≥6 to <12 years with severe atopic dermatitis (AD) inadequately controlled with topical corticosteroids (TCS). In this double-blind, phase 3 trial (LIBERTY AD PEDS; NCT03345914), patients (pts) were randomized to receive either subcutaneous dupilumab 300mg every 4 weeks (q4w) regardless of baseline body weight; or every 2 weeks (q2w) as 100mg for pts <30kg and 200mg for pts ≥30kg; or placebo (PBO), for 16 weeks. All pts received concomitant medium potency TCS from Day –14.122 pts received 300mg q4w, 63 received 100mg q2w, 59 received 200mg q2w, and 123 received PBO. At Week 16, the proportion of pts <30kg who reached Investigator Global Assessment scores of 0/1 (IGA [0,1]) was 29.5% in 300mg q4w and 20.6% in 100mg q2w groups vs 13.1% in the PBO group. Among pts ≥30kg, 36.1% in 300mg q4w and 39.0% in 200mg q2w groups achieved IGA [0,1] compared with 9.7% in the PBO group. Among pts <30kg, 75.4% (300mg q4w) and 60.3% (100mg q2w) achieved ≥75% improvement from baseline in Eczema Area and Severity Index (EASI-75) vs 27.9% in the PBO group. In pts ≥30kg, 63.9% (300mg q4w) and 74.6% (200mg q2w) achieved EASI-75 vs 25.8% in the PBO group. Significant improvements in pruritus were also observed in pts receiving either dupilumab regimen compared with PBO. Dupilumab was well tolerated, and data were consistent with the known safety profile. In children aged ≥6 to <12 years with severe AD, dupilumab showed clinically meaningful and statistically significant improvement in AD signs and symptoms. Differences were observed in several key efficacy parameters when dupilumab dose regimens were evaluated by baseline weight strata, particularly IGA and EASI-75 improvement in children weighing <30kg treated with 300mg q4w compared to those treated with 100mg q2w.

LB943

Calcipotriol and 5-fluorouracil as neoadjuvant therapy for mohs micrographic surgery in treatment of cutaneous squamous cell carcinoma

Y. Humeda[1], L. Angnardo[1], E. Corty[2], L. Hyder[1], M. Hoffmann[1], A. Cognetta[3]

[1]Dermatology, Florida State University College of Medicine, Tallahassee, Florida, United States, [2]Johns Hopkins School of Public Health, Baltimore, Maryland, United States, [3]Department of Dermatology, Mohs Micrographic Surgery and Cutaneous Oncology Division, Florida State University College of Medicine, Tallahassee, Florida, United States

Cutaneous squamous cell carcinoma (cSCC) often occurs intermixed with numerous actinic keratoses (AK) within a field of significant actinic damage. When used together, calcipotriol and 5-Fluorouracil (C5FU) synergistically activate a $CD4^+$ T cell-mediated immunity against AK resulting in a mean reduction in AK on the face and scalp when compared to the use of 5FU with Vaseline. We frequently encounter subclinical spread of cSCC in situ beyond the initial biopsied lesion. We investigated the use of C5FU preoperatively in patients with biopsy-proven cSCC compared to Mohs Micrographic surgery (MMS) alone. We hypothesized that those pretreated with C5FU would require fewer MMS layers to obtain clear margins and thereby a potentially smaller final defect size. A retrospective chart review was conducted between December 2014 and October 2018 using an electronic database. We screened 500 patients that underwent MMS. Data were collected from 20 MMS patients pretreated with C5FU and 20 patients who did not undergo neoadjuvant treatment. Inclusion criteria were biopsy-proven cSCC in situ on the face or scalp with no previous documented field therapy for actinic damage within the past 5 years. The C5FU group was associated with fewer MMS layers compared to the MMS only group (1.2 vs. 1.4, respectively). The final defect size was larger in the C5FU group than in the MMS only group (204.9 mm^2 vs.198.4 mm^2, respectively). However, the initial defect size was also larger in the C5FU group compared to the MMS only group (79.7 mm^2 vs. 72.3 mm^2, respectively). Although not statistically significant, these results may be clinically significant, and highlight the potential of C5FU as a beneficial neoadjuvant therapy for cSCC and suggest the need for a larger-scale, higher-powered, prospective study to further establish its value, as it has not previously been investigated.

LB944

The impact of dermatology consultation for dermatology-related admissions in oncology patients

A. Y. Ning[1], M. Levoska[2], S. Hill[2], M. Beveridge[2]

[1]Case Western Reserve University SOM, Cleveland, Ohio, United States, [2]Dermatology, University Hospitals Cleveland Medical Center, Cleveland, Ohio, United States

Dermatologic toxicities in oncology patients may result in unnecessary cessation of cancer therapy. Early diagnosis by dermatology can minimize treatment disruption. We aimed to characterize inpatient oncology admissions for dermatologic issues and the impact of dermatology consultation on management. We performed a retrospective chart review of inpatient dermatology consultations from 7/01/2018 to 1/22/2020 at University Hospitals Cleveland Medical Center. Of 794 consultations,138 were for oncology patients. Eighteen oncology patients were admitted for dermatologic issues related to their cancer/cancer therapy and were included in our study. Of these, fifteen (83%) were admitted for rashes and three (17%) for skin/soft tissue infections. Primary teams held cancer therapy in eight patients (44%) prior to consulting dermatology. Dermatology agreed with holding therapy in six patients (75%). In the others (n=2, 25%), dermatology recommended resuming treatment; however, primary team management did not change. The remaining ten patients continued cancer therapy, consistent with dermatology recommendations. Overall, we found good agreement between dermatology and primary team management (kappa=0.769). Primary teams often started therapy for dermatologic issues prior to dermatology evaluation (n=13, 72%), most commonly with systemic antimicrobials (n=6, 33%) or systemic corticosteroids (n=4, 22%). Only two patients (11%) were recommended by dermatology to continue initial treatment. Two patients started on systemic corticosteroids (50%) were switched to topical corticosteroids, and systemic antimicrobials were discontinued in three patients (50%). Dermatology and primary teams often agreed on cancer therapy management. When in disagreement, dermatology recommended restarting therapy. Dermatologists often used non-systemic therapies, limiting toxicities in this medically complex group. Dermatologists can provide value through an oncodermatologic service with a collaborative approach for patient management.

LB945

Using artificial intelligence to understand patient perspectives towards treatment of dermatological diseases

J. Jueng[1], L. Dupuis[1], A. Su[1], A. Kunadia[1], R. Dellavalle[2], I. Brooks[3], R. Sinha[3], B. Maner[4], F. Siddiqui[5], V. Burton[6], J. Seyffert[7], J. Solomon[1, 4, 7]

[1]University of Central Florida College of Medicine, Orlando, Florida, United States, [2]University of Colorado Health Science Center, Aurora, Colorado, United States, [3]University of Illinois Center for Health Informatics, Champaign, Illinois, United States, [4]Ameriderm Research, Ormond Beach, Florida, United States, [5]Alabama College of Osteopathic Medicine, Dothan, Alabama, United States, [6]Clemson Cyber Institute, Clemson, South Carolina, United States, [7]Department of Dermatology, Kansas City University of Medicine and Biosciences-Advanced Dermatology and Cosmetic Surgery, Orlando, Florida, United States

Patients with dermatologic conditions frequently use social media, generating millions of posts in which they share about their experience with disease and treatment. Using Artificial Intelligence (AI), we analyzed public social media posts and identified associations between Patient Global Impression of Change (PGIC) and patient sentiments which could provide insight into disease burden. Using the Crimson Hexagon (CH) AI-powered social media database, millions of publicly available social media posts were identified. CH classified the emotion of the posts as either anger, disgust, fear, happiness, sadness, or surprise. Machine learning through natural language processing was trained to analyze emotional response in relationship to PGIC based on treatment. We used patient sentiments towards alopecia areata, acne, melanoma, and psoriasis treatment(s) as representative tests of our methodology. For example, JAK inhibitors are a treatment for alopecia areata. Our analysis showed that of 239 posts about JAK inhibitors, 210 indicated a positive outcome of treatment based on the PGIC scale. However, of the 210 posts noting treatment effectiveness, 191 indicated negative sentiment (fear, anger, disgust) towards treatment. Analysis was performed similarly for the other diseases. Our analysis will provide insight into treatment efficacy and subsequent effects on patient wellbeing. This insight could help providers improve patient centered care and allow pharmaceutical companies to directly address patient needs in novel therapeutics.

