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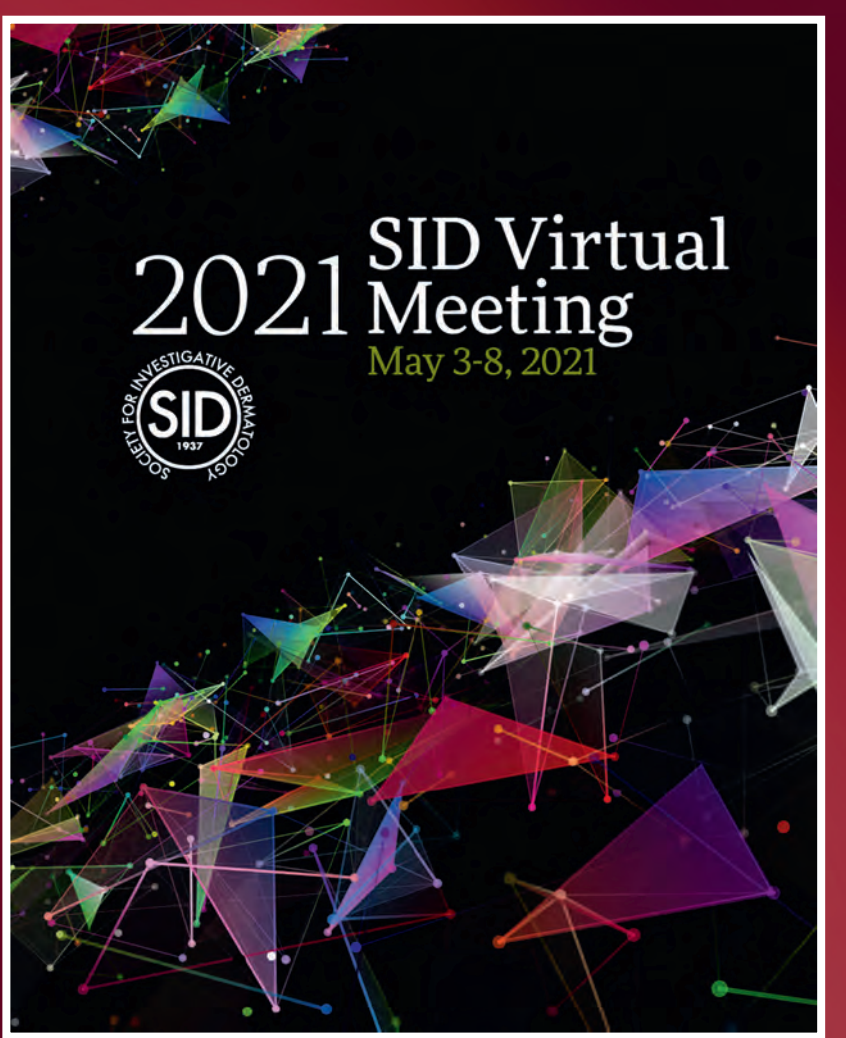

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001

Biasing of the outcome of antigen (Ag) presentation by calcitonin gene-related peptide (CGRP)-exposed endothelial cells (ECs) requires CGRP-induced expression of IL-6 by ECs and trans-presentation of IL-6 to T cells

W Ding, LL Stohl, J Lam, R Ahmed, V Isak, Z Bulmer and RD Granstein *Weill Cornell Medicine, New York, New York, United States*

Exposure of dermal microvascular ECs to CGRP endows them with the ability, acting as bystanders, to bias the outcome of Langerhan cell (LC) Ag presentation to T cells away from the Th1 pole and toward the Th17 pole. IL-6 production by ECs mediates much of this effect and, accordingly, CGRP treatment of ECs from IL-6 knock-out mice failed to bias the outcome of Ag presentation. In this regard, pre-exposure of LCs to IL-6, but not pre-exposure of T cells, biased the outcome of Ag presentation in this manner. Exposure of LCs to anti-CD126 antibodies before and during IL-6 treatment inhibited much of this effect, indicating that the IL-6 receptor α-chain on LCs is involved in this process. Also, exposure of LCs to IL-6 upregulated LC IL-6 production. To examine if IL-6 trans-presentation by CD126 on LCs to T cells is responsible for these observations, cultures were set up as follows: (1) BALB/c LCs were treated for 3 hrs with IL-6, then washed x 4 and co-cultured with DO11.10 T cells (which spontaneously respond to $cOVA_{325-339}$, BALB/c background) and $cOVA_{325-339}$. (2) This group was set up identically except that LCs were pretreated with medium alone. (3) Cultures were set up identically except that LCs were not pretreated but, rather, responding T cells were pretreated with a CD126-IL-6 chimeric molecule and washed x 4 prior to setting up cultures. After 48 hrs, supernatants (SUPs) were harvested and cytokine content quantified by ELISA. SUPs from wells containing IL-6-pretreated LCs showed significantly decreased IFNγ content and significantly increased IL-17A content compared to control cultures. Cultures in which T cells were pretreated with the chimeric molecule showed similar changes in IFNγ and IL-17A production. These results strongly indicate that the effect of IL-6 treatment of LCs on biasing the outcome of Ag presentation results from trans-presentation of IL-6 by LCs to responding T cells.

002

Colitis alters the antigen-specific response to skin commensal bacteria and predisposes to neutrophilic skin inflammation

GR Merana, M Dhariwala and T Scharschmidt *Dermatology, University of California San Francisco, San Francisco, California, United States*

The gut and skin are major barrier sites that house microbial communities capable of influencing host immunity. Under homeostatic conditions, resident microbes are thought to have a dominant impact on local immune cell function. However, the prevalence of neutrophilic skin disorders among patients with IBD suggests that this compartmentalized control may not hold under disease conditions. We hypothesize that an altered immune response to gut-resident microbes during colitis may facilitate excessive inflammation directed at skin commensals. Our lab has previously shown that colonization of neonatal mice with *Staphylococcus epidermidis* engineered to express the model antigen 2W (*S. epi*-2W) results in establishment of antigen-specific tolerance. This tolerance is denoted by a higher percentage of 2W-specific regulatory T cells (Tregs) in the skin and skin-draining lymph nodes (SDLNs) and fewer skin neutrophils upon later-life skin barrier disruption plus *S. epi*-2W re-exposure. To test whether colitis perturbs tolerance to commensal skin bacteria, we colonized mice with *S. epi*-2W as neonates and subjected them to chemically-induced colitis as adults in conjunction with tape-stripping and *S. epi*-2W re-exposure. Colitic mice exhibited increased skin neutrophils and reduced percentages of *S. epi*-specific Tregs in skin and SDLNs compared to controls. Notably, no difference in *S. epi*-specific Tregs was noted in mice subjected to LPS-induced sepsis, suggesting that *gut* inflammation specifically was needed. Intra-intestinal presence of *S. epi*-2W and reduced percentages of *S. epi*-specific Tregs in the colon and gut-draining LNs during colitis indicate initiation of this altered response in the gut. Consistent with this, adoptive transfer experiments revealed a colitis-induced increase in CD4+ T cell trafficking from gut-draining LN to SDLNs. Recovery of *S. epi*-specific Tregs in colitic $Cd4^{Cre}Il1r1^{flox/flox}$ mice indicates an additional role for circulating IL-1 cytokines in shaping the skin commensal response during colitis.

003

***In vitro* genetic reprogramming increases MHC-I expression and ameliorates resistance to an antitumor immune response in Merkel cell carcinoma**

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Merkel cell carcinoma (MCC) is a rare but aggressive skin cancer with half of patients unresponsive to immune checkpoint inhibitors (ICIs). A primary resistance mechanism driving ICI resistance in MCC in particular and cancer immune evasion in general is downregulation of tumor MHC class I (MHC-I) expression, thereby limiting cytotoxic cellular immune responses. Assessment of three patient-derived MCC cell lines, MCC13, MCC26, and UISO, demonstrated low baseline MHC-I expression in 2 of the 3 cell lines (MCC13 and UISO), with higher expression in MCC26. We used biodegradable polymeric nanoparticles based on poly(beta-amino ester)s to co-deliver DNA plasmids encoding a co-stimulatory molecule (4-1BBL) and an immunostimulatory cytokine (IL-12) to reprogram the cancer cells, allowing them to interact productively with cytotoxic lymphocytes and elicit a targeted anti-tumor immune response. Following nanoparticle administration, 22, 12, and 42% of MCC13, MCC26, and UISO cells expressed surface-bound 4-1BBL and produced 1460, 14, and 1000 ng/mL secreted IL-12, respectively. This was accompanied by a 2.5- and 2.3-fold increase in MHC-I expression (mean fluorescence intensity) in MCC13 and UISO 3 days after transfection. Without nanoparticle-based reprogramming, co-culture of MCC13 and UISO with unselected human CD8+ T-cells did not result in T-cell activation or cancer cell death. Following nanoparticle transfection with 4-1BBL and IL-12, however, MCC13, MCC26, and UISO co-culture with CD8+ T-cells led to 1300-, 4.4-, and 86-fold increased IFNg production and 3.6-, 1.6-, and 2.8-fold CD8+ T-cell expansion, respectively, as well as significant cancer cell death in MCC13 and UISO ($p<.0001$). These *in vitro* results represent proof of concept of a new nanotechnology to genetically reprogram MCC cells, allowing localized T cell activation while also increasing MHC-I expression, helping to address a major mechanism of ICI resistance.

004

Immunoregulatory roles of IFNg signaling in non-T and B cell population is important for suppression of interface dermatitis in mouse

M Mukai[1], H Takahashi[1] and M Amagai[1,2] *1 Dermatology, Keio University School of Medicine, Tokyo, Japan and 2 Laboratory for Skin Homeostasis, RIKEN IMS, Yokohama, Japan*

IFNg is one of the most characterized pro-inflammatory cytokines produced by type 1 T helper (Th1) cells and crucial for the defense against viral infections and autoimmune disease development. We previously established Th1-mediated interface dermatitis model that was induced by adoptive transfer of Dsg3-specific TCR transgenic (H1) $CD4^+$ T cells into $Rag2^{-/-}$ mice. In this model, H1 T cells directly infiltrated into the epidermis and damaged keratinocytes, inducing interface dermatitis. In addition, pivotal pathogenic roles of IFNg produced from H1 T cells were elucidated since $IFNg^{-/-}$-H1 T cells did not cause interface dermatitis after transfer into $Rag2^{-/-}$ mice. In this study, we aimed to further investigate the roles of IFNg signaling in the dermatitis by using IFNg receptor $(IFNgR)^{-/-}$ mice. When naïve $IFNgR^{-/-}CD4^+$ T cells were stimulated *in vitro* by anti-CD3 and anti-CD28 antibodies with IL-12, Th1 differentiation was suppressed compared to WT T cells. ($6.7 \pm 0.79\%$ vs $1.2 \pm 0.35\%$, $P=0.002$). Adoptive transfer of H1-$IFNgR^{-/-}$ T cells into $Rag2^{-/-}$ did not cause the dermatitis. On the other hand, whenH1 T cells were transferred into $IFNgR^{-/-}$-$Rag2^{-/-}$ mice (n=3), the dermatitis unexpectedly appeared earlier with more severe phenotype than when $Rag2^{-/-}$ mice were used as recipients (n=3) (clinical score, $22 \pm 2.9\%$ vs $4 \pm 1.3\%$, $P<0.0001$). Flow cytometric analysis revealed that IFNg-producing H1 cells was more abundant in the lesional skin of $IFNgR^{-/-}$-$Rag2^{-/-}$ recipients, compared to $Rag2^{-/-}$ recipients ($36\pm 0.55\%$ vs $13 \pm 0.66\%$, $P<0.0001$). These results indicated that IFNg signaling in H1 T cells is indispensable for inducing interface dermatitis, but the signal in non-T and B cell population present in $Rag2^{-/-}$ mice functions as an immunoregulatory pathway. Thus IFNg signaling exerts not only pro-inflammatory but also anti-inflammatory actions depending on the types of signal-accepting cells.

005

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a novel psoriasis susceptibility locus

A Merleev, A Toussi, L Downing, M Tran, J Nava, S Le and A Marusina *Dermatology, University of California Davis, Sacramento, California, United States*

Proprotein convertase subtilisin/kexin type-9 (PCSK9) variants have been linked to hypercholesterolemia, atherosclerosis, and inflammation. Psoriasis is an inflammatory disease that is associated with increased atherosclerotic risk. Here we demonstrate that *PCSK9* is upregulated across multiple psoriasis RNA-Seq datasets and that its expression strongly correlates with *LDLR* and *PLA2G3*. Furthermore, we identify a *PCSK9* variant (rs662145) that is associated with psoriasis susceptibility in two independently acquired genetic datasets (OR = 4.65 and 6.67). Parsing a large psoriasis RNA-Seq dataset for this variant revealed that it is associated with increased *PCSK9* expression. Constructing a gene-gene 2D expression map of the psoriasis transcriptome reveals that *PCSK9* closely clusters with *PLA2G3*, a member of the phospholipase A2 family that has been linked to inflammation and psoriasis. Single cell sequencing reveals that PCSK9 and PLA2G3 are co-expressed in basal, differentiating, and keratinized keratinocytes. Finally, immunohistochemistry confirmed keratinocytes as the primary cell type expressing PCSK9. Together these data support a role for PCSK9 in the pathophysiology of psoriasis and provide a causal link between psoriasis and atherosclerosis.

006

Opsin expression in human Langerhans cell-like cell line, ELD-1

T Ye, Y Lan, Y Wang, Z Liu and H Lu *Department of dermatology, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, China*

Background: Langerhans cell (LCs) is the most powerful antigen-presenting cells (APCs), which plays a vital role in inducing and maintaining the primary immune response. Opsins are typically associated with the retina in mediating vision. Recently, the studies of other groups and ours have shown that opsin is expressed in human skin cells (Keratinocytes, Melanocytes, Fibroblasts) and performs light and no light mediated physiological functions. However, the expression and it's function of Langerhans cells have not been reported. Objective: To determine the mRNA and protein expression of opsin in human Langerhans cell-like cell line, ELD-1. MethodsELD-1 was cultured in 1640 medium supplemented with 10% FBS. The mRNA level and protein content of opsins in ELD-1 were detected by fluorescent quantitative real-time PCR and western blotting analysis respectively. The expression of opsins in ELD-1 were analyzed by the immunofluorescence technique under the fluorescence microscope and the confocal microscope in vitro. Results Immunofluorescence analysis showed that in LCs, the expression of Opsin1-SW, Opsin2, Opsin3, Opsin4 and Opsin5 can be detected mainly on the cell membrane. The results of real-time fluorescence quantitative PCR demonstrated that the expression of Opsin1-SW, Opsin2, Opsin3, Opsin4 and Opsin5 mRNA were detected in ELD-1 and opsin3 mRNA were significantly more abundant than other opsins ($p<0.05$). The results were consistent with that of opsins protein by western blot analysis. Conclusion: Our study is the first report on the expression of opsins in ELD-1, and opsin may play an important role in LCs.

007

Health supplement Spirulina induces inflammatory cytokine production via monocyte derived dendritic cells and classical monocyte activation in Dermatomyositis

D Diaz[1,2], C Bax[1,2], Y Li[1,2], M Grinnell[1,2], T Vazquez[1,2], E Keyes[1,2] and V Werth[1,2] *1 Dermatology, Corporal Michael J. Crescenz VAMC, Philadelphia, Pennsylvania, United States and 2 Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States*

The popular herbal supplement Spirulina has previously been shown to stimulate inflammatory cytokine production in Dermatomyositis (DM) patients *in vitro*. We sought to evaluate whether Spirulina's immunostimulatory effects differ in healthy controls (HCs) compared to DM. We performed ELISA on Spirulina stimulated HC and DM PBMC supernatants, demonstrating similar effects in both HCs and DM with Spirulina significantly increasing TNFα and IFNγ levels. Inhibition of TLR4 or TBK1 significantly decreased Spirulina's immunostimulatory effects on both TNFα ($p<0.0001$) and IFNγ ($p<0.05$) at 0.3 mg/ml Spirulina. Using flow cytometry, we investigated Spirulina's immunostimulatory effects at the cellular level, demonstrating that for TNFα and IFNγ secretion, Spirulina has the greatest effect on monocyte-derived dendritic cells (moDC) and classical monocytes (CM) in DM patients. With stimulation at 0, 0.3, and 1 mg/mL of Spirulina, the percent of moDCs secreting IFNγ increased from a mean (SEM) of 1.01% to 96.40% and 96.90% (1.80) ($p<0.0001$), respectively and the median fluorescent intensity (MFI) increased similarly. ($n = 3$, $p <0.01$). The mean percent of CMs secreting IFNγ also increased ($p<0.0001$), and pre-treatment with TLR4 inhibitor suppressed CM activation ($p<0.05$). Moreover, the MFI of CMs secreting IFNγ increased significantly ($p<0.005$). TLR4 or TBK1 inhibition decreased MFI for both moDC and CM's ($p<0.05$ and $p<0.001$, respectively).TNFα+ moDCs increased from 1.14% of total moDCs with no stimulation to 49.10% (12.4) at 0.3 mg/mL Spirulina ($p<0.05$). TLR4 and TBK1 inhibition suppressed the percentage of Spirulina-induced moDCs secreting TNFα ($p<0.05$); TLR4 inhibition trended towards significance in CMs ($p=0.053$). These data demonstrate that Spirulina induces CM and moDC activation in DM, likely via TLR4 or TBK1 activation.

008

Multiplexed skin immunophenotyping of new-onset dermatomyositis lesions following first time use of *Spirulina platensis*

T Vazquez[1,2], J Patel[1,2], C Bax[1,2], D Diaz[1,2], M Grinnell[1,2], E Keyes[1,2], M Sharma[1,2] and V Werth[1,2] *1 Corporal Michael J. Crescenz VAMC, Philadelphia, Pennsylvania, United States and 2 Dermatology, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, United States*

The rise in the use of natural supplements to improve wellbeing and boost immune function has led to a rise in the use of herbal products such as *Spirulina platensis*. Our group has previously shown that Spirulina use is temporally associated with both new-onset and acute exacerbations of dermatomyositis (DM). We have also previously shown that Spirulina is capable of activating the TLR4 and STING pathways, as well as inducing TNFa, IFNb, and IFNγ production. Here, we sought to characterize the cutaneous inflammatory infiltrate in Spirulina-induced dermatomyositis (Spir-DM). We performed high-plex, *in situ*, single-cell level analysis of lesional biopsies of DM and Spir-DM skin using Imaging Mass Cytometry (IMC). We utilized two separate panels of 37 metal-conjugated antibodies against various surface markers, intracellular cytokines, and phosphorylated signaling molecules of interest. Significance was determined by the Mann-Whitney test. Our data show similar dermal counts of 17 cell populations, including macrophages, dendritic cells, T and B cells ($p>0.05$). Total cytokine and activated pathway signal intensity was also similar between both groups for type I IFN and JAK-STAT pathways ($p>0.05$). Using a heatmap of cell types plotted against intracellular markers, we sought to identify cytokines or inflammatory pathways that may be differentially up- or down-regulated in Spir-DM patients. We similarly found no significant differences at the canonical cell-type level; however, there was notable heterogeneity in both groups. While the precise trigger for autoimmunity induced by Spirulina requires further interrogation, we believe these data suggest Spirulina induces immunophenotypically similar DM when compared to DM triggered by other causes, with little difference in the inflammatory infiltrate.

009

Hyperthermia controls DAB2 transcription in macrophage through inducing the separation of cJun and cFos heterodimers

S Zhao, R Qi, X Gao and H Chen *The First Affiliated Hospital of China Medical University, Shenyang, Liaoning, China*

Sporotrichosis is an emergent subcutaneous mycosis of humans and some animals caused by dimorphic fungi of the *Sporothrix schenckii*. Hyperthermia can effectively treat sporotrichosis by regulating the phenotypic regulation of macrophages which is critical for controlling tissue inflammation and resolution. The adaptor protein disabled homolog 2 (DAB2), a regulator of phenotypic polarization in macrophages, have been identified to inhibit an inflammatory phenotype of the macrophages. However, whether and how hyperthermia act on the immune regulation of macrophages through DAB2 needs more research and complement. In this study, mouse bone marrow extracted primary macrophages (BMDMs) and cell line ANA-1 were used to investigate the regulation of DAB2 gene transcription by hyperthermia in vitro. Immunofluorescence was used to examine sub-localization of AP-1 complex within the cells under the 42°C conditions. Chromatin immunoprecipitation was used to detect whether 42°C stimulation affect the binding of AP-1complex to DAB2 gene. Conditional DAB2 knockout mice were used to evaluate the role of DAB2 in Sporotrichosis. Our results show that 42°C stimulation downregulated the expression of c-Jun and c-Fos, and led to the separation of c-Jun and c-Fos dimers, causing the downregulated transcription of DAB2. In conclusion, topical hyperthermia treatment can inhibit the transcription of DAB2 gene, promote macrophage M1 polarization, and promote the treatment of sporotrichosis via AP-1 complex.

010

Demystification of the effects of docosahexaenoic acid on the PPAR signaling pathway in psoriasis

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Psoriasis is a multifactorial skin disease that is distinguished histologically by the hyperproliferation of keratinocytes. n-3 polyunsaturated fatty acids (n-3 PUFAs), particularly docosahexaenoic acid (DHA), are known to have numerous anti-inflammatory effects in several pathologies, including psoriasis. The beneficial actions of DHA in psoriasis are primarily mediated by its interactions with the receptors activated via peroxisome proliferators (PPARs), as well as by its secretion of active anti-inflammatory metabolites. The aim of this study was therefore to assess the influence of DHA on the main characteristics of psoriasis, namely hyperproliferation and abnormal cell differentiation of lesional keratinocytes, through the PPAR signaling pathway, using a tissue-engineered psoriatic model. Psoriatic skin substitutes were produced according to the self-assembly method, using culture medium supplemented with 10 µM DHA in comparison with regular medium. Three different psoriatic cell populations were used. The supplementation of the culture media with DHA regulated the expression of cell differentiation proteins in psoriatic substitutes. Moreover, the added DHA was correctly incorporated into the membrane phospholipids of the epidermis and metabolized into eicosapentenoic acid (EPA) in psoriatic substitutes supplemented with DHA. Also, the addition of DHA to the culture medium decreased the synthesis of lipid mediators derived from n-6 PUFAs, known to be overexpressed in psoriasis. Finally, DHA supplementation positively restored the expression of PPAR receptors, which is deregulated in the pathology and causes a decrease in the synthesis of TNF-α. Ultimately, our results show that DHA has beneficial effects in attenuating the psoriatic features which are achieved through the signaling pathway of PPARs.

011

Systemic hyperinflammation as a driver of maculopapular drug exanthema in severely ill COVID-19 patients?

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Coronavirus disease 2019 (COVID-19) has been associated with cutaneous findings, some being the result of drug hypersensitivity reactions. Here, we utilize imaging mass cytometry (IMC) to characterize the cutaneous immune response in maculopapular drug rashes (MDR), including those associated with COVID-19 infection (COVID MDR). For comparison, skin from healthy controls and patients with drug rash with eosinophilia and systemic symptoms (DRESS) was analyzed. Results demonstrated that COVID MDR are characterized by a more prominent infiltration of cytotoxic $CD8^+$ T cells and highly activated, phenotypically shifted monocyte/macrophage (Mo/Mac) clusters in comparison to MDR and DRESS. RNA sequencing transcriptome of the affected skin also demonstrated a more robust cytotoxic response in lesional COVID MDR skin. Serum proteomic profiling of COVID MDR patients revealed up-regulation of various inflammatory mediators (IL-4, IL-5, IL-8, IL-18, IL-6, TNF, and IFN-γ), eosinophil and Mo/Mac -attracting chemokines MCP-2, MCP-3, MCP-4 and CCL11. Analyses of cytokine networks demonstrated a relatively milder cytokine storm in DRESS compared to COVID MDR, while MDR did not exhibit such features. These results suggest that a massive systemic cytokine storm promotes activation of Mo/Mac and cytotoxic $CD8^+$ T cells, which impacts MDR development in severely ill COVID-19 patients.

012

FABP5-induced Th17 polarization in atopic march

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Atopic March (AM) represents a typical progression of allergic diseases that often begin early in life, which has a role for the strongest evidence for systemic involvement of atopic dermatitis (AD). However, the mechanism underlying the development of AM in patients with AD is still unknown. To elucidate the possible mechanisms which might be engaged in AM, whole-transcriptome analysis was done with the skin biopsy specimens, blood samples in AD, AM, and healthy controls. Metabolic pathways-related genes were one of the most enriched in AM samples compared with AD and healthy controls. Interestingly, the genes which were related to fatty acid metabolism were elevated in AM skin than AD skin. Furthermore, we found that increased fatty acid binding protein 5 (FABP5) expression was observed in human skin samples and T cells with AM patients, in accordance with increased IL-17A level, when compared with AD samples and healthy controls. Knock-down of FABP5 in T cells inhibited IL-17A expression. Direct correlation was observed between FABP5 expression and IL-17A level. Taken together, the results indicate that 'fatty acid binding protein 5' might be as a possible biomarker to explain the progression of atopic march in atopic dermatitis patients, acting by directly promoting Th17 inflammation.

013

Different effects of combined blockade of IL-4/IL-13 and selective inhibition of IL-13 in *in vitro* model systems for atopic dermatitis with allergen stimulated lymphocytes

T Honstein, S Evers, LM Roesner, J Zeitvogel and T Werfel *Department of Immunodermatology, Medizinische Hochschule Hannover, Hannover, Lower Saxony, Germany*

New strategies for the management of atopic dermatitis have been focused on the development of humanized antibodies directed against Th2 cytokines for the last few years. Dupilumab is the only antibody approved for the treatment of AD so far and blocks the IL-4Rα receptor subunit, inhibiting IL-4/IL-13 signaling pathways. Two specific anti-IL-13 antibodies have recently also shown promising results in clinical trials. The cytokines IL-4 and IL-13 have similarities, in part because they share the same receptor subunits and thus signal through similar pathways. Our work focuses on the question of whether combined blockade of IL-13 and IL-4 or selective inhibition of IL-13 have different functional effects on lymphocytes from sensitized patients with atopic dermatitis. After stimulation of mononuclear cells from the blood of patients sensitized via IgE against house dust mite or against autoantigens, antigen induced proliferation and cytokine production were measured after IL-4 and/or IL- 13 blockade. T-cell subtypes were determined and B-lymphocytes were examined with regard to IgE production. Surprisingly, combined IL-4/IL-13 blockade led to an increase in antigen-specific growth of mononuclear cells in short-term cultures over 7 days. This effect was not caused by IL-13 inhibition alone. The investigations with long-term cultures over 3 weeks showed a suppressive effect on the growth of the antigen-specific T cell lines by both the selective IL-13 and the combined IL-4/IL-13 blockade. Moreover, specific aIL-13 treatment had an effect on IL-5 and IL-17 levels whereas aIL-4Ra treatments affected IL-5 levels only. IgE monitoring showed that both forms of IL-4/IL-13 inhibition reduced in vitro IgE production in anti-CD40 plus IL-13 stimulated cells by more than 70%. Our work shows different functional effects by combined IL-4/IL-13 resp. by selective IL-13 blockade raising the question of the benefit of additional IL-4 inhibition in Th2 directed treatments of AD.

014

A novel Pemphigus vulgaris patient-derived antibody with sequence homology to antibodies directed against desmosomal and non-desmosomal targets induces keratinocyte dissociation

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The predominant autoantigenic targets in the autoimmune blistering skin disease Pemphigus vulgaris (PV) are the keratinocyte associated desmosomal proteins desmoglein (Dsg) 3 and 1. However, the presence of autoantibodies to these targets does not fully explain disease activity and phenotype. We and others have described the presence of numerous non-Dsg antibodies in the context of disease. However, the scope, specificity, and particularly functionality of non-Dsg autoantibodies has not been fully defined. Our group has previously reported the discovery of a patient derived antibody (AtS13) that bears 74% heavy-chain homology to anti-thyroid peroxidase (TPO) antibody and 86% light-chain homology to an anti-desmosome antibody as per BLAST alignment. While this antibody did not bind to Dsg3, -1 or TPO by ELISA and Western Blot and did not stain intercellular regions on monkey esophagus by IIF we did observe binding to a 55-60kDa protein in HaCaT keratinocyte lysates. Additionally, immunofluorescence revealed a cytoplasmic target within HaCaT keratinocytes but no co-localization with the cell membrane or any component thereof, including Dsg3. In order to investigate the functional role of this novel antibody and its potential to induce keratinocyte dissociation, HaCaT keratinocytes were grown to confluence and subjected to treatment with increasing concentrations of Ak23, an established mouse anti-human Dsg3 antibody, and/or AtS13. We show that while AtS13 induces a strong dose-dependent dissociation of keratinocytes in vitro, the rate of fragmentation is lower than that of Ak23 alone. AtS13 in combination with Ak23, however, induces an approximately 3-fold higher fragmentation rate than Ak23 alone, indicating a synergistic effect of these autoAbs in vivo. While the exact epitope target of the AtS13 antibody is yet to be defined, our data suggests a functional activity of this novel patient-derived antibody in the skin with potential disease relevance.

015

Role of hippo signaling in apoptosis of lupus keratinocytes

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Skin inflammation and photosensitivity are common manifestations of systemic lupus erythematosus (SLE), yet mechanisms underlying heightened cell death and epidermal inflammation following UV light remain unclear. We performed genome-wide DNA methylation analysis on keratinocyte (KC) DNA from non-lesional, non-sun exposed skin of SLE patients and healthy controls and identified Hippo signaling as the top canonical pathway. Hippo mutations increase cell proliferation in oncogenesis models, including in UV-induced neoplasms. However, this pathway has not been studied in inflammatory skin disease. YAP is a critical component in the regulation of the Hippo pathway. Through a kinase cascade that includes LATS1/2, TAZ and WWC1, the Hippo pathway targets YAP for phorphorylation, preventing nuclear translocation and transcriptional activation of TEAD. This pathway may play a role in heightened epidermal cell death in SLE. We found significant hypomethylation of LATS1/2 and WWC1 in SLE KC compared to control ($\Delta\beta$ = 0.17 and $\Delta\beta$ = -0.15, respectively), both phosphorylate YAP and thus cause cytosolic sequestration and inhibtion of TEAD signaling. To determine functional relevance of our methylation data, we compared paried RNA-seq samples stimulated with IFNγ and IFNα. We found a negative correlation between IFN induced genes and methylation signatures, suggesting methylation changes result in functional expression differences in vivo. To further evaluate *in situ*, we analyzed expression data and localization of these proteins using immunofluorescent microscopy of lesional biopsies and found a significant increase in cytooplasmic retention of phosphorylated YAP in SLE compared to control. Collectively, our work describes a novel mechanistic paradigm for how Hippo signaling through restriction of YAP transcriptional activity is a mechanism of dysregulated apoptosis and photosenstivity in lupus skin.

016

Three North American cases of cutaneous Pemphigus vulgaris with no history of mucosal disease

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Pemphigus is a group of autoimmune blistering diseases including Pemphigus vulgaris (PV) and Pemphigus foliaceus (PF). Explanation of lesion morphology has been elegantly proffered by the Desmoglein Compensation Hypothesis (DCH) based on the epidermal distribution of desmoglein (Dsg) proteins and autoantibody profiles. In this theory, PF is characterized by subcorneal lesions in the presence of only anti-Dsg1 antibodies, while PV lesions are suprabasilar and associated with anti-Dsg3 in mucosal PV, or anti-Dsg1 and -Dsg3 in mucocutaneous PV. However, logical inconsistencies in the DHC have emerged and exceptions have been published in multiple small-scale studies. One of these inconsistencies described by our group and others is that some PV patients present with solely cutaneous disease (cPV) without concomitant mucosal lesions, in violation of the tenets of the DHC. To date, cPV patients reported in the literature have been classified as such based on their lesion status at time of presentation. We report here three cases of clinically and histologically confirmed cPV without any history of mucosal lesions (cPVwohm). Of these patients, two do not carry the most common PV associated HLA alleles, DRB1*04:02 or DQB1*05:03. The same two patients also tested negative for the primary PV associated autoantibodies, anti-Dsg 3 and anti-Dsg 1, while in active disease status. We confirm the first documented individual cases of cPVwohm in North America, supporting the existence of PV patients that develop cutaneous disease without a history of mucosal lesions, further challenging the fidelity of the DCH. Two of the 3 patients reported did not type for the common PV-associated HLA genes or display anti-Dsg autoantibodies while in active disease, suggesting some cPV patients may develop Pemphigus via genetic and immune mechanisms that differ from typical mucosal or mucocutaneous PV.

017

Endotypes of mucous membrane pemphigoid predict disease severity

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Mucous membrane pemphigoid (MMP) is an autoimmune bullous disease predominantly involving mucosae and is caused by autoantibodies directed against BP180, Collagen VII, Laminin 332, or α6β4 integrin. Oral/pharyngeal lesions are the most common, but any mucous membrane can be involved. The potential long-term consequences are devasting, including blindness, airway compromise, loss of dentition and strictures. Despite its morbidity, therapies specific for MMP have not been developed. Our goal was to determine if MMP can be resolved into distinct disease endotypes based on the autoantibody target. Seventy-one patients who met clinical, histological and immunologic criteria for MMP were enrolled prospectively. Demographics, clinical information, and course of the disease were all recorded. Sera were obtained and the relevant antigen was determined using a combination of indirect immunofluorescence, ELISAs and immunoblotting. BP180 was the most common primary autoantibody target, identified in 51 patients (73.5%) followed by α6β4 in 5 (7.4%), COLVII in 4 (5.9%), and LAM332 in 3 (4.4%). Autoantibodies targeting LAM332 or COLVII predicted more severe disease that was associated with an increased number of sites affected, involvement of high-risk sites (larynx, eye), need for rituximab for disease control, and a decrease in long-term remission. A propensity for specific sites of involvement based on target autoantigen (LAM332/larynx, COLVII/ocular) was also observed, but was not exclusive. Our findings suggest that MMP can be sorted into endotypes based on the autoantibody target. These endotypes can improve disease outcomes by facilitating selection of the most efficacious treatment and will aid in the development of type-specific targeted therapies.

018

TSST-1+ *Staphylococcus aureus* in bullous pemphigoid

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Bullous pemphigoid (BP) is an autoimmune blistering disease that is treated with high dose immunosuppression due to lack of specific targets. *Staphylococcus aureus* is a commensal bacterium implicated inflammatory and autoimmune disorders because of its secretion of toxins with superantigen effects. We prospectively evaluated *S. aureus* colonization and its production of toxic shock syndrome toxin-1 (TSST-1) in 28 new onset BP patients. Inclusion criteria were active blistering and linear basement membrane IgG/C3 or a serum ELISA >14 for BP180 IgG. Bacterial swabs were obtained from the lesion interior, nares and unaffected skin of BP patients and nares and anatomically matched skin of 28 age- and sex-matched controls. Staphylococcal growth was assessed on blood agar, and TSST-1 production by cultured *S. aureus* isolates and in blister fluid was evaluated by immunoblot. *S. aureus* colonization of BP lesions was 3-6-fold higher than the nares or unaffected skin from the same patients ($p \leq 0.300$) and 6-fold higher than control nares or skin ($p \leq 0.0015$). Evaluation of superantigen gene profiles using PCR indicated that 96% of BP patients are colonized with a clonal strain of TSST-1+ *S. aureus*. Colonization and circulating levels of TSST-1 neutralizing antibodies, measured by ELISA, did not correlate. Interestingly, *S. aureus* colonization was not observed in patients who had received prior antibiotics. In colonized patients with severe disease, addition of anti-staphylococcal antibiotics resulted in clinical improvement and eliminated lesional colonization. This study shows that BP lesions harbor a clonal strain of TSST-1 producing *S. aureus* that is not evident in the general elderly population. Thus, immunosuppressive therapies should be balanced with the knowledge that *S. aureus* is likely present in BP lesions and the knowledge that antibiotics may play an important therapeutic role through bacterial clearance.

019

Vgll3 causes discoid lupus-like fibrosis in a mouse model of lupus

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Fibrosis is an abnormal wound healing process characterized by collagen deposition, myofibroblast accumulation, and extracellular matrix remodeling. Fibrosis can also be seen in autoimmune diseases, where it may be widespread and affect organs beyond the skin including lungs and kidneys. Skin and organ fibrosis is often associated with high morbidity and even mortality, and there is no effective treatment. Recent work from our laboratory has shown that epidermal-directed overexpression of murine *Vgll3* causes severe lupus-like skin lesions reminiscent of discoid lupus erythematosus (DLE), as well as systemic autoimmune disease with end-organ damage. Given the apparent fibrotic nature of the skin lesions in transgenic (TG) *Vgll3* mice, we wanted to determine whether *Vgll3* induced fibrosis. We analyzed male and female TG and wild-type (WT) mice aged 2-3 months, comparing fibrotic biomarkers of human DLE and scleroderma. Here, we demonstrate that epidermal *Vgll3* overexpression causes development of not only cutaneous inflammation but also severe fibrosis. Changes include increased infiltration of granulocytes/monocytes accompanied by significant expression of fibrotic biomarkers (*Acta2*, *Col1*, *Tgfb1*, and *Ccn2*, also known as connective tissue growth factor (*Ctgf*) and pro-fibrotic cytokines (*Il4* and *Il13*) in TG mice. The detection of high expression of *Ccn2* and *Tgfb1* as well as *Col1* mRNA and protein in the skin of TG mice, as is seen in skin of human scleroderma and DLE patients, suggests that skin-directed overexpression of *Vgll3* may impact fibrosis development, and there may be a role for targeting *CTGF* in early autoimmune fibrosis. Further studies will need to elucidate the specific mechanisms that may be at play.

020

Induction of hair loss by expanded CD4 T cells from previously affected AA mice

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Alopecia Areata (AA) is a common autoimmune disease characterized by infiltration of the hair follicle by T cells, resulting in nonscarring hair loss. Our published work suggested an increased representation of IFN-γ-producing, activated CD4 T cells in the skin-draining lymph nodes of AA mice when compared to unaffected (UA) controls. Our objective was to determine the contribution of CD4 T cells to AA pathogenesis. We adapted a recently described model of mouse AA induction whereby adoptive transfer of in vitro expanded bulk lymph node (LN) cells from previously affected AA mice induced disease in previously unaffected mice. To address the role CD4 T cells play in AA pathogenesis, we first sorted CD4 T cells and assessed their ability to induce AA. Mouse recipients of in vitro expanded CD4 T cells isolated from the LNs of AA mice developed AA at a substantially increased rate compared to mouse recipients of in vitro-expanded CD4 T cells from LNs of UA mice. CD4-mediated AA induction was found to be dose-dependent, with larger numbers of CD4 T cells inducing disease in recipient mice at a higher rate. Using congenic markers, we found that the transferred CD4 T cells were present in the skin draining LNs of recipient mice at three weeks following transfer but largely absent at 16 weeks, suggesting these cells may be conferring their effect early during disease development. Additionally, we found that the CD4 T cell population is critically dependent on the presence of endogenous CD8 T cells in order to transfer disease. Our data suggests that CD4 T-helper type 1 cells contribute to the activation of CD8 T cells to enable autoimmune attack on the hair follicle. Further studies are needed to further dissect how CD4 T cells, and IFN-γ, lead to AA.

021

Multidimensional *in situ* immune profiling of discoid and subacute cutaneous lupus erythematosus

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Cutaneous lupus erythematosus (CLE) can be subdivided into acute cutaneous (ACLE), subacute cutaneous (SCLE), and chronic cutaneous LE (of which discoid lupus [DLE] is the predominant subtype). Previous studies using RNA extracts or traditional immunostaining have demonstrated subtle differences between the subtypes; however, no multiplexed, single-cell analyses have been conducted. We profiled the immune infiltrate of DLE and SCLE using Imaging Mass Cytometry, an unbiased, high-plexed, *in situ* technique for cellular level analysis. 19 SCLE and 18 DLE, treatment-naïve FFPE biopsies were stained with 37 metal-conjugated antibodies. Slides were ablated on the Hyperion Imaging System (Fluidigm). Cells were segmented using a nuclear based algorithm on Visiopharm and imported into histoCAT where cell mean pixel intensity data was obtained to cluster cells using the Phenograph algorithm based on cell markers. Significance was determined by the Mann-Whitney test, bivariate correlations were determined by Pearson's r. We found 9 unique populations consisting of dermal CD4 T, CD8 T, CD14+CD16+ macrophages, CD68+ macrophages, B cells, CD56+ Cells, Tregs, conventional dendritic cells (cDC), and plasmacytoid dendritic cells (pDC) with similar percentages between DLE and SCLE ($p>0.05$). 16 cytokines and phosphorylated inflammatory signaling pathways were included and the data revealed higher pTBK1 in DLE compared to SCLE ($p<0.05$). At the cell type level, the data showed increased pIRF3 in DLE pDCs compared to SCLE ($p<0.05$). Overall, these results suggest substantial overlap between DLE and SCLE, with a potential role for pTKB1 and pIRF3 in DLE. Future studies are needed to investigate the potential suitability of these pathways as targeted therapies for DLE.

022

UHRF1 downregulation promotes T follicular helper cell differentiation by increasing BCL6 expression in SLE

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Background: Transcription factor B cell lymphoma 6 (BCL6) is a master regulator of T follicular helper (Tfh) cells, which play a crucial role in the pathogenesis of systemic lupus erythematosus (SLE). However, the mechanisms by which BCL6 expression is regulated are poorly understood. Ubiquitin-like with PHD and RING finger domains 1 (UHRF1) is an important epigenetic factor that regulates DNA methylation and histone modifications. In the present study, we assessed whether UHRF1 can regulate BCL6 expression and influence the differentiation and proliferation of Tfh cells. Results: Compared to healthy controls, the mean fluorescence intensity of UHRF1 (UHRF1-MFI) in Tfh cells from SLE patients was significantly downregulated, whereas that of BCL6 (BCL6-MFI) was significantly upregulated. In vitro, UHRF1 knockdown led to BCL6 overexpression and promoted Tfh cell differentiation. In contrast, UHRF1 overexpression led to BCL6 downregulation and decreased Tfh cell differentiation. In vivo, conditional UHRF1 gene knockout (UHRF1-cKO) in mouse T cells revealed that UHRF1 depletion can enhance the proportion of Tfh cells and induce an augmented GC reaction in mice treated with NP-keyhole limpet hemocyanin (NP-KLH). Mechanistically, UHRF1 downregulation can decrease DNA methylation and H3K27 trimethylation (H3K27me3) levels in the *BCL6* promoter region of Tfh cells. Conclusions: Our results demonstrated that UHRF1 downregulation leads to increased BCL6 expression by decreasing DNA methylation and H3K27me3 levels, promoting Tfh cell differentiation in vitro and in vivo. This finding reveals the role of UHRF1 in regulating Tfh cell differentiation and provides a potential target for SLE therapy.

023

Single-cell composition and architecture of cutaneous lupus

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Cutaneous lupus erythematosus (CLE) is an incompletely understood autoimmune disease that can occur in isolation or in the context of systemic lupus erythematosus (SLE). CLE is often disfiguring, and no FDA-approved therapies for CLE exist. Further, evidence suggests skin inflammation in CLE can provoke systemic autoimmune disease, including precipitating dangerous kidney inflammation. Thus, understanding CLE pathogenesis has great potential to alleviate lupus morbidity and even mortality. We employed single-cell RNA-sequencing (scRNA-seq) and spatial sequencing to investigate the transcriptomes and arrangement of the cellular players in CLE. 7 patients with active CLE were enrolled. 6/7 carried a diagnosis of SLE. Lesional and nonlesional sun-protected skin biopsies and peripheral blood mononuclear cells (PBMCs) were subjected to scRNA-seq on the 10X platform. Comparison to control cells derived from 14 healthy skin biopsies and PBMCs from 4 healthy donors revealed dramatic transcriptomic differences between healthy, nonlesional CLE, and lesional CLE keratinocytes, fibroblasts, and immune cell subsets. Additionally, subclustering of skin biopsy-derived immune cells and PBMCs identified potential circulating precursors to the immune cells that infiltrate the skin and give rise to CLE lesions. Finally, integration of the scRNA-seq data with spatial sequencing revealed a complex architecture of immune cells, stromal cells, and keratinocytes, with spatially distinct inflammatory responses. Collectively, these data provide deep characterization of skin alterations and inflammation in CLE and offer a resource for further interrogation of the roles of constituent cell types in CLE pathogenesis.

024

Immune microenvironment deep profiling of cutaneous lupus erythematosus skin stratified by patient response to antimalarials

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Lupus erythematous (LE) is a systemic autoimmune disease with a variety of cutaneous manifestations. Antimalarials are first-line systemic therapy, yet not all patients respond to hydroxychloroquine (HCQ), quinacrine (QC), or either (NR). Our group has previously shown that QC responders demonstrate increased conventional dendritic cells (cDC) and TNFa relative to HCQ responders. Here, we investigated the differences between these patients using Imaging Mass Cytometry (IMC), an unbiased multiplexed technique. 12 HCQ, 11 QC, and 20 NR treatment-naïve FFPE samples were stained with 37 metal conjugated antibodies and ablated on the Hyperion Imaging System (Fluidigm). Images were segmented using a nuclear app-based algorithm in Visiopharm and imported into histoCAT where single cell mean pixel intensity data was obtained to cluster cells using the Phenograph algorithm. One-way ANOVA, Kruskal-Wallis, and post-hoc Tukey/Dunn's tests (per data normality) were performed. Correlations were determined by Pearson's r. NR patients were found to have a decreased percentage of Tregs compared to QC responders ($p<0.05$). QC responders had a higher expression of pSTING and IFNκ compared to HCQ responders ($p<0.05$). The total expression of pSTING and IFNκ was found to positively correlate and colocalize in skin ($p<0.0001$, $r=0.676$). CD14+CD16+/CD68+ macrophages and cDCs were the predominant cell types found to express pSTING and IFNκ. These data may suggest a relative dysregulation in tolerance due to decreased Tregs in patients refractory to antimalarials. Our results show that activated STING correlated with IFNκ, suggesting co-regulation in macrophages and cDCs that may be responsive to QC. This analysis on treatment naïve biopsies may lead to further discovery of biomarkers that may predict patient response to therapy and direct targeted treatment.

025

ALA-PDT inhibits skin squamous cell carcinoma (cSCC) via regulating formation of tertiary lymphoid structures

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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. 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. Here, we found that tertiary lymphoid structures (TLSs), which are ectopic lymphoid organs that develop in non-lymphoid tissues at sites of chronic inflammation including tumors, play a pivotal role in anti-tumor immune function of ALA-PDT. We analyzed 77 samples of cSCC patient in our hospital. TLS was observed in 79% of the patient samples, and the density of TLS is negatively correlation with the Brodes classification of cSCC. Intriguingly, immunohistochemistry showed that ALA-PDT could promote the formation of TLS in vivo. We also found that promotion effect of ALA-PDT on TLS is mediated by M1 macrophage, which function as lymphoid tissue inducer cells in TLS formation and recruited by ALA-PDT. Further investigation substantiated that PDT-secreted exosomes were involved in M1 macrophage polarization. Our study elucidated a mechanism that ALA-PDT influence M1 macrophage polarization via PDT-secreted exosomes, which could facilitate the formation of TLS. Thus, our research may contribute to in-depth molecular understanding of the ALA-PDT on anti-tumor immune function.

026

CXCR4$^+$ skin-resident natural killer T cells participate in cutaneous allergic inflammation in atopic dermatitis

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Natural killer T (NKT) cells is an unconventional subset of lymphocytes that bridge innate and adaptive immunity. However, the role of NKT cells in the development of atopic dermatitis (AD) has not been well understood. So, we aimed to investigate the function of NKT cells in the cutaneous allergic inflammation in atopic dermatitis. In global transcriptomic and proteomic analyses, CXCR4 and CXCL12 were significantly upregulated in human AD skin and CXCR4$^+$ NKT cells were enriched in AD skin and were consistently elevated in AD mouse models. Skin-resident NKT cells uniquely expressed CXCR4, unlike NKT cells in liver, spleen and lymph nodes. Interestingly, skin fibroblasts were the main source of CXCL12. In addition, the adoptive transfer of allergen-induced NKT cells in Rag1$^{-/-}$ mice, which do not have conventional T cells, also developed significant cutaneous allergic inflammation. By using parabiosis technique and intravital imaging, CXCR4$^+$ NKT cells preferentially trafficked to CXCL12-rich areas, forming an enriched CXCR4$^+$ NKT/CXCL12$^+$ cell cluster, which developed in acute and chronic allergic inflammation in our AD mouse models. Taken together, CXCR4$^+$ skin-resident NKT cells may form a niche that contributes to atopic dermatitis, where CXCL12 is highly expressed.

027

Langerhans cells rely on good neighbors to overcome gene deficiencies

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We recently identified that epidermal resident Langerhans cells (LCs) acquire gene expression fingerprints from surrounding keratinocytes (KCs) in the form of mRNA and protein. In the present study, we aimed to determine whether this transfer can also overcome gene deficiencies. For this purpose, using the Cre/Lox system, we specifically deleted genes for connexin 43 (Cx43), MyD88, and MHC-II in LCs. While all three genes underwent recombination, reduced protein levels were only observed for MHC-II, whereas Cx43 and MyD88 protein levels remained unaltered. Considering that KCs lack MHC-II, but express Cx43 and MyD88 at high levels, we posit that LCs can acquire gene products from surrounding KCs to overcome their own deficiencies if those products are available. Preliminary experiments suggest that LCs can also provide, though to a lesser extent, to KCs in need. In summary, we present evidence that cells can compensate for gene deficiencies if the surrounding cells can provide. These findings highlight the limitations of cell-specific gene deletion and could provide an explanation as to why certain gene deletions do not lead to measurable deficiencies.

028

Use of systemic immunosuppressive treatment is not related to COVID-19 infection in a retrospective review of patients in Massachusetts

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Importance: It is unclear if systemic immunosuppression for chronic conditions modifies patients' risk of contracting COVID-19, leading to uncertainty among patients and dermatologists treating immune-mediated skin conditions during the pandemic. Methods: We partnered with the Massachusetts Department of Public Health to identify COVID-19 positivity and mortality for patients treated at the Mass General Brigham who were prescribed a systemic immunosuppressant from 07/01/19-02/29/20. We excluded biologics, steroids, and antirheumatic drugs from the analysis. Patients were compared with exact matched controls using a multivariable logistic regression for infection and multivariable Poisson regression for mortality, adjusting for demographics, comorbidity score, and local infection rate. Results: The most common medications identified were Methotrexate (23.5%), Mesalamine (19.2%), Paclitaxel (8.3%), Mycophenolate (7.8%), Hydroxyurea (6.0%), and Tacrolimus (5.3%). 218 of 14,865 (1.5%) patients prescribed systemic immunosuppressants and 1,368 of 80,318 (1.7%) controls were identified as COVID-19 positive. Of these, 26 (0.2%) patients prescribed immunosuppressants and 162 (0.2%) controls died after diagnosis. Patients prescribed immunosuppressants were not more likely to have a COVID-19 diagnosis (OR 0.91, 95% CI 0.79-1.05, p=0.22) or die after diagnosis (OR 0.95, 95% CI 0.62-1.44, p=0.80) after adjusting for demographics, comorbidity score, and local infection rate. Conclusions and Relevance: We found no evidence that systemic immunosuppression preceding the COVID-19 pandemic increased risk of contracting COVID-19 or risk of mortality among COVID-19 positive patients.

029

Topical xenobiotics promote oral food allergy

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Allergic disease is increasing in prevalence in industrialized nations. Children with atopic dermatitis are at an increased risk of developing food allergies. The prevailing theory to explain this relationship is termed epicutaneous sensitization, whereby chronically scratched skin comes into contact with food allergens like peanut and this leads to the development of an allergic reaction to the food when eaten. Our preliminary work has shown that certain chemicals, including food preservatives and over-the-counter drugs promote the development of oral food allergy. The ability for xenobiotics to promote allergic sensitization may in part explain the rise in prevalence in allergic disease in industrialized nations where these agents have become ubiquitous. We hypothesized that the immune system may be similarly shaped by exposure to substances within topicals frequently in contact with the skin (like soaps, emollients, detergents), such that upon ingestion of certain foods, primed individuals will develop an allergic response. To test this, mice were sensitized by intradermal injection of the xenobiotic, sulforaphane, concurrently with oral gavage of the model food antigen ovalbumin (Ova). We then assessed for Ova-specific IgE and IgG1 and tested whether mice would anaphylax to Ova re-exposure. We have found that topically applied xenobiotics can induce allergic sensitization to orally administered Ova which leads to an anaphylactic response upon re-challenge. This suggests that topical xenobiotics are sufficient to induce allergic sensitization to orally ingested allergens.

030

Defining adaptive and innate immune cell profiles in Hidradenitis Suppurativa at the single cell resolution

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Hidradenitis suppurativa (HS) is a severe chronic inflammatory skin disease lacking effective therapeutic options due to little understanding of the complex immune response within the lesional skin. Using single-cell transcriptomic analyses, we examined the signature changes in each immune cell types during HS progression, as well as *in silico* ligand-receptor predictions between different immune cell types to construct the interaction network that contribute to HS pathogenesis. Our results revealed a predominant Th17 response, as well as a distinct regulatory T cells existing in the lesional skin. We found that M1-polarized macrophages likely facilitate chemotaxis and IL1B responses in perilesional skin, while regulate lymphocyte activation and tissue remodeling in the lesional skin. In addition, we identified a significant increase of CCR7 expressing dendritic cells, as well as activated stromal fibroblasts expressing CCR7-ligand CCL19, which together support the organization of tertiary lymphoid organ (TLO)-like aggregates that contribute to persistent local inflammation. Importantly, we demonstrated a dense infiltration of plasma cells near sinus tracts, and that clonal expansion of the plasma cells frequently exists in HS patients. Together, our work provides a comprehensive understanding of immune responses and cytokine networks defining disease chronicity in HS, as well as significant implications for future therapeutics.

031

Single cell transcriptomic analysis of cutaneous T cells in psoriasis

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Psoriasis is an immune-mediated disease characterized by skin and systemic inflammation that affects 125 million people worldwide. However, the underlying pathways contributing to psoriasis pathogenesis have not been fully elucidated. This project utilizes single-cell transcriptomes of T cells from healthy and psoriatic skin in an effort to identify key biomarkers and pathways of psoriasis. T cells were clustered into subtypes and differential gene expression analysis was performed between lesional and healthy skin to identify psoriatic marker genes in each T cell subtype. Regulatory CD4+ T cells in psoriasis lesional skin were found to upregulate cytokines such as IL-32, as well as genes in the interferon-gamma-mediated signaling, NF-κB signaling, and putrescine catabolic pathways. As a result, psoriatic Tregs may amplify several of the pathways behind psoriasis and drive inflammation via IL-32, which has been previously found to be significantly upregulated in plaque psoriasis. Future work includes using VDJ analysis to more closely investigate psoriatic TCR abnormalities and to incorporate more patient data.

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A multicomponent skin-targeted COVID-19 vaccine elicits robust humoral and cellular immune responses

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Considerable progress has been made toward development of COVID-19 vaccines in the past year. However, there is still a need for painless, self-administered, shelf-stable, and efficacious SARS-CoV-2 vaccines to enable sustainable immunization programs against COVID-19. To address this need, we hypothesized that harnessing the immune-responsive cutaneous microenvironment using microarray patches (MAPs) to deliver integrated SARS-CoV-2 vaccine components would bring together biologic advantages of targeting the endogenous immune circuitry of the skin with a thermostable and user-friendly cutaneous vaccine delivery platform. We show that immunologically rich cutaneous microenvironments in both murine and human skin can be efficiently targeted using our 3D printing-enabled dissolving MAPs to deliver a recombinant SARS-CoV-2 protein antigen, with or without an innate immune agonist. Immunization of mice with vaccine-loaded MAPs generates robust antibody and cellular immune responses, and multicomponent (antigen plus adjuvant) MAP vaccination improves the induced antigen-specific immune responses, such as virus-specific Th1 and IgG2c responses, which are vital for control of SARS-CoV-2 viral infection. Notably, multicomponent MAP vaccination results in increased immune responses compared to immunization via traditional intramuscular injection, and MAP immunization obviates adverse effects of intramuscular delivery of adjuvants, suggesting improved safety and efficacy compared to conventional vaccination routes. These results are supported by our translational studies utilizing freshly-excised human skin, suggesting that multicomponent MAPs induce greater expression of co-stimulatory molecules by human skin-migratory DCs, which may contribute to enhanced immune responses. Ultimately, the simplicity, thermostability, immunogenicity, and versatility of MAPs may enable novel vaccination strategies and increase the effectiveness of global immunization campaigns against SARS-CoV-2 and other existing or novel pathogens.

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IL-15 is an unexpected guardian of hair follicle immune privilege and promotes human hair growth *ex vivo*

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Successful, long-lasting alopecia areata (AA) treatment requires targeting of the key pathomechanisms, i.e. collapse of hair follicle (HF) immune privilege (IP) and premature catagen induction. Recent research has suggested that the pleiotropic cytokine, interleukin-15 (IL-15), is involved in AA pathobiology and that inhibiting IL-15-induced signaling may be beneficial in AA therapy. Yet, this concept has not yet been assessed in human scalp hair follicles (HFs). Specifically, since HF-IP restoration is required for re-initiating hair growth and for preventing relapse of AA, it is crucial to clarify the impact of IL-15 on human HF-IP and HF cycling. Here we show that IL-15+ cell number is increased while IL-15 receptor alpha protein expression is decreased in AA-affected human scalp HFs compared to healthy human scalp skin. When organ-cultured, healthy human anagen scalp HFs were treated with recombinant human IL-15 (rhIL-15), anagen was significantly prolonged and hair matrix keratinocyte apoptosis inhibited. Moreover, expression of MICA and MHC class I was reduced while hair bulb expression of the potent IP guardian, a-MSH, was increased by 50 and 100 ng/mL rhIL-15 *ex vivo*. Importantly, if rhIL-15 was administered before the HF IP collapse induction by IFNg, the increased expression of the NKG2D-activating "danger" signal, MICA, and MHC class I as well as the decreased expression of a-MSH induced by IFNg were all prevented. Taken together, despite its involvement in autoimmune diseases, IL-15 operates as an IP guardian and hair growth promoter in human HFs, while IL-15Ra signaling is defective in AA. Therefore, selective stimulation, rather than inhibition, of IL-15Ra-mediated signaling is likely to be beneficial in the future management of AA and possibly other inflammatory hair loss disorders.

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Dysregulation of VISTA expression and functionality in psoriatic monocytes and Mo-MDSCs

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V-domain Immunoglobulin Suppressor of T cell activation (VISTA) is an inhibitory B7 family immune-checkpoint molecule. VISTA is highly expressed on myeloid, hematopoietic and cancer cells and participates in T cell-mediated autoimmunity and antitumor immunity, playing a broad role in regulation of myeloid- and T cell-mediated immunity. VISTA is upregulated on myeloid-derived suppressor cells (MDSCs) from AML patients. We previously reported MDSCs are increased but functionally impaired in psoriasis (Pso); VISTA knock-out (KO) mice exhibit Pso-like inflammation. VISTA-KO mice exhibit Pso-like inflammation. Whether VISTA signaling is related to Pso MDSC dysfunction is unknown. We analyzed VISTA expression on CD14+ Pso and healthy control (HC) monocytes (Mo) using flow cytometry. Mo-MDSC (CD14+HLA-DR^{neg}) were elevated in Pso patients, and, as hypothesized, VISTA surface expression was elevated (1.6±0.9 % vs 13.2±4.0 % of Mo in HC vs Pso, n=4, 3, $p<0.01$). Innate signaling for human Mo activation via LPS attenuated VISTA gene expression in HC and Pso patients, suggesting VISTA expression is sensitive to inflammatory status. A novel VISTA ligand is V-Set and Immunoglobulin domain containing 3 (VSIG-3); consistent with a functional role for VISTA in human Mo, we found that VSIG-3 stimulation of CD14+ Mo attenuates IL-6 expression. In Pso patients, VSIG-3 was less effective in reducing IL-6 in Pso-Mo compared to HC (average IL-6 after VSIG-3 relative to LPS alone of 66±7.1% in HC versus a minimal effect on IL-6 of 89±7.0% in Pso, n=2, 3, $p<0.05$). Thus, in addition to T cell signals, VISTA expression/signaling is implicated in human Mo and dysregulated in Pso. VISTA pathway targeting may represent a novel immune rebalancing approach in Pso and related inflammatory diseases whose engagement inhibits T-cell proliferation as well as cytokine and chemokine production, demonstrated previously by VSIG-3 inhibition of anti-CD3-induced IL-17 secretion on PBMCs

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Expansion of bacterial phosphatidylglycerol reactive CD4+ T cells in atopic dermatitis

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CD1a, a lipid antigen-presenting molecule structurally related to MHC class I, is constitutively expressed on Langerhans cells in human epidermis. Studies in recent years have suggested a pathogenic role for CD1a in inflammatory and allergic skin disease. We have recently detected a subset of CD1a-restricted CD4+ T cells that specifically responds to bacterial phosphatidylglycerols. In particular, lysyl-phosphatidylglycerol (LPG), an aminoacylated membrane lipid present in many gram-positive bacteria, including *S. aureus*, binds to CD1a and is recognized by these T cells. Using CD1a tetramers loaded with LPG, we detected CD1a-LPG staining T cells in the peripheral blood of multiple donors, and were able to isolate and expand these T cells in vitro. The majority of tetramer+ T cells were CD4+ αβ T cells, and responded in a dose-dependent manner to LPG. CD1a-LPG reactive T cell lines showed a predominantly Th2 cytokine profile, with abundant IL-4 and IL-13 release. Beyond the recognition of purified lipid antigen, CD1a-LPG reactive T cells also responded to whole bacteria, as CD1a-expressing dendritic cells pre-incubated with *S.aureus* induced IL-13 release from the T cell lines. The increased bacterial skin colonization in atopic dermatitis (AD), specifically with *S.aureus*, prompted us to investigate the presence of CD1a-LPG reactive T cells in AD patients. A pilot study in atopic dermatitis patients and healthy controls showed a significantly increased frequency of CD4+ CD1a-LPG tetramer+ T cells in the blood of AD patients. Ongoing work aims to understand the contribution of CD1a-LPG reactive T cells to Th2 mediated pathology in AD.

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IL-23 maintains tissue resident memory Th17 cells in murine and psoriatic skin

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Tissue resident memory Th17 cells (T_{RM17}) are the key cell type driving the chronic skin inflammation of psoriasis. Although IL-23 is strongly associated with autoimmunity and chronic inflammatory disorders including psoriasis, and anti-IL-23 biologic agents have remarkable efficacy in the treatment of psoriasis, the precise role of IL-23 in supporting IL-17-mediated skin inflammation remains unclear. In mice, we found that circulating memory T cells are dispensable for anamnestic protection from *C. albicans* skin infection, and T_{RM17} mediated protection from *C. albicans* reinfection requires IL-23. Administration of anti-IL-23R antibody to dual *Il17a*^{Cre} *Rosa26*$^{CAG\text{-}fl/fl\text{-}tdTomato}$*Il17f*$^{Thy1.1/Thy1.1}$ (17Fate) fate reporter mice following resolution of primary *C. albicans* infection resulted in a selective reduction in the number of CD69$^+$CD103$^+$ T_{RM17} cells in skin compared with isotype controls. T_{RM17} proliferation was reduced and survival was unaffected. CD301b+ dermal dendritic cells (dDC) are an obligate source of IL-23 that supports T_{RM17} maintenance in skin after *C. albicans* challenge. These data demonstrate that locally produced IL-23 promotes *in situ* T_{RM17} proliferation to support their long term retention in skin. In human skin, we identified dermal cDC2 as the principal source of IL-23, although keratinocytes and CD4$^+$ T cells were additional sources of IL-23 in psoriasis skin. Analysis of human psoriasis skin before and after clinical anti-IL-23 therapy revealed reduced retention of T_{RM17} in association with reduced T_{RM17} proliferation, suggesting that targeted depletion of pathogenic T_{RM17} is the major mechanism by which anti-IL-23 therapy induces uniquely durable disease-free intervals in psoriasis patients.

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Functional interrogation of immune cell types identified by single-cell RNA sequencing in alopecia areata

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Alopecia areata (AA) is an inflammatory disease in which autoreactive CD8+ T cells attack the hair follicle (HF) and result in non-scarring alopecia. Previous work from our lab, using both human samples and the graft-induced C3H/HeJ mouse model, established that CD8+ NKG2D+ T cells are the major pathogenic drivers of AA. However, the role of other immune cell types in AA such as regulatory T cells (Tregs), non-Treg CD4+ T cells, and myeloid lineages have not been fully defined. To investigate the complex immune environment underlying AA, we performed single cell RNA-sequencing (scRNAseq) of CD45+ immune cells in skin harvested from affected and unaffected mice. We also performed antibody-based depletion of major immune cell populations to functionally interrogate their role in disease onset, in which 7-week-old C3H/HeJ mice were treated with antibodies one week prior to disease induction via engraftment, followed by continued antibody treatment for two weeks. Consistent with our previous work, scRNAseq revealed a massive expansion of CD8+ T cells in AA, and depletion of CD8+ T cells resulted in complete disease prevention. Non-Treg CD4+ T cells comprised a minor population in our dataset, and accordingly, their depletion resulted in only a slight delay in disease onset with the graft model. Depletion of other minor cell types in our dataset, such as γδ-T cells, NK cells, and B cells, had no effect on the kinetics of AA disease onset. Interestingly, depletion of CSF1R+ myeloid cells delayed disease onset in similar extent as non-Treg CD4+ T cell depletion. Although CSF1R+ cells comprised a smaller proportion of the immune landscape in AA compared to control skin, they showed upregulation of inflammatory cytokines such as Ccl5 and Cxcl10, which are known to activate T cells. Functional dissection of immune cell populations in AA supported that CD8+ T cells are the major pathogenic cell type in disease, and uncovered novel roles for myeloid lineages in AA onset.

038

High-throughput single-cell αβ TCR sequencing identifies pathogenic CD8+ T cell clones that are sufficient to induce alopecia areata in a C3H/HeJ retrogenic model

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Alopecia areata (AA) is an autoimmune disease in which CD8+ T cells attack hair follicles and leads to nonscarring hair loss. AA is postulated to be an antigen-driven disease, however, whether pathogenesis requires T cell-mediated antigen recognition, and if so, what epitope(s) the T cells recognize, remain unclear. Our previous work showed that clonal expansion of CD8+ T cells coincides with AA disease onset, and we identified dominant T cell receptor (TCR) sequences shared among independent samples from the C3H/HeJ mouse model of AA, supporting the antigen-driven nature of AA. To understand whether specific subsets of CD8+ T cells undergo clonal expansion and drive AA pathogenesis, here we performed parallel single-cell RNA-sequencing and single-cell VDJ-sequencing on individual immune cells harvested from the skin and lymph nodes of affected vs. unaffected C3H/HeJ mice. In AA mice, the clonal repertoire of CD8+ T cells in the skin becomes significantly restricted, whereas cells in the lymph node retain clonal diversity, suggesting that pathogenic CD8+ T cells undergo clonal expansion in the skin after antigen recognition. This independent dataset validated our previously reported TCR sequences, and we found that the degree of clonal expansion correlated with gene signatures suggestive of T cell activation and pathogenicity. Using one of the highly expanded αβ TCR pairs, we generated TCR retrogenic mice, in which SCID C3H/HeJ mice devoid of endogenous T cells were reconstituted with bone marrow cells expressing only the TCR sequence of interest. TCR retrogenic mice displayed normal T cell development, including CD8+ T cells, and by 7 weeks post-transplantation over 80% of mice developed AA-like hair loss. Our results indicate that this pathogenic CD8+ T cell clone is sufficient to induce spontaneous AA in C3H/HeJ mice, and strongly supports the antigen-driven nature of AA.

039

IL-7 regulates the PD-1 signaling pathway via degradation by E3 ubiquitin ligase F-Box Protein 38

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Alopecia areata (AA) is a T cell-mediated autoimmune disease attacking the hair follicle (HF). The IL-7 signaling pathway plays an important role in T cell survival and it has been therapeutically targeted in several T cell-dependent autoimmune disease models. Here, we showed that C3H/HeJ mice with AA exhibited hair regrowth after anti-IL7Ra treatment. Mechanistically, we observed that IL-7Ra blockade significantly reduced the number of alopecic effector $CD8^+$ T cells. We also found that C3H/HeJ mice treated with anti-IL-7Rα showed a significant increase in the frequency of $PD\text{-}1^+CD44^+$ T cells within SDLNs compared to controls. Our previous results indicated that IL-7 may antagonize the function of PD-1 by downregulating the expression of PD-1 in T cells, however, the mechanism of this downregulation remains unclear. Recently, the F-box protein FBXO38, a member of the SKP1−CUL1−F-box protein family of E3 ubiquitin ligases, has shown to interact with PD-1 and decrease PD-1 cell-surface expression via degradation. Based on this findings, we postulated that IL-7 might decrease PD-1 expression through upregulation of FBXO38. We found that IL-7 significantly increased the transcription levels of FBXO38 and concurrently decreased the expression of PD-1 on the surface of T cells in vitro. We further observed that FBXO38 knockdown using siRNA increased the PD-1 expression on the surface of T cells compared to control, and moreover, this effect could not be rescued by IL-7 treatment. Our results indicate FBXO38 as a critical mediator of PD-1 degradation and suggests that targeting IL-7-mediated regulation of FBXO38 expression may represent a potential strategy to enhance PD-1 signaling in T cell-mediated autoimmune diseases including AA.

040

Single-cell RNA sequencing identifies a disease-dominant CD8+ T cell population co-expressing both activating and inhibitory receptors of the NKG2 family

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Alopecia areata (AA) is a cell-mediated, autoimmune form of hair loss characterized by lymphocytic infiltration of the bulb region of the hair follicles (HFs), inflammation, and destruction of the HF. We previously showed that CD8+ NKG2D+ T cells are necessary and sufficient to induce AA in the graft-induced C3H/HeJ mouse model. Here, we used single-cell RNA sequencing to comprehensively profile the T cell component of the inflammatory infiltrate in AA. We first isolated CD45+ cells from the skin of affected and unaffected control mice and focused our analysis on CD8+ T cells. We observed a marked expansion of all CD8+ T cells in AA mice (41% versus 4% of all CD45+ T cells in control mice). CD8+ T cells in both AA and control mice were clustered into 5 distinct populations, each with an associated set of marker genes. Shared CD8+ populations included antigen-experienced effector cells with high expression of IFNG, GZMA, and a memory T cell population with high CD69 and CD40LG, both of which were expanded in AA mice. Interestingly, we discovered a population of CD8+ T cells that is largely predominant in AA mice, marked by increased expression of not only NKG2D, an activating receptor, but also NKG2A, a known inhibitory receptor of the NKG2 family. These cells were also characterized by increased expression of T cell exhaustion markers (PD1, TIM3, CTLA4) as well as the co-stimulatory markers CD137 and ICOS. To probe the NKG2A receptor pharmacologically, we found that treatment of mouse T cells *in vitro* with an NKG2A agonist antibody resulted decreased IFNG production, and treatment of C3H/HeJ mice with a blocking antibody against Qa-1 (an NKG2A ligand) prior to disease induction via engraftment resulted in earlier AA onset and accelerated disease progression *in vivo*. Therapeutic manipulation of the NKG2 family of activating and inhibitory receptors represents a novel treatment approach in AA.