Pharmacology and Drug Development

LB946

Improvements in acne-prone skin quality correlate with a reduction in saliva cortisol levels after use of an 8-week 3-step topical regimen

C. Skobowiat[1], J. S. Craw[1], R. Bianchini[1], K. Fields[1, 2], K. Rodan[1, 2], T. Falla[1]

[1]Rodan + Fields LLC, San Francisco, California, United States, [2]Department of Dermatology, Stanford University, Stanford, California, United States

It is well established that both UV exposure and emotional stress can lead to an activation of stress response via the Hypothalamic Pituitary Adrenal (HPA) axis resulting in elevated cortisol levels negatively affecting skin. [a, b, c] In this study, we found that subjects using a three-step skincare regimen not only experienced the expected skin benefits but also significantly lower cortisol levels after eight weeks. Thirty women, ages 18-45, applied a three-step skincare regimen featuring antioxidants, electrolytes, prebiotics and a sunscreen to their faces twice a day for eight weeks. Saliva samples along with clinical grading and TEWL measurements were taken at baseline and at week eight. Subjects also completed a questionnaire on skin quality at the beginning and end of the study. Over the duration of the study, cortisol levels, determined from saliva using ELISA, decreased from an average of 435 ng/dL at baseline to 73.3 ng/dL ($p \leq 0.005$). Concurrently, a statistically significant increase in barrier function (TEWL) corresponded to a clinical improvement in healthy glow. Subjects reported a significant improvement in pore appearance and decreased number of acne lesions at the end of the study. In addition, 76% of the subjects felt more confident about posting a selfie at the end of the study compared to 53% at the beginning. We suggest that one of the factors responsible for the observed decrease in saliva cortisol levels may be a reduction of emotional stress due to improved skin quality. References [a] Br J Dermatol. 2013 Mar;168(3):595-601; [b] J Invest Dermatol. 2006 Aug;126(8):1697-704; [c] Sci Rep. 2018 Apr 20;8(1):6334.

LB947

Development of LY3454738, an agonistic antibody to human CD200R

S. C. Potter, K. D. Werle, S. P. Bauer, D. I. Ruiz, J. Rhoden, S. Demarest, D. Witcher, A. Koester

Eli Lilly, San Diego, California, United States

CD200R is an immune receptor of the IgG family that is primarily expressed on cells of the myeloid lineage and was recently identified as a marker for Th2 biology (Blom et al 2017). In vivo studies with knockout mice of either the receptor or its ligand, CD200, have demonstrated that it is an inhibitory receptor capable of negatively regulating immune responses. Previous work using agonistic antibodies to mouse CD200R showed inhibition of mast cell activation in vitro and in vivo (Cherwinski et al 2005) as well as efficacy in multiple preclinical models of autoimmune diseases. We developed an agonistic antibody to the human CD200 receptor to downregulate the immune system in multiple human inflammatory conditions. LY3454738, is a humanized IgG4 monoclonal antibody that was derived from a rabbit antibody discovered by immunizing rabbits with alternating soluble extracellular domain (ECD) of hCD200R and cyno CD200R protein. The antibody was selected based on desired properties for agonism and cross-reactivity to cyno CD200R. In vitro LY3454738 demonstrated inhibition of FcγR induced cytokine secretion from a human myeloid cell line as well as inhibition of primary mast cell activation. In vivo the antibody demonstrated efficacy in a humanized mouse model of contact hypersensitivity as well as passive cutaneous anaphylaxis in cynomolgus monkeys. After demonstrating safety and tolerability in a phase 1 trial in healthy volunteers, LY3454738 is currently being studied in patients with atopic dermatitis and chronic spontaneous urticaria. The poster will describe properties and functional activities of the clinical drug candidate.

LB948
Evaluation of creams containing ozonated sunflower oil
P. Brizzi, M. Andreassi, L. Furi, J. Zanardi, V. Travagli
Dipartimento di Biotecnologie, Chimica e Farmacia - Dipartimento di Eccellenza 2018-2022, Università degli Studi di Siena, Siena, Italy
This study concerns the evaluation from the physical point of view of preparations for topical application containing sunflower oil (Helianthus annuus) as such and after ozonation. Six different creams with different amounts of either untreated or ozonated sunflower oil (3%, 5% and 10%) with respect to the original one without sunflower oil have been evaluated. Two different preparation methods have been adopted: i) adding the relative percentages of oil in the base cream; ii) replacing a part of a lipophilic component of the base cream. The various preparations have been evaluated for apparent viscosity (T = 35 °C; n=3) by both vibrational (VV) and torsional oscillation viscometers (TOV). For creams obtained by i): viscosity values decrease as oil amounts increase with increasing oil concentration. Moreover, the ones with ozonated oil were slightly less viscous than the corresponding creams with non-ozonated oil, with the exception of 10% ozonated oil cream (442 mPa·s vs 555 mPa·s,VV; 145 mPa·s vs 210 mPa·s, TOV). However, the creams prepared with i) proved to be unstable, with a temperature-dependent phase separation with the appearance of oil droplets on the surface (especially for 10% and 5%). For creams obtained by ii): viscosity values proportionally increase with oil concentration (2.13 Pa·s at 10% ozonated oil, VV). On the contrary, they did not give stability problems to the heating, maintaining intact consistency and appearance even after 3 months from the preparation. Obtained results suggested that the method of inserting the oil into the base cream cannot be exploited as it brings the formulation into an excess of lipophilic phase that the various emulsifying components are not able to stabilize. Despite the higher viscosity, the creams obtained by using the replacement method always show both suitable spreadability for the type of topical preparation and excellent stability over time, even in the presence of temperature fluctuations.

LB949
Development of topical MEK inhibitor, NFX-179, as a chemopreventive agent for squamous cell carcinoma
A. Shah[1], B. Sell[2], W. Sun[1], M. Duncton[3], J. Banoo[1], J. Kincaid[3], K. Sarin[1], K. Y. Tsai[2]
[1]Dermatology, Stanford University, Redwood City, California, United States, [2]H. Lee Moffitt Cancer Center, Tampa, Florida, United States, [3]NFlection Therapeutics, Wayne, Puerto Rico, United States
Cutaneous squamous cell carcinoma (cSCC) is the second most common skin cancer comprising over one million cases and contributing up to 9,000 deaths annually in the United States. We recently demonstrated that blockade of RAS signaling by MEK inhibitors reduces the formation of cSCCs in mice highlighting the potential of MEK inhibitors in chemoprevention of cSCC. However, the systemic toxicities of MEK inhibitors preclude their chronic usage in chemoprevention. To that end, NFlection Therapeutics has developed a topically formulated, novel and potent MEK inhibitor, NFX-179, designed to selectively inhibit MEK in SCC tumor tissue but with a high rate of clearance from plasma to limit systemic exposure. In human tissue, topical application of NFX-179 was shown to penetrate the stratum corneum and concentrate in the epidermis and dermis and potently suppress phosphorylation of ERK (p-ERK), a key downstream component of the RAS signaling pathway, in a dose related fashion. NFX-179 also demonstrated suppression of p-ERK in ex-vivo human cSCC tumor explants. Application of NFX-179 to the back skin of a UV-driven mouse model reduces cSCC formation compared to vehicle in a dose dependent manner. Lesion development was reduced by 27%, 73% and 92% at 0.01%, 0.15%, and 0.5% dosages, respectively. These results support the development of topical NFX-179 for as a chemopreventative agent for SCC. Plans are underway to conduct human clinical trials in 2021.

Pigmentation and Melanoma

LB950
Geographic variations in cutaneous malignant melanoma distribution in Russian Federation
A. Muntyanu[1], E. Savin[1], F. Ghazawi[2], A. Alakel[1], A. Zubarev[1], I. Litvinov[1]
[1]Dermatology, McGill University, Toronto, Ontario, Canada, [2]Dermatology, University of Ottawa, Montreal, Quebec, Canada
Cutaneous Malignant Melanoma (CMM) incidence has been rising around the world and over the last three decades at rates greater than for any other malignancy. Our objective was to describe geographic trends in incidence and mortality of CMM in Russia between 2001 and 2017 using geo-informatics technique (mapping) and descriptive statistical analysis. Additionally, we aimed to study the associations between ethnicity, geographic latitude/longitude and CMM incidence/mortality rates. We retrospectively analyzed the data from the Moscow Oncology Research Institute, Ministry of Health of Russian Federation for the period of the study. International Classification of Diseases (ICD) C43 code (comprising C43.0-C.43.9) was used to identify cutaneous melanoma cases. Routine methods of descriptive epidemiology were used to study incidence and mortality rates by age groups, years, and jurisdictions (i.e., Federal Districts and Federal Subjects of Russia). In total 141,597 patients were diagnosed with melanoma in Russia over the period 2001-2017, of which 62% were women ($p<0.001$). The overall age-standardized incidence and mortality rates were 4.27/100,000 and 1.62/100,000, respectively. Geographic mapping revealed North-to-South and East-to-West gradients across the country. Intrinsic patient characteristics such as the skin phenotype and the climate zones of the country could be an important risk factors for melanoma development. This study, for the first time, reports the burden and geographic distribution of CMM in Russia and the trends correlate with observations in countries with similar geography.

LB951
Interpretable pathologist-level classification of melanoma disease pathologies with a convolutional neural network pipeline
P. Xie[1], F. Li[5, 3, 4], S. Zhao[5, 3, 4], J. Liu[1], K. Lu[1], Y. Zhang[2], Z. Ke[1], X. Chen[5, 3, 4]
[1]National University of Defense Technology, Changsha, Hunan, China, [2]Xiangya Hospital, Changsha, Hunan, China, [3]Hunan Key Laboratory of Skin Cancer and Psoriasis, Changsha, Hunan, China, [4]Hunan Engineering Research Center of Skin Health and Disease, Changsha, Hunan, China, [5]Dermatology, Xiangya Hospital, Central South University, Changsha, Hunan, China
For diagnosing melanoma, hematoxylin and eosin (H&E) stained tissue slides remains the gold standard. Accurate diagnosis of melanoma pathology images plays an important role in early diagnosis and successful treatment for melanoma. Deep convolutional neural networks (CNNs) show strong discrimination power for complex medical image recognition tasks. However,the black box nature of CNNs limits their role in clinical settings, where interpretable diagnosis is indispensable. In this paper, we build a deep learning pipeline to precise diagnosis of melanoma using CNNs and localize possible malignant melanoma tissue by the gradient-weighted class activation mapping (Grad-CAM). First, we have built the largest multi-center melanoma database that contains 2241 digital whole-slide images from 1321 patients (1806 melanomas and 435 nevus). Further, CNN model was trained and tested on our database.The CNN model achieved AUC of 0.985, sensitivity of 93.6% and specificity of 95.9%. The accuracy of the algorithm was compared with that of 20 pathologists (0.933 vs 0.732). Finally, Grad-CAM was used and accomplished accurate localization of the melanoma salient feature area. The salient area agrees with the regions of interest detected by the pathologists. The method would help doctors understand how the model makes decisions based on the patient's pathological data. This study is the first work that combines interpretability with a deep learning model for the classification of nevus and melanoma in histopathology. These findings suggest that our approach can provide experts with an explainable, rapid, and accurate diagnosis of melanoma.Thus have a great influence on the deep integration of deep learning technology with clinical practice.

LB952

Multi-scale network for melanoma histopathology image analysis

P. Xie[1], F. Li[3, 4, 5], S. Zhao[3, 4, 5], Q. Li[6], J. Liu[1], K. Lu[1], Y. Zhang[2], T. Li[1], J. Zhou[1], Z. Ke[1], X. Chen[3, 4, 5]

[1]National University of Defense Technology, Changsha, Hunan, China, [2]Xiangya Hospital, Changsha, Hunan, China, [3]Dermatology, Xiangya Hospital, Central South University, Changsha, Hunan, China, [4]Hunan Key Laboratory of Skin Cancer and Psoriasis, Changsha, Hunan, China, [5]Hunan Engineering Research Center of Skin Health and Disease, Changsha, Hunan, China, [6]Pathology, Xiangya Hospital,Central South University, Changsha, Hunan, China

Histopathology images have distinct histomorphological features on different scales. In the pathological image analysis, pathologists can only make proper diagnosis by comprehensively analyzing histomorphological features of dif-ferent scales. However, the current convolutional neural network with single-scale image input does not have the powerful ability to merge features of different scales. Accordingly, we propose a multi-scale convolutional network architecture for merging features of different scale images, with refer-ence to the doctors' diagnosis process. Specifically, we create the multi-scale network search (MSNS) algorithm to further optimize the architecture . The architecture can be used in pop convolutional neural networks(CNNs).To develop and evaluate the proposed methods, a melanoma dataset was constructed within 634 digital whole-slide images (185 melanomas, 188 intradermal nevus, 119 compound nevus, 142 junctional nevus). We combined the MSNS with stae-of-the-art CNNs, such as ResNet, EfficientNet and InceprionNet. The model with multi-scale convolutional network architecture is superior to the original network with relative accuracy gains of 2%~3%. Experimental results on melanoma dataset imply that incorporating diagnosis can help neural network identify more adequate features, by which we can achieve higher diagnosis accuracy than the original neural network.

LB953

Melanoma cell invasion is inhibited in the absence of α3β1 integrin mediated paracrine signaling from keratinocytes

K. J. Bommareddy[1], A. Dabaghian[1], A. Ndoye[1], L. Davis[1], C. DiPersio[1, 2]

[1]Surgery, Albany Medical College, Albany, New York, United States, [2]Department of Regenerative and Cancer Cell Biology, Albany Medical College, Albany, New York, United States

Our previous work established a critical role for the laminin-binding integrin α3β1 in regulating the ability of epidermal keratinocytes to modify the cutaneous microenvironment during skin development, tumorigenesis and wound healing. These modifications occur through direct modulation of extracellular matrix (ECM) and paracrine signals to stromal cells (e.g., endothelial cells, fibroblasts) that facilitate angiogenesis, inflammation, or ECM remodeling. Mass spectrometry of the keratinocyte secretome identified a number of α3β1-dependent secreted proteins that modulate the microenvironment including ECM proteins (e.g., fibulin-2), proteases (e.g., MMP-9), and growth factors/cytokines (e.g., MRP-3). Epidermal keratinocytes also communicate with melanocytes through cell-cell contact and paracrine signals, raising the intriguing possibility that they modulate melanoma progression and invasion. In this study, we seek to determine if α3β1-dependent paracrine signals from keratinocytes can modulate melanoma cell invasion. To test this hypothesis, we are using mouse keratinocyte (MK) cell lines engineered to express or lack α3β1 (MKα3+ or MKα3-, respectively). To assess invasion of human A375 melanoma cells in response to factors secreted by these MK variants, we performed transwell Matrigel assays in the presence of culture medium that was conditioned for 24 hours by MKα3+ or MKα3- cells. Preliminary results suggest that A375 cells are less invasive when exposed to medium from MKα3- cells, compared with medium from MKα3+ cells, or unconditioned medium, suggesting that α3β1-deficient keratinocytes may secrete a factor(s) that reduces melanoma cell invasion. We are using this model to identify specific α3β1-dependent factors that modulate melanoma cell invasion. Understanding how integrin α3β1 controls keratinocyte communication with melanoma cells may provide new mechanistic insights into melanoma tumorigenesis and identify novel therapeutic targets.

LB954

Identification and characterization of patients with vitiligo treated with ruxolitinib cream based on the achievement of F-VASI50

M. Howell, H. Liu, S. Owens, K. Sun, F. Kuo, K. Butler

Incyte Corporation, Wilmington, Delaware, United States

Vitiligo is a chronic, inflammatory skin disease characterized by increased interferon-gamma signaling through Janus kinase (JAK) 1 and JAK2 and subsequent activation of CD8+ T cells, which target melanocytes resulting in areas of depigmentation. Ruxolitinib cream, a JAK1/JAK2 inhibitor, is under investigation for vitiligo treatment in a 52-week, randomized, double-blind, phase 2 study (NCT03099304). Significantly more patients treated with ruxolitinib cream vs vehicle achieved ≥50% improvement in facial Vitiligo Area Scoring Index (FVASI50) at Week 24 (primary endpoint); at Week 52, patients treated with ruxolitinib cream 1.5% twice daily (BID) attained the highest FVASI50 response (57.6%). This analysis investigated the differences between patients that achieved or did not achieve FVASI50 at week 24 following treatment with ruxolitinib cream. Patients were classified as FVASI50≤24 if they achieved FVASI50 or greater at Week 24; all other patients were classified as FVASI50>24. Stratification identified 15 FVASI50≤24 and 16 FVASI50>24 in the ruxolitinib cream 1.5% BID group. In FVASI50≤24, FVASI improved by 79.9%±4.0% and 91.9%±1.5% at Weeks 24 and 52, respectively. In contrast, FVASI improved by 1.1%±7.3% and 25.1%±13.4% in FVASI50>24 at Weeks 24 and 52. RNA sequencing of noninvasive skin samples from 33 patient pairs (baseline and Week 24) identified >400 differentially expressed genes between FVASI50≤24 and FVASI50>24 at baseline; >300 genes were modulated between baseline and Week 24 following treatment with ruxolitinib cream. Overall, this analysis identified significant differences between patient groups which require deeper interrogation to understand the clinical significance for patients with vitiligo.