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CXCR3 blockade reduces skin germinal center B cells and autoantibody titers in murine cutaneous lupus erythematosus

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Cutaneous Lupus Erythematosus (CLE) describes a broad range of autoimmune dermatologic diseases that are characterized histopathologically by interface dermatitis and autoantibody deposition. Racial and ethnic disparities have been reported in disease activity and outcomes in CLE, with African Americans presenting with greater disease damage. Treatment options available to patients with CLE are unfortunately limited. It is important to understand the pathogenesis of CLE to develop effective treatments for patients. One of the most highly upregulated chemokine families in CLE is the CXCR3 chemokine family. The interaction between CXCR3-expressing T-cells and its ligands have been associated with tissue damage in CLE subtypes. To further understand the role of CXCR3 in CLE immunopathogenesis, we performed studies using a mouse model of CLE and human tissue. Here, we characterize CXCR3-bearing immune cells in the skin of this mouse model and in blister biopsies obtained from CLE patients. We observed higher expression of CXCR3 on T cells and B cells, supporting the role of CXCR3 in the pathogenesis of CLE. We then wanted to determine whether CXCR3 blockade with a monoclonal antibody could prevent CLE disease development. We show that CXCR3 blockade in CLE mouse models stabilized skin lesions and helped reduce autoantibody titers and germinal centers. This suggests that blockade of CXCR3 may have prevented the stimulation of autoantibody-secreting B cells, resulting in reduced autoantibody titers. These results provide further rationale for targeting CXCR3 in CLE.

042

Competition for active TGFβ augments accumulation of antigen-specific CD8+ T cells in murine melanoma

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Melanoma is the least common skin cancer but is responsible for the majority of skin-cancer related mortality. Successful anti-tumor immunity is mediated in part by tumor antigen specific CD8+ T cells. Our group has shown that competition for limiting amounts of active TGFβ allows antigen-specific CD8+ resident memory T cells (Trm) to persist in the epidermal niche at the expense of bystander Trm. Whether or not competition for active TGFβ affects T cells in the context of chronic antigen is unknown. Using a model which renders CD8+ T cells independent of TGFβ (E8iCreER^{T2} TGFβRCA), we explored the effects of constitutive TGFβ signaling in CD8+ tumor infiltrating lymphocytes (TIL) in an intradermal B16 melanoma model. We found that while the total number of CD8+ TILs was unaffected, the percentage of CD8+ T cells expressing TGFβRCA was increased in the tumor but not spleen or lymph nodes thereby demonstrating a tumor selective enrichment of these cells. Enrichment was most evident in CD8+ T cells expressing the resident memory markers, CD69 and CD103. Moreover, rendering CD8+ T cells independent of TGFβ resulted in more rapid growth of implanted B16 tumors. A survey of human biopsy samples also revealed that melanomas express higher levels of the TGFβ-activating integrins avβ6 and avβ8 when compared to melanocytic nevi. Taken together, these data indicate that CD8+ T cell competition for active TGFβ occurs in the context of persistent tumor antigen and suggest that increased expression of TGFβ-activating integrins in melanoma may prevent the accumulation of antigen specific CD8+ TILs , representing a novel mechanism of tumor immune-evasion.

043

Loss of DLX3 tumor suppressive function is associated with poor prognosis in human SCCs

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Development and progression of cutaneous squamous cell carcinoma (cSCCs) is known to be regulated by traditional oncogenic and tumor suppressive proteins; however, new evidence suggests that homeoproteins can act as cancer modulators through regulation of proliferation, migration and survival. We carried out a human clinicopathologic analysis of DLX3 expression in 121 cSCCs and 6 benign skin tumors. Correlation analysis showed that tumors of increased pathologic stage had diminished levels of DLX3 expression. Kaplan-Meier analysis of overall survival (OS) revealed a statistically significant difference between patients with high DLX3 expression and low DLX3 expression. We then used a two-stage dimethylbenzanthracene (DMBA)/12-O-tetradecanoylphorbol 13-acetate (TPA) mouse skin carcinogenesis model to observe Dlx3 function in vivo. Dlx3 knockout mice (Dlx3KO) presented with higher numbers of tumors compared to wild type (WT). It is generally accepted that treatment with tumor initiator (DMBA) does not produce papillomas without a chemical promoter (TPA). We next examined the effect of DMBA-only treatment on Dlx3-deficient skin tissue. In Dlx3KO mice, papillomas began to appear at ∼16 weeks after DMBA treatment whereas all WT mice were tumor free over the entire experiment course (∼31 weeks). Thus, we found that Dlx3KO skin is self-promotive for DMBA-initiated tumorigenesis. Our study also showed that a single DMBA application was sufficient to produce tumors in DLX3-deficient skin. Whole transcriptome analysis (RNA-seq) of tumor and skin tissue from our mouse model uncovered a molecular dependence on the proliferation regulators responsible for tumor promotion, supporting a tumor suppressive function for DLX3 in skin.

044

Nonmelanoma skin cancer in children and young adults with iatrogenic risk factors

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Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are rare in pediatric patients and not well characterized. A multicenter retrospective case-control study by colleagues recently demonstrated most affected patients have at least one identifiable risk factor, either a predisposing genetic condition or iatrogenic exposure (prolonged immunosuppression, radiation therapy, chemotherapy, and/or voriconazole use). Different factors were associated with the development of BCC versus SCC. We hypothesize the mechanism of tumorigenesis is distinct in these patients due to differing immunologic microenvironments of these cancers. Our study aims to characterize and compare the immunogenicity of BCC and SCC in order to better understand the disparate mechanisms of cancer development. We have obtained and banked tissue samples of SCC and BCC from pediatric patients from Boston Children's Hospital and Massachusetts General Hospital. In this cohort, SCC and BCC did not co-occur in the same patients, with SCC found in all patients with prolonged immunosuppression and BCC more common in patients who had undergone radiation therapy and chemotherapy. We characterized these samples using immunohistochemical staining for antibodies identifying T cells and antigen presenting cells. Our preliminary findings clearly show that SCC is infiltrated by CD8+ T cells, which can explain the dependency of SCC development on immunosuppression. Our data suggest that even in patients with low UV burden and chronic immunosuppression, resident T cells may be present and active, serving as a target for cancer directed therapy. In contrast, BCC has minimal T cell infiltrate and therefore can develop in immunocompetent patients with other iatrogenic risk factors. Radiation therapy has been shown to activate Sonic hedgehog signaling pathway and may be the mechanism for BCC induction in children. We plan to expand our analysis to include other centers for optimal characterization.

045

Glucose-6-phosphate dehydrogenase is a promising predictor of immunotherapy response for Merkel cell carcinoma

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Immune checkpoint therapies(ICI), such as PD-L1 or PD-1 blockade therapy, for Merkel cell carcinoma (MCC), have recently shown successful results. Approximately 50% of patients, however, remain without durable benefit from these epochal treatments. Further treatment strategies are, therefore, still required. We collected 90 specimens from 71 patients and 53 blood serum samples from 21 patients with MCC at 10 facilities. RNA sequencing was performed to evaluate patients' immune activity and classified tumors into 2 types: the "immune active type" and the "cell division type". Expression of the glucose-6-phosphate dehydrogenase (G6PD) gene was highly significantly upregulated in the "cell division type". Among 395 genes, G6PD expression correlated with the presence of lymph node or distant metastases during the disease course and significantly negatively correlated with PD-L1 expression. Immunohistochemical staining of G6PD also correlated with disease-specific survival and PD-L1 expression. A blood serum test could measure G6PD activity. The detection values significantly increased in the non-responder to ICI and decreased in the responder to ICI. G6PD expression was an immunohistochemically and serum-detectable prognostic marker that negatively correlated with immune activation and PD-L1 levels and could be used to predict the immunotherapy response.

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Knockdown of IGF2BP1 reduces the tumorigenicity of basal cell carcinoma cells in mice

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More than four million Americans each year are affected by basal cell carcinoma (BCC). Anyone with a history of sun exposure can develop BCC. However, people who are at highest risk are the ones in the fair skinned populations. Immunocompromised patients have also been reported to have a 10 fold higher risk of developing BCC than the general population and BCCs appear to show a more aggressive behavior in these patients. Although most of BCCs are not life threatening, this malignancy if untreated can destroy the tissues, causing ulceration and disfigurement. BCC therefore causes considerable morbidity and places a huge burden on healthcare service worldwide. The cost of care for BCCs is the fifth highest for all cancers in the Medicare population in the United States. Constitutive activation of Hh signaling pathway drives the development of BCC through activation of Gli1 which is the transcription factor mediating the Hh pathway. We previously demonstrated that Gli1 was regulated by the Wnt signaling through activation of its target, IGF2BP1. Moreover, the regulation of Gli1 by the Hh upstream signal appears IGF2BP1-dependent as well. We hypothesized that IGF2BP1-dependent regulation of Gli1 expression and activities was important in the development of BCC. We used the CRISPR/Cas9 approach to knock down IGF2BP1 in UW-BCC1 cells and test our hypothesis. Two million UW-BCC1 cells with conditional knockdown of IGF2BP1 were injected subcutaneously in the flank of immunocompromised mice. Tumor growth was monitored weekly for a period of eight weeks. We observed that knockdown of IGF2BP1 in UW-BCC1 cells significantly reduced tumor growth in mice compared to controls ($P< 0.001$). In addition, a reduction in the expression of Wnt and Hh targets was observed in the tumors. Interestingly, a gender disparity in the development of tumors using UW-BCC1 cells was observed. IGF2BP1 appears to contribute to BCC development and might represents a novel target in the treatment of basal cell carcinoma.

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Parallels between wound healing and cancer: An avenue to cancer therapeutics

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Adult stem cells (SCs) located in barrier tissues such as the skin must endure repeated bouts of inflammation over an organism's lifetime in order to maintain tissue homeostasis. During skin wound repair, hair follicle stem cells (HFSCs) enter the highly inflammatory wound, where they must survive to fuel epithelial regeneration. On the other hand, skin squamous cell carcinoma (SCC) arises when tumor-initiating SCs (tSCs) acquire mutations that allow survival and immune evasion. Cancer, then, can be thought of as a wound that never heals, especially given that patients suffering from chronic wounds have increased risk of developing malignancy. Recent work has confirmed that wounded and tumorigenic SCs indeed display common gene signatures, leading us to hypothesize that common signaling pathways may be activated in the wound and tumor environment and that those mechanisms promote SC survival in the face of inflammation. To test this, we studied HFSCs, which are responsible for cutaneous wound repair as well as give rise to skin SCC upon acquiring mutations that elevate RAS/MAPK signaling. Using a sophisticated genetic reporter system, we found that both normal HFSCs during wounding and tSCs initiating oncogenic Hras^{G12V}-driven SCC respond to similar signals in the microenvironment. Comparisons of transcriptome data revealed a short-list of genes that are activated in both wounded HFSCs and SCC-tSCs, which can be induced by these signals in vitro. This short-list provided guidance for functional studies that identify key genes involved in the process. Overall, these data show that whether native or tumorigenic, stem cells exploit their microenvironment to obtain a survival advantage during inflammatory pathology. Our findings suggest that intercepting this exploitation could be an important avenue for effective immunotherapy.

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Human protein SLURP-1 inhibits melanoma cells migration by interaction with a7-nAChRs

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Activation of the a7 type nicotinic acetylcholine receptors (a7-nAChRs) promotes proliferation and migration of cancer cells and suppresses apoptosis. The secreted human Ly-6/uPAR protein-1 (SLURP-1) inhibits a7-nAChRs and down-regulates proliferation of carcinoma cells. SLURP-1 expression is reduced in melanoma cells compared to healthy tissues, as well as in metastatic melanoma cells compared to the primary tumor. In the present study we analyzed a SLURP-1 and α7-nAChR expression in tumor and normal tissue samples from the TCGA Melanoma database and revealed the association between the low expression of α7-nAChR and higher survivability of melanoma patients. We produced a recombinant analog of human SLURP-1 (rSLURP-1) and studied its antiproliferative activity on a set of primary melanoma cells including one low differentiated cell line (mel P) and four moderately differentiated cell lines (mel Kor, mel Cher, mel H, mel P, mel Gi). We found that rSLURP-1 had no antiproliferative effect in all cell lines studied. At the same time, rSLURP-1 selectively inhibited migration of mel P cells with $EC_{50} = (67,8 \pm 0,4)$ nM. To understand a mechanism of the rSLURP-1 selectivity, real-time PCR analysis was performed. All investigated cells demonstrated a similar mRNA expression level of both *CHRNA7* and *SLURP-1* genes, while the cell-surface expression of α7-nAChR was significantly higher in mel P cells, that was confirmed by flow cytometry using fluorescently labeled α-Bgtx, the specific α7-nAChR ligand. Knockdown of the α7-nAChR expression in mel P cells by α7-siRNA completely abolished the rSLURP-1 effect on a cell migration. Thus, rSLURP-1 inhibits migration of low differentiated melanoma by interaction with a7-nAChRs on a surface of cancer cells.

049

Personal history of rosacea and risk of basal cell carcinoma of the face: A regional analysis of Rhode Island

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Basal cell carcinoma (BCC) and rosacea are two entities that affect the face. BCC is the most common form of skin cancer and most frequently occurs in sun-exposed regions, while rosacea is a chronic inflammatory skin condition that primarily affects the central face. Immune system dysregulation has been implicated in the development of rosacea, which is exacerbated by ultraviolet (UV) radiation. This study retrospectively assesses associations between personal history of rosacea with development of BCC of the face versus other body sites through a regional analysis of Rhode Island patients. A total of 4,537 patients with a diagnosis of BCC from 2016-2020 were identified. 2,455 of them presented with a BCC of the face. A total of 270 patients had a prior history of rosacea according to available medical records. A multivariate logistic regression analysis adjusted for patient age, sex, smoking history, and skin of color was used to estimate the associations between rosacea and BCC on the face. Among patients with BCC, a history of rosacea was inversely associated with BCC of the face; odds ratios (OR) and 95% confidence intervals were 0.75 (0.58, 0.95). Female sex and patient's age were each associated with significantly increased risk of BCC of the face. History of smoking and skin of color were not significantly associated with risk of BCC of the face. These results suggest that a history of rosacea may be a protective factor against development of BCC of the face. Behavioral and biological factors may contribute to the findings. For example, patients with rosacea are advised to decrease sun exposure, which might subsequently lower the risk of skin cancer on the face. On the other hand, the increased sebum production and sebaceous hyperplasia that occur with rosacea may affect BCC development due to the antioxidative properties of sebum. Limitations of our study include lack of non-cancer controls and age of rosacea onset. Concurrent mild rosacea cases may have also not been recorded.

050

Loss of retinoic acid receptor-related receptor alpha (Rorα) promotes the progression of UV-induced cSCC

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Cutaneous squamous cell carcinoma (cSCC) is prevalent in the world, accounting a huge part in non-melanoma skin cancer. Most cSCCs are associated with a distinct pre-cancerous lesion, the actinic keratosis (AK). However, the progression trajectory from normal skinto AK and cSCC has not fully demonstrated yet. To identify genes involved in this progression trajectory and possible threptic targets for cSCC, here we constructed a UV-induced cSCC mouse model covering the progression from normal skinto AK to cSCC, which mimicked the solar UV radiation perfectly using the solar-like ratio of UVA and UVB, firstly. Then, transcriptome analysis and a series of bioinformatics analysesand cell experiments proved that Rorα is a key transcript factor during cSCC progression.Rorα could down-regulate the expressions of S100a9 and Sprr2f in cSCC cells, which can inhibit theproliferation and migration in cSCC cells, but not the normal keratinocyte. Finally, further animal experiment confirmed the inhibitory effect of cSCC growth by Rorα in vivo.Our findings showed that Rorα would serve as a potential novel target for cSCC, which will facilitate the treatment of cSCC in the future.

051

CD271 activation reduces SCC spheroid aggressiveness, modulates keratinocyte differentiation and favors response to therapy

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Cutaneous squamous cell carcinoma (cSCC) is the second most frequent form of skin cancer showing a rapidly increasing incidence worldwide. In the skin, neurotrophins (NTs) and their receptors form a complex network with regulatory functions. CD271, the NT low affinity receptor, is implicated in the switch between stem and early progenitors, thus playing a key role in keratinocyte differentiation. However, the expression and function of CD271 in tumors are controversial and its role in cSCC has to be functionally elucidated. We showed that CD271 receptor modulation by γ- or α-secretase inhibitors affects cSCC cell behavior and NT signaling activation increase the number of CD271 nuclear positive cSCC cells. By the novel flow-based W8^{TM} machine analysis, we present evidences that CD271 overexpression significantly increases cSCC spheroid mass density, while it reduces their weight and diameter. In addition, RNAseq analysis of CD271 overexpressing spheroids showed a major fold-enrichment in cell differentiation and keratinization genes, further corroborated by the histological and molecular analysis. CD271 overexpression or activation in SCC-xenografting zebrafish models increases the number of mpeg-1 positive cells, indicating that CD271 activity might be able to stimulate the innate immune response. Upon CD271 overexpression, photodynamic therapy or chemotherapy (5FU or cisplatin) reduce cSCC spheroid size and viability to a higher degree as compared to the effect in mock spheroids. Therefore, our data strongly indicate that CD271 signaling favors the inhibition of cSCC development and may be considered as a potential target of therapy for this type of cancer.

052

Staphylococcal enterotoxin promotes the development and maintenance of the skin lesions in cutaneous T cell lymphoma

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Cutaneous T cell lymphoma (CTCL) has long been known to have a strong association with *Staphylococcus aureus* (SA) colonization. Eradication of SA is beneficial to CTCL patients. However, how SA colonization contributes to the pathogenesis of CTCL is poorly characterized. Here, we evaluated the SA colonization in the lesional and nonlesional skin of a cohort of 67 CTCL patients. Skin bacterial culture showed SA colonization in 34.3% of patients. The percentage of SA colonization increased along with the progression of the disease stage. The clinicopathological analysis revealed a positive correlation between SA colonization and dermal eosinophil infiltration in CTCL skin lesions. In most CTCL patients, SA simultaneously presented on the lesional and nonlesional skin and produced the same types of enterotoxins. Further skin microbiome analysis by 16S ribosomal DNA sequencing revealed that the abundance of *Staphylococcaceae* was markedly greater on lesional than on nonlesional skin. An inverse correlation was identified between the abundance of *Staphylococcaceae* and the microbial diversity. Furthermore, staphylococcal enterotoxin B (SEB) was the most frequently detected enterotoxins in our patients and presented in 17.4% SA isolates. Recombinant SEB triggered remarkable cell growth of peripheral blood mononuclear cells (PBMCs) from both healthy donors and leukemic CTCL patients. However, the transcriptional programs induced by SEB were completely different between PBMCs from healthy donors and leukemic CTCL patients. SEB stimulated an increase in $CD4^+$ memory T cells and IL13 expression in CTCL patients, whereas an increase of $CD8^+$ memory T cells and γδ T cells coupled with an anti-bacterial response was seen in healthy donors upon SEB stimulation. These findings confirmed that SA colonization is common in CTCL patients and suggested that SA contributed to the development and persistence of CTCL skin lesions via enterotoxin. These results provide new insights into the association between SA and CTCL and may pave the way for future anti-SA treatment in CTCL.

053

The inhibitory role of ganglioside GD3 on the functional activities of benign T cells in cutaneous T-cell lymphoma

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In cutaneous T-cell lymphoma (CTCL), the malignant T cells and benign T cells are confined in the same skin lesions. It is thus difficult to evaluate the phenotypical characteristics and functional activities of the malignant and benign T cells separately. In various solid malignant tumors, ganglioside GD3 is upregulated on the surface of malignant cells and takes part in tumor progression and the inhibition of antitumor activities. GD3 is also expressed in T cells and is involved in the modulation of their effector functions. However, the role of GD3 in CTCL has not been well-understood. Herein, we distinguished the malignant and benign T cells in the skin lesions of 12 CTCL cases by flow cytometry and compared their phenotypical characteristics with that of T cells from control skin specimens (Ctl). We revealed that benign T cells in CTCL skin lesions consisted of less Th17 cells compared to Ctl (% IL-17A in CD4: 5.9 ± 5.1 % in Ctl vs 1.3 ± 1.6 % in CTCL benign, $p = 0.0006$). Besides, the malignant CD4 T cells showed significantly stronger expression of GD3 than benign CD4 T cells and Ctl (% $GD3^+$ in CD4: 79.8 ± 9.0 % in Ctl vs 68.7 ± 17.8 % in CTCL benign vs 95.7 ± 4.4 % in CTCL malignant, $p = 0.0041$ between Ctl and CTCL malignant, $p < 0.0001$ between CTCL benign and CTCL malignant). IL-17A production from Ctl CD4 T cells was downregulated by culture in the presence with GD3 (% IL-17A in CD4: 6.2 ± 2.6 % in +GD3 vs 10.9 ± 6.6 % in -GD3, $p = 0.0313$), and the expression of siglec-7, an inhibitory ligand of GD3, turned out to be upregulated in CTCL benign CD4 T cells (% siglec-7^+ in CD4: 2.7 ± 2.2 % in Ctl vs 9.8 ± 6.5 % in CTCL benign, $p = 0.0112$). Our results suggest that the malignant T cells suppress Th17 activity of the benign counterpart T cells in CTCL lesions possibly via the signaling by GD3. These results imply the possibility of GD3 as a potential treatment target in CTCL.

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Phenformin promotes keratinocyte differentiation via the calcineurin/NFAT pathway

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Phenformin is a drug in the biguanide classify that was previously used to treat type 2 diabetes. We have reported the anti-tumor activities of phenformin to enhance the efficacy of BRAF-MEK-ERK pathway inhibition and to inhibit myeloid-derived suppressor cells in various melanoma models. Here we demonstrate that phenformin suppresses tumor growth and promotes keratinocyte differentiation in the DMBA/TPA two stage skin carcinogenesis mouse model. Moreover, phenformin enhances the suspension-induced differentiation of mouse and human keratinocytes. Mechanistically, phenformin induces the nuclear translocation of NFATc1 in keratinocytes in an AMPK-dependent manner. Pharmacological or genetic inhibition of calcineurin/NFAT signaling reverses the effects of phenformin on keratinocyte differentiation. Taken together, our study reveals an anti-tumor activity of phenformin to promote keratinocyte differentiation that warrants future translational efforts to repurpose phenformin for the treatment of cutaneous squamous cell carcinomas.

055

Epidermal integrin α3β1 promotes a tumor-supportive protease secretome

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As the major cell surface receptors for the ECM, integrins regulate adhesion and migration, and have been shown to drive tumor growth and progression. The development of integrin-targeted cancer therapies is hindered by incomplete understanding of integrin function in tumor cells and the microenvironment. Previous studies showed that mice with epidermis-specific deletion of the α3 integrin subunit fail to form skin tumors during two-step chemical tumorigenesis, indicating a pro-tumorigenic role for integrin α3β1. We therefore generated mice with tamoxifen-inducible, epidermis-specific α3 knockout to determine the role of α3β1 in the maintenance of established tumor cells and/or the associated stroma. We recently showed that genetic ablation of α3 in established skin tumors caused their rapid regression, indicating that epidermal α3β1 is essential to maintain tumor growth. Interestingly, a robust increase in stromal apoptosis was observed prior to increased apoptosis in α3β1-deficient tumor cells, indicating that alteration of the tumor microenvironment preceded the effect on tumor cells. Consistently, MS analysis of conditioned medium from immortalized keratinocytes showed that α3β1 regulates a substantial fraction of the keratinocyte secretome, to include several matrix proteases. Our current studies confirm, with a panel of immortalized and transformed keratinocytes, the integrin α3-dependent regulation of BMP-1, MMP-9, and MMP-3. RNA *in situ* hybridization showed that expression of these genes was reduced in α3β1-deficient tumor cells *in vivo*. Bioinformatic studies show that expression of BMP-1, MMP-9, and MMP-3 correlate with expression of ITGA3 (the gene encoding the integrin α3 subunit) in human SCC, and that ITGA3, BMP-1 and MMP-3 associate with poor survival outcome in these patients. Overall, our findings identify α3β1 as a pleiotropic regulator of the keratinocyte protease secretome, and as a potential therapeutic target.

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Testosterone signaling through ZIP9 renders melanoma more aggressive in males than in females

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Melanoma and most other cancers occur more frequently, and have worse prognosis, in males compared with females. Though sex steroids are thought to be involved, classical androgen and estrogen receptors are not detectable in most melanomas. Here we show that testosterone promotes melanoma proliferation by activating ZIP9 (*SLC39A9*), a zinc transporter that is not intentionally targeted by available therapeutics, but is widely expressed in human melanoma. This testosterone activity requires zinc influx, MAPK activation and YAP1 nuclear translocation. We demonstrate that FDA approved inhibitors of the classical androgen receptor also inhibit ZIP9, and thereby antagonize the pro-tumorigenic effects of testosterone in melanoma. In male mice, androgen receptor inhibitors suppressed growth of ZIP9-expressing melanomas, but had no effect on isogenic melanomas lacking ZIP9, nor on melanomas in females. These data suggest that ZIP9 might be effectively targeted in melanoma and other cancers by repurposing androgen receptor inhibitors that are currently approved only for prostate cancer.

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Understanding the distinct roles of p53 family of transcription factors through identification of protein-protein interactions

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The p53 family of transcriptions factors — p53, p63, and p73 — share a high degree of homology, however members are activated in response to different stimuli, have distinct (sometimes opposing) roles and are differentially expressed. The complexity is increased further by transcription of multiple isoforms, which may interact and impact cellular outcome. While p53 deficiency leads to high incidence of spontaneous tumors in mice, this is not seen in p63 or p73 deficient mice, which instead display severe developmental defects in skin and nervous system, respectively. *p53* gene mutations are commonly seen in human cancers, but *p63* and *p73* are rarely mutated. P63 levels are elevated in squamous cancers of the skin, head/neck, and lung and p73 is overexpressed in neuroblastomas and in other malignancies. Proteins function via interacting with other proteins and/or with nucleic acids. Therefore, identification of binding partners and their 3D interactions is essential to fully comprehend protein activity and mechanism. We applied a novel powerful *in silico* protein-protein interaction prediction method — HMI-PRED — to predict interaction partners of p53 family members, and modeled 3D structures of these protein interaction complexes. As proof of principle, our method recovered experimentally known interactions for p53, p63, p73 (60, 26, 20, respectively) but also identified novel candidate partners (377, 594, 969 for p53, p63, p73). Of these, 95 are common to all members, however, unique interactions for individual family members were also identified (168, 232, 398 for p53, p63, p73). This approach can also be applied to predict the effects of oncogenic mutations on these interactions. The similarities and differences seen among the interaction partners can help to elucidate distinct functions of p53 family members as well as how they can lead to distinct/opposing outcomes, and yield novel strategies in developing highly efficacious cancer therapies.

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Identification of novel gene classifiers to non-invasively diagnose non-melanoma skin cancer

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Non-melanoma skin cancers (NMSC) are the most common types of skin cancer and include both basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). NMSC primarily form on sun exposed skin including the head, face, neck, arms, and hands. BCC accounts for >75% of NMSC cases; however, SCC is more aggressive and may occur in other locations as well. Combined, BCC and SCC are responsible for >15,000 deaths each year in the US alone, which exceed deaths due to melanoma. Current diagnosis of NMSC relies on an in-depth visual assessment of the lesion in question followed by a surgical skin biopsy for histopathologic review. This analysis investigated whether the non-invasive collection of skin tissue with 'smart stickers' and subsequent genomic analysis could properly classify NMSC. Adhesive skin collections kits were used to collect the lesional skin from 58 patients with BCC, 41 patients with SCC, and 42 patients with non-cancerous skin diseases. Whole transcriptomic analysis was conducted on each sample and differentially expressed genes were determined by comparing BCC and/or SCC with non-cancerous skin disease (other) using multiple comparisons. Eighteen genes were significantly (fold change >1.5; $p<0.1$) increased in BCC compared to other skin diseases while 14 genes were increased in SCC (fold change >1.5; $p<0.1$). Further analysis identified 12 genes that were differentially expressed in both lesional BCC and lesional SCC compared to other skin diseases. These results require further investigation but suggest that "smart sticker" enabled non-invasive skin sampling and genomic analysis may provide an opportunity to identify patients with NMSC earlier and without the need for surgical biopsy.

059

Single-cell RNA sequencing reveals tissue compartment-specific plasticity of mycosis fungoides tumor cells

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While mycosis fungoides (MF) is usually restricted to the skin during early disease, malignant cells can appear in blood, bone marrow and secondary lymphoid organs in later disease stages. However, only little is known about phenotypic and functional properties of malignant T cells in relationship to tissue environments over the course of disease progression. We thus profiled the tumor micromilieu in skin, blood and lymph node in a patient with advanced MF using single-cell RNA sequencing combined with V-D-J T-cell receptor sequencing. In skin, we identified clonally expanded T-cells with characteristic features of tissue-resident memory T-cells (T_{RM}, *CD69*$^+$*CD27*$^-$*NR4A1*$^+$*RGS1*$^+$*AHR*$^+$). In blood and lymph node, the malignant clones displayed a transcriptional program reminiscent of a more central memory-like phenotype (*KLF2*$^+$*TCF7*$^+$*S1PR1*$^+$*SELL*$^+$*CCR7*$^+$), while retaining tissue-homing receptors. The skin tumor microenvironment contained potentially tumor-permissive myeloid cells producing regulatory (*IDO1*) and Th2-associated mediators (*CCL13, CCL17, CCL22*). Given their expression of *PVR, TNFRSF14* and *CD80/CD86*, they might be under direct control by *TIGIT*$^+$*CTLA4*$^+$*CSF2*$^+$*TNFSF14*$^+$ tumor cells. This work demonstrates adaptive phenotypic and functional plasticity of MF tumor cell clones. Thus, the T_{RM}-like phenotype allows them to reside within the skin for long periods of time, while adopting a T_{CM}-like phenotype with skin homing molecule retention in peripheral blood. Furthermore, understanding the mechanisms underlying the plasticity of MF cells could be relevant for the migratory behavior of regular memory T cells.

060

Toll-like receptor 4 activity in the tumor microenvironment promotes cutaneous T-cell lymphoma

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How microenvironmental factors such as skin extracellular matrix (ECM) and resident immune cells contribute to cutaneous T-cell lymphoma (CTCL) is not well understood. Here we report a ligand-receptor interaction connecting the ECM and immunosuppressive macrophages in the CTCL microenvironment. We find that expression of a fibronectin splice variant encoding extra domain A (EDA), a hallmark of ECM dysregulation in cancer, occurs in mouse CTCL tumors, and we confirm this finding in human CTCL lesions. Notably, EDA is a ligand for toll-like receptor 4 (TLR4), a pattern recognition receptor with roles in regulating immunity within tumor microenvironments. We find that CD206+ immunosuppressive macrophages within the lesional skin of CTCL patients express high levels of TLR4. Similarly, tumor-localized CD206+ macrophages are TLR4-high in our immune-competent CTCL mouse model. Lastly, we observe the abrogation of CTCL tumor growth in TLR4 deficient mice compared to wild-type. Together, these data suggest that TLR4 activity in the CTCL microenvironment promotes tumor growth, and EDA-TLR4 interactions may drive immunosuppressive macrophages. Our results uncover a link between the extracellular matrix and anti-tumor immune response that may reveal new therapeutic targets in CTCL.

061

Absence of Adrb2 minimally affects UV-induced immunosuppression and skin cancer development

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Excessive ultraviolet (UV) exposure has been associated with majority cases of non-melanoma skin cancer and is identified as the main causative factor for skin cancer. Chronic stress has also been associated with increased risk for skin carcinogenesis and reduced antitumor immunity. The effects of stress are partially mediated through activation of the sympathetic nervous system that results in the release of the catecholamine hormones, which act through the β-adrenergic receptors (β-ARs). Preclinical studies have demonstrated that the nonselective β-AR antagonist carvedilol exhibits chemopreventive activity on skin cancer, but the mechanism is unknown. The present study was aimed at investigating the role of β2-AR (Adrb2) on UV-induced skin damage, inflammation, immunosuppression and carcinogenesis. In both wild-type and whole-body Adrb2 knockout mice on an SKH-1 background, single dose UV radiation at the minimal erythema dose (224 mJ/cm^2) induced DNA damage (CPD and 6-4PP) and inflammation markers (IL-6, IL-1β, IL-10, TNF-α) to a similar degree in both genotypes. When mice were exposed to seven repeated doses of UV radiation, time-dependent sunburn, and trans-epidermal water loss (TEWL) were observed in both wild-type and knockout mice, while the knockout mice showed diminished sunburn and TEWL. Chronic UV radiation exposure for 25 weeks induced the formation of skin tumors in both wild type and knockout mice, although the knockout mice showed slightly earlier and heavier tumor burdens but without statistical significance. Furthermore, although the knockout mice showed enhanced contact hypersensitivity (CHS) reactions to dinitrofluorobenzene, in a local immunosuppression model, UV radiation suppressed the CHS reaction in wild-type and knockout mice equally. Based on these results, we conclude that Adrb2 plays only a minor role in UV-induced skin carcinogenesis and immunosuppression.

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Eco-evolutionary aspects of UV-induced clonal dynamics during skin carcinogenesis

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The question of how the sequence of cancer development progresses – from normal tissue to carcinogen-damaged tissue to precancerous lesion and finally to malignant tumors — remains largely unanswered. Classically, these steps are attributed to the sequential acquisition of discrete genetic events such as driver mutations. However, in humans, the clonal dynamics governing cancer development happen over years, remain largely invisible even in model systems, and have been difficult to link to specific molecular changes. This rubric fails to account for clonal dynamics in the context of tissue architecture and fails to explain the consequences of large numbers of mutations present in normal tissue. Here we characterize clonal dynamics and transcriptional signatures during the skin carcinogenesis by multicolor lineage tracing. We generated a K14Cre-ERT2; Confetti mice with inducible fluorophore expression, which were then UV-irradiated for 3 months. Clones were visualized using in-vivo confocal microscopy and scRNAseq was performed comparing UV-exposed (EXP) vs. non-exposed (NON) epidermis vs. tumors. There were significantly fewer labeled clones in UV-exposed areas, but their mean sizes differed by some 15-fold, with an over 6-fold increase in variance. By all three ecological metrics (clone size, clone number, CoV) we observe phase shifts marking key transition points in clone structure. scRNAseq of EXP/NON epidermis and tumors revealed differential representation of 16 clusters. EXP clusters were associated with altered keratinocyte differentiation, increased inflammation, and upregulation of metabolic regulators. These changes were maintained during tumor formation, suggesting that they are likely to be important in carcinogenesis. Mutational analysis suggests that the emergence of large clones is not driven by mutations but rather by stochastic expansion of clones in the absence of selection. Our findings have important implications for establishing fundamental principles of carcinogenesis and cancer prevention.

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WITHDRAWN

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Genome analysis reveals UV signature mutations in sun-exposed skin tumors in tuberous sclerosis complex

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Tuberous sclerosis complex (TSC) is a tumor syndrome caused by pathogenic variants in *TSC1* or *TSC2*. Individuals with TSC develop skin tumors with second-hit somatic mutations in *TSC1* or *TSC2* in fibroblast-like tumor cells. These somatic mutations in facial angiofibromas, but not fibrous tumors in non-sun-exposed areas, are commonly UV signature mutations, prompting recommendations to limit UV exposure to reduce the severity of TSC skin tumors. In order to investigate whether UV light induces additional genetic alterations that promote TSC tumor pathogenesis, we performed whole genome sequencing of DNA from patient peripheral blood and fibroblast-like cells grown from TSC skin tumors. Unique dual-indexed PCR-free libraries were sequenced as pools on an Illumina NovaSeq 6000 generating 2x150 read pairs with a Picard mean coverage of ~38X for blood samples and ~63X for tumor samples. *TSC1* and *TSC2* germline mutations were identified in 39 out of 48 patients (5 *TSC1*, 34 *TSC2* including 12 mosaic). *TSC1* and *TSC2* somatic mutations were found in 27 out of 45 tumor samples (2 *TSC1*, 25 *TSC2*), including copy neutral LOH, point mutations, large deletions, and intra- and inter-chromosomal gene fusions. The mutation rate per Mb was higher in sun-exposed (median 5.7/Mb, IQR 4.1-11.8) than non-sun-exposed locations (median 1.7/Mb, IQR 1.5-2.0). C>T mutations and a UV signature were more frequent throughout the genome in sun-exposed than other sites, but there were no recurrent mutations in known driver genes. In summary, TSC skin tumors have relatively low somatic mutation rates that are increased in sun-exposed sites; however, somatic alteration of *TSC1* or *TSC2*, whether random or UV-induced, combined with a *TSC1/TSC2* germline mutation, appears to be sufficient to drive tumorigenesis.

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C-FOS drives reversible basal to squamous cell carcinoma transition

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While squamous transdifferentiation may arise within various subpopulations of adenocarcinomas, its' underlying mechanisms remain poorly understood. In the skin, the occasional transition of basal cell to squamous cell carcinoma upon Hedgehog-targeting therapies strongly suggests that squamous transdifferentiation is critical for resistance and thereby represents a major therapeutic issue. Here, using previously identified surface markers of resistant skin basal cell carcinomas (BCC-RM) and patient single cell and bulk transcriptomic data, we uncover the dynamic epigenetic roadmap of basal to squamous cell carcinoma transition (BST). Experimentally induced BST identifies AP1 family members in regulating tumor plasticity. In particular, c-FOS plays a central role in BST by regulating the accessibility of distinct AP-1 regulatory elements and the expression of associated target genes. Remarkably, despite prominent changes in cell morphology and BST marker expression, we show using inducible *in vitro* and *in vivo* model systems that c-FOS-mediated BST remains a reversible process. Preventing the activation of the EGFR pathway after c-FOS induction reverts BST in both mouse models and human tumors. Thus, by identifying the molecular basis of BST, our work reveals a therapeutic opportunity targeting plasticity as a mechanism of tumor resistance.

066

Role of TGF-β1 in the resistance of squamous cell carcinoma to photodynamic therapy

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Squamous cell carcinoma (SCC) can be treated by using different strategies, including photodynamic therapy (PDT). PDT is a non-invasive therapy that consists in the combination of visible light, molecular oxygen and a photoactive compound (photosensitizer) that leads to the formation of reactive oxygen species, inducing cell death. Tumoral microenvironment (TME) has gained prominence over the last few years since it has been indicated to be crucial for tumor progression as well as for the response to treatments. One of the TME components are cancer-associated fibroblasts (CAFs). CAFs are activated fibroblasts characterized by the expression of specific proteins, such as α-SMA, endoglin, CD10 and vimentin. It has been indicated that CAFs influence the response to therapies due to the secretion of different factors such as transforming growth factor β1 (TGF-β1). TGF-β1 has a dual role in the tumor progression, being considered a crucial element in skin cancer progression. In this sense, we evaluated the influence of CAFs in the efficiency of photodynamic therapy. Thus, CAFs obtained from donors with in situ SCC were characterized by western blot and indirect immunofluorescence by evaluating the proteins indicated above. Also, two established human SCC lines, A-431 and SCC-13, were used to evaluate the impact of TGF-β1 in the response to PDT. Cells were treated with exogenous TGF-β1 or with conditioned medium obtained from isolated CAFs for 48h and then exposed to PDT. After treatment, cell viability was evaluated by MTT. The results showed that TGF-β1 was capable of inducing morphological and proliferation changes in these cell lines and a resistance to the therapy was also observed. Through flow cytometry, cell cycle was evaluated, confirming that the observed changes in proliferation were due to the reversible arrest of the cycle caused by TGF-β1. Hereby, we consider that TGF-β1 constitutes a potential target for the optimization of PDT in the treatment of SCC.

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Resistance metabolic markers to PDT in squamous cell carcinoma

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Squamous Cell Carcinoma (SCC) is the second most frequent type of skin cancer among the population. Within the clinically approved treatments, photodynamic therapy (PDT) with the methyl aminolevulinate acid (MAL) is an extended non-invasive modality. Nevertheless, PDT is not always effective and resistant cells may appear after treatment. In this study, the SCC-13 human line was used as model for SCC. This cell line was called parental (P) and it was subjected to 10 PDT cycles to obtain resistant cells, which were inoculated in immunosuppressed mice; the induced tumors were sub-cultured and a cell population called 10GT was obtained. In order to determine metabolic factors responsible of PDT resistance and their cellular consequences, we analyzed differences between the P and resistant cells referring to therapy sensitivity, proliferation, spheroid formation and genomic variations through a CGH array. Interestingly, 10GT line was the most resistant to PDT; it formed larger colonies and higher number of spheroids. CGH array revealed alterations in multiple pathways, including metabolism, cell cycle and programmed cell death, among others. The expression of metabolic genes of interest such as PKM2 and β-F1-ATPase, as well as p53, Ki67, CCND1, was altered. These changes were validated by immunohistochemistry in tumors induced by inoculation of 10GT and P cells in immunosuppressed mice. In addition, we also evaluated the expression of these markers in SCC carcinomas induced in mice exposed chronically to UV and treated or not with PDT. In conclusion, we can indicate that the process of PDT resistance entails alterations in mitochondrial and glycolytic metabolic markers, particularly PKM2 and β-F1-ATPase, that could be proposed as predictor markers of MAL-PDT resistance.

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Differential expression of keratins during cutaneous squamous cell carcinoma progression

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Squamous cell carcinoma (SCC) is the second most frequent skin cancer after basal cell carcinoma. Actinic keratosis (AK) arises within the sun exposed skin, eventually develops SCC in situ, and invasive SCC. Cancer cells may change their phenotypes along with tumor progression. However, the change of keratin (KRT) expression during this process is not fully understood. Previous studies showed increased expression of KRT6 and KRT16 in SCC, though these keratins are found in proliferating keratinocytes as found in psoriasis, thus not cancer specific. We have previously performed laser capture microdissection on AK, in situ SCC, and invasive SCC tissues. Messenger RNA extracted form those tissues were subjected to genechip analysis. Various keratin expression was altered between AK, in situ SCC, and invasive SCC. KRTs 6A and 6B were the most highly expressed compared to normal epidermis, with increased expression as cancer progression. Whereas KRTs 2 and 10, known as markers for differentiated keratinocytes, were down regulated in SCCs compared to normal epidermis. Among differentially expressed KRTs, KRT13 was slightly but significantly elevated in AK (FCH in AK vs. epidermis; 4.12) and further increased in in situ and invasive SCC (FCHs 30.54 and 40.47, respectively). KRT19 was not differentially expressed in AK region (FCH; 1.17), but increased in in situ and invasive SCC (FCHs; 2.80 and 11.33 respectively). Interestingly, KRT9, positive for differentiated keratinocytes in palms and soles, were detected in AKs (FCH in AK vs. epidermis; 17.25) but decreased in in situ and invasive SCCs. Immunohistochemistry of KRTs 9 and 13 were examined in AK and SCC tissues, as well as BCC and seborrheic keratosis to evaluate the disease specificity. Our results suggested that KRT13 was expressed even earlier stage of this malignancy. In addition, KRT19 might be a novel marker of malignant transformation of epidermal keratinocytes and acquisition of invasion capacity.

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Oncogenic *JAK3* mRNA isoform in cutaneous T-cell lymphoma

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Mycosis fungoides (MF) and Sézary Syndrome (SS) are the most common cutaneous T-cell lymphomas (CTCLs). The exact carcinogenesis of CTCL is still unknown which limits our ability to prevent, detect, and treat CTCL. Janus kinase 3 (JAK3), a non-receptor tyrosine kinase, has been found constitutively activated and implicated in pathogenesis of CTCL. There are 3 splice isoforms or variants, *JAK3S*, *JAK3B*, and *JAK3M*, previously reported. The commonly described isoform or variant, *JAK3S* or *JAK3*, encodes an 1124-amino acid protein and is predominately expressed in hematopoietic cells. Recently, our transcriptome analysis by RNAseq identified a new *JAK3* mRNA isoform, variant, or in-frame fusion in SS patients. This study was to validate and determine the prevalence and oncogenic role of this new *JAK3* mRNA isoform in CTCL. Total RNA were extracted from sorted malignant T-cells from 33 SS patients. RT-PCR and Sanger sequencing were performed to confirm the existence of new *JAK3* mRNA isoform. The expression level of new *JAK3* mRNA isoform was assessed by QPCR using customized probe and primers. The Kaplan Meier survival analysis was done and correlated with the expression level of new *JAK3* mRNA isoform. As a result, a new isoform *JAK3* mRNA was confirmed in 13 of 33 SS patients by RT-PCR and Sanger sequencing. Its expression was detected in all 33 SS patients by QPCR, with a heterogeneous expression levels (range: 0.14 ~ 35.43 fold-change; mean±SD: 5.25±6.85 fold-change). In contrast, only 2 out of 7 healthy donors were positive for the new *JAK3* mRNA isoform, with a zero to low expression (0.08±0.06 fold-change, $p<0.001$). There were 19 patients with >2 fold-change gene expression while 14 patients with ≤2 fold-change. The 5-year survival of patients with higher level expression (>2 fold-change) was 42.1% in comparison with 78.6% in patients with lower expression (≤2 fold-change) (Gehan-Breslow-Wilcoxon test, $p=0.0359$). Our results suggest that the new *JAK3* mRNA isoform may have an oncogenic role in CTCL. The study of its oncogenic function in CTCL *in vitro* and *in vivo* is ongoing.

070

Comprehensive single-cell analysis of Sézary syndrome reveals novel expression and therapeutic biomarkers

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Cutaneous T cell lymphomas (CTCL) are a group of malignancies thought to derive from a clonal proliferation of skin-tropic T cells. Diagnosis is often delayed several years due to variations in presentation and lack of definitive biomarkers, which result in missed early intervention opportunities that may prevent disease progression. In the setting of more aggressive forms of CTCL, most treatments can initially decrease the tumor burden, but often ultimately fail to prevent the selective outgrowth of a resistant population. The lack of effective therapies is due in part to an incomplete understanding of tumor biology. Using single-cell mRNA and adaptive immune receptor sequencing of peripheral blood immune cells in SS, transcriptomic variations were quantified of almost 50,000 malignant and nonmalignant T cells across six SS patients. Within the population of single cells, we identified both quiescent and proliferative populations across multiple patients, and were able to newly identify a large number of genes associated with malignancy. Furthermore, in a single patient, we characterized differences in cell populations comparing malignant T cells after disease progression in the setting of treatment with histone deacetylase inhibition (HDACi) and photopheresis. HDACi therapy led to new transcriptional profiles in a subset of SS cells, characterized by the increased expression of the transcriptional factor *FOXP3*. These data are critical in defining a compendium of gene expression profiles and intratumoral heterogeneity in SS and CTCL, which are pivotal in our understanding of CTCL biology and in the development of targeted treatments.

071

RET is a therapeutic target in cutaneous squamous cell carcinoma

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Somatic rearrangements and activating germline mutations in the RET proto-oncogene are known to contribute to the development of thyroid and lung malignancies; however, its role in keratinocyte cancers has not been characterized. We identified RET as a potential therapeutic vulnerability in cutaneous squamous cell carcinoma (cSCC) through transcriptome analyses comparing human cSCC to the gene signatures of MAB21L4 and CacyBP, two protein interaction partners that we have shown are required for full induction of the terminal differentiation program in epidermis and whose loss accelerates conversion to invasive neoplasia. RET expression is increased in cSCC, consistent with published data in breast cancer as well as head and neck SCC. Disruption of the MAB21L4-CacyBP interaction reduced RET ubiquitination by CacyBP-associated Siah1, an E3 ubiquitin ligase, and subsequently stabilized RET expression. Both genetic ablation of RET and its selective inhibition by pralsetinib increased expression of differentiation markers in human skin organoids, demonstrating a role for RET in maintaining the progenitor cell state in the epidermis. Loss of RET function by CRISPR/Cas9 gene editing as well as pralsetinib treatment effectively suppressed transformation of normal human skin organoids into invasive neoplasia. Pralsetinib also reduced the invasive capacity of human cSCC cells in vitro and blocked the progression of established cSCC xenograft tumors in vivo. Antitumor activity correlated with RET inhibition and increased expression of differentiation markers. Taken together, these data identify an unexpected and important connection between the MAB21L4-CacyBP relationship and RET in cSCC development and progression. Our findings indicate that a drug repurposing strategy to target RET dependence in cSCC may be warranted.

072

Targeting field cancerisation in epidermal cancers

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Ultra-Violet (UV) radiation from sunlight is the main carcinogen driving epidermal cancers of the skin. The accumulation of UV-induced mutations on sun-exposed areas leads to field cancerisation. Knowing the regenerative property of the skin, we investigated if epidermal ablation removes mutations on the epidermis and allows less mutated cells from the deeper part of the skin to repopulate the ablated surface. Patients with history of multiple epidermal cancers were subjected to epidermal ablation on their forearms with three different depths of laser (600nm, 400nm and fractional laser). Deep targeted DNA sequencing of 151 cancer related genes had revealed the mutation burdens on ablated and non-ablated epidermis. There were 12.9 mutations per Megabase (Muts/Mb) found in non-ablated control epidermis compared to 2.55 Muts/Mb in 600nm, 4.45 Muts/Mb in 400nm and 3.58 Muts/Mb in fractional laser ablated and regenerated epidermis (n=7). To evaluate the efficacy of epidermal ablation on epidermal cancers formation, we utilised a UVB inducible Basal Cell Carcinomas (BCC) murine model (K14Cre/ER::Ptch1lox/+). These mice were subjected to a total of 20 weeks of UVB radiation. Half of the dorsal epidermis was dermabrased after the first 10 weeks of radiation. Wholemount staining of a BCC biomarker, Keratin-17 (K17), revealed a significant reduction in the number of K17 patches in dermabrased area (0.35 patches/mm^2 $\pm$ 0.04 compared to 0.07 patches/mm^2 $\pm$ 0.03) suggesting dermabrsasion can be used to reduce BCC occurrence. Overall, our findings propose a potential technique to reverse the photo-damage process by removing the mutations accumulated on the epidermis. This study may also pave the way to a larger clinical trial of epidermal ablation as an adjuvant therapy for high-risk epidermal cancer patients.

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A non-invasive genomic test for early assessment of UV damage in human skin

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UV-exposure leads to mutations in skin cells, which may clonally expand and ultimately turn into cancerous lesions ('field cancerization'). Early detection of low-frequency cancer-causing UV-signature mutations in still normal-appearing skin remains a challenge. We have developed a non-invasive genomic test capable of quantifiably detecting UV-signature mutations with high sensitivity to change this paradigm. This genomic test combines DermTech's non-invasive skin sample collection technology 'adhesive skin collection kits' with an ultrasensitive and multiplexed MALDI-TOF mass spectrometry-based mutation detection platform, 'the MassARRAY' from Agena Bioscience. The test includes over two dozen DNA driver mutations in genes such as *TP53*, *CDKN2A*, *NOTCH1*, and *NOTCH2*, known to be associated with the development of non-melanoma skin cancers. In a proof-of-concept study, contralaterally collected skin samples from sun-exposed body sites in subjects of varying race, age, and gender were tested. UV-signature mutations were detected in sun-exposed skin, with the numbers of mutations increasing with the subjects' age. In our reference control neonatal keratinocytes, no UV signature mutations were detected. This genomic test demonstrates reliable detection of UV-signature mutations in sun-exposed skin samples collected using DermTech's non-invasive skin sample collection technology and offers the opportunity to non-invasively assess UV-damage of skin that lacks visual elements of UV damage. Additional studies will help understand the correlation of UV-damage detected in normal-appearing skin to future non-melanoma skin cancer risk. We envision this proactive genomic approach to measure UV damage to change how photoaging is assessed and treated.

074

Cutaneous T-cell lymphoma and canine epitheliotropic lymphoma: A comparative analysis

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Cutaneous T-cell lymphoma (CTCL) is a rare type of skin cancer involving T lymphocytes in the skin. Canine epitheliotropic lymphoma (EL) is a spontaneous cutaneous lymphoma in dogs also arising from the T lymphocytes in the skin and mucosa. Many studies have identified immune genes, pathways and cells that drive the pathogenesis of CTCL, including interleukins, chemokines, cell cycle control/oncogenes, and other leukocytes. Data suggest that similar processes are involved in the pathogenesis of EL in canines. Here, we present case studies of 6 canines with EL which occurred spontaneously in client-owned companion dogs. We performed comparative transcriptomics studies on 160 genes from lesional skin biopsies from these cases and from cases of 5 healthy canines, in order to identify any significant differences that may reflect oncogenesis and immunopathogenesis. We further sought to determine if the oncogenic processes of EL and CTCL are conserved across humans and canines by comparing our Nanostring data to previously published datasets. Similar chemokine profiles were observed in dog EL and human CTCL, and we are performing ongoing analyses to validate potential biomarkers and drivers of disease. Future studies exploring the oncogenesis of spontaneous malignancies in companion animals will expand our understanding of these disorders, and will be useful in developing targeted therapies, repurposing drugs for veterinary and human medicine, and predicting disease prognosis and treatment response.

075

PTCH1 mutations in high-frequency basal cell carcinoma patients without Gorlin stigmata

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Gorlin syndrome is an autosomal dominant disorder characterized by tumor preponderance and developmental defects. Diagnosis is traditionally made based on clinical criteria, including the presence of major features, such as multiple basal cell carcinomas (BCCs), palmar and plantar pits, jaw keratocysts, and falcine calcification, as well as minor features, including skeletal and radiologic abnormalities. Gorlin syndrome is predominantly caused by mutations in patched 1 *(PTCH1)*, a tumor suppressor gene in the hedgehog signaling pathway. Although penetrance appears to be complete in Gorlin syndrome, there is variable expressivity, and monozygotic twins with the same mutation have been documented to demonstrate varying clinical features. In this study, we perform germline exome sequencing in 73 individuals with high-frequency BCCs, defined as 6 or more over a 10-year period, to characterize rare germline variants that contribute to the development of high-frequency BCCs. We identify 3 subjects who harbored frameshifts or deletions in PTCH1, predicted to result in premature truncation of the PTCH1 protein. Notably, all 3 patients lack a family history suggestive of Gorlin syndrome and lacked any other clinical skin features associated with Gorlin syndrome. Currently, genetic testing for PTCH1 is suggested only as diagnostic confirmation in patients lacking sufficient clinical criteria or as testing in known familial cases. Our data suggest that this approach may miss a number of individuals with Gorlin syndrome. A genotype-first approach may help clarify the true prevalence of Gorlin syndrome in the population. In addition, the advent of genetic sequencing may facilitate a necessary expansion beyond clinical criteria in the diagnosis of Gorlin syndrome.

076

Subtype specific analyses reveal infiltrative basal cell carcinoma are highly interactive with their environment

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Little is known regarding the molecular differences between BCC subtypes, despite clearly distinct phenotypes and clinical outcomes. In particular, infiltrative BCCs have poorer clinical outcomes in terms of response to therapy and propensity for dissemination. In this project we aimed to use exome sequencing and RNA sequencing to identify somatic mutations and molecular pathways leading to infiltrative BCCs. Using whole exome sequencing of 36 BCC samples (8 infiltrative) combined with previously reported exome data (58 samples), we determine that infiltrative BCC do not contain a distinct somatic variant profile and carry classical UV induced mutational signatures. RNA sequencing on both datasets revealed key differentially expressed genes such as POSTN and WISP1 suggesting increased integrin and Wnt signalling. Immunostaining for POSTN and WISP1 clearly distinguished infiltrative BCCs and nuclear beta-catenin staining patterns further validated the resulting increase in Wnt signalling in infiltrative BCCs. Of significant interest, in BCCs with mixed morphology, infiltrative areas expressed WISP1 while nodular areas did not, supporting a continuum between subtypes. In conclusion, infiltrative BCCs do not differ in their genomic alteration in terms of initiating mutations. They display a specific type of interaction with the extracellular matrix environment regulating Wnt signalling.

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Isoform-specific aPKC maintains Hedgehog signaling in the absence of primary cilia

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Primary cilia loss is a common feature of advanced cancers. While primary cilia are necessary to initiate Hedgehog (HH)-driven cancers, how HH pathway activity is maintained in advanced cancers devoid of primary cilia is unclear. Here, we find that HH-driven basal cell carcinoma (BCC) and medulloblastoma accumulate mutations in the Alström and Usher syndrome genes. Loss of Alström and Usher syndrome gene expression, which are common underlying causes of deafness and blindness, suppresses ciliogenesis and HH signaling. Loss of primary cilia also enhances atypical protein kinase C iota/lambda (aPKC) expression, a GLI1 kinase necessary for advanced BCC growth. We show that aPKC expression is inversely correlated with primary ciliogenesis and that superficial BCCs display less primary cilia and higher aPKC expression, with the opposite true in nodular BCC subtypes. Surprisingly, a constitutively active isoform of aPKC drives HH pathway activity but not full-length protein. Overexpression of the constitutively active aPKC variant can maintain HH pathway activity in the absence of primary cilia and can drive resistance to the SMO antagonist vismodegib regardless of cilia status. Our results suggest tumors enhance isoform-specific expression of aPKC to prevent mutation-induced cessation of tumor growth and that aPKC may serve as a biomarker for SMO inhibitor sensitivity.

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Drug targeting and comparative transcriptomics of alpha-beta subcutaneous panniculitis-like T-cell lymphoma and primary cutaneous gamma delta T-cell lymphoma

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Background: Subcutaneous panniculitis-like T-cell lymphoma (abSPTCL) exhibits an alpha/beta phenotype and is often indolent. By comparison, primary cutaneous gamma-delta T-cell lymphoma (PCgdTCL) is highly aggressive. This study identified key inflammatory and oncogenic pathways in abSPTCL and PCgdTCL to determine mutational or expression patterns that may contribute to disease formation, as well as identified potential drug targets. Methods: We performed transcriptomic profiling using RNA sequencing of lesional skin biopsies from patients with abSPTCL (n=10), PCgdTCL (n=9), and controls (n=5). Differential analysis, enrichment pathway analysis, and relational drug database query were performed on the RNA-seq data. Results: The top significantly upregulated gene pathways in both abSPTCL and PCgdTCL were those involving immune and inflammatory response, viral defense, type I interferon signaling, interferon gamma-mediated signaling, and positive regulation of T cell proliferation. In 286 unique genes involved in the top 8 enriched immune response pathways in abSPTCL, there were 79 genes with >50 interactions with other immune genes. Using these 286 genes as input to query the Target Central Resource Database (TRCD) database, 59 genes were matched to 170 (132 unique) FDA-approved drugs. Of the 284 unique genes in the top 8 immune pathways in PCgdTCL, 81 had >50 interactions with other genes within the group. Similarly, querying the TRCD database, 45 genes were matched to 85 (56 unique) FDA-approved drugs. Conclusion: Our transcriptomic profiling pinpoints disease-related enrichment pathways and gene functions in abSPTCL and PCgdTCL, which may reveal molecular drivers of disease activity. Hub genes and the matched drugs represent potential mechanistic targets for future development of novel therapies.

079

Somatic mutation of the OXA1L 5′UTR enables cutaneous squamous cell carcinoma

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Cancer-enabling mutations that alter the amino acid sequences of protein-coding genes have been extensively studied; however, the role of non-coding mutations in tumor development has not been fully characterized. We performed whole exome sequencing that included the untranslated regions (UTRs) in 37 high-risk cutaneous squamous cell carcinomas (cSCC), including 17 patient-matched metastases and normal skin as control. Two adjacent bases in the 5′UTR of OXA1L, which encodes a mitochondrial translocase, were somatically mutated in 27% (10 of 37) of cSCC and also detected in other ultraviolet radiation-associated skin cancers. OXA1L is decreased in cSCC, and the most common cancer-associated OXA1L UTR mutation reduced gene expression in reporter assays. Human cSCC harboring this somatic variant expressed lower levels of the OXA1L transcript, providing further support that OXA1L expression is affected by this single base-pair substitution in its 5′UTR. Enforced expression of OXA1L in primary human keratinocytes transduced with oncogenic Ras attenuated tumor growth in vivo and base-editing normal keratinocytes to cancer-associated mutant OXA1L using a hybrid adeno-associated virus-recombinant Cas9/sgRNA approach accelerated neoplastic invasion in human skin organoids. Reversion of the OXA1L UTR mutation back to wild type in melanoma cells also effectively suppressed tumorigenesis in vivo. To define how the mutant OXA1L UTR enables tumorigenesis, we performed proteomic profiling in wild type and edited human keratinocytes. In parallel, we also analyzed the transcriptomes of neoplastic human skin organoids comprised of keratinocytes with wild type or mutant OXA1L UTRs. The resulting data demonstrate that mutagenesis of the OXA1L UTR results in upregulation of genes involved in glycolysis and downregulation of oxidative phosphorylation genes. These findings identify a previously unrecognized role for OXA1L in cSCC development and highlight how mutations arising in the non-coding genome can contribute to tumorigenesis.

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A novel role for keratin 17 during DNA damage response and tumor initiation

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High levels of the intermediate filament protein keratin 17 (K17) are associated with poor prognoses for several human carcinomas. Studies in mouse models have shown that K17 expression is positively associated with growth, survival, and inflammation in skin, and that lack of K17 delays onset of tumorigenesis. K17 occurs in the nucleus of human and mouse tumor keratinocytes, where it impacts chromatin architecture, gene expression, and cell proliferation. We report here that K17 is induced following DNA damage and promotes keratinocyte survival. Presence of nuclear K17 is required at an early stage of the double-stranded break (DSB) arm of the DNA damage and repair (DDR) cascade, consistent with its ability to physically interact with key DDR effectors including γ-H2AX, 53BP1 and DNA-PKcs. Mice lacking K17 or with attenuated K17 nuclear import showed curtailed initiation in a two-step skin carcinogenesis paradigm. The newly found impact of nuclear-localized K17 on DDR and cell survival provides a basis for the link between K17 induction and poor clinical outcomes for several human carcinomas.

081

Ultraviolet light-induced collagen degradation inhibits melanoma invasion

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Ultraviolet radiation (UVR) increases the incidence of cutaneous melanoma. The ageing, sun-exposed dermis accumulates UVR damage, and older patients develop more melanomas at UVR-exposed sites. As fibroblasts play key roles in the stromal response to UVR and in cancer progression, we investigated how long term UVR modifies dermal fibroblast function and how this affects melanoma invasion. Chronic UVR exposure on dermal fibroblasts showed that extracellular matrix pathways, particularly those involved in collagen catabolism, were upregulated in the absence of acute UVR. Importantly, the expression of collagen-cleaving matrix metalloprotein-1 (*MMP1*) was persistently upregulated. This resulted in persistent degradation of collagen 1, and an overall degraded and disorganised matrix. Collagen degradation by MMP1 decreased melanoma invasion *in vitro*. Conversely, both inhibiting extracellular matrix degradation and MMP1, or higher collagen 1 expression, restored the invasion of melanoma through collagen. Primary cutaneous melanomas of aged humans confirmed these *in vitro* findings, revealing significantly fewer cancer cells invade as single cells at the invasive front of melanomas arising in chronic sun damaged skin. We show high collagen deposition and melanoma cell invasion in the dermis are robust predictors of poor melanoma-specific survival in 3, international cohorts of primary melanoma. Thus, melanomas arising over UVR-damaged, collagen-poor skin are less invasive, and this reduced invasion improves survival. However, we discovered a subset of melanomas arising over collagen-poor, UVR-damaged dermis have a poor outcome, and found that increased new collagen synthesis by melanoma-associated fibroblasts at the invasive front in these cases restores melanoma single cell invasion and drives poor outcome. Finally, we demonstrate high *COL1A1* gene expression is an early stage biomarker of poor outcome across a broad range of primary cancers.

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The immune microenvironment of cutaneous squamous cell carcinoma *in situ* contains suppressive phenotypes

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Squamous cell carcinoma in situ (SCCIS) is a common precancerous lesion that may progress to squamous cell carcinoma (SCC). The tumor immune microenvironment (IME) regulates cancer progression by promoting immunosuppression associated with suppressive tumor associated macrophages (TAMs) and myeloid derived suppressor cells (MDSCs). Changes in IME in early carcinogenesis are not clear but key for tumor immune evasion. Our RNA seq studies showed that the largest group of differentially expressed genes in SCCIS are immunoregulatory that likely promote a pro-carcinogenic IME. To determine if the IME of SCCIS differs from that of adjacent skin, we utilized image mass cytometry (IMC). SCCIS tissue sections containing epidermis were stained with metal conjugated antibodies to detect key immune cell populations and processed using the Hyperion Imaging System. Images were segmented using a nuclear app-based algorithm in Visiopharm and imported into histoCAT where single cell mean pixel intensity data were obtained to cluster cells using the Phenograph algorithm based on cell markers and predefined epidermal nuclei. Statistical analysis with using the Mann-Whitney test identified nine immune cell populations in SCCIS: CD14+ MDSCs, keratinocytes, CD204+ TAMs, CD4+ T cells, CD8+ T cells, myeloid dendritic cells (mDCs), Tregs, endothelial, and plasmacytoid dendritic cells (pDCs). SCCIS had more suppressive immune cells such as CD14+ MDSCs, CD204+ TAMs, and Tregs ($p<0.05$) as well as CD4+ T and mDCs ($p<0.05$, $p<0.01$) with no differences in CD8+ T and pDCs. Increased intraepithelial CD204+ TAMs were found in SCCIS ($p<0.01$). Neighborhood analysis in SCCIS reveals interactions between CD204+ TAMs-keratinocytes, CD204+ TAMs-Tregs, and mDCs-Tregs with avoidance in adjacent normal skin between CD204+ TAMs-keratinocytes ($p<0.05$). CD8+ T cells avoided keratinocytes in SCCIS ($p<0.05$) compared to insignificant interactions in normal skin. These data indicate increased suppressive immu

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Chemically-induced cutaneous neoplasms spontaneously regress in mice lacking autoimmune regulator

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Cutaneous squamous cell carcinomas (cSCCs) frequently arise from precancerous lesions known as actinic keratoses (AKs). Factors that determine whether an AK will progress to a cSCC, remain stable, or regress to normal tissue are poorly defined. In AK and cSCC mouse models upregulation of autoimmune regulator (*Aire*) is associated with skin inflammation and tumor onset and the genetic lack of *Aire* attenuates the early stages of skin tumorigenesis. Here, to better assess the role of Aire in the onset and promotion of AK- and cSCC-like lesions, we subjected germline Aire null mice ($Aire^{-/-}$; FVB/NJ or C57BL/6J backgrounds) to a classic two-step chemical carcinogenesis protocol [1x 7,12-dimethylbenz[a]anthracene (DMBA), 2x/wk 12-O-Tetradecanoylphorbol-13-acetate (TPA)], which resulted in papilloma formation in 92% of all mice (n=36/39). Aire deficiency resulted in reduced papilloma burden and smaller sized papillomas compared to $Aire^{+/+}$ control mice. Unexpectedly, we observed that all papillomas that formed in 12 out of 22 $Aire^{-/-}$ mice rapidly and synchronously regressed despite continued tumor promotion with TPA. Histopathology indicated that regressed papilloma tissue from $Aire^{-/-}$ mice more closely resembled normal mouse skin architecture than non-regressed papilloma tissue from $Aire^{-/-}$ mice or from $Aire^{+/+}$ papilloma tissue. After 15 weeks of TPA treatment, CD8+ cells consistent with cytotoxic T cell infiltrate were observed in the stroma surrounding the hyperproliferative epithelium in non-regressed papilloma tissue from $Aire^{-/-}$ mice or $Aire^{+/+}$ mice. However, these cells were markedly absent from regressed papilloma tissue in $Aire^{-/-}$ mice, suggesting that an absence of functional Aire may improve the anti-tumoral immune response during the early stages of skin tumorigenesis. Altogether, this study indicates that Aire contributes in multiple ways to the development of skin neoplasms (onset, promotion, and regression) and suggests that the targeting of Aire function may provide a therapeutic benefit to patients at risk for skin cancers.

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A case of primary subcutaneous leiomyosarcoma of the lower extremity

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Subcutaneous leiomyosarcomas (LMS) are a rare smooth muscle neoplasm representing only 2-3% of all superficial soft tissue sarcomas. The purpose of this study was to provide a clinical case that focuses on proper management and follow-up care due to the high rate of recurrence. A middle-aged Caucasian man with past medical history of chronic obstructive pulmonary disease was referred for a suspicious skin lesion measuring 1.4 cm on his right anteriolateral thigh due to increasing pain. The patient denied medications except 325mg of acetaminophen. The patient denied any associated systemic findings. In social history, patient reports an 88-pack year of smoking. The area around the lesion was anesthesized with 1ml of 2% xylocaine with epinephrine and a subsequent 8 mm shave biopsy of the lesion was taken by an electrodessication and curettage (ED&C) technique. The section showed a biopsy extending from epidermis to deep dermis. The section showed highly atypical spindle cells with pleomorphic nuclei and mitotic activity. The tumor cells were arranged in long and short fascicles. Positive staining was present for smooth muscle actin, vimentin, and desmin. Histology in conjunction with immunostaining supported a grade I LMS tumor. Right femur MRI with contrast showed enhancing skin lesion in the anterolateral distal thigh extending into the subcutaneous soft tissues with skin thickening measuring 2.6 x 1.4 x 3.0 cm. CT imaging studies of the chest, abdomen, and pelvis showed no evidence of metastatic disease. Patient underwent wide local excision (WLE) of the right lower extremity under general anesthesia with a circumscribed mass obtained. Pathology confirmed 1cm negative margins. At one-week follow up, the incision appeared to be healing well and the patient was scheduled for 3 month follow up. Due to a recurrence rate of 8-40% in subcutaneous LMS, close surveillance is recommended at 3-6 month intervals for the first 2 years as well as long-term care thereafter. As the strongest predictor of recurrence is adequacy of margins, we recommend 1cm margins for adequate outcomes.