LB955

Predicting metastatic melanoma from melanoma pathological images using a Convolutional Neural Network: A Multicenter Study

P. Xie[1], F. Li[3, 4, 2], S. Zhao[3, 4, 2], Q. Li[5], J. Liu[1], K. Lu[1], Y. Zhang[6], T. Li[1], J. Zhou[1], Z. Ke[1], X. Chen[3, 4, 2]

[1]National University of Defense Technology, Changsha, Hunan, China, [2]Hunan Engineering Research Center of Skin Health and Disease, Changsha, Hunan, China, [3]Dermatology, Xiangya Hospital, Central South University, Changsha, Hunan, China, [4]Hunan Key Laboratory of Skin Cancer and Psoriasis, Changsha, Hunan, China, [5]Pathology, Xiangya Hospital,Central South University, Changsha, Hunan, China, [6]Xiangya Hospital, Changsha, Hunan, China

Pathological images are the gold standard for the diagnosis of melanoma.Recent studies have shown that convolutional neural networks(CNNs)' ability to diagnose melanoma from pathological images is as good as or slightly better than that of pathologists.However, few studies have verified whether CNN can directly predict the metastasis of melanoma from pathological images, which is an experiment of great medical significance. In the present study, We investigated whether CNNs can predict the metastasis of melanoma from pathological images.First, We collected 472 digital whole-slide images (metastatic melanoma 143, primary melanoma 329) to develop the model. Of these, 85 whole-slide images were from xiangya hospital and 387 whole-slide images were from Yale medical center.Furthermore, we trained a classical convolutional neural network model VGG19, and evaluated its performance. Our model showed 93.5% of accuracy, 98.6% of specialty, 89.3% of sensitivity. The results showed that our model forecast precisely. These findings suggest that CNN is able to predict whether melanoma is metastatic directly from pathological images.Thus will accelerate clinical trial design and reduce patient pain.

LB956

Adherence to adjuvant therapy in patients (pts) with resected melanoma

C. Beisel[1], T. Poretta[2], M. Burke[2], H. Sipsma[3], E. Fuqua[3], B. Stwalley[2], M. Yang[3]

[1]Melanoma Research Foundation, Washington, District of Columbia, United States, [2]Bristol Myers Squibb, Princeton, New Jersey, United States, [3]Analysis Group, Inc., Boston, Massachusetts, United States

Treatment adherence can be impacted by several factors, including route of administration, dosing schedule, and pt perceptions. This study assessed pt-reported adherence and associated barriers in pts with melanoma treated with adjuvant therapy. Adult pts with resected stage 3/4 melanoma with no evidence of disease were recruited by a pt panel and the Melanoma Research Foundation to participate in an online survey. Pts were required to have received adjuvant therapy with nivolumab or pembrolizumab (intravenous [IV] cohort) or dabrafenib+trametinib (oral cohort). Adherence was estimated using self-reported overall number of infusions missed for the IV cohort and self-reported adherence level in the past 2 months for the oral cohort. Of 184 eligible pts (127 IV, 57 oral), mean age was 45 years, 44% were female, and 78% were white. Most pts were engaged in treatment decision-making (86%) and considered their well-being to be “good” (46%), “very good” (26%), or “excellent” (9%). Compared with the oral cohort, more pts in the IV cohort were employed full time (67% vs 47%; *P*=0.012) and had commercial insurance (76% vs 47%; *P*<0.001). Mean time on the current adjuvant treatment was similar between the IV (8.3 months) and oral (7.7 months) cohorts. Adherence was relatively high, with pts following their regimens always (81% IV, 58% oral; *P*=0.002) or almost all of the time (17% IV, 33% oral; *P*=0.002). Nonadherence behavior was lower in the IV cohort than in the oral cohort (19% vs 42%; *P*<0.001), with forgetfulness (54% vs 46%), affordability (0% vs 46%; *P*<0.001), and safety concerns (29% vs 42%) listed as common reasons for nonadherence. Many pts did not expect to follow future regimens as instructed (37% IV, 46% oral), primarily due to affordability and safety concerns, which were similar in both cohorts. This study found that pt-reported adherence to adjuvant therapy was relatively high among those with advanced resectable melanoma but also presented potential areas for further improvement.

LB957

Skin cancer recognition for whole slide histology images with state-of-the-art Convolutional Neural Networks

P. Xie[1], F. Li[2, 3, 4], S. Zhao[2, 3, 4], Q. Li[5], J. Liu[1], K. Lu[1], Y. Zhang[5], T. Li[1], J. Zhou[1], Z. Ke[1], X. Chen[2, 3, 4]

[1]National University of Defense Technology, Changsha, Hunan, China, [2]The Department of Dermatology, Xiangya Hospital, Central South University, Changsha, Hunan, China, [3]Hunan Key Laboratory of Skin Cancer and Psoriasis, Changsha, Hunan, China, [4]Hunan Engineering Research Center of Skin Health and Disease, Changsha, Hunan, China, [5]Xiangya Hospital, Changsha, Hunan, China

Whole Slide histology image remains the gold standard. But recent research reveals a high discordance between individual pathologists. In order to reduce the misdiagnosis made by doctors, the aim of this study is to using deep learning to assist pathologists for skin cancers diagnosis. 626 whole slide images were collected (162 melanoma, 115 basal cell carcinoma, 349 nevus) from Xiangya Hospital. All the lesions in the WSIs were selected and random sampled as patches. The resulting patches of 500 WSIs were used for the training of a convolutional neural network (CNN). The resulting patches of addition 126 WSIs were used to test the performance of the CNN. We trained in ResNet50, MobileNet and InceptionV3 by transfer learning. The result shows that the InceptionV3 model achieves superior performance than other models (InceptionV3: 0.923 accuracy, MobileNet: 0.921 accuracy, ResNet50: 0.919 accuracy.) in the classification task. To locate the lesion of skin cancer, we visualized the prediction confidence map of the model in WSIs. The result showed that the model can successfully located the lesion area in three diseases, which could offer more reference to pathologists . In conclusion, CNNs indicate to be a valuable tools in diagnosing skin cancer and can provide more support for specialists beyond just the diagnosis.

LB958

Imiquimod treatment of lentigo maligna with positive margins, close margins, or field of dysplasia

R. Kwak[2, 1], C. Joyce[3], A. Werchniak[4], H. Tsibris[1, 2], J. Lin[1, 2]

[1]Harvard Medical School, Boston, Massachusetts, United States, [2]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States, [3]Department of Public Health Sciences, Loyola University Chicago, Chicago, Illinois, United States, [4]Department of Dermatology, Wentworth-Douglass Hospital, Dover, New Hampshire, United States

Lentigo maligna (LM) is a subtype of melanoma in situ more commonly found in sun-exposed areas. LM can occur with an extension of atypical melanocytes into the periphery known as the “field effect” that makes surgical resection alone challenging.[1] For this reason, primary or adjuvant imiquimod therapy is commonly practiced in treating LM. Though its efficacy is evident in the literature, little is currently known about its efficacy in treating the cases with positive (PM) or close margins (CLM) or with field of dysplasia (FD). We performed a retrospective chart review of all patients at Brigham and Women’s Hospital and at Dana-Farber Cancer Institute between 01/2011 and 8/2019 diagnosed with LM. We identified 121 patients who were treated with topical imiquimod (5% cream) as a primary (24.8%) or adjuvant (75.2%) therapy. Patients undergoing primary therapy had a significantly higher mean age compared to the adjuvant group (74.4 vs. 65.9 years, p<0.01). The recurrence rate (RC) was 20.0% for primary therapy (median follow-up 49 months [mo], average time-to-recurrence 21 mo) compared to 13.2% for adjuvant therapy (49 mo, 15.4 mo, respectively). In the adjuvant group, cases with PM or CLM (< 1cm) had a trend toward higher RC (20.5%) than the rest of the group (6.4%) (p=0.06). There was no statistically significant difference between PM and CLM. CLM had a trend toward higher RC (25.0%) compared to wider margins (WD) (> 1cm) (0.0%, p=0.09). FD demonstrated a lower RC (7.3%) when compared to CLM (25.0%) or PM (19.4%) (<0.01). No patient with WD treated with adjuvant imiquimod had a recurrence in the study period (median follow-up of 53 mo). 1. Swetter SM, Chen FW, Kim DD, Egbert BM. Imiquimod 5% cream as primary or adjuvant therapy for melanoma in situ, lentigo maligna type. J Am Acad Dermatol. 2015 Jun;72(6):1047-53.

LB959

Lesion location for melanoma pathology analysis

P. Xie[1], F. Li[2, 3, 4], S. Zhao[2, 3, 4], Q. Li[5], J. Liu[1], K. Lu[1], Y. Zhang[5], T. Li[1], J. Zhou[1], Z. Ke[1], X. Chen[2, 3, 4]

[1]National University of Defense Technology, Changsha, Hunan, China, [2]The Department of Dermatology, Xiangya Hospital, Central South University, Changsha, Hunan, China, [3]Hunan Key Laboratory of Skin Cancer and Psoriasis, Changsha, Hunan, China, [4]Hunan Engineering Research Center of Skin Health and Disease, Changsha, Hunan, China, [5]Xiangya Hospital, Changsha, Hunan, China

Pathological imaging is the gold standard for the diagnosis of melanoma.Recent studies have shown that convolutional neural networks(CNNs) is effective on the diagnosis of melanoma from pathological images. However, CNN could only predict whether it was a melanoma, not locate the lesion. In this paper, we propose lesion location class activation mapping (LLCAM) method to precisely locate the lesion of melanoma in pathological images. First, we collect more than 300 skin digital whole-slide images, including 188 melanomas. Further, we trained a convolutional neural network ResNet50 to distinguish melanoma lesion from other skin tissue.ResNet50 achieved 96.7% of accuracy, 96.9% of sensitivity, 96.6% of specialty. Finally,we use LLCAM to locate the lesion of melanoma on this well trained model. Experimental results show that LLCAM accomplished accurate localization of melanoma lesions, outperforming other competitive methods. More importantly, Our method solved the problem of whether there is a lesion in the detected pathological image, compared with other state-of-the-art weakly supervised localization method. In conclusion, accurate localization of melanoma lesions can better assist experts in diagnosis . This will help doctors build more trust in computer-aided systems.

Skin of Color

LB960
Oncodermatologic conditions in skin of color
B. Olamiju, J. S. Leventhal
Yale School of Medicine, New Haven, Connecticut, United States
Purpose: Dermatologic toxicities from anticancer treatments are an increasing challenge for patients and their providers. Effective prevention and clinical management of such toxicities can allow patients to remain on life-saving oncotherapeutics. The purpose of this study was to perform an analysis of cutaneous toxicities in skin of color patients that were referred to an oncodermatology clinic at a single academic institution. Methods: A chart review was conducted to investigate dermatologic toxicities in patients with skin of color on cancer treatment who were referred over the course of three years. Clinical variables extracted from the electronic record review included patient age, sex, race and ethnicity, primary cancer diagnosis, cancer treatment drug class, type of dermatologic toxicity, and management course. Results: Fifty-five patients (17 men, 38 women) with mean age of 58.2 years were included in the analysis. Seventy-six cutaneous toxicities were recorded. 49.1% of patients identified as Black of African American, 36.4% as Hispanic or Latino, and 14.5% as Asian. The most common primary cancer diagnoses of patients were breast (34.5%), lung (25.5%), and gastric (9.1%). Chemotherapy-induced toxicities were the most common toxicities, among which targeted drugs were the most frequent inciting agents. Treatment modifications due to skin toxicities occurred in 12.0% of all patients and were more common in patients treated with targeted agents and immune checkpoint inhibitors in comparison to traditional chemotherapy. The most common categories of dermatologic toxicities in our cohort were acneiform eruptions (21.3%), dermatitis (20.0%), and hand foot syndrome (20.0%). Conclusion: Anticancer treatments can prompt a diverse range of cutaneous toxicities among skin of color patients. This is the first study to analyze dermatologic toxicities associated with cancer treatment exclusively within a skin of color patient cohort. It is important that patients of color are referred early to a dermatologist if a dermatologic toxicity develops in the setting of oncologic therapy in order to mitigate anticancer treatment reductions or interruptions.

LB961
Disparities in dermatology referral and consultation patterns in the emergency department
R. Kwak[1, 2], D. Bartenstein[1, 2], D. Rubins[1, 3], M. H. Noe[1, 2], A. P. Charrow[1, 2]
[1]Harvard Medical School, Boston, Massachusetts, United States, [2]Department of Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States, [3]Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts, United States
While the Emergency Department (ED) frequently places outpatient follow-up referrals and in-ED consultations to dermatology, little is currently known about whether demographic factors such as race and sex impact these rates. Research in other contexts such as admission to cardiology has already demonstrated ED triage is impacted by race when controlled for other factors. We performed a retrospective chart review of all patients seen in the ED at two university hospitals between 1/1/2017 and 7/1/2019 for rash or dermatitis. Our search identified 6279 patients, of which 1239 (19.7%) received either a dermatology referral (n=753, 12.0%) or a consult (n=487, 7.8%). There were 687 Caucasian (CA) (53.7%), 174 African American (AA) (13.6%), 85 Asian (AS) (6.6%), 77 (8.1%) Hispanic (HI) patients, and 189 other (14.6%) patients. Most patients had a private health insurance (44.1%), followed by Masshealth (33.2%), Medicare (18.6%), and Medicaid (3.8%). The mean age was significantly higher for the consult group (50.2 yrs) compared to the referral group (39.2 yrs) ($p<0.01$). Females received both more consults and referrals than males ($p<0.01$). AA received fewer dermatology consults (5.4%) compared to CA (10.2%) and AS (8.7%) ($p<0.05$), as well as fewer referrals (11.0%) compared to CA (13.4%) ($p<0.01$). Regarding the type of service, AA were more likely to receive referrals (71.3%) over consults (28.7%), compared to CA, who had an even distribution between the two services (52.0% and 48.0%, respectively) ($p<0.01$). There was no significant difference between HI and non-HI, or between CA and AS. Overall, the results suggest a troubling disparity in access to dermatologic subspecialty services among patients in the ED. Further analysis on insurance type, area deprivation index, and language will be beneficial in understanding the differential access to dermatologic care in the ED.

LB962
Evaluation of time of presentation of central centrifugal cicatricial alopecia and outcomes of disease
T. Onamusi, A. McMichael
Wake Forest School of Medicine, Winston-Salem, Ohio, United States
Background: Central centrifugal cicatricial alopecia (CCCA) is a form of scarring hair loss that predominantly affects women of African descent. Although the etiology remains unknown, the pathogenesis involves inflammatory mediated destruction of the hair follicles. The management of CCCA is approached symptomatically rather than through evidence-based recommendations. Since treatment options do not provide a cure and are only aimed at halting disease progression, it is important to understand how time to presentation and symptomatology relates to treatment outcomes. Objective: To assess the relationship between the presentation and symptomatology of CCCA and the outcome of disease. Methods: We retrospectively reviewed the complete medical records of patients seen at the Wake Forest Dermatology Clinic from January 2014-December 2019. Data obtained from the chart review included duration of hair loss reported by the patient and by the provider, duration & type of symptoms, hair care practices, common co-morbid conditions, exam findings, prior treatments and treatments after diagnosis. Treatment outcomes were assessed 1 year after treatment using the Central Scalp Alopecia Photography Scale. Results: A total of 100 patient records were included in this review. Patients' ages ranged from 26-74. The duration of hair loss ranged from 6 months-25 years. The mean duration of hair loss reported by patients was 6.8, while the mean duration of hair loss reported by the provider was 6.5 years. Symptom duration ranged from 0-16 years, with an mean duration of 4.2 years. History of thyroid disease, sitting under hot dryers, wearing natural hair, use of braids, washing hair once weekly, and scaling were found to have a statistically significant effect on the outcome of treatment. Conclusion: CCCA is a form of scarring alopecia that is progressive in nature. The findings indicate that some factors can have a significant effect on treatment outcomes. This study provides information that can be used to guide treatment approach and inform patients on disease prognosis.