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CCN1-induced age-related dermal microenvironment promotes skin cancer development

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Aging is a major risk factor for keratinocyte skin cancer. Here we investigate the impact of Age-Related alterations in the Dermal Microenvironment (ARDM) on keratinocyte skin cancer initiation/development, using a transgenic mouse model of accelerated dermal aging. This model expresses CCN1, a secreted extracellular matrix (ECM) associated protein, driven by the fibroblast collagen1A2 promoter. CCN1 is significantly elevated in dermal fibroblasts in aged human skin and is a key driver of ARDM. By six months of age, *Col1a2-CCN1* mice exhibited accelerated dermal aging including dermal thinning (reduced 39%, N=6, $p<0.05$), loss of collagen protein content and gene expression (reduced 51% and 63%, respectively, N=5, $p<0.05$), and increased degradation of collagen fibrils, measured by immunostaining and atomic force microscopy, compared to control littermates. Furthermore, *Col1a2-CCN1* mice exhibited increased expression of multiple matrix metalloproteinases, hepatocyte growth factor, and cytokines and reduced expression of TGF-β type II receptor (N=5, $p<0.05$), as observed in aged human skin. Importantly, six-month-old *Col1a2-CCN1* mice displayed dramatically increased susceptibility to skin papilloma/tumor formation in two different skin tumor models; two-stage chemical carcinogenesis (N=5) and keratinocyte-targeted inducible oncogenic HRas (N=4-5, $p<0.05$). In both of these models, multiple papillomas/tumors occurred only in *Col1a2-CCN1* mice. No papillomas/tumors were formed in littermate controls. In stark contrast, two-month-old *Col1a2-CCN1* mice, before the development of ARDM, were completely resistant to papilloma/tumor formation, similar to controls. These data demonstrate that ARDM creates a tissue milieu that actively promotes keratinocyte cancer. *Col1a2-CCN1* mice provide a powerful model for investigating the mechanisms by which age-related changes in the stromal microenvironment contribute to cutaneous carcinoma development in the elderly.

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Identification of novel basal cell carcinoma susceptibility loci in a multiethnic cohort

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Basal cell carcinoma (BCC) is one of the most common malignancies worldwide and has a moderate genetic component with an array-heritability estimate of 0.17. Previously published genome-wide association studies (GWAS) have reported 33 loci associated with BCC, explaining 10.98% of BCC heritability, suggesting that additional loci remain to be discovered. Most published studies have exclusively utilized European descent populations, and the impact of genetic factors on BCC risk in a multiethnic population has not been explored. We conducted a multiethnic GWAS of BCC in the large and ethnically diverse Kaiser Permanente Genetic Epidemiology Research on Adult Health and Aging (GERA) cohort consisting of 21,247 BCC cases and 76,681 controls from four race/ethnicity groups (non-Hispanic whites, Hispanic/Latinos, East Asians, and African-Americans). Cases were identified from electronic pathology records using a validated SNOMED code-based algorithm. We conducted a logistic regression of BCC and each SNP using adjusting for age, sex, and ancestry principal components in each race/ethnicity group, followed by a meta-analysis combining the results across the four race/ethnicity groups. Our multiethnic GWAS meta-analysis identified 38 genome-wide significant ($P<5.0\times10^{-8}$) BCC-associated loci, of which 12 were novel. Most of them replicated in an external independent sample. Identified novel loci are implicated in the adaptive response to hypoxia pathway (*ARNT*), immune regulation (*CLTA4*), lipid metabolism (*CYP1B1*), tissue remodeling and tumor invasion (*MMP24)* or elastic fiber formation and connective tissue development (*EFEMP2*). Interrogation of loci uncovered by our GWAS using functional annotations integrative tools also prioritized biological pathways underlying BCC. Study findings provide new insight into the genetic basis of BCC susceptibility and may help identify individuals at higher BCC risk.

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Expression of opsin 3 in skin tissues and hemangioma vessels and may be involved in vascular development

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Background: OPN3 is a non-visual opsin, it also belongs to the GPCRs Superfamily. Previous studies have found that OPN3 is expressed in a variety of peripheral tissues, including placenta, retina, liver, heart, lung, skeletal muscle, pancreas, etc., and regulating a variety of cellular physiological functions. OPN3 not only regulates the change of skin pigment and cell senescence, but also affects the proliferation, apoptosis and autophagy of liver cancer, colon cancer and melanocyte, etc. However, it is not clear whether OPN3 can participate in the regulation of angiogenesis. Objective: To explore whether OPN3 can regulate angiogenesis. Methods: Immunofluorescence was used to observe the expression of several opsins in normal skin tissues and hemangioma vessels, including OPN1,OPN2,OPN3,OPN4 and OPN5, but the expression of OPN3 is the most enriched. Then we also demonstrated that OPN3 was also highly expressed in vascular endothelial cells through real-time fluorescence quantitative PCR, Western blot and immunofluorescence localization. SiRNA mediated opsin3 gene expression silencing was then assess opsin3 knockdown and changes in endothelial cell proliferation, migration, and angiogenesis related protein expression and function. Results: Immunofluorescence, real-time fluorescence quantitative PCR and Western blot all showed that Opsin3 was highly expressed in hemangioma, blood vessels of skin tissue and endothelial cells. We used siRNA mediated OPN3 silencing to demonstrate its ability to regulate endothelial cell proliferation and migration through its interaction with VEGFR-2. Conclusion: Our study is the first to demonstrate that OPN3 may play a role in endothelial cell angiogenesis by inhibiting and down-regulating VEGFR2 expression.

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Down-regulation of OPN3 expression affects the adhesion and migration of human melanocytes

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Background: Opsin3 (OPN3) is a transmembrane heptahelical G protein−coupled receptor (GPCR) with the potential to produce a nonvisual photoreceptive effect. Interestingly, some studies have shown that Opn3 mRNA is highly expressed in human skin tissues. Recent studies have focused on the role of OPN3 in human melanocytes. The function of OPN3 in melanocytes is not fully understood. Objective: To explore the regulation of OPN3 on the adhesion and migration of melanocytes. Method: Primary human epidermal melanocytes (HEM) from neonatal foreskin were cultured in 254 medium containing human melanocyte growth supplement (HMGS2, Cascade Biologics/Invitrogen). The protein and mRNA expression of OPN3 were detected by Western blot analysis and real-time fluorescent quantitative PCR (qRT-PCR), respectively. Knockdown of OPN3 in NHMs was performed using siRNA technology according to the manufacturer's protocol. Three pooled siRNA oligos targeting OPN3 or negative control siRNAs were purchased from ViewSolid Biotech (Beijing, China). 48 hours after transfection, the silencing efficiency of the siRNA-OPN3 sequence was analyzed by quantitative RT-PCR compared with a negative control siRNA that did not target any known genes. Transwell cell invasion test and scratch test were used to analyze cell migration and adhesion capabilities. Simultaneously, Western blotting was used to detect the expression levels of adhesion and migration related proteins ICAM-1 and E-cadherin. Results: Both real-time fluorescent quantitative PCR and Western blotting showed that Opsin3 is highly expressed in skin melanocytes. Compared with the control group, in melanocytes transfected with OPN3 siRNA, cell adhesion and migration, as well as the levels of ICAM-1 protein and E-cadherin were significantly reduced ($P<0.05$). Conclusions: Down-regulation of OPN3 expression may affect the adhesion and migration ability of melanocytes.

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The mevalonate pathway enzyme HMGCS1 upregulation in keratinocytes contributes to psoriasis by promoting IL-23 expression

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Psoriasis is an autoinflammatory disease characterized by the excessive proliferation of keratinocytes (KC), requiring a large amount of cholesterol to form cell membranes. 3-hydroxy-3-methylglutaryl coenzyme A (HMGCS1) is a catalytic enzyme for the first step of cholesterol synthesis and the mevalonate pathway. Using R language to analyze two Gene Expression Omnibus(GEO) data sets, we discovered a more abundance of HMGCS1 in psoriatic lesions and confirmed this in psoriatic patients and psoriatic animal models by immunohistochemistry. In TNF-α stimulated psoriatic cell model, HMGCS1 and some cytokines like IL-23, IL1-β, IL-8 overexpressed. After knocking down HMGCS1, the migration speed of Hacat cells decreased, the cell cycle altered, and the percentage of apoptotic cells increased. We also found that suppressing HMGCS1 can downregulate the expression of IL-23 and the phosphorylation level of signal transducer and activator of transcription 3 (STAT3). When inhibiting the phosphorylation of STAT3, the expression of IL-23 decreased. Interestingly, the mRNA level of HMGCS1 significantly correlated with STAT3 in the GEO datasets mentioned above. The expression of HMGCS1 reduced in STAT3 small interfering RNA-treated Hacat cell. In conclusion, TNF-α regulates the expression of HMGCS1, which leads to an increase of STAT3 phosphorylation and the production of more IL-23. STAT3 might be the transcription factor of HMGCS1. Taken together, we provide evidence suggesting that the mevalonate pathway enzyme HMGCS1 upregulation in keratinocytes contributes to psoriasis by promoting IL-23 expression.

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White pine bark extract modulates cell-to-cell communication pathway for a lighten skin and age spot benefit

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In skin, pigmentation is mainly due to melanin content, produced by melanocytes through the mechanism called melanogenesis. However, many external factors modulate melanin production and impact pigmentation mechanism, leading to the increase of esthetical disorders as hyperpigmentation or age spots. Until now, the melanocytes were the main target to modulate the pigmentation, but new studies have demonstrated the importance of internal communication factors in melanogenesis. Some of them, produced by surrounded keratinocytes improve the expression of gene encoding for melanocytes-specific proteins involved in melanogenesis. The crucial role of keratinocytes is among other things underlined by their abundance into the skin compare to melanocytes cells. By reducing cells cross talk, a white pine bark extract has shown beneficial effects to modulate skin clarity and age spot appearance on Asian women. *In vitro*, 0.2% of extract on NHEK culture decreased melanogenic cytokines, by 67% for EDN-1 and 56% for SCF ($p<0.001$), as well as carbonylated proteins, by 51%. On innovative *ex vivo* studies on pigmented and non-pigmented RHE white pine extract inhibited melanin production, by 4 and 7% ($p<0.005$), showing direct effect (topic application) and indirect effect (supernatant application) through the crosstalk between melanocytes and keratinocytes cells, respectively. In a 28-day double-blind placebo-controlled clinical study on Asian women with pigmentary spots on face, 1% of extract in cream modulated skin complexion parameters, as brightening and luminosity ($p<0.05$). Extracts also modulated these parameters on age spots as well as spot sizes($p<0.05$). These findings demonstrate the extract ability to modulate crosstalk between keratinocytes and melanocytes cells through the decreases of melanogenic cytokines synthesis. The extract has a high capacity to illuminate and even out skin tone, improves skin clarity and reduces the appearance of dark spots.

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MARCH family E3 ubiquitin ligases selectively target cadherin family proteins for degradation

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Cadherins play a central role in the assembly and adhesive function of adherens junctions (AJ). Adhesion and signaling functions of cadherins regulate epidermal and vascular barrier function, development, and tumor metastasis. Our group previously showed that a viral ubiquitin ligase K5, which is encoded in Human Herpesvirus 8 genome and involved in Kaposi Sarcoma, causes endocytosis and degradation of VE-cadherin (VE-cad). K5 is homologous to endogenous human membrane associated RING-CH-type ubiquitin ligase (MARCH) proteins. However, the contribution of endogenous MARCH family proteins to cadherin regulation is unclear. To assess the effect of MARCH family protein expression on epithelial and endothelial cadherins, MARCH proteins were expressed in epithelial and endothelial cells and the effect on E-cadherin (E-cad), VE-cad, and N-cadherin (N-cad) was monitored by western blot and immunofluorescence microscopy. MARCH1 had no discernable impact on E-cad, VE-cad or N-cad. MARCH8 downregulated E-cad and slightly downregulated VE-cad but not N-cad. In contrast, MARCH2, MARCH3 and MARCH4 transfection markedly downregulated VE-cad. Interestingly, MARCH2 and MARCH3 did not downregulate E-cad and N-cad, but MARCH4 downregulated E-cad, VE-cad and N-cad. These results revealed that different MARCH proteins have different cadherin specificities. To determine the amino-acid sequences within VE-cad that dictate this specificity, we generated VE-cad/N-cad chimeras by swapping the cadherin transmembrane and juxta-membrane domains (VE-cad-NcadTMDJMD, N-cad-VEcadTMDJMD). Interestingly, MARCH2 downregulated N-cad-VEcadTMDJMD but not VE-cad-NcadTMDJMD. These results indicate that MARCH proteins exhibit selectivity for different cadherins and that the amino acid sequence within the transmembrane and juxta-membrane domains is essential for this selectivity. These findings suggest that MARCH proteins may differentially regulate cadherins in epithelial and endothelial cells to modulate cell migration and barrier function during development and inflammation.

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Reciprocal control of epidermis and sensory neurons in juvenile and adult human skin in a re-innervated epidermis model

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Skin is in constant evolution from the embryo state, reciprocal control between epidermis and neurons in the different ages of life is little known. Our objective was to characterize epidermal and neuronal interaction in juvenile or adult using a new model of re-innervated epidermis. Human sensory neurons were obtained from iPS cells, their differentiation and functionality were validated by observation of neuronal morphology, neuronal markers expression, and increase of intracellular calcium following lactic acid (LA) stimulation. Human sensory neurons were cultured in presence of epidermis obtained from juvenile and adult donors. After 3 or 6 days of co-culture, the reinnervation of the epidermis was confirmed by PGP9.5 immunostaining revealing the presence of nerve fibers in the epidermis. The reinnervated epidermis model was able to response to a neuronal stimulation, induced by LA, by increasing substance P production. Further investigations were performed to characterize innervated epidermis from juvenile or adult donors. Those possess different homeostasis traits with or without innervation, as shown by TUNEL, KI67 and p63 staining: non-innervated condition or longer culture time induced a non-significant increase of apoptotic cells, innervated juvenile epidermis showed a decrease of proliferative cells compared to adults, innervation non-significantly increased stem cells number. Gene expression of barrier function markers by qPCR highlighted a distinct transcriptomic profile as most of the barrier markers were more expressed in juvenile epidermis. We developed a reinnervated epidermis model and demonstrated that innervation and age of epidermis induce different epidermal characteristics. In addition to our previous work showing that juvenile keratinocytes possess trophic properties on sensory neurons, we suggest that reciprocal interactions between skin cells and sensory neurons could control epidermal homeostasis.

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Dissecting intercellular communication in adult human skin with single-cell and spatial transcriptomics

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Human skin contains distinct spatial compartments that cooperate in functional barrier maintenance, including the interfollicular epidermis (IFE), dermis, and the pilosebaceous unit (PSU). Yet, cellular communication within and among these compartments is largely unexplored. To address this, we performed single-cell RNA-sequencing (scRNA-seq) of 10 adult patient hair-bearing normal skin tissues along with spatial transcriptomics on a subset of these. We obtained ~21,000 single-cell transcriptomes and ~13,000 spot transcriptomes and integrated these data with previously published scRNA-seq from human and mouse skin to deconvolve cell types and subpopulations. Cycling cell analysis suggested spinous keratinocytes (KCs) maintain proliferative capacity prior to terminal differentiation, in contrast to mouse IFE. Spinous KCs with proliferative potential displayed a transcriptional signature indicative of heavy metal processing, suggesting one pathway responsible for increased stratification of human skin. Ligand-receptor analyses mapped intercellular communication involved in epidermal homeostasis, including known mediators such as DLL1-NOTCH1 and AREG-EGFR and lesser studied pairs such as APOE-LDLR and EFNB1-EPHB6. To shed light on potential etiologies of dermatologic genetic disease, we identified cell type-specific expression of mutated genes, implicating specific cell types and their communication networks in pathogenesis. In inflammatory skin diseases with putative multicellular communication, such as hidradenitis suppurativa and generalized pustular psoriasis, we further nominate therapeutics to reverse these phenotypes, leveraging our crosstalk analyses and drug perturbation databases. This work provides a resource for advancing the study of human skin homeostasis and disease.

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Cytokine and chemokine factors regulate HIV-1 *trans*-infection from dendritic cells to CD4+ T-cells

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Dendritic cells (DC) is one of the earliest immune cells to encounter HIV-1 during acute infection at mucosal surfaces. They act as a critical interface between the innate and adaptive immune systems through mechanisms of antigen-presentation. HIV-1 subverts the immune system by hijacking DCs, impairing cellular function to promote survival and propagation by trans-infection to target CD4 T-cells. 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 highly efficient viral dissemination and accelerated disease progression almost exclusively driven by cell-to-cell transmission. Our previous findings have shown 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 our model of cell-cell HIV-1 transfer from primary monocyte-derived DCs (MDDC) to CD4 T-cells, we used high-throughput siRNA screening against known cytokines, chemokines, cytokine and chemokine receptors. Using the Human ON-TARGET plus SMARTpool cytokine & chemokine siRNA library, we screened 319 individual genes and investigated their differential effects on HIV-1 transfer from monocyte-derived DCs (MDDC) to a CD4+ T cells. Our data identified several cytokines restricting against HIV-1 cell-to-cell transfer in DC to T cell assays. These candidates were further analyzed using loss-of-function assays, genetic downregulation and neutralisation assays. Disruption of specific cytokine-driven mechanisms in MDDC results in dramatic changes in the capacity for cell-to-cell transfer to CD4+ T-cells. Taken altogether, these data continue to support DCs as a therapeutic target during early-stage HIV-1 infection linking the cytokine network and informing potential novel therapeutic strategies against early-stage HIV-1 infection and transmission.

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Bioengineering a complex skin equivalent for skin care applications

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Bioengineered skin constructs provide a physiologically relevant platform for fundamental and translational research. We have developed a complex, robust and reproducible full thickness skin equivalent, which has been validated against *in vivo* skin using in-depth analyses. To mimic the dermis *in vitro*, primary fibroblasts were cultured within the Alvetex® Scaffold to produce endogenous extracellular matrix proteins. The robust dermal compartment supports the generation of an organised, stratified and keratinised epidermis. Additional cell types have also been incorporated, such as melanocytes and immune cells, to increase the skin equivalent complexity. The human skin equivalent recapitulates the organised structure of human skin *in vitro*. Skin constructs that represent different ages have been developed using neonatal, young or ageing cells. The use of ageing and senescent cells recreates the ageing skin phenotype *in vitro*, with regards to epidermal thinning, decreased keratinocyte proliferation and reduced extracellular matrix synthesis. Pigmented skin equivalents include melanocytes, which localise to the basal layer and transfer melanin to neighbouring keratinocytes to form a protective supranuclear cap. The pigmented skin equivalents are functional, and exposure to ultraviolet radiation induces a tanning effect. Langerhans cells have also been incorporated into the epidermis to produce an immune-competent skin equivalent, which is responsive to topical allergens and irritants. We have developed advanced, robust and reproducible skin constructs that resemble the structure of human skin. These skin equivalents have multifaceted research applications such as investigating the underpinning mechanisms of skin ageing, assessing the effect of exogenous stressors and evaluating cosmetic formulations.

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Architectural changes in desmosomes during assembly and maturation

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Desmosomes are macromolecular junctions important in maintaining adhesion and resisting mechanical stress in epithelial tissue. Dysregulation of desmosome function occurs in multiple genetic and acquired skin diseases and squamous cell carcinomas. While the structure of mature desmosomes is generally understood, desmosome architecture during assembly, disassembly, and recycling remains unexplored. The dynamic nature of the maturation process combined with the biochemical intractability and diffraction limited size of desmosomes make it challenging to study. To overcome this, we used super-resolution direct STochastic Optical Reconstruction Microscopy (dSTORM) and quantified the architectural changes during desmosome assembly. We employed cell lines representing simple (MDCK), transitional (HUC), and stratified (NHEK) epithelia to determine universal features. Assembly was synchronized by a low to high calcium switch and desmosomes were tracked for 36 hours. Over the course of maturation, we found a significant decrease in the distance between desmoplakin (DP), but not plakoglobin (PG) plaques suggesting a shift in DP arrangement. This coincided with increased adhesive function and decreased E-cadherin (E-cad) enrichment. We then explored the architecture of individual desmosomes characterized as E-cad + or -. Regardless of time post-calcium switch, E-cad + desmosomes had wider DP plaques. This indicates that nascent desmosomes, even those at later timepoints, go through the same architectural changes. Our ability to map protein localization throughout assembly at single desmosome resolution revealed a universal correlation between DP architecture and maturation, suggesting that DP arrangement may have functional implications. Our novel approach provides vital insight into the structure-function relationship in desmosome dynamics and has potential to shed light on maintenance of epithelial integrity in wound repair, development, and disease states.

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Adipose triglyceride lipase dependent adipocyte lipolysis inhibits dermal fibrosis

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Tissue fibrosis in many organs results from altered and excessive extracellular matrix (ECM) protein deposition. Concomitant with ECM expansion, resident lipid-filled cells including mature adipocytes are lost inhuman and mouse fibrosis, yet the mechanisms that drive lipid loss and their contribution to tissue fibrosis are unknown. Using bleomycin induced skin fibrosis, we explored the timing and function of adipocyte lipid loss in fibrosis progression and identify an early, fibro-protective role of mature adipocyte lipolysis during fibrosis onset in the skin. We find that mature adipocyte size is reduced after 5 days of bleomycin induced skin fibrosis, and small mature adipocytes display reduced or lost markers of the lipid droplet perilipin. Genetic lineage tracing experiments of mature adipocytes reveal that dermal adipocytes persist in the dermal white adipose (DWAT) layer even after significant lipid loss and loss of perilipin. Analysis of skin sections of control and bleomycin treated skin at 5 days revealed that control adipocytes contain the typical unilocular lipid droplet, while adipocytes in bleomycin skin contained lipid-filled vesicles budding from the large lipid droplet, indicating that the reduced lipid content likely results from lipolysis. To analyze the function of adipocyte lipolysis in skin fibrosis, we analyzed the response of mice lacking the lipolytic rate-limiting enzyme adipocyte triglyceride lipase (ATGL) in adipocytes in murine back skin by crossing AdiponectinCre mice to Atgl floxed mice (Adiponectin-Cre; $Atgl^{fl/fl}$). Consistent with inhibition of lipolysis, Adiponectin-Cre; $Atgl^{fl/fl}$ mice retain their perilipin+ lipid droplets. Surprisingly, Adiponectin-Cre; $Atgl^{fl/fl}$ mice display precocious expansion of dermal thickness and increased collagen remodeling. These data indicate that fibrotic stimuli induce adipocyte lipolysis and that fatty acids are protective against excessive ECM production during skin fibrosis development.

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Age-related reduction of fibroblast size induces hepatocyte growth factor expression in a YAP/TAZ dependent manner

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Fragmentation of collagen fibrils, which form the bulk of the dermal extracellular matrix (ECM), is a prominent feature of human skin aging. This fragmentation disrupts the binding of dermal fibroblasts to the ECM, thereby reducing fibroblast mechanical force/spreading/stretching. Fibroblasts become rounder and smaller and undergo functional adaptive responses that give rise to an aged phenotype. In human skin, hepatocyte growth factor (HGF) is primarily produced by dermal fibroblasts and acts in an autocrine/paracrine manner to suppress collagen production. HGF protein and mRNA are significantly elevated 4.2-fold and 3.1-fold, respectively, in aged (80+ years old), compared to young (20-30 years old) sun-protected human dermis. We have investigated the mechanistic connections between fibroblast spreading/size and HGF expression. Treatment of primary cultured adult human dermal fibroblasts with latrunculin-A (Lat-A) causes rapid depolymerization of the actin cytoskeleton with consequent reduction of cell size. This reduction of fibroblast size (50% less surface area) results in upregulation of HGF mRNA (15.2-fold, $p<0.05$, $n=5$) and protein (5.5-fold, $p<0.05$, $n=5$). YAP/TAZ are transcriptional co-factors whose activities are regulated by cellular mechanical force/spreading. Reduction of fibroblast size causes translocation of YAP/TAZ from the nucleus into the cytoplasm, thereby abolishing transcriptional activity. Gain and loss of function studies revealed that fibroblast size-dependent regulation of HGF expression is mediated by YAP/TAZ. Expression of constitutively active mutant YAP/TAZ, which localizes to the nucleus, prevented the induction of HGF in fibroblasts with reduced size. Restoration of fibroblast size, by removal of Lat-A, downregulates HGF expression. However, siRNA-mediated knockdown of YAP/TAZ abrogates this downregulation. These data reveal a novel mechanism by which age-related reduction of fibroblast spreading/mechanical force leads to elevation of HGF in a YAP/TAZ -dependent manner.

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Retromer-dependent Dsg1 trafficking promotes epidermal differentiation and is enhanced by a small molecule chaperone

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The epidermis is a regenerating epithelial tissue that forms a multi-cellular barrier protecting against water loss, pathogens, and allergens. Essential for the generation of this barrier is an intercellular desmosomal cadherin, Desmoglein 1 (Dsg1). Dsg1 first appeared during evolution in terrestrial vertebrates where it is restricted to stratified tissues. We previously showed that the proper trafficking and positioning of Dsg1 on the plasma membrane in cells committing to transit into superficial layers is required for normal epidermal morphogenesis. Here, through two independent BioID screens and proximity ligation assays, we show that Dsg1 associates with VPS35, an essential component of a multi-subunit endosomal trafficking complex known as the retromer. Using antibody based recycling and biochemical stability assays we showed that pharmacological disruption of endosomal trafficking or genetic depletion of retromer components VPS35 and VPS29, block Dsg1 plasma membrane localization and result in its mistargeting to the lysosome without affecting the adherens junction cadherin, E-cadherin. Consistent with the idea that cadherin remodeling is important in tissue morphogenesis, loss of retromer function also disrupts Dsg1-mediated sorting of keratinocytes into superficial epidermal layers in an in vitro competition assay. Further, VPS35 depleted cultures displayed signs of aberrant differentiation and morphogenesis in 3D organotypic epidermal culture. These findings could be clinically important, as a pharmacological chaperone that enhances retromer function (R55) is sufficient to enrich the plasma membrane localization of wild-type and a disease-associated Dsg1 mutant that results in Severe dermatitis multiple Allergies and Metabolic wasting (SAM syndrome). Altogether, our data support the importance of the retromer's role in proper keratinocyte differentiation and epidermal morphogenesis via the regulation of Dsg1 trafficking.

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Pathogenic CD8+ T cells form cytolytic immune synapses to mediate hair follicle destruction in Alopecia Areata

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We previously reported CD8+NKG2D+ T cell-mediated cytolysis in hair follicle (HF) destruction in Alopecia Areata (AA), however, the molecular mechanism by which target cell killing occurs in AA is not understood. Cytotoxic CD8+ T cells form immune synapses (IS) to recognize antigen and specifically kill target cells. To identify the cytolytic IS that are formed between CD8+ T cells and HF target cells in AA, we defined the spatial localization and recruitment of key proteins involved in IS formation using high-resolution skin imaging of the C3H/HeJ mouse model of AA. We observed CD8+ T cells located within AA HF that displayed polarization of three IS components at the site of contact with the target cell, including the TCR signaling kinase Lck, cytolytic machinery consisting of the microtubule organizing center (MTOC), and lytic granules. MTOC/Lck colocalization demonstrates that CD8+ T cells assemble IS that bind antigen and recruit the cytolytic machinery aimed towards the target cell. Most CD8+ T cells were already detached from TUNEL+ dead target cells after killing, except for a few that maintained synaptic contact with target cells capturing the site of active IS *in vivo*. To complement skin imaging, we modeled T cell cytotoxicity in an *in vitro* system wherein C3H/HeJ CD8+ T cells were used to target cells presenting mismatched MHC. Alloreactive C3H/HeJ T cells assembled IS in vitro, which involved polarization of Lck and MTOC at the synaptic interface, corroborating our findings obtained from in situ AA mouse skin imaging. We measured the relative distance between the MTOC and IS in which a shorter distance reflects the cytotoxic success of synapsing CD8+ T cells and found that the MTOC distance was shorter in synapsing CD8+ T cells compared to control. Spatial localization of key IS molecules Lck, MTOC, and granzymes in CD8+ T cells define the cytolytic IS between HF and CD8+ T cells that mediate HF destruction in AA.

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Upregulated immune response networks in granuloma annulare

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Background: Granuloma annulare (GA) is a chronic granulomatous skin disease of unknown etiology. A comprehensive catalog of gene expression profiles generated by RNA sequencing of GA samples is essential to understand etiopathogenesis and to develop effective therapies. We aimed to identify key inflammatory and oncogenic pathways in GA to determine which transcriptomic expression patterns are associated with this disorder. Methods: We performed transcriptomic profiling analysis using RNA sequencing on skin biopsies of GA (n=22) and normal skin (controls, n=10). Differential analysis was performed on transcriptomic profiles. Results: Transcriptomic profiling highlighted notable differences between GA and controls that may elucidate unique pathogenic pathways in this disorder. Compared to controls, GA samples revealed 1,560 upregulated genes and 1,901 downregulated genes of 41,783 genes/transcripts with non-zero reads in RNA-seq data, with FDR adjusted p-values < 0.001 and log2 fold change > 1. These differential genes demonstrated upregulation of immune response, T cell proliferation and co-stimulation, signal transduction, and interferon-gamma-mediated signaling pathways, and downregulation of pathways involving cell adhesion, nervous system development, muscle contraction, and angiogenesis. The top 10 upregulated immune response hub genes identified by pathway enrichment analysis and gene interaction network analysis included: PTPRC, TNF, CD86, IL6, IL10 TLR2, TLR8, CTLA4, TYROBP, and CCR5. Conclusion: Our comprehensive transcriptomic profiling pinpoints disease-related enrichment pathways and gene functions in GA, which may shed light on molecular drivers of disease. Identified hub genes represent potential drug targets for further development of novel therapies.

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Transcriptomic profiling of cutaneous sarcoidosis

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Background: Cutaneous sarcoidosis (CS) occurs in up to 1/3 of systemic sarcoidosis cases and can be treatment-refractory. The goal of this study was to identify key inflammatory and oncogenic pathways in CS to determine expression patterns that contribute to disease formation. Methods: RNA-seq was performed on skin biopsies of CS (n=13), and normal skin (controls, n=10). Differential analysis was performed on the entire transcriptomic profiles between groups, followed by gene function enrichment analysis (GFEA), and network hub gene analysis to investigate genes associated with disease development. Results: Our transcriptomic data highlighted key expressional differences between CS and controls. Of 38,631 transcripts/genes with nonzero total read count, there were 2,162 upregulated and 2,542 downregulated genes in CS. The most significantly upregulated gene pathways in CS were those involving immune and inflammatory response, interferon-gamma-mediated signaling, T cell related functions, such as T cell costimulation, positive regulation of T cell proliferation, and T cell receptor signaling pathway. The most significantly downregulated pathways were cell adhesion, angiogenesis, flavonoid glucuronidation, epoxygenase P450 pathway, and extracellular matrix organization. Based on the top 10 closely related upregulated immune and inflammatory response pathways identified by GFEA, interaction network analysis elucidated the following hub genes: TNF, PTPRC, IL6, CD86, IL10, TLR2, CTLA4, TLR8, CD80, and TLR7. Conclusion: Our transcriptomic profiling identified disease-related enrichment pathways and gene functions in CS, including elevated cellular immune and inflammatory activities and repressed cell adhesion and extracellular matrix organization, which may reveal molecular drivers of disease. The identified hub genes represent possible diagnostic biomarkers and potential drug targets for future development of novel therapies.

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The contact hypersensitivity defect in mice lacking epidermal *Pparg* requires signaling through TNFR1, TNFR2, and tryptophan hydroxylase 1

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Loss of epidermal *Pparg* (*Pparg*-/-^{epi}) in mice is associated with a marked defect in contact hypersensitivity (CHS) responses that is dependent on increased TNFα signaling. TNFα exists as both a full-length transmembrane form that activates both type 1 & 2 TNFα receptors (TNFR1 & 2) and a cleaved soluble form that acts primarily through TNFR1. We show that neutralizing antibodies to both TNFR1 & 2 completely restore CHS responses in *Pparg*-/-^{epi} mice. We also examined differentially expressed (DE) transcripts from epidermal scrapings of *Pparg*-/-^{epi} mice relative to their wildtype (WT) littermates. Interestingly, tryptophan hydroxylase 1 (*Tph1*) was markedly elevated in *Pparg*-/-^{epi} mice (17.04-fold change, FDR = 7.8E-09). This increase was verified by qRT-PCR of whole skin RNA extracts. We therefore treated WT and *Pparg*-/-^{epi} mice with the Tph1 inhibitor, fenclonine. Fenclonine had no effect on CHS responses to DNFB in WT mice but completely restored CHS responses in *Pparg*-/-^{epi} mice. Other investigators have shown that mast-cell derived Tph1 is an important regulator of cutaneous immunologic tolerance. We show that in *Pparg*-/-^{epi} mice have an approximately 2-fold increase in mast cells, as well as 2.18 to 4.94-fold increases in multiple mast cell markers in the DE analysis. Regulatory T-cells can also inhibit CHS responses. DE analysis showed a 3.23-fold increase in *Foxp3* transcripts in *Pparg*-/-^{epi} mice. RT-PCR also showed a similar increase in *Foxp3* & *Il2ra* (CD25) transcripts in *Pparg*-/-^{epi} mice. Finally, treatment with neutralizing CD25 antibodies partially but significantly restored CHS responses in *Pparg*-/-^{epi} mice. Our data indicate that epidermal PPARγ acts to regulate immune function through down-stream TNFα/TNFR1 & 2 signaling, Tph1, regulatory T cells, and possibly mast cells.

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Comparative transcriptomic profiles of cutaneous sarcoidosis and granuloma annulare

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Background: Cutaneous sarcoidosis (CS) and granuloma annulare (GA) are chronic granulomatous skin diseases which are often refractory to therapy. The goal of this study was to identify key regulatory pathways and gene function networks in GA and CS to determine expression patterns differentiating these conditions. Methods: RNA-seq was performed on skin biopsies of CS (n=13), and GA (n=22). Differential analysis was performed on the entire transcriptomic profiles of each group. Results: Although the transcriptomic profiles of GA and CS samples had similarities, there were notable differences between these groups which may elucidate unique pathways that differentiate each disease. Our differential analysis identified 408 genes highly expressed in CS samples and 328 genes highly expressed in GA samples with p value ≤ 0.001 and log2 fold change ≥ 1. The highly expressed genes in GA samples were involved in the pathways including cellular glucuronidation, keratinization and keratinocyte differentiation, establishment of skin barrier, extracellular matrix disassembly. The most highly expressed pathways in CS group were those involving immune and inflammatory response, tryptophan catabolic process, T cell proliferation, response to interferon-gamma, and signal transduction. The top 10 significantly expressed genes in CS and GA are CD38, ITGAL, PRSS21, FMO1, SLC6A7, KAL1, CD22, CD6, DPEP1, GRAMD1B and CTTNBP2, GFRA2, VWC2, PLP1, GPSM2, ELL3, PLCD3, PCSK2, SLC6A15, MUC12, respectively. Conclusion: Our comparative transcriptome findings pinpoints disease-specific enrichment pathways and gene functions in CS and GA patients, which may shed light on different molecular mechanisms that contribute to disease progress. The top differential genes represent potential drug targets to develop novel therapies for these chronic granulomatous disorders.

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Wnt signaling stimulates ATGL-regulated lipolysis in dermal fibrosis

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Fibrotic disorders, characterized by the deposition of excessive extracellular matrix in all organs and the loss of lipid-filled cells in several organs, contribute to approximately 45% of deaths in Europe and North America. Skin has a distinct dermal white adipose tissue (DWAT) compartment, making dermal fibrosis a good model for studying fibrotic fat loss. DWAT adipocytes perform various functions impacted by their lipid content. A primary process by which adipocytes homeostatically modulate their lipid content and mobilize stored lipid is adipose triglyceride lipase (ATGL)-regulated lipolysis. The mechanisms underlying fibrotic fat loss in DWAT and the impact of lipid depletion in fibrosis are unknown. Wnt signaling is dysregulated in human fibrosis and has known anti-adipogenic roles. We hypothesize that dermal Wnt signaling activation stimulates ATGL-regulated lipolysis leading to fibrotic lipid depletion. Using a genetically inducible and reversible mouse model of dermal Wnt activation, we show Wnt activation is sufficient to cause dermal fibrosis. The ATGL-regulated lipolysis axis is activated in DWAT adipocytes during the onset and progression of Wnt-induced fibrosis. Dermal Wnt activation leads to elevated phosphorylated hormone sensitive lipase and phosphorylated perilipin**,** lipolytic proteins downstream of ATGL, preceding fibrotic fat loss *in vivo*. Wnt activation also leads to a reduction in the size of perilipin-positive adipocyte lipid droplets *in vivo*. Thus, stimulated ATGL-regulated lipolysis occurs as an early event in dermal fibrosis. Consistently, murine primary intradermal adipocytes release more glycerol, a product of lipolysis, upon Wnt activation *in vitro* indicating that Wnt signaling has cell-autonomous lipolytic effects. ATGL enzymatic inhibition is sufficient to rescue Wnt-induced lipolysis. Current studies focus on the role of ATGL in Wnt-induced dermal fibrosis *in vivo*. Our results implicate lipolysis as a novel therapeutic target for fibrosis treatment.

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CO-Detection by indexing (CODEX) reveals clinically distinct classes of eczematous rashes

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Spongiotic rashes are known to be more heterogeneous than psoriasiform rashes, but a more precise molecular classification has yet to be established. We performed CO-Detection by indexing (CODEX), which utilizes DNA-barcoded antibodies visualized by cyclic addition and removal of fluorescently labeled complementary DNA oligos, to perform highly multiplexed immunofluorescence on 12 samples of histopathologically spongiotic dermatitis, ranging from allergic contact dermatitis to endogenous eczema. This type of systems-level approach utilizing highly multiplexed spatial imaging, which captures many antigens on a single cell basis, has not been previously utilized in spongiotic dermatitis, nor in any other rashes. We utilized a customized 40 antibody panel allowing enumeration of key APC and T cell types while ascertaining immune cell functional states/signaling status and spatial orientation to skin anatomic structures and cell types. We identified recurrently aberrant immune cell subpopulations enabling subclassification of these rashes into distinct classes correlating with etiology and anatomic site ($p < .007$). Our findings point to a broadly applicable technology capable of stratifying histopathologically indistinct rashes.

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IL-33 signaling in sensory neurons promotes dry skin itch

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Chronic itch is a highly debilitating and often intractable condition that affects up to 20% of the population. Despite recent advances in uncovering the key cellular and molecular pathways that underlie chronic itch, most studies have focused on itch that arises in the setting of inflammatory skin disorders. Thus, how itch is elicited in conditions that lack overt inflammation, such as in dry skin (xerosis) and chronic pruritus of unknown origin (CPUO), remains poorly understood. IL-33 has recently been implicated in a mouse model of dry skin itch. Additionally, we find that patients with CPUO exhibit elevated levels of serum IL-33 compared to control subjects. However, the precise mechanisms by which IL-33 promotes itch remains unclear. Although a well-known driver of cutaneous inflammation, we hypothesized that IL-33 can promote itch independently of immune cells. Indeed, we demonstrate that IL-33 activates sensory neurons from the dorsal root ganglia of mice and humans. Further, in a mouse model of dry skin, we find that both mast cells and lymphocytes are dispensable for the development of chronic itch. Instead, mice that conditionally lack IL-33 receptor (ST2) on sensory neurons exhibit attenuated dry skin itch. Collectively, these findings indicate that sensory neuron-specific IL-33 activity is required in the context of dry skin itch.

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Antimicrobial peptide hBD-3 improves Th2 cytokine-mediated impairment of tight junction barrier through autophagy activation

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Th2 cytokines such as IL-4 and IL-13 attenuate the skin barrier function and modulate autophagy. We previously showed that the antimicrobial peptide human β-defensin (hBD)-3 improves the tight junction (TJ) barrier function in keratinocytes; however, its effect on Th2 cytokine-mediated impairment of TJ barrier function remains unclear. We aimed to evaluate the effects of hBD-3 on IL-4- and IL-13-mediated impairment of TJ barrier in human keratinocytes and explore the underlying mechanism. We assessed the expression of autophagy marker LC3, and TJ-related proteins and the signaling pathways in keratinocytes by western blot, autophagosome/autolysosome formation by immunofluorescence and electron microscopy, expression and distribution of TJ-related proteins by real-time PCR and immunofluorescence, respectively. We found that hBD-3 increased the expression of LC3, and enhanced the formation of autophagosomes/autolysosomes in keratinocytes. Besides, hBD-3 induced activation of mTOR and MAPK signaling pathways, which were required for the hBD-3-mediated activation of autophagy, as evidenced by the inhibitory effects of their specific inhibitors. hBD-3 also rescued the downregulation of TJ proteins, including claudin-1 and -4 in IL-4+IL-13-treated keratinocytes. Interestingly, autophagy deficiency following infection of keratinocytes with Atg3 mutant adenovirus abolished hBD-3-mediated TJ barrier improvement, suggesting that hBD-3 regulates TJ barrier function through autophagy activation. Our findings provide novel evidence that hBD-3 might be a therapeutic target for the treatment of skin diseases that are characterized by dysfunction of autophagy and skin barrier.

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IL-4 and IL-13 cytokines drive sex steroid hormone synthesis and lipid abnormalities in sebocyte during atopic dermatitis pathogenesis

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One of the hallmark features of atopic dermatitis (AD) is an elevation of the type 2 cytokines, IL-4 and IL-13. Thus, the development of a monoclonal antibody against the IL-4 receptor, Dupilumab, has revolutionized therapy for AD patients. Though the central role of IL-4 and IL-13 in AD is clear, we still have an incomplete understanding of how these immune cytokines drive changes in the skin epithelium. Sebaceous glands are specialized sebum-producing epithelial cells that release a mixture of lipids, free fatty acid, and antimicrobial proteins to the skin surface. Little is known about how sebocyte biology changes in AD. Here we show the impact of type 2 cytokines on sebocytes and find that IL-4 and IL-13 stimulate the expression of 3-beta hydroxysteroid dehydrogenase (HSD3B1). HSD3B1 is a rate-limiting enzyme in sex steroid hormone synthesis. Using liquid chromatography-tandem mass spectrometry, we demonstrate that IL-4 and IL-13 can enhance HSD3B1 dependent androgen production. Further, in an HSD3B1 dependent manner, IL-4 and IL-13 drive lipid abnormalities in human sebocyte cells through regulation of INSIG1 expression. Consistent with our findings in sebocytes, the expression of HSD3B1 is highly elevated in the skin of AD patients and can be restored by Dupilumab treatment. Taken together, these data suggest that targeting sex steroid hormone synthesis pathway could be a potential therapeutic avenue to restore normal skin barrier function in AD patients.

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Deletion of *TNFAIP6* gene in human keratinocytes by CRISPR/Cas9 edition demonstrates a role for TSG-6 to retain hyaluronan inside epidermis

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TNFα-stimulated gene 6 (TSG-6) protein is found in human skin, where it exerts anti-inflammatory properties and contributes to wound healing. In human epidermis, TSG-6 is mainly secreted in the extracellular matrix between keratinocytes where it interacts with HA. This work aims at better understanding TSG-6 and HA functions in epidermal physiology. Reconstructed human epidermis (RHE) incubated with Th2 interleukins to mimic atopic dermatitis (AD) or RHE infected with *Trichophyton rubrum* dermatophytes were compared with RHE cultured in normal conditions. In both pathological conditions, mRNA expression levels and protein release of TSG-6 were strongly upregulated in parallel to HA production, suggesting that they might play a role together in challenged epidermal tissues. Potential role was investigated by creating TSG-$6^{-/-}$ cells using the CRISPR/Cas9 system to edit *TNFAIP6* gene in N/TERT keratinocytes, an immortalized human cell line which produces keratinized layers in tissue reconstruction. Two TSG-$6^{-/-}$ clones harboring major deletions in both alleles of the target gene were used to reconstruct RHE. TSG-$6^{-/-}$ RHE exhibit normal epidermal morphology with efficient barrier and typical localization of HA and differentiation markers. Their phenotype was further analyzed through RNA sequencing. Despite no alteration in the expression of genes involved in HA metabolism, an increased amount of HA was detected in medium underneath TSG-$6^{-/-}$ RHE in concomitance with a reduced epidermal HA content, especially in conditions that mimic AD and dermatophytosis. This enhanced HA leakage from either challenged and untreated TSG-$6^{-/-}$ epidermis is reversed when TSG-6 expression is reintroduced in the tissue, suggesting TSG-6 critical involvement to cross-link and thus retain HA in epidermal extracellular matrix. In addition to other organs and tissues, this work demonstrates overexpression and function of TSG-6 in challenged epidermis.

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Type XVII collagen modulates epidermal patterning

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Many mammals show epidermal patterning, as seen in alternating mouse tail scales and in the microtopography of the human skin surface. However, the mechanisms underlying the development of such patterning are poorly understood. Here, we show that type XVII collagen (COL17), a niche for epidermal stem cells, is a factor in determining epidermal patterning. Col17-null mice exhibit slender epidermal tail scales, a condition that is alleviated by human COL17 overexpression. Aberrant cell polarity, a feature of Col17-null mouse epidermis, does not account for the slender-scale phenotype. Skin regeneration in mice after wounding is accompanied by slender-scale epidermis, a condition that is rescued by human COL17 overexpression. The revertant mosaicism and diseased skin seen in human junctional epidermolysis bullosa demonstrate that COL17-positive and negative skin harbor mutually distinct epidermal patterns. These results demonstrate that COL17 defines mouse tail scale morphology and human skin microtopography. Our study sheds light on the role of the stem cell niche in tissue pattern formation.

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Identification of a desmoglein-1 reducing component of human stratum corneum contained in wild thyme (*Thymus serpyllum*) extract

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The outermost layer of the skin, the stratum corneum (SC) has the very important function of preventing excessive transpiration via the epidermis (inner-to-outer barrier) and preventing invasion by foreign substances (outer-to-inner barrier). The SC also contributes to the softness and mechanical strength of the skin. In the histological examination of normal human skin, when observing thin skin sections with hematoxylin/eosin staining, the SC consists of two layers, an outer layer with a basket-weave (BW) structure, and an inner layer with a compact structure. Reportedly, the layer with the BW structure contributes to the barrier function and to SC flexibility. We hypothesized that by identifying the relevant component that develops the BW structure, the barrier function and flexibility of the SC might be restored, leading to healthy skin. Since it is known that a major component of corneodesmosomes is desmoglein 1 (Dsg1), the degradation of which is a necessary process for generating the BW structure of the SC, we performed a quantitative and distribution pattern analysis of Dsg1 as a marker of the completeness of BW structure formation in the SC. Dsg1 is involved in adhesion between SC cells, so we investigated its components after treatment with wild thyme (*Thymus serpyllum*) extract, which was found to be effective in a preliminary experiment. In analyses conducted using our established Dsg1 reduction evaluation method, an 80% ethanol extract prepared from wild thyme was found to have a Dsg1 reducing effect in the human SC. There was also a Dsg1 reducing effect with a 60% methanol eluate obtained by separation of the 80% ethanol extract using a HP-20 column. Subsequently, the 60% methanol eluate was purified using silica gel and an ODS column, and six compounds were isolated. Evaluation of the Dsg1 reducing effect of these six compounds showed that eritrichin and salvianolic acid A have a Dsg1 reducing effect in the human SC. Therefore, applying an extract containing eritrichin or salvianolic acid A to the skin can be expected to improve skin health.

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Encapsulated Activated-Grape Seed Extract (ACTIVITIS™) inhibits demethylation of PP2A promoting anti-aging benefits and barrier repair for human skin

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Cumulative oxidative stress and chronic inflammation are critical during skin aging. One pathway that regulates both processes involves Protein phosphatase 2A (PP2A), a serine/threonine phosphatase. Reversible methylation of the C-terminal leucine of the PP2A catalytic subunit (PP2Ac) plays a crucial role in regulating PP2A function. Oxidative stress has been previously shown to dramatically decrease methylation of the C-terminal leucine of the PP2A catalytic subunit (PP2Ac) in dermal fibroblasts. Previously, we developed a novel, proprietary grape seed extract called Activated-Grape Seed Extract, or ACTIVITIS™ (INCI name: anthocyanins and hydrolyzed proanthocyanidins) which is enriched for PP2A-activating flavonoids with increased potency in preventing PP2A demethylation when compared to commercial Grape Seed Extract. To enhance skin delivery and other potential benefits of ACTIVITIS™, we developed a chemical encapsulation formulation utilizing a liposome-based approach. Results demonstrate that this novel encapsulated form of ACTIVITIS™ retains PP2A demethylating activity with similar potency as the non-encapsulated form. To explore the potential benefits of encapsulated ACTIVITIS™, we performed *in vitro* assays utilizing 3D epidermal skin tissue models and UVB irradiation. We found encapsulated ACTIVITIS™ significantly increases both cell proliferation (Ki67) and skin barrier (Filaggrin) marker expression after UVB irradiation. Moreover, encapsulated ACTIVITIS™ formulation decreases DNA damage marker Cyclobutane pyrimidine dimers (CPD), thus enhancing epidermal integrity and barrier repair after UVB-induced effects. Lastly, clinical results in human subjects demonstrates that encapsulated ACTIVITIS™ is well tolerated and provides anti-aging benefits such as improving fine lines and wrinkles when applied topically.

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Involucrin deficiency results in decreased vitamin D receptor-mediated inflammation and Csnk1e isoform bias

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We previously identified increased *involucrin* (*IVL*) expression for human skin barrier evolution that arose out-of-Africa. This led us to re-examine the function of involucrin in both adult and newborn *Ivl*-/- mice. We investigated the inflammatory responses in adult mice using the MC903 (vitamin D agonist) inducible model for atopic dermatitis. Unexpectedly, *Ivl*-/- mice exhibited reduced ear thickness compared to WT mice ($p<0.001$). Underlying this decreased ear skin inflammation was a comparative decrease in *thymic stromal lymphopoietin* (*Tslp*) expression in *Ivl*-/- treated ears. Flow cytometry analysis to determine innate and adaptive immune cell phenotypes identified a notable decrease in CD4+ T cell infiltrate in *Ivl*-/- treated ears ($p<0.05$). We investigated a potential mechanism for the reduced MC903-induced inflammation and identified reduced vitamin D receptor (Vdr) expression in *Ivl*-/- versus WT skin. Thus far, we have identified a new phenotype for *Ivl*-/- mice with reduced Vdr-mediated inflammation and decreased adaptive CD4+ T cell response as a result of decreased Vdr. We further examined the impact of involucrin deficiency in the skin using a comprehensive multi-omics approach (ATAC-seq, RNA-seq, and LC/MS proteomics) to determine chromatin accessibility, transcriptomic, and proteomic changes in *Ivl*-/- and WT newborn epidermis ($q<0.05$). Involucrin was identified at the intersection of all three datasets thus demonstrating the validity and rigor of the approach. Five potential targets at the intersect of ATAC-seq differentially accessible regions and LC/MS differentially expressed proteins were determined. Of most interest, Csnk1e, known to regulate circadian clock, was found to have less accessible chromatin and reduced protein expression. DEXseq analysis identified a bias for *Csnk1e* alternative transcripts in *Ivl*-/- mice. Together our findings reveal a functional role for the evolutionarily selected involucrin to regulate VDR-mediated inflammation and potentially for the circadian response in the skin.

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Aged human keratinocytes have protein coding and noncoding RNA signatures indicative of inflammation, defective proliferation, and barrier deficiency

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Skin is in direct interface with the environment, subjecting it to extrinsic damage and intrinsic metabolic processes that result in aging. Long noncoding RNAs (lncRNA) are regulatory molecules, impacting transcription, translation, and macromolecular organization. Unlike other classes of RNAs, lncRNAs are understudied in most fields, including aging. This study investigates the role for lncRNA and protein coding RNA in aging using NGS by comparing transcriptomes from paired neonatal and adult keratinocyte donors. The biological impact of the genes correlated with and differentially expressed during aging were identified using pathway analysis and GWAS. Age positively correlated (correlation coefficient ≥ 0.6) with expression of 925 protein coding and 181 lncRNA genes, and negatively correlated with 739 protein coding and 168 lncRNA genes. Both classes of genes have single nucleotide polymorphisms associated with inflammatory disorders, including psoriasis and IBD. The differentially expressed genes play roles in barrier function, adhesion, cytokine-receptor interactions, and cancer-associated pathways. Surprisingly, senescence associated lncRNAs showed variable regulation with age: NORAD and MIAT had weak negative correlations, yet PINT and PVT1, components of the p53/Myc pathway, are enriched with age. Comparison to publically available skin suction blister and dermal fibroblast aging datasets revealed a core set of age-associated protein coding genes, which may underlie skin aging regardless of the layer or embryonic origin on the cell. In contrast, lncRNAs were notably lineage specific and may represent a modality for cell-type specific targeting of the aging process. Given the finding of altered barrier function in aged human keratinocytes, these findings will be compared to psoriasis, atopic dermatitis, and ichthyosis, diseases in which barrier dysfunction contributes to pathogenesis.

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SDR9C7 catalyzes the critical dehydrogenation of acylceramides for skin barrier formation

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The corneocyte lipid envelope, composed of covalently bound ceramides and fatty acids, is important to the integrity of the permeability barrier in the stratum corneum, and its absence is a prime structural defect in various skin diseases associated with defective skin barrier function. *SDR9C7* encodes short-chain dehydrogenase/reductase family 9C member 7 (SDR9C7), which was recently found to be mutated in ichthyosis. In a patient with an *SDR9C7* mutation and in a mouse *Sdr9c7*-knockout model, we show that the loss of covalent binding of epidermal ceramides to protein appears as a structural fault in the barrier. For unresolved reasons, protein binding requires the lipoxygenase-catalyzed transformation of linoleic acid (18:2) esterified in omega-O-acylceramides. In *Sdr9c7*-knockout epidermis, quantitative liquid chromatography−mass spectometry (LC-MS) assays revealed the almost complete loss of a species of omega-O-acylceramide esterified with linoleate-9,10-*trans*-epoxy-11*E*-13-ketone; other acylceramides related to the lipoxygenase pathway were more abundant. Recombinant SDR9C7 catalyzed the NAD^+-dependent dehydrogenation of linoleate 9,10-*trans*-epoxy-11*E*-13-alcohol to the corresponding 13-ketone, while ichthyosis mutants were inactive. We propose, therefore, that the critical requirement for lipoxygenases and SDR9C7 is in producing acylceramide containing the 9,10-epoxy-11*E*-13-ketone, a reactive moiety known for its non-enzymatic coupling to protein. This suggests a mechanism for the coupling of ceramides to protein and provides important insights into skin barrier formation and pathogenesis.

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Effect of the antimicrobial peptide derived from insulin-like growth factor-binding protein 5 on skin barrier regulation

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Background: A novel antimicrobial peptide derived from insulin-like growth factor-binding protein 5 (AMP-IBP5) displays both antimicrobial and immunomodulatory properties. AMP-IBP5 is involved in the regulation of cutaneous immunity through cytokine/chemokine production and promotion of keratinocyte migration and proliferation. However, the role of AMP-IBP5 in the regulation of skin barrier remains unclear. Objective: To investigate the effects of AMP-IBP5 on skin barrier function and the underlying mechanisms. Methods: Normal human epidermal keratinocytes were stimulated with AMP-IBP5 and Western blot was used to analyze the protein expression of various differentiation markers and tight junction (TJ)-related proteins. Immunofluorescence microscopy was used to examine the intercellular distribution of TJ proteins. Activation of aPKCζ, Rac1 and GSK 3α/β on transepithelial electrical resistance (TER) was evaluated. Results: AMP-IBP5 increased the expression of various TJ proteins including claudin-1, -2, -4, -7, occludin, zonula occludens 1 and enhanced their distribution along the cell-cell borders. AMP-IBP5 also enhanced the expression of differentiation markers such as filaggrin, loricrin, transglutaminase 1 and keratin 1, and improved the TJ barrier function by increasing TER. In addition, AMP-IBP5 increased the phosphorylation of aPKCζ, Rac1 and GSK 3α/β which are required for AMP-IBP5-mediated improvement of TJ barrier, as evidenced by the inhibitory effects of their respective inhibitors on TER. Conclusion: Our findings suggest that AMP-IBP5 might be a therapeutic target for skin diseases with skin barrier defects such as atopic dermatitis and psoriasis through improvement of the skin barrier function.

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Ichthyosis transcriptome reveals increased atherosclerosis markers and immune and barrier differences amongst subtypes

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Preliminary studies have suggested upregulation of inflammatory pathways in the skin and circulation of patients with common forms of ichthyosis. However, a complete characterization of various ichthyosis subtypes remains unavailable, precluding development of better treatments. We thus aimed to characterize the common ichthyosis skin transcriptome, as well as the unique profiles of various subtypes in the largest cohort to date of 54 ichthyosis patients (7 Netherton syndrome/NS, 13 epidermolytic ichthyosis/EI, 16 lamellar ichthyosis/LI, 18 congenital ichthyosiform erythroderma/CIE) and 40 healthy controls using RNA-seq. Differentially expressed genes (DEGs) were defined as fold changes/FCH>2 and false discovery rate/FDR<0.05. We found robust and significant Th22/Th17 (i.e. PI3, IL17A/F, S100A7) skewing in all subtypes, with greatest changes observed in NS, CIE, and EI. The Th2 pathway showed only modest changes, particularly in NS. Several Th1-related genes were upregulated primarily in CIE (e.g. IL1B, IL12B, CXCL9). Across all subtypes (less evident in EI), lipid metabolism (e.g. FADS1/2, FAR2, FA2H, ELOVL3, HAO2) and barrier junction (e.g. CLDN8, CLDN23, CDH10) markers were downregulated, whereas epidermal differentiation genes (e.g. SPRR1A/1B/2C/2G, IVL, PGLYRP3/4, LCE3D) were upregulated. A striking number of DEGs were associated with atherosclerosis/cardiovascular processes (e.g. VNN1, PCSK9, and IL36A/B/G [up in all], HBEGF and SELE [up in NS, CIE, EI], ACE2 [up in CIE, EI], and ApoE [down in all]). Our findings suggest that the common ichthyosis variants share aberrations in Th17/Th22, lipid metabolism, and atherosclerosis-linked products while exhibiting variations in Th2 and Th1 pathways. This may help elucidate the pathogeneses of these subtypes and inform the development of subtype-specific treatments.

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Proteomics and lipidomics reveal the protective mechanism of dietary n-3 PUFA supplementation for photoaging

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Chronic ultraviolet radiation (UVR) could induce photoaging, even carcinogenesis. Dietary omega-3 polyunsaturated fatty acid (n-3 PUFA) supplementation has been proved to alleviate photoaging and cutaneous carcinoma. Whereas the exact mechanism remains poorly elucidated, accumulated evidence suggested that the alleviation effect of n-3 PUFA for photoaging is a multifactorial procession characterized by different pathways. Here, we performed a whole-genome proteomics and lipidomics analyses using a self-constructed photoaging mouse model with n-3 PUFA or n-6 PUFA supplementation. Significant alleviation of photoaging was observed and a total of 88 differentially expressed proteins and 152 differentially expressed lipids were identified in mice with n-3 PUFA supplementation. We found that n-3 PUFA may alleviate photoaging by upregulating Hmmr (hyaluronic acid receptor) expression, which can decrease Mmp9 expression, reducing collagens degradation. As most proteins were associated with lipogenesis and lipids metabolism, we further analyzed the lipidomics data, most triglyceride (93%) showed a significant increase in the n-3 PUFA supplementation group. Our proteomics and lipidomics results indicate that the protective mechanism of n-3 PUFA for photoaging is complicate, the effect of elevated triglyceride by n-3 PUFA supplementation in counteracting skin photoaging should cannot be overestimated, which will become a new prime target in fighting photoaging.

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Keratin switching modulates cellular mechanical properties to balance epidermal strength and plasticity

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The epidermis must balance the ability to resist mechanical stress with the plasticity required for tissue remodeling during growth and wound healing. The keratin intermediate filament (KF) network, linked between adjacent cells through desmosomes, has long been recognized as a key mediator of epidermal integrity. Disruption of the KF network leads to epidermal fragility in a number of diseases. However, the contribution of the KF network to epidermal plasticity remains poorly defined. In contrast to the actin and microtubule cytoskeletal networks, KFs are notable for their molecular diversity, with expression of individual keratin genes determined by tissue, anatomic site, and cell differentiation. Following epidermal wounding, expression of five wound-associated keratins increases relative to steady-state epidermal keratins. This suggests that changing KF composition may alter KF mechanics or function in order to support increased epidermal remodeling. To test this hypothesis, we expressed different levels of steady-state keratin 5 (K5) or wound-associated keratin 6A (K6A) in cultured human keratinocytes. Live imaging revealed that increasing K6A expression decreased KF motion dynamics compared to increasing K5 expression. Changing KF composition also altered keratinocyte mechanical properties, with $K6A^{high}$ cells generating increased traction stress compared to $K5^{high}$ cells. Furthermore, in monolayer migration assays, $K6A^{high}$ cells reached faster peak migration speeds than $K5^{high}$ cells when combined in a mixed population. Together, these results demonstrate that changes in KF composition modulate KF network dynamics and cellular mechanical properties, revealing mechanisms for regulation of epidermal plasticity during tissue remodeling.

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Unbearable transepidermal water loss (TEWL) experimental variability: Why?

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Purpose: Despite the wide breadth of research, much disparity exists in transepidermal water loss (TEWL) research data - possibly due to uncontrolled experimental variables. We determined whether such experimental variables significantly impact TEWL studies and cause this disparity. Methods &Materials: An initial literature search regarding TEWL was performed to determine potential confounding variables. A subsequent search procured relevant and representative studies investigating the impact of these variables on TEWL. Results: Variables, such as age, anatomic site, and temperature, impact TEWL and should be controlled for in TEWL studies. Other variables, such as smoking and menstrual cycle, have inconclusive results or do not provide sufficient data breadth to make a conclusion regarding its effect, if such an effect exists, on TEWL metrics. Therefore, these variables require further research to determine their potential impact on TEWL. Conclusion: Matching for as many experimental variables as possible may reduce the disparity in TEWL data / conclusions, a major tool in dermtaologic research.

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Identification of novel molecular markers of disease severity and skin itchiness in children with atopic dermatitis

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Atopic dermatitis (AD) is a globally prevalent skin disorder affecting millions of children. AD causes severe discomfort, skin irritation, itchiness and social stigmatization. The heterogeneous presentation of AD together with the invasiveness of skin biopsy sampling – especially from children – limits the acquisition of clinical samples. Consequently AD remains to be fully elucidated. In this study, we established a robust, minimally invasive skin tape-stripping method that enabled in-depth characterization of the skin transcriptome in healthy children (n=15, age 1 months to 16 years old), and in the lesional and non-lesional skin of children with AD (n=39, age 1 to 14 years old), using RNA sequencing. Transcripts from skin of healthy individuals differed markedly from that of AD patients; however, there were only minor differences between non-lesional and lesional AD skin. Transcripts that defined AD lesional skin were mainly associated with immune regulation, skin tissue remodeling and cell cycle control, and represented novel and established markers that pointed to the involvement of monocytes, mast cells and neutrophils. Notably, these markers differentially linked to clinical disease manifestations as assessed by the Eczema Area and Severity Index and Pruritus score. Taken together, the results support the identification of novel molecular skin markers of pediatric AD that are selectively implicated in clinical outcomes related to disease severity and skin itchiness.

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Skin-resident immune cells actively coordinate their distribution with epidermal cells during homeostasis

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Our organs consist of multiple cell types that ensure proper architecture and function. How different cell types coexist and interact to maintain their homeostasis *in vivo* remain elusive. The skin epidermis comprises mostly epithelial cells, but also harbors Langerhans cells (LCs) and Dendritic Epidermal T cells (DETCs). In response to injury or infection, LCs and DETCs become activated and play critical immunological roles. During homeostasis, they coexist with epithelial cells in the basal layer of the epidermis. Whether, and how, distributions of LCs and DETCs are regulated during homeostasis is unclear. Here, we addressed this question by tracking LCs, DETCs and epithelial basal cells over time within the skin of live adult mice. We show that LCs and DETCs maintain their overall position despite continuous turnover of neighboring basal epithelial stem cells. Moreover, LCs and DETCs rapidly and maximally explore basal epithelial cell junctions through their dendritic extensions. Altering the epithelial cell density triggers corresponding changes in the immune cell density, but not vice versa, suggesting that epithelial cells determine immune tissue composition in the epidermis. Moreover, LCs and DETCs are organized in a tiling pattern that is actively maintained. When LCs or DETCs are ectopically removed, neighboring epidermal LCs or DETCs, respectively, move into the emptied spaces and re-establish the tiling pattern. Finally, LCs require the GTPase Rac1 to maintain their positional stability, density and tiling pattern. Overall, we discovered that epidermal cells regulate the density of immune cells during homeostasis, and that immune cells actively maintain a non-random spatial distribution, reminiscent of neuronal self-avoidance. We propose that these cellular mechanisms provide the epidermis with an optimal response to environmental insults.

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Lipidomic analysis of congenital ichthyotic skin suggests disruption in ceramide catabolism

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Congenital ichthyoses are a group of genetic disorders that share skin barrier impairment and generalized scaling and erythema. We found reductions in several lipid-modifying enzymes by RNA-Seq, but epidermal lipid alterations in ichthyosis are poorly understood. Stratum corneum lipids were extracted from tape strips of 85 ichthyosis patients (mean age, 23 yrs) and matched healthy controls (mean age, 32 yrs). Based on targeted lipidomics, ichthyotic skin had lower levels of total ceramides and dihydroceramides, including their monohexyl and lactosyl derivatives (all $p<0.001$). Consistent with reduced expression of *ELOVLs*, ichthyotic skin also had fewer very long chain (>C24) ceramides/ dihydroceramides compared to control skin ($p<0.05$ to $p<0.001$). Using Spearman correlation coefficients, Ichthyosis Area and Severity Index (IASI) and subscore IASI-scaling (IASI-S) showed significant correlations with expression of ceramides and dihydroceramides, especially for lamellar ichthyosis (IASI: $\rho=0.52$, $p=0.008$; IASI-S: $\rho=0.59$, $p=0.002$), implying a compensatory role for scaling to increase lipid expression. In contrast, sphingosine was increased in all subgroups of ichthyosis ($p<0.001$) and significantly correlated with increases in IASI and subscore IASI-erythema (IASI-E), especially for Netherton syndrome (IASI-E: $\rho=0.71$, $p=0.009$). The increased total sphingosine, metabolized from ceramide by ceramidase, suggests high ceramidase activity and not just decreased de novo synthesis to explain the lipid profiles, raising the possible use of ceramidase inhibitors as a novel therapeutic option.

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HSP27 a key element of skin regulation subjected to climate variations

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Humidity, along with other climatic factors such as temperature, can impact the skin health. Indeed, our skin is daily exposed to humidity and temperature variations. All around the world, the daily humidity varies from low to high relative humidity (45% to 90%). In addition, the skin temperature can reach 44°C under sun exposure. The rare studies available show that low humidity causes several changes in the skin (dryness, itchiness, impairment of the desquamation process, skin roughness, and loss skin elasticity). Unfortunately, as most of the studies were performed on murine models, they remain inconclusive about the real effects of humidity and temperature variations on the human skin health. Thus, we developed human skin explant models (heat stress, humidity stress and a combined heat and humidity stresses model) in order to understand the effect of climate on skin physiology. We analyzed epidermis thickness and the expression profiles of loricrin (LOR), filaggrin (FLG), keratin 10 (K10), and HSP27. These markers were chosen upon their relevance in skin homeostasis. In all conditions studied an epidermis thickening was observed suggesting keratinocyte proliferation. In skins exposed to a heat stress, the differentiation process is delayed and decreased. In skins exposed to low humidity an early expression of K10 expression occurs suggesting a disorganized start of the differentiation process. In skins exposed to heat and low humidity the differentiation process of keratinocytes seems to be sustained and overstimulated suggesting an uncontrolled differentiation process. We found that the different environmental conditions studied modulate the expression of HSP27, a central protein regulating LOR and K10 (differentiation process), and the synthesis and processing of proFLG and FLG (epidermis physiology). Our results showed that climatic factors (humidity and/ or temperature) through the modulation of HSP27 expression are responsible in the weakening of skin barrier function due to impairment of skin differentiation process.

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EphA2 is a novel regulator of autolysosome recycling at end-stage autophagy and a key regulator in epidermal proliferation

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Abnormal autophagy is observed in several skin diseases associated with hyperproliferation. We have shown that Beclin1 is increased in psoriatic lesions and when Beclin1 is conditionally knocked out in the epidermis, imiquimod(IMQ)-induced hyperproliferation is markedly attenuated. This indicates that autophagy plays a positive role in psoriasis and contributes to epidermal hyperproliferation in psoriatic lesions. However, how autophagy is regulated in the epidermis of psoriatic lesions is a knowledge gap that needs to be filled. We show that loss of EphA2, a receptor tyrosine kinase, reduces proliferation in mouse epidermis and human keratinocytes. Importantly, expression of EphA2 was shown to be increased in psoriatic lesions. We demonstrate that knockdown of EphA2 in HEKs results in an increase in: (i) large vacuoles; and (ii) LC3II protein, a marker for autophagosomes. To further explore this defect in autophagy, we measured autophagy flux by two well-established approaches: LC3II turnover and p62 levels, and found that loss of EphA2 reduced LC3II turnover in HEKs; and increased p62 in HEKs as well as in mouse epidermis, indicating an inhibition of autophagy flux. To determine the novel interactors of EphA2 in autophagy, we conducted BioID and co-immunoprecipitation assays and confirmed that among the novel binding partners of EphA2 was phospholipase D1 (PLD1). We have shown that PLD1 is a critical regulator of autolysosome recycling at end-stage autophagy. We have also demonstrated that the activity of dynamin1 (one of key players in autolysosome recycling at end-stage autophagy) and its association with autolysosomes is inhibited by PDL1/PKC pathway in HEKs. EphA2 affects autolysosome recycling via inhibiting PLD1/PKC signaling and thus ensuring dynamin1 activity and its association with autolysosome. Thus, we conclude that upregulation of EphA2 in the "activated" epidermis contributes to enhanced autophagy and consequently a hyperproliferative state.

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Using a cosmetic technology to increase expression of 25-hydroxyvitamin D3 1-α hydroxylase in human keratinocytes

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Vitamin D3 is made in the skin through the photolysis of 7-dehydrocholesterol by UVB between 295-303 nm or obtained through food or supplements. Vitamin D3 does not have significant biological activity and must be converted to a biologically active form in a multistep reaction by either entering the blood stream where activation occurs in the liver then the kidneys or activation in the skin to be used locally. Interestingly, keratinocytes are the only cells in the body that can perform this process from start to finish. Active Vitamin D3 or calcitriol is important for proper skin function and 25-hydroxyvitamin D3 1-α hydroxylase (CYP27B1), a rate-limiting enzyme in the formation of active form of vitamin D3, was shown to be required for optimal epidermal differentiation and permeability barrier homeostasis. Therefore, identifying compounds that can increase expression of 25-hydroxyvitamin D3 1-α hydroxylase in skin is a viable approach to develop cosmetic products aiming to increase synthesis of active form of Vitamin D3 in skin, which could lead to barrier homeostasis. Human keratinocytes were treated *in vitro* with a cosmetic technology. After 24 hours of treatment, these keratinocytes exhibited an 86% increase in 25-hydroxyvitamin D3 1-α hydroxylase expression ($p<0.05$), normalized to GAPDH, compared to the untreated control. This increase suggests that the cosmetic technology could help facilitate the formation of Vitamin D produced in the skin, which has implications in barrier improvement. In-house clinical study of immediate barrier shows that a formulation containing patented technology blend significantly improve skin's barrier 15 minutes after a single application compared to baseline and untreated control, as shown via TEWL measurements. This further suggest the role of this cosmetic technology in supporting skin barrier homeostasis.