LB963
Perceptions of clinical trials among patients with atopic dermatitis: An analysis by race
A. Bazen, A. Sevagamoorthy, F. K. Barg, J. Takeshita
University of Pennsylvania, Philadelphia, Pennsylvania, United States
There is a lack of diversity among participants in dermatologic clinical trials. In order to gain an understanding of reasons for poor racial/ethnic diversity in dermatologic clinical trials, we performed a qualitative pilot study of adults with atopic dermatitis (AD) to identify their perceptions of clinical trials. We performed semi-structured interviews of 26 adults with AD. We used a purposive sampling approach to enroll a similar number of white (N=8), black (N=8), and Asian (N=9) adults. Interview responses were independently coded by two research assistants using a grounded theory approach. Median (interquartile range, IQR) age was 28 (24 - 47) years; 76% were female. Median (IQR) duration of AD was 20 (10-27) years; 60% had moderate-to-severe AD. While understanding of clinical trials was similar across racial groups, our data suggest differences in motivations for and barriers to participating in clinical trials by race. Among white patients, motivations to participate in clinical trials were primarily driven by potential direct benefits such as new treatment options in the setting of multiple prior treatment failures and drug safety. Among black and Asian patients, similar direct benefits were voiced as motivating factors, however, an additional motivator included the potential to improve the lives of others. Barriers to participation in clinical trials included side effects and risks of the intervention among all participants. Particularly among black participants, a greater sense of fear of clinical trial procedures such as invasiveness of the intervention and testing procedures (e.g., “radiation” from x-rays) as well as concerns about “pain” and “death” were noted. Our findings suggest different motivators for and barriers to clinical trial participation by race that may be helpful in guiding future clinical trial recruitment efforts among minority groups. Additional studies across a broader range of dermatologic conditions are needed to better understand how to improve minority enrollment in dermatologic clinical trials.

Skin, Appendages, and Stem Cell Biology

LB965
Follicular trochanters: A potential mechanism for stem cell depletion in scarring alopecia
F. C. Roche[1, 2], A. Fischer[1], K. J. Gaddis[3], T. Dentchev[1], S. C. Taylor[1], J. T. Seykora[1]
[1]Dermatology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]University of Rochester School of Medicine & Dentistry, Rochester, New York, United States, [3]Dermatology, University of Minnesota, Minneapolis, Minnesota, United States
The follicular bulge is a stem cell niche where the outer root sheath (ORS) abuts the arrector pili muscle insertion site in both murine and human hair follicles. Prior studies have recognized an uncommon structure termed the “follicular trochanter” which was described as an epithelial protrusion of the outer root sheath in anagen follicles. It has been shown that these structures prominently express keratin 15, a stem cell marker. We sought to determine if trochanters embedded in the fibrotic stroma contribute to follicular scarring in cicatricial alopecias. We retrospectively evaluated ten random cases of central centrifugal cicatricial alopecia (CCCA), lichen planopilaris (LPP) and discoid lupus erythematous (DLE). After confirmation of the diagnosis on H&E-stained slides, recut slides from the formalin-fixed, paraffin-embedded blocks underwent anti-keratin 15 (K15) immunofluorescence staining. Overall, 10/30 scarring alopecia cases had K15-positive trochanters. They were most frequent in CCCA (60% of cases) as compared to DLE and LPP (20% each). The total number of trochanters per follicle was greater in CCCA than in DLE and LPP (P<0.05). In addition, there was a greater number of K15 stem cells found in the trochanters of CCCA compared to DLE and LPP (P<0.05). Our immunofluorescence studies for keratin 15 staining show that fibrosis-associated trochanters are comprised of hair follicle stem cells. Overall, these findings provide insights into the pathomechanism of CCCA.The fibrotic trochanters could represent a disrupted stem cell niche that may be separated from the follicles by the perifollicular fibrosis associated with scarring alopecia.

Tissue Regeneration and Wound Healing

LB966
Transcriptional regulation of migrating-keratinocyte adhesion during cutaneous wound healing
G. Kashgari[1], S. Venkatesh[1], S. Gacias[1], A. Bayat[1], R. Herndon Klein[1], A. Paul Ta[3], R. Ramos[2], M. V. Plikus[2], P. H. Wang[3], B. Andersen[1, 3]
[1]Biological Chemistry, University of California Irvine, Irvine, California, United States, [2]Developmental and Cell Biology, University of California, Irvine, Irvine, California, United States, [3]Medicine, University of California Irvine, Irvine, California, United States
The migration of wound-edge keratinocytes is part of the wound response, crucial for complete wound closure. Despite significant advances, the molecular mechanisms that orchestrate cell-cell adhesion between migrating keratinocytes are not fully characterized. During wound re-epithelization, keratinocytes at the wound edge undergo series of cellular modifications. These cellular modifications require a loosening of cell-cell adhesion for effective migration. Mice lacking the epidermal transcription factor Grainyhead Like-3 (GRHL3) exhibit impaired wound healing and an increased adhesion between keratinocytes at the wound edge. The increased cell-cell adhesion in *Krt14-Cre Grhl3fl/fl* wounds coincides with high expression of the adherens junction protein E-Cadherin and downregulation of the newly identified wound-response gene Fascin (FSCN1). Gene expression analysis of isolated wound-edge keratinocytes shows significant downregulation of Fscn1 mRNA expression in *Krt14-Cre Grhl3fl/fl* wounds. In addition, ATAC-seq on *Krt14-Cre Grhl3fl/fl* wound-edge keratinocytes shows loss of wound-specific peaks near Fscn1 gene, in a region that is highly enriched for GRHL3 motifs. Together, these data elucidate a novel wound-specific FSCN1-E-cadherin pathway controlled by GRHL3 that is required for cell-cell loosening between migrating keratinocytes during wound re-epithelization. This pathway is altered in chronic diabetic wounds in mice.

LB967
Hemorrhagic complications in outpatient dermatological surgery
A. Chandra[1], M. Manway[2], G. Egnatios[2]
[1]Midwestern University, Phoenix, Arizona, United States, [2]Affiliated Dermatology, Scottsdale, Arizona, United States
The increasing incidence of cutaneous neoplasms in the Caucasian population of the United States has made Mohs micrographic surgery (MMS) and surgical excision with wide margins the standard of care for the treatment of melanoma and non-melanoma skin cancers. These commonly performed surgical modalities promote high cure rates and conservative treatment methods, sparing the maximal amount of normal tissue. Although there is a growing body of evidence regarding the efficacy of dermatological surgery for the removal of cutaneous malignancies, there is still some debate regarding the guidelines for maintaining safety and avoiding complications of MMS and other dermatologic procedures. The most common postoperative complications with dermatological surgeries are infections (61.1%), dehiscence and partial or full necrosis (20.1%), and bleeding and hematoma (15.4%). Postoperative bleeding is especially imperative to predict as it further increases the risk of potentiating hematoma formation, dehiscence, infection, and eventually tissue necrosis. By providing a comprehensive report of common risk factors for postoperative bleeding, this literature review aims to educate dermatologists and other physicians about high-risk patients in order to better predict, prevent, and manage postoperative complications.

LB968

Inhibition of sonic hedgehog signalling via MAPK activation controls chemotherapy-induced alopecia

I. S. Haslam[2, 1], G. Zhou[6], G. Xie[6], X. Teng[6], X. Ao[6], Z. Yan[6], E. Smart[2], D. Rutkowski[2], J. Wierzbicka[2, 5], Y. Zhou[7], Z. Huang[8], Y. Zhang[8], N. Farjo[3], B. Farjo[3], R. Paus[4, 2], Z. Yue[9]
[1]Dept of Biological Sciences, University of Huddersfield, Huddersfield, United Kingdom, [2]Centre for Dermatology Research, MAHSC and NIHR Manchester Biomedical Research Centre, University of Manchester, Manchester, United Kingdom, [3]The Farjo Institute, Manchester, United Kingdom, [4]Dept of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, Miami, Florida, United States, [5]Medical University of Gdansk, Gdansk, Poland, [6]Fuzhou University, Institute of Life Sciences, Fujian, China, [7]Department of Oncology, Union Hospital of Fujian Medical University, Fujian, China, [8]Southern Center for Biomedical Research and Fujian Key Laboratoy of Developmental and Neural Biology, Fujian Normal University, Fujian, China, [9]Guangdong Key Laboratory for Genome Stability and Disease Prevention, Department of Cell Biology and Medical Genetics, Shenzhen University School of Medicine, Guangdong, China

Chemotherapy-induced hair loss (alopecia, CIA) remains a major unsolved problem in clinical oncology. CIA is often considered to be a consequence of the anti-mitotic and apoptosis-promoting properties of chemotherapy drugs acting on rapidly proliferating hair matrix keratinocytes. Here we show that in a mouse model of CIA, down-regulation of Shh signalling in the hair matrix is a critical early event. Inhibition of Shh signalling recapitulated key morphological and functional features of CIA, whereas recombinant Shh protein partially rescued hair loss. Phospho-proteomics analysis revealed that activation of the MAPK pathway is a key upstream event, which can be further manipulated to rescue CIA. Finally, in organ-cultured human scalp hair follicles as well as in patients undergoing chemotherapy, reduced expression of SHH gene correlates with chemotherapy-induced hair follicle damage or the degree of CIA, respectively. Our work revealed that Shh signalling is an evolutionarily conserved key target in CIA pathobiology. Specifically targeting the intra-follicular MAPK-Shh axis may provide a new strategy to manage CIA.

Translational Studies

LB969

Analysis of epidermal gene expression profiles in patients with allergic contact dermatitis using non-invasive skin tape stripping

I. Tam[1, 3], J. Park[2], J. Yu[3]
[1]Tufts University School of Medicine, Boston, Massachusetts, United States, [2]Cutaneous Biology Research Center, Massachusetts General Hospital, Boston, Massachusetts, United States, [3]Department of Dermatology, Massachusetts General Hospital, Boston, Massachusetts, United States

Allergic contact dermatitis (ACD) affects 20% of patients and combined with irritant contact dermatitis (ICD) account for more than 90% of occupational skin diseases. Patch testing is the gold standard for diagnosis of ACD but result interpretation may be patient and physician dependent. The purpose of this study is to examine whether noninvasive tape stripping can be used to differentiate ACD, ICD, and normal skin. We examined 39 immune and barrier genes expressed in various skin layers. Patients referred to the Massachusetts General Hospital Contact Dermatitis Clinic with confirmed diagnosis of ACD through patch testing were recruited. 100% petrolatum vehicle served as control and 2% and/or 4% sodium lauryl sulphate was used to induce ICD. 20 consecutive tape strips were collected on sites of ACD, ICD, and vehicle-control skin. Tape strip samples were extracted to isolate total RNA and profiled by quantitative real-time PCR to analyze molecular biomarkers. A total of 9 patients, 7 females and 2 males; mean age, 38.6 years (range, 24-72 years), had at least one ACD reaction on patch testing. 13 ACD, 10 ICD, and 9 vehicle-control samples were obtained from 9 patients. All 39 biomarkers were detected amongst the samples with 4/39 markers significantly different between ACD and vehicle-control samples. Our analysis revealed that CD1A (Langerhans cell marker) was significantly increased, whereas loricrin (skin barrier component), KRT1 (suprabasal epidermal keratin), and KRT14 (basal epidermal keratin) were significantly decreased in ACD relative to vehicle-control samples ($p<.05$). In comparison of ACD and ICD samples, loricrin and KRT1 demonstrated significant differences ($p<.05$). These findings illustrate divergent epidermal molecular differences associated with ACD, ICD, and normal skin. We propose skin tape stripping as a novel strategy that potentially enables non-invasive and molecular marker-based diagnosis of ACD.

LB970

Dermoscopy during treatment of early Mycosis Fungoides

G. Chaidemenos[2], T. Spiliopoulos[1], E. Balaskas[3], G. Kontochristopoulos[4]
[1]Patras Hospital, Patra, Greece, [2]Private Practice, Thessaloniki, Greece, [3]General Hospital, Creta, Greece, [4]A. Syngros Hospital, Athens, Greece

The type of dermoscopy pattern in post-therapy remaining plaques of early Mycosis Fungoides, might help physicians in selecting the most appropriate therapeutic measures. Dermoscopy is a noninvasive popular technique enhancing the diagnostic ability in Derrmatological practice. “Spermatozoa-like” structures and fine short linear vessels are the most specific dermoscopic diagnostic features of early Mycosis Fungoides. Little is known about their modification following treatment. In a pilot study we prospectively investigated the modifications, if any, of dermoscopy features in 8 patients, who had been treated for at least 1 year. All cases were verified by (immune-)histology. Contact non-polarized X10 working magnification dermoscopy, was performed. For reasons of better reproducibility of results, “spermatozoa-like” structures and fine, short, linear vessels were included in the term of “fine-short-undulating” vessels. Treatment included a class I topical steroid, followed by class II steroids and calcitriol ointment. X^2 test was used for pre- and post-treatment incidence comparison. The ratio of women to men was 5:3. Dermoscopy pattern was not uniform in all plaques of each patient. Exclusively Dotted vessels existed in 66/167 (40%) plaques, “fine-short-undulating” vessels in 59 (35%) and a mixed form in 42 (25%). Dermoscopic features changed significantly ($p :.05$) after therapy of 27 (12-39) months: Only 3/42 (7%) remaining plaques had a dotted picture. Instead, 23 (55%) consisted of the “fine-short-undulating” and 16 (38%) of the mixed pattern. Overall, dermoscopic “fine-short-undulating” vessels were almost invariably present in the persisting disease plaques, indicating their relative resistance to therapy and the probable ineffectiveness of topical potent steroids in subpapillary plexus

LB971

Type I and II interferon signaling differentially associated with histopathologic findings in dermatomyositis skin

T. Wu[1], M. Tabata[1], L. Mushila Hodgkinson[1], K. Page[2], C. Huard[2], J. Buhlmann[2], K. Sarin[1], D. Fiorentino[1]
[1]Dermatology, Stanford University, Cupertino, California, United States, [2]Development & Medicine, Pfizer Worldwide Research, Cambridge, Massachusetts, United States

Upregulation of interferon (IFN) signaling is well documented in the skin, as well as blood and muscle of dermatomyositis (DM) patients. However, it is unclear if IFN signaling is related to the pathologic features commonly found in DM skin biopsies. Here, we investigate the association of Type I and II IFN gene signatures with cardinal histologic findings seen in DM skin to better understand the contribution of IFN signaling to key pathological features of DM. Histological staining and RNA sequencing were performed on 113 skin biopsies from 99 patients with DM. Biopsies were scored for severity (low vs. high) of fifteen histopathologic features by a blinded dermatopathologist. Genes selectively induced by IFN-alpha or IFN-gamma[1] were selected to represent Type I (IFN1) and II (IFN2) IFN signaling gene sets. IFN1and IFN2 scores were then calculated using the average expression values for the genes in each gene set. In univariate analysis, perivascular inflammation (IFN1 $p=0.0066$, IFN2 $p<0.0001$), extravasated red blood cells ($p=0.0349$, $p=0.0089$), dyskeratosis ($p<0.0001$ for both), and basal vacuolization ($p<0.0001$ for both) were associated with higher IFN1 and IFN2 scores. Mucin deposition was only associated with a higher IFN1 score ($p=0.002$). Periadnexal inflammation ($p=0.0498$) and neutrophils ($p=0.0137$) were only associated with a higher IFN2 score. Parakeratosis was associated with a lower IFN1 score ($p=0.0381$). Our results suggest that IFN dysregulation is closely related to DM skin pathology. In addition, Type I and II IFN signaling pathways may be associated with distinct pathologic processes in DM skin, which can have important implications for future targeted treatments in DM. [1]J.C. Hall et al., Proc Natl Acad Sci USA 109(43):17609-14, 2012, doi:10.1073/pnas.1209724109

LB972

IL-17 inhibitor-induced remission of guttate psoriasis

A. Fogel, B. Strober

Dermatology, Yale University, New Haven, Connecticut, United States

Effective, targeted treatments for guttate psoriasis are lacking. Drugs that block the interleukin-17 (IL-17) pathway are highly effective in the treatment of plaque psoriasis. Given the overlap of immunopathogenic features between guttate and plaque psoriasis, including an over activation of Th17 cells, it is plausible that IL-17A inhibition would be an effective treatment for guttate psoriasis. Ixekizumab, a humanized IgG4 monoclonal antibody that targets interleukin-17A (IL-17A) is FDA-approved for the treatment of moderate-to-severe plaque psoriasis in adult patients who are candidates for systemic therapy, but to our knowledge has never been reported in the treatment of guttate psoriasis. Here we report three cases of guttate psoriasis successfully treated with ixekizumab. Three patients presented with guttate psoriasis involving 15-40% body surface area (BSA) and with psoriasis physician global assessment (PGA) scores ranging from 3-6. Two patients developed lesions idiopathically, and one developed lesions after a documented episode of streptococcal pharyngitis. Lesions had been present for 7-90 days at the time of presentation, and prior ineffective treatment attempts across patients by primary care providers included courses of keflex, triamcinolone cream, betamethasone ointment, oral prednisone, oral acyclovir, and expectant management. Patients were treated with standard ixekizumab dosing regimens for plaque psoriasis. Starting doses of 160mg subcutaneous injection and induction doses of 80mg every two weeks thereafter were given. Two patients achieved complete clearance after 2 doses of ixekizumab (clearance documented in clinic at 42 and 86 days after treatment initiation), and the third achieved 1% BSA involvement and PGA score of 1 after 3 doses (documented 61 days after treatment initiation). No adverse effects of treatment were noted. This report provides strong evidence for the potential efficacy of IL-17 inhibition in guttate psoriasis. Additional follow-up to assess for duration of clinical remission and progression to plaque psoriasis, as well as larger, randomized, controlled studies are needed.