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Mapping of the biophysical properties of pregnant women abdomen skin: A pilot study

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During pregnancy mechanical stretching of abdomen skin due to baby growth is very important and could lead to skin breakage (also known as striae distensae or stretch mark). Recent work demonstrated that biomechanical properties of healthy abdomen skin change drastically during pregnancy and that these properties remain altered 4 months after delivery. It remains unclear if these observed modifications are homogeneous on the abdomen area or if a specific area is more affected. The aim of this pilot study is to perform a mapping of abdomen skin properties of a woman at 8 months of pregnancy using various non-invasive techniques in order to evaluate if gradient or specific pattern of these properties exist. 25 measurement points have been defined on one half of the abdomen. Assessments performed included hydration (Corneometer CM825), Transepidermal Water Loss (TEWL, Vapometer SWL-5), mechanical properties (Cutometer SEM 575) and thickness and echogenicity of the skin (Dermascan 20 MHz). Mapping of each property has been performed by interpolation algorithm using Octave software. Results obtained show that skin biophysical parameters were not homogeneous on abdomen as illustrated by figure 1 with skin thickness. Moreover, specific locations exhibited particular properties. High variations of the measurements between the 25 points were observed: 104% for hydration, 40% for TEWL, 134% for echogenicity, 47% for thickness and 30% to 160% for biomechanical properties. Study of the relationships between all measured parameters demonstrated that some skin properties are significantly correlated while others are independent. This pilot study demonstrated that the skin properties of women at 8 months of pregnancy are not homogeneous on the abdomen, tending to show that specific area could be more affected and therefore more subjected to stretch mark formation. A future study will compare women at different stages of pregnancy to quantify the variations of skin properties on the abdomen along pregnancy time.

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Tulsi extract induces a global defense and protection against endogenous and exogenous stresses contributing to skin aging

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Tulsi, or « sacred basil », has been traditionally used in ayurvedic medicine, in which this adaptogen plant is considered as an elixir of life. We developed a new active ingredient rich in polyphenols extracted from the leaves of *Ocimum sanctum*. Here, we have investigated the biological efficacy of the extract *in vitro*. We show that *O. sanctum* leaf extract significantly inhibited the production of reactive oxygen species by normal human keratinocytes stimulated by hydrogen peroxide. The production of nitric oxide induced by cortisol in keratinocytes was also significantly reduced by the extract. Cell bioenergetics as well as autophagy/mitophagy processes have been evaluated in primary human fibroblasts exposed to standardized urban pollutants. The extract was able to normalize energy efficiency by restoring oxygen consumption rate and ATP level that were decreased by the pollutants. It also reversed the pollution-induced increase in damaged mitochondria and normalized mitophagy and autophagy in pollution-exposed cells. Taken together, these results suggest a protection of the mitochondrial network. Finally, the extract was evaluated in a model reproducing inflammation processes observed during aging and known as inflamm'aging. Conditioned medium obtained from reconstructed epidermis subjected to inflammatory stimulation was applied on dermal fibroblasts. Typically, in these conditions, the expression of a number of genes linked to ageing processes is altered in fibroblasts, in particular markers of inflammatory processes, stress response and dermal matrix. The extract was able to normalize the modulation of gene expression in fibroblasts induced by inflamm'aging. Overall, these results suggest that the newly developed *Ocimum sanctum* leaf extract contributes to the skin global defense and helps it fight against exogenous and endogenous stress. The extract was able to neutralize the impact of these stresses which, in the long-term, can accelerate skin aging process.

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Development of a minimally-invasive method, guided by *in vivo* non-invasive imaging, to sample atopic skin

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The application of molecular profiling techniques, typically performed on punch biopsies, has been an important recent advance in skin research. This has enhanced the understanding of the pathophysiology of major skin diseases such as atopic dermatitis (AD), and the development of novel targeted therapeutics. AD is a highly prevalent, relapsing, chronic inflammatory skin disease, characterized by eczematous plaques and intense pruritus, more frequent in children than in adults. In children, however, punch biopsies are neither feasible nor desirable and minimally- or non-invasive techniques are required to assess AD severity and treatment effectiveness. We have developed a novel skin sampling method using minimally-invasive, adhesive tape-stripping, guided by *in vivo* imaging with dermoscopy, reflectance confocal microscopy, and optical coherence tomography. The goal was to demonstrate the reliable and reproducible acquisition of RNA and proteins from the skin removed on a limited number of tape-strips. Method optimization involved range-finding experiments sampling the skin of 30 healthy volunteers (5 adults, 25 children). With our refined approach, which required acquisition of only 11 tapes, the amounts of RNA and protein extracted were equivalent to those previously reported with techniques that, in our experiments, resulted in non-acceptable skin erosions. Our minimally-invasive method enables sample collection to be performed faster and more comfortably for the patient, which is crucial in clinical practice.

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Enzymatically-derived hydroxy-lumisterols regulate epidermal keratinocytes and act as agonists on the aryl hydrocarbon receptor (AhR)

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We recently discovered pathways of lumisterol (L3) activation by CYP11A1 and CYP27A1, with resulting CYP11A1-derived hydroxymetabolites being detectable in the human serum and epidermis. CYP11A1-derived hydroxylumisterols induced keratinocyte differentiation and photoprotective actions against UVB. In follow-up studies we detected hydroxyproducts of CYP27A1 action on L3, 25(OH)L3, 25R27(OH)L3 and 25S27(OH)L3, in human epidermal keratinocytes and serum. They inhibited cell proliferation and stimulated keratinocyte differentiation. Previously we made the unexpected discovery that vitamin D3 hydroxyderivatives act as ligands for the AhR. Therefore, we investigated possible interactions of both CYP11A1 and CYP27A1 hydroxylumisterols with AhR. Molecular docking using crystal structures of the ligand binding domains (LBDs) of AhR revealed high docking scores for L3 hydroxyderivatives that were even better than their natural ligands, predicting tight binding of L3-derivatives to the receptor. Functional studies using a human AhR reporter assay system revealed marked activation of AhR by a series of L3-hydroxyderivatives. Finally, L3-hydroxyderivatives stimulated the expression of *CYP1A1* and *CYP1B1* genes, which are downstream targets of the AhR signaling pathway. These results are consistent with translocation of the AhR from the cytoplasm to the nucleus. Molecular dynamics simulations predicted that binding of the L3 derivatives induced conformational changes to AhR which could mediate AhR-dependent signaling. Thus, the discovery of these pathways not only challenges existing dogmas that lumisterol is biologically inactive, but together with our identification of L3 derivatives as ligands for AhR, they open new possibilities for regulation of epidermal barrier functions and nuclear receptor signaling in general.

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Metabolomic identification of an essential glucose-IRF6 axis in differentiation

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Advances in high throughput metabolomics enable discovery of new essential roles for biomolecules in processes such as epidermal differentiation. Metabolomic analysis during keratinocyte differentiation that detects >14,000 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 showed that intracellular glucose elevation was essential for differentiation. Metabolites in glucose catabolic pathways were unchanged in differentiation, suggesting that the accumulated pool of glucose itself was required. Consistent with this, decreasing cellular glucose levels, by restricting available glucose or by increasing intracellular glucose catabolizing enzymes, HK1/2 and G6PD, blocked differentiation. Knockout and pharmacologic inhibition studies demonstrated that 3 glucose transporters, GLUT1, GLUT3 and SLGT1, were essential for glucose accumulation and differentiation. Furthermore, RNAseq analysis of glucose-depleted epidermal tissue revealed 34% of the genes downregulated by glucose are part of the epidermal differentiation gene signature. ATACseq identified candidate transcription factors (TFs) that may act on glucose-regulated genes, including ZNF750, NFE2L2, and IRF6. Glucose affinity chromatography followed by mass spectrometry identified the IRF6 TF as a glucose binding protein. IRF6 was essential for epidermal differentiation and was verified to bind glucose directly at high affinity. Glucose was found to enhance 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 free intracellular glucose, which in turn binds to IRF6 and enables IRF6 DNA binding and IRF6-driven differentiation gene induction.

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Unbound corneocyte lipid envelopes in 12R-lipoxygenase deficiency support a direct role in lipid-protein crosslinking

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Loss-of-function mutations in arachidonate lipoxygenase 12B (ALOX12B) are an important cause of autosomal recessive congenital ichthyosis (ARCI). 12R-lipoxygenase (12R-LOX), the protein product of ALOX12B, has been proposed to covalently bind the corneocyte lipid envelope (CLE) to the proteinaceous corneocyte envelope (CE), thereby providing a scaffold for the assembly of barrier-providing, mature lipid lamellae. To test this hypothesis, we performed an in-depth ultrastructural examination of CLEs in ALOX12B-deficient human and mouse epidermis, extracting samples with pyridine to distinguish covalently attached CLEs from unbound (i.e., non-covalently bound) CLEs. ALOX12B-/- stratum corneum contained abundant pyridine-extractable (i.e., unbound) CLEs, compared to normal stratum corneum. These unbound CLEs were associated with defective post-secretory lipid processing, and were specific to 12R-LOX deficiency, since they were not observed with deficiency of the related ARCI-associated proteins, patatin-like phospholipase 1 (PNPLA1)- or abhydrolase domain containing 5 (ABHD5). These results suggest that 12R-LOX contributes directly to CLE-CE crosslinking, which appears to be a prerequisite for post-secretory lipid processing, and provide insights into the pathogenesis of 12R-LOX deficiency in this subtype of ARCI, as well as other conditions that display a defective CLE.

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Correlation of 12r-lox activity and hsp70 with barrier function

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The evaluation of the skin health is an essential step in the skincare industry in order to assess the safety and the efficacy of active ingredients. 12R-LOX (gene ALOX12B) is a lipoxygenase expressed in keratinocytes and is implicated in the oxygenation of the esterified omega-hydroxyacyl-sphingosine (EOS) ceramides, a required process to their covalent linkage to proteins of the cornified envelope. It is an important step in establishing the water barrier by preventing unnecessary evaporation through epithelial cells. HSP70 family members are among the most abundant HSPs in the skin expressed constitutively within keratinocytes. HSP70 expression is elevated in both epidermis and dermis for cytoprotection after skin samples are heat shocked or after stressors like UVB. Traditionally transepidermal water loss (TEWL) has been widely used as a way to evaluate skin barrier function and tape stripping as a way to evaluate biological markers (biomarkers). Our aim was to understand how TEWL, 12R-LOX and HSP70 could be correlated clinically. Subjects were treated with a hydrating cream for 3 weeks on their lower inner forearm. After treatment, TEWL was measured and tape strips were collected from the treatment area and untreated site. 12R-LOX enzyme activity was measured with a fluorescence-based assay and HSP70 by ELISA using the protein samples isolated from the tape stripes. The 12R-LOX enzyme activity had a negative correlation with TEWL as previously described and had a positive correlation with HSP70. Moreover, HSP70 also had a negative correlation with TEWL confirming the correlation between the two biomarkers 12R-LOX and HSP70 with barrier function. The study of these two biomarkers is a new interesting and non-invasive approach for studying skin under different conditions.

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Comparing hydration levels in healthy normals vs. atopic dermatitis and xerosis cutis using a novel wireless, non-invasive sensor

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The objective measurement of skin barrier function, essential in the management of atopic dermatitis (AD) and xerosis cutis (XC), are limited to bulky, expensive, and user-dependent tools such as corneometers. Herein, we report the development and validation of a novel, wireless, low-profile, and flexible skin hydration sensor able to directly measure skin water content using thermal conductivity. The aim of this study was to show a measurable difference in skin hydration of healthy normal subjects vs. subjects affected by AD and XC using this novel sensor. The novel device uses a thermal actuator and multi-sensor module to apply thermal energy to the skin and capture the corresponding temporal changes in temperature. The temperature difference between when the actuator is on and off is input to a thermal transport model, where computational modeling connects the temperature change with hydration levels of the skin. A total of 44 subjects were recruited for this study, including 25 healthy normal subjects, 5 subjects with AD, and 14 subjects with XC. Subjects were placed in the XC group if their overall dry skin score was $\geq$1. Measurement sites included the arm, leg, and forehead. These locations were selected for accessibility with the forehead as an internal control known to have higher hydration. A one-way analysis of variance was done to compare hydration readings. Hydration readings were higher in healthy normal subjects, both overall and at each site. The overall mean hydration was 78.3% (CI 77.2 − 79.4) in healthy normal subjects vs. 61.9% (CI 59.4 − 64.4) for subjects with AD and 53.5% (CI 51.3 − 55.7) for subjects with XC ($p < 0.001$). Using a novel sensor, the skin hydration of subjects with AD and XC is demonstrably lower compared to healthy normal subjects. A wearable, accurate, and low-burden skin hydration sensor would support drug development, detect small but clinically meaningful changes in disease severity, and track response to treatment.

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Physiological function of Krox20 (Egr2) in epithelial stem cells

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Resident stem cells (SCs) within tissues are important for normal homeostasis maintenance and wound repair. This is mediated by the ability of SCs to properly self-renew, maintain their identity, and differentiate; hence the importance of understanding the key mechanisms underlying SC physiology. Krox20, a zinc finger-containing transcription factor, is well known for mediating stem and progenitor cell activation and differentiation in a variety of tissues. In a recent study, we reported for the first time a population of epithelial-derived Krox20-expressing keratinocytes in the hair follicle that ultimately terminally differentiate to form the structural component of the hair shaft. These Krox20 lineage cells in the hair follicle also mediate melanocyte differentiation via Stem Cell Factor production for hair pigmentation. In light of the importance of Krox20 in other cells types, the role of Krox20 cells in epidermal and hair development warrants the elucidation of Krox20 function in epithelial cells. We report here that ablation of Krox20 in skin epithelial cells caused spontaneous hair loss, correlated with increased epidermal differentiation. On the other hand, the overexpression of Krox20 in epithelial cell lines resulted in the upregulation of various epidermal SC markers, suggesting maintenance of stemness as a potential role of Krox20 in epithelial cells. Moreover, we also observed a reduction of apoptosis in Krox20-overexpressing cells, pointing to an additional Krox20 function in regulating cell survival. Analysis of the molecular mechanisms underlying these observations showed that they occur through the modulation of Notch and Wnt/βcatenin pathways. These results highlight the importance of Krox20 in regulating epidermal homeostasis, through the maintenance of a resident SC population.

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The long noncoding RNA *PRANCR* regulates epidermal homeostasis and wound healing through alternative splicing of fibronectin-1

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Most human genes undergo alternative splicing (AS), but the molecular mechanisms controlling AS are largely unknown; and the functional consequences of most AS events remain uncharacterized. AS regulation is critical for skin development. For example, expression of specific isoforms of the epidermal transcription factor *TP63* are required for epidermal stratification. Over the recent years, long noncoding RNAs (lncRNAs) are emerging as novel regulators of AS. Here, we investigated whether *PRANCR*, a lncRNA that we recently discovered as essential for epidermal progenitor renewal, functions by regulation of AS. Using transcriptome-wide analysis, we demonstrated that *PRANCR* controls 238 AS events in epidermal keratinocytes. Specifically, we show that *PRANCR* promotes expression of an mRNA isoform containing extra-domain A (EDA) in the keratinocyte cell fate gene fibronectin-1 (*FN1*). Inclusion of the EDA domain (EDA+) is promoted by the serine/arginine-rich splicing factors (*SRSF*s) 1 and 7 and *PRANCR* is required for full expression of these factors. Depletion of *PRANCR* or the FN1 EDA+ isoform both lead to equivalent proliferation defects and severe delays in *in vitro* keratinocyte migration, consistent with skin wound healing defects reported in FN1-EDA deficient mice. Aberrant AS of FN1 EDA+ isoforms have been associated with fibroblasts in psoriasis and scar formation during wound repair, and our results indicate intrinsic AS of FN1 is also important in epidermal keratinocytes. Collectively, we identify an epidermal lncRNA that regulates epidermal proliferation and migrations by controlling AS of an important keratinocyte cell fate gene.

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Environmental stress protection and inflammaging prevention: A novel synergistic antioxidant blend

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We present an efficient approach to slowing the progression of skin damage using a multi-mechanistic antioxidant blend suitable for cosmetic formulations and validate its performance *in vitro*. Three different mechanisms were targeted simultaneously: scavenging reactive oxygen species (ROS), mitigating intracellular ROS and reducing ROS-induced inflammaging markers. Using a Design of Experiment approach, a novel, non-phototoxic synergistic blend of antioxidants was identified and characterized *in vitro* for its ability to quench free radicals (DPPH assay) and intracellular ROS generated by UVA (DCFH assay). Further, we demonstrated that use of the blend results in a mitigation of markers correlated with inflammaging (photoaging and hyperpigmentation) caused by environmental oxidative stress (PGE_2, IL-8, MMP-1).[3] These results support the notion that a multi-mechanistic antioxidant blend may effectively alleviate environmentally induced skin damage and be easily incorporated into skin care formulations providing an anti-inflammaging benefit. Brewer, M.S. "Natural Antioxidants: Sources, Compounds, Mechanisms of Action and Potential Applications." *Comprehensive Reviews in Food Science and Food Safety*, vol. 10, 2011; McMullen, Roger L. *Antioxidants and the Skin*. Allured Books, 2013; Oswald, T., Crane, C.M., Dueva-Koganov, O., Bianchini, R. Design of Experiments to Optimize a Novel Antioxidant Blend [conference presentation]. Innovations in Dermatological Sciences, Rutgers, NJ 2018.

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Heterochromatin maintenance is crucial for terminal keratinocyte differentiation and inhibition of inflammatory responses in the epidermis

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Mammalian genome is largely populated with transposable elements (TEs) including endogenous retroviruses (ERVs), largely distributed in the constitutive and facultative heterochromatin. TE silencing in the genome include DNA methylation and repressive post-translational histone modifications. Aberrant reactivation of transposable elements has significant impact on normal mammalian development and pathobiology of multiple immunological disorders in many organs. H3K9me3 methyltransferase SETDB1 and SWI/SNF chromatin-remodeling protein LSH regulating heterochromatin maintenance are both expressed in the epidermal keratinocytes (KCs). Conditional Krt14-driven *Setdb1* and *Lsh* gene ablation leads to marked alterations in the epidermal structure, development of skin inflammation, and premature death. These phenotypes were associated with de-repression of repetitive sequences, increased expression of ERV-specific dsRNAs and, as a result, induction of interferon-mediated activation of innate immune response in skin. ATAC-seq and ChIP-seq analyses of primary KCs isolated from LshKO and Setdb1KO mice showed alterations in distribution of heterochromatin domains compared to controls. RNA-seq analysis showed upregulation of multiple antiviral response pathways activated by ERVs including cytoplasmic RNA sensor MDA5, helicases LGP2, RIG-I and proinflammatory cytokines. In addition, loss of SETDB1 in KCs leads to significant upregulation of proteins interacting with short chain non-coding regulatory RNAs, such as PIWIL2, TDRD1, TDRD12, RNF17 and RNF165. Thus, these data reveal distinct mechanisms of heterochromatin maintenance and retrotransposon silencing in the epidermis mediated by Setdb1 and Lsh and suggest their role in the control of epidermal homeostasis and inflammatory skin conditions.

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The clock protein BMAL1 maintains the diploid status of human keratinocytes via a functional interaction with c-myc

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A diverse array of biological processes is under the control of the circadian clock composed of four core proteins, including BMAL1, CLOCK, PERs, and CRYs. These circadian oscillators are present in major cell types within different skin compartments and regulate diverse aspects of skin homeostasis at the local level. We found that loss of BMAL1 promoted differentiation in human keratinocytes. This effect was accompanied by a significant increase in the cell population with polyploidy and strong induction of r-H2AX, a marker for DNA damage. These results indicate that BMAL1 is crucial for maintaining genome stability and proliferation potential in human keratinocytes. Mechanistic studies showed that loss of BMAL1 enhanced the expression c-myc, a pro-oncogene with the pro-differentiation function in keratinocytes. More importantly, co-depletion of c-myc with BMAL1 genes could reverse premature differentiation and polyploidy caused by the loss of BMAL1. These data suggest that the clock protein BMAL1 plays an essential role in maintaining the diploid stem cell potential by suppressing the expression and activity of c-myc in human keratinocytes.

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Translation and growth pathways are directly influenced by autoimmune regulator (Aire) in skin keratinocytes

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Autoimmune regulator, Aire, is commonly known to function as a transcriptional regulator in medullary thymic epithelia. However, we and others have identified Aire to express and function in non-thymic tissues, including the skin, where it greatly influences inflammation and tumorigenesis. We report here that, within skin keratinocytes, Aire participates in a variety of cellular processes outside of its classically defined role in the nucleus. We employed a proximity based biotinylation screen (BioID) to examine the localization of Aire and its binding partners within keratinocytes. Aire is observed by structured illumination microscopy to localize in distinct subcellular compartments within the nucleus, cytoplasm, and along cytoskeletal tracks. Disease causing and function blocking mutations in Aire substantially alter these distribution patterns in keratinocytes, suggesting a link between Aire subcellular compartmentalization and pathogenesis. Using an isobaric labeling method (iTRAQ) coupled to tandem mass spectrometry, we identified 295 common Aire binding partners (99.0% probability), most of them novel, and quantitatively assessed the impact of Aire mutations on binding partner associations. Specifically, Aire binding partners associated with protein translation and cell growth (e.g. multiple 40S and 60S ribosomal protein subunits, cell growth-regulating nucleolar protein, eukaryotic translation initiation factors 4 and 5, elongation factor 1 alpha 1) were significantly altered in cells expressing mutant Aire compared to wild-type Aire. Serum pulse assays confirm a positive correlation between loss of wild-type Aire expression in keratinocytes and increased translation associated signaling ($P\text{-}AKT^{S473}$, $P\text{-}S6K^{T389}$, $P\text{-}S6^{S235/S236}$, $P\text{-}4EBP1^{S65}$, $P\text{-}eIF4B^{S422}$, and $P\text{-}eEF2K^{S366}$). These newly identified partners for Aire expand our understanding of Aire function in the skin and may provide a basis for the pro-tumorigenic role for Aire in skin cancers.

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Optimization of the barrier function of a tissue-engineered skin model through supplementation of cell culture media with docosahexaenoic acid

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Percutaneous absorption studies showed that tissue-engineered skin models are more permeable than normal human skin. These observations were partly explained due to the lower levels of polyunsaturated fatty acids, such as docosahexaenoic acid (DHA), found in the epidermal phospholipids of the skin model. In this study, we investigated the impact of a supplementation of the culture media with DHA on the barrier function of a reconstructed skin model. To this end, tissue-engineered human skin substitutes were produced according to the self-assembly method using culture media supplemented with 10 mM DHA and compared with their respective counterparts. The skin substitutes produced with or without DHA presented similar skin morphology, as they both displayed a differentiated epidermis. Moreover, the supplementation with DHA did not influence the skin substitute thickness. Percutaneous absorption of testosterone assayed using a Franz cell diffusion system was significantly decreased in skin substitutes produced with DHA, showing that addition of DHA into the culture media can affect skin impermeability in vitro. The incorporation of DHA into the phospholipid fraction of the epidermis was evaluated using gas chromatography analyses. According to these analyses, higher levels of DHA were measured in the epidermal phospholipids of the supplemented skin models, showing successful incorporation of DHA. Furthermore, retroconversion of DHA was registered in the skin substitutes as increased levels of eicosapentaenoic acid were measured in the epidermis after DHA supplementation. Taken all together, these results showed that the addition of DHA into the culture media modulates the lipid profile of the skin models, leading to an improved skin barrier function.

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Skin barrier dysfunction initiates psoriasis inflammation via activating FPR1-ER stress-NLRC4 axis in keratinocytes

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Decreased skin barrier function may aggravate or even initiate psoriasis, and aiding barrier reestablishment using emollients helps improve the psoriatic symptoms, but the mechanisms for such changes have remained unclear. Here we show that epidermal barrier defect caused by tape stripping or topical use of acetone exacerbates the psoriasis-like inflammation, such as increasing the expressions of inflammasome NLRC4, its downstream cytokines, and other pro-inflammatory mediators, in IMQ-induced mouse model. And FLG deficiency mice also exhibit severer psoriasis-like inflammation when treated with IMQ. In turn, topical application of emollients rescues the epidermal barrier injury and skin inflammation. Moreover, silencing NLRC4 also markedly reduces the psoriasis-like inflammation *in vivo*. Through RNA-sequencing analyses we show that the epidermis from FLG deficiency mice overexpresses the pattern recognition receptor FPR1, activating which will up-regulate NLRC4, IL1B, IL18, and other immune-related genes. In *in vitro* experiments, we further show that FPR1 regulates the PERK-eIF2α (ER stress) pathway to modulate NLRC4 expression and activation, thus contributing to the immune responses of keratinocytes. Importantly, FPR1 antagonist also attenuates the skin symptoms and normalizes the barrier dysfunction in psoriasis-like mice. Taken together, our findings suggest that epidermal barrier dysfunction aggravates inflammation by activating FPR1-ER stress-NLRC4 in keratinocytes, which is responsible for the feed-forward amplification of inflammatory responses in psoriasis. This work identifies FPR1 or NLRC4 as a novel potential therapeutic target for psoriasis and other inflammatory skin diseases involving the skin barrier homeostasis.

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Barrier disruption and inflammatory skin models to evaluate cosmetic formulations on lipid metabolism

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Primary function of the skin is to protect the body from environment and excessive water loss. Skin is characterized by active lipid metabolism and lipids play crucial roles both in terms of structural integrity and functionality. Lipid metabolism can be modified by skin barrier alteration and inflammation leading to modification of ceramides and free fatty acids ratio inducing barrier dysfunction. Aim of this study was to investigate, on 3-dimentional skin model, barrier alteration effect and inflammation on lipid metabolism (enzymes expression and lipid levels) and to evaluate different formulations in order to balance negative impact of the stress. *Ex vivo* human skin model exposed to SDS and reconstructed epidermis model with TNFα stress were used. Enzyme expression (ALOX12B and EVOL1) was performed by immunofluorescence staining with confocal microscopy. Ceramides, free fatty acids and cholesterol synthesis were analysed using chromatographic method. Mass spectrometry imaging (MSI) allowed to follow skin penetration of lipophilic ingredients of formulations. SDS barrier alteration decreased significantly ALOX12B expression and increased EVOL1 expression. Epidermal lipids synthesis was also modified after inflammatory stress, in particular, ceramides ratio leading to impairment of the barrier function. Same trends were described *in vivo* for pathologies like ichthyosis, atopic dermatitis and psoriasis. The different lipidic constituents of formulations penetrated and were localized into the SC and viable epidermis by MSI. Rhealba® oat plantlets extract formulation permitted to restore lipid metabolic disorders and balance ceramides ratio. Skin models demonstrated the impact of barrier alteration and inflammation on lipid metabolism. These skin models can be used to evaluate cosmetic formulations on lipid metabolism to preserve barrier function with can be specifically altered in different inflammatory pathologies.

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Cytokine milieu of inflamed skin regulates peptidylarginine deiminases and deimination in keratinocytes

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Protein post-translational modifications form a vital component of inflammatory microenvironment and are critical in the pathogenesis of multiple skin disorders. One among them is protein deimination or citrullination, catalyzed by peptidyl-arginine deiminases (PADs). Expression of these enzymes in tightly controlled during differentiation of keratinocytes, however the regulatory elements are still unknown. Here we show that the cytokines infiltrating inflamed skin, regulate PADs expressed by keratinocytes. Transcription profiling by qRT-PCR in 3D skin equivalents and differentiated epidermal keratinocytes revealed that pro-inflammatory cytokines suppress the expression of PADs at RNA ($p<0.0001$) level. Western blotting and antibody-based PAD activity assay ($p<0.05$) in primary keratinocytes revealed decrease in enzymatic activity of PADs in presence of high concentrations of cytokines. Considering lesional psoriatic skin is enriched with immune cells, we hypothesized that PAD expression would be lower in patients compared to healthy controls. To test this, we checked the expression of PAD isotypes in biopsies isolated from paired lesional and non-lesional skin of patients suffering with plaque psoriasis and from healthy controls. We saw a decreased expression of PADs in lesional epidermis ($p<0.037$) as compared to healthy controls. Further, decrease in PAD expression in keratinocytes had a functional impact on overall deimination of proteins, especially filaggrin ($p<0.05$) and keratin ($p<0.05$), known to be crucial for epidermal differentiation. Drugs used to treat skin disorders involving keratinization defects could turn around this deleterious effect, by boosting overall deimination.These data not only support the important role played by deimination in skin barrier function, they identify a potential effect of cytokine environment on epidermal keratinocytes.

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A two-phase model of epidermal stratification: Lessons from centrosomes

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The stratified skin epidermal barrier in mammals develops from a single-layered epithelium. The mechanisms initiating epidermal stratification and how the process is fueled are still not well-understood. Previous studies proposed a central role for perpendicular/asymmetric cell division orientation of the basal keratinocyte progenitors in epidermal stratification. Here, we used centrosomes, that organize the mitotic spindle, to test whether cell division orientation and stratification are linked. Genetic ablation of centrosomes from the developing epidermis led to the activation of the p53-dependent mitotic surveillance pathway resulting in reduced epidermal thickness and hair follicle arrest. Double mutant keratinocyte progenitors lacking both centrosomes and p53 rescued the epidermal phenotypes. Importantly, the mutant progenitors significantly altered their division orientation in the later developmental stages without affecting stratification or differentiation. Based on time-lapse imaging and analysis of tissue growth dynamics, our data suggested a two-phase model of epidermal stratification. The first and major phase of epidermal stratification is characterized by highly proliferating basal and suprabasal transit-amplifying cells as well as cell delamination, while the second phase may be uncoupled from the division orientation of the basal progenitors. The data provide insights for tissue homeostasis and hyperproliferative diseases that may recapitulate developmental programs.

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Role of voltage-gated T-type calcium channel in acute and chronic itch

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Peripheral signaling of itch begins from the activation of nerve endings via itch mediators that leads to membrane depolarization by opening of transient receptor potential channels that results in the opening of voltage-gated ion channels, which will trigger an action potential. T-type voltage-gated Ca^{2+} channels control the action potentials in excitable cells and are expressed in nociceptive fibers and well known in pain processes, but little is known about their role in itch. Here, we demonstrate that the Cav3.2 subunit of T-type Ca^{2+} channels are expressed in the small sized mouse dorsal root ganglia (DRG) and co-localized with Mrgprs, G protein-coupled receptors expressed exclusively in peripheral sensory neurons, function as itch receptors. Cav3.2 T-type calcium channel is expressed in the sensory nerve endings in the intraepidermal nerve fiber in normal human skin and the density of Cav3.2 T-type channel positive nerves are increased in the lesional skin of atopic dermatitis patients. Pharmacological inhibition of T-type channels by mibefradil attenuated nonhistaminergic itch mediators such as CQ, BAMB8-22, PAR2 agonist peptide, IL-31, and TSLP-evoked scratching behavior in mice. DRG and trigeminal ganglia in the MC903 mouse model of atopic dermatitis showed increased expression of Mrgprs and Cav3.2 and enhanced membrane localization of Cav3.2. Pharmacological inhibition of T-type channels also significantly attenuated itch-evoked scratching in the MC903 mouse model of atopic dermatitis. Together, we demonstrate that T-type calcium channel plays an important role in acute and chronic itch.

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Disruption of nucleolar functions variably affect epidermal differentiation

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Nucleoli are multifunctional organelles and the site of ribosome synthesis, which is critical for protein translation. A hallmark of epidermal differentiation is the expression of a defined set of proteins in suprabasal keratinocytes where each epidermal layer has a unique identity and protein profile. However, the link between nucleolar function and cell differentiation remains unexplored. To determine whether nucleoli play a role in the process of epidermal differentiation, we examined nucleoli number and area in different epidermal layers of mature 3D human skin equivalents (3D HSE). As keratinocytes became more differentiated, nucleolar number (fibrillarin punctae) and area (relative to the nucleus) decreased ($P \leq 0.1$). Blocking differentiation by silencing EPHA2 receptor tyrosine kinase reduced the number of nucleoli in the basal layer and increased their area ($P < 0.0001$). These results demonstrate the dynamics of nucleoli during keratinocyte differentiation. To address whether nucleolar structure and/or ribosome synthesis are important for differentiation, we knocked down POLR1A (segregating nucleolar structure) or UTP4 (disrupting processing of rRNA without segregating nucleoli) in 3D HSE. Knockdown of either protein reduces ribosome synthesis. Assessment of tissue morphology revealed both knockdowns reduced epidermal stratification. However, there were contrasting effects on the extent of differentiation. POLR1A knockdown accelerated differentiation with increased deposition of keratohyalin granules and enhanced cornification. In contrast, loss of UTP4 impaired these aspects of terminal differentiation. Further, filaggrin expression was enhanced in siPOLR1A 3D HSE tissue sections, but reduced in UTP4 knockdown samples. The changes in nucleolar characteristics and the variable effects of nucleolar segregation on keratinocyte stratification and protein expression suggest a functional role for nucleoli in the process of epidermal differentiation. Future studies will tease out the mechanisms of nucleoli in keratinocyte differentiation.

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Dermatologic manifestations of *PIK3CA*-related Overgrowth Spectrum (PROS)

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Background: *PIK3CA*-related overgrowth spectrum (PROS) describes a diverse group of rare syndromes caused by postzygotic, somatic mutations in the phosphatidylinositol-3-kinase (PI3K) signaling pathway. PROS is characterized by complex vascular malformations associated with soft tissue and bony overgrowth. In PROS, inconsistent nomenclature, siloed awareness among experts, and the lack of effective treatment options greatly impact diagnosis, treatment, and patient quality of life. Here we detail the dermatologic manifestations of PROS and current clinical management options, highlighting areas for further investigation. Methods: A review of literature was conducted before 12/14/20. Search terms included "*PIK3CA*" or "*PIK3R1*" and various related overgrowth disorders, with a focus on dermatologic findings. Findings were supplemented with expert clinical experience. Results: The literature search returned an initial 184 publications; 90 of these included dermatologic findings. Dermatologic manifestations include vascular stains, venous malformations, lymphatic malformations, and epidermal nevi. The clinical management of patients with PROS focuses primarily on symptoms: pain, swelling, and lymphedema, and on treatment and prevention of hematologic abnormalities, including thrombosis and pulmonary embolism. Current interventions include laser therapy, surgical debulking, intravascular ablation (sclerotherapy, laser, and embolization), anticoagulation therapy, pain management, and oral sirolimus. These treatments have limited efficacy and symptom recurrence is common. No FDA-approved pharmacologic treatments for PROS exist, and topical and systemic agents targeting the PI3K pathway are being investigated. Conclusions: Diagnosis, classification, and treatment of PROS remain challenging due to phenotypic heterogeneity and existing treatment options with limited efficacy. Ongoing genomic advancements may lead to promising future targeted therapies.

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Peripheral nervous system degeneration in patients with xeroderma pigmentosum

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Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder with defective DNA nucleotide excision repair (NER). XP is characterized by photosensitivity with a greater than 2,000-fold increased frequency of sunlight induced skin cancer. Approximately 25% of XP patients develop progressive neurological degeneration. XP patients have defects in 8 complementation groups (XP-A to G and XP variant). Patients in complementation groups XP-A and XP-D have absent deep tendon reflexes, loss of cognition, cortical and cerebellar degeneration, progressive neurosensory hearing loss and peripheral neuropathy (PN). The frequency of PN in different XP complementation groups is unknown. We retrospectively reviewed nerve conduction studies (NCS), hearing evaluations and brain imaging studies from XP patients evaluated at the NIH from 1986 to 2015. There were 33 patients [20.7±13.6 years (range 3-54 years): XP-A (9 patients), XP-C (7 patients), XP-D (10 patients), XP-E (1 patient), XP-V (4 patients), and XP with unknown mutations (2 patients). None of the XP-C patients demonstrated sensorineural hearing loss, imaging abnormalities or PN. The XP-A and XP-D patients had similar sensorineural hearing loss and brain imaging findings. However, the PN observed in XP-A and XP-D patients differed: 78% (7/9) of XP-A patients had sensory and motor neuropathy but 50% (5/10) of XP-D patients had sensory neuropathy and none (0/10) had motor involvement ($p<0.01$). This suggests that DNA repair plays a role in the maintenance of the nervous system both centrally and peripherally.

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Complex phenotypes in trichothiodystrophy patients with *XPD (ERCC2)* mutations

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Trichothiodystrophy (TTD), Cockayne syndrome (CS) and Cerebro-Oculo-Facial-Skeletal syndrome (COFS) are rare, autosomal recessive disorders with defective DNA repair. Overlaps of different DNA repair disorders in the same patient were reported including xeroderma pigmentosum (XP)/CS complex and XP/TTD. We describe 6 TTD patients in 5 families with *XPD (ERCC2)* mutations and clinical features of CS or COFS. All presented with major features of TTD ("tiger tail" banding on polarized microscopy, skin abnormalities, short stature and developmental delay). Three patients (TTD406BE and TTD407BE, 9 yo (d19 yo) and 7 yo sisters; and XP624BE, 5 yo boy) also had CS features including deep-set eyes and postnatal growth failure. TTD406BE and TTD407BE had CS type pigmentary retinopathy. Three patients (TTD373BE 13 mo girl with early death; TTD522BE 10 yo girl; and, TTD633BE 13 mo girl) also had features of COFS with microcephaly, congenital cataracts, facial dysmorphism and skeletal abnormalities. DNA sequencing revealed each patient was a compound heterozygote with mutations in *XPD*; TTD406BE and TTD407BE had R112H (reported in TTD, XP/TTD, XP/CS) and L461V,V716_R730del (reported in XP,TTD, XP/CS). XP624BE had L461V,V716_R730del and G675R (reported in XP/CS). TTD373BE had R616W and D681N, both reported in COFS, TTD and XP. TTD633BE had D681N and L461V,V716_R730del. TTD522BE had F568YfsX2 (reported in TTD, XP/TTD) and a novel mutation, D240G. Different XPD repair/ transcription gene defects are associated with complex clinical phenotypes and may reflect interactions of different alleles or of modifier genes. These findings may give insight into the genotype-phenotype relationship.

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LETR1 is a lymphatic endothelial-specific lncRNA governing cell proliferation and migration through KLF4 and SEMA3C

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Recent studies have revealed the importance of long noncoding RNAs (lncRNAs) as tissue-specific regulators of gene expression. There is ample evidence that distinct types of vasculature undergo tight transcriptional control to preserve their structure, identity, and functions. We determine a comprehensive map of lineage-specific lncRNAs in human dermal lymphatic and blood vascular endothelial cells (LECs and BECs), combining RNA-Seq and CAGE-Seq. Subsequent antisense oligonucleotide-knockdown transcriptomic profiling of two LEC- and two BEC-specific lncRNAs identifies LETR1 as a critical gatekeeper of the global LEC transcriptome. Deep RNA-DNA, RNA-protein interaction studies, and phenotype rescue analyses reveal that LETR1 is a nuclear trans-acting lncRNA modulating, via key epigenetic factors, the expression of essential target genes, including *KLF4* and *SEMA3C*, governing the growth and migratory ability of LECs. Together, our study provides several lines of evidence supporting the intriguing concept that every cell type expresses precise lncRNA signatures to control lineage-specific regulatory programs.

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A specific mutation in TRPM4 predisposes mice to psoriasiform dermatitis (PsD)

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A heterozygous (het) gain-of-function (GoF) mutation (I1033M or I1040T) of TRPM4, a calcium-activated nonselective monovalent cation channel, has been reported as the cause of progressive symmetric erythrokeratodermia (PSEK). The disease is characterized by red, scaly plaques in periorificial and acral regions only during childhood, suggesting involvement of one or more regulating factor(s). To determine if the TRPM4 mutation alone is sufficient to lead to skin disease, we created a mouse (I1029M, genetically equivalent to the human I1033M mutation) using CRISPR-Cas9 methods. From birth to 3 months of age, homo- and het I1029M mice displayed no apparent skin changes compared to wild type (WT) littermates. Since PSEK skin features resemble those observed in psoriasis, we sought to compare the response of I1029M and WT littermates to topical imiquimod (IMQ) application, a model of PsD. Het I1029M mice showed enhanced skin inflammation with greater clinical severity scores (200% vs WT), thicker epidermis, more infiltration of CCR6+ γδ low T cells, higher expression of p-STAT3 protein, and higher mRNA levels of IL17a (1.5-fold) compared to WT mice. In vitro, we found using patch-clamp that the resting membrane potential of primary keratinocytes from I1029M mice (-22.2 ± 3.7 mV) was elevated vs. those from WT (-54.6 ± 5.8 mV), consistent with the GoF mutation phenotype. As dynamic membrane potential is critical for cell cycle, cell-volume control, proliferation, and wound healing in various kinds of cells, the TRPM4 GoF mutation may result in cellular conditions that amplify signaling in keratinocytes. Indeed, inhibition of I1029M-expressing keratinocytes by Compound 5, a known inhibitor of TRPM4, partially reversed membrane depolarization. In summary, the I1029M TRPM4 mutation alone is insufficient to trigger skin changes in mice, but results in changes in membrane depolarization that might leave keratinocytes more susceptible to pathogenic environmental stimuli such as IMQ application.

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Glucocorticoids promote inflammation by induction of CCL20 expression in keratinocytes

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Glucocorticoids (GC) are widely used to treat autoimmune and inflammatory skin diseases and are generally envisioned as powerful immunosuppressants. However, in conditions such as rosacea and perioral dermatitis, GCs can paradoxically lead to increased skin inflammation. The molecular mechanisms by which GCs promote inflammation in these clinical contexts are not understood. Recently it was observed that GCs lead to increased expression of the pro-inflammatory cytokine, CCL20, in lung epithelia of steroid-resistant asthma. CCL20 is also expressed at high levels by keratinocytes in papulopustular rosacea, promoting inflammation by recruiting T-lymphocytes and dendritic cells. Here, we investigated how GCs affect CCL20 expression in human keratinocytes. In contrast to suppression of other pro-inflammatory cytokines, treatment of primary human keratinocytes with GCs led to 2 to 3-fold higher expression of CCL20. In addition, while GCs prevented the induction of inflammatory genes in response to tumor necrosis factor-α stimulation, CCL20 expression was amplified even further. Mechanistically, GCs repressed activity of inflammation-related signaling pathways including NFkB and p38/MAPK, but these inhibitory effects were counterbalanced by binding of activated glucocorticoid receptor to the CCL20 enhancer and promoter, which directly induced CCL20 expression. These results indicate that GCs directly promote CCL20 expression and provide new insight to how GCs may contribute to the inflammation observed in steroid-exacerbated and steroid-resistant skin conditions such as rosacea and perioral dermatitis.

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Assessment of safety in repeat dosing of an *in vivo* topical gene therapy for the treatment of recessive dystrophic epidermolysis bullosa (RDEB) in a phase I/II trial

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RDEB, a devastating mechanobullous skin disease caused by COL71 mutations and collagen VII (C7) deficiency, is without approved corrective therapies. Following successful COL7A1 gene transfer and C7 re-expression in preclinical RDEB animal models, we initiated a first in human randomized single center placebo-controlled phase 1/2 trial conducted between May 2018 and March 2020 to demonstrate safety and efficacy of beremagene geperpavec (B-VEC), a replication-defective, nonintegrating HSV1 vector encoding human COL7A1, to durably heal and molecularly correct RDEB skin following repeat topical applications to wounds. Other in vivo vectors such as AAV and adenovirus are not amenable to repeat dosing. To evaluate safety and tolerability of repeat B-VEC dosing, we assessed AEs, including clinically significant changes in laboratory results, vitals, and physical exam findings as well as examining pre- and post-treatment COL7 and HSV-1 antibodies. In addition, blood, urine, and skin swabs were collected throughout the course of the trial to address safety concerns associated with viral shedding. Nine adult and pediatric RDEB patients enrolled in the trial. Three of the nine patients re-enrolled at a later stage, rendering twelve total participants. Out of 129 B-VEC doses given, twenty mild adverse events (AEs) and only one moderate AE were noted, most of which were deemed unrelated to study drug. No deaths, serious or significant adverse events (SAEs) were reported. HSV-1 and COL7 antibodies, when present, were detected at variable levels before and after B-VEC treatment and demonstrated no negative correlation with clinical outcome. In this trial, repeat dosing of B-VEC, a novel in vivo topical gene therapy delivering COL7A1 to RDEB skin, was associated with no SAEs. Unlike other gene therapies, in vivo repeat dosing with B-VEC was safe and well-tolerated.

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MYC-CPSF-HNRNPA3 cooperation promotes epidermal progenitor maintenance through modulating intronic transcription termination

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Epidermal progenitors need to suppress terminal differentiation to maintain their regenerative capacity. In our search for new mechanisms regulating differentiation, we mapped transcriptome-wide transcription termination sites using 3′ region extraction and deep sequencing (3′READS). We identified 428 transcription termination sites located in the introns that are differentially used between the progenitor state and the differentiation state. Keratinocyte differentiation is also accompanied by the downregulation of the Cleavage and Polyadenylation Specificity Factor (CPSF) complex, a key player in recognizing the transcription termination signal in nascent RNA. We found that CPSF expression in the progenitor state is dependent on the intact function of MYC. MYC directly binds to CPSF1 promoter, and MYC knockdown downregulates CPSF1 expression. CSPF knockdown, using RNAi or CRISPRi, consistently induced premature differentiation, impaired epidermal tissue regeneration, and affected 42% of the differentially used termination sites located in the introns. These sites include a termination site located in the first intron of the differentiation activator GRHL3. CRISPR knockout of GRHL3 intronic termination site increased full-length GRHL3 mRNA expression, as well as GRHL3 target gene expression. Using complex purification coupled with mass spectrometry, we identify multiple RNA-binding proteins binding to CPSF only in the progenitor state, but not in the differentiated state. A genetic screen, using double RNAi of CPSF and its interacting RNA-binding proteins, revealed HNRNPA3 as a key interactor that enhances GRHL3 intronic termination. HNRNPA3 promotes GRHL3 intronic termination through suppressing splicing between the first two exons. Our data suggest a model where the interaction between CPSF and RNA-binding proteins, such as HNRNPA3, promotes specific intronic termination and maintains the regenerative capacity of epidermal progenitors.

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A comparison study of Outcome Measures for Epidermolysis Bullosa; Epidermolysis Bullosa Disease Activity and Scarring Index (EBDASI) and the Instrument for Scoring Clinical Outcomes of Research for Epidermolysis Bullosa (iscorEB)

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The success of clinical trials in Epidermolysis Bullosa (EB) is dependent upon the availability of a valid and reliable scoring tool that can accurately assess and monitor disease severity. The Epidermolysis Bullosa Disease Activity and Scarring Index (EBDASI) and Instrument for Scoring Clinical Outcomes of Research for Epidermolysis Bullosa (iscorEB) were independently developed and validated against the Birmingham Epidermolysis Bullosa Severity Score (BEBS), but have never been directly compared. Our objective was to compare the reliability, convergent validity and discriminant validity of the EBDASI and iscorEB scoring tools. An observational cohort study was conducted in 15 patients with EB. Each patient was evaluated by six dermatologists with expertise in EB using the EBDASI and iscorEB-clinician scoring tools. Quality of life was assessed using the iscorEB-patient and Quality of Life in EB measures. The intraclass correlation coefficients (ICC) for inter-rater reliability were: EBDASI 0.942 and the iscorEB-clinician 0.852. The ICC for intra-rater reliability was 0.99 for both scores. The two tools demonstrated strong convergent validity with each-other. In conclusion, both scoring tools demonstrate excellent reliability. The EBDASI appears to better discriminate between EB types and disease severities.

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Generation of junctional epidermolysis bullosa model mice with revertant mosaicism

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Revertant mosaicism (RM) is a naturally occurring phenomenon involving spontaneous correction of pathogenic mutations in somatic cells, that is known as a potential "natural gene therapy." RM has been observed in several inherited conditions, including junctional epidermolysis bullosa (JEB)-a group of inherited skin fragility syndromes. Recently, CRISPR/Cas9 was revealed to increase gene recombination via double-strand breaks (DSBs) by the mechanisms of RM. Thus, the purpose of this study is to create a JEB model mouse with RM to clarify the mechanism by which RM occurs and spreads. We first generated JEB model mice with frameshift mutations in exon2 (*Col17a1*^{exon2fs}) or exon3 (*Col17a1*^{exon3fs}) of *Col17a1* leading to dysfunctional COL17 protein. Then, compound heterozygous model mice (*Col17a1*$^{exon2fs/exon3fs}$) were produced by cross-breeding these mice. They showed clinical phenotype that resembled those seen in patients with JEB, generalized intermediate. To confirm DSBs-induced mitotic recombination can lead to RM, we generated CRISPR/Cas9 targeting intron 2 of *Col17a1* and created DSBs in epidermal keratinocytes obtained from the compound heterozygous mouse cells (*in vitro*) and in the skin of these mice (*in vivo*) by intradermal injection. *In vitro*, recovered high expression of Col17 cells were observed in the compound heterozygous keratinocytes after CRISPR/Cas9 treatment not only by immunofluorescence (IF) staining but also by flow cytometry. In the treated compound heterozygous cell population, the high expression of Col17 was seen at a greater rate than non-treated cells significantly. *In vivo*, one month after injecting CRISPR/Cas9 vectors into the skin, the high expression of Col17 cells with apical-lateral structure was observed by IF. In conclusion, our results demonstrated that this model mouse may present RM cells *in vitro* and *in vivo*.

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Congenital leukonychia caused by a mutation in the *GJB2* gene

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Congenital leukonychia is a rare nail disorder with white appearance of nails. *PLCD1* is considered as causative gene and congenital leukonychia with *PLCD1* mutation shows either autosomal dominant or recessive inheritance trait. While leukonychia was also observed in the patients with hearing loss caused by the variant in *GJB2*. In the family, there are patients with leukonychia without hearing loss, while other unrelated patients who suffer only from hearing loss also exist. To the best our knowledge, only one case of leukonychia caused by *GJB2* gene has been reported. A 13-year-old girl had white nail since birth. Her bilateral 2-5 fingernails showed smooth and white color. Her bilateral toe fingers were observed as mild white color. In addition to nail abnormalities, she did not show any other associated findings and there is no known family history. The pathogenic mutation was not identified in *PLCD1*. We then performed whole-exome sequencing, and c. 408C>A (p.Tyr136*) and c.134G>A (p.Gly45Glu) in the *GJB2* gene were identified. We searched for the mutations in the patient's parents and the patient's mother without white nail also had the same 2 variants in *GJB2*. Based on these findings, 2 variants of the patient in *GJB2* exist in the same allele originated from the patient's mother. Previously and our case reports demonstrated that *GJB2* variant cause congenital leukonychia. While there are individual without white nail in the family members with *GJB2* variant. Therefore, several elements including the *GJB2* variants and permeability and/or the modifier of *GJB2* gene might be involved with congenital leukonychia. *GJB2* gene is involved with various diseases such as KID syndrome and Vohwinkel syndrome and *GJB2* related skin and nail disorders are autosomal dominant form. Therefore, the nail condition in our patient was induced by either *GJB2* variant. Our case report supported that *GJB2* variant cause congenital leukonychia.

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Replacing *COL7A1*-deficient epidermis over the entire body by autografting cultured revertant keratinocytes in severe recessive dystrophic epidermolysis bullosa

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Biallelic *COL7A1*-null variants cause severe recessive dystrophic epidermolysis bullosa (RDEB). Patients develop chronic ulcers, scarring, and contractures of the hands, feet, and axillary vaults, and are at high risk of developing squamous cell carcinoma (SCC). Revertant skin, where the causative germline variants are corrected by somatic eventsand bullae no longer form, can appear in junctional and dystrophic epidermolysis bullosa. Here, we attempted to replace the epidermis of the entire bodybyautograftingcultured revertant keratinocytesin a 17-year-old girl with severe RDEB. She was a compound heterozygote of the c.2005C>T (p.R669*) and c.6573+1G>C variants of *COL7A1*. At the age of 15 years, she noticed that a small area on her right forearm never formed bullae. Immunofluorescence with LH7.2 antibody stained for COL7A1 in this area, despite the complete absence of LH7.2staining in other skin areas. Sanger sequencing showed a reverse somatic mutation of the c.2005C>T variant in the epidermis, but not in the dermis. We cultured keratinocyte cell sheets from the revertant skin and verified that there was no*de novo* mutation of cancer-related genes by whole-exome sequencing. Under general anesthesia, we mechanically peeled ~2,000 $cm^2$of epidermis and autografted the revertant keratinocyte cell sheets. Biopsy of the autografted skin showed positive LH7.2 staining and loss of the c.2005C>T variant, confirming successful replacement of the epidermis. To obtain sufficient revertant keratinocytes to replace the entire epidermis, we established revertant keratinocytes from the autografted skin and re-autografted them on the affected skin after peeling the epidermis. Repeated operations have replaced ~80% of the skin area with revertant keratinocytes, with few bullae forming after re-epithelialization. This not only improved the patient's quality of life, but probably reduced her risk of developing SCC in the future.

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Pharmacogenetics study of different psoriasis treatments

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Responses to psoriasis treatment vary dramatically among patients. Pharmacogenetic studies seek to identify genetic variations that are associated with treatment response. In this study, we assessed the responses to nine treatment options (acitretin, adalimumab, coal tar, calcipotriol, dithranol, etanercept, methotrexate, photochemotherapy, and ultraviolet B radiation) among 1,692 patients that were genotyped in our recent GWAS. The number of patients administered for each treatment ranged from 127 to 1,182. Among these treatment options, the rate of self-reported treatment effectiveness had a wide range from 19.4% to 62.3%. For instance, the response rates for etanercept, adalimumab, and calcipotriol were 62.3%, 52.2%, and 19.4%, respectively. Associations between genetic markers (SNPs and INDELs) and self-reported treatment response were evaluated after adjusting for population stratification using principal components. In total, 6,502,658 common ($\geq$ 5% minor allele frequency), well imputed ($R^2 \geq 0.7$), markers were included. We identified two loci achieving suggestive significance ($p = 5 \times 10^{-6}$) located in previously identified psoriasis-associated loci: 13q14.3 ($p = 2.6 \times 10^{-6}$) was associated with response to coal tar (978 patients), and 6p22.2 ($p = 3.4 \times 10^{-6}$) was associated with response to calcipotriol (1,182 patients). We further revealed that multiple markers in the 13q14.3 locus overlap with an active enhancer region ($p = 8.6 \times 10^{-5}$). This study provides support for the hypothesis that psoriasis-associated loci are also associated with treatment responses.

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Application of microdissection-based spatial transcriptomics for mechanistic and biomarker investigations in dermatology

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The skin acts as the first barrier to our body, and its structure is closely associated with the expression of various cutaneous phenotypes. Consequently, the three layers and the appendages of skin vary significantly in their anatomy and function. Therefore, understanding the spatial landscape of the skin should be essential for mechanistic and biomarker discoveries in dermatology. To address this issue, here, we have established a spatial transcriptome (ST) system by using 100 μm-diameter punch microdissection (md) in frozen skin sections (hereafter 'mdRNA-seq'). The advantages of this system are, namely, 1) high resolution: expression profile of more than 10,000 genes in φ100 μm spot is detectable, which provides much better resolution than the conventional ST method; 2) native detection: no enzymatic digestion step is required; and, 3) targeted sampling and low cost: this enables collection of the regions of interest such as skin appendages. We confirmed the accuracy of the mdRNA-seq method by comparing the distribution of the cell type-specific genes using single-cell RNA-seq data. We next applied the mdRNA-seq to analyze skin specimens from atopic dermatitis (AD) patients and AD-model mice. The dermal transcriptional profiles exhibited species difference; however, the epidermal gene signature showed common changes between human and mouse along with the progression of AD. Taking advantage of this technique, we finally identified a set of epidermal biomarkers of AD that reflects the local inflammatory status. In summary, mdRNA-seq provides a powerful tool for elucidating complex skin pathologies by revealing site-specific gene expressions that are undetectable by bulk and single-cell transcriptomics.

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Further mechanistic studies of BET and HDAC inhibition for blocking proliferation and inducing apoptosis in CTCL

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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 21: 82-92, 2019). Mediators of apoptosis (cleaved caspases 8 and 9) were increased and proliferative drivers (NFkB, cyclin D1, c-MYC) were decreased. Initial gene expression array studies of CTCL lines treated with BETi/HDACi (OTX015/Romidepsin) at nanomolar levels showed suppression of survival factors (AKT, NFkB) and upregulation of pro-apoptotic factors (multiple caspases, death receptors/ligands, BCL2 family inhibitory factors). AKT inhibitor (MK2206) partially mimicked the anti-CTCL effects of BETi/HDACi. Further gene expression studies showed several proliferation drivers were down-regulated including c-MYC, JUND, and STAT3. Other genes were upregulated including HIC1, RB1, ATM, BRCA1/2, and E2F1. BETi/HDACi reduced pro-oncogenic DNp73 especially in CTCL and reversed the DNp73/TAp73 ratio in CTCL but not in CD4+ T cells. DNp73/AKT1 dual over-expression induced a modest (15-20%) reduction in CTCL apoptosis caused by BETi/HDACi treatment. BETi/HDACi increased H3K27 acetylation around transcription start sites. Nevertheless, RNA polymerase II binding was reduced at these sites in c-MYC, AKT1 and DNp73. Reduced expression of these factors was confirmed at the transcript and protein levels. Knockdown of BET2 and BET4 but not BET3 suppressed c-MYC. Knockdown of all three suppressed GATA3. Our results further define the anti-CTCL mechanisms of BETi/HDACi treatment and support continued exploration of this combinatorial approach as novel therapy for advanced CTCL.

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***ENPP1* variants in patients with GACI and PXE: Genotype/phenotype overlap of heritable disorders with ectopic mineralization**

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Pseudoxanthoma elasticum (PXE) is an autosomal recessive disorder characterized by late onset and progressive calcification and pleomorphic elastic fibers, resulting in skin, eye and cardiovascular manifestations. To date, PXE has been associated with biallelic inactivating sequence variants in the *ABCC6* gene encoding a transmembrane transporter ABCC6 expressed predominately in the liver and kidney. *ABCC6* pathogenic variants are also present in some patients with generalized arterial calcification of infancy (GACI), an often fatal disorder associated with calcification and intimal proliferation within large and medium-sized arteries that lead to cardiovascular collapse in early childhood. GACI is more commonly caused by pathogenic variants in *ENPP1*, which encodes ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1). In our study genotyping 436 PXE patients by a gene-targeted panel of next generation sequencing, we identified two adult patients with biallelic variants in *ENPP1* and normal *ABCC6* sequences. These patients, 57 and 27 years of age, developed classical signs of PXE in their teens, including retinal angioid streaks, pseudoxanthomatous skin lesions, and calcium deposits in biopsies of lesional skin. This study provides evidence that in addition to GACI, the *ENPP1* gene can also cause a classic form of PXE, and thus expands the genotypic and phenotypic overlap between PXE and GACI.

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Elucidating the METTL3-m^{6}A epitranscriptome in epidermal development and carcinogenesis

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Epidermal differentiation requires highly coordinated changes in gene expression, disruption of which may drive the development of keratinocyte cancers (KCs), which collectively outnumber all other human malignancies combined. One emerging area of gene regulation is that of epitranscriptomics (regulated RNA modifications), which offers an additional layer of gene regulation in a spatiotemporal- and signal-dependent manner. However, its significance in healthy and diseased epidermis is poorly understood. m^{6}A is the most abundant internal modification in eukaryotic mRNAs and is found to facilitate rapid transcriptome turnover during cell differentiation to maintain homeostasis. Its deposition on nascent pre-mRNA is carried out by a multicomponent writer complex that consists of catalytic subunit METTL3. Recent evidence suggests that depletion of m^{6}A levels by inactivating METTL3 promotes differentiation in certain cellular contexts. Consistent with this, here we show that global m^{6}A levels are significantly reduced during normal epidermal differentiation and that METTL3 depletion in human keratinocytes promotes a downregulation of anti-apoptotic genes as well as those that promote cell cycle progression. As it has been shown that METTL3 is frequently overexpressed in human cancers (including KCs) and has been functionally linked to the survival, proliferation, and growth of tumor cells, our data supports a model in which dysregulation of the METTL3-m^{6}A epitranscriptome may prevent proper epidermal development and differentiation, leading to carcinogenesis. Moreover, it suggests that an inhibition METTL3-m^{6}A may protect against tumorigenesis by promoting differentiation and apoptosis.

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Functional characterization of missense variants in *ABCC6*, the gene responsible for pseudoxanthoma elasticum

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Inactivating mutations in the *ABCC6* gene cause pseudoxanthoma elasticum (PXE), a prototype of heritable ectopic mineralization disorders. Without a practical biomarker and assessment platform, the evaluation of pathogenicity of *ABCC6* missense variants relies solely on inconsistent bioinformatics predictions. In this study, we utilized an adenovirus-mediated expression system and an *Abcc6*$^{-/-}$ mouse model of PXE to characterize the pathogenicity of ten human *ABCC6* missense variants. Intravenous administration of recombinant adenovirus expressing the wild-type human *ABCC6* cDNA reconstituted the ABCC6 protein, raised levels of plasma pyrophosphate (PPi) that serves as a systemic biomarker of hepatic ABCC6 activity, and prevented ectopic calcification in the *Abcc6*$^{-/-}$ mice. In contrast, two variants, p.S400F and p.G1302R, had similar abundance and cellular localization at the basolateral plasma membrane of hepatocytes to the wild-type ABCC6 but failed to normalize plasma PPi levels and prevent ectopic calcification. Variants p.T364R and p.R1138W were localized exclusively intracellularly in hepatocytes while variants p.R518Q, p.R760W, and p.R807Q showed both plasma membrane and intracellular localization. These five variants also demonstrated dramatic reduction in ABCC6 expression and stability and failed to normalize plasma PPi levels and prevent ectopic calcification. Three variants, p.R391G, p.L420V, and p.R1064W, were likely to be benign as they acted similarly to the wild-type ABCC6, restored plasma PPi levels and prevented ectopic calcification. Our results demonstrated that the adenovirus-mediated liver-specific expression in a mouse model of PXE provides a useful *in vivo* platform for characterization of *ABCC6* missense variants with multifaceted consequences.

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INZ-701 prevents ectopic mineralization in an *Abcc6*$^{-/-}$ mouse model of pseudoxanthoma elasticum

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Pseudoxanthoma elasticum (PXE), a heritable ectopic mineralization disorder, affects the skin, eyes and the cardiovascular system. PXE is predominantly caused by biallelic inactivating mutations in *ABCC6* encoding a putative efflux transporter, ABCC6, expressed primarily in the liver. Recent studies demonstrated that the absence of ABCC6-dependent adenosine triphosphate release from the liver and consequently reduced plasma inorganic pyrophosphate (PPi) levels are critical pathogenic features of PXE. As PPi is a potent mineralization inhibitor, this study examined whether restoration of plasma PPi levels by ENPP1, the principal enzyme that generates PPi from adenosine triphosphate, could prevent ectopic mineralization in ABCC6 deficiency. INZ-701, a recombinant human ENPP1-Fc fusion protein that is being developed as an enzyme replacement therapy for the treatment of ENPP1 deficiency, was tested for prevention of ectopic mineralization in an *Abcc6*$^{-/-}$ mouse model of PXE. *Abcc6*$^{-/-}$ mice, at 5-6 weeks of age, the time of earliest stages of ectopic mineralization, were treated with INZ-701 at 2 and 10 mg/kg administered by subcutaneous injection every other day. Administration of INZ-701 showed a dose-dependent increase in plasma ENPP1 activity and plasma PPi level both 2 and 8 weeks after initiation of treatment. Histopathologic examination of vehicle-treated *Abcc6*$^{-/-}$ mice revealed extensive mineralization in the muzzle skin containing vibrissae, a biomarker of the mineralization process in these mice, while significantly reduced mineralization was detected in mice treated with INZ-701. Quantitative calcium assay demonstrated that the amount of calcium in the muzzle skin biopsies were reduced by 68% and 74% in mice administered with INZ-701 at 2 and 10 mg/kg, respectively. These results suggest that INZ-701 might provide a promising treatment strategy for PXE, a disease with high unmet need and no approved treatment.

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STING-IFN-κ-APOBEC3G pathway mediates resistance to CRISPR transfection in keratinocytes

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CRISPR-Cas9 has been proposed for treatment of genetically inherited disorders including of the skin. However, a limitation for widespread use of CRISPR for correction of inherited skin diseases is the poorly understood transfection resistance of keratinocytes (KCs). Here we report that CRISPR transfection activates STING dependent antiviral responses in KCs, resulting in heightened endogenous interferon (IFN) responses ($p < 0.01$) through induction of IFNκ ($p < 0.001$), and decreased plasmid stability secondary to induction of the cytidine deaminase *APOBEC3G*. Notably, CRISPR generated KO KCs had permanent suppression of IFNκ ($p < 0.001$) and IFN stimulated genes (ISGs) expression ($p < 0.001$), secondary to hypermethylation of the *IFNK* promoter region by the DNA methyltransferase DNMT3B. Pretreatment with the JAK1/JAK2 inhibitor, baricitinib prior to CRISPR transfection led to enhanced transfection efficiency ($p < 0.001$), absence of *IFNK* promoter hypermethylation, and normal IFNκ activity and ISG responses. These results provide insights into the transfection resistance of KCs and indicate that CRISPR mediated gene-correction can lead to permanent alteration of antiviral responses in skin, which can be prevented by JAK1/JAK2 inhibition. This work has major implications for future gene therapy of inherited skin diseases using CRISPR technology.

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First-in-human safety and mechanism of action (MOA) analyses of repeatedly dosed *in vivo* gene delivery for directed human type III collagen (COL3) expression in aesthetics

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Due to the essential role collagen plays in skin biorejuvenation, collagen stimulation has been a focus of recent cosmetic product development. However, directed expression of full-length human COL3, produced by and secreted from the subject's own cells, has not been explored clinically. To this end, we engineered KB301, a replication-defective HSV-1 gene therapy vector, for delivery of COL3. Preclinically, KB301 was shown to transduce clinically relevant skin cells and secrete COL3 *in vitro* and express dermally localized COL3 without toxicity *in vivo*. Interim results from the safety arm of this phase I clinical trial establish KB301 as a safe *in vivo* approach to targeted COL3 supplementation. Healthy adult subjects were enrolled in Cohort 1, which was an open-label evaluation of safety, tolerability, and MOA of repeat dose KB301. Each subject had a region of healthy buttock skin selected to receive two intradermal injections of low-, mid-, or high-dose KB301 spaced 30 days apart. Parallel sites of non-treated or placebo-treated skin were used as intra-subject controls. Adverse events (AEs), including clinically significant changes in laboratory results, vitals, and physical exam findings were monitored. Blood, urine, and skin swabs were collected to evaluate viral shedding. MOA was assessed by *COL3A1* transcript analysis in full-thickness skin tissue biopsies harvested 48 hours after the first or second dose. AE's were limited to transient edema and erythema at the injection site, and no serious AEs were reported. No vector shedding was detected. Inter-subject analysis indicated KB301 similarly expressed *COL3A1* after first and repeat dosing, even in previously anti-HSV-1 seropositive subjects. Taken together, KB301 was shown to be both safe and efficacious for *COL3A1* supplementation in healthy human subjects, supporting clinical progression of KB301 for the treatment of superficial skin depressions and acne scarring.

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Psoriasis and coronary artery disease share a genetic risk factor through *IFIH1*

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Psoriasis patients are believed to be at higher risk of coronary artery disease (CAD), a leading cause of death in the USA, compared to the general population. We used data from 32,309 patients in the Michigan Genomics Initiative (MGI), with demographics from a recent health system encounter, to confirm psoriasis is significantly associated with CAD (OR=1.40, $p=7.5\times10^{-5}$, adjusting for age, gender, BMI, race and socioeconomic disadvantage). To investigate the potential impact of shared genetics, we trained a 524-marker polygenic risk score (PRS) for psoriasis by applying PRSice-2 on GWAS from 7,161 cases and 12,309 controls, then applied it to MGI (including 1,104 patients with a diagnosis of psoriasis). We used our PRS to model psoriasis comorbidities through logistic regression, along with the number of psoriasis diagnostic codes (a surrogate for symptom severity and health system engagement). The psoriasis PRS was nominally significant for CAD (OR=1.04, p=0.04) independent of the number of diagnostic codes (OR=1.05, $p=7\times10^{-4}$), suggesting a shared genetic risk. We then applied trans-disease meta-analysis (TDMA) to compare the summary statistics from our full psoriasis meta-analysis (combining the training and left out data) with a recent GWAS for CAD (122,733 cases and 424,528 controls). TDMA found a significant signal in chromosome 2 ($p=4.1\times10^{-15}$ in psoriasis, 2.4×10^{-7} in CAD and 2.0×10^{-19} in TDMA) that is in high LD ($r^2=0.90$ in Europeans) with a missense variant for *IFIH1* and is a cis-eQTL ($p=1.2\times10^{-8}$) for the same gene in IFN-γ stimulated primary monocytes. Interestingly, *IFIH1* is associated with Singleton-Merten syndrome, a rare genetic disease that induces early arterial calcification. Our results suggest a potential link between CAD and psoriasis through the *IFIH1* locus.

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Hidradenitis suppurativa genome-wide association study

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Hidradenitis suppurativa (HS) is a prevalent inflammatory skin disease. HS causes deep, painful, recurrent abscesses. 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 ($\lambda=1.005$). Our cohort recapitulated HS race and gender predilections with genetically African female participants accounting for 35% of cases, but only 10% of controls. Genotype data for 6 million variants was tested for association, adjusting for five principal components. No locus exceeded our threshold for statistical significance. Importantly, there was no evidence for HLA association supporting classification of HS as inflammatory rather than autoimmune. Several loci approached the significance threshold, suggesting that an expansion in cohort size is needed to provide adequate power to detect associations. Interestingly, the lead SNP at one of the most significant loci (rs11075745; $p=8\times10^{-7}$) 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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Inherited STK4/MST1 deficiency in two unrelated families with atypical epidermodysplasia verruciformis

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Epidermodysplasia verruciformis (EV) is an autosomal recessive disorder characterized by chronic cutaneous lesions caused by human β-papillomavirus (β-HPV), with flat warts that often evolve into non-melanoma skin cancers. There are two main types of EV: (a) "typical" with manifestations restricted to the skin, and (b) "atypical" or syndromic form with extracutaneous infections. Typical EV is caused by bi-allelic mutations in TMC6, TMC8, or CIB1 which impair keratinocyte-intrinsic immunity to β-HPV. Atypical EV is caused by single-gene defects impairing T-cell development or function. In the cohort of 47 EV families, we disclosed unreported homozygous loss-of-function mutations in STK4 in two unrelated consanguineous families with atypical EV. STK4 encodes a serine/threonine kinase, which regulates innate and adaptive immune responses. The gene set enrichment analysis demonstrated highly positively enriched sets of genes in chemokine signaling, cell cycle and Ras signaling, and highly negatively enriched genes of inflammatory response and TGF-beta signaling. The immunophenotyping and T-cell proliferation assay of the patients showed low count of blood CD3+ T lymphocytes, with a disrupted balance of T cell subsets consistent with T-cell lymphopenia and poor response to PHA stimulation. Collectively, finding provide corroborating evidence for the pathogenicity of sequence variants in STK4 as the culprit gene in our patients with atypical EV.

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Patient reported outcomes following EB-101 treatment of recessive dystrophic epidermolysis bullosa (rdeb) wounds showed durable wound healing and reduction in disease burden

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RDEB is an ultra-rare, severe, inherited collagen disorder caused by the absence of type VII collagen. Manifestations include fragility of stratified squamous epithelium, painful blistering, delayed or impaired wound healing, large, chronic severely painful wounds, increased risk of infection, malnutrition, invasive squamous cell carcinoma and premature mortality. We report long-term patient-reported outcomes following treatment of large, chronic RDEB wounds with the gene-corrected autologous cell therapy EB-101. Large RDEB wounds that remained unhealed for ≥12 weeks were treated with 35 cm2 gene-corrected keratinocyte sheets (EB-101). Up to 6 sheets were transplanted in each of 7 adult participants. 3 to 6 years after initial treatment, participants were asked to rate change in wound pain, for treated and control wounds, compared with their pre-treatment state using a seven-point scale, ranging from 1 (very much improved) to 7 (very much worse). Investigator assessment of wound healing data from the last visit was recorded for each responder. Responses were received from 5 participants with 27 treated chronic wounds and 5 chronic, untreated (control) wounds. 59% (16) of treated wounds had > 50% healing, of which 75% (12/16) had healing of > 75%. Compared to pre-treatment state, 67% (18/27) of treated wounds had improved pain (scores < 4), with much or very much improved pain (scores of 1-2) reported for treated wounds with > 50% healing (9/16, 56%). Pain improvement was not reported for control wounds, with 4/5 wounds having no change and 1/5 wounds having worse pain (score of 5). Four responders (4/5) reported willingness to undergo another EB-101 treatment, and 1 responder did not as he did not experience improvement. EB-101 treatment resulted in significant long-term wound healing and patient-reported reductions in wound-related pain in most treated large, chronic RDEB wounds.

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Knock-down of *SDR9C7* impairs epidermal barrier function

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The Mendelian Disorders of Cornification consist of a highly heterogeneous group of disorders, and the majority of non-syndromic cases belong to the family of autosomal recessive congenital ichthyosis (ARCI). Mutations in *SDR9C7* have been associated with ACRI, and clinical manifestations include mild to moderately dry, scaly skin with or without hyperkeratosis, palmoplantar keratoderma, and erythroderma. SDR9C7, with short-chain dehydrogenase/reductase activity, is known as NAD- or NADP-dependent oxidoreductase and has been shown to be involved in the final step of epidermal lipid barrier formation by covalent binding of acylCer to the cornified envelope. Here, we present the clinical and molecular description of 19 ARCI patients in five consanguineous families with *SDR9C7* mutations. We also downregulated the expression of *SDR9C7* in keratinocytes by siRNA technique in 3D organotypic skin constructs. Our results demonstrated morphological and histological abnormalities in these constructs *ex vivo*, similar to those observed in ichthyosis patients. Moreover, the results from keratinocyte migration and epidermal dye penetration assays provided evidence for the role of SDR9C7 in the disease pathomechanism. Collectively, our results indicate that *SDR9C7* deficiency by itself is sufficient to disrupt epidermal barrier function leading to ichthyotic phenotype.

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Dynamic transcriptional and epigenetic regulation through vitamin D receptor and p63/p53 signaling in epidermal keratinocytes

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Accumulated evidence has shown that the vitamin D receptor (VDR) with its ligand 1,25hydroxyvitamin D_3 ($1,25D_3$) regulates epidermal stem cell fate, such that with its deletion (VDR KO) the skin changes epidermal lineage and predisposed to tumor formation. Here, we have explored the direct VDR genomic targets and potential signal pathways mediating VDR function. Expression profiling demonstrate that VDR regulates fate transcription factors through the p53/p63 pathway. Epigenetic studies using chromatin immuno-precipitation and next generation DNA sequencing (ChIP-seq) demonstrated that VDR is directly recruited into regulatory regions of fate transcription factor genes including *FOS*, *MYC*, and *SOX7* especially into large clusters of enhancers called super-enhancer (SE) that are postulated to support high level transcription of cell fate genes. ChIP-seq also showed that VDR is colocalized with p63 in the SEs, and bioinformatic analysis predicts functional annotation of VDR overlapping with p63 including stem cell differentiation, DNA repair, and tumor growth. Knockdown of p63 and mutant p53 blunts $1,25D_3$ induced VDR target gene expression including *FOS*, *JUN*, *SOX7*, and DNA repair genes such as *XPC* and *GADD45A*. In addition, VDR is required for DNA repair as *VDR* deletion delays the clearance of UVB induced photoproducts. These results suggest that VDR drives normal epidermal stem cell fate while suppressing cutaneous tumorigenesis by regulating fate driving transcription factors and DNA repair by cross-talking with p53/p63 signaling. Lack of VDR may result in defects in epidermal stem cell function through inappropriate epigenetic regulation of fate driving transcription factors leading to tumor formation.

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Transethnic analysis of psoriasis susceptibility in South Asians and Europeans enhances fine-mapping in the MHC and genomewide

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We performed a GWAS of psoriasis in 2,590 cases and 1,720 controls of South Asian descent (SAS). Compared to our existing European-origin (EUR) GWAS, the effect sizes of known psoriasis signals were highly correlated in SAS and EUR (Spearman $r = 0.78$; $p < 2 \times 10^{-14}$). We then conducted a transethnic meta-analysis, identifying two novel non-MHC psoriasis loci (1p36.22 and 1q24.2). rs2103876 (1p36.22) is a cis-eQTL in blood for *MTHFR*, whose expression is positively correlated ($p=4.2x10^{-97}$) with the psoriasis risk allele (T) and overexpressed in psoriatic lesional (PP) skin ($p=3.06x10^{-23}$; FC $=1.7$). The only significant blood eQTL target for rs12046909 (1q24.2) is *XCL1*, a chemokine receptor ligand that is also overexpressed in PP skin ($p=0.011$; $FC=1.95$). For these two loci, the transethnic GWAS provided higher genetic resolution (mean reduction in 95% CI= 20 kb) and a reduction in the number of potential causal variants (mean = 10) than EUR alone. We explored multiple strategies to develop reference panels for accurately imputing MHC genotypes in both SAS and EUR. HLA-C*06 was the top-ranking MHC locus in both populations but was even more prominent in SAS. Transethnic modeling also substantially boosted the probability that HLA-C*06 is causal. Analysis of the extended MHC region uncovered 5 independent psoriasis loci in SAS, 14 in EUR, and 17 in transethnic, all of which map to the classical MHC. Secondary MHC signals included coding variants of HLA-C and HLA-B, but also potential regulatory variants of these two genes as well as HLA-A and several HLA class II genes, with effects on both chromatin accessibility and gene expression. This study highlights the value of transethnic meta-analysis for discovery and fine-mapping of susceptibility loci.

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Integrative computational analysis identifies eQTL-lincRNA pairs associated with alopecia areata

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Alopecia Areata (AA) is an autoimmune disorder characterized by hair loss ranging from patchy disease to total body hair loss. AA is one of the most prevalent autoimmune diseases in the US, with a lifetime risk of 1.7%. Our previous GWAS studies and subsequent meta-analysis in AA patients revealed many SNPs associated with increased disease risk. In complex polygenic diseases like AA, >90% of GWAS SNPs map to non-coding regions, and at least 60% of them reside in cis-regulatory elements (CREs) such as lincRNAs, miRNA binding sites and CTCF sites. Recently, multiple studies reported autoimmune disease associated GWAS SNPs in lincRNAs impacting disease through reshaping three dimensional (3D)-genome architecture. However, the impact of GWAS SNPs in lincRNAs and their impact on 3D-genome structure in AA has not been evaluated. We systematically screened AA associated GWAS SNPs in CREs using publicly available studies in the GWAS catalog and retrieved 44 SNPs. We selected 27 AA GWAS SNPs with *p*-value < 5E-8 for downstream analysis, suggesting these SNPs may function as eQTLs. These SNPs were further screened for CREs in the UCSC genome browser. A total of 5 AA SNPs (rs7299099, rs2155219, rs694739, rs4348998, rs9479482) were found in the CREs and exhibited chromatin signatures for enhancer RNAs. Further, single-tissue eQTL analysis using GTEx portal identified 3 eQTL-lincRNAs pairs, out of which 2 (rs694739-AP003774.2 and rs9479482-AL355497.2) exhibited the highest *p*-values, P=5.37E-21 and P=1.12E-12 for skin, respectively. By using publicly available chromatin conformation capture studies in dermal fibroblasts, we visualized these eQTL-lincRNAs pairs in Juicebox and found them located in the same topological associated domain (TAD). It is known that CREs in the same TAD tend to operate together at the 3D-genome level, predicting that these SNPs may influence the 3D genome structure in AA. To validate our computational findings, we are performing hi-C sequencing in AA scalp skin to define the impact of GWAS SNPs in CREs on chromatin looping in AA.

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Expression of active matrix metalloproteinase-1 in dermal fibroblasts: A novel mouse model of accelerated human dermal aging

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Fragmentation, disorganization, and depletion of the collagen-rich dermal extracellular matrix (ECM) are characteristic features of aged human skin. These deleterious alterations are thought to critically mediate many of the prominent clinical attributes of aged skin including thinning, fragility, impaired wound healing, and propensity for carcinoma. Matrix metalloproteinase-1 (MMP1), which is a secreted enzyme that initiates cleavage of collagen fibrils, is significantly increased in dermal fibroblasts in aged human skin. To investigate the potential role of elevated MMP1 in skin aging, we have generated a Cre-inducible mouse model, using CRISPR/Cas9 technology, that expresses full-length, catalytically-active human MMP1 (MMP1*) in dermal fibroblasts, driven by a *Pdgfra-Cre* transgene. Immunohistology of *Pdgfra;MMP1** bitransgenic mice revealed MMP1* expression by fibroblasts throughout the dermis in dorsal, ear, tail, and volar skin. MMP1 activity in dorsal skin, measured by ELISA, was significantly greater (39-fold, n=6, p<0.01) in *Pdgfra;MMP1** mice than littermate controls. MMP1* protein was similarly increased (7.6ng/g, n=6) vs. not detectable in controls. Importantly, by the age of six months, dorsal skin of *Pdgfra;MMP1** mice revealed physical, histological, and molecular alterations that closely resembled those seen in aged human skin. These features included increased fragility, thinning of the dermis (reduced 35%, n=5, p<0.01), fragmentation and disorganization of collagen fibrils, visualized multiphoton confocal second harmonic generation microscopy, reduced fibroblast spreading, and altered gene expression, including reductions of type I, III, IV, and V collagens and upregulation of IL1-beta and IL-6. The above data demonstrate that elevated dermal expression of MMP1 is a key driver of many aspects of skin aging. Therapeutic intervention to reduce/inhibit MMP1 activity during aging may help to prolong skin health throughout life.

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The pivotal role of the autotaxin/lysophosphatidic acid pathway in RDEB fibrosis and scarring

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Lysophosphatidic acid (LPA) is a class of bioactive phospholipids displaying a wide range of cellular effects via LPA receptors (LPAR). LPA is produced primarily by the hydrolysis of lysophosphatidylcholine by the autotaxin (ATX) enzyme. The ATX/LPA axis plays a significant role in the pathophysiology of several fibrotic diseases. Patients with recessive dystrophic epidermolysis bullosa (RDEB) develop numerous skin wounds that heal with extensive scarring, contractures and deformities. Here, we sought to evaluate the role of the ATX/LPA pathway in RDEB fibrosis and scarring. We found that ATX is markedly increased in the skin and sera of 20 RDEB patients. Immunoblot analysis revealed ATX protein levels in RDEB fibroblasts to be elevated 3 − 10 folds compared to normal human fibroblasts. RT-PCR showed elevated mRNAs of LPAR1, LPAR2, and LPAR4 in RDEB fibroblasts. Treatment of RDEB fibroblasts with Ki (LPAR inhibitor) and AM966 and PF (ATX inhibitors) reduced the expression of multiple fibrosis markers [collagen I, connective tissue growth factor, alpha smooth muscle actin, periostin, and tenascin C]. Ki and PF also reduced mRNA levels for fibrosis genes and IL-6, a pro-inflammatory mediator known to stimulate fibrosis. In addition, Ki and PF treatment reduced protein levels of pro-fibrogenic TGF-β in the media of RDEB fibroblasts. Furthermore, inhibition of ATX/LPA by Ki and PF functionally reversed the collagen lattice hypercontractability characteristic of the RDEB cellular phenotype. Lastly, we demonstrated via LPA induction that LPA-LPAR binding activates the PI3K/AKT intracellular signaling pathway to increase the expression of TGF-β and relevant fibrotic markers. AKT inhibition via chemical inhibitor or siRNA abolished this activation. In conclusion, our data demonstrate the involvement of the ATX/LPA pathway in RDEB fibrosis and scarring and also suggest a molecular basis for the therapeutic use of small molecules that target this pathway to treat fibrotic diseases associated with excessive TGF-β activity such as RDEB.

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***ASPRV1* mutations cause dominantly inherited ichthyosis**

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Via exome sequencing we show that mutations in *ASPRV1* cause a dominant form of palmoplantar keratoderma and lamellar ichthyosis, a phenotype that has otherwise been exclusively recessive. *ASPRV1* encodes aspartic peptidase retroviral-like 1, a mammalian-specific and stratified epithelia-specific protease important in processing of filaggrin, a critical component of the uppermost epidermal layer. The full-length protein is 343 amino acids and 37 kDa, but auto-cleavage generates a 14 kDa active enzyme (residues 191-326), with an active site at Asp212 essential to proteolytic activity. Eleven affected subjects in five unrelated ichthyosis kindreds were shown to be heterozygous for one of four previously unreported and damaging *ASPRV1* missense mutations. Two subjects have a mutation that arose *de novo*, and eight are within extended kindreds in which the mutation and disorder cosegregate, conclusively establishing that *ASPRV1* mutations cause this dominant form of ichthyosis. Mutation p.Lys199Glu is eight residues distal to the N-terminal auto-cleavage site, mutation p.Arg208Trp is four residues proximal to the active site, and mutations p.Arg311Pro and p.Pro314Thr are 12-15 residues proximal to the C-terminal auto-cleavage site. Crystal structure modeling of the active enzyme shows all four mutation sites are on the same surface and tightly clustered within the three-dimensional protein. Expression of mutant proteins demonstrates that *ASPRV1* mutations disrupt auto-cleavage and filaggrin processing, a function vital to epidermal barrier integrity. Because the skin disorder in subjects with *ASPRV1* mutations is a desquamation defect, keratolytic agents such as lactic acid and urea could be employed as pathogenesis-driven therapy. These agents have been used by two subjects so far, with almost complete resolution of non-palmoplantar scale.

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Tristetraprolin family members repress early T cell cytokine production and are recurrently downregulated in diverse human rashes

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Human inflammation activates dynamically, but the mechanisms underlying such sensitive kinetics remain incompletely understood. We single-cell profiled transcriptomes and epitopes from 24 diverse rashes in adults, including psoriasis, atopic dermatitis, lichen planus, and bullous pemphigoid. The tristetraprolin family members *ZFP36* and *ZFP36L2* were identified as recurrently, highly repressed transcripts in T cells ($\log_e$FC = -0.47 and -0.85 respectively; adjPvals < 0.001). CRISPR-mediated knockdown of these genes induced a greater than 2-fold secretion of multiple inflammatory cytokines in T cells, including TNF-α ($p = 0.008$), GM-CSF ($p = 0.002$), IFNγ ($p = 0.030$), and IL13 ($p = 0.044$). Interestingly, cytokine induction was greatest preceding stimulation, suggesting tristetraprolins amplify signaling at the onset of inflammation. Supporting this temporal model, we also found that attenuation of *ZFP36L2* mRNA precedes induction of cytokine transcripts in stimulated T cells. Collectively, our data implicate tristetraprolins as early, central regulators of inflammation in human skin and suggest an actionable point for therapeutic modulation.

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Functional genomic analysis of STX17 in alopecia areata reveals a novel role in melanocyte function

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Alopecia Areata (AA), one of the most prevalent autoimmune diseases with a lifetime risk of 2.1%, is a complex genetic disease characterized by autoimmune-directed hair loss of the scalp and body. In AA, pigmented hairs are preferentially attacked, suggesting a role for hair follicle (HF) melanocytes, as cellular targets harboring AA antigens. Our previous genome-wide association study (GWAS) and meta-analysis identified an AA-risk locus that contains *Syntaxin17* (*STX17*), an autophagy-related gene with a reported role in hair pigmentation. To define the role of *STX17* variants in AA, we performed targeted genomic sequencing across the 550 kb GWAS locus containing *STX17* in 849 AA patients. We defined a risk haplotype that carried the GWAS risk allele together with 34 additional variants associated with AA. Using *in silico* and multi-dimensional patient data, we discovered that 33 of these variants were eQTLs associated with decreased expression of STX17 in AA scalp skin. Additionally, AA HFs revealed dysregulated expression of STX17 proximal to dysmorphic follicular melanocytes. We identified an autophagy-independent role for STX17 in melanogenesis, and found that knockdown of STX17 resulted in accumulation of the melanocyte antigen, MART1. In this study, we used functional genomics to identify functional variants on a common AA risk haplotype that significantly downregulated skin expression in AA patients, and found that reduced levels of STX17 in melanocytes resulted in increased antigen expression, our findings provide a mechanistic link between genetic susceptibility to AA and the preferential attack of pigmented hair follicles in disease.

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Differential gene expression in psoriatic vs. normal T-cells is enhanced by CD3-CD28 activation

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We performed RNA-seq on blood-derived, flow-sorted CD4 and CD8 memory T cells and myeloid dendritic cells (mDC) either directly upon isolation or after 24h of CD3-CD28 stimulation from 126 subjects (79 females, 47 males, 58 normal, 68 psoriatic), totaling 955 libraries. The filtered and aligned RNA-seq reads (>25M per sample) were analyzed by PCA, revealing clear separations on the basis of activation, CD4/CD8, gender, and skin homing (CLA), with lesser discrimination between psoriatic and healthy individuals. To focus on psoriasis-related differences, we utilized DESeq2 to identify differentially expressed genes (DEGs) as function of psoriatic phenotype in the context of CD4/CD8, T-cell activation, skin homing, and gender (FDR < 0.05, |log2 FC| > 0.585). We identified 139 psoriasis-related DEGs across all cell types, which were most significantly enriched for "IL-17 signaling pathway" (KEGG, adj. p=0.0086). Notably, IL-17 pathway genes such as IL17A, IL22, and CCL20 were more strongly induced by CD3/CD28 in CLA+ vs. CLA- T-cells (~2-4-fold). 37 DEGs demonstrated an interaction between disease status and activation (FDR <0.05), including key immune genes IFNG, IL2RA, IFI44L, and FCGR3A. 132 psoriasis DEGs were found in CD3/CD28-stimulated CD4 T-cells vs. only 27 in resting CD4 T-cells (63 and 39, respectively, for CD8). In mDC, 276 psoriasis DEGs were most significantly enriched for "Cytokine-cytokine receptor interaction" (KEGG, adj. $p=4.14\times10^{-5}$). We identified 670 gender-related DEGs across all cell types. Top-ranking DEGs included Y-linked genes like TMSB4Y, and X-linked genes such as TSIX (expressed only on the inactive X in humans) and X-inactivation "escapees" including chromatin remodelers KDM6A and KDM5C. These results identify psoriasis-related, T-cell activation-enhanced DEGs in blood-derived immunocytes, highlighting the subtle but important systemic component of psoriatic immune-mediated inflammation.

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Whole-transcriptome analysis by RNA-Seq for genetic diagnosis of Mendelian skin disorders in the context of consanguinity

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Among the ~8,000 Mendelian disorders, over 1,000 of them have cutaneous manifestations. In many of these conditions, the underlying mutated genes have been identified by DNA-based techniques, which, however, can overlook certain types of mutations, such as exonic-synonymous and deep-intronic sequence variants. Whole-transcriptome sequencing by RNA-Seq can identify such mutations and provide information of their consequences. We have analyzed the whole transcriptome of 40 families with different types of Mendelian skin disorders with extensive genetic heterogeneity. The RNA-Seq data were examined for variant detection and prioritization, pathogenicity confirmation, RNA expression profiling as well as genome-wide homozygosity mapping in the case of consanguineous families. Among the families examined, RNA-Seq was able to provide information complementary to DNA-based analyses for exonic and intronic sequence variants with aberrant splicing. In addition, we tested the possibility of using RNA-Seq as the first-tier strategy for unbiased genome-wide mutation screening without information from DNA analysis. We found pathogenic mutations in 35 families (~88%) with RNA-Seq in combination with other NGS methods, and we successfully prioritized variants and found the culprit genes. In addition, as a novel concept, we propose a pipeline that increases the yield of variant calling from RNA-Seq by concurrent use of genome and transcriptome references in parallel. Our results suggest that "clinical RNA-Seq" could serve as a primary approach for mutation detection in inherited diseases, particularly in consanguineous families, provided that tissues and cells expressing the relevant genes are available for analysis.

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GJB2 mutations in patients with ichthyosis follicularis and histopathology of porokeratotic adnexal ostial nevus

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Ichthyosis follicularis (IF) is a distinct cutaneous entity reported in combination with atrichia and photophobia. Mutations in *SREBF1* and *MBTPS2* have been associated with IF. We sought the genetic cause of IF in two distinct families from a cohort of 180 patients with clinical manifestation of Mendelian disorders of cornification (MeDOC) or ichthyosis. In Family 1, the proband presented with IF, bilateral sensorineural hearing loss (SNHL), and keratitis was found to have a *de novo* heterozygous *GJB2*: c.148G>A, p.Asp50Asn in genomic DNA extracted from the peripheral blood lymphocytes. In Family 2, manifested by IF, bilateral SNHL, and punctate palmoplantar keratoderma, compound heterozygous mutations in the *GJB2* were discovered: a pathogenic c.526A>G; p.Asn176Asp, and a common frameshift mutation, c.35delG; p.Gly12Valfs*2. Histopathology from both patients was compatible with porokeratotic adnexal ostial nevus (PAON). Interestingly previously, somatic mutations in the *GJB2* were shown in association with PAON. Due to shared histopathology between segmental and mosaic PAON and the PAON in association with ichthyosis syndromes such as Keratitis-ichthyosis-deafness (KID) syndrome, it was suggested that KID syndrome is the constitutional form of PAON. Accordingly, our finding supports this hypothesis by finding *GJB2* mutations in the DNA of lymphocytes in MeDOC patients with PAON histopathology. These findings attest to the complexity of the clinical consequences of different mutations in *GJB2*.

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Kindler epidermolysis bullosa-like skin phenotype and downregulated basement membrane zone gene expression in poikiloderma with neutropenia and a homozygous *USB1* mutation

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A distinct form of epidermolysis bullosa, Kindler EB (KEB), manifests with blistering and poikiloderma caused by mutations in *FERMT1* encoding Kindlin-1. Here we report a patient clinically diagnosed as KEB with substantially reduced immunostaining for Kindlin-1, however, no mutations were identified in *FERMT1*. Instead, whole-exome sequencing and homozygosity mapping identified a sequence variant at +4 position of intron 2 of *USB1*, which encodes an exoribonuclease required for processing of U6 snRNA, a critical component of spliceosomes. RNA-Seq confirmed the pathogenicity of this variant causing aberrant splicing, predicted to result in loss of function of USB1. Mutations in this gene have been reported in patients with poikiloderma and neutropenia, a condition distinct from KEB. Whole transcriptome analysis revealed that several genes expressed in the cutaneous BMZ and associated with different subtypes of EB were downregulated at the mRNA level and confirmed at protein level by skin immunofluorescence. These observations provide a novel mechanism for blistering in the skin as a result of reduced presence of adhesion complexes critical for stable association of epidermis and dermis at the level of cutaneous BMZ.

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Viral reactivation and severe systemic syndromes in post-stem cell transplant patients

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Patients undergoing stem cell transplant (SCT) may develop severe systemic syndromes with cutaneous findings, such as graft-versus-host disease (GVHD) and drug hypersensitivity syndrome (DHS). These conditions are associated with viral reactivation and can be challenging to differentiate. We aim to characterize the post-SCT relationship between viral reactivation and these multiorgan diseases. Human herpesvirus 6 (HHV6) and cytomegalovirus (CMV) titers were collected from the Johns Hopkins post-SCT cohort over five years. Detailed clinical and laboratory data was obtained by chart review. Of 1379 total records, 393 patients were scheduled for 8 weeks of weekly HHV6 titers, with clinically indicated CMV titers. In this group, 45% developed post-SCT viremia. Skin eruption incidence was significantly higher in viremic vs aviremic patients (72% vs 51%, $p<0.01$). Skin GVHD incidence was significantly higher in viremic vs aviremic patients (41% vs 34%, $p<0.01$). Mean time from SCT to rash did not significantly differ between groups. Cutaneous drug eruption and DHS incidence did not significantly differ between groups. Rash duration and severity did not significantly differ between viremic and aviremic patients. Post-SCT viremic patients were significantly more likely to develop a cutaneous eruption, including GVHD, than aviremic patients, reaffirming GVHD's association with viral reactivation. In our cohort, we found that RegiSCAR criteria was suboptimal for differentiating GVHD and DHS. Distinguishing these diseases is crucial for optimal management, so potential reduced efficacy of diagnostic criteria in this population reinforces the importance of managing these patients in multidisciplinary teams of experts familiar with the clinical features and skin biopsy findings of these conditions. In our continuing efforts, we are investigating clinical and laboratory markers regarding patterns of viral reactivation to facilitate differentiation between GVHD and drug eruption syndromes in post-SCT patients to improve patient management and outcomes.

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IL-36β or IL-36γ induces peeling skin syndrome-like symptom by suppressing *TGM5* and *CDSN* in a three-dimensional human epidermis model

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The inhibitor of IL-36 receptor (IL36R) is known to have effects on generalized pustular psoriasis (GPP), and the gene mutation of *IL36RN*, which is an IL36R antagonist is often reported in GPP patients. We previously reported that IL-17A which is well known as a psoriatic target cytokine is directly downregulates the gene expression of pro-filaggrin, and suppressed epidermal granular layer formation in a three-dimensional (3D) human epidermis model. This time, we investigated the direct roles of IL36R agonists (IL-36α, IL-36β and IL-36γ) on epidermal development using the 3D model. Histopathological findings showed that IL-36α caused mild parakeratosis, whereas IL-36β and IL-36γ caused marked parakeratosis and extensive detachment of the stratum corneum directly above the stratum granulosum. Global gene expression analysis of those 3D models revealed that both IL-36β and IL-36γ significantly suppressed the gene expression of transglutaminase 5 (*TGM5*) and corneodesmosin (*CDSN*) which have been reported in peeling skin syndrome (n=3, $P<0.01$). The same tendency could be confirmed by qRT-PCR (n=3, $P<0.05$, $P<0.001$ respectively). The GO enrichment analysis of those DEGs (differentially expressed genes) were revealed the TOP1-GO term whose genes were upregulated by IL-36β was GO: 0042517 (positive regulation of tyrosine phosphorylation of Stat3 protein), and those upregulated by IL-36γ was GO: 0030198 (extracellular matrix organization). Furthermore, the TOP 6 of upregulated-DEGs by IL-36γ was GO: 0031581 (hemidesmosome assembly), suggesting that IL-36γ may have some influence on the composition of the papillary dermis and induces Auspitz's sign. Finally, the results of our research suggest that both IL-36β and IL-36γ may play important roles in dyskeratosis such as psoriasis and peeling skin syndrome.

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Microbial expression of lantibiotics may explain discrepancies between S. aureus culturability and metagenomics in atopic dermatitis subjects and healthy controls

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Atopic Dermatitis (AD) is an inflammatory skin disease exacerbated by Staphyloccocus aureus (S. aureus) skin colonization. The Atopic Dermatitis Research Network performed taxonomic analysis of skin swabs from 20 disease severity-matched AD S. aureus culture+ (n=10) and AD culture- (n=10) and 9 culture-, nonatopic (NA) subjects. Metagenomic analysis revealed discrepancies between molecular and culture evidence of S. aureus, suggesting that S. aureus skin burden may not be fully reflected by culture results. We hypothesized that production of lantibiotics by cutaneous microbes, which inhibit S. aureus growth, may explain high genomic S. aureus abundance in culture- samples and vice versa. Since each lantibiotic class (I, II, III & IV) has specific biosynthesis genes (lanB/C, lanM, labKC & lanL, respectively), Hidden Markov Models, previously used to assess oral and stool samples, were used to search metagenomes for these genes. We observed 190 total hits (AD [nonlesional & lesional]: 122, median 1/sample; NA: 68, median 7/sample). Significantly fewer lantibiotic hits were found in S. aureus culture+ AD nonlesional than their metagenomic counterparts ($P<0.01$) but no difference in lesional samples. BLAST search of peptide sequences revealed 17.0% of lantibiotic hits were from Malassezia restricta, 8.9% from coagulase-negative Staph, 8.9% from Cutibacterium acnes and 7.9% from Malassezia globosa. Our findings suggest that expression of lantibiotics may reduce S. aureus viability and highlights the potential importance of bacterial-fungal dynamics in the skin ecosystem.

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Commensal microbiota regulates skin barrier function and repair via signaling through the aryl hydrocarbon receptor

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Commensal microbes are critical in maintaining skin homeostasis. However, their mechanisms of crosstalk with host epithelia remain poorly defined, especially during barrier disruption and repair. The goal of this study is to understand mechanisms by which skin microbiome impact barrier repair. We demonstrate that microbiota is necessary and sufficient for proper differentiation and repair of the epidermal barrier. RNAseq was used to compare epithelial transcriptomes of germ free (GF) mice to specific pathogen free (SPF) mice to identify microbially-regulated genetic pathways. Epithelial development and differentiation genes were downregulated in GF mice. Electron microscopy and immunofluorescence-based analyses revealed that GF mice have abnormal epidermal ultrastructure. GF mice were deficient in barrier repair compared to SPF mice following tape-stripping, as measured by transepidermal water loss ($p<0.0001$). A similar effect was observed after antibiotic depletion of microbiota in SPF mice. We identified the aryl hydrocarbon receptor (AHR) pathway as downregulated in GF epidermis ($p=0.0033$). Since AHR is a known regulator of epidermal differentiation, we hypothesized that skin microbiota engages in crosstalk with keratinocytes via the AhR to promote barrier repair. Murine skin lacking keratinocyte AHR was more susceptible to barrier damage and infection, during steady state and epicutaneous sensitization. Colonization with a defined consortium of human skin isolates restored barrier competence in an AHR-dependent manner. We reveal a fundamental mechanism whereby the microbiota regulates skin barrier formation and repair, with far-reaching implications for the numerous skin disorders characterized by epidermal barrier dysfunction.

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Small proline-rich proteins (SPRRs) function as antimicrobial proteins in the skin

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Human skin, the body's largest organ, functions as a physical barrier to prevent the entry of foreign pathogens, while providing home to a myriad commensals. To maintain homeostatic bacterial population, a number of antimicrobial mechanisms have evolved to protect against continual microbial challenges. To understand the role of sebaceous gland in skin host defense, we performed whole transcriptome RNA-sequencing on immortalized human sebaceous gland cells under uninfected conditions, with comparison to sebocytes treated with the bacterial cell wall product, lipopolysaccharide (LPS). We identified a family of genes, small proline-rich protein (SPRR) family, that are up regulated in sebocytes by bacteria signals. We also demonstrate that mouse Sprr1a and Sprr2a are up regulated in mouse skin by bacterial cell wall components. SPRR family proteins have been shown to function as cross-linking proteins in the development of the differentiated cornified envelope of squamous epithelium. Our in vitro studies suggest SPRR proteins also have potent bactericidal activity against MRSA (methicillin-resistant *Staphylococcus aureus*), *Pseudomonas aeruginosa* and other bacteria including both skin commensals and skin pathogens. Mechanistic studies established that SPRRs bactericidal activity is mediated by bacterial membrane binding and disruption. In accordance with these in vitro results, *Sprr1a*$^{-/-}$*Sprr2a*$^{-/-}$ mice are more susceptible to topical MRSA and *Pseudomonas aeruginosa* skin infection. Taken together, our findings reveal a novel antimicrobial function of SPRR proteins and provide insight into the role of sebaceous gland during skin infection. Future work will further investigate the role of SPRR proteins in skin host defense.

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Bile acids improve psoriasiform dermatitis with inhibition of IL-17A production and CCL20-CCR6 mediated trafficking of T cells

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Recent studies suggest a role of bile acids (BAs) in regulating Th17 cells, but their therapeutic value in treating psoriasis and the underlying mechanism whereby BAs regulating T cells remains unclear. Using IL-23 minicircle DNA (MC) based murine model of psoriasiform dermatitis (PsD), we showed that secondary BAs significantly improved PsD without obvious systemic side effects. Administration of lithocholic acid (LCA) and other BAs by oral gavage ameliorated IL-23 MC induced-PsD, as evidenced by decreased ear thickness, epidermal thickness, and infiltration of IL-17A+ T cells in the skin draining lymph nodes. BAs treatment did not induce apparent hepatoxicity examined by histology and serum levels of ALT and AST. Of the BAs we tested, LCA possessed the greatest potency in treating PsD. IV administration of LCA at much lower dosing (compared to oral treatment) showed a similar anti-psoriatic effect in the IL-23 MC model. Ex vivo experiments revealed that LCA reduced IL-17A production in the IL-23-stimulated murine T cells (>90% reduction) without inducing apoptosis. The presence of two main BA receptors,TGR5 or FXR, was not required for the inhibitory effect of BAs on IL-17A because T cells from TGR5- or FXR-deficient mice exhibited similarly impaired IL-17A production. Instead, the decreased expression of IL-17A in T cells was associated with early suppression of RORγt by BAs. CCL20 is a chemokine critical for blood to skin trafficking of IL-17A-producing T cells. Strikingly, BAs inhibited CCL20 expression in keratinocytes and reduced the ability of supernatant from BA-treated HaCat cells to stimulate migration of CCR6-overexpressing Jurkat cells but not control CCR6-low Jurkat cells. We conclude that LCA improves PsD with minimal toxicity via direct inhibition of IL-17A production in T cells and by blocking CCL20-mediated trafficking. Administration of BAs or strategies that regulate BAs metabolism may provide beneficial effects in patients with psoriasis.

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Epidermal invasion by Malassezia spp. yeasts in 3D-reconstructed tissue

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Being usually harmless commensal components of the skin microbiome, Malassezia spp. sometimes become involved in human disorders like pityriasis versicolor where yeasts proliferate and invade the cornified layer, leading to alterations in pigmentation and mild itching. Mechanisms underlying the shift from commensal to pathogenic yeasts are still unclear. Experimental tools that could enable studies of the infection process used by Malassezia are required in order to understand their interactions with keratinocytes and better manage these kind of diseases and their treatments. Here, an in vitro model using reconstructed human epidermis (RHE) is described, based on topical application of Malassezia furfur individuals grown in suspension. Seeded on RHE preincubated with olive oil, M. furfur is able to proliferate and invade the tissue, as assessed respectively by quantitative PCR of yeast-specific DNA and histology of RHE using PAS-staining. This model exhibits lesions mimicking human pityriasis versicolor, with invasion limited to the cornified layer. Most notably, the formation of hyphae is observed. Simultaneous with the developing infection, the integrity of the epidermal barrier of RHE is altered, as revealed by lower trans-epithelial electrical resistance and increased permeability to Lucifer Yellow. Keratinocytes in infected RHE react to the presence of yeasts by enhancing mRNA expression and release of several pro-inflammatory cytokines (i.e. IL-8, TSLP, TNFα, IL-1α, IL-1β, IL-17C) and antimicrobial peptides (AMPS; i.e. hBD2, hBD3, Ribo7, S100A7) into the culture medium underneath the tissue. Overexpression of Toll-Like Receptor (TLR) 2 by keratinocytes in infected RHE suggests involvement of this TLR in the detection of Malassezia by epidermal cells, as well as in triggering expression and release of cytokines and AMPs. This model provides experimental conditions to further investigate changes in Malassezia's phenotype, when individuals behave as pathogens, and to identify how keratinocytes perceive and react when intimately exposed to them.

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Female hormones enhance HIV-1 acquisition in female Langerhans cells, but not in male Langerhans cells

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Hormonal contraceptives, which are composed of progesterone with or without estrogen, are used world-widely. Several published epidemiologic reports have elucidated that usage of hormonal contraceptives increased the risk of HIV acquisition about two-fold in women of heterosexual HIV-1-serodiscordant couples. However, the underlying mechanism is not determined. Langerhans cells (LCs) distributed in vaginal epithelium and skin epidermis are the initial cellular target of HIV-1. HIV-1 productively infects LCs through CD4 and CCR5 on LCs, followed by migration of HIV-1-infected LCs to draining lymph nodes. Dendritic cells (DCs) distributed in vaginal lamina propria and skin dermis could be a target of HIV-1 in the case that epithelium is ablated. To examine whether 17β-estradiol and progesterone modulate HIV-1 infection in LCs and DCs, we generated monocyte-derived LCs and DCs (mLCs and mDCs, respectively). Pretreatment of female mLCs, but not male mLCs, with 17β-estradiol or progesterone increased HIV-1 infection about two-fold. Pretreatment of female and male mDCs with 17β-estradiol or progesterone did not modulate HIV-1 infection. Both 17β-estradiol and progesterone increased HIV-1 infection in mLCs from both premenopausal and postmenopausal female, suggesting that female hormones enhance the risk of HIV-1 acquisition in female mLCs. Interference of receptors for estrogen and progesterone did not inhibit the increased risk of HIV-1 acquisition by female hormones in female mLCs. Collectively, female hormones enhance risk of HIV-1 acquisition in female LCs thought unknown putative signaling pathway.

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Comprehensive succinylome profiling reveals the pivotal role of lysine succinylation in energy metabolism and quorum sensing of *Staphylococcus epidermidis*

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Lysine succinylation is a newly identified PTM, which exists widely from prokaryotes to eukaryotes and participates in various cellular processes, especially in the metabolic processes. *Staphylococcus epidermidis* is a commensal bacterium in the skin, which attracts more attention as a pathogen. However, the significance of lysine succinylation in proteins of *S. epidermidis* has not been investigated. We performed the first comprehensive succinylome analysis of *S. epidermidis*(ATCC 12228) using the LC-MS/MS technology and in-depth bioinformatics analysis. A total of 1557 succinylated lysine sites in 649 proteins were identified in *S. epidermidis*. Gene Ontology, KEGG enrichment, and PPI analysis suggested that lysine succinylation played a pivotal role in energy metabolism, especially the glycolysis/gluconeogenesis process. One succinylation site(K144) was identified in S-ribosylhomocysteine lyase, a key enzyme in the quorum-sensing system, indicating the regulatory role succinylation may play in bacterial processes. In terms of the succinylation features, we conducted a conservation analysis of succinylome profiles in multiple organisms and concluded that lysine succinylation widely existed and met the law of evolution. Moreover, 13 conserved motifs were identified. The specific motif KsuD was conserved in model prokaryotes and eukaryotes. Succinylated proteins with this motif were highly enriched in the glycolysis/gluconeogenesis pathway. 15 succinyltransferases and 18 desuccinylases were predicted that could be pivotal regulators of succinylated events in this organism. These results not only provide a novel insight into the survival, metabolism, virulence, and cell-to-cell communication of *S.epidermidis* but also potentially confer the innovation of new drugs and therapies for related diseases.

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Rare presentation of Vesiculobullous Lyme Disease: A case series

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Early stage Lyme-associated erythema migrans most commonly manifests with a "targetoid" appearance. However, several erythema migrans variants have been reported, which may result in misidentification and delayed diagnosis and treatment. This case series presents a rare variant of bullous erythema migrans. Case 1: A 54-year-old woman presented with a worsening lesion on her leg. She reported a sudden onset of stinging in the area while walking outdoors. A darkening vesiculobullous rash appeared soon after. A differential of atypical Lyme, Sweet's syndrome, and Herpes Zoster was considered. Her symptoms improved rapidly with empiric doxycycline treatment and serology at three weeks confirmed a Lyme disease diagnosis. Pathology findings showed hyperkeratosis and acanthosis with prominent papillary edema along with a histiocyte-rich inflammatory infiltrate. Case 2: A 49-year-old female presented four days after the appearance of an enlarging, darkening rash on her leg. The rash developed vesiculobullous changes soon after it appeared. The patient was empirically treated with a 21-day course of doxycycline and rapidly improved. Several weeks later, positive IgM Lyme studies confirmed an atypical Lyme diagnosis. Case 3: A 65-year-old female presented with a three day history of a swollen, painful rash on the left flank. Central dusky papulovesicles developed soon after the appearance of the rash. The patient was treated empirically for herpes zoster with valacyclovir and for Lyme disease with doxycycline. Lyme serologies drawn at 21 days were positive for IgM, confirming an atypical Lyme diagnosis. All three cases exhibited rapidly developing bullous lesions in the absence of more common Lyme symptoms. Clinicians in Lyme-endemic areas should be aware that Lyme disease may exhibit a broad range of clinical and histologic findings, including bullous presentations. Thus, a low threshold to consider Lyme disease in the differential diagnosis of bullous lesions is warranted in endemic areas.

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Investigation of vitamin B_{12} sharing within the skin microbiome

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The skin microbiome has a key role in supporting necessary functions that promote skin health, including protection from pathogens and immune homeostasis. To more fully understand microbiome function in the context of skin health, it is necessary to characterize the fundamental biological and ecological interactions that occur within the skin microbiome. Using comparative genomics across 71 species of the *Corynebacterium* genus, a dominant skin taxon, we identified that skin-associated species encode for *de novo* biosynthesis of cobamides (vitamin B_{12}), a cofactor essential for enzymatic reactions across all domains of life. Because a much larger fraction of bacteria require B_{12} than are able to synthesize it, we hypothesize that B_{12} sharing occurs within the skin microbiome and that these interactions mediate microbial community composition. To assess B_{12} biosynthesis and use by skin microbiota, 906 skin metagenomes were queried for conserved sequences of B_{12} biosynthetic and dependent enzymes and B_{12} regulatory elements. Reads mapping to B_{12} dependent and regulatory sequences were classified to 1462 species, while *de novo* cobamide biosynthesis was restricted to 23 species. This suggests B_{12} is utilized throughout the phylogenetic diversity of the skin microbiome while only produced by a small number of taxa. In addition, we analyzed published relative abundance data from 594 skin metagenomes and found that in skin sites such as the occiput and inguinal crease, increased microbiome diversity was associated with a higher abundance of B_{12}-producing Corynebacteria (rho=0.36-0.79, $p<0.05$). Loss of diversity is associated with dermatological disease, therefore our results suggest that B_{12} sharing plays a role in mediating microbiome structure and ultimately may promote skin health by promoting diversity. In the future, we will test this with *ex vivo* live human skin and synthetic skin microbial communities consisting of B_{12} producers and users to examine the effects on community dynamics when B_{12} biosynthesis is depleted.

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TNF directs protective neutrophil and IL-17$^+$ γδ T cell responses against *Staphylococcus aureus* skin infections

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Staphylococcus aureus is the leading cause of skin and soft tissue infections and has become a major health burden due to the emergence of antibiotic-resistant strains. To develop alternative therapies to antibiotics, we sought to understand the protective immune mechanisms against *S. aureus* skin infections mediated by tumor necrosis factor (TNF). TNF is a proinflammatory cytokine that is rapidly induced upon *S. aureus* exposure and whose inhibition is associated with increased risk of *S. aureus* infections in humans. However, the contribution of TNF or the cognate receptors, TNFR1 and TNFR2, to host defense against *S. aureus* skin infections is unclear. Therefore, to determine the host defense role of TNF, we used an *in vivo* mouse model of *S. aureus* skin infection whereby TNF, TNFR1, or TNFR2 deficient mice and wildtype (wt) mice were intradermally injected with bioluminescent *S. aureus* and monitored for 14 days. TNF, TNFR1, and TNFR2 deficient mice exhibited increased lesion sizes and bacterial burden compared to wt mice, suggesting TNF is important against *S. aureus* skin infections and TNFR1 and TNFR2 have non-redundant roles in TNF-mediated immunity. Since TNF has been reported to contribute to neutrophil trafficking and IL-17 expression, we hypothesized that TNF promotes the previously identified protective neutrophil abscess formation and IL-17+ γδ T cell responses against *S. aureus* skin infection. In fact, TNF deficient mice had significantly impaired neutrophil recruitment, abscess formation, and diminished skin-infiltrating IL-17$^+$ γδ T cells compared to wt mice. Taken together, these findings indicated that differential TNF signaling by TNFR1 and TNFR2 directs protective neutrophil and IL-17$^+$ γδ T cell responses during *S. aureus* skin infections, which has implications in the development of novel immune-based therapies as alternatives to antibiotic treatment against *S. aureus* and potentially other bacterial infections.

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Activity of sarecycline in murine models of infection and inflammation

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Sarecycline (SAR) is a tetracycline-class antibiotic FDA-approved for treatment of moderate-to-severe acne. *In vitro* studies demonstrated a narrow-spectrum antibacterial activity, targeting clinically relevant Gram-positive bacteria with reduced activity against Gram-negative bacteria commonly found in the human gastrointestinal tract. A murine systemic (intraperitoneal) infection model was used to assess the *in vivo* efficacies of SAR, doxycycline (DOX), and minocycline (MIN) against *S. aureus* and *E. coli*. At 48 hours after systemic infection with *S. aureus*, SAR, DOX, and MIN had a protective dose required to achieve 50% survival (PD_{50}) of 0.25, 0.3, and 0.03 mg/kg, respectively. In contrast, SAR did not demonstrate *in vivo* efficacy against *E. coli* even at the highest dose (PD_{50} >40 mg/kg), while DOX and MIN had a PD_{50} of 5.72 and 6.95 mg/kg, respectively. A murine neutropenic thigh wound infection model was used to model tissue-based infection to assess efficacies of SAR and DOX against *S. aureus*. At 24 hours post infection, SAR achieved a 2-log^{10} reduction of thigh bacterial burden comparable to DOX, with 50% effective dose (ED_{50}) values of 8.23 and 8.32mg/kg, respectively. The anti-inflammatory effects of SAR, DOX, or MIN, were tested in male, Sprague Dawley rats using a carrageenan-induced footpad edema model. Mean percent inflammation at a dose of 100 mg/kg was 53.1, 36.0, and 20.5, respectively. SAR demonstrated *in vivo* efficacy against *S. aureus* but not *E. coli* in animal models of infection, confirming the narrow-spectrum activity observed *in vitro*. SAR also showed an anti-inflammatory effect comparable to DOX and MIN.

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Phenotypic heterogeneity of heterozygous dedicator of cytokinesis 8 (DOCK8) mutations

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Background: Dedicator of cytokinesis 8 (DOCK8) deficiency leads to a combined immunodeficiency characterized by cutaneous and systemic infections, atopy, and autoimmunity. Although there is an understanding of homozygous autosomal recessive DOCK8 deficiency, there is limited information about simple or complex heterozygous mutations in the DOCK8 region or whether heterozygosity for DOCK8 is associated with cutaneous or systemic infections. Inclusion of heterozygous mutation analysis may improve the collective understanding of DOCK8 genetic variants. Objective: The purpose of this review is to provide useful insights into the clinical features, genetic analysis and genetic variants of DOCK8 deficiency, with particular emphasis on heterozygous DOCK8 mutations. Methods: PubMed, NCBI, and Medline were searched for scientific articles that provide information on DOCK8 deficiency. The keywords queried included "DOCK8 deficiency", "mutations", "heterozygous", and "genetics". Results: Due to the vital role that DOCK8 plays in the immune system as well as the actin cytoskeleton, the clinical hallmarks of DOCK8 deficiency are recurrent upper respiratory tract, cutaneous infections and eczema. Although most symptomatic individuals have homozygous autosomal recessive mutations, this review revealed similar clinical findings in patients with heterozygous DOCK8 mutations. Conclusion: Although individuals with heterozygous DOCK8 mutations are generally asymptomatic, recent literature highlights the vast phenotypic heterogeneity of these individuals.

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A basophil-neuronal axis promotes itch

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Atopic dermatitis (AD) is a chronic and relapsing inflammatory skin disorder associated with severe, chronic itch. In addition to chronic itch, patients with AD often suffer flares of acute itch. Although recent seminal discoveries have unearthed the neuroimmune circuitry of itch, mechanisms underlying acute itch exacerbations remain overlooked. Herein, we identify that a large proportion of patients with AD harbor allergen-specific IgE and exhibit a propensity for acute itch flares, provoking the hypothesis that acute itch flares in AD may be driven by allergen-specific IgE. To definitively address this, we developed novel murine models of IgE-mediated itch and found that, although in the steady-state, IgE indeed stimulates acute, mast cell-dependent, histaminergic itch, in the context of AD-like disease, both mast cells and histamine become entirely dispensable for acute itch flares that happen on top of chronic itch. Rather, we strikingly found that this form of itch was critically dependent on the presence of basophils. Inflammatory basophils were prone to release cellular contents and intravital imaging revealed basophil-sensory neuron interactions within the skin that was provoked by cutaneous allergen exposure. Functionally, allergen-stimulated basophils exhibited enhanced production of leukotriene C4 (LTC4). The presence of CysLTR2 on neurons, a receptor for LTC4, was critically required for acute itch flares in AD-associated inflammation**.** Collectively, our study demonstrates a previously unrecognized form of acute itch flare that emerges in the context of chronic skin inflammation to activate a non-canonical basophil-neuronal circuit.

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Genotyping of 30 kinds of cutaneous human papillomaviruses by a multiplex microfluidic loop-mediated isothermal amplification and visual detection method

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Human papillomaviruses (HPVs), a group of non-enveloped small viruses with double-stranded circular DNA which lead to multiple skin diseases such as benign warts, are commonly seen in clinics. The current HPV detection systems aim mainly at mucosal HPVs, however, an efficient clinical approach for cutaneous HPVs detection is lacking. To establish a rapid detection system for cutaneous HPVs, we used a colorimetric loop-mediated isothermal amplification (LAMP) with hydroxynaphthol blue (HNB) dye in combination with microfluidic technology. The lower detection limit of the LAMP assay was 10^7 viral DNA copies/μl when tested on synthesized L1 DNA sequences, which was better than the conventional PCR. Compared to PCR sequencing, the sensitivity of HPV27, HPV2, HPV1, HPV57, HPV3, HPV4, HPV7 and HPV75 genotypes detections were 100%, whereas the specificity was 34.55%, 45.12%, 95.83%, 98.59% and 97.62% respectively, when tested on clinical samples. The new cutaneous type HPV detection system is characterized by both a good sensitivity and specificity compared to conventional methods.

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Heightened levels of microvesicle particles resulting from combination of ethanol and thermal burn injury

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Ethanol, in combination with thermal burn injury, is a clinically significant problem resulting in an increase in morbidity and mortality due to acute multi-organ toxicity from excess systemic cytokine release. Moreover, murine models of intoxicated burn injury replicate the acute toxic effects as well as a delayed systemic immunosuppression. Almost half of the admitted hospital patients with burn injuries were alcohol intoxicated at the time of admission. Our group has demonstrated that the lipid mediator Platelet-activating factor (PAF) plays an important role in the delayed immunosuppressive effects of intoxicated thermal burn injury. As PAF receptor signaling causes generation of subcellular microvesicle particles (MVP), the objective of the present studies is to define the role of MVP in the toxicity associated with EtOH + burn injury. Using HaCaT keratinocyte-derived cell line, we demonstrate that both thermal burn injury and EtOH alone generate increased release of MVP into the supernatant. Combining the two agents results in an increased generation of MVP in at least an additive fashion. These studies suggest that MVP might play a role in the augmented toxicity of intoxicated thermal burn injury.

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Age and circadian regulation of cutaneous innate antiviral immunity at homeostasis

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Neonates and the elderly are at increased risk for skin viral and bacterial infections that have the potential to cause systemic malaise and death. Innate antiviral proteins (AVPs), a component of the natural antimicrobial defense program, are expressed in the skin and protect the host against potential viral infections. Interestingly, newborns and the elderly have immature diurnal circadian rhythms. Unknown to us is whether and how changes in circadian regulation throughout age alter homeostatic innate antiviral immune responses in the skin. We hypothesize that age-related modulation of the daily circadian rhythm via the Period circadian regulatory axis facilitates transcriptional regulation of innate antiviral host defense molecules in the skin. *In silico* analysis of murine and non-human primate skin reveals a subset of cutaneous AVPs that display distinct circadian oscillation at homeostasis. Notably, skin from *Bmal1*$^{-/-}$ mice show reduced antiviral transcription compared to heterozygous littermates, and siRNA knockdown of *CLOCK* in human keratinocytes leads to reduced basal AVP mRNA expression ($p<0.05$). Intriguingly, a subset of cutaneous AVP mRNAs are not expressed in neonatal mice, but peak AVP expression induction instead coincides with increased transcript expression of core circadian gene, *Per2*, in adult mice ($p<0.05$). Moreover, elderly mice show reduced constitutive AVP transcript ($p<0.05$) and protein expression compared to adult mice. These findings suggest that variability in AVP expression across age may be due to changes in circadian regulation of keratinocyte innate immune programs. Future studies are directed to investigate whether age and associated changes in circadian regulation similarly impact anticipatory innate immune responses in the setting of wounds.

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Evaluation of SARS-CoV-2 spike protein response on PI3K agonist-mediated IL-8 release

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A novel coronavirus related to a condition known as a severe acute respiratory syndrome (SARS) was termed as SARS Coronavirus-19 (SARS-CoV-2 or COVID-19), which has caused an unprecedented global pandemic. While the mode of COVID-19 infection, its structural configuration, and multiple mechanisms of action including the critical roles of spike proteins have been substantially explored, elucidation of signaling pathways regulating its cellular responses is yet to be fully determined. Among major signaling cascades, phosphoinositide 3-kinases (PI3K) and its downstream pathways have been exploited as the potential therapeutic targets for COVID-19, and its activation induces the release of cytokines such as interleukin-8 (IL-8). To that end, the current studies were sought to determine SARS-CoV-2 spike S1 subunit protein (referred to as COVID-19) on PI3K agonist, phorbol myristate acetate (PMA)-mediated IL-8 release. Given that multiple cell types including epithelial lining of the nasal, bronchial and alveolar cells have been found to be primarily affected by COVID-19, we used naso-pharyngeal carcinoma, KBP and non-small cell lung cancer, A549 cell lines for our studies. We observed that treatments with only PMA but not COVID-19 were able to induce dose-dependent IL-8 release from both KBP and A549 cell lines. Our next studies determined the effects of COVID-19 pretreatment with PMA and *vice versa* to evaluate if any of this combination would exert synergistic effect on IL-8 release. We observed no significant differences in IL-8 release with either of these combinations when compared with PMA-alone group. However, significantly increased IL-8 release was noticed by PMA + COVID-19 combination when compared with COVID-19-alone group. Overall, these studies indicate that PI3K signaling does not directly mediate COVID-19-induced IL-8 release in these cellular models.

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The distinct skin microbiota of congenital ichthyoses

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The ichthyoses are genetic keratinizing disorders with an impaired epidermal barrier and increased risk of microbial infections. Congenital forms have recently been found to have a Th17 immune signature with increased antimicrobial peptides, but the skin microbiota is largely unexplored. We analyzed the metagenome profile of the skin microbiome for major congenital ichthyosis subtypes. Body site-matched skin surface samples were collected from the scalp, upper arm, and lower back of 23 adult patients with ichthyosis and 16 healthy controls for whole metagenomics sequencing analysis. Taxonomic profiling showed changes in bacteria, fungi, and virus abundance across the subtypes. *Cutibacterium acnes* and *Malassezia* were significantly reduced, consistent with skin barrier disruption and depletion of lipids as a carbon source for lipophilic microbes (p-value <0.01). Microbial richness was also reduced, with specific increases in *Staphylococcus* and *Corynebacterium* (preferential inhabitants of dry skin), as well as shifts in fungal species, including among *Malassezia*. *M. globosa* was reduced at all body sites, while *M. sympodialis* was reduced in the ichthyotic upper arm and lower back. Relative abundance of *M. slooffiae*, by contrast, was increased in all body sites of lamellar ichthyosis/LI and congenital ichthyosiform erythroderma/CIE. A previously undescribed *Trichophyton* species was also detected as sporadically colonizing LI, epidermolytic ichthyosis, and CIE ichthyotic subtypes. The ichthyosis skin microbiome is altered from healthy skin with some specific changes that distinguish among ichthyosis subtypes. Understanding microbial colonization in ichthyosis could facilitate development of targeted treatments.

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Targeting of HDAC8 and HDAC9 in keratinocytes to enhance skin immune defense

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We recently reported that short-chain fatty acids (SCFA) promote an inflammatory response in keratinocytes by suppression of HDAC8 or HDAC9, specific histone deacetylases whose activity increases tolerance of the skin to inflammatory signals. Upon silencing of HDAC8 or 9 in keratinocytes, subsequent exposure to TLR2/6, TLR3, or TLR7 ligands enhances inflammatory cytokine production in keratinocytes, but this effect does not occur in bone-marrow derived cells, thus demonstrating epidermal specificity of this mechanism. Chip-Seq and signal pathway analysis by RNA-Seq identified MAP2K3 as a key intermediate in this process, with increased acetylation at H3K9 and H3K27 in the MAP2K3 promoter after silencing HDAC8 and HDAC9 or inhibition of HDAC activity by SCFA butyrate. Antibody pull-down and mass spec analysis showed that HDAC8 and HDAC9 bind the FACT complex to drive gene elongation. These responses were ablated with MAP2K3 knock down. Furthermore, HDAC8 and HDAC9 silencing in keratinocytes lead to IFN-β-dependent activation of antigen presentation ability in cultured dendritic cells and enhanced T cell proliferation in culture. Increased immune reactivity of keratinocytes was also seen in K14Cre-HDAC8/9flox mice in response to UV radiation or imiquimod application, thus validating the critical role of this epigenetic mechanism in the skin. To exploit a potential benefit of HDAC8 and HDAC9 inhibition, we evaluated the impact of HDAC inhibition by topical application of SCFAs on survival of *S. aureus*. Topical treatment of mice with butyrate upregulated antimicrobial peptide production (Camp and mBD4) and subsequently inhibited *S. aureus* in mice despite elevated Th2 cytokines generated in an MC903-induced AD mouse model. These observations show a novel approach to enhance host defense against pathogens on human skin.

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Sarecycline demonstrates reduced activity against representative fungal and bacterial species commonly found in the human gastrointestinal tract

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Use of broad-spectrum antibiotics (e.g., doxycycline, minocycline) significantly alters the gut and skin microbiome leading to dysbiosis, resulting in microbial imbalance and has been associated with exacerbation of inflammation. Sarecycline was developed as the first narrow-spectrum tetracycline-class antibiotic to treat acne. Narrow-spectrum antibiotics are hypothesized to cause minimal interference with endogenous gastrointestinal (GI) tract microbiota, thereby maintaining innate microbial diversity. To examine the breadth of this effect, a panel of microorganisms that reflect the diversity of the gut microbiome were evaluated with sarecycline compared to the broad-spectrum minocycline using *in vitro* susceptibility testing and time-kill assays. Sarecycline had a lower minimum inhibitory concentration (MIC) against 3 out of 4 isolates from *Actinobacteria* phylum, 10 out of 12 isolates from *Bacteroidetes*, and 5 out of 7 isolates from the *Firmicutes*. Furthermore, sarecycline was less active against *E. coli*, and significantly less active against *P. freudenreichii* when compared *to minocycline*. Against fungi, sarecycline showed less activity against 4 representative *Candida* species. Time-kill curves for *E. coli* and *C. tropicalis* showed significantly less activity against *E. coli* for sarecycline compared to minocycline at all time-points (*p*-values <0.05). Similarly, sarecycline was significantly less effective in inhibiting *C. tropicalis* compared to minocycline at 20 and 22 hours exposure. Overall, sarecycline showed reduced antimicrobial activity against 79% of gut microflora tested compared to minocycline, suggesting that it has less potential to cause dysbiosis. Further *in vivo* testing is warranted.

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Epidermal interferon production is positively regulated by *Staphylococcus aureus* in SLE and involves the STING pathway

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Cutaneous inflammation is exhibited by many systemic lupus erythematosus (SLE) patients. Keratinocytes are an important source of type I interferons (IFNs), which play a pathogenic role in cutaneous and systemic SLE. However, drivers of cutaneous IFN production are not well understood. Microbial dysbiosis is an underexplored aspect of cutaneous IFN production. We recently demonstrated that SLE lesional skin is highly colonized by *Staphylococcus aureus* (50%) secondary to effects of elevated type I IFN signaling. In this work we examined whether *S. aureus* can also induce keratinocyte IFN production, and we identified the specific intracellular signaling pathway utilized by *S. aureus* in this process. Immortalized keratinocytes (N/TERTs) were exposed to live and heat killed (HK) *S. aureus* followed by gene expression analysis via quantitative real time PCR (qRT-PCR). IFNβ expression occurred rapidly (1 hour) in N/TERTs while other IFNs such as IFNκ were produced with longer exposure. Only live, not HK *S. aureus*, induced IFN transcription. Notably, *Staphylococcus epidermidis*, the ubiquitous skin colonizer, did not induce IFN production in keratinocytes. Also, co-culturing *S. aureus* with *S. epidermidis* decreased IFN production in keratinocytes in a dose-dependent manner suggesting that *S. epidermidis* can potentially regulate inflammation induced by *S. aureus*. In order to identify signaling pathways leading to IFN upregulation by *S. aureus*, *MyD88* and *TMEM173* (STING) knockout keratinocyte cell lines, generated by CRISPR-Cas9, were treated with live *S. aureus*. Loss of *TMEM173*, but not *MyD88*, eliminated *S. aureus*-induced IFN production. This indicates that STING is essential for keratinocyte IFN production in response to *S. aureus* colonization. In summary, *S. aureus*, but not *S. epidermidis*, generates an epidermal IFN response in a *TMEM173*-dependent manner. Further study of the impact of microbial dysbiosis will be important for understanding the pathobiology of IFN-driven skin diseases.

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Eosinophil-derived IL-17 protects against epicutaneous *Staphylococcus aureus* infections

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Staphylococcus aureus is the predominant cause of skin and soft tissue infections in humans and with the emergence of methicillin-resistant strains, has prompted the need for alternatives to antibiotics through a better understanding of host immune responses. IL-17-mediated immunity is crucial for host defense against *S. aureus* skin infections, but the immune cells involved are not entirely defined. Recently, eosinophils were identified to produce IL-17 during fungal infections. However, the role of eosinophils in IL-17-mediated immunity against *S. aureus* skin infections is unexplored. To investigate the role of eosinophils against *S. aureus* skin infections, wildtype (wt) and eosinophil-deficient mice were epicutaneously exposed to a bioluminescent strain of *S. aureus* and bacterial burden monitored over time. Eosinophil-deficient mice had increased bacterial burden compared to wt mice, suggesting that eosinophils contributed to host defense against S. aureus. Next, we used an IL-17A-tdTomato x IL-17F-GFP dual reporter mouse with flow cytometry to determine whether eosinophils produced IL-17A or IL-17F during *S. aureus* infection. Interestingly, ~50% of IL-17A-producing cells in the *S. aureus*-exposed skin were eosinophils, whereas eosinophils did not produce IL-17F. To test whether eosinophil-derived IL-17 protects against *S. aureus* skin infections, eosinophils were adoptively transferred into IL-17A/F-deficient mice treated with neutralizing mAb against IL-17A+F or an isotype control. Indeed, eosinophils restored the host defense defect in IL-17A/F-deficient mice, but not in the presence of IL-17A+F mAbs, indicating that eosinophil-derived IL-17 promoted host defense. Taken together, we uncovered a novel mechanism whereby eosinophil-derived IL-17 protects against *S. aureus* skin infections, which has implications in the development of immune-based therapies against *S. aureus* and potentially other skin infections.

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Tight junction changes associate with increased epidermal susceptibility to viruses

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Atopic dermatitis (AD) is characterized by skin barrier dysfunction, increased type 2 immunity, and an altered skin microbiota with high *Staphylococcus aureus* abundance. These factors contribute to enhanced susceptibility to viral skin infections. We hypothesize that both type 2 immunity (IL-4/13) and *S. aureus* colonization alter the epithelium to make it highly permissive to viral infections. Using human keratinocytes (KCs), we observed that the state of differentiation significantly affected susceptibility to vaccinia virus (VV). Similar trends were observed with herpes simplex virus. Undifferentiated KCs were relatively resistant to VV infection, whereas infection at the time of differentiation or up to 2 days later increased susceptibility by 13- and 6-fold, respectively (% area with plaques; n=5-9, $p<0.001$). This highlights a narrow window in which KCs are highly susceptible and implicates a role for tight junctions (TJ) in KC infectivity. We found that KCs exposed to *S. aureus* USA300 supernatant or IL-4/13 (50 ng/mL) had increased KC susceptibility to VV (7 & 8-fold increases in plaque number; $p<0.01$ & $p<0.05$ respectively) at day 2 post-differentiation. To further explore this observation of infectivity associated with changes in differentiation, we determined whether these stimuli had an effect on TJ function (Transepithelial Electrical Resistance [TEER]). USA300 induced a decrease in TEER (>80%, $p<0.02$) and reduced accumulation of the TJ protein, occludin ($p<0.01$). The effect of IL-4/13 on TEER were highly variable. We are currently exploring whether IL-4/13 and USA300 alter the expression of KC differentiation markers and are using laser capture on full thickness human epidermal explants to identity strata that support viral replication. These findings will clarify which epidermal layers (differentiation states) are most susceptible to viral infections and whether this is modified by exposure to *S. aureus* virulence factors and/or type 2 cytokines.

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Skin controls the gut immune response through innate ECM cross talk

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Inflammatory bowel diseases (IBD) associate with skin inflammatory diseases but why this occurs is unknown. Inflammation or wounding of the skin induces *Cemip* hyaluronidase in the dermal extracellular matrix (ECM) and production of HA fragments that have been shown to be recognized by TLR4. We hypothesized that such HA fragments may enable the skin to promote inflammation in the gut. To test this, mice expressing hyaluronidase in the skin (K14/Hyal1 mice), or mice with skin wounds (Wd), were compared to their littermate controls. Both groups showed HA digestion in the dermis but expression of Hyal1 did not induce skin inflammation. Remarkably, both skin interventions enhanced disease in the colon when mice were fed DSS, seen histologically, by increased weight loss ($p<0.0001$), lower survival rates (Control 100% survived, K14/Hyal1: 20%, Wd; 80%), and FACS. Even in the absence of DSS challenge, scRNA Seq of colons from K14/Hyal1 revealed large changes in the abundance of stromal fibroblast subsets; cluster 5 increased from 1.21% to 43.2%, clusters 0, 2 and 7 decreased from 29.8 to 4.16%, 18.9 to 0% and 4.5 to 0%, respectively). Pseudotime analysis distinguished three lineages within these populations with HA-induced shift from Cluster 2 toward 5 in lineage 3 most associated with fat cell differentiation. Genes altered in these subsets were validated by whole tissue RNA Seq and qRT-PCR. Colon fibroblasts from TLR4-/- mice failed to respond to HA fragments. DSS challenge in mice further induced genes related to adipogenesis. Reanalysis of scRNA-Seq data from healthy human subjects and patients with newly diagnosed IBD was consistent with our observations in mice of increased fat cell differentiation. Taken together, these results show the role of fibroblasts and reactive adipogenesis in tissue inflammation and directly demonstrate how the skin can control intestinal inflammation.

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A multitargeted approach for soothing irritated skin

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Effective prevention and treatment of skin inflammation requires a multi-pronged approach targeting modulation of skin inflammation, protection from environmental aggressors and restoration of microbiome balance. This study evaluates the effectiveness of a novel soothing complex on the production of pro-inflammatory cytokines and the ability to protect from external stressors. Results indicate that the complex reduces the level of cytokines in cells following induction of an inflammatory state. The blend also decreases reactive oxygen species (ROS) in a DPPH assay and intracellular ROS in a DCFH assay following ROS propagation by urban dust and UV. Inclusion of an effective probiotic is shown to support growth of normal flora *S. epidermidis,* which has been previously demonstrated to induce host immunity *in vivo*. Together these *in vitro* results indicate that carefully selected skin soothing ingredients in an optimized combination may effectively mitigate stress-induced skin irritation while also protecting from external aggressors.

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Inflammatory changes of the small intestinal microenvironments in the murine model of psoriasis

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Despite a well-known relationship between psoriasis and inflammatory bowel diseases, changes in intestinal microenvironment in psoriasis remain largely unknown. In this study, we examined the inflammatory responses of the skin and intestine of an imiquimod (IMQ)-induced murine model of psoriasis, using ΔdblGATA mice, which have a systemic eosinophil deficiency. IMQ-treated mice showed significantly decreased weight and energy intake, while showing increased fecal protein and intestinal permeability. The composition of microbiome was also altered with increased Bacteroidetes and decreased Firmicutes. On RNA sequencing, in small intestine, the expression of genes related with apoptosis, protein digestion and absortion was significantly upregulated, whereas in the skin, that of NOD-like receptor pathway, and Th17 signaling pathway was markedly increased. With IMQ-induced stimulation, IL-17-producing T cells were significantly increased in the skin, but not in the small intestine. In small intestine, IL-17 expression was significantly decreased while the expression of IL-1beta, IL-6, TNF, and S100a8 was upregulated with decreased intestinal tight junction molecules. These changes were not observed in large intestine. Interestingly, the small intestine of IMQ-treated mice showed markedly decreased eosinophils, with a significant upregulation of the eosinophil degranulation markers. The underlying pathologic role of degranulated small intestinal eosinophils in psoriatic inflammation was further substantiated by significantly upregulated tight junction and mucus molecules in the small intestine of IMQ-treated ΔdblGATA mice. These collective data suggest that degranulation of small intestinal eosinophils accelerate pathogenesis of psoriatic inflammation by damaging intestinal barrier integrity.