LB973

Immunologic profiling of fixed drug eruptions

E. Schunkert[1], G. Romar[1], P. Hsieh[1], R. Foreman[2], V. Barrera[3], S. J. Divito[1]

[1]Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States, [2]Pathology, Massachusetts General Hospital, Boston, Massachusetts, United States, [3]Biostatistics, Harvard Chan School of Public Health, Boston, Massachusetts, United States

Fixed drug eruptions (FDE) are a unique form of drug reaction in which a rash recurs at the same skin site(s) with each repeated drug exposure. This intriguing reaction has been postulated to be due to skin resident memory T cells, yet investigation into T cell phenotype and function and the concomitant inflammatory milieu are surprisingly limited. To elucidate disease pathobiology, we performed Nanostring transcriptional profiling on RNA extracted from formalin fixed paraffin embedded skin specimens of active FDE lesions and healthy control skin. Analysis of 200 genes demonstrated that 52 genes were significantly upregulated and 3 genes downregulated (adjusted p value < 0.05, fold change ≥ 1.5) and suggested involvement of not only CD8+ T cells, but also NK cells, monocytes and dendritic cell subsets. Data supported recruitment of central or peripheral type memory T cells, with increased CD45RO, CD62L, CCR7, and CD127, and no difference in CD69, CD103, Hobit, CX3CR1, KLRG1, CD28 or CD27. Effector function favored Th1/Tc1 polarization. Notably, while perforin and granzyme B were upregulated, granulysin and Fas/FasL, potentially key mediators in SJS/TEN, were not differentially expressed in FDE. Several chemokines were significantly increased, though not CCR4 or CCR10, which are often considered the main T cell recruiting chemokines in skin. Finally, several surprising genes demonstrated differential expression raising possible roles for NK cell receptors in disease pathogenesis and for the IDO/AHR pathway, possibly in an attempt to quell inflammation. Tissue staining confirmed that the majority of T cells were αβ not γδ type, and were CD45RO+ yet CD103-, as well as the presence of a robust mononuclear CD3- infiltrate. This data suggest FDE are much more immunologically complex than previously appreciated and provide a strong framework upon which to build future studies.

LB974

Donor-derived T cells accumulate in skin during resolution of acute GVHD

L. Khatib, E. Schunkert, P. Hsieh, S. J. Divito

Dermatology, Brigham and Women's Hospital, Boston, Massachusetts, United States

Graft-versus-host disease is a significant cause of morbidity and mortality following stem cell transplantation. Donor T cells are believed to be the main mediators of disease, however we recently identified that host T cells survive stem cell transplant conditioning and are present in high percentages in skin during active acute GVHD. Host T cells appear to contribute to disease pathology in both human studies and a humanized mouse model. Data further suggested that after resolution of acute GVHD, T cell chimerism in skin shift dramatically to donor T cells. In this study, we confirmed using fluorescence in situ hybridization concurrently with immunofluorescence staining for CD3, a T cell marker, that the percent host T cells decreased significantly after resolution of acute skin GVHD compared to active disease. Moreover, we observed that during severe acute skin GVHD, the percent donor T cells was significantly increased in patients who had received systemic steroids prior to skin biopsy compared to patients who had not received treatment prior to biopsy. The increase in percent donor T cell chimerism was secondary to a significant increase in the number of donor T cells, rather than to a decrease in the number of host T cells in treated patients. In fact, treatment with systemic steroids was associated with an increase in the total number of T cells in skin. Previous publications have reported that systemic steroids promote regulatory T cell development/recruitment in inflammatory skin disease, raising the possibility that systemic steroids treat GVHD by recruiting donor regulatory T cells into skin. In analysis of a subset of patients, we did not observe a difference in either the percentage or frequency of CD3+CD4+Foxp3+ regulatory T cells in skin biopsies taken after treatment with systemic steroids versus those taken without preceding treatment. Taken together, the data suggest that accumulation of donor-derived T cells in skin mediates resolution of acute skin GVHD, however further studies are necessary to ascertain the mechanism by which this occurs.

2020 Keywords

Acne
Adherens Junctions
Adhesion
Adipocytes
Adnexae (other than hair)
Aging
Allergy
Alopecia
Angiogenesis
Antimicrobial Peptides
Apoptosis
Atopic Dermatitis
Autoimmunity
Autoinflammation
B Cells
Barrier Function
Basal Cell Carcinoma
Basement Membrane
Basophils
Bioinformatics
Biologics
Biomechanics
Bullous Disease
Cadherins
Cancer Biology
Cancer Genetics
Carcinogenesis
Cell Adhesion
Cell Biology
Cell Migration
Cell-based Therapy
Chemokines
Chemotaxis
Chromatin
Clinical Research
Clinical Trials
Collagen
Connective Tissue Diseases
Contact Sensitivity
Cutaneous T Cell Lymphoma (CTCL)
Cytokines
Cytoskeleton
Dendritic Cells
Desmosomes
Developmental Biology
Differentiation
DNA Repair Disorders
Drug Development
Drug Reactions
Drug Resistance
Dysplastic Nevi
Eccrine Glands
Ectodermal Dysplasia
Eczema
Elastin
Endocrine Regulation
Endothelial cells
Eosinophils
Epidemiology
Epidermal Structure
Epidermolysis Bullosa
Epigenetics
Evolutionary Development (Evo-Devo)
Extracellular Matrix
Gene Regulation
Gene Therapy
Genetic Diseases
Genetics
Genetics, Human
Genetics, Molecular
Genetics, Mouse
Genome-wide Association Studies (GWAS)
Genomics
Glycobiology
Graft versus Host Disease (GvHD)
Growth Factors
Hair Biology
Health Services Research
Hematopoiesis
Hemidesmosomes
Heterogeneity
Hidradenitis Suppurativa
HIV/AIDS
Ichthyosis
Imaging
Immunity, Adaptive
Immunity, Innate
Immunodeficiencies
Immunomodulatory Therapy
Immunotherapy
Induced Pluripotent Stem (iPS) Cells
Infection, Bacteria
Infection, Fungal
Infection, Papillomavirus

Infection, Parasitic
Infection, Viral (non-HIV/HPV)
Inflammasome
Inflammatory Skin Diseases
Integrins
Intercellular Junctions
Interleukins
Interventional Trials
Intravital Imaging
Itch
Keratinization Disorders
Keratinocyte Biology
Keratinocyte Differentiation
Keratinocytes
Keratins
Langerhans Cells
Laser
Lichen Planus
Lipidomics
Lymphatics
Lymphoma
Macrophages
Mast Cells
Matrix Biology
Melanocytes
Melanoma
Merkel Cell Carcinoma
Merkel Cells
Metabolism
Metabolomics
Metagenomics
Metastasis
Methods/Tools/Techniques
Microbiology
Microbiome
Microscopy
Models
Models, animal
Models, mouse
Models, zebrafish
Mycology
Nail
Natural Killer (NK) Cells
Neurobiology
Neurophysiology
Neutrophils
Oncogenes
Optics
Patient Outcomes Research
Pediatrics
Peripheral Nervous System
Personalized medicine
Pharmacology
Photobiology
Photochemistry
Photodynamic therapy
Phototherapy
Pigmentation and Pigment Cell Biology
Plasma cells
Plasmacytoid dendritic cells
Proteases
Proteomics
Pruritus
Psoriasis
Public Health Research
Regenerative Medicine
Regulatory T Cells
Repository
Retinoids
RNA Biology
Rosacea
Scar
Scleroderma
Sebaceous Glands
Signal Transduction
Single cell genomics
Squamous Cell Carcinoma
Statistics
Stem Cells
Steroids
Systems biology
T Cells
Thrombosis/Coagulopathy
Tissue Regeneration
Toxicology
Transcription
Transcription Factors
Tumor Biology
UV Radiation
Vaccines
Vascular Biology
Vascular Tumors
Vasculitis
Vitiligo
Wound Healing