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Reactive adipogenesis in the perifollicular stroma is a component of the host immune response in acne

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Cutibacterium acnes (*C. acnes*) is a major causative agent of acne vulgaris, an inflammatory disease of the pilosebaceous gland, however the pathophysiology of acne is not well understood. Recent studies have shown that dermal fibroblasts actively contribute to innate cutaneous immunity but their function in acne pathogenesis has not been elucidated. In response to bacterial infection, dermal fibroblasts differentiate into adipocytes and acutely synthesize cathelicidin antimicrobial peptide (Camp) in a process termed reactive adipogenesis. Analysis of human acne and murine acne-like skin by single-cell RNA-seq analysis identified an increase in pro-adipogenic and pro-inflammatory shift in fibroblast populations in lesional skin. Acne skin had elevated PREF1 and CAMP staining and increased expression of several adipogenic markers. Peaking on the first day of infection, *C. acnes* infected mice had ~3-fold higher *Pref1* and ~17-fold higher *Camp* expression respectively and higher expression than uninfected mice through day 6. Treatment with retinoic acid (RA) resulted in ~2 fold higher *Camp* expression on day 6 vs. vehicle treated infected mice ($p < 0.05$). Mice treated with RA also reduced lesion size. In contrast, lesions in *Camp*$^{-/-}$ mice were larger than WT mice and were unaffected by RA treatment. *In vitro*, addition of sterile *C. acnes* supernatants to mouse 3T3-L1 preadipocytes enhanced *Camp* expression by itself (~31-fold respectively, $p < 0.0001$) and was significantly elevated when compounded with RA (~164-fold respectively, $p < 0.0001$). Similar results were observed in mouse primary fibroblasts with a ~185-fold increase in *Camp* expression when treated with supernatants alone (~185 fold respectively, $p < 0.0001$), however these effects were lost in *Tlr2*$^{-/-}$ cells. Taken together, these results provide new insight into acne infection and identify reactive adipogenesis as a previously unrecognized, yet significant component of acne pathophysiology.

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TNF-α upregulates WNT5A that induces MCP-1 production in osteoclasts, leading to recruitment of circulating monocytes in psoriatic arthritis

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Psoriatic arthritis (PsA) is a chronic inflammatory joint disease with bone erosions mediated by osteoclasts. WNT signaling is an important regulator of active osteoclastogenesis. We aimed to investigate how WNT signal pathways regulate active osteoclasticlastogenesis in PsA. The circulating CD14+ monocytes from 12 PsA patients (average age 45.2 years, M/F=8/4) and 12 healthy controls (HCs) (average age 47.4 years, M/F=7/5) were obtained and differentiated into monocyte derived osteoclasts (MDO) by TNF-α and RANKL in vitro. We profiled the transcriptional levels of 20 WNT ligands in MDO by PCR. The chemokine and cytokine levels in the MDO supernatants from PsA patients and HCs were measured by multiplex ELISA. The function of candidate WNTs in MDO was investigated using RNA interference. MDO in PsA patients were treated with TNF-α inhibitor to investigate how TNF-α regulates WNT ligand and/or production of chemokines in these cells. The results showed that, among 20 WNT ligands, the relative expression of WNT5A was selectively increased in MDO from PsA patients (n=12) as compared to that from HCs(n=12) (9.6 ±2.8 and 1.0 ±0.53, $p<0.01$). MCP-1 expression is selectively increased among 31 chemokines and 36 cytokines in the condition medium in MDO from PsA patients. Interestingly, the increased MCP-1 level in MDO from PsA was abrogated when these cells were transfected with WNT5A siRNA. The ratio of CD14+ monocytes migration in the MDO supernatants from HCs and PsA patients was 1 ±0 and 2.4±0.6 ($p< 0.01$). Furthermore, WNT5A expression and MCP-1 production were reduced in MDO from PsA patients receiving TNF-α blockers for treatment. We concluded that TNF-α upregulates WNT5A that induces MCP-1 production in osteoclasts, leading to recruitment of circulating monocytes in PsA.

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The dynamic change of the skin microbiome in severe hidradenitis suppurativa after short term effective treatment with adalimumab

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Background: The composition and diversity of microbiome community has been implicated in several immune mediated diseases such as atopic dermatitis. The dysbiosis usually recovered along with clinical symptoms after treatment. The involvement of bacteria in the pathogenesis of hidradenitis suppurativa has been postulated, although the roles of bacteria remain unclear. In the present study, we aimed to investigate the skin microbiome in severe HS patients and its dynamic change after effective treatment with adalimumab. Methods: We prospectively recruited 13 severe HS patients and 10 healthy controls from September 1st, 2019 to April 30th, 2020 in Chang Gung Memorial Hospital, Kaohsiung branch and Chiayi branch. We sampled the skin lesions in HS patients and normal skin in axilla or buttocks in healthy control group and performed next-generation sequencing on 16S ribosomal RNA for the skin microbiome analysis, including the composition, alpha diversity, and beta diversity, and compared these parameters after treatment. Results: A total of 13 HS patients, 10 healthy controls and 6 post-treatment HS patients were included in the final analysis. Corynebacterium and Staphylococcus were frequently identified in healthy controls, while Prevotella, Peptoniphilus, Porphyromonas, Finegoldia, Anarococcus, and Ezakiella were significantly dominant in HS patients ($P<0.05$). Although alpha diversity was similar across 3 groups, there were significant differences in the composition of bacteria between healthy control group and HS group, and in that between healthy control group and post-treatment group ($P<0.05$). However, there was no difference between the HS group and post-treatment HS group despite successful clinical responses ($P=0.56$). Conclusion: Severe HS patients featured with characteristic skin microbiome on the lesional surface that differed significantly from healthy controls. After successful treatment with adalimumab, the composition of the distinct skin microbiome did not alter significantly despite evident clinical improvement.

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Neutrophil-specific defensin receptors that prevent skin dysbiosis and bacterial infection

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Defensins are a large family of positively-charged antimicrobial peptides that have been implicated innate immunity. Although known for their ability to directly kill bacteria, the mechanisms by which defensins regulate host-pathogen interactions remain elusive. Herein, we have deleted the entire *Defensin* gene cluster (3 million base pairs) in a keratinocyte-intrinsic manner and observed enhanced susceptibility to infection with *Staphylococcus* aureus. Strikingly, we identified previously unrecognized roles for neutrophil-specific orphan G protein-coupled receptors, Mrgpra2a/b, as defensin receptors in this context. Disruption of the defensin-Mrgpr axis resulted in the impaired ability of the epithelium to mobilize neutrophils to release IL-1β and prevent skin dysbiosis and *S. aureus* infection.

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Isotretinoin disrupts skin microbiome composition and metabolic function after 20 weeks of therapy

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Despite being the gold standard treatment for severe acne for nearly 40 years, how isotretinoin influences the entire microbiome, the strain level composition of *C. acnes,* and the metabolic function of the microbiome throughout treatment is still not clear. We collected cyanoacrylate glue follicular casts from the cheek of acne patients (14 M, 4 F; ages 14-29 yrs.) throughout isotretinoin treatment (0wk, 1wk, 4wks, 8wks, 20wks and 6 months after cessation) followed by whole genome sequencing to determine how the skin microbiome and strain composition of *C. acnes* is influenced by isotretinoin. As expected, isotretinoin significantly decreased sebum levels by 4wks of treatment and disease severity scores significantly decreased by 8wks of treatment. α-diversity (Shannon Diversity Index) was not significantly impacted by isotretinoin treatment. However, β-diversity (VSAT) was significantly altered at both 8wks and 20wks ($p<0.05$) of isotretinoin compared to pretreatment. The relative abundance of *C. acnes* significantly decreased after 20wks of treatment ($p=0.03$) and analysis of *C. acnes* strains showed that SLST cluster A strains (phylotype IA) were most affected and decreased by 20wks ($p<0.05$). We identified 989 KEGG orthology (KO) terms enriched at 20wks compared to 0wks. Pathway analyses revealed significant decreases in 3 pathways: amino acid biosynthesis ($q=.00055$), peptidoglycan biosynthesis ($q=.0143$), and folate biosynthesis ($q=.05$). Down regulation of these three pathways likely reflects the change in energy sources (decreased sebum) and decreased levels of *C. acnes.* Six months after isotretinoin, sebum and *C. acnes* levels, microbial composition and metabolic pathways returned to pretreatment levels, indicating that isotretinoin does not permanently alter the skin microbiome. In sum, 20 weeks of isotretinoin treatment is necessary to induce simultaneous changes in the critical factors of acne pathogenesis.

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A retrospective study of acute graft-versus-host disease and its mimickers in the post-transplant period

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Acute Graft-versus-Host Disease (GVHD) is a potentially fatal immune response that requires differentiation from other hypersensitivity responses occurring after allogeneic stem cell transplantation. Our study retrospectively investigates a wide array of variables in 69 patients that underwent an allogenic stem cell transplant and were discharged with a GVHD or rash-related ICD9 code within 100 days between 2012-2014 at The Ohio State University Wexner Medical Center. When compared to non-GVHD rashes, GVHD had 7.4 times the odds to occur between days 20-40 than in 0-20 ($p=0.004$). GVHD was also associated with a higher body surface area affected (43.3% in GVHD vs. 29.7% in non-GVHD, $p=0.031$). When including any organ GVHD diagnosis, the rash was 9.2 times the odds to occur between days 20-40 ($p=0.001$). Furthermore, patients diagnosed with acute GVHD had 4.4 times the odds to experience diarrhea ($p=0.032$). While GVHD still poses a diagnostic challenge, this study reinforces the extreme importance of the timeline for the eruption to occur in comparison with other demographic and clinical factors and also introduces the potential value in the extent of involvement. Further studies should be conducted to increase the power of these findings as well as investigate further relationships regarding minor morphologic differences, body sites, and coexisting symptoms by only the most high-risk time period after transplant.

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Pharmacological properties of Myrtacine and Celastrol extracts on *Cutibacterium acnes* and immuno-inflammatory cascade involved in acne

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Acne vulgaris is a chronic and recurring skin disease affecting many adolescents and adults throughout their lifetimes. The pathogenesis of acne involves an interplay of several factors including sebum production increase and follicular hyperkeratinization. More recently, another key factor has been identified: the microbiome, and more particularly *Cutibacterium acnes* (*C. acnes*) specie, and its impact on the local Th17-mediated immuno-inflammation. In this study, the pharmacological properties of Myrtacine®, Myrtus communis extract, and Celastrol enriched plant cell culture extracts, alone or in association, have been investigated. We studied their effect on IA1 *C. acnes* pathogen phylotype and the immuno-inflammatory cascade on cellular and tissue acne-mimicking models. Myrtacine (0.001%), showed an IA1 *C. acnes* anti-biofilm activity by inhibiting its formation and promoting its destruction. Moreover, Myrtacine had an anti-virulence effect by the significant inhibition of several virulence factor gene expression. Myrtacine (0.001%) and Celastrol (0.0025%) significantly and synergistically inhibited pro-inflammatory cytokines (IL 6, IL-8, IL10, IL12p40 and TNFα) produced in response to IA1 *C. acnes* phylotype. Moreover, an inhibitory effect of Celastrol, in solution or formulated at 0.3%, was demonstrated specifically on IL17 released by immune cells *in vitro* but also by Th17-lymphocytes integrated in a 3-dimensional skin model. Our results demonstrate the strong regulatory properties of Myrtacine and Celastrol on *C. acnes* and Th17-mediated immuno-inflammation. Therefore, it shows the real interest of these two active ingredients for a targeted therapy of inflammatory acne disease

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Whole-blood immune profile in hidradenitis suppurativa

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Hidradenitis suppurativa (HS), a chronic inflammatory skin condition with a multifactorial etiology, has a complex cutaneous immune reaction localized around the hair follicles in intertriginous skin. HS pathogenesis remains enigmatic, although some hypotheses have been proposed. Increasing evidence of the association between HS and other inflammatory conditions (e.g. inflammatory bowel disease) and cardiovascular disease suggests that patients with HS have underlying systemic inflammation. To date, few studies have sought to understand the systemic changes that occur in the immune system of HS patients. One recent study performed bulk RNA-sequencing on peripheral blood mononuclear and showed minor differences in transcriptomes of peripheral blood mononuclear cells, but bulk RNA-sequencing does not have the capacity to identify specific changes in cellular subsets. To determine whether specific systemic changes occur in HS patients we performed CyTOF using a standardized panel that identifies 37 immune cell subpopulations in whole blood. We analyzed whole blood samples from 8 HS and 7 healthy controls. Compared to healthy controls, HS patients had an increased frequency of plasmablasts and a decreased frequency of $CD66b^-$ neutrophils. Furthermore, marked differences in monocyte subclasses showed a shift from classical monocytes ($CD14^+$ $CD16^-$) towards intermediate ($CD14^+$ $CD16^+$) and non-classical subsets ($CD14^{dim}$ $CD16^+$) in HS. We also identified a large population of $CDR45RO^+$ $CCR6^+$ $CD38^+$ intermediate monocytes in HS, which was largely absent in healthy controls. Taken together our results support previous studies highlighting the role of neutrophils and B cells in HS pathogenesis, and identify newly discovered monocyte dynamics in peripheral blood of HS patients further supporting widespread inflammation as a feature of HS.

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Murine cutaneous microbiota composition is largely mouse strain determined with microbiota changes during acute wound healing showing mouse strain specific responses

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To date little research has investigated the genetic determinants of cutaneous microbiota composition and how microbiota composition can effect acute wound healing. Here we used 114 mice totalling 30 different mouse strains from an advanced cross-breeding program, The Collaborative Cross, and performed large, 1.5x1.5cm, full excisional wounds of mouse dorsal skin. 16sRNA sequencing immediately before wounding and at days 3 and 10 post-wounding show microbiota compositional changes are largely strain dependent with different mouse strains showing different changes in microbiota composition as a result of wounding. Principle component regression of centred-log ratio abundances and wound healing speed, time-to-wound closure, show an adjusted R-squared <5%. PERMANOVA analysis suggests <4% of variance in microbiota compositional changes during healing are explained by wounding alone with >40% variance explained by mouse strain and mouse strain specific responses to wounding. Murine microbiota composition is largely mouse strain dependent with microbiota changes during wound healing largely determined by mouse strain specific responses.

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Rho Kinase deficiency protects mice from UVB-induced skin inflammation by inhibition of neutrophil NETosis

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Background: Ultraviolet B (UVB) is an important risk factor for lupus. UVB induces skin inflammation with recruitment of neutrophils that become NETotic and exhibit NET-associated proinflammatory cytokines. We have recently identified that PKCα-mediated nuclear lamin B phosphorylation is responsible for nuclear envelope rupture, nuclear DNA release and NET formation. Interestingly, inhibition of Rho Kinase (ROCK) with its inhibitor HA1077 attenuates actin assembly, PKCα nuclear translocation, and NETosis in vitro. Intraperitoneal application of HA1077 alleviates NETosis in vivo and NET-associated proinflammatory cytokines in the skin of UVB-irradiated C57/BL6 wildtype (WT) mice. Here, we sought to further study the causal role of ROCK in NET formation and UVB-induced skin inflammation. Method: We have generated CD45.1 mice with hematopoietic specific ROCK1 deficiency by bone marrow transplantation (BMT) of hematopoietic stem cells (HSCs) from ROCK1 deficient mice, followed by UVB exposure (150 mJ/cm^2, 5 consecutive days). We examined neutrophil NET formation in vitro and in vivo, as well as NET-associated IFNα, IFNγ, TNFα, IL-17A expression and exhibition in skin of the UVB-irradiated mice. Results: We found that ROCK1 deficiency decreases NET formation in vitro in neutrophils from ROCK1 deficient mice as compared to those from WT mice. Very importantly, NET formation and NET-associated IFNα, IFNγ, TNFα, IL-17A were significantly attenuated in the skin of UVB-irradiated BMT mice with ROCK1 deficiency as compared to those transplanted with WT HSCs. In an ex vivo experiment, exhibition of neutrophil NET-associated IFNγ, TNFα, IL-17A were significantly reduced in PAF-treated neutrophils from ROCK deficient mice as compared to those from WT mice. Conclusion: Hematopoietic specific ROCK1 deficiency attenuates neutrophil NET release and NET-associated proinflammatory cytokine display in UVB-induced skin inflammation.

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Induction of protective antimicrobial responses mediated by NOD2 as a treatment for wounds infected with multidrug-resistant bacteria

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Of critical concern is the increasing antimicrobial resistance of bacteria and the decreasing availability of effective treatments for these multi-drug resistant (MDR) strains. The antimicrobial activity of the pattern recognition receptor NOD2 is increased by the pyrimidine synthesis inhibitor *N*-phosphonacetyl-l-aspartate (PALA) *in vitro*. We investigated the efficacy of a topical PALA formulation to enhance NOD2-mediated protective immune responses as novel therapeutic approach for wounds infected with MDR bacteria. In a mouse model of MRSA infected biopsy wounds, topical application of PALA increased bacterial clearance from wound tissue as early as day 4 post-infection without impairing wound healing kinetics. PALA enhanced bacterial clearance corresponded with increased epidermal and dermal production of antimicrobial peptides (AMPs), including Reg3β, Reg3γ, mBD14 and mBD3, as well as increased levels of TNF-α, IL-1β, IL-17A, and IL-22, indicating an involvement of the IL-17/IL-22 axis in this response. NOD2 expression was required for this response, as *Nod2*$^{-/-}$ mice exhibited a loss of PALA-enhanced MRSA clearance, AMP expression, and Th17 cytokine expression. Molecular analyses *in vitro* demonstrate that PALA treatment increased NOD2 activation of the transcription factor IRF1 and upregulation of IL-17C, IL-22, and NOD2 expression. PALA-enhanced IRF1 activation required the expression of NOD2 and the protein kinase RIP2, but not the adaptor protein MAVS, as demonstrated by siRNA-mediated knockdown analysis. However, PALA enhanced clearance of MRSA required expression of all three proteins, suggesting crosstalk between canonical and non-canonical NOD2 signaling pathways enhances protective antimicrobial responses. These results indicate that induction of protective antimicrobial immune responses mediated by NOD2 is achievable and well tolerated, suggesting this could be an effective therapeutic approach to MDR bacterial infections.

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A positive feedback loop between mTORC1 and cathelicidin promotes skin inflammation in rosacea

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Rosacea is a chronic inflammatory skin disorder whose pathogenesis is unclear. Here, we focus on addressing the role of mTOR signaling in the pathogenesis of rosacea. We report that mTORC1 signaling is hyperactivated in both rosacea patients and mouse models. Functionally, both mTORC1 ablation and pharmacological inhibition by its specific inhibitors restrained the development of rosacea in an LL37-induced rosacea-like mouse model. On the contrary, hyperactivation of mTORC1 signaling in TSC2$^{+/-}$ mice exacerbated rosacea development. Furthermore, we revealed a positive feedback circuit between mTORC1 signaling and cathelicidin, in which LL37 activates mTORC1 signaling by binding to TLR2, which in turn enhances the expression of cathelicidin. Subsequently, cathelicidin LL37 derived from this loop stimulates NF-κB signaling, cytokines and chemokines production which are key factors associated with rosacea development through mTORC1 signaling. Finally, our pilot clinical study showed that topical application of rapamycin had a significant curative effect on rosacea patients. Collectively, these findings suggest a pivotal role for mTORC1 signaling in the pathogenesis of rosacea and reveal a potential therapeutic target for rosacea treatment.

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Effect of triamcinolone & manuka honey on *Staphylococcus aureus* growth & hemolytic activity

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Atopic dermatitis (AD) is an inflammatory skin disease commonly treated with topical corticosteroids (TCS). Manuka honey (MH) is an alternative therapy made from nectar of the *Leptospermum scoparium,* native to New Zealand. The high abundance of *Staphyloccocus aureus* (*S. aureus*) on AD skin enhances disease severity, in part due to the effects of hemolysins. To address the effect TCS & MH have on *S. aureus,* we quantified growth & hemolytic activity of USA300 (MRSA strain) with & without exposure to triamcinolone acetonide (TAC) or MH. USA300 was cultured in Tryptic soy broth (TSB) containing 0.1% w/v TAC or MH (Manukora^{TM}; 1.25%, 2.5%, 5% & 10% w/v). Growth after MH exposure was evaluated by measuring optical density at 600nm (OD_{600}). Due to TAC's low solubility which interfered with OD readings, we evaluated growth by colony forming units (CFUs) after 5 hrs. After 24 hrs, supernatants were filtered (0.22-μm) and spotted onto rabbit blood agar (RBA) to quantify hemolytic activity (diameter of zones of clearance). TAC exposure resulted in a slightly lower CFUs (avg 0.82 x 10^9) than TSB alone (avg 1.26 x 10^9 CFU; n=2/condition), suggesting that TAC did not significantly inhibit USA300 growth. In contrast, MH markedly reduced OD_{600} values by 5, 23, 82 & 92% in 1.25, 2.5, 5, & 10% MH, respectively, compared to TSB alone (mean; n=3, p<0.03) after 5 hrs. This inhibitory effect of MH on USA300 growth was notable with the most significant reduction (mean 35%; n=3, p=0.001) observed at the highest concentration (10%) that was still observed at 24 hrs. Supernatants from TAC-incubated USA300 reduced clearance zones on RBA (avg 1.8±0.4 mm) compared to TSB alone (avg 5.8±0.4 mm; n=2/condition). Whereas, MH, inhibited all hemolytic activity, even at the lowest concentration 1.25% (n=3). Collectively, these findings demonstrate that at even at low MH concentrations, commonly found in cosmetic products, there is a greater effect on *S. aureus* growth as well as nearly complete inhibition of hemolytic activity compared to TAC, the most commonly used topical TCS.

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Shifts in the skin bacteria and fungi in healthy children transitioning through puberty

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While skin microbial shifts among different age groups are known from several culture- and sequencing-based studies, the influence of sexual maturation on the skin microbiome in individuals followed longitudinally has not been studied as systematically. In this prospective and longitudinal study, twelve healthy children were evaluated up to 6 years to investigate puberty-associated skin microbial shifts. Using 16S rRNA (V1-V3) and ITS1 amplicon sequencing with DADA2 analysis, bacterial and fungal communities of five different skin sites were analyzed and compared with serum hormone levels. The composition and the diversity of skin microbial communities transitioned toward a more 'adultlike' microbiome. The microbial shifts were associated with Tanner stages and showed sex-specific differences. Female children demonstrated increasingly predominant lipophilic *Cutibacterium* and *Malassezia* in early Tanner (2-3) versus late Tanner (4-5) stages (mean relative abundances of *Cutibacterium*, $P < 0.001$ [15.9% to 32.2%] for antecubital fossa; *Malassezia*, $P = 0.03$ [28.6% to 53.3%] for antecubital fossa and $P < 0.001$ [24.5% to 58.5%] for volar forearm). Furthermore, the microbial communities in female children converged to be more similar to one another with increasing Tanner stages, but not in male children. The higher relative abundances of lipophilic species, *C. acnes* and *M. restricta*, were strongly associated with levels of serum androgens which are known to influence sebaceous gland activity (testosterone, $P < 0.001$ for *C. acnes* and *M. restricta*; androstenedione, $P = 0.11$ for *C. acnes* and $P = 0.16$ for *M. restricta*), suggesting hormonal effects on the skin microbial communities. In summary, these results signify complex interrelationships of sexual maturation, skin microbiome, and skin physiology.

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Single cell transcriptomics identifies a potential role for Arg1+ macrophages in alopecia areata pathogenesis

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Alopecia Areata (AA) is an autoimmune disease that attacks the hair follicle and leads to hair loss ranging from small patches to total scalp and body hair loss. We previously showed that AA pathogenesis is predominantly mediated by CD8+ T cells, however, the role of other immune cells in AA has not been widely studied. We performed single cell RNA-seq on CD45+ immune cells harvested from the dorsal skin of AA-affected and control C3H/HeJ mice. Unbiased clustering revealed three distinct subsets of macrophages, two of which were shared between AA and control mice. The third cluster was comprised of cells that predominantly originated from AA mice, suggesting that these cells may be involved in AA pathogenesis. Interestingly, this cluster of macrophages exhibited significant upregulation of genes involved in arginine metabolism, including Arginase 1 (Arg1), the main enzyme involved in arginine catabolism. Although Arg1 was previously associated with suppressive M2 macrophages, the AA-enriched Arg1+ macrophages also showed upregulation of pro-inflammatory cytokines as well as multiple glycolytic genes that were associated with an inflammatory phenotype in macrophages. Flow cytometry and immunohistochemistry validation showed an increased frequency of Arg1+ macrophages in AA skin, localized near the hair follicle bulb, the site of autoimmune attack in AA. Further, treating C3H/HeJ mice prior to disease onset with nor-NOHA, an arginase inhibitor, reliably resulted in a delay in disease onset, consistent with recent studies showing that arginase inhibitors demonstrated therapeutic efficacy in pre-clinical models of other inflammatory disorders such as systemic lupus erythematosus and inflammatory bowel disease. The identification of a unique population of Arg1+ macrophages delivers novel insight into metabolic control of macrophages in AA, and reveals potential novel therapeutic targets in metabolic pathways.

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***Bacteroides* colonization is associated with reduced depigmentation in vitiligo**

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Perinatal administration of antibiotics influences vitiligo kinetics in FH mice prone to depigmentation. Depigmentation in FH mice is mediated by tyrosinase-reactive CD8 T cells. Depigmentation was accelerated over the first 6 months by ampicillin, and trended towards delayed development by neomycin administered through the drinking water. Neomycin administration was associated with increased cutaneous Treg infiltration. 16S rRNA sequencing revealed an increased prevalence of *Pseudomonas* in response to ampicillin, and increased *Bacteroides* in response to neomycin. We questioned whether gut colonization by *Bacteroides* predicts delayed vitiligo development, and initiated a clinical study to analyze the microbiome of human vitiligo patients compared to healthy controls. DNA extracted from fecal samples of 10 subjects were submitted for microbiome analysis. Again a trend towards (50%) increased prevalence of *Bacteroides* was observed among healthy controls, whereas subjects with vitiligo trended towards a (50%) increase in *Faecalibacteria*. Congruent with our mouse studies, vitiligo patients generally exhibit fewer skin Tregs than controls. Finally we subjected vitiligo prone mice to topical antibiotics. Over-the-counter neosporin or bacitracin ointment, or petroleum jelly were applied to denuded dorsal skin of pmel-1 mice that develop gradual depigmentation, mediated by gp100-reactive CD8 T cells. Ointments were applied for a total of 6 weeks in 2 periods separated by a short antibiotic holiday, to one side of the dorsum; the other was treated with petroleum jelly. Controls were treated with petroleum jelly alone. Neomycin rich ointment significantly delayed bilateral vitiligo development compared to mice treated with petroleum jelly alone. Topical treatment did not have a remarkable impact on the gut microbiome. It may be possible to identify and exploit protective *Bacteroides* species for vitiligo treatment.

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Utilizing in vivo proximity labeling combined with affinity purification-mass spectrometry to investigate interacting partners of vaccinia dsRNA-binding protein E3

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Proximity labeling coupled with affinity purification-mass spectrometry is a newly developed method for studying protein-protein interaction in vivo. APEX is an ascorbate peroxidase that oxidizes biotin phenol to generate short-lived radicals, which react with side chains of amino acids in proximity. Streptavidin beads are then used to pull doen biotinylated proteins, which are subjected to mass spectrometry. Vaccinia virus is a large cytoplasmic DNA virus that belongs to the poxvirus family. The vaccinia E3L gene encodes a key virulence factor that is composed of Z-DNA-binding and dsRNA-binding domains. E3 targets multiple pathways including IFN production and stress responses. E3 is localized to the virosome, where viral DNA replication and intermediate and late gene transcription occur. To investigate E3-binding partners in the host, we generated two recombinant modified vaccinia virus Ankara (MVA) viruses- one with E3L tagged with FLAG-APEX2 and the other with FLAG-APEX2 replacing the E3L gene. In both cases, the expression of either E3L-FLAG-APEX2 or FLAG-APEX2 is under the control of endogenous E3L promoter. Imaging analyses of MVA-E3L-FLAG-APEX2 or MVAΔE3L-FLAG-APEX2 infected B16-F10 murine melanoma cells at 8 h post-infection revealed that E3-FLAG-APEX2 is located in the virosomes. Consistent with that, mass spectrometry of streptavidin-pulled down proteins showed that several viral proteins are enriched, including E3, major core proteins 4a and 4b, B1 kinase, H1 dual-specificity protein phosphatase. For host proteins, the majority of E3-interacting host proteins include nucleolysin TIA, RNA-binding protein 3, Caprin, RNA-binding protein 3, Caprin 1, and G3BP1. Imaging studies demonstrate that MVAΔE3L infection in B16-F10 cells induces the formation of stress granules, whereas MVA does not. These results provide strong evidence that E3 interacts with proteins involved in stress-granule formation in the virosomes.

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The association between topical calcineurin inhibitor use and risk of cancer: A systematic review and meta-analysis

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Topical calcineurin inhibitors (TCI) are commonly used for atopic dermatitis and other inflammatory dermatoses. The U.S. Food and Drug Administration issued a black box warning in 2006 indicating a potential risk of malignancy with TCI use based primarily on case reports, animal studies, and systemic tacrolimus use in organ transplant recipients. Since then, large epidemiologic studies have examined the association between TCIs and cancer; we conducted this systematic review and meta-analysis of observational studies to synthesize the evidence. We searched Medline, Embase, and Web of Science from inception to August 2020. We included observational studies investigating the association between treatment with TCIs (tacrolimus, pimecrolimus) and development of cancer, with non-active or active comparators. A total of 8 cohort studies (408,366 treated participants, 1,764,313 non-active comparator controls, 1,067,280 controls using topical corticosteroids) and 3 case-control studies (3,898 cases and 14,026 cancer-free controls) were included. There was no association between TCI use and cancer overall compared to non-active comparators (relative risk (RR) 1.03, 95% confidence interval 0.92 to 1.16). Lymphoma risk was elevated with TCI use in studies with non-active (RR 1.86, 1.39 to 2.49) more than topical corticosteroid comparators (RR 1.35, 1.13 to 1.61), suggesting the relationship may be partly confounded by indication. No significant association was found between TCI use and skin cancer. In summary, we found TCI use to be associated with a modestly elevated risk of lymphoma but not with other cancers. Given the low absolute risk of lymphoma, patients and clinicians should be reassured by these findings.

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Differences in outpatient dermatology encounter work Relative Value Units by patient race, sex, and age

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In the United States, persons of color have less access to dermatologic care than whites. Financially undervaluing medical conditions that disproportionately affect specific patient groups may perpetuate inequitable healthcare. The objective of this retrospective cross-sectional single institution study was to determine whether patient race and sex influence the work relative value units (wRVUs) generated in outpatient dermatology encounters. 66,463 outpatient dermatology encounters from 2016-2020 among 30,036 unique adult patients were analyzed. Patients had mean age of 55.9 (SD: 18.5) years and were predominantly white (70.1%) and female (59.6%). The average wRVUs/encounter was 1.40 (SD: 0.71). In adjusted analysis, non-white race, female sex, and younger age were associated with significantly fewer wRVUs per encounter. Dermatology visits with Black patients generated 0.267 (95% CI: 0.254-0.280) fewer wRVUs/encounter compared to white patients. Female sex was also associated with 0.111 (95% CI: 0.101-0.122) fewer wRVUs/encounter, and wRVUs/encounter increased by 0.06 (95% CI: 0.06-0.06) with each 10-year increase in age. Destruction of premalignant lesions and biopsies were strong mediators of the observed race, sex, and age differences. In conclusion, dermatology encounters among persons of color and women generate fewer wRVUs than those with white males. Dermatologist compensation based on wRVUs may incentivize provision of specific services, exacerbating differential access to care and disparities in healthcare outcomes.

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Cross sectional descriptive study: First adult atopic dermatitis clinic in Canada

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The natural history of atopic dermatitis (AD) in adulthood remains poorly understood. Adults with AD struggle chronically thoughout their lives, and often have severe or resistant dermatitis. In North America, adult patients were previously orphaned from on-label systemics, with cohort and real-world studies remaining limited. This led to the establishment of the first adult AD-dedicated Canadian tertiary clinic in 2018, the McGill University Hospital Network Centre of Excellence for Adult AD (McGill COE-AD), with a need to describe its patient population. The purpose of this study was to characterize the first adult AD clinic in Canada and its population. A cross-sectional questionnaire was administered to 122 patients at McGill COE-AD from April 2018 to November 2020. Patient age, gender, age at diagnosis, atopic history, skin hygiene, and AD severity (EASI) were collected. Descriptive statistical analyses were performed. The population, 56% female, had age distributed as: 27% age 18-29; 29% age 30-39; 19% age 40-49; and 25% age >49. 77% had childhood eczema and 18% were diagnosed after age 21. 40% of patients self-reported having asthma and 60% self-reported having seasonal allergies. Notably, 94% routinely applied emollient and 72% had consulted Dermatology prior to visiting COE-AD for uncontrolled disease, while 45% had a primary care provider. Despite this, 85% of patients reported washing their eczema each time they bathe, most often with liquid soaps. AD severity was distributed as follows: 11.1% mild (EASI 1-7.0); 41.7% moderate (EASI 7.1-21); 9.7% severe (EASI 21.1-50); and 37.5% very severe (EASI 50.1-72). Our findings are consistent with an increasing recognition of adult-onset AD, with nearly one-fifth of this Canadian cohort being diagnosed after age 21. A majority of patients had previously consulted with Dermatology but remained uncompliant or unaware of general measures such as avoidance of soaping. Our findings highlight both a high prevalence of adult AD and unmet needs for knowledge translation.

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Association of stroke with psoriasis in end-stage renal disease patients

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Previous research in the general population suggests that the inflammatory skin disease psoriasis is associated with an increased risk of vascular events, such as stroke. Thus, psoriasis may also represent a significant risk factor for stroke in dialysis patients. We queried the United States Renal Data System for incident dialysis patients between 2004 and 2015. Psoriasis was defined as having at least two ICD-9 or ICD-10 diagnosis codes. ICD codes were also used to query the outcome of interest, stroke, as well as other clinical risk factors. Logistic regression was used to examine the association of psoriasis and other risk factors with stroke. Of 966,399 end-stage renal disease (ESRD) patients, we identified 89,700 (9.3%) subjects with stroke and 6,286 (0.7%) with psoriasis. Of the 6,286 patients with psoriasis, 796 (0.9%) also had a stroke. Psoriasis was associated with an increased risk of stroke in an unadjusted model [odds ratio (OR)=1.16; confidence interval (CI)=1.08-1.25]. However, after controlling for age, race, sex, ethnicity, dialysis modality, access type, congestive heart failure, myocardial infarction, pulmonary disease, connective tissue disease, peptic ulcer disease, liver disease, diabetes, paraplegia, cancer, metastatic cancer, AIDS, and tobacco and alcohol dependence, the final adjusted model showed that psoriasis was not associated with stroke (OR=0.96, CI=0.88-1.04). Congestive heart failure was found to be a confounder of the association of psoriasis with stroke, with a final adjusted OR of 1.79 (CI=1.75-1.83). Thus, contrary to prior research in the general population, in ESRD patients, psoriasis was not associated with an increased risk of stroke after controlling for various demographic and clinical parameters. Our finding emphasizes the importance of controlling for a wide variety of confounders in population studies examining associations between diseases and risk factors.

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Opiate use in dermatology in the United States: A population-based study using the national ambulatory medical care survey

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The role of dermatologists in the opioid epidemic is unknown. While dermatologists perform many procedures and treat many painful skin conditions, there is a knowledge gap in understanding their pain medication management. This study sought to analyze patterns in oral pain medication prescribed by dermatologists. We conducted a cross-sectional, population-based study using the National Ambulatory Medical Care Survey from 2009 to 2016. Among 288,462,610 weighted visits, 3,650,070 (1.3%) of visits included an oral opiate prescription. Other oral analgesics were also analyzed, with 13,638,281(4.7%) of visits including non-steroidal antiinflammatory prescriptions, 1,932,515 (0.7%) of visits including acetaminophen prescriptions, and 1,593,134 (0.6%) of visits including gabapentin prescriptions. Overall, 43.1% of all opiates prescribed were for dermatological procedures. Additionally, diagnoses with the highest rates of opiate prescribed included vitiligo (10.3%), hemangioma (3.8%), and pemphigus (3.6%). Increasing patient age ($p<0.001$) and Northeast and West geographic regions ($p=0.02$) were significantly associated with increased opiate prescribing. Opiates were prescribed at higher rates for patients with certain risk factors, such as concurrent benzodiazepine use (adjusted OR 8.17, 95% CI 5.3-12.7), depression (adjusted OR 3.28, 95% CI 2.0-5.4), substance use disorder (adjusted OR 9.40, 95% CI 2.0-44.4), and tobacco use (adjusted OR 1.09, 95% CI 1.0-1.1), after adjusting for socio-demographic factors. In conclusion, while opiate prescribing among dermatologists is low, dermatologists need to be aware of factors associated with their use and consider non-narcotic analgesics when appropriate.

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Psychiatric comorbidity in prurigo nodularis and the impact of socioeconomic status

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Prurigo nodularis (PN) is a poorly understood chronic condition of intense pruritus. Psychiatric disorders may perpetuate the disease and pose a potential therapeutic target given the paucity of efficacious PN treatments. Understanding the impact of socioeconomic status (SES) may help clinicians address mental health in this population. This study assessed the relationship between SES and incidence of psychiatric comorbidity among PN patients and quantified the utilization of mental health services. A retrospective chart review of PN patients from 2007-2019 was performed. Per capita income by zip code (US Census Bureau) was compared to the Livable Income Threshold by county (MIT Living Wage Calculator) to determine SES as follows: below the LIT, <100% above LIT, >100% above LIT, >200% above LIT and >300% above LIT. Wilcoxon, Chi-square, and Fisher's exact tests were used to test the associations of continuous and categorical variables, respectively. Of 288 patients, 57% were female and significantly more likely to have a psychiatric disorder than men (51% vs 32%, $p=0.001$). 44% of patients had at least 1 psychiatric comorbidity, with mood (75%) and anxiety (63%) disorders being most common. Median income was $34,775 and 25% of incomes were below the LIT. Lower SES groups had a higher incidence of psychiatric disorders (48% vs 43%, $p=0.46$) and utilization of mental health services (60% vs 46%, $p=0.23$). 41% of patients with a psychiatric disorder had no record of psychiatrist or psychologist visits. Previous studies have shown that PN patients with psychiatric symptoms often require medication and hospitalization. Our results highlight a gap in care, as a significant proportion of patients lacked psychiatric evaluation regardless of SES. Women and lower SES groups may be vulnerable populations given the higher incidence of psychiatric comorbidities. These results emphasize the need for vigilant psychosocial evaluation and support in the PN population.

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Pemphigus and bullous pemphigoid in the United States: A population-based study evaluating patient clinical characteristics and treatment trends

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Pemphigus and Bullous Pemphigoid (BP) are blistering skin diseases that have been increasing in incidence. Determining patient characteristics and medication prescribing patterns for pemphigus and BP can help dermatologists better care for this medically complex population. This study aimed to examine patient characteristics and treatments prescribed at pemphigus and BP visits in the United States. We performed a cross-sectional, population-based analysis using the National Ambulatory Medical Care Survey (NAMCS) database from the years of 1995-2015. During the study years, there were 453,348 (weighted) pemphigus visits. Female patients accounted for the majority (61%). Patients between the ages of 60-69 years accounted for 33% of the visits. Dermatologists conducted 92% of pemphigus visits, and primary care physicians (PCP) conducted 6%. The most common treatments prescribed at pemphigus visits were prednisone (44%), topical corticosteroids (27%), trimethoprim/sulfamethoxazole (13%), and tetracycline (2%). Pemphigus visits were associated with diagnoses such as urinary tract infections (15%), diabetes (15%), and gastroesophageal reflux disease (12%). Additionally, from 1995-2015 there were 1,020,457 (weighted) BP visits. The majority of visits were from females (68%). Individuals 70 years and older accounted for 68% of all BP visits. Dermatologist conducted 96% of BP visits, and PCP did not conduct any visits. The most common treatments prescribed at BP visits were prednisone (32%), topical corticosteroids (22%), methotrexate (6%), minocycline (6%), and doxycycline (5%). BP visits were associated with the diagnosis of hypertension (5%). In conclusion, this study demonstrates that both pemphigus and BP predominantly affect an older population. Notably, oral prednisone was the most commonly prescribed treatment for pemphigus and BP.

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Cutaneous squamous cell carcinoma and mortality in end stage renal disease

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Multiple factors related to immunosuppression are associated with cutaneous squamous cell carcinoma (cSCC), for example, the increased risk of cSCC observed in immunosuppressed organ transplant recipients, which in turn can lead to death. Patients with end-stage renal disease (ESRD) are known to have dysregulated immune systems; however, the impact of cSCC on mortality in ESRD has not been reported. Therefore, we addressed this question by analyzing the mortality of adult ESRD patients in the US Renal Data System between 2004 and 2014, excluding organ transplant recipients. A Cox Proportional Hazards (CPH) analysis controlling for demographic and clinical parameters was used to determine if cSCC was a risk factor for mortality in ESRD. Of the 1,035,193 patients analyzed, 624 patients (0.1%) were diagnosed with cSCC after initiation of dialysis. The median survival time for those with cSCC was 3.91 years [95% confidence interval (CI)=3.67-4.15] while the median survival for those without cSCC was 2.92 years [95% CI=2.92-2.93]. ESRD patients with cSCC were at lower risk of death [adjusted hazard ratio=0.75; 95% CI=0.69-0.82] compared to those without. Decreased risk of death was also associated with Black race, Hispanic ethnicity, increasing Charlson Comorbidity Index scores, and a diagnosis of tobacco dependence or actinic keratosis. Increased risk of death was associated with increasing age, male sex, hemodialysis (vs peritoneal dialysis) and alcohol dependence. Thus, ESRD patients who develop cSCC have decreased mortality risk relative to those without cSCC, in contrast to the association between cSCC and increased risk of death in the general population and in organ transplant recipients. The reason for this difference remains unclear and suggests the need for further study of cSCC in ESRD patients.

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Differences in acne therapy prescribing patterns between pediatricians and dermatologists

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Many children and adolescents with acne seek care from both pediatricians and dermatologists. However, the acne treatments that patients receive may differ between these two specialties. This study sought to determine if differences exist in acne prescribing patterns between pediatricians and dermatologists. We conducted a population-based, cross-sectional analysis using data from the National Ambulatory Medical Care Survey from 2002 to 2016 for pediatric patients (ages $\leq$18). There were approximately 45.8 million (weighted) outpatient acne visits between 2002 and 2016 for pediatric patients; 54% of visits were conducted by dermatologists, 28% by pediatricians, and 18% by other providers. Compared to pediatricians, dermatologists saw older patients (mean age 15.4 $\pm$ 2.22 vs 13.1 $\pm$ 4.49; p<.001), as well as a higher proportion of white patients (92.8% vs 79.7%; p<.001), non-hispanic patients (86.8% vs 76.9%; p<.001), and patients with private insurance (83.1% vs 69.5%; p<.001). The most frequently prescribed medication classes by dermatologists and pediatricians were topical retinoids (41.1%) and topical combination therapies (20.1%), respectively. Compared to patients seen by dermatologists, patients seen by pediatricians were 71% less likely to receive topical retinoids (OR 0.29, 95%CI 0.21-0.40), 48% less likely to receive topical antibiotics (OR 0.52, 95%CI 0.35-0.77), and 53% less likely to receive oral antibiotics (OR 0.47, 95%CI 0.34-0.66). Our findings demonstrate that pediatricians prescribe topical retinoids, topical antibiotics, and oral antibiotics less frequently compared to dermatologists. It is important to understand these differences in prescribing patterns for acne care and to identify potential educational gaps.

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Association of ruxolitinib with NMSCs risk in patients with polycythemia vera and myelofibrosis

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The risk of skin cancer in patients with polycythemia vera (PV) or myelofibrosis (MF) taking ruxolitinib has not been systematically studied outside of the clinical trial setting. To evaluate the risk of developing non-melanoma skin cancer (NMSC) after ruxolitinib exposure in a real life setting, we conducted a retrospective cohort study for patients diagnosed with PV or MF between January 1, 2010 to January 1, 2020 at a single academic referral center. Eligible patients (n=2,563) were matched on age, gender, race, Charlson comorbidity index, disease diagnosis (PV or MF), and follow-up time to create the study cohort (n=564). MF/PV patients exposed to ruxolitinib had a hazard ratio (HR) of 2.70 (95% CI, 1.06-6.92) for NMSC compared to unexposed patients. In particular, HR of 3.13 (95% CI, 1.35-7.26) was observed for SCC, adjusted for age, gender, transplant history, chemotherapy history, radiation history, immunosuppression history, and hydroxyurea exposure. Increased age, immunosuppression history, and white race were additional independent risk factors. The increased risk of NMSC suggests the importance of regular skin cancer screening for patients taking ruxolitinib.

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The epidemiology of atopic eczema in older adults: A population-based study in the United Kingdom

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Increasingly, studies have focused on atopic eczema beyond childhood but there remains a gap in the literature on older adults. We sought to characterize the epidemiology of atopic eczema among older adults in comparison to other age groups by investigating how patient characteristics, patterns of disease activity and severity, and calendar trends varied across the life course. Atopic eczema was identified using a validated algorithm in a cohort of 9,154,936 individuals aged 0-99 registered in a UK electronic health records database (The Health Improvement Network) from 1994-2013. Cross-sectional analyses of disease prevalence were conducted at each age, and logistic mixed effect regression models were used to identify predictors of active disease over time among children (ages 0-17), adults (ages 18-74), and older adults (ages 75-99). We found that physician-diagnosed atopic eczema increased across the 2-decade period and was most common in children (18.3%) and older adults (11.6%). While atopic eczema in children and adults ages 18-74 was significantly associated with female sex, in older adults, atopic eczema was less common among females (AOR 0.73, 95% CI 0.70-0.76). It was also more likely to be active and of higher severity in older adults compared with the other age groups. While atopic eczema has been understudied in older adults, this study demonstrates that atopic eczema is increasingly common, active, and severe among those ages 75 to 99 and underscores the need for greater evaluation of the disease in the growing older adult population.

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Clinical outcomes in COVID-19 patients with varicella zoster virus

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Varicella Zoster Virus (VZV) causes shingles in adults and may be reactivated in stress related states such as inflammation. There is minimal literature on whether patients with VZV who contract COVID19 are at higher risks of complications and therefore the aim was to examine this. A retrospective cohort study was done using TriNetX, a national federated real time database of 63 million records. COVID patients were identified by validated ICD-10 and serology codes per CDC guidelines. An 1:1 matched propensity score analysis was conducted, adjusting for comorbidities and demographics, to calculate adjusted Risk Ratios (aRR) with 95% CI. 45-day COVID complications were examined with severe COVID being defined as a composite of mortality and ventilation. Subgroup analyses were also performed for VZV patients with a one-year history of antivirals. In a matched sample of 3493 patients in each cohort, there was no statistically significant difference between VZV-COVID patients and non-VZV COVID patients in outcomes such as hospitalization (1.01[0.83-1.22]), acute respiratory distress syndrome (ARDS) (1.41[0.96-2.07]), mechanical ventilation (0.98[0.75-1.28]), mortality (1.04[0.82-1.31]), and severe COVID (1.01[0.83-1.22]). VZV-COVID patients were at a statistically significant higher risk for sepsis (1.64[1.25-2.16]). Subgroup analysis revealed that VZV-COVID patients with a history of antiviral use were at statistically significant higher risks for hospitalization (1.37[1.07-1.74]) and severe COVID (1.65[1.01-2.75]) than VZV-COVID patients not on antivirals. No differences between the cohorts were seen in ARDS, sepsis, mechanical ventilation, and morality. VZV patients with COVID are not at higher risk for COVID complications compared to COVID patients without VZV. However, history of antiviral use in VZV-COVID patients has a higher risk for severe COVID compared those without antivirals. Additional research is needed to visit the longer term impacts of COVID on VZV patients.

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The infodemiology of hyperhidrosis: Examining trends and seasonality in public interest

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Hyperhidrosis is a condition that causes excessive sweating and is estimated around 15.3 million people in the USA suffer from it. However, the current knowledge on public interest and the seasonality of hyperhidrosis is limited. Therefore, the aim was to evaluate the infodemiology of hyperhidrosis. Google Trends was searched between Jan 2004- Dec 2019 for "Hyperhidrosis" in the USA and worldwide to gain information on public interest. Relative search volume (RSV) data from Northern Hemisphere countries (i.e. USA) and southern hemisphere countries (i.e. Australia) were also examined to assess trends in seasonality by a cosinor analysis to calculate amplitude {A}, phase month {P}, low point month {L}, and trend significance. Hyperhidrosis had an overall increasing trend in search volume in USA and worldwide. The 5 countries with the highest RSV was Philippines, United States, Australia, Canada, the United Kingdom. Within the USA, the 5 states highest RSV was South Dakota, North Dakota, West Virginia, Illinois, and Connecticut. The cosinor analysis revealed a statistically significant seasonal variation in RSV of hyperhidrosis in the USA ({A}=14.4, {P}=6.7, {L}=12.7, $p<0.0001$) and in Australia ({A}=9.34, {P}=12.9, {L}=6.9, $p<0.0001$). A pattern out of phase by 6 months was observed between the USA and Australia with peaks in the summer and troughs in the winter, confirming that the pattern is truly seasonal as opposed to being calendar-driven. Public interest in seeking hyperhidrosis information in the USA and worldwide has increased in recent years while also displaying a true seasonal pattern.

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Factors associated with in-hospital mortality in mycosis fungoides patients

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Mycosis fungoides (MF) is the most common cutaneous T-cell lymphoma. Early on, it is generally characterized by an indolent course, but over time can eventually become refractory to treatment. The 10-year survival rate can be as low as 20% for MF patients with advanced stage. We aim to characterize the factors associated with emergency department visits associated with in-hospital mortality in MF patients. We examined cases of MF from 2006-2015 in the Nationwide Emergency Department Sample (NEDS) and identified visits associated with an outcome of death. Baseline demographic, socioeconomic factors, and hospital characteristics were compared between MF patients that did and did not die during their visit using the chi-square test for categorical variables, and the Mann Whitney U or analysis of variance tests for continuous variables. Multivariate logistic regression was used to identify factors associated with in-hospital mortality. The same analysis of ED visits of all cancer types (ICD Clinical Classifications Software codes 11 to 45) was performed as well. A trend analysis was performed by comparing year categories in the multivariate. There was a total of 57,665 ED visits for MF from 2006-2015. The mean age of the cohort was 61.1 and 7.2% of the cohort died during their visit. On multivariable analysis, it was found that age (OR 1.76; 95% CI 1.38-2.23), Sézary syndrome (OR 1.75; 95% CI 1.02-3.00), sepsis (OR 9.69; 95% CI 8.24-11.40), and anemia (OR 1.18; 95% CI 0.98-1.42) were associated with in-hospital mortality in MF patients. Female sex (OR 0.75; 95% CI 0.63-0.89) and neutropenia (OR 0.58; 95% CI 0.43-0.78) was negatively associated with in-hospital mortality. An analysis of all cancer patients yielded similar results with the exception that neutropenia was not significantly associated with in-hospital mortality. There appears to be a decreasing trend of in-hospital mortality among MF patients and overall cancer patients presenting to the ED.

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The association between adenotonsillectomy and alopecia areata in childhood: A nationwide population-based retrospective cohort study

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Recent studies suggest that adenotonsillectomy may disrupt the development of a healthy immune system and lead to an increased long-term risk of autoimmune disorders. However, there is a paucity of literature on the association between the surgery and skin disorders. In the present study, we aimed to determine the risk of autoimmune skin disorders in pediatric patients who received adenotonsillectomy. A nationwide population-based retrospective cohort study was performed using the Korean National Health Insurance claims database. A birth cohort (n = 2,347,591) from January 2002 to December 2006 was evaluated up to December 2019. Patients who received adenotonsillectomy within the first 9 years of life were matched with the control population in a 1 to 2 ratio. The incidence rate ratios (IRRs) of alopecia areata, psoriasis, and vitiligo compared to the control group were calculated, and multivariable stratified Cox proportional hazards regression analysis was performed to evaluate the risks of target skin diseases after adenotonsillectomy. A total of 2,331,360 children were included in the study, and 73,637 subjects were identified as the study population. There was a statistically meaningful association between adenotonsillectomy and alopecia areata (IRR 1.238; 95% CI, 1.104-1.388). This association was also maintained in subgroup analysis regardless of sex or age group at surgery. There was no significant association between the surgery and psoriasis (IRR, 0.934; 95% CI, 0.752-1.160) or vitiligo (IRR, 1.094; 95% CI, 0.939-1.273). The findings from our study revealed an increased risk of alopecia areata in patients who received adenotonsillectomy within the first 9 years of age. Although adenotonsillectomy is essential for certain patients, our results suggest that adenotonsillectomy may inflict a lasting effect on the occurrence of alopecia areata, warranting prudence before making the decision to remove the adenoids and tonsils.

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Four childhood atopic dermatitis subtypes identified from trajectory and severity of disease

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Atopic dermatitis (AD) disease activity and severity is highly variable during childhood. Prior attempts to identify subtypes based on disease trajectory have assessed AD activity over time without incorporating severity. We identified and internally validated four childhood AD subtypes in 11,866 children from an English birth cohort using both activity and severity of disease: Severe-Frequent (4%); Moderate-Frequent (7%); Moderate-Declining (11%); and Mild-Intermittent (12%). In all subtypes, the probability of reporting severe symptoms declined with age; within the Frequent subtypes symptom patterns were relatively homogeneous. We classified the remaining children (66%) as Unaffected/Rare. The associations of filaggrin null mutations (*FLG*), an AD polygenic risk score (PRS), and comorbid asthma with disease subtype were stronger in the subtypes with more severe and active disease, with *FLG* and AD-PRS being significantly different between subtypes. We found little to no evidence that patient characteristics and early life risk factors were associated with disease subtypes or able to differentiate between them. Conclusions: Both AD activity and severity are important for defining disease trajectory subtypes; genetic risk factors have potential to differentiate between subtypes.

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The clinical spectrum of primary cutaneous CD4$^+$ small/medium-sized pleomorphic T-cell lymphoproliferative disorder: An updated systematic literature review and case series

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Background: Primary cutaneous CD4$^+$ small/medium pleomorphic T-cell lymphoproliferative disorder (SMPLPD) is a provisional entity within the 2016 World Health Organization classification of primary cutaneous lymphomas. The condition is currently classified as a lymphoproliferative disorder to emphasize its benign course and discourage aggressive, systemic, treatment modalities. Methods: We conducted an updated systematic literature review and a retrospective chart review of diagnosed cases of SMPLPD from two Canadian academic cutaneous lymphoma centers. Results: A total of 23 studies with 136 cases were extracted from the systematic review and 24 patients from our retrospective chart review. SMPLPD proved relatively common accounting for 12.5% of all cutaneous T-cell lymphomas encountered in our cutaneous lymphoma clinics, second in frequency only to mycosis fungoides. The typical clinical presentation was that of an older individual (median age 59 years) with an asymptomatic solitary lesion on their upper extremity. The most common clinical differentials were cutaneous lymphoid hyperplasia, basal cell carcinoma, and lymphoma unspecified. T follicular helper markers were reliably detected. The main treatment modalities were surgical excision, local radiation therapy, and topical or intralesional steroids. Cure was achieved in the vast majority of cases. Conclusions: SMPLPD is an underdiagnosed T-cell lymphoma with an overtly benign clinical course. The condition has an excellent prognosis and responds well to skin-directed therapies. Practitioners should be aware of this condition to avoid aggressive systemic treatments.

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Increased susceptibility to sunburn occurs during the first three years of hydrochlorothiazide use: Results from the National Health and Nutrition Examination Survey, 2009-2018

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Hydrochlorothiazide (HCTZ) has been associated with increased photosensitivity and development of non-melanoma skin cancer. Given the Food and Drug Administration's recommendation of increased sun protection in patients taking HCTZ, we investigated the relationship between HCTZ use, photoprotection behaviors, and sunburn using the National Health and Nutrition Examination Survey (NHANES). We combined NHANES data from 2009-2018 (931 taking HCTZ, 18148 not taking HCTZ) and used multiple logistic regression to calculate adjusted odds ratios (aOR) of staying in shade, wearing long sleeves, and using sunscreen. We used quasi-Poisson regression with linear spline to model incidence rate ratios (IRR) of sunburns in the past year associated with duration of HCTZ use. Models were adjusted for survey cycle, sex, age, race/ethnicity, education level, income to poverty ratio, history of skin cancer, and time spent outdoors. We controlled for photoprotection behaviors when examining sunburn as the outcome. Current HCTZ use was not associated with change in odds of frequently staying in shade (aOR 1.11, CI 0.93 − 1.32, P=0.253), wearing long sleeves (aOR 1.38, CI 0.99 − 1.91, P=0.063), or using sunscreen (aOR 1.11, CI 0.89 − 1.39, P=0.363). Among participants who took HCTZ for 3 years or less, each additional year of HCTZ use was associated with a 38% increase in incidence rate of sunburns in the past year (IRR 1.38, CI 1.12 − 1.70, p=0.004). Among participants who took HCTZ for more than 3 years, no statistically significant association between duration of HCTZ use and incidence rate of sunburns was found (IRR 0.98, CI 0.93 − 1.04, p=0.526). In summary, we found areas for improvement in sun protection among patients taking HCTZ and increased susceptibility to sunburn during the first 3 years of HCTZ use. Education on photoprotection methods, especially with initiation of HCTZ, is important in this population to prevent photodamage and the development of skin cancer.

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Direct healthcare cost of atopic dermatitis in the Swedish population

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Data quantifying population-based direct healthcare costs (DHCC) for atopic dermatitis (AD) by severity are limited. This study was designed to provide estimates for these costs. Patients were identified at first AD diagnosis in the National Patient Registry (secondary care) or in primary care (national coverage: 31%) (International Classification of Diseases-10 L20) or first dispensation of topical calcineurin inhibitor or topical corticosteroid (Anatomical Therapeutic Chemical code D11AH01/02 once; D07 twice in a year) in the Prescribed Drug Registry in 2007-17 (index) and followed until death, emigration, 31 Dec 2018 or adulthood. Patients without AD diagnosis with a record of diagnoses/treatment for other non-AD skin conditions were excluded. Patients were matched 1:1 on age, gender and region to controls. 1-year DHCC for secondary and primary care visits and filled prescriptions were compared with controls (2020€). Disease severity (mild-to-moderate [M2M] vs severe) using AD treatment and visits as proxies was assessed between index to 30 days after. 187,338 M2M (48% female; mean age 4) and 46,754 severe children (51%; 8), while 445,317 M2M (55%; 55) and 11,640 severe adults (57%; 53) were included. In children vs. controls, 1-year DHCC for secondary care, primary care and medications were respectively €72, €23, €33 million (mn) higher in M2M and €26, €4, €13 mn higher in severe; in adults vs. controls, €353, €68, €182 mn higher in M2M and €21, €2, €17 mn higher in severe (all comparisons significant, $p<0.05$). On population level, AD is associated with substantial economic burden, which is higher in M2M vs severe AD partially due to higher prevalence of M2M.

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Dermatologist preferences regarding implementation strategies to improve statin use among patients with psoriasis

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Patients with psoriasis are at increased risk of cardiovascular (CV) disease, but are less likely to have high cholesterol identified and treated with statins. Since many patients with psoriasis do not routinely see primary care, involving dermatologists to screen for cholesterol and potentially prescribe statins has promise to improve CV outcomes. To evaluate dermatologist preferences for strategies to improve statin use among psoriasis patients, a survey consisting of a best-worst scaling choice experiment of 8 implementation strategies and items on willingness to screen and manage CV risk factors was fielded among dermatologists recruited through the National Psoriasis Foundation from Oct-Dec 2020. Ratio-scaled preference scores for each strategy were generated using hierarchical Bayes analysis in Lighthouse Studio. In these preliminary results among 69 dermatologists, 44% were male and 25% practiced in an academic setting. Overall, 64% agreed that checking a lipid panel and calculating a CV risk score seems doable and 32% agreed that prescribing statins seems doable. Additionally, 68% agreed that they would consider changing their practice if a trial demonstrated that psoriasis patients achieved better CV prevention when their dermatologists screened for high cholesterol and prescribed statins. In the best-worst scaling experiment, the highest ranked strategies included clinical decision support (preference score, 23.2), patient educational materials (15.7), and physician educational outreach (15.4). Our results highlight that dermatologists are willing to consider lipid screening and prescribing statins in those with psoriasis. These findings will guide the design of a future trial to evaluate strategies to improve lipid screening and statin use among psoriasis patients.

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Dermatology on TikTok: Analysis of content and creators

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Background: Dermatology patients and practitioners use social media for rapid dissemination of health information. TikTok, a short-form video sharing platform, is the fastest growing social media network and represents a novel, unsupervised source of medical information. Methods: Top dermatologic diagnoses and procedures from publicly available survey data were queried as TikTok hashtags. Content of the first 40 videos for each hashtag were analyzed from July 10 to 13, 2020, and classified by creator (healthcare professional, personal, business, professional organization), content (education, promotional, patient experience, entertainment), and impact (views, likes). Results: A total of 544 videos were analyzed. Laypeople created the most videos (45%), followed by healthcare professionals (HCPs) (39%). Board-certified dermatologists (BCDs) accounted for a minority of total posts (15.1%). BCDs accounted for the most videos made by a HCP (33%). Predominant content was educational (40.8%), followed by entertainment (26.7%). Videos from laypeople received the largest percentage of views (50.68%). The most-liked (66.9 million) and most-viewed (378 million) posts were both related to #skincare, but only 2.5% of analyzed #skincare videos were produced by BCDs. Conclusion: A majority of dermatology-related videos on TikTok are produced by laypeople. However, the top 5 dermatologists on TikTok have a combined following of 5.2 million (M), with over 600M views and 80M likes, illustrating the wide reach and potential opportunity for education using this novel platform. TikTok has a large audience interested in skin-related education. This highlights a potential role for BCDs to engage in this space as educators and viewers, both to provide accurate information and to be aware of skincare trends our patients are exposed to.

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Racial differences in cutaneous sarcoidosis

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A substantial percentage of sarcoidosis patients experience cutaneous symptoms. Racial differences in systemic involvement, cutaneous presentation, and prognosis remain understudied. We conducted a retrospective chart review from a population-based sample of 240 patients diagnosed with cutaneous sarcoidosis at the Johns Hopkins Hospital aged 18+ from 2015-2020. Multiple logistic regressions were conducted to assess differences in disease characteristics by race after adjusting for insurance type, age at diagnosis, and sex. More black patients than white patients had cutaneous sarcoidosis (B=183, W=47). Compared to white patients, black patients were more likely to be female (F=75.4%, M=24.6%, p=.005), were diagnosed earlier (B=41.7, W=49.8, p<.0001), and had longer follow-up time (B=14.2 yrs, W=8.3 yrs, p=.004). Although black patients had more progressive disease (p=.033), this association was not significant when controlling for age of diagnosis (p=.37). Thus, earlier age of diagnosis was associated with worse prognosis (p<.001). Blacks with cutaneous sarcoidosis were less likely to also have ocular sarcoid involvement (OR .108, 95% CI .014-.836; p=.033). Among patients with pulmonary involvement, blacks were more likely to have restrictive lung disease (OR 3.15, CI 1.15-8.7; p=.007) and decreased diffusing capacity (OR 3.12, CI 1.15-8.67; p=.026). Black patients were more likely than white patients to have cutaneous manifestations of plaques (OR 3.94, CI .1.69-9.17; p=.002) and lupus pernio (OR 4.67 CI 1.34-16.36; p=.016). Here we demonstrate racial differences in sarcoid prognosis, systemic, and cutaneous involvement. Racial differences in systemic and cutaneous involvement may be related to differences in differential disease prognosis, potentially serving as indications for more extensive therapy to combat progressive sarcoid.

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COVID-19 related outcomes in psoriasis and psoriasis arthritis patients

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Psoriasis is a systemic chronic inflammatory disorder that affects the skin and is associated with other disorders. There is scan literature on outcomes of COVID19 patients with Psoriasis (Pso) and Psoriasis Arthritis (PsoA), especially from multicenter data. Therefore, the aim was to examine investigate the risk of COVID complications in these two groups. A retrospective cohort study was done using TriNetX, a federated real time database of 63 million records. COVID patient cohorts were identified by validated ICD-10/serology codes per CDC guidelines. An 1:1 matched propensity score analysis was conducted, adjusting for comorbidities and demographics, to calculate adjusted Risk Ratios (aRR) with 95% CI. 45-day COVID complications were examined with severe COVID defined as a composite of mortality and ventilation. Subgroup analyses were also performed for Pso and PsoA patients on systemic immunosuppressants. In a matched sample of 2288 patients in each cohort, there was no differences between Pso-COVID patients and non-Pso COVID patients in hospitalization (0.90[0.78-1.03]), sepsis (0.78[0.54-1.14]), mortality (0.82[0.57-1.19]), and severe COVID (0.77[0.58-1.03]). Pso-COVID patients had statistically significant lower risk of acute respiratory distress syndrome (0.51[0.30-0.90]) and mechanical ventilation (0.65[0.45-0.95]). In a matched sample of 502 patients in each cohort, PsoA-COVID patients had no differences in any of the listed outcomes. A subgroup analysis revealed that Pso-COVID and PsoA-COVID patients with a one-year history of systemic immunosuppressant use also had no differences in COVID outcomes compared to Pso-COVID patients and PsoA-COVID patients without immunosuppressants respectively. Pso-COVID and PsoA-COVID patients were not at higher risk for severe COVID complications. History of immunosuppressant use in both cohorts also revealed no higher risk in COVID complications. Additional studies are warranted to visit the longer-term impacts of COVID on Pso and PsoA patients.

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The risk of contracting COVID-19 after dermatological procedures compared with other medical procedures

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During the COVID19 pandemic, research has shown that many patients have decided to delay elective procedures, even if available, to reduce COVID exposure. There is scant literature that demonstrated the risk of COVID after dermatological procedures and whether these risks are higher compared to other medical procedures. This study aims to investigate these risks. A retrospective cohort study was done using TriNetX, a federated real time database of 63 million patient records. Patients undergoing any procedure were identified by CPT codes from Jan 2020-Nov 2020. ICD-10 and serology codes were used to identify 30-day risk of post-procedural COVID diagnosis per CDC guidelines. A 1:1 matched propensity score analysis was conducted, adjusting for comorbidities and demographics, to calculate adjusted Risk Ratios (aRR) with 95% CI. 224,536 dermatological procedures were conducted during the timeframe. Overall, there was a 2% risk of 30 day COVID diagnosis after a dermatological procedure. After matching, patients had a lower risk of contracting COVID after undergoing dermatological procedures when compared to urinary procedures (aRR[95%CI])=(0.56 [0.54-0.58]), gastrointestinal procedures (0.61[0.59-0.63]), cardiovascular procedures (0.56 [0.55-0.58]), respiratory procedures (0.42[0.40-0.43]), hemic/lymphatic procedures (0.49 [0.46-0.53]), musculoskeletal procedures (0.75[0.73-0.78]), and nervous procedures (0.86 [0.83-0.89]). There was no difference in COVID risk compared with reproductive procedures. Dermatological procedures presented a higher COVID risk when compared with endocrine procedures (1.46[1.17-1.82]), ophthalmic procedures (1.23[1.15-1.32]), and auditory procedures [1.52[1.41-1.64]). There is a minimal risk of contracting COVID after dermatological procedures, even when compared to other medical procedures. Risks can be further mitigated by following proper guidelines by public health officials.

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Female sex and white race are associated with Hidradenitis Suppurativa diagnostic delay

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Background: Although Hidradenitis Suppurativa (HS) is a chronic, inflammatory skin disease estimated to have a prevalence of 1%, the time from onset of symptoms to diagnosis -termed *diagnostic delay*- is 7 years on average. Late diagnosis may delay disease course-altering treatments and comorbidity management. We aimed to determine if demographic characteristics such as race, gender, median income, and access to dermatologists are associated with HS diagnostic delay. Methods: We conducted a single-center study of patients treated in an HS specialty clinic and included those who met HS diagnostic criteria, provided demographic characteristics and history. Data were summarized using medians and interquartile ranges (IQR) for continuous variables, and frequencies and counts for categorical variables. We used Spearman correlation to examine the relationship between diagnostic delay and continuous variables, and Wilcoxon rank sum tests to compare delay time with categorical variables. Results: Of 221 eligible HS patients, the majority were female (73%) and White (42%) (Black 23%, Asian 9%, Hispanic 12%, other 8%). Median (IQR) age at onset was 19 years (14, 26). Median diagnostic delay was 4 years (1, 10). Younger age at symptom onset correlated with a longer diagnostic delay ($p<0.001$). Female patients had longer diagnostic delay compared to males (6 years (1, 13) vs. 2 years (0, 6), $p=0.01$). White patients had longer median diagnostic delay compared to patients of other racial and ethnic backgrounds combined (5 years (2, 14) vs. 3 years (0, 8), $p= 0.004$). We found no significant correlations between diagnostic delay and either annual household income or density of dermatologists in county of patient residence. Limitations include recall bias and single center data collection. Conclusion: This study identifies demographic characteristics that may be associated with diagnostic delay in HS, including female sex, white race and younger age. Additional studies with larger cohorts are needed to better understand the relationship between demographic characteristics and HS diagnostic time

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Examination of characteristics and treatments in pediatric and adult hidradenitis suppurativa

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Hidradenitis Suppurativa (HS) is a chronic inflammatory disease affecting pediatric and adult patients. Analysis of real-world data that address the clinical treatment of pediatric HS patients and how treatments compare with adult HS patients has not been reported. HS adult (>18 years) and pediatric (< 18 years) subjects were identified in 3 US administrative claims databases, [IBM MarketScan® Commercial Claims and Encounters (CCAE), Multistate Medicaid (MDCD), and Optum's de-identified Clinformatics® Data Mart Database (Optum)] transformed into the Observational Medical Outcomes Partnership Common Data Model. Subjects were required to have 2 SNOMED codes for HS and at least 365 days of prior observation time to the first HS diagnosis. Demographics, associated diseases, and use of therapies (topical treatments, oral antibiotics, biologics, and surgical treatments) were evaluated. A total of 2047, 2522, and 709 pediatric and 21158, 12666, and 11061 adult HS subjects with mean ages of 15, 15, and 15 (pediatric) and 38, 36, and 44 (adult) years, with female predominance (ranges pediatric: 84-86%; adult: 73-83%), were identified from CCAE, MDCD, and Optum. Furuncles had similar prevalence in pediatric and adult subjects (ranges pediatric: 7-9%; adult: 7-8%). Pilonidal cysts were infrequent in both groups (ranges pediatric: 2-4%; adult: 3-5%). The most common first line pediatric treatment was topical treatment and oral antibiotic combination (36-38%); utilized in 22-26% of adults. The most common first line adult treatment was an oral antibiotic alone (38-43%), utilized in 31-33% of pediatrics. This study illustrates that associated conditions and therapeutic treatment of HS in adults and pediatrics is very similar. These data support similarity of disease treatment practices which may inform development of drugs for the pediatric population.

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Seasonal variation, climate factors and squamous cell carcinoma

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The impact of climate factors on the seasonality of squamous cell carcinoma (SCC) diagnoses is not well understood. We identified 1061 SCC patients (median age=78) in Rhode Island, a state with four distinct seasons, from 2017-2019. Due to known effects of organ transplants and indoor tanning on SCC development, patients with past medical history of either were excluded; recurrent SCC cases were also not included. Dates of 1338 diagnoses from 996 patients were collected to calculate the proportion of diagnoses per month. Relationships between average monthly values of climate factors from 2017-2019 and SCC diagnoses were evaluated with Spearman's rank-order correlations. Climate factors evaluated included UV index, ambient temperature, humidity, cloudiness, air pressure, number of days with sun, hours of sunlight, and volume of precipitation. Our results show that SCC diagnoses rose beginning in February and peaked in May, with consistent levels throughout mid-summer to late fall (July-November). There were significant positive correlations ($p<0.05$) between the hours of sun of the month of diagnosis ($r=0.77$) and month prior ($r=0.75$) with SCC diagnosis. Other weather factors from these months, including temperature and UV index, were also significantly positively correlated with SCC diagnosis. When analyzed separately, diagnoses in high UV-exposed sites, including head and neck and distal extremities (n=1031), showed a similar positive correlation with hours of sun in the same month ($r=0.71$) and following month ($r=0.66$). There was a slight delay in the influence of hours of sun on low UV-exposed body sites, including the trunk and proximal extremities, (n=307); while hours of sun of the month of diagnosis were not significantly correlated, levels of the variable at one ($r=0.77$) and two months ($r=0.83$) prior were positively correlated with diagnosis frequency. These results suggest that SCC diagnoses have seasonal variation and some climate variables, particularly hours of sun, may play a role in the seasonality patterns of SCC.

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Incidence and prevalence of granuloma annulare in the United States: A cohort study

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Given the lack epidemiologic data available for granuloma annulare, which is limited to small single-center studies, the burden of disease has not been well established. The purpose of this study was to estimate the population-based incidence and prevalence of granuloma annulare in the United States. This retrospective cohort study used the Optum de-identified Clinformatics Data Mart Database between January 1, 2017 and December 31, 2018. We have previously validated the use of ICD-10 codes to identify patients with granuloma annulare. The primary outcome was age-, sex-, and race/ethnicity-specific annualized incidence and prevalence of granuloma annulare. We also evaluated treatments used within 6 months of the first diagnosis. The entire population was considered at risk while continuously enrolled in the dataset. Confidence intervals for prevalence and incidence estimates were computed using the Wilson score method. We identified 11,608 cases of incident granuloma annulare and 17,862 cases of prevalent granuloma annulare over the study period. The overall annualized incidence of granuloma annulare was 0.04% or 37.9 (36.9-38.9) per 100,000 and the overall annualized prevalence of granuloma annulare was 0.06% or 58.3 (57.1-59.5) per 100,000. The incidence and prevalence of granuloma had a 3:1 female:male predominance. Granuloma annulare was more common in white individuals, as well as in those in their 5th and 6th decades of life. Within 6 months of their first diagnosis, 42.0% of patients filled a prescription for a topical steroid and 9.6% of patients received an intralesional injection. Oral tetracycline prescriptions were filled by 7.1% of patients and hydroxychloroquine prescriptions by 2.3% of patients. Phototherapy and TNF-inhibitors were rarely used. In this study, we have estimated that granuloma annulare is an uncommon disease in the United States that is more common in women. These findings are an important step to understanding the basic epidemiology and disease burden of granuloma annulare.

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Sun protection attitudes and behaviors among minority groups in a low socioeconomic community

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Background: Although skin cancer is less common among minority groups, they often present at later stages and have worse outcomes. Literature on this disparity is limited. Objective: To evaluate the attitudes influencing sun protective behaviors, skin cancer risk perception, and dermatologist access among an underserved, racially and ethnically diverse community. Methods: A cross-sectional survey of adult patients at five student-run, free primary care clinics in Sacramento, California. Results: 390 surveys were collected with a response rate of 86.4%. Overall, respondents did not use sunscreen, rarely sunburned, were unsure or perceived themselves at low risk for skin cancer and reported limited access to dermatologists. Compared to Whites, Latinos were likely to believe it was not worth getting sunburned to be tan (OR = 24.43, 95% CI: 9.37 to 63.3, P < 0.001). Whites were more likely than Asians (OR = 3.69, 95% CI: 1.50 to 9. 11, P = 0.004) and Latinos (OR = 4.83, 95% CI: 1.83 to 12.8, P = 0.001) to perceive having access to a dermatologist. Conclusions: Ethnic groups differ in their knowledge of sun protection and self-perceived skin cancer risk. The Latino community showed discrepancies between sun protection knowledge and practices, serving as a possible interventional target.

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A national, retrospective cohort study to estimate survival and standardized mortality in tuberous sclerosis complex (TSC) patients: Late disease diagnosis is significantly associated with increased mortality

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Tuberous sclerosis complex (TSC) is a genetic disease with systemic manifestations. Population-based epidemiological studies on TSC mortality and survival, especially in Asians, remain scarce. This study aims to estimate the life expectancy and mortality statistics in Asians with TSC, and prognosis and TSC mortality based on demographic factors. The TSC Catastrophic Illness Certificate holders during 1997−2010 were identified from Taiwan's National Health Insurance. Queries on diagnosis and endpoint age, sex, and comorbidities were made. 471 patients were identified, of which 14 died. Compared to literature, patients had similar demographics (including manifestations) and standardized mortality ratio (4.99), and lower prevalence (1/63,290) and mortality (0.21%/year). All-cause mortality risk was higher (HR=6.54) with late-diagnosis (>18). Average remaining lifetime was lower than general population, decreasing with age. This study highlights the importance of diagnosis age in prognosis. Physician vigilance, early diagnosis, and careful monitoring are beneficial for patient outcome and survival.

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Association of dermatologic manifestations of IBD with natural history and biomarkers of severity

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Dermatologic inflammatory conditions are one of the most common extraintestinal manifestations of inflammatory bowel disease (IBD), yet risk factors and association with IBD natural history and biomarkers of IBD severity have not been fully described. We sought to characterize the prevalence, risk factors, and biomarkers of severity associated with cutaneous inflammatory conditions in a prospective cohort of IBD patients followed over a multiyear time period. In a cohort of 4,215 IBD patients, 313 (7.4%) patients had an inflammatory dermatologic condition. Dermatologic conditions included eczema (34.9%), psoriasis (23.9%), erythema nodosum (22.5%), pyoderma gangrenosum (11.8%), hidradenitis suppurativa (6.1%), and pemphigus and bullous pemphigoid (0.9%). IBD patients carried one (89.7%), two (9.3%), or three (1.0%) dermatologic diagnoses. Skin involvement was significantly associated with female gender (P<0.001), Crohn's disease (CD) (P=0.03), increased CD activity (Harvey-Bradshaw index) (P=0.003), lower quality of life (short inflammatory bowel disease questionnaire (P=0.013), requirement for more aggressive medical therapy (systemic steroids, immunomodulators, and biologics) (P<0.001), history of intestinal resection (P<0.001), peripheral blood eosinophilia (P<0.001), peripheral blood monocytosis (P<0.001), low vitamin D (P=0.008), albumin (P<0.001), and hemoglobin (P<0.001), and elevated C-reactive protein (P<0.001) and erythrocyte sedimentation rate (P<0.001). IBD patients with dermatologic manifestations represent a distinct subgroup with increased inflammatory activity, more aggressive multiyear trajectories, and an increased association with novel biomarkers of IBD severity including peripheral blood eosinophilia and monocytosis, highlighting the need for individualized treatment approaches.

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Atopic dermatitis and the risk of developing rheumatoid arthritis - A population-based cohort study

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Atopic Dermatitis (AD), a prevalent and often persistent skin disease, is associated with immune-mediated inflammation and skin barrier dysfunction. It is now recognized as a systemic disease linked to a larger issue of immune dysfunction. Data is scarce on its association with other chronic inflammatory conditions such as rheumatoid arthritis (RA), particularly in both adults and children. We aimed to assess the risk of RA in patients with AD, stratified by age, after adjusting for traditional risk factors, using a previously validated algorithm. A population-based longitudinal cohort study from 1994 -2015 was performed using a UK based electronic medical records database generalizable to the general population [The Health Improvement Network (THIN)]. A total of 625,083 adult patients with AD and 409,431 pediatric AD patients were matched on age, practice, and index date to 2,678,888 adult and 1,809,029 pediatric unexposed controls. Hazard ratios (HRs) were calculated using Cox regression models. Covariates included age, sex, Townsend index, allergic rhinitis, and asthma (for both age groups) and body mass index, smoking, and drinking (for ≥18y stratum). We observed an increased risk of incident RA in AD patients (<18y HR:1.38; 95% CI 1.14 -1.67); (≥18y HR: 1.18, 95% CI 1.13-1.22). Further stratifying by the severity of AD, estimated by treatments prescribed, the risk of developing RA was higher in adults and children with severe AD compared to controls (HR: 5.64; 95% CI 5.189-6.13) and (HR: 8.35; 95% CI 5.63-12.38) respectively.Effects were attenuated in both pediatric and adults patients with mild (<18 y HR :1.16 ; 95% CI 0.94-1.44) (≥18y HR 0.95; 95% CI 0.90-1.01) and moderate AD (<18y HR 1.17; 95% CI 0.72-1.91) (≥18y HR 1.03; 95% CI 0.97- 1.10). Our findings from a large population-based cohort suggest an overall increased risk of RA in patients with AD, with the association primarily limited to patients with severe AD. This sets the stage for further studies on potential underlying mechanisms, such as overlapping therapies or shared pathophysiology

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Understanding diversity in eczema clinical trial participation

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Eczema clinical trials (CT) are rapidly increasing in number, yet clinical trial participation (CTP) is low with motivations and considerations for CTP poorly understood. Diversity and representation in CT is also a challenge across many diseases, including eczema, limiting application of CT findings to underrepresented groups. To address these gaps, the National Eczema Association administered a 46-question online survey, collecting data from 1,285 adult eczema patients and caregivers of children age 0-17 (respondents: 72% White, 10% Black, 10% Asian, 8% Multiracial/Other) on CT interest, literacy, and factors of importance for CTP. While previous CTP and previous CT consideration/attempted enrollment did not vary by respondent race or Hispanic ethnicity (range 8.8-11.6% and 12.1-19.8% respectively), mean rank of future likelihood to participate in CT was lowest for respondents of Asian race and highest for multiracial/other respondents (n=859, p=.049). Likelihood to participate in CT was most strongly positively correlated with self-reported understanding of the term "randomization" in White (p=.039) and Black (p=.042) respondents and "inclusion" in Black (p=.025) respondents. Of the top 5 most important factors when considering eczema CT, Black respondents more highly rated the potential to receive better care (p=.002) and having in depth knowledge about the drug (p=.009; n=596) while Asian respondents rated these factors lower. Trust in CT doctor/site, potential side effects, and having rescue therapy did not significantly differ with race. Non-hispanic respondents rated several factors lower than hispanics; ability to be compensated, approval from family and friends, and having a supportive community (all p=<.001). While this study did not corroborate known disparities in previous or interest in eczema CTP, it does provide insights into universally important topics for eczema patients and caregivers regarding interest and motivators of CTP as well as areas of potential emphasis by race that may support strong recruitment strategies and CTP design to improve CTP and diversity in eczema clinical trials.

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A meta-epidemiologic study of power and sample size in randomized controlled trials published in dermatology journals

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Background: Randomized controlled trials (RCTs) are considered the gold-standard for evaluating the effectiveness of an intervention, but not all RCTs are well-conducted or well-reported. Reporting *a priori* power and sample size calculations for the primary outcome can help increase a clinician's confidence in the results of an RCT. Objectives: We aimed to assess the methodologic quality of power, sample size, and outcome reporting in RCTs. Methods: We conducted a meta-epidemiologic review of sample size calculations and primary outcome reporting in RCTs published in the ten highest-impact dermatology journals according to the 2019 Science Citation Index, from 2015 - 2019. Results: We screened 2,939 articles and included 205 from eight journals in the final analysis. The majority of studies (N = 155, 76%) reported power and sample size calculations, though only 7 studies (3%) explicitly reported calculating sample size *a priori* and 147 studies (72%) were unclear in their reporting. Most studies (143/205, 70%) reported a clearly defined primary outcome with an associated timeframe. Of the studies that reported power and sample size calculations, 87 (42%) were reported in a reproducible and complete manner (with full reporting of α, β, effect size, and standard deviations for continuous outcomes). Of 146 studies that reported both a calculated and final sample size, 124 (85%) achieved at least their calculated sample size. Conclusions: Sample size calculation and primary outcome reporting in RCTs in the dermatology literature is suboptimal. Improved reporting of these methodological parameters will help clinicians evaluate and interpret the results of RCTs and apply their findings to their patients.

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Risk of opportunistic, viral, and hospitalized infections in atopic dermatitis

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Atopic dermatitis (AD) is classically linked to Staphylococcal and herpes simplex virus (HSV) infections but the incidence of opportunistic, hospitalized and other viral infections is less clear. In a cohort study using U.K. population-based electronic health data, we examined the association between AD and opportunistic infections (e.g. invasive mycoses, tuberculosis, pneumocystis), viral infections (HSV, cytomegalovirus [CMV], varicella zoster [VZV] and Epstein-Barr virus [EBV]) and hospitalized infections. AD severity was time-updated using treatments as a proxy; moderate AD was defined by ≥2 potent topical steroid or calcineurin inhibitor prescriptions within 1 year and severe AD by systemic medication or phototherapy use. A total of 381,678 children (<18 years old) with mild AD, 22,433 with moderate AD and 5,320 with severe AD and 410,867 adults (≥18 years old) with mild AD, 196,101 with moderate AD and 18,115 with severe AD were identified using a validated algorithm and matched on age, practice and index date with 1,809,029 pediatric and 2,678,888 adult controls. In Cox regression analysis adjusted for sociodemographics and comorbidities, both children and adults with AD were at greater risk for hospitalized infection compared to controls (HRs 1.40 [95% CI 1.38-1.43] and 1.25 [1.24-1.27], respectively), with increasingly greater risk among those with worse AD severity. Adults with AD were more likely to develop opportunistic infections than controls (mild AD: HR 1.12 [0.998-1.26]; moderate AD: 1.41 [1.25-1.59]; severe AD: 2.54 [2.00-3.22]). Children and adults across all AD severities were at greater risk for any viral infection (HRs 1.37 [1.36-1.38] and 1.26 [1.25-1.28], respectively) including VZV and HSV. CMV risk was also elevated in children of all AD severities. Our results suggest an increased risk of opportunistic, hospitalized and viral infections in patients with AD, even among those not receiving immunosuppressive therapies. Future studies are needed to dissect the mechanisms driving infection risk in AD.

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Comorbidities among children with hidradenitis suppurativa

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Comorbidities among adults with hidradenitis suppurativa (HS) have been extensively explored, but there is limited data regarding comorbidities among children with HS. The objective of this study was to determine the prevalence of comorbidities among pediatric patients with HS using an aggregated, de-identified patient database. Pediatric patients (<18 years old) with HS were identified in Explorys, a cloud-based electronic health record database composed of over 360 hospitals. Pediatric patients between 5 and <18 years old were used as controls, as all children with HS were ≥5 years old. Of the 8,856,840 children identified, 1,590 children had a diagnosis of HS. The most common comorbidities were obesity (29.56%, OR 27.31, 95%CI 24.52-30.42, p<0.0001), asthma (27.67%, OR 4.04, 95%CI 3.62-4.51, p<0.0001), contact dermatitis (25.16%, OR 3.63, 95%CI 3.24-4.06, p<0.0001), acne vulgaris (15.09%, OR 26.36, 95%CI 22.97-30.25, p<0.0001), seasonal allergic rhinitis (15.72%, OR 5.49, 95%CI 4.80-6.28, p<0.0001), atopic dermatitis (14.47%, OR 5.44, 95%CI 4.73-6.26, p<0.0001), acanthosis nigrans (12.58%, OR 63.53, 95%CI 54.74-73.73, p<0.0001), attention deficit hyperactivity disorder (11.32%, OR 3.21, 95%CI 2.74-3.74, p<0.0001), major depressive disorder (10.06%, OR 16.70, 95%CI 14.18-19.66, p<0.0001), and verruca vulgaris (9.43%, OR 5.37, 95%CI 4.54-6.35, p<0.0001). Children with HS were also more likely to have hyperlipidemia (5.66%, OR 13.82, 95%CI 11.17-17.10, p<0.0001), essential hypertension (3.14%, OR 13.43, 95%CI 10.13-17.81, p<0.0001), metabolic syndrome (3.14%, OR 64.29, 95%CI 48.43-85.33, p<0.0001), and generalized anxiety disorder (4.40%, OR 9.34, 95%CI 7.35-11.87, p<0.0001). These findings suggest children with HS are more likely to have certain dermatologic, inflammatory, metabolic, and psychiatric conditions.

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Analysis of association between variation in ambient solar ultraviolet exposure and disease severity for patients with moderate-severe psoriasis

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Evaluating the placebo response in clinical trials of moderate-severe psoriasis affords the opportunity to assess the effect on psoriasis of temporal changes in ambient solar ultraviolet radiation exposure (UVR), without confounding from use of systemic, ultraviolet, or potent topical therapies, which are prohibited during trial conduct. Anonymized placebo-treated subject data, including dates and PASI scores for baseline and Week 16 visits and approximate investigator location [i.e., first 3 zip code digits, which served as a proxy for subject location], pooled from the placebo-controlled double blinded periods (Weeks 0-16) of 3 moderate-severe psoriasis clinical trials (NCT00237887, NCT02684370, NCT02684357), were accessed through the Vivli data platform. Investigator location was manually geocoded and linked to estimated mean daily ambient erythemally weighted UVR for the months corresponding to baseline and Week 16, using data from the Ozone Monitoring Instrument on board the NASA EOS Aura spacecraft, and the German climate center. Simple linear regression of the percentage change in PASI score from baseline to Week 16 on percentage change in UVR between the months of the two visits was performed using the R statistics package. Simple linear regression of percentage PASI change from 538 placebo-treated subjects with non-missing observations yielded a beta coefficient for percentage change in UVR of -0.009 (standard error = 0.008), with a p-value of 0.243 and adjusted R-squared value of 0.0007. Lack of statistical significance was observed across multiple regression analyses adjusting for baseline covariates and for interaction terms, and for mean difference analysis testing whether PASI50 responders versus nonresponders had a significantly greater increase in UVR. Multiple analyses of this dataset failed to demonstrate evidence that temporal variations in ambient solar UVR are associated with clinically relevant or statistically significant variations in psoriasis severity.

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Incidence of bullous pemphigoid and pemphigus vulgaris in a nationwide study of United States veterans

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Recent studies suggest that the incidence of bullous pemphigoid (BP) has increased. These studies are drawn from European populations, and there is little data on the incidence trend for BP or pemphigus vulgaris (PV) in the United States (US) The objective of this study was to evaluate the trend in incidence for BP and PV using the Veterans Health Administration's (VHA) nationwide health care database. We conducted a retrospective cohort study of veterans diagnosed with BP or PV in VHA dermatology clinics between January 1, 2005 and December 31, 2017. Patients were included in the study if they had 2 separate International Classification of Diseases, 9th or 10th Revision (ICD-9 or -10) codes for BP or PV obtained from a VHA dermatology encounter during the study period. To capture only incident cases, we excluded patients with a preceding BP or PV ICD-9 or -10 code or those without a VA encounter of any type in the year prior to diagnosis. We identified 2,430 individuals with BP and 548 with PV during the study period. The mean ages were 75.6 and 65.0 for BP and PV, respectively. The majority of patients were male (96 & 93%) and white (75 & 70%). The incidence of BP in 2005 was 52.6 per 100,000 dermatology patients versus 38.7 in 2017. The incidence of PV in 2005 was 17.8 per 100,000 dermatology patients versus 9.1 in 2017. Overall, for both diagnoses there was a decreased incidence at the end of the study period compared to the beginning. However, the trend over time did not demonstrate a gradual downtrend. There were a few interval upticks in incidence for BP and downticks for PV. Despite the limitations of this study drawn from a predominately male population, these data help to support the idea that the incidence of BP and PV are not increasing in the US.

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Association of lichen planus with cardiovascular disease: An international cohort study

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Introduction: Lichen planus (LP) is an inflammatory dermatosis that has been associated with cardiac risk factors such as hyperlipidemia, however evidence is lacking on its relationship to cardiovascular disease (CVD). We sought to determine whether LP is associated with CVD in two population-based cohorts, the UK Biobank and *All of Us*. Methods: We performed a three-stage study: first, we tested for association of LP with CVD in the UK Biobank. Second, we conducted an independent replication in the US-based *All of Us* cohort. Third, we meta-analyzed the results using an inverse variance weighted random effects model. We used multivariable logistics regression to determine whether LP (ICD10 L43) was associated with CVD, defined as a composite of coronary artery disease, myocardial infarction, and stroke, after adjusting for vascular risk factors including hyperlipidemia. Results: In the UK Biobank, we included 502,536 participants with available data, of which 792 had LP (mean age 59 [SD 7], 33% male, 95% white). CVD was more common among those with LP than those without (20% vs. 12%, p <0.001, unadjusted odds ratio [OR] 1.83). LP was associated with CVD in multivariable analyses (OR 1.67, 95% CI 1.37-2.02, p<0.001) adjusted for age, sex, race, and vascular risk factors. From the *All of Us* cohort, we included 230,577 participants with available data, of which 788 had LP (age 65 [12], 26% male, 61% white). CVD was more common among those with LP than those without (19% vs. 9%, p<0.001, unadjusted OR 2.21). LP was associated with CVD in multivariable analyses (OR 1.23, 95% CI 1.02-1.49, p=0.03). Meta-analysis showed that LP was associated with a 44% increase in the odds of having CVD (OR 1.44, 95% CI 1.09-1.92, p=0.01; heterogeneity I^2 =76%; Cochran Q, p=0.04). Conclusions: LP is independently associated with CVD. Further studies are needed to determine whether this association is causal and if it is mediated by traditional vascular risk factors.

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"Moisturize and get off any steroid cream": An analysis of social media posts regarding TCS use in AD

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Analyses of online interactions among atopic dermatitis (AD) patients are limited. This study aims to analyze the content and engagement of online posts on topical corticosteroid (TCS) use in AD. Of 2047 posts extracted from 5 social media groups, 949 referenced TCS safety, defined as a higher-order theme (HOT). Within the HOT of TCS safety, 11 lower-order themes (LOTs) were identified: TCS use and Addiction/Withdrawal (36.7% of posts), Patterns of TCS use (15.0%), TCS use is harmful (9.8%), Avoidance of TCS use (9.0%), TCS use and Skin Thinning (7.9%), Other side effects of TCS use (7.4%), Fear of using TCS (4.0%), Overuse of TCS (3.6%), Questions about TCS use (2.8%), TCS use on the face (2.6%), and Benefits and Risks of TCS use (1.2%). Posts were assigned positive, negative, or neutral sentiment and an engagement score. Negative sentiment was more prevalent (n=655; 69.0%) compared to positive (n=60; 6.3%) or neutral sentiment (n=234; 24.7%). Sentiments were given a value of -1 (negative), 0 (neutral), and 1 (positive). All subgroups had negative mean sentiment scores (FB_EczemaSupportGroup -0.93, 95% CI [-1.01–.84]; r/eczema -.60 [-.64–.56]; r/SkincareAddiction -.50 [-1.01–.006]; r/TS_Withdrawal -.92 [-1.00–.84]; r/EczemaCures -.39 [-.74–.04]). Mean engagement was significantly higher (p<0.001) in subreddits compared to the Facebook group (FB_EczemaSupportGroup 0.9, 95% CI [0.37-1.42]; r/eczema 4.75 [3.97-5.54]; r/SkincareAddiction 7.3 [2.16-12.4]; r/TS_Withdrawal 9.16 [7.05-11.27]; r/EczemaCures 7.39 [3.66-11.1]). The information online regarding TCS use in AD is overwhelmingly negative; education efforts should incorporate concerns determined by sentiment and theme analysis.

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Access and usage of technology among patients with dermatologic conditions

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While telemedicine has allowed for continued care for patients with dermatologic conditions, the degree of technology access and usage for health-related reasons in this population has been understudied. The aims of this study are 1) to describe the level of technology access and usage for health-related reasons in the U.S. population with dermatologic issues and 2) identify the demographics of those with low technology access and usage. We used the National Health Interview Survey (NHIS) database to assess technology usage and access among participants with skin conditions in 2017. Participants were queried on history of skin conditions for themselves or their children including skin cancer, functional limitations due to skin issues, and eczema. Level of technology access and usage was obtained. Technology access was defined by internet and cellphone access. Technology usage was defined by use of technology for health reasons such as using computers for scheduling appointments and searching health information. Comparisons between groups were performed based on the weighted sample using t-tests for continuous variables and Wald chi-square tests for categorical variables. In 2017, 27,742 adults responded to the NHIS representing a weighted estimate of 246,657,271 adults in the United States. In this population, 7.8% reported having a skin issue for themselves or their children. Among this group with skin issues, 67.3% had low usage of technology for health-related reasons and 23.3% had low technology access. 49.0% of Hispanics and 46.3% of Blacks in this group had high technology usage for health-related reasons compared to 30.5% of white respondents (p<0.001). There was no significant difference in access to internet or cellphone by race or ethnicity (p=0.157). This study shows that there are racial and ethnic differences in how patients with dermatologic issues use technology for health-related reasons. Further research is needed to understand how this information relates to disparities in care.

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Reluctance towards digital image sharing and challenges for teledermatology

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Background: Patient reluctance to engage in telemedicine remains a key challenge to digital expansion in the era of COVID-19. Teledermatology, in particular, is heavily impacted by this, given its' foundation in visual assessments. An understanding of patient attitudes towards digital image sharing and determinants of these attitudes is necessary to address patient-centered barriers to teledermatology adoption. Objective: To evaluate digital image sharing preferences and predictors of patient preferences. Methods: We conducted a secondary analysis of pooled data from the Health Information National Trends Survey 4, Cycle 3 and 4, a cross-sectional survey of 6,437 US adults. Differences in willingness to electronically exchange digital images/videos (e.g., skin lesions) with providers were compared by patient characteristics and beliefs. Results: Overall, 53.5% of US adults reported disinclination towards digitally exchanging images and videos with their providers. Disinterest was greater in adults aged 75 or above (70.9%), retired (67.3%), with less than a high school education (65.1%), with less than $20,000 annual income (60.9%), and limited English proficiency (63.3%). Further, aversion was also higher among adults who distrust health information from doctors (75.4%), lack mobile device ownership (77.1%), and have fair or poor health (60.4%). Conclusion: Disinclination towards digital image sharing may pose challenges for teledermatology adoption among certain groups during this period of telehealth growth. Improved efforts targeting barriers to adoption, including older age, lower socioeconomic status, language barriers, worse health, and poorer physician-patient relationship dynamics, are needed to ensure vulnerable groups are not left without needed dermatologic care.

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The impact of mental health comorbidities on patient satisfaction: A population study among U.S. adults with dermatitis

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The association between mental health comorbidities and patient satisfaction is rarely studied in adults with dermatitis. Treatment non-compliance and negative perceptions of providers may be associated with low satisfaction. Patient satisfaction can be measured using patients' perception of patient-provider communication. We sought to determine the association between patients' mental health comorbidities and their perception of patient-provider communication quality among U.S. adult patients with dermatitis. We performed a cross-sectional study using the Medical Expenditure Panel Survey from 2004-2017. Among 24,386,994 (weighted) U.S. adult (≥18 years) patients with dermatitis pooled during the 14-year period, 15,482,175 (63%), 6,852,026 (28%), and 2,052,794 (9.0%) had no-to-mild, moderate, or severe symptoms of psychological distress, respectively. Additionally, 17,373,888 (71%), 3,583,468 (15%), and 3,429,638 (14%) had no-to-mild moderate, or severe symptoms of depression, respectively. We adjusted for socio-demographic characteristics and comorbidities and used validated instruments, patient-provider communication composite score, K6, and PHQ2. Compared to patients with no-to-mild symptoms, patients with moderate or severe psychological distress symptoms reported lower satisfaction with providers (b=-0.821; p<0.001) and b=-1.362; p<0.001, respectively) and were 3.1 times and 6.9 times more likely to report low satisfaction [AOR: 3.14 (1.84-5.37); p<0.001; AOR: 6.86 (3.17-14.85); p<0.001, respectively]. Compared to patients with no-to-mild symptoms, patients with moderate or severe depression symptoms reported lower satisfaction with providers (b=-0.709; p<0.001 and b=-1.084; p<0.001, respectively) and were 3.0 times and 3.9 times more likely to report low satisfaction [AOR: 3.03 (1.60-5.77); p=0.001 and AOR: 3.93 (2.19-7.02); p<0.001, respectively]. In conclusion, dermatitis patients' baseline mental health status may be associated with their satisfaction of the provider.

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Personal history of rosacea and risk of head and neck squamous cell carcinoma among women in the US

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Rosacea is a chronic cutaneous inflammatory disease that presents primarily in the central face. Its pathophysiology may be partially explained by a dysregulation in immune response, as well as factors including UV radiation. This study prospectively evaluates the relationship between personal history of rosacea with risks of squamous cell carcinoma (SCC) based on body site using the all-female Nurses' Health Study II (NHSII) cohort. In the NHSII, participants completed biennial questionnaires that gathered medical history, including clinician-diagnosed rosacea and SCC. Our study was comprised of 90,238 white participants with no skin cancer history at baseline that were followed for 20 years (1991-2011). Cox proportional hazard models were applied to estimate the associations between history of rosacea and SCC risk. Multivariate models were adjusted for age and other skin cancer risk factors. During the follow-up period, 525 SCC cases were documented. Rosacea was associated with an increased SCC risk; relative risks (RR) and 95% confidence intervals were 1.33 (0.94, 1.87). When the SCCs were grouped by head and neck (HN) vs. non head and neck (non-HN) sites, there was a significantly increased risk of HN SCC (n=245; RR= 1.83 [1.15, 2.92]), but no significant associations with non-HN SCC (n=280; RR= 0.97 [0.57, 1.63]). When evaluating HN SCCs further to compare face versus non-face HN SCC, participants with a history of rosacea were at a significantly increased risk of non-face HN SCC; RR was 3.12 (1.30, 7.51). There were no significant associations between history of rosacea and risk of HN SCC of the face; RR was 1.67 (0.96, 2.89). These results suggest that history of rosacea is significantly associated with head and neck SCC, particularly in non-face regions. Inflammation plays a significant role in SCC development. The associations found in this study could be attributed to the inflammatory nature of rosacea. The resulting inflammation may lead to immunologic and neurovascular changes in the region that promote development of SCC.

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Risk of headache and migraine in patients with atopic dermatitis- A population based cohort study

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There are several known comorbidities of atopic dermatitis (AD) yet there is still little known about AD and some non-allergic disorders. Migraine is of interest as it has a similar genetic expression profile as AD and potential mechanisms of action including increased cytokines and mast cell activation. To assess the risk of headache/migraine among AD patients, we performed a population-based cohort study using a U.K.-based electronic medical record database (The Health Improvement Network). We identified a total of 1,034,514 AD patients, both adult (≥18y) and children (<18y), that were matched on age, practice, and index date with 4,487,917 controls. We determined that both adults and children were at greater risk for headache (≥18y HR 1.19, 95%CI 1.18-1.21; <18y HR 1.10, 95%CI 1.09-1.12) and specifically migraine (≥18y HR 1.14, 95%CI 1.11-1.16; <18y HR 1.05, 95%CI 1.03-1.08) using a Cox regression adjusting for age, sex, Townsend score, hormone therapy, allergy and asthma for all, adding BMI, smoking, and drinking for adults. We further stratified by disease severity including mild, moderate, and severe. Severity was assessed through the established method of using proxy measures of treatment such that those using systemic therapies or phototherapy are defined as severe, those with 2 or more potent topical steroids or topical calcineurin inhibitor prescriptions within 1 year are moderate, and are considered to have mild disease by default. Although similar risks are seen overall in children when compared to adults, results by disease severity show different trends. Among children, only mild AD increases risk of migraine (HR 1.08, 95%CI 1.05-1.10) and severe AD exhibits protectiveness (HR 0.84, 95% CI 0.74-0.95) where for adults, risk remains consistent. The excess risk of migraines was 1 in 594 per year in patients with AD. The indication of increased risk of headache and migraine for both adults and children with AD and the unique presentation in children across severity calls for further research investigation.

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Platelet-rich plasma for treating female androgenic alopecia: A systematic review

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Androgenic alopecia (AGA) is a common form of hair loss that affects males and females. Hormonal, environmental, and genetic factors all play a role in its pathogenesis, oftentimes affecting patients' quality of life due to diminished self-esteem. Treatment options are limited, thus driving the demand for hair restoration options. Platelet-rich plasma (PRP) has shown promising results for the AGA population. This literature review was conducted to assess the effectiveness of PRP treatment for AGA in female subjects. A total of sixteen studies were found that included females, but only five studies met our inclusion criteria of evaluating only female subjects. These five studies from PubMed between 2011 to 2020 were evaluated and grouped by study features, preparation methods, and treatment protocols. A total of 136 female subjects with AGA between the ages of 18-64 were treated with PRP or placebo, or both. The following metrics were evaluated pertaining to hair: count, thickness, growth, volume, mass index, and caliber. Some studies also included subjective assessment reports. Four of the five studies showed significance in effectiveness of PRP in increasing hair thickness/density while subjective improvement in hair quality was noted in all five studies. More studies are needed to assess the advantage of PRP treatment in order to mitigate the negative psychological factors of AGA impacting the female population.

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Caffeinated or decaffeinated coffee consumption and risk of cancers: A meta-analysis

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Cancer is the second leading cause of death globally. Coffee consumption has been reported to reduce the incidence of various types of cancers; however, previous studies showed variable results, and few studies have addressed the effect of caffeinated versus decaffeinated coffee on cancer incidence. We performed a meta-analysis to systematically assess what types of cancer are prevented by caffeinated or decaffeinated coffee. We used PubMed, Scopus, and Embase databases to comprehensively identify peer-reviewed prospective cohort studies that associate coffee consumption with risk of cancers. The Newcastle-Ottawa Scale was used to assess the quality of nonrandomized studies. Summary relative risk (RR) was calculated by using the DerSimonian and Laird random effects model. Dose response was analyzed by using linear regression. A total of 65 studies for 10 major cancer types were used for our meta-analysis (bladder, breast, colorectal, endometrial, hepatocellular, lung, ovarian, pancreatic, prostate, and skin cancers). Caffeinated coffee consumption (≥2 cups per day) significantly reduced the risk of hepatocellular, endometrial, and skin cancers by 46% (RR 0.54; 95% confidence interval (CI) 0.39−0.74), 39% (RR 0.61; 95% CI 0.44−0.84), and 17% (RR 0.83; 95% CI 0.74−0.92), respectively, whereas decaffeinated coffee had less or no effect in these three cancer types. Significant dose-response effects of caffeinated coffee were observed in hepatocellular, endometrial, and skin cancers with 9.9%, 7.4%, and 7.8% risk reductions per cup, respectively. Intriguingly, decaffeinated coffee (≥2 cups per day) may reduce the risk of colorectal cancer by 12% (RR 0.88; 95% CI 0.73−1.07). Coffee consumption had no association with risks of breast and prostate cancers. Our meta-analysis demonstrates that caffeinated coffee consumption decreases the risk of hepatocellular, endometrial, and skin cancers in a dose-dependent manner. Further investigations are needed to elucidate molecular mechanisms by which caffeine prevents different types of cancer.

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Proportions of biologic discontinuation among psoriasis patients with metabolic comorbidities

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Background: The purpose of the study was to analyze the proportions of biologic discontinuation among psoriasis patients with and without metabolic comorbidities, and stratified by drug class, using real-world data. Methods: The Corrona® Psoriasis Registry is a prospective, multicenter, non-interventional registry in North America. Patients with plaque psoriasis who initiated a biologic therapy (5/2015 to 12/2019) and had a 6-month follow-up visit were included (N=2,924). The proportion of biologic discontinuations by 6 months post-initiation were calculated by metabolic comorbid status (current obesity and histories of hypertension [HTN], diabetes [DM], and hyperlipidemia [HLD]) and by drug class (tumor necrosis factor [TNF] inhibitors, interleukin [IL]-17 inhibitors, IL-23 or IL-12/23 inhibitors). Results: Higher frequencies of patients with obesity (17% vs. 13%) and with DM history (20% vs. 14%) discontinued compared to those without, while discontinuations were similar between those with and without HTN and HLD history. Patients initiating TNFs had higher proportions of discontinuation than the IL-17 and IL-23/IL-12/23 groups. Among patients initiating TNFs, those with obesity, DM history and HTN history had higher proportions of discontinuation (30%, 34%, 34%, respectively) vs. those without (22%, 24%, 22%, respectively), while among IL-23 or IL-12/23 initiators, compared to patients without, patients with obesity (11% vs. 7%) or DM history (13% vs. 8%) had slightly higher proportions of discontinuations. Discontinuations did not differ between obesity or comorbidity groups in IL-17 initiators. Conclusion: In these real-world psoriasis patients, those with obesity and history of DM had higher proportions of biologic discontinuations 6 months following initiation, except in the IL-17 class. Metabolic comorbidities should be considered when choosing biologics.

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Epidemiology of alopecia areata in black patients

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Black patients have greater odds of alopecia areata when compared to whites (odds ratio, 1.77; 95% confidence interval, 1.37-2.28).[1] Few studies in the literature have examined the epidemiology of alopecia areata exclusively in Black patients, demonstrating an increased need for understanding in this area. To investigate the epidemiology of alopecia areata in Black patients, a retrospective analysis was conducted in 265 pediatric and adult patients diagnosed and treated for alopecia areata at Wake Forest Baptist Health between January 2015 and December 2020. Patients were assessed according to distribution by age, sex, medical and autoimmune comorbidities. 190 (71.7%) of patients were female (female-to-male ratio, 2.5:1). The largest age group presenting for care was the 18-34 year age group (35.8%) followed by the 10-17 year age group (15.1%). These results suggest a female predominance and increased prevalence of disease in younger patients. Further evaluation of epidemiology can provide greater understanding of alopecia areata in Black patients. 1. Lee H, Jung SJ, Patel AB, et al. Racial characteristics of alopecia areata in the United States. J Am Acad Dermatol. 2020 Oct;83(4):1064-1070.

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Racial and language disparities in teledermatology visits for acne during the COVID-19 pandemic

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Background: Teledermatology has emerged as an essential model of care during the COVID-19 pandemic. However, the impact of the rapid transition to teledermatology on patients of certain racial/ethnic and language groups due to differential access to technology, lower digital health literacy, language barriers, or cultural perceptions is unknown. We sought to identify race and language disparities in teledermatology utilization for patients with acne, one of the most frequent diagnoses seen via teledermatology, before and during the pandemic. Methods: A retrospective chart review of all in-person and virtual visits for acne at a large academic dermatology department from March-May 2019 and March-May 2020 was conducted. Virtual visit types included video- or audio-only visits. Chi-squared analyses were performed to compare populations across visit types. Results: 3544 visits were analyzed. Virtual visits accounted for 1229/1630 (75.6%) during- pandemic visits. Racial and language distributions of patients for overall visits were not statistically significantly different pre- and during-pandemic. However, video visits (versus audio-only) comprised a greater proportion of virtual visits during-pandemic for White (86.9%) compared to non-White patients (82.0%), and for English-speaking (86.2%) compared to non-English-speaking patients (60.5%) (both $p<0.001$). During-pandemic, interpreters use was documented for 23.1% of all non-English-speaking patients for in-person visits versus 9.3% of virtual visits ($p<0.001$). Conclusions: Non-White and non-English-speaking patients were less likely to use video visits for acne during the pandemic than White and English-speaking patients. Non-English-speaking patients were less likely to receive interpreters in virtual visits than in-person visits. These findings suggest underlying disparities in teledermatology access and barriers to interpreter use during virtual visits.

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Lifestyle and demographic risk factors in mycosis fungoides and Sezary syndrome: A single institution cohort study

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Background: Mycosis fungoides (MF) and Sezary syndrome (SS) are common subtypes of cutaneous T-cell lymphoma (CTCL). Risk factors and impact on quality of life (QoL) are poorly understood. Previous studies of CTCL risk factors have not analyzed disease stage, severity, and QoL together. We explored associations between demographic and lifestyle factors and these parameters. Methods: A cohort study was conducted at a large CTCL multidisciplinary clinic from April 2019 to December 2020. REDCap surveys were administered to 115 MF/SS patients, investigating 11 demographic and lifestyle factors. QoL was evaluated using Skindex-29; pain and itch with Likert scales. Disease severity was assessed using the modified Severity Weighted Assessment Tool (mSWAT). Factors were compared using t-test, chi-squared, and linear or logistic regression models. Results: History of chemical exposures was associated with greater disease severity ($p=0.034$) and worse QoL ($p=0.005$), but not with pain/itch severity ($p=0.118$). Disease severity and stage were associated with worse QoL (both $p<0.001$). There were significant racial differences in early (IA-IIB) versus late (IIIA-IV) stage disease ($p=0.034$) and QoL ($p=0.039$). There was a significant relationship between smoking and disease stage ($p=0.028$) but not severity ($p=0.360$). Obesity was correlated with disease severity ($p=0.021$), but not with stage or QoL ($p=0.582$; 0.232). Conclusion: We provide an analysis of patient lifestyle and demographic factors in the context of MF/SS severity, stage, and QoL. We identified race and smoking as potential risk factors for advanced disease, and chemical exposures and obesity for increased disease severity. Worse QoL was significantly associated with a history of chemical exposure, severe pain/itch, race, and stage. Identification of demographic and lifestyle associations in MF/SS will enable physicians to provide more individualized patient care and education.

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Cumulative ultraviolet radiation exposure is associated with both increased melanoma and non-cutaneous cancer risk

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Ultraviolet radiation (UVR) exposure is associated with increased risk of skin cancer. However, mixed evidence suggests a protective, inverse relationship between UVR and risk of certain non-cutaneous cancers, depending on temporality of exposure. To address this discrepancy, we examined three ongoing U.S. prospective cohort studies, the Health Professionals Follow-Up Study (HPFS) and Nurses' Health Study (NHS) I and II, to identify associations between cumulative UV exposure and cancer risk. We used an established spatiotemporal exposure model to calculate cumulative time-varying average UV exposure, defined as average July noon-time erythemal UVR. Our sample included 47,714 males from HPFS, and 112,507 and 99,940 females from NHS I and II, respectively. In each study, participants were stratified into quintiles by cumulative UVR exposure, using the first quintile as reference for Cox proportional-hazards modeling. In the meta-analysis of all three cohorts, we found increased total cancer risk across all UVR exposure quintiles after controlling for potential confounders [highest quintile Hazard Ratio (HR), 1.04; 95% Confidence Interval (CI), 1.01-1.07; $p=0.002$; P for heterogeneity=0.49]. All UVR exposure quintiles were also associated with increased risk of total cancer excluding melanoma (highest quintile HR, 1.03; 95% CI, 1.01-1.60; $p=0.02$; P-het=0.56). Lastly, UVR was associated with the highest risk for melanoma (highest quintile HR, 1.20; 95% CI, 1.07-1.34; $p=0.002$; P-het=0.70). These findings suggest that UVR is associated with increased risk of both melanoma and non-cutaneous cancers. Despite emerging data for the protective benefits of UVR against cancer, further research is necessary to understand the health effects of sun exposure and underlying mechanisms.

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Rates, characteristics, and comparison of hidradenitis suppurativa readmissions in the united states: A national population-based study

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This study aims to describe the rates and characteristics of non-elective 30-, 90- and 180-day readmissions for adult patients hospitalized for Hidradenitis suppurativa (HS) in the United States (US). We also aim to compare HS 180-day readmission rates to that of heart failure (HF), the most common cause of readmissions for Medicare patients. We analyzed the 2017 National Readmission Database (NRD). We included hospitalizations for all adult HS and HF patients ($\geq$18 years) and excluded elective or planned readmissions. Chi-square tests were used to compare baseline characteristics between readmissions and index hospitalizations. Multivariate cox regression was used to identify independent predictors of readmissions. A total of 2204, 1719, and 1053 index hospitalizations with a primary diagnosis of HS, that were discharged alive, were included in the 30-, 60- and 90-day HS readmission analysis. Among these, 392 (17.8%), 582 (33.9%), and 512 (48.6%) were readmitted within 30, 60, and 180 days, respectively. For all three readmission timeframes, HS, followed by sepsis, were the two most common reasons for readmission. The 180-day readmission rate of HS patients was comparable to that of heart failure (48.6% vs 48.0%). HS Readmissions within 90-days were associated with a total of 3,823 hospital days and 33 million US dollars in hospital charges. Compared to index hospitalizations, the readmissions cohort had a higher Charleston comorbidity index score, severe or extreme loss of function, electrolyte disturbance, anemia, and sepsis. The leading reason for readmissions in patients with HS is the skin disease itself. We found that HS readmissions at 6 months are similar to HF. Interventions aimed at improving access to early dermatological care are essential in preventing unnecessary readmissions of HS patients.

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Age of melanoma diagnosis in patients with limited English proficiency

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Patients with Limited English Proficiency (LEP) often receive substandard care. The United States LEP population was 8% as of 2013 and continues to grow. We aim to estimate the association between stage of melanoma diagnosis and LEP by comparing age of melanoma diagnosis between patients with differing self-reported household English-use from a nationally representative sample. We performed a retrospective cross-sectional study with pooled data from the 1999/2000 through 2017/2018 National Health and Nutrition Examination Surveys (NHANES). Demographics and self-reported age of melanoma diagnosis were compared between non-LEP and LEP patients, defined as speaking some English versus no English in the household, respectively. Frequencies and means were compared between groups using Rao-Scott $\chi 2$ and design-based t-tests, respectively, using a weighted-subject, stratified design. A total of 314 unweighted adult melanoma patients were identified from 1999/2000 to 2017/2018, of whom 4 were LEP (1.2%). This amounted to 1,708,858 weighted adult melanoma patients (95% CI: 1,438,719-1,978,998), of whom 4,297 were LEP (95% CI: 0-9,388). Compared to non-LEP adult melanoma patients, LEP melanoma patients were more likely to be of Hispanic ethnicity ($p<0.001$) or other/mixed/Asian American race ($p<0.001$). Mean age of diagnosis of non-LEP adult melanoma patients (51.9 years, 95% CI: 49.9-53.9 years) was less than LEP adult melanoma patients (63.4 years, 95% CI: 54.1-72.6), with an 11.5 year estimated difference in means (95% CI: 2.0-21.0 years, $p=0.02$). Our results indicate the need for melanoma screening and awareness in LEP populations for earlier detection of melanoma. Limitations to the study include not having the patient's stage of melanoma, self-reported data, and a small sample size.

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Dermatology visits account for a majority of dermatologic diagnoses: A representative sample of U.S. outpatient visits

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Given their prevalence, skin diseases are an important public health issue. In 2013, over 25% of the US population was impacted by dermatologic diseases, resulting in $75 billion in direct healthcare costs. Through 2010, non-dermatologists diagnosed a majority of skin diseases in outpatient visits. We sought to assess whether this was still true in 2016 and to determine the most common dermatologic diagnoses seen in dermatology and non-dermatology practices. We assessed visits in the 2016 National Ambulatory Medical Care Survey, an annual representative survey of visits to U.S. outpatient physicians. We analyzed all diagnosis codes reported at visits with dermatologists and non-dermatologists to determine the most common dermatologic diagnoses. Observed visits were weighted to obtain a nationally representative estimate of all visits in the U.S. There were an estimated 49.9 million visits to dermatologists with 107 million dermatology diagnoses and 834 million visits to non-dermatologists with 106 million dermatology diagnoses. The top 5 diagnoses for dermatologists were actinic keratosis, seborrheic keratosis, acne vulgaris, unspecified melanocytic nevi, and unspecified external cause. The top 5 dermatology diagnoses for non-dermatologists were unspecified dermatitis, rash and other nonspecific skin eruption, unspecified viral infection, unspecified atopic dermatitis, and unspecified chalazion. Seborrheic keratosis, malignant neoplasm of the skin, melanin hyperpigmentation, melanocytic nevi, and actinic keratosis were the most commonly referred diagnoses to dermatologists. In 2016, dermatologists diagnosed a majority (50.2%) of skin diseases in the outpatient setting. The skin conditions most commonly seen by non-dermatologists differ from those seen by dermatologists. These differences as well as the top diagnoses and referrals can be used as a foundation for tailoring dermatology training for non-dermatologists.

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A retrospective study of cellulitis outcomes in Ohio hospitals with or without access to dermatology residency programs

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There is a substantial body of work suggesting that cellulitis is over-diagnosed, partly due to the lack of inpatient skin specialists familiar with alternative etiologies, resulting in costly readmissions. The goal of this study was to determine whether there was a difference in number of cellulitis discharges adjusted for bed size in Ohio hospitals with and without access to dermatology residency programs. This was a retrospective chart review that looked at Inpatient Utilization and Payment Public Use File datasets from the Centers for Medicare & Medicaid Services website. The datasets included information on hospital size, location, total Medicare costs from 2011-2017, common diagnosis related groups (DRG)s, inpatient discharges, and the number of diagnoses of cellulitis with major complications (MCC), controlled for bed size. There were 14 hospitals with dermatology residency programs and 135 without them. Overall, cellulitis hospitalizations per bed size from 2011-2017 have decreased in both hospitals with and without dermatology residency programs ($R = -0.109$, $n = 1042$, $P = 0.0004$), while the total Medicare costs have slightly increased for both ($R = 0.041$, $n = 1095$, $P < 0.0001$). Hospitals with dermatology programs have a greater variety of DRGs ($P < 0.0001$). Hospitals with dermatology residency programs also have significantly lower cellulitis discharges with MCC controlled for bed size ($P < 0.0001$). Previous studies have shown that dermatologists can differentiate and diagnose cellulitis and its mimics with a reasonable agreement rate even through telemedicine. This study emphasizes the importance of access to dermatology consults via dermatology residency programs for skin-related diseases, whether through an increased investment in inpatient dermatology services or teledermatology.

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Characterizing risk factors for hospitalization for psoriasis patients

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Psoriasis is a chronic autoimmune disease with a large economic impact. The objective of this retrospective study was to characterize patients who are hospitalized for psoriasis, and differentiate features for patients with a single hospitalization from those who are hospitalized multiple times during the study period. Hospitalized psoriasis patients were identified from an inpatient database at a single academic institution. Differences between psoriasis patients with one hospitalization and those with multiple hospitalizations were characterized, as were differences between patients who were hospitalized primarily for psoriasis and those who were admitted primarily for other reasons. Patients with multiple hospitalizations had a higher mean Charlson comorbidity score (3.9 vs. 2.8, $P < 0.05$). They had a higher death rate during index hospitalization (7% vs. 2%) and a longer mean length of index hospitalization (15 days vs. 8 days), but these differences were not statistically significant. Patients who were primarily hospitalized for psoriasis had a lower mean Charlson comorbidity score (1.8 vs. 3.4, $P < 0.05$), shorter hospitalizations (0.4 days vs. 3.3 days, $P < 0.05$) and a lower death rate (0% vs. 4.7%, $P < 0.05$) than those hospitalized for other reasons. Patients with a primary discharge diagnosis of psoriasis also had a trend toward lower average income by zip code, though this value was not statistically significant. Our findings affirm the importance of regular dermatologic care for psoriasis patients in preventing hospitalizations. Dermatologists should be aware of the risk factors for hospitalization for psoriasis patients and work to mitigate them, as well as encourage patients to seek dermatologic care.

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Wrong-site surgery in medicine and dermatology: Analysis of data from the Joint Commission and from the Patient Safety Authority of Pennsylvania

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Wrong-site surgery procedures (WSS) are patient safety events which are underreported and may result in patient harm. WSS are typically grouped to include wrong-site, wrong-side, wrong-person and wrong-procedure errors. Despite the attention given to these preventable errors by professional organizations and development of the Universal Protocol, WSS still occurs and reliable data on their frequency is lacking. We analyzed publicly available data on WSS from the Joint Commission (JC) and from the Patient Safety Authority (PSA) of Pennsylvania. JC data is national and all specialty with most reported voluntarily. From 2005-18 there were 1501 WSS cases reported to JC. A breakdown of 2017-2020 Q2 data revealed 309 cases with 233 wrong-site, 40 wrong-procedure and 36 wrong-patient. Pennsylvania is among a small group of states which legally mandate the reporting of all safety events including near misses. According to PSA from 2015-2019 there were 368 WSS reported from 178 licensed facilities, excluding private offices. Dermatology accounted for 9 (2%) of the 368 cases, 8 of which were wrong-site and one wrong-side. Of the 9 procedures, 2 were biopsies, 4 excisions, 2 Mohs and 1 curettage. Five involved the head with 1 each from the chest/thorax, upper extremity, and spine and 1 was unspecified. Root causes of WSS identified by both the JC and PSA were accuracy and verification issues in procedure scheduling, failure to follow the three-part Universal Protocol and organizational safety culture issues. Additional strategies for dermatology include accurate biopsy site identification utilizing high-quality scanning and close-up photographs and specific and consistent anatomic designations. In conclusion WSS data may not reflect their absolute frequency. Health care facilities should conduct gap analyses of their existing procedures to minimize the occurrence of WSS.

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Evaluating clinical features and the presence of eosinophilia in pityriasis rubra pilaris

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Pityriasis rubra pilaris (PRP) is a rare disease presenting with orange to salmon-colored folliculocentric papules on the trunk and extremities, waxy palmoplantar keratoderma, and hyperkeratotic nails. PRP literature remains limited, and its pathogenesis remains unclear, often resulting in missed or delayed diagnosis. Further, although a case study found eosinophilia in a PRP patient, hematologic abnormalities have not been extensively examined, sparking the interest to evaluate for an association between eosinophilia and PRP to enhance diagnosis. PRP patients from 1980-2020 at Mass General Brigham were identified. Demographics, disease presentation, and laboratory and pathology data were recorded. This study was approved by the Brigham and Women's Hospital IRB. Student t-test and chi-square analysis were conducted to evaluate for differences; $p<0.05$ was considered significant. 142 PRP patients were identified (55% male, 85% white); 82% were categorized as Type 1. 19.7% had eosinophilia in serum or skin biopsy. Age at presentation for patients with and without eosinophilia was 60.8±14.6 and 53.1±19.7, respectively ($p=0.03$). Presenting symptoms included pruritus (33%), ocular dryness (4%), and hair thinning (4%). Lesions were present in the extremities (49%), trunk (43%), and head and neck (33%). Common biopsy findings included sparse superficial dermal perivascular lymphohistiocytic infiltrate (39%) and alternating ortho-/parakeratosis (37%). There were no significant differences in patient sex, race, disease presentation, or biopsy findings between the eosinophilia and non-eosinophilia cohorts. This study is the largest PRP study to date. Nearly 20% of PRP patients had eosinophilia at diagnosis and therefore, the presence of peripheral or biopsy-eosinophilia at diagnosis highlights an association that may suggest potential treatment strategies or help elucidate the underlying pathophysiology of this rare condition.

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Demographic and clinical factors associated with patient-reported remission in psoriatic arthritis

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Achievement of remission in psoriatic arthritis (PsA) is a key goal for patients and clinicians, yet definitions of remission may vary. Treat-to-target initiatives in PsA have utilized multidomain measures such as Minimal Disease Activity that incorporate the status of joints, skin, and function. The goal of this study is to identify factors associated with patient-reported PsA remission. The National Psoriasis Foundation conducted a survey within a random stratified sample of 1,570 individuals with psoriatic disease in the United States. Participants provided demographics and were asked about a provider diagnosis of psoriasis, PsA, or both. All participants with PsA were asked whether they felt their PsA was in remission, and PsA severity was assessed using the Psoriatic Arthritis Impact of Disease-9 (PsAID-9) questionnaire. Participants provided information on treatments and quality of life (QoL). Multivariate logisitic regression was used to identify factors associated with remission. Of the 834 participants reporting PsA, 144 (17.3%) reported that their PsA was in remission with an average duration of remission of 43 months. Of those in remission, 65 (78.4%) reported current treatment for PsA. Multivariate regression revealed that presence of remission was independently associated with non-white race, PsAID-9<=4 (acceptable disease state), less impairment on the PsA Global QoL scale, and psoriasis remission. Factors not associated with PsA remission included age, sex, BMI, and biologic use. These results indicate that patient-reported remission in PsA is not solely associated with PsA severity, but encompasses other factors such as race, achievement of skin remission, and quality of life.

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Health information technology utilization among skin cancer patients

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Background: Health information technology (HIT) refers to the use of online platforms to perform tasks related to one's medical care. HIT utilization is linked to improved patient outcomes, however, relatively little is known regarding HIT utilization in the context of skin cancer. Methods: We conducted a retrospective cross-sectional review of the National Health Interview Survey (NHIS) from 2011-2018. With summary statistics and multivariable logistic regression, we analyzed associations between sociodemographic characteristics and HIT utilization among patients reporting a skin cancer diagnosis. The primary outcome was whether patients scheduled healthcare appointments online. Secondary outcomes were whether patients looked up health information online, communicated with healthcare providers by e-mail, and filled prescriptions electronically. Results: From 2011-2018, the proportion of patients who scheduled healthcare appointments online increased from 4.36% to 21.35%. The proportion of patients who looked up health information online, communicated with a healthcare provider by e-mail, and filled a prescription electronically increased from 48.89% to 58.21%, 6.32% to 26.01%, and 12.05% to 17.86%, respectively. Logistic regression revealed that uninsured skin cancer patients were less likely to schedule appointments online, communicate with providers by email, or fill prescriptions electronically ($p<0.05$ for all). Patients with income below 200% of the federal poverty level and patients without a bachelor's degree were less likely to look up health information online, communicate with a healthcare provider by e-mail, or fill a prescription electronically ($p<0.05$ for all). Conclusions: There are substantial differences in HIT utilization of skin cancer patients across sociodemographic lines. Interventions aimed at increasing HIT utilization among disadvantaged groups may reduce health disparities related to skin cancer.

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Patients' attitudes towards active surveillance for basal cell carcinoma

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Basal cell carcinoma (BCC) is the most common cancer in the US. 30-50% of BCCs may remain stable or shrink in size. Most BCCs are managed surgically regardless of life-expectancy. More than 40% of patients with limited life-expectancy die within 5-years of their BCC treatment, rarely from skin cancer, and likely do not live long enough to benefit from their treatment. Active surveillance has been proposed for some of these patients. The objectives of this study were to determine patients' attitudes and concerns regarding active surveillance, and to evaluate the effect of an educational video on patients' attitudes and concerns. We conducted a pre/post survey study of 203 patients in the dermatology clinic at the Minneapolis VA Medical Center from August 2019 to October 2020. An educational video on BCC was created, and reviewed/accepted by the Minneapolis VA IRB to ensure educational rather than coercive content. The primary study outcomes were change in the number of patients with concerns regarding BCC active surveillance, change in specific concerns, and the percent of patients comfortable in participating in a study for BCC active surveillance pre- and post-video. Significantly less respondents were concerned with their doctor monitoring their BCC post- versus pre-video (61% vs 48%; p= 0.0065). Most respondents felt comfortable, very comfortable, or neutral in participating in a study for active surveillance, and no significant differences found between pre- and post-video (73% vs 75%; $p = 0.5517$). Respondents were most concerned with tumor growth (54%) and metastases (40%). Post-video, significantly more patients were concerned with frequent doctor visits (0% vs 9%; $p = <0.0001$) and making the wrong decision (15% vs 26%; $p = 0.0070$). No significant difference in comfort level with BCC active surveillance was noted between older (≥75) versus younger (<75) respondents. Majority of patients are comfortable with active surveillance of BCCs. Providing education on BCCs may alleviate patients' concerns regarding active surveillance.

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Association of occupational exposures with disease manifestations in systemic sclerosis

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Systemic sclerosis (SSc) is thought to be induced by an environmental trigger in a genetically predisposed host and leads to significant mortality from internal organ involvement. In this study, we compare the SSc disease features for different occupational exposures of SSc patients in Canada. Data on 1525 patients was extracted from the Canadian Scleroderma Research Group cohort over the years 2003-2019. Gender, occupational exposure history, symptom severity, antibody profile and mortality data were collected. Logistic regression models were used to determine clinical characteristics associated with each occupational exposure. Occupational exposures were reported in 494 patients, predominantly to organic solvents (307), industrial fumes (139), silica (101), heavy metals (93), asbestos (87) and epoxy resins (83). Occupational SSc was more prevalent in males compared to non-occupational SSc (1:1.5 *vs* 1:6 male to female ratio). Silica exposure was associated with higher prevalence of diffuse SSc (OR 1.19, CI 1.08-1.32) and increased mortality (OR 1.15, CI 1.06-1.25). Exposure to organic solvents was associated with renal disease (OR 1.05, CI 1.01-1.08) and asbestos with increased mortality (OR 1.16, CI 1.06-1.26). In addition, industrial fumes and heavy metal exposure were associated with higher prevalence of interstitial lung disease (ILD), renal disease and mortality. Epoxy exposure was associated with ILD, renal disease, diffuse SSc and anti-RNA polymerase III antibody positivity. Consistently, lower frequency of anti-centromere antibody was noticed in patients exposed to silica, heavy metal or industrial fumes. This study revealed that SSc patients with previous occupational exposure to organic solvents, industrial fumes, silica, heavy metals, asbestos and epoxy resins are predominantly males and exhibit more severe disease phenotype and/or mortality. While effective workplace protection strategies are needed, it remains imperative to obtain a detailed occupational history in SSc patients to focus on secondary prevention and risk education.

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Multimodal skin lesion classification in dermoscopy and clinical images using a hierarchical attention fusion network

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Recently, convolutional neural networks (CNNs) have achieved remarkable success in skin lesion classification. However, most existing methods are limited to relying only on dermoscopy or clinical images, with the exception of a few multimodal methods that simply integrate the extracted clinical and dermoscopy image features in the later stages of the network. The existing methods can not learn good representations from the multimodal image pairs. In this paper, we propose a novel hierarchical attention fusion network for the multimodal skin lesion classification in dermoscopy and clinical images. Compared to existing multimodal CNNs, our method has an attention fusion block to learn the refined single-modality features through the attention information in the complementary imaging modality and integrate the extracted single-modality features hierarchically in each stage of the network. To validate the effectiveness of our method, we have constructed a skin lesion dataset containing 1907 sets of multimodal image pairs (dermoscopy and clinical images). This dataset is collected from Xiangya Hospital of Central South University in recent 10 years, covering five common skin diseases: basal cell carcinoma, melanoma, nevus, squamous cell carcinoma, and seborrheic keratosis. On the collected dataset, our method can achieve the average accuracy of 81.2%, while the average accuracy of the simple late-fusion based multimodal method is only 77.3%. The experimental results demonstrate that our method can achieve more accurate classification results than the simple late-fusion based multimodal method. We believe that our method will contribute to the establishment of the multimodal skin disease diagnosis system, and assist dermatologists to diagnosis in the practical clinical workflow.

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Statistical study, of adverse drug reactions, of patients with melanoma, treated with biological drugs

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The aim of the work was to analyze ADRs from more innovative drugs used in oncological immunotherapy for the treatment of melanoma, in particular Nivolumab and Pembrolizumab. The ADRs were compared, detected by the analysis of medical records, and reported to the Pharmacy of the Hospital "Santa Maria alle Scotte" of University of Siena in the period January 2019-October 2020. Furthermore, the ADRs extracted from the Italian National Pharmacovigilance Network have been reported. The study population consisted of 263 patients, where 43 ADRs were reported, of which 37.21% is represented by ADRs in patients with melanoma. Melanoma patients treated with Nivolumab and Pembrolizumab globally represent 42.20% of treated patients (of which, 72% are treated with Nivolumab and 28% with Pembrolizumab). In patients treated for melanoma, there are a general prevalence of the male subject (66.14%), in line with literature data; in particular, 70% of melanoma patients treated with Nivolumab and 68% of patients treated with Pembrolizumab. From the analysis of all treatments carried out for melanoma, 32.55% ADR was detected with Nivolumab and Pembrolizumab. By re-elaborating the ADRs data, it appears, therefore, that the serious reactions are 33.33% for Pembrolizumab and 62.50% for Nivolumab. According to the above, there is a need to further sensitize health professionals to the consultation of IME lists at the time of the reporting of ADRs, in order to avoid differences in the reporting of similar ADRs, aimed at improving good clinical practice, of strategic drugs, for the management of serious pathologies such as melanoma.

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Risk stratification of patients with stage I cutaneous melanoma (CM) using 31-gene expression profiling (GEP)

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Introduction: Up to fifteen percent of patients with AJCC stage I CM are at risk for metastasis. The 31-GEP test for CM stratifies metastatic risk into low (Class 1A), intermediate (Class 1B/2A), or high (Class 2B), is an independent prognostic factor for CM recurrence, and when used with clinical factors, informs sentinel lymph node biopsy (SLNBx) recommendations and subsequent surveillance decisions. Methods: The 31-GEP test was performed on primary tumors from 850 patients with stage I CM. A subset of 325 patients received a negative SLNBx result. Kaplan-Meier and Cox proportional hazards analyses were used to assess 5-year recurrence-free survival (RFS). Results: Patients with stage I CM and a Class 1A 31-GEP result had a 98% 5-year RFS vs. 76% (Class 2B; $p<0.001$). Multivariable analysis showed that a Class 2B GEP result was an independent predictor of recurrence (HR, 5.8; $p<0.001$). Class 2B result had a sensitivity of 42%, and Class 1A had an NPV of 98%. In the SLNBx-assessed subset (N=325), the NPV for SLNBx was 91% (30 of 325 patients with a negative SLNBx experienced a recurrence) compared with an NPV of 95% for a Class 1A 31-GEP result (N=222, 68%). Conversely, patients with a Class 2B result had a 29% recurrence rate. Conclusions: The 31-GEP test stratifies 5-year metastatic risk in patients with stage I CM with a higher NPV than SLNBx. While most stage I patients have a favorable 5-year prognosis, 31-GEP can identify those who may benefit from increased surveillance.

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Quality and readability of online health information on eosinophilic fasciitis

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Eosinophilic Fasciitis (EF) is a rare fibrosing disorder with an unknown etiology. Its rarity limits its research and presents diagnostic and therapeutic challenges. Increasing the availability of patient-facing information may shorten time to accurate diagnosis and treatment, improving outcomes. The current state of such publicly available information is uncharacterized. Google was queried for "eosinophilic fasciitis" and "Shulman syndrome" to replicate potential searches performed by patients with EF, and "thickening of arm and leg skin" and "inflammation of fascia" to replicate queries of common EF symptoms. Websites were screened for credibility using the Health-on-the-Net (HON) Foundation criteria, and were assessed for relevance, quality, and readability by two raters using a modified DISCERN (mDISCERN) checklist. A two-tail t-test compared HON and non-HON-accredited websites (p values<0.05 were significant). Of the 419 websites screened, 36 met eligibility criteria; 28% were HON-accredited (n=10). Average readability grade level was 11.1±2.1 and 9.5±2.4 for HON and non-HON-accredited websites, respectively (p=0.023), with 8% (n=3) of websites at or below the 6th-grade level. The total mDISCERN score for all eligible websites was 40.7±13.2 [15-75], with 58% of websites scoring poor or very poor (p<0.001). Further, only four websites were identified using symptom-related search terms. Cohen's kappa interrater reliability was 0.69, indicating substantial agreement. Our results demonstrate a lack of reliable, high-quality, and readable online health information on EF. Fewer than 30% of websites were HON-accredited, and most scored as poor quality. Further, the lack of symptom-related search results indicates the dearth of information available to patients experiencing symptoms without an established diagnosis. Nearly all websites evaluated far exceeded the recommended 6th-grade reading level. Given that EF outcomes benefit from earlier detection, diagnosis, and treatment, a need for more accessible patient education materials exists.

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Epidemiology of leukocytoclastic vasculitis: A multicenter retrospective review of 440 patients

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Leukocytoclastic vasculitis (LCV) may be idiopathic or associated with underlying disorders. The aim of this study was to evaluate the demographics, comorbid diseases, and causes of LCV in a large cohort. A multicenter retrospective review at Massachusetts General Hospital, Brigham and Women's Hospital, and the Hospital of the University of Pennsylvania between the years of 2000 and 2014 identified 440 patients with LCV confirmed by histology. 56.1% of patients were male, and 43.9% were female. Age ranged from 18 to 93 years (mean, 55). The most common comorbidities at the time of diagnosis were active cancer (13.2%), renal insufficiency (8.9%), and hepatitis B or C (7.5%). Recent past medical history included antibiotic use (46.8%), upper respiratory infection (8.0%), and pneumonia (5.0%). Common symptoms at presentation included fever (24.8%), pruritus (22.7%), and joint pain (19.3%). 80.2% (353/440) of patients were diagnosed with skin-limited LCV. The remaining patients were diagnosed with systemic vasculitis, of which the most common subtype was IgA vasculitis (12.0%), or underlying connective tissue disease (3.0%). Of patients who had skin-limited LCV, the etiology was most commonly "idiopathic" (60.3%, 213/353), followed by medication exposure (27.5%, 97/353). Less common etiologies of skin-limited LCV included infections (9.9%) and malignancy (2.0%). This study demonstrates that over 80% of initial episodes of LCV are skin-limited, and approximately 60% of these cases are idiopathic. The prevalence of known etiologies can help dictate the evaluation and treatment of patients presenting with LCV. Future studies should focus on establishing a protocol for evidence-based evaluation of patients presenting with LCV.

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Comparison of ItchyQuant, KidsItchyQoL and TweenItchyQoL: Pruritus assessment tools for 6-7-year-olds vs. 8-17 year olds

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In an effort to address the dearth of quantitative pruritus assessment tools for children, we developed and validated pediatric adaptations of the ItchyQoL and ItchyQuant. TweenItchyQoL, a 35-item survey, was developed for children ages 8-17. Due to difficulties in language and comprehension in children younger than 6 years, the 14-item cartoon-annotated KidsItchyQoL was developed specifically for children aged 6-7. The ItchyQuant is a cartoon-annotated numeric rating scale for self-reported itch severity. TweenItchyQoL and KidsItchyQoL were administered in 175 and 100 children, respectively, and evaluated for reliability, validity and responsiveness; ItchyQuant was administered to all. We compared the psychometrics of these novel pediatric pruritus assessment tools. Both TweenItchyQoL and KidsItchyQoL demonstrated reliability (Cronbach's alpha > 0.84), reproducibility (ICC > 0.7 and >0.66, respectively) and validity. Only the TweenItchyQoL demonstrated statistically significant responsiveness, although KidsItchyQoL demonstrated a strong correlation between the scores and self-reported changes in itch (improvement, no change, worsening). Finally, the ItchyQuant demonstrated responsiveness in both pediatric populations with statistical significance. From this head-to-head comparison, we hypothesize that the stronger psychometrics in TweenItchyQoL may be due to the larger number of items. The lower psychometrics for KidsItchyQoL may be attributable to the fact that 6-7-year-olds are a more diverse population in cognition and literacy, with children ranging from kindergarteners to third grade. In summary, all three tools are promising to assess chronic pruritus in children ages 6-7 and 8-17.

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Psoriatic arthritis risk in psoriasis patients in the Corrona® Psoriasis Registry

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Background: Understanding characteristics that predict the onset of psoriatic arthritis (PsA) in psoriasis (PsO) patients would help effectively direct treatment. We aimed to develop a model to predict the 2-year PsA risk for real-world PsO patients. Methods: Patients in the prospective, multicenter, non-interventional Corrona Psoriasis Registry without PsA at enrollment and with a 24-month follow-up visit (FU) were included. Demographic and clinical variables collected at enrollment were used to construct logistic regression models to predict PsA diagnosis at FU. Data were randomly partitioned into training (70%) and testing (30%) sets. Models were developed using stepwise forward selection, backward elimination, and elastic net to select predictors. Performance was compared using area under the receiver-operating-characteristic curve (AUC), sensitivity (SE), and specificity (SP). Results: 1489 patients were analyzed (n=1042, training; n=447, test). In the training set, mean age was 49 years, 43% were female, and 119 (11%) developed PsA; those who developed PsA had higher mean Psoriasis Epidemiology Screening Tool (PEST) scores (2.8 vs 1.8), and patient-reported fatigue (33 vs 25, VAS-100) and skin pain (23 vs 19, VAS-100) at enrollment vs those who did not. Nine unique models were constructed; PEST and BMI were common in all. In the testing set, the most predictive model included PEST, BMI, modified Rheumatic Disease Comorbidity Index, work status, alcohol use, and fatigue (AUC= 68.9%, SE=82.9%, SP=48.8%). A more parsimonious model (PEST and BMI only) performed similarly (AUC=68.8%; SE=92.7%, SP=36.5%). Conclusions: While predictive ability was limited, and further refinement and external validation are needed, our findings provide insight for developing a tool to evaluate PsA risk in PsO patients.

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Improved survival outcomes in Merkel cell carcinoma on the basis of anatomic location

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Background: Merkel cell carcinoma is a rare, aggressive neuroendocrine cancer of the skin with poor prognosis. As with other skin cancers, the anatomic site of presentation of the primary MCC tumor may be important for prognosis. Previous studies have investigated how the location of MCC impacts survival. Given the rarity of MCC, our study used data from the National Cancer Database (NCDB), which captures approximately 70% of all cancer diagnoses in the US. Objectives: This study compared survival rates between patients with primary MCC of the head/neck, trunk, upper extremities, and lower extremities. Methods: Data from a final sample of 7,858 MCC patients from 2004-2016 NCDB were analyzed. One and three-year all-cause mortality were calculated by the Kaplan-Meier estimator. A univariate and multivariate Cox proportional hazards model was generated based on three-year all-cause mortality. Using the multivariate Cox model, three-year all-cause survival by anatomic site was plotted. Results: The unadjusted all-cause survival at 1 year was greatest for upper and lower extremity tumors (87.9% and 88.7%, respectively) and lower for head/neck (80.7%) and trunk (80.8%) tumors. Similarly, greater 3-year all-cause survival was observed for upper extremity (66.1%) and lower extremity (63.8%) tumors than head/neck (55.0%) or trunk (52.1%) tumors. When adjusted for covariates in the Cox proportional hazard model, plotted survival 36 months after diagnosis was greater for upper (67.6%) and lower extremity tumors (67.8%) than head/neck (60.0%) and trunk (58.6%) tumors. Limitations: NCDB reports long-term all-cause mortality, not disease-specific mortality beyond 90 days after diagnosis. Conclusion: In a large, multi-year, nationally diverse population of MCC cases, patients with primary tumors of the upper or lower extremities have a 3-year all-cause mortality survival advantage relative to patients with primary tumors of the head/neck or trunk. The results illustrate that anatomic location may be an important prognostic factor for patient care.

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No difference in skin cancer rates by transplanted organ type after the initial skin cancer

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Background: Organ transplant recipients (OTR) develop skin cancers at high rates. Although these patients often develop multiple skin cancers, few studies account for the total number developed. Due to skin cancer's morbidity and mortality in the transplant population, it is critical to identify high-risk patients prior to developing multiple skin cancers. Methods: We examined differences in rates of skin cancer development in a validated cohort of OTR at a single tertiary-care hospital. We used data from the electronic health record both to identify patients and to count skin cancers. We compared rates of skin cancer development based on organ type, age at transplant, and other factors using log-rank tests and Kaplan-Meier plots. Results: There were 5,190 OTR included. Seven hundred patients had at least one skin cancer, and there were 6,864 skin cancers overall. Seventy patients contributed a total of 3,319 skin cancers, which was 48.4% of all skin cancers. Lung transplant recipients had the highest proportion of skin cancer cases (69/398, 17.3%), but the lowest mean number of skin cancers per patient (4.6 ± 4.8). Compared to liver transplant recipients, heart, lung, or kidney recipients were more likely to develop at least one skin cancer ($p < 0.0001$). There was no difference by transplant type in the rate of developing a second ($p = 0.07$) or third skin cancer ($p = 0.50$); the rates remained unchanged when stratified by age group at transplant ($p < 0.0001$). There was an increased rate of developing a first skin cancer with increasing age at transplant. Conclusions: Organ transplant recipients have different risks for first skin cancer development based on transplanted organ type and age at transplantation. Age at transplant remained strongly associated with the risk of subsequent skin cancers; however, transplanted organ type did not impact subsequent skin cancer risk.

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Lifestyle modifications associated with symptom improvement in Hidradenitis Suppurativa patients

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Many hidradenitis suppurativa (HS) patients are interested in implementing lifestyle modifications, such as following particular diets or avoiding specific products, in an attempt to alleviate their symptoms. However, insufficient research has been conducted to support well-informed lifestyle modification counseling, and patients frequently defer to anecdotal endorsements of various interventions. Therefore, we sought to clarify which lifestyle modifications were capable of improving HS symptoms. We conducted a survey-based study to examine modifiable risk factors and their association with the severity of HS. Five hundred and ninety-one patients with HS participated in an online survey asking them to rate the severity of their HS before and after various modifiable lifestyle changes using a combination of 5-point patient global assessment of disease (no disease, mild, moderate, severe, or very severe disease) and self-identification of Hurley stage using the previously validated Hidradenitis Suppurativa Symptom Assessment tool. Average improvements in both subjective and objective ratings of symptom severity were calculated and Chi square testing revealed statistically significant differences between the proportion of patients who endorsed subjective improvement ($P < 0.0001$), and Hurley improvement ($P < 0.0001$) across all interventions, indicating that not all lifestyle modifications were associated with the same improvement. Numerous lifestyle interventions including substantial weight loss, smoking cessation, use of gentle skin and depilatory products, and menstrual regulation were associated with both subjective and objective improvements in symptom severity. These results suggest that patients affected by HS may experience clinically significant improvement from a variety of lifestyle modifications.

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A machine-learning modified CART algorithm informs Merkel Cell Carcinoma prognosis

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Background: Merkel cell carcinoma (MCC) is a rare neuroendocrine skin cancer with a high rate of mortality. MCC staging is currently based on tumor primary size, clinical detectability of lymph node metastases, performance of a lymph node biopsy, and presence of distant metastases. We aimed to use a modified classification and regression tree (CART) algorithm using available data points in the National Cancer Database (NCDB) to elucidate novel prognostic factors for MCC. Methods: Retrospective cohort study of the NCDB and Surveillance, Epidemiology, and End Results (SEER) registries. Cases from the NCDB were randomly assigned to either the training or validation cohorts. A modified CART algorithm was created with data from the training cohort and used to identify prognostic groups that were validated in the NCDB validation and SEER cohorts. Results: A modified CART algorithm using tumor variables available in the NCDB identified prognostic strata as follows: I: local disease, II: $\leq$3 positive nodes, III: $\geq$4 positive nodes, and IV: presence of distant metastases. Three-year survival for these groups in the NCDB validation cohort were 81.2% (SE: 1.7), 59.6% (SE: 3.0), 38.0% (SE: 6.0), and 20.2% (SE: 7.0) respectively. These strata exhibited greater within-group homogeneity than AJCC groups and were more predictive of survival. Conclusions: Risk-stratified grouping of MCC patients incorporating positive lymph node count were strongly predictive of survival and demonstrated a high degree of within-group homogeneity and survival prediction. Incorporation of positive lymph node count within overall staging or sub-staging may help to improve future MCC staging criteria.

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Outcomes of surgical correction of facial morphea: A cross-sectional analysis

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Surgical procedures are often recommended for morphea, but their timing is controversial. A cohort of 17 patients who underwent 43 procedures were followed for 1-60 months. These patients had mostly facial linear morphea (94%). The most common procedure was fat grafting for facial morphea (67%, n=29), but others included free tissue transfer (7%) and filler injections (7%). Recurrent disease, as determined via clinical examination and/or imaging using validated measures, was noted 8-36 months post-procedure in 3 patients after free tissue transplant, 2 after fat transfer, and 1 after osteoplasty. Patients with disease recurrence were less likely to be following with a dermatologist prior to procedure when compared to patients without (50% vs 100%, p=0.004), and were less likely to have a preceding MRI to assess activity (25% vs 78%, p=0.04). We highlight in particular cases of recurrence after free tissue transfer, as recent studies suggest this to be therapy for active disease. One patient underwent parascapular flap transfer shortly after diagnosis of Parry Romberg Syndrome (PRS) at age 18. He never underwent medical therapy, and experienced recurrence shortly after the procedure. Ultimately, his facial asymmetry improved with immunotherapy and fat transfer. The other two patients underwent microvascular free flap transplant at ages 9 and 16, within 1 year of PRS diagnosis. They experienced recurrence within 1 year, likely due to inadequately treated disease activity at time of procedure. While free tissue transfer and other procedures can be appropriate treatments for the cosmetic and functional impact of morphea, these are not definitive therapy for active disease. Results of this small study indicate the importance of a multi-disciplinary approach in ensuring morphea inactivity through both clinical evaluation and objective imaging measures prior to recommending surgical procedures.

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Melanoma survival in Canada: A national population-based study elucidating healthcare and socioeconomic barriers affecting patient care

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Background: Cutaneous melanoma (CM) accounts for the majority of skin cancer deaths. While morbidity due to this cancer can be largely prevented, disparities in melanoma survival persists. The mortality to incidence ratio (MIR) is a method to approximate case-based survival and is a useful tool to compare CM survival between geographic areas. We aim to investigate CM survival through MIR analysis in Canada over a 25-year period and determine healthcare and socioeconomic factors associated with poor survival.Methods: Data was obtained from the Canadian Cancer Registry and Canadian Vitals Statistics patient databases from 1992-2016 for all Canadian provinces. Age-standardized incidence and mortality rates, and MIR were calculated per province, per year. Healthcare and socioeconomic factors associated to MIR were determined using a generalized linear mixed model while adjusting for province and year. Results: Our analysis identified 106,015 CM cases and 20,570 CM deaths between 1992 to 2016. Plotted annual national MIR from 1992 to 2016 demonstrated a significant linear increase (p-value <0.0001). Saskatchewan (0.187), Ontario (0.185) and Manitoba (0.183), were among the provinces with the highest overall MIR, while New Brunswick had the lowest (0.152). Increased median household income, percentage of individuals with tertiary education, density of dermatologists and family physicians per 100,000 individuals, were factors negatively associated to MIR (i.e. better disease outcome) (p-value <0.05). Interestingly, healthcare spending per capita and number of visible minorities were not significantly associated with MIR. Conclusions: Public health resources should be oriented towards providing adequate primary and specialty healthcare services in high-risk provinces to improve CM survival.

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Risk factors associated with detection of osteomyelitis on magnetic resonance imaging in patients with cellulitis

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Background: Cellulitis is a skin infection whose presentation can involve associated conditions such as osteomyelitis. Guidelines for radiologic examination of cellulitis are limited, and magnetic resonance imaging (MRI) in particular is overused for detection of possible osteomyelitis. Objective: To assess clinical factors associated with detection of osteomyelitis on MRI in cellulitis cases. Methods: Single-center, retrospective chart review of adult patients treated for cellulitis between 2017 and 2018 was conducted. Patients with cellulitis undergoing MRI were identified as the study population. A set of possible clinical risk factors were evaluated for association with detection of osteomyelitis via univariate chi-square analysis. Factors meeting a significance threshold ($p<0.1$) were used in a model for detection of osteomyelitis via multivariate logistic regression. Results: Of 778 patients with cellulitis reviewed, 89 patients (11.4%) received 95 MRIs. 28 patients (31.5%) had 29 MRIs demonstrating osteomyelitis. History of diabetes (OR 2.68; CI 1.02, 7.05), erythrocyte sedimentation rate (ESR) above 70 mm/hr (OR 2.27; CI 0.88, 5.85), and age more than 50 years (OR 2.40, CI 0.85, 6.73) emerged as notable predictors for osteomyelitis detection in multivariate logistic regression. Images acquired in diabetic patients older than 50 years with elevated ESR have a likelihood of 59.4% (CI 2.7%, 98.7%) compared to a baseline likelihood of 9.0% (CI 3.5%, 21.7%) for detecting osteomyelitis. Conclusions: Osteomyelitis is detected by MRI in a minority of cellulitis cases with more likely detection in patients with diabetes as well as those with advanced age or elevated ESR. These results may predicate an effective scoring system for indication of MRI in cellulitis.

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Predictors of sentinel lymph node biopsy positivity in cutaneous adnexal tumors

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Objective: Malignant cutaneous adnexal tumors are rare and often aggressive tumors. Although sentinel lymph node biopsy (SLNB) can help guide management and selection of adjuvant therapies, guidelines for the appropriate use of SLNB for cutaneous adnexal tumors are lacking. The objective of this study was to evaluate risk factors for SLNB positivity in patients with malignant cutaneous adnexal tumors in order to guide clinical decision-making. Methods: The National Cancer Database (NCDB 2004-2015), containing >70% of newly diagnosed cancers in the United States, was used to identify patients with malignant cutaneous adnexal tumors who underwent SLNB. Multivariable logistic regression was used to determine risk factors for SLNB positivity after controlling for sociodemographic characteristics, primary site, and comorbidities. Results: In total, 1,109 patients with malignant cutaneous adnexal tumors who underwent SLNB were identified from 2004-2015, most of whom had eccrine porocarcinoma (19.3%), eccrine adenocarcinoma (15.0%), or hidradenocarcinoma (13.2%). 287 (25.9%) patients had a positive SLNB. In multivariable analyses controlling for sociodemographic factors, primary site, and comorbidities, older age (adjusted odds ratio [aOR] 2.03, 95% CI 1.08-3.84), Black race (aOR 2.05, 95% CI 1.26-3.33), and Medicaid (aOR 2.55, 95% CI 1.26-5.15) were independently associated with increased odds of SLNB positivity. Female sex (aOR 0.73, 95% CI 0.53-0.99) and primary site on the head (aOR 0.43, 95% CI 0.3-0.62) or limbs (aOR 0.26, 95% CI 0.18-0.37) compared to the trunk were associated with lower odds of SLNB positivity. Conclusion: Differences in SLNB positivity by socioeconomic characteristics, race, and primary site may be due to a combination of healthcare disparities and differences in tumor biology. Given the cost and morbidity associated with SLNB, individualized risk estimates for SLNB positivity are critical in facilitating thorough decision-making for healthcare providers and patients with malignant cutaneous adnexal tumors.

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Risk factors associated with detection of cutaneous abscess on ultrasonography in patients with cellulitis

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Background: Cellulitis is a skin infection that can be complicated by cutaneous abscess. Guidelines for radiologic examination of cellulitis are limited. In particular, ultrasonography (US) may be overused for detection of possible cutaneous abscess. Objective: To assess clinical factors associated with detection of cutaneous abscess on US in cellulitis cases. Methods: Single-center, retrospective chart review of adult patients treated for cellulitis between 2017 and 2018 was conducted. Patients undergoing US for evaluation of cellulitis were identified as the study population. A set of possible risk factors were evaluated for association with detection of cutaneous abscess via univariate chi-square analysis. Factors meeting a significance threshold ($p<0.1$) were used in a model for detection of cutaneous abscess via multivariate logistic regression. Results: Of 778 patients with cellulitis reviewed, 244 patients (31.4%) received 300 USs. 31 patients (12.7%) had 34 USs demonstrating cutaneous abscess. Multivariate logistic regression revealed infection affecting the groin and buttocks (OR 6.86; CI 1.92, 24.5), infection overlying permanent hardware (OR 10.5; CI 2.51, 43.9), and intravenous drug use (OR 2.44; CI 1.14, 5.22) as significant predictors of cutaneous abscess detection on US. Conclusions: Cutaneous abscess is detected by US in a minority of studies acquired for the evaluation of cellulitis. Infection affecting the groin and buttocks, infection overlying permanent hardware, and intravenous drug use emerged as significant predictors of cutaneous abscess detection. The latter two factors may indicate distinct populations for whom US may be warranted in the evaluation of cellulitis. These results further may predicate an effective scoring system for indication of US in evaluation of cellulitis.

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Effects of ruxolitinib cream in patients with atopic dermatitis with head and/or neck involvement

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Atopic dermatitis (AD) often involves the head and/or neck (HN). In two phase 3 studies (TRuE-AD1, TRuE-AD2), 1249 patients (≥12 y) with AD for ≥2 y, an Investigator's Global Assessment (IGA) score of 2/3, and 3%−20% affected body surface area were randomized (2:2:1) to twice-daily 0.75% or 1.5% ruxolitinib (RUX) cream (Janus kinase [JAK] 1/JAK2 inhibitor) or vehicle cream for 8 weeks of double-blind treatment. In this analysis, pooled efficacy at Week 8 was assessed by the proportion of patients achieving IGA treatment success (IGA of 0/1 and ≥2-grade improvement from baseline), ≥75% improvement in Eczema Area and Severity Index (EASI-75) vs baseline, and ≥4-point improvement in itch Numerical Rating Scale score (NRS4) in patients who had HN involvement at baseline (n=696 [55.7% of randomized patients]); data in the overall population (OP) were also included. Safety and application site tolerability were assessed. Week 8 response rates were numerically greater among patients with HN involvement vs the OP for IGA treatment success (0.75% RUX/1.5% RUX/vehicle, 54.3%/56.5%/8.1% vs 44.7%/52.6%/11.5%), EASI-75 (62.6%/67.2%/18.4% vs 53.8%/62.0%/19.7%), and NRS4 (48.1%/59.4%/13.3% vs 41.5%/51.5%/15.8%) with significant differences for both RUX doses vs vehicle (all $P<0.0001$). Application site reactions were reported in 14/555 patients (2.5%) with HN involvement and 19/999 patients (1.9%) in the OP who applied RUX cream vs 13/141 (9.2%) and 18/250 (7.2%) for vehicle. Application site pain (ie, stinging/burning) was reported in 5/555 patients (0.9%) for HN and 7/999 (0.7%) for OP among those who applied RUX vs 8/141 (5.7%) and 12/250 (4.8%) for vehicle; none were serious. In summary, RUX cream demonstrated good efficacy and tolerability (low rates of stinging/burning) in patients with HN involvement.

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Gender disparities in health-related quality of life (HRQoL) in patients with cutaneous T-cell lymphoma

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Patients with cutaneous T-cell lymphoma may experience impaired HRQoL due to pruritus, physical disfigurement, changes in functional status and distress associated with an incurable disease. Existing evidence is conflicting with comparison of HRQoL by gender. We performed a cross-sectional study to assess HRQoL in patients with CTCL by partnering with the Cutaneous Lymphoma Foundation to distribute an electronic survey from February-April 2019. A total of 292 patient responses (66% female, mean age 57y) were included in the analysis. Most of the cohort had early-stage (IA − IIA) (74%; 162/203) mycosis fungoides (MF) (87%; 241/279), followed by Sézary (SS) (12%; 33/279). As assessed by the Skindex-16 and Functional Assessment of Cancer Therapy-General (FACT-G), women with CTCL experience significantly worse HRQoL compared with men (Skindex-16*: 51±26 vs. 36±26, P=<.001; FACT-G: 69±21 vs. 77±16, P=.005). Women experience worse HRQoL in all three of the Skindex-16 subscales (symptoms: β=14.0, P=<.001; emotions: β=15.1, P=<.001; functioning: β=11.3, P=.006) but only two of the four FACT-G subscales (physical: β=-2.8, P=<.001; emotional: β=-2.0, P=.004). Women with CTCL experience a significantly worse HRQoL compared with men. This gender difference was present even when controlling for stage of disease. Further research is needed to disentangle what specific factors contribute to this gender disparity in CTCL. *In Skindex, *higher* is worse HRQoL; in FACT-G, *lower* is worse HRQoL.

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Itch-free state in patients with atopic dermatitis treated with ruxolitinib cream

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Atopic dermatitis (AD) is a highly pruritic, inflammatory skin disease. In two phase 3 studies (TRuE-AD1, TRuE-AD2), 1249 patients (≥12 y) with AD for ≥2 y, an Investigator's Global Assessment (IGA) score of 2 or 3, and 3%−20% affected body surface area were randomized (2:2:1) to twice-daily 0.75% or 1.5% ruxolitinib (RUX) cream (Janus kinase [JAK] 1/JAK2 inhibitor) or vehicle cream for 8 weeks. In this pooled analysis, effects of RUX on itch were assessed by the proportion of patients achieving an itch Numerical Rating Scale score of 0 or 1 (NRS 0/1) and no days of itch per Item 1 (frequency of itch) of the Patient-Oriented Eczema Measure (POEM). At Week 8, more patients who applied RUX (0.75%/1.5%) vs vehicle achieved NRS 0/1 (45.5%/51.5% vs 23.1%; $P<0.0001$); median time to NRS 0/1 was significantly shorter with RUX vs vehicle (12/8 days vs 51 days; $P<0.0001$). More patients achieved no days of itch per POEM with RUX (28.3%/32.9%) vs vehicle (9.0%; both $P<0.0001$). As assessed by NRS 0/1 or POEM, more patients achieved itch-free status at Week 8 with RUX vs vehicle (47.7%/52.0% vs 23.4%; both $P<0.0001$) regardless of baseline itch score (NRS <6: 57.4%/58.1% vs 27.5%, $P<0.0001$; NRS ≥6: 34.2%/41.3% vs 17.7%, $P<0.01$). In summary, a significant number of patients with AD treated with RUX cream achieved and sustained an itch-free state.

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Comorbidities of pediatric hidradenitis suppurativa: A retrospective analysis

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Introduction: Adult hidradenitis suppurativa patients have a high burden of comorbidities; however, data on comorbidities in pediatric HS patients is limited. We aimed to characterize physical and psychosocial comorbidities of pediatric HS patients. Methods: A retrospective chart review of all HS patients (identified using ICD-9/10 codes) aged 25 and younger at time of data pull in April 2020 in the UCLA health system was performed. Inclusion criteria included a diagnosis of HS prior to 18 years old. Data on demographics and pediatric comorbidities were extracted. Results: A total of 73 patients (80.8% female) met inclusion criteria. One third (35.8%) were White, 28.3% Hispanic, 18.9% Black, and 5.7% Asian. Mean age of symptom onset was 12.6 years (range 6-17) and of HS diagnosis was 14.3 years (range 7-17). The majority of patients (68.1%) were overweight or obese. The most common cutaneous comorbidity was acne vulgaris (37.0%) followed by acanthosis nigricans (23.3%) and atopic dermatitis (17.8%). Metabolic disorders were present, including hypercholesterolemia (16.4%), pre-diabetes and diabetes (6.8% and 2.7% respectively), and hypertension (4.1%). Six (10.2%) female patients had a diagnosis of PCOS, and two (2.7%) patients had precocious puberty. Other medical comorbidities included anemia (9.6%) and thyroid disease (2.7%). Two patients had Down's syndrome. Psychiatric comorbidities affected over a quarter (27.4%) of patients, and were more common in female patients (25.4% with anxiety, 22% with depression) compared to male patients (7.1% each with anxiety or depression). Notably, five patients (6.8%) had a history of suicidal ideation. Discussion/Conclusion: HS pediatric patients have a significant comorbidity burden including both medical and psychiatric conditions. Our study highlights the need for further large-scale studies on comorbidities in pediatric HS and development of consensus guidelines on comorbidity screening in this population.

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Acral lentiginous melanoma: Presentation and outcomes in the era of effective melanoma therapy

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Background: Acral lentiginous melanoma (ALM) is a genetically distinct melanoma subtype, classically associated with later stage presentation and worse overall survival (OS) than non-ALM variants. Over the past decade, novel therapies, including immunotherapy and targeted therapy, have led to improved melanoma survival; however, ALM typically does not harbor the mutations necessary for targeted therapy effectiveness, and limited data exist describing the clinical features and outcomes of ALM in the modern therapeutic era. Methods: A retrospective analysis was performed using the National Cancer Database comparing a historical cohort (HC) (2004-2006) to a modern cohort (MC) (2013-2015) of ALM patients. Survival analysis was performed using the Kaplan-Meier method and log-rank test, and outcomes were compared to those for non-ALM patients among the same time periods. Results: A total of 126,501 patients were studied, including 1,960 with ALM. Among ALM patients, compared to the HC, the MC had thicker tumors (median 1.7mm vs. 1.4mm, $p<0.01$), more node-positive disease (31.3% vs. 21.2%, $p<0.01$), and more advanced (stage III/IV) disease (31.8% vs. 22.2%, $p<0.01$). Among all stages, there was a clinically small but statistically significant improvement in 5-year OS over time for non-ALM patients (80.0% MC vs. 79.1% HC, $p<0.001$), but not for ALM patients (70.4% MC vs. 68.6% HC, $p=0.39$). Among stage III/IV patients specifically, rates of immunotherapy treatment were similar for ALM and non-ALM patients (23.8% ALM vs. 24.3% non-ALM, $p=0.23$); 5-year OS significantly improved for non-ALM patients (60.4% MC vs. 53.6% HC, $p<0.001$), but not for ALM patients (45.6% MC vs. 49.6% HC, $P=0.55$). Conclusion: Despite the advent of novel melanoma therapies, outcomes for advanced stage ALM have not improved, and ALM thickness and stage of presentation have increased over time. These findings highlight the critical importance of increased efforts for raising awareness of ALM and its early detection.

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The impact of COVID-19 on gender representation in academic dermatology publications

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COVID-19 has disproportionately affected female academics, where publications by women authors in many fields have decreased during the pandemic. This trend has yet to be examined in dermatology. We surveyed the recent representation of total female authors, female first authors (FFA), and female senior authors (FSA, a potential indicator of career advancement) in the dermatologic literature. Publications from five top h-index Web of Science dermatology journals (Journal of Investigative Dermatology, Journal of the American Academy of Dermatology, JAMA Dermatology, Dermatologic Surgery, Lasers in Surgery and Medicine) were analyzed. Genderize.io predicted binary gender by author first name. Surprisingly, the total proportion of female authorship increased from 44% (2018) to 46% (2020), and similarly for FFA (2018: 46%, 2019: 48%, 2020: 53%) and FSA (2018: 38%, 2019: 38%, 2020: 40%). Many possible explanations exist for this trend. The proportion of women board-certified in dermatology has grown substantially in recent years, possibly exceeding any detrimental impact of COVID-19. Decreased patient capacity at dermatology clinics, suspension of elective procedures, and a prominent shift to telemedicine may provide more time for research. Our study was limited to five top influential dermatology journals, and prediction of gender using genderize.io's database. Due to indexing delays, some 2020-indexed publications may have been generated before the pandemic, necessitating further study. While it is promising that FFA data suggests proportional contributions from female lead authors, FSA percentages are still lagging behind, corroborating patterns of female underrepresentation in senior faculty positions and ongoing gender disparities in research funding and academic promotion. Future analysis and discussion will be necessary to strengthen support for female academic dermatologists.

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Geographical distribution of systemic sclerosis in Canada: A large Canadian database study

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Systemic sclerosis (SSc) leads to significant morbidity as a result of skin fibrosis and mortality due to internal organ involvement. While the pathogenesis is not fully elucidated, it is believed to be induced by an environmental trigger in individuals with a genetic predisposition. Data on 1506 patients was extracted from the Canadian Scleroderma Research Group cohort over the years 2003-2019. Forward Sortation Area (FSA) was used to map the 17-year prevalence with geographic information systems. Simulations were created to evaluate the expected prevalence per FSA based on age and sex of the population alone and compared to observed values, with a binomial probability test, to identify high prevalence areas. Environmental exposures were assessed using the Canadian Urban Environmental Health Research Consortium and DMTI Enhanced Point of Interest. The 1506 patients were distributed over 665 FSAs with 60% of cases found in Quebec and Ontario. Analysis revealed 19 FSAs with significantly elevated prevalence compared to expected. Among these, 6 were in rural regions and often far from recruitment centers. They were situated in close proximity to metal mining/transformation, international airports or large train terminal, where metal in airborne nanoparticles and diesel exhaust have been reported, and near salt mine and uranium mine operations. In this exploratory study, we present preliminary findings of the uneven geographic distribution of SSc cases in Canada. Heavy metal and benzene air pollution appeared to be a common theme among hot spots. This preliminary data is highly relevant and forms grounds to initiate populational studies in Canada and abroad to confirm our findings of geographic clustering and to focus on identified putative environmental triggers with plausible.

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Second intention healing is the second most common method of reconstruction for skin cancer excisions

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Importance: The frequency of second intention healing (SIH) after conventional excision of skin cancer had not been studied. Objectives: To characterize the frequencies of SIH versus linear closure and clinical factors associated with each method of wound management. Design, Setting, Participants: Adult patients with one skin cancer and one malignant excision procedure per claim in Optum, a large insurance claims database including nationwide commercially insured patients. SIH was defined as having a malignant excision claim without an associated reconstruction claim in the following 7 days. Multivariable regression was used to correlate SIH or linear closure with year, diagnosis, lesion location, width of the excision, geographic location, sex, age, annual income, education, and race. Results: After malignant excisions of the skin, SIH was the second most common (19.9%) overall wound management method after linear reconstruction (60.5%). A major factor associated with SIH use compared to linear closure was width of the excision ($p<0.001$). The rate of SIH was highest after small excisions (74.1% after excisions <0.5cm; 46.3% after excisions 0.6-1.0 cm), compared to larger excisions (18.1% after excisions 2.1-3.0 cm; 22.6% after excisions 3.1-4.0cm; and 27.3% after excisions larger than 4 cm). Conclusions and Relevance: According to a large claims database, SIH is was the second most common strategy to manage wounds after malignant excisions. SIH was more common with excisions <1 cm but also used in 27% of excisions >4 cm. Additional study is warranted to determine indications, outcomes, and cost for this common method to manage malignant excision wounds.

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Acne, androgenetic alopecia, and atopic dermatitis in transgender patients on hormone therapy: A retrospective comparative cohort study

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To determine the prevalence of acne, androgenetic alopecia (AGA), and atopic dermatitis (AD) among transgender/genderqueer (TG) patients on gender-affirming hormone therapy (GAHT) compared to cisgender patients, we conducted a retrospective comparative cohort study via electronic health records of TG adults on masculinizing or feminizing hormone therapy (MHT, FHT) seen at Fenway Health between August 1, 2014 and August 1, 2020. Outcomes of acne, AGA and AD were identified via International Statistical Classification of Diseases, 9th/10th Revisions, Clinical-Modification (ICD-9-CM, ICD-10-CM) codes. Odds ratios (OR) and 95% confidence intervals (95% CI) were used to compare the risk of disease for TG patients on GAHT to cisgender patients. The sample ($n = 3618$) included 1,465 patients on FHT, 1,614 patients on MHT, 265 ciswomen, and 285 cismen, with median ages of 24 (interquartile range (IQR) 21-30), 23 (IQR 21-32), 25 (IQR 20-28), 29 (IQR 22-33), and 26 (IQR 23-44) years, respectively. Patients on MHT were significantly more likely to have acne compared to ciswomen (OR: 1.91 (95% CI: 1.33, 2.82), $p = 0.0003$). We did not find a significantly different risk of acne among patients on FHT compared to cismen (OR: 1.02 (95% CI: 0.56, 1.98)). Whereas patients on FHT were significantly less likely to have AGA compared to cismen (OR: 0.40 (95% CI: 0.21, 0.79), $p = 0.0023$), patients on MHT were significantly more likely to have AGA compared to ciswomen (OR: 3.3 (95% CI: 1.06, 16.50), $p = 0.0343$). There was no significant difference in risk for AD between patients on FHT versus cismen (OR: 0.80 (95% CI: 0.52, 1.26)). However, the risk of AD significantly decreased for patients on MHT compared to ciswomen (CI: 0.50 (95% CI: 0.33, 0.76), $p = 0.0004$). Healthcare providers should consider the impact GAHT may have on dermatologic conditions.

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Dupilumab in Canadian eczema clinic

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Atopic dermatitis (AD) is a chronic Th2-mediated inflammatory condition. Dupilumab is an interleukin (IL) receptor antagonist that inhibits IL-4 and IL-13 type 2 immune signaling. In North America, dupilumab remains the only approved systemic treatment for adults with moderate-to-severe AD. Despite positive trial outcome data, real-world evidence is still needed. As the first tertiary AD clinic in Canada, the McGill University Centre of Excellence for Atopic Dermatitis (McGill COE-AD) identified a need to characterize usage of this on-label therapy in its patient cohort with moderate-to-severe AD. The study objective was to describe the AD patient population currently on dupilumab in the McGill COE-AD. Clinical data was gathered from 52 patients undergoing dupilumab therapy at the COE-AD from April 2018 to November 2020. Patient age, gender, AD severity (EASI), and AD burden (Dermatology Life Quality Index, DLQI) were collected. Descriptive statistical analyses were performed. The COE-AD's patient population on dupilumab is 52% male with average age 40 (range 23-72 years). Regarding AD severity: 61% of patients showed an overall decrease in EASI scores from treatment commencement; 10% showed an overall increase in EASI; and 29% showed an initial decrease but then increase in EASI. All patients showed an increase in DLQI with an average increase of 74% (95% CI 68%, 80%). The McGill COE-AD cohort findings are consistent with trial data for dupilumab efficacy, with 61% of patients showing an improvement in EASI scores. Notably one third of dupilumab patients showed an initial fall but then rise in AD severity, and one tenth showed an overall rise in AD severity. These discrepancies may reflect variation in treatment course (from 1 month to 16 months), as well as real-world confounders such as variance in withdrawal time from off-label cyclosporine, methotrexate or mycophenylate mofetil use. Patients showed an overwhelming improvement in AD quality of life burden on dupilumab. Ongoing studies are needed to determine long-term efficacy and quality of life for this population.

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The frequency and utility of drug cessation trials in older adults with chronic eczematous dermatitis of unknown etiology

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Eczematous dermatitis is a major cause of recalcitrant pruritic eruptions in older adults. While some medications such as calcium channel blockers have been indirectly implicated in eczematous eruptions, there are no data demonstrating the utility of medication changes in this condition. We performed a retrospective cohort study of adults older than 65 with chronic eczematous eruptions of unknown etiology to evaluate the frequency and effectiveness of medication cessation trials. Out of 650 adults older than 65 presenting to our clinic with new onset eczematous eruptions, 86% (561) had identifiable eczematous disorders not known to be associated with medications such as nummular dermatitis, contact dermatitis or classic atopic dermatitis. Out of the remaining 14% (89) patients with new onset eczematous eruptions with no underlying identifiable cause, 33 had undergone a drug cessation trial prior to their first visit and 18 stopped a medication after their first visit to our institution. Although there was mention of improvement in 22% of cases, all patients still sought tertiary care for their persistent rash despite stopping a medication. Negative outcomes (including 4 hospitalizations) occurred in 18 of the drug cessation trials, all of which were due to exacerbation of the underlying comorbidity that the medication was prescribed to treat. Our single-institution experience suggests that the majority of patients with chronic eczematous conditions of unknown etiology in the aging population undergo drug cessation trials that ultimately do not improve their dermatitis. Drug cessation trials are not benign and can lead to dangerous worsening of underlying conditions. Further study of the etiology of eczematous eruptions in older age is needed.

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The impact of comorbidity identification on outcomes of pyoderma gangrenosum: A retrospective cohort study of previously hospitalized patients

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Pyoderma gangrenosum (PG) carries a substantial comorbidity burden. While the effect of underlying disease state on in-hospital outcomes has been evaluated, long-term outcomes remain unclear. The objective of the present study was to determine the effect of identified comorbidities on long-term outcomes and evaluate the current practice of comorbidity identification in PG patients. A retrospective cohort study of PG patients seen in The Ohio State University (OSU) hospital system was performed. Comorbidity testing, treatment and long-term outcomes were compared between groups with and without identified comorbidities. We identified 55 unique hospitalized patients with PG between 2011-2017. The presence of an identified comorbid disease state was associated with superior outcomes regarding ulcer resolution within one year ($p = .030$) and while in the OSU system ($p = .047$), PG recurrence after prior resolution ($p = .019$) and PG-related readmission ($p = .017$) despite less aggressive initial systemic treatment recommendations. Inflammatory bowel disease was the most common identified comorbidity and was associated with greater ulcer resolution at one year and while in the OSU system, as well as a lower rate of readmission, than those without identified comorbidities. Consistent comorbidity evaluation, however, is often incomplete in those with and without identified disease associations. In conclusion, a thorough evaluation to identify associated comorbidities of PG patients is important to prognosticate, improve patient outcomes, and reduce the cost burden placed by PG on the healthcare system.

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Access to dermatologic care in urban underserved communities

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Research on geographic access to dermatologic care has focused on urban-rural differences, while little is known about disparities in geographic access in urban populations. There are known disparities in access to dermatologic care, and counties with higher median incomes tend to have more dermatologists. Conversely, counties with a population majority of African Americans, Hispanics, and/or Native Americans are more likely to have zero dermatologists as compared to counties with a white majority. This study aims to evaluate differences in the geographic distribution of dermatologic care between urban medically underserved areas (MUA) and urban non-MUA in the United States. We conducted a spatial analysis using geolocated practice locations (N=13,593) from the American Academy of Dermatology (AAD) member directory in March 2020 and urbanized census tracts from the 2014-2018 American Community Survey (N=36,602). We use the US Census Bureau definition of urbanized areas, and the Health Resources and Services Administration (HRSA) definition of MUA. A zero-inflated negative binomial model was used to compare the number of dermatologists per census tract in MUA and non-MUA tracts. The median number of dermatologists per urban MUA census tract was 0 (Range 0-134). The median number of dermatologists per urban non-MUA census tract was 0 (Range 0-70). Urban MUA were more likely to have zero dermatologists though not statistically significant (coefficient: 10.32, p=0.680). In contrast, among census tracts with at least 1 dermatologist, urban MUA had an average of 2.6 times more dermatologists as compared to urban non-MUA (p= <0.0001). Among urban areas with at least 1 dermatologist, urban MUA had significantly more dermatologists than urban non-MUA. Further research is needed to characterize whether the geographic location of dermatologists in MUA correlates with true access to dermatologic care among these urban communities.

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Epidemiologic trends of mycosis fungoides and sezary syndrome in Arkansas reveals increasing incidence and disparities

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Cutaneous T-cell lymphomas (CTCL) are rare non-Hodgkin's lymphomas from skin-homing T cells, most commonly represented by mycosis fungoides (MF) and Sezary syndrome (SS). With fluctuating population demographics in the US, we compare the population of MF and SS from 2013-2020 in Arkansas in relationship to SEERs cohort representing 36% of the US population from 2000-2017 to gain insight into trends in prevalence and incidence. The UAMS database included 150 patients, 58% male, 42% female; 70% White , 28.6% Black. There is a discordance between the Blacks (26.4%) in the patient population to the general Arkansas population (15.7%) ($P = 0.0004$), highlighting the disparity in increase incidence in Blacks. The youngest mean age was in Black males (57.6 yrs), while the oldest mean age was in White females (67.0 yrs). The average age for advanced stage (≥IIB) for Blacks (59.8 yrs) versus Whites (70.1 years) differ by 10 years. These findings are consistent with data derived from the SEER based on 3278 patients. The CTCL mortality rates are highest among Black Males (0.5) [CI, 0.44-0.57] and Black Females (0.28) [CI, 0.25-0.32]. There was a significant increase in mortality rates of CTCL in the US; APC, 6.19% (95% CI, 4.19-8.22, $p < .001$) between 2000-2017, with a male predominance in all sex groups and races, but disproportionately affecting young Black males. An analysis of patient residence reveal that most live near major interstates and near chemical emitting facilities. Population-adjusted rates indicate that in Arkansas, CTCL is 2.23 times more prevalent in metropolitan than non-metropolitan areas, an observational estimate consistent with the 2.38 ratio calculated using SEERS data. The geographic patterns support potential environmental exposures that may be a risk factor in MF/SS. In conclusion, we present demographics analysis from a single academic center on MF and SS in relation to SEER database with focus on updating trends to better understand geographic and variables that affect disparities of this rare malignancy.

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Ruxolitinib cream rapidly decreases skin pain in atopic dermatitis

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Atopic dermatitis (AD) is an inflammatory skin disease associated with skin pain. Efficacy outcomes, with a focus on skin pain, from a pooled analysis of two phase 3 studies (TRuE-AD1, TRuE-AD2) in 1249 patients (≥12 y) with AD for ≥2 y with Investigator's Global Assessment (IGA) score 2 or 3 and 3%−20% affected body surface area are reported. Patients were randomized (2:2:1) to twice-daily 0.75% or 1.5% ruxolitinib (RUX; Janus kinase [JAK] 1/JAK2 inhibitor) or vehicle cream for 8 weeks. At Week 8, more patients who applied RUX (0.75%/1.5%) achieved IGA treatment success (IGA of 0/1 and ≥2-grade improvement from baseline) vs vehicle (44.7%/52.6% vs 11.5%; $P<0.0001$). Significantly greater reductions in itch numerical rate scale (NRS) score were observed within 12 h of first application (mean change from baseline, −0.4/−0.5 vs −0.1; $P<0.02$). Greater mean reductions from baseline in skin pain NRS scores were observed within 12 h with RUX vs vehicle (−0.3/−0.3 vs −0.04; $P<0.03$); further reductions were observed over time (Week 8, −2.5/−2.6 vs −1.3; $P<0.0001$). A substantial proportion of patients who applied RUX achieved 'no pain/discomfort' per EQ-5D-5L (fourth dimension) at Week 2 (57.8%/61.8% vs 36.3%), which was sustained through Week 8 (67.0%/69.4% vs 42.8%). In summary, RUX cream significantly decreased inflammation, itch, and skin pain in patients with AD.

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Clinical trial publication trends and disease focus: 2015-2019

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Dermatology is a broad specialty, and clinical trial resources are limited. We sought to describe current trial disease focus and resource allocation. We tabulated clinical trials from 5 dermatology journals (JAMA Dermatology, BJD, JID, JAAD, and JEADV) from 2015-2019. Study characteristics, funding, and disease focus were extracted. Results are descriptive, associations with industry funding were investigated with logistic regression. 265 clinical trials met inclusion criteria. Trials targeted inflammatory (n=156, 58.9%), neoplastic (n=60, 22.6%), surgical techniques/outcomes (n=19, 7.17%), cosmetic (n=19, 7.17%), and infectious (n=11, 4.15%) conditions. Fifty-eight specific conditions were identified. The most common were psoriasis (n=75, 28.3%), actinic keratoses (n=30, 11.3%), atopic dermatitis (n=23, 8.68%), and surgical techniques (n=13, 4.91%). Other diseases constituted <5% of trials. Most were randomized (n=245, 93.5%) and controlled (n=243, 92.4%). Active controls were prevalent (n=104, 39.5%), but less in psoriasis (n=18, 24.7%) and atopic dermatitis (n=4, 18.2%). Most were industry-funded (n=156, 58.87%). Most industry funding was for inflammatory disease (OR 10.2, 95% CI: 3.20-32.4, reference = surgical studies). Most funding and clinical trials are focused on a small number of diseases. Other common disease processes such as pruritus, wounds, and hidradenitis suppurativa, carry significant morbidity but have relatively minimal representation in trials. We hope these data offer perspective and help identify under-represented needs.

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Food insecurity in wound healing

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The United States Department of Agriculture (USDA) defines food insecurity (FI) as the lack of consistent access to sufficient food in order to sustain a healthy lifestyle. Food insecure individuals experience poorer health outcomes and higher rates of chronic diseases, such as type 2 diabetes mellitus, hypertension, childhood asthma, and migraine headaches, compared to their food secure counterparts. There is no data about FI in dermatology. We hypothesized that FI is prevalent in people with chronic wounds and investigated the burden of FI in a dermatologic patient population with wounds. We designed a single-center, cross-sectional, rolling enrollment study in 50 respondents, aged ≥18 years, from January 2020 to November 2020. After ethical approval, subjects with chronic wounds of varying etiologies were recruited from outpatient wound clinics. All subjects completed the US Adult Food Security Survey Module, (US-AFSSM, USDA), and a demographics questionnaire. These demographics were adapted from the National Health and Nutrition Examination Survey to a 6th grade reading level. FI status was assessed on a scale of 0 to 10 per the US-AFSSM, (score of 0 = low FI, score of 10 = very high FI). Out of 50 subjects, 36% (95% CI: .2292, .5081) screened positive for food insecurity, which is significantly more than the Miami-Dade County proportion of 11.8%. From our total cohort, 36% of people identified as White, 24% as Black, and 38% as Hispanic/Latino. Similar to national findings, reported race was significantly associated with FI: non-White race patients were associated with FI, unlike White race patients (OR: 8.0, 95% CI: 1.575 − 40.633). 30% had a high school education or lower. 50% of our total cohort had an income of <34,999/year. Reported income <34,999/year was associated with FI (OR: 17.33, 95% CI 1.984 − 151.406). From our preliminary analysis, this data suggests that FI is prevalent in people with chronic wounds especially in racial minorities and low-income subjects. Data on wound healing in food insecure patients is forthcoming and may impact clinical care.

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Data driven approach identifies hidradenitis suppurativa subtypes in electronic health records

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Hidradenitis suppurativa (HS) is a prevalent inflammatory skin disease that is associated with a high burden of comorbidities. Heterogeneity in clinical presentation coupled with variation in treatment response suggests that among people who share an HS diagnosis there exist different biological causes of disease. Obscure etiological heterogeneity creates inefficiencies in healthcare and attenuates power in clinical trials and research studies. While previous studies have attempted to identify HS subtypes on the basis of characteristics primarily related to skin lesions, our group hypothesizes that distributions of comorbidities can be used to identify medically relevant HS subtypes. We implemented machine learning algorithms to investigate comorbidity patterns using longitudinal data from the eMERGE consortium (project NT227) that contains 368,331 diagnosis codes from 668 HS participants capturing on average 16 years of observations per person. Using a tensor factorization (TF) method we identified five disease subtypes characterized by the onset of different sets of diseases prior to an initial HS diagnosis, including (1) neuropsychiatric, (2) joint, (3) metabolic, (4) cardiopulmonary, or (5) obesity and acne. We next leveraged the feature weighting scheme identified by TF to develop subtype phenotype scores (SPS) for research participants. Unsupervised clustering of participant SPS in the eMERGE cohort and in an independent cohort both indicate that most HS participants can be assigned to a single subtype. This work suggests that patterns of HS comorbidities can be used to identify disease subtypes. Future studies are aimed at determining the biological and clinical relevance of our work.

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Efficacy of ruxolitinib cream in adults and adolescents with atopic comorbidities

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Atopic dermatitis (AD) is an inflammatory skin disease associated with atopic and nonatopic comorbidities. Efficacy outcomes in patients with or without atopic comorbidities from a pooled analysis of two phase 3 studies (TRuE-AD1 [NCT03745638], TRuE-AD2 [NCT03745651]) in 1208 evaluable patients (≥12 y) with AD for ≥2 y with Investigator's Global Assessment (IGA) score 2 or 3 and 3%−20% affected body surface area are reported. Patients (N=1249) were randomized (2:2:1) to twice-daily 0.75% or 1.5% ruxolitinib cream (RUX; Janus kinase [JAK] 1/JAK2 inhibitor) or vehicle cream for 8 weeks. Outcome measures included the percentage of patients achieving IGA treatment success (IGA-TS; IGA of 0/1 and ≥2-grade improvement vs baseline); ≥50%, ≥75%, and ≥90% improvement in Eczema Area and Severity Index (EASI-50, -75, and -90, respectively) vs baseline; and a ≥4-point improvement in itch Numerical Rating Scale score (NRS4). Overall, 51.1% of efficacy-evaluable patients had atopic comorbidities at baseline. At Week 8, among patients with comorbidities, substantially more who applied RUX (0.75%/1.5%) vs vehicle achieved IGA-TS (50.4%/57.4% vs 10.5%), EASI-50 (75.8%/82.1% vs 33.3%), EASI-75 (58.3%/67.7% vs 19.3%), EASI-90 (42.5%/50.6% vs 5.3%), and NRS4 (47.0%/55.5% vs 9.9%). Greater mean change (reduction) from baseline in itch NRS scores occurred within 12 h of first application of RUX vs vehicle (−0.4/−0.4 vs −0.1). Similar results were observed among patients without comorbidities. In summary, patients with AD treated with RUX cream had markedly improved AD signs and symptoms (itch relief within hours of first application) regardless of atopic comorbidities.

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Smoking cessation is significantly correlated with disease manifestation in Pemphigus vulgaris

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Pemphigus vulgaris (PV) is a potentially fatal autoimmune blistering skin disease characterized by the prominence of anti-desmosomal antibodies anti-desmoglein 3 and 1 leading to intraepidermal blistering. However, other antibodies including those targeting acetylcholine receptors have also been implied in disease. The etiology of PV is multifactorial with a strong genetic association with certain HLA alleles, but it is clear that additional environmental and immune trigger factors are needed to induce disease. Among these trigger factors, an inverse relationship between smoking and PV has been noted in several case-control studies, i.e. PV occurs less frequently in current and former smokers when compared to controls. It has been speculated that the putative protective action of smoking is due to the anti-acanthotylic action of triggering acetylcholine receptors by their agonist nicotine. However, no study to date has directly addressed the temporal relationship between smoking and disease, specifically whether patients with a history of smoking ceased to smoke before or after disease onset. Thus, we designed a study in which patients with a biopsy confirmed diagnosis of PV were asked whether they had a history of smoking and whether they quit smoking before or after disease onset in addition to other constant and variable disease parameters. Out of 78 PV patients and 38 age- and sex-matched controls, approximately 50% were former or current smokers in both groups. Of the former/current smokers in the PV group, however, 94% of patients quit smoking before study inclusion, with 81% quitting before the onset of disease, while in the control group only 56% of subjects quit smoking before enrollment (OR 0.59 and 0.69; 95% CI 0.24-1.45 and 0.28-1.72, respectively). Our data suggests that it is not the mere absence of smoking, but the lack of smoking-associated molecules (presumably nicotine) in subjects previously exposed to these molecules (in other words, smoking cessation), that can be considered to be a trigger factor in PV.

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Trends and outcomes in patients with malignant skin cancers during the COVID pandemic

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The COVID-19 pandemic has forced healthcare organizations to reduce the provision of non-emergent services leading to delays in diagnoses and treatment. There is scant literature regarding the impact of COVID in malignant skin cancer (MSC) patients. A retrospective analysis was done from Apr-July 2020 using TriNetX, a national federated database of 63 million records. The incidence of new MSC-related codes was estimated during the pandemic period and compared to a corresponding pre-pandemic period. Medical encounter visits utilized by MSC patients during the pandemic was also recorded. 30-day COVID complications were compared among COVID patients with and without history of confirmed MSC. 1:1 propensity score matching for comorbidities and demographics was performed to estimate adjusted risk ratios with 95% CI. Kaplan Meir's (KM) analysis was done to calculate survival probability in 30 days among the matched cohorts. The incidence of malignant melanoma, malignant non-melanoma skin cancers (NMSC), and overall MSC dropped by 40%(p=0.001), 48%(p<0.001), and 53%(p=0.001) respectively when compared to a similar pre-pandemic period. Skin cancer screenings dropped by 37%(p=0.04). Telehealth services utilized by MSC patients increased from 100 encounters total in 2018 to 23279 encounters from Jan-July 2020. After matching, COVID-19 patients with a history of MSC had a significantly lower risk of hospitalization (RR [95%CI])=(0.8[0.71-0.90]) and severe outcomes=(0.8[0.64-0.98]) compared to COVID-19 patients without history of MSC. Matched KM analysis revealed MSC-COVID patients had a statistically significant lower cumulative probability of getting severe COVID compared to Non-MSC COVID patients (p=0.004). MSC related codes have decreased whereas telehealth visit has increased. COVID- 19 patients with a history of MSC demonstrated a significantly reduced risk of 30-day COVID complications compared to COVID-19 patients without MSC.

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National trends of dermatology procedures during the COVID pandemic

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The COVID19 pandemic has caused a major disruption in healthcare delivery as restrictions have limited the ability to seek elective procedures. This investigation aimed to assess the pandemic impact on the incidence of dermatology procedures compared to the pre-pandemic period. TrinetX, a national federated healthcare database comprised of 61 million patient records, was used to identify the incidence of new dermatology procedures performed each month for two periods of the pandemic. The first period was from Apr-July 2020, and the second was from Aug-Nov 2020. The mean for each variable was then compared with the pooled monthly incidence from similar periods between 2018-2019 before the pandemic. Descriptive analyses were performed, and comparisons were made using a student's t-test. Procedures were identified by CPT codes and categorized into groups. In the Apr-July 2020 period, 11 groups of procedures showed a significant statistical decrease in incidence by up to 47.3%. They included: Excision of benign lesions, shaving of epidermal/dermal lesions, skin tag removal, skin biopsy, paring/cutting procedures, surgical procedures on nails, surgical procedures on pilonidal cyst, destruction of malignant lesions, destruction of benign/premalignant lesions, Mohs surgery, and incision and drainage procedures. The incidence of debridement procedures during the initial COVID period was similar to overlapping months in 2018 and 2019. In the late pandemic period, 10 procedure groups demonstrated a significant statistical decrease in incidence. The incidence of debridement procedures and surgical procedures on pilonidal cysts during the later COVID period was similar to overlapping months in 2018 and 2019. The statistically significant decrease in dermatology procedures during the COVID-19 pandemic may suggest that the general public is postponing skin care placing a greater burden on healthcare.

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Atopic dermatitis: Patient characteristics and patient-reported outcomes on topical therapy

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Topical treatments are standard of care for many adults and adolescents with atopic dermatitis (AD). We determined real-world control/satisfaction and patient-reported outcomes related to topical AD treatment. Data were from the AD Adult/Pediatric Disease Specific Programs, point-in-time surveys of US physicians and their patients conducted in 2018/2019, respectively. Physicians reported treatment, disease control (deteriorating/changeable = uncontrolled, stable/improving = controlled), and satisfaction with current control. Patients completed a matched questionnaire including the Patient-Oriented Eczema Measure (POEM), the Dermatology Life Quality Index (DLQI and child-equivalent cDLQI), and the Work Productivity and Activity Impact (WPAI) questionnaire (adults only). Independent sample t-tests compared uncontrolled vs controlled patients. Of 575 patients (424 adults/151 adolescents 12-17 yrs), 398 (69%) received topicals only (TOP) and 140 (24%) received topicals with a systemic (steroid, immunosuppressant or biologic) (T+S) for at least a month. On TOP, 21% of adults and 24% of adolescents were uncontrolled, rising to 26% and 50%, respectively, on T+S. This aligned with physicians being dissatisfied with control achieved for 27-31% of adults and 40-50% of adolescents, for TOP/T+S, respectively. For adults/adolescents, respectively, receiving TOP: POEM scores were 7.9/8.6 for controlled vs 11.3/12.4 uncontrolled (p=0.010/0.013); DLQI/cDLQI scores 5.5/6.2 for controlled vs 8.3/8.6 uncontrolled (p=0.004/0.054). For WPAI, % overall work impairment scores for adults receiving TOP: controlled 13.8% vs uncontrolled 22.5% (p=0.083). Similar trends between controlled and uncontrolled patients using T+S were observed. In conclusion, many patients on topical AD therapies are uncontrolled and report decreased quality of life and impaired work productivity, while physicians often report dissatisfaction related to control. There is a need for treatments to improve disease control and patient outcomes.

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The risk of Mohs surgery complications in patients with pre-operative opioid use

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Previous research has shown that opioid use may delay wound healing by disrupting myofibroblast and immune cell recruitment. As the opioid crisis continues to grow and impact the USA, little is known about the risk of complications after Mohs Micrographic Surgery (MMS) in patients with a history of opioid use. A retrospective cohort study was done using TriNetX, a federated real time database of 61 million patient records from 2006-2020 nationally. Cohorts were stratified by opioid use and no opioid use within one year preceding MMS. CPT and ICD-10 codes were determined a priori to identify MMS and complications of interest. A 1:1 matched propensity score analysis was conducted, adjusting for comorbidities and demographics, to calculate adjusted Relative Risks (aRR) with 95% confidence intervals. A matched cohort of 52,243 patients revealed that while the overall complication rates are low, opioid use is associated with a significantly higher risk for developing 15 post- operative complications in 30 day follow up. These include cellulitis/lymphangitis (aRR [95% CI])= (1.98[1.58-2.49]), any cutaneous infections (1.47[1.30-1.67]), hypertrophic scars (1.62[1.19-2.20]), hematomas (1.88[1.15-3.09]), wound dehiscence (2.29[1.77-2.94]), hemorrhage (1.66[1.23-2.25]), pruritus (2.00[1.28-3.14], muscle weakness (2.37[1.39-4.06]), anesthesia of skin (1.79[1.17-2.73]), paresthesia of skin (1.55[1.00-2.41]), gangrene (2.31[1.10- 4.84]), skin graft complications (3.00[1.74-5.20]), rash (2.55[1.77-3.69]), localized swelling (2.46 [1.83-3.29]), and pigmentation changes (1.82[1.37-2.42]). Patients with prior opioid use have a significantly higher risk of developing post- operative complications after MMS. Greater care should be taken in patients with opioid history use to mitigate negative outcomes.

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Trends in dermatological prescribing patterns during the COVID pandemic

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This investigation aimed to assess the pandemic impact on the incidence of newly prescribed dermatological agents compared to the pre-pandemic period. TrinetX is a real-time, federated healthcare database that was used in the retrospective review comprised of 61 million patient records at the time of the analysis. This database was used to identify the incidence of newly prescribed drugs each month for two periods of the pandemic. Medications were categorized by LOINC codes and categorized into groups. The first period was from April-July 2020, and the second was from August-November 2020. The mean for each drug group was then compared with the pooled monthly incidence from similar periods between 2018-2019 before the pandemic. Descriptive analyses were performed, and comparisons were made using a student's t-test. 11 groups of dermatological agents were analyzed in both periods. In the early pandemic period, 7 of 11 groups of agents showed a statistically significant reduction of up to 32.1% in prescription. These agents included anti-psoriatic, anti-acne, topical anti-neoplastic, topical anti-viral, topical anti-inflammatory, topical anti-fungal, and all dermatological agents as a whole. Newly prescribed topical analgesics, keratolytic, anti-perspirant, and anti-bacterial agents did not show a statistically significant decrease in prescription. During the later pandemic, five of the dermatological agents showed a statistically significant decrease in prescription. Anti-psoriatic, topical analgesics, topical antineoplastics, topical keratolytic, topical anti-perspirant, and topical anti-bacterial agents did not show a significant reduction. This study shows that there has been a significant reduction in incidence of prescribed dermatological agents during the COVID19 pandemic. Further research is needed to determine the future impact of this disruption in the dermatological care.

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Risks of COVID-19 infection and mortality for patients on biologics

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During the ongoing coronavirus disease 2019 (COVID-19) crisis, data on risks of immunomodulatory biologics have been limited, causing uncertainty for patients and providers whether to continue biologic therapy for chronic skin disease. We aimed to investigate if patients treated with biologics were at an increased risk for COVID-19 infection and all-cause mortality once infected. We performed a retrospective study of 7,361 patients prescribed biologics and 74,910 matched controls, cross-referenced with the Massachusetts Department of Public Health COVID-19 infection and all-cause mortality data through June 19, 2020. We included patients in the Mass General Brigham system with at least 1 prescription for a biologic between July 1, 2019 and February 29, 2020. Multivariable logistic regression was used on matched data to calculate the odds ratio (OR) for COVID-19 infection between patients on biologics and controls, adjusting for age, gender, race, Charlson Comorbidity Index (CCI) severity grade, median income, and local infection rate. Multivariate Poisson regression was performed on COVID-19 positive patients to compare all-cause mortality, adjusting for gender, CCI severity, income, and local COVID-19 rate. 7,361 patients treated with biologics and 74,910 matched controls were included in the analysis (mean age, 50.6 years; 56.0% women, 84.5% white; mean age adjusted CCI 2.8). There were 87 (1.2%) infections and 7 deaths (8.0%) in patients treated with biologics and 1063 (1.4%) infections and 71 deaths (6.7%) in the control group. Patients treated with immunosuppressive biologics were not at increased risk of COVID-19 diagnosis (OR 0.88, 95% CI 0.71-1.09, p=0.25) or subsequent mortality (OR 1.38, 95% CI 0.62-3.07, p=0.43). Given an absence of evidence that patients treated with biologics are more susceptible to COVID-19, patients should be encouraged to continue their therapy to prevent disease progression during this pandemic.

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Estimating patient demand for Mohs surgery in the United States using Google search trends

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Mohs surgeons (MS) who undergo an American College of Mohs Surgery (ACMS) accredited fellowship have intensive training needed to handle the complexities of skin cancer care. As the incidence of skin cancer rises yearly in the USA, these patients may use search engines to seek potential physicians that can help with their needs. However, this public demand for well-trained MS is currently not known. Google Trends was queried from 2004-2019 to find the average relative search volume (RSV) for the topic "Mohs Surgery" for each state. The number of unique ACMS trained Mohs Surgeons by primary mailing address was acquired and then divided by the 2019 Census Bureau population estimates to find the concentration of MS per capita values. The RSV values were then divided by the per capita values to estimate the demand index of MS for each state. The demand index was highest in Delaware (7887), Mississippi (2797), and West Virginia (2329) and lowest in Washington (914), South Dakota (897), and DC (511). The greatest MS concentration per 10,000 people was in DC (0.11), Vermont (0.08), and Colorado (0.079) and lowest in Indiana (0.03), Mississippi (0.02), and Delaware (0.01). The highest search volumes were in New Hampshire (100), Rhode Island (96), and Florida (95) and the lowest volumes were in Kansas (50), Mississippi (47), and Alaska (42). Alaska was the only state without any registered ACMS Mohs surgeon. The findings highlight which markets may be saturated and which may have a significant unmet need for ACMS trained Mohs surgeons.

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Clinical outcomes in COVID-19 patients with dermatopolymyositis

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Dermatopolymyositis (DPM) is chronic inflammatory disorder that not only affects the skin and muscles but is also associated with malignancies and other disorders. There is currently scant literature on the outcomes of COVID19 patients with DPM and aim was to examine investigate the impact of AD on COVID complications. A retrospective cohort study was done using TriNetX, a national federated real time database of 63 million records. COVID patient cohorts were identified by validated ICD-10 and serology codes per CDC guidelines. An 1:1 matched propensity score analysis was conducted, adjusting for comorbidities and demographics, to calculate adjusted Risk Ratios (aRR) with 95% confidence intervals (CI). 45-day COVID complications were examined with severe COVID being defined as a composite of mortality and ventilation. Subgroup analyses were also performed for Dermatopolymyositis patients on systemic immunosuppressants. In a matched sample of 177 patients in each cohort, there was no statistically significant difference between DPM-COVID patients and non-DPM COVID patients in any of the outcomes examined such as hospitalization (0.97 [0.63-1.49]), acute respiratory distress syndrome (1.01[0.42-2.34]), sepsis (1.1[0.48-2.52]), mechanical ventilation (1.01[0.43-2.34]), mortality (1.2[0.53-2.71]), and severe COVID (1.5 [0.69-3.25]). Subgroup analysis also revealed that DPM-COVID patients with a one-year history of immunosuppressant use had no significant difference in any of the listed outcomes compared to DPM-COVID patients without history of immunosuppressants. DPM patients who contract COVID are not at higher risk for more severe COVID outcomes compared to COVID patients without DPM. Systemic immunosuppressants in DPM-COVID patients also did not lead higher risk for COVID complications compared to DPM-COVID patients without a history of systemic immunosuppressants. Continuing research on the long term impacts of COVID on DPM patients is needed.

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Impact and associations of atopic dermatitis out-of-pocket healthcare expenses in the United States

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Atopic dermatitis (AD) is associated with substantial financial cost including increased out-of-pocket (OOP) expenses. However, associations and impact of OOP cost on household finances are not well understood. To characterize the impact and associations of elevated OOP healthcare expenses for AD management, a 25-question voluntary online survey was administered to National Eczema Association members (n=113,502). Inclusion criteria (U.S. residents age ≥18 years who either self-reportedly had AD or were primary caregivers of individuals with AD) was met by 77.3% (1,118/1,447) of respondents. Respondents with monthly OOP expenses for co-pays and/or deductibles for AD-related HCP office visits >$200 were more likely to have increased AD severity, poor disease control, increased flares, number of healthcare provider (HCP) office visits, prescription polypharmacy, use of step-up therapy, comorbid food allergy, and frequent skin infections (P<0.005 for all). Respondents with total OOP yearly expenditures >$1,000 had similar associations and additionally increased rates of comorbid asthma, allergic rhinitis, anxiety and/or depression (P<0.005 for all). Approximately two-thirds (n=624, 64.6%) reported a moderate, significant, or devastating impact of OOP expenses on household finances. Predictors of harmful financial impact included severe AD (adjusted odds ratio [95% confidence interval]: 2.62 [1.11-6.19], P=0.04), comorbid asthma (1.42 [1.07-1.87], P=0.03), ≥5 HCP visits in the past year (2.80 [1.62-4.82], P=0.0007), >$200 OOP monthly expenditures (2.16 [1.45-3.22], 0.0006), and ≥$1,000 annual expenditures for AD (4.56 [3.31-6.27], P<0.0001). OOP healthcare expenses for AD significantly impact household finances. Clinical interventions are needed to minimize OOP expenses for AD patients while striving for optimal care outcomes.

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Using Google trends to calculate patient demand for general dermatologists in the United States

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There is a predicted projected shortage of over 400 full-time board-certified dermatologists by 2025 while demand continues to rise. Patients may use search engines to identify potential accessible dermatologists for their skincare needs but the regional public demand for well-trained dermatologists by state remains unknown. Google Trends was queried from 2004-2019 to find the average relative search volume (RSV) for the term "Dermatologist" of each state. The number of dermatologists from each state in the Medicare Physician Compare National Database was acquired and then divided by the 2019 Census Bureau population estimates to find the concentration of specialists per capita values. The RSV values were then divided by the per capita values to estimate the relative demand index of dermatologists for each state. The relative demand index was highest in Texas (100), Georgia (93), and Idaho (92.5) and lowest in New Hampshire (24), Wyoming (23), and Montana (8). The greatest specialist concentration per 10,000 people was in Montana (2.3), Wyoming (0.74), and New Hampshire (0.64) and lowest in Arkansas (0.27), Hawaii (0.26), and Texas (0.24). The highest relative search volumes (RSV) were in New Jersey (100), New York (89), and Maryland (87) and the lowest volumes were in Wisconsin (40), Oregon (39), and North Dakota (34). The findings highlight which markets may be saturated and which may have a significant unmet need for trained dermatologists.

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Determining the risk of post-operative complications in essential hypertension patients undergoing Mohs surgery

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Essential Hypertension in the post-operative period is a risk factor for increased cardiovascular events, bleeding and mortality. Hypertension may impair tissue perfusion and increase wound drainage leading to higher risks of wound contamination. Surgical operations on hypertensive patients are associated with greater risks of cardiovascular compromise, delayed wound healing, and infection. Our goal was to study whether individuals with Essential Hypertension have an increased risk of post-operative complications after undergoing Mohs surgery. A retrospective cohort study was carried out using TriNetX, a national federated real time database of over 61 million electronic medical records from 2006-2020. Patients were queried by ICD 10 and CPT to find variables of interest. A 1:1 matched propensity score analysis was conducted, adjusting for comorbidities and demographics. Adjusted Risk Ratios with 95% confidence intervals were generated. A matched cohort of 59,264 patients revealed Essential Hypertension patients were at a significantly higher risk for developing 12 post-operative complications. These include Cellulitis/Lymphangitis (Adjusted Relative Risk [95% CI])=(2.51[1.93-3.27]), Any Cutaneous Infection (1.32[1.15-1.50]), Hematoma (1.70[1.05-2.76]), Wound Dehiscence (1.51[1.17-1.95]), Hemorrhage (2.89[2.04-4.09]), Pain (4.39 [3.46-5.55), Pruritus (2.57[1.53-4.32], Muscle weakness (6.44[3.59-11.57]), Anesthesia of Skin (4.92[3.00-8.08]), Paresthesia of Skin (2.96[1.88-4.68]), Rash (3.18[2.21-4.56]), and localized swelling (3.67[2.67-5.04]). Patients with Essential Hypertension have a statistically significant increased risk of developing post-operative complications after Mohs Surgery. Management of hypertensive patients may be required due to the greater risk of delayed wound healing complicated by increased wound drainage and infection. Patients may benefit from adequete blood pressure control and increased monitoring in the post-operative period.

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Clinical outcomes in COVID-19 patients with Atopic dermatitis

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Atopic Dermatitis (AD) is a systemic inflammatory disorder that not only affects the skin but is also associated with numerous other disorders. There is currently scant literature on the outcomes of COVID19 patients with atopic dermatitis and aim was to examine investigate the impact of AD on COVID complications. A retrospective cohort study was done using TriNetX, a national federated real time database of 63 million records. COVID patient cohorts were identified by validated ICD-10 and serology codes per CDC guidelines. An 1:1 matched propensity score analysis was conducted, adjusting for comorbidities and demographics, to calculate adjusted Risk Ratios (aRR) with 95% confidence intervals (CI). 45-day COVID complications were examined with severe COVID being defined as a composite of mortality and ventilation. Subgroup analyses were also performed for AD patients on systemic immunosuppressants. In a matched sample of 2408 patients in each cohort, there was no statistically significant difference between AD-COVID patients and non-AD COVID patients in any of the outcomes examined such as hospitalization (0.89[.075-1.04]), acute respiratory distress syndrome (1.05[0.55-2.1]), sepsis (0.98[0.66-1.46]), mechanical ventilation (0.73 [0.43-1.23]), mortality (1.02[0.62-1.62]), and severe COVID (0.8[0.54-1.19]). Subgroup analysis also revealed that AD-COVID patients with a one-year history of immunosuppressant use had no significant difference in any of the listed outcomes as well compared to AD-COVID patients without history of immunosuppressants. AD patients who contract COVID are not at higher risk for more severe COVID outcomes compared to COVID patients without AD. Likewise, AD patients with a history of systemic immunosuppressants are also not at a higher risk for COVID complications compared to AD patients without a history of systemic immunosuppressants. More research may be needed to visit the longer term impacts of COVID on AD patients.

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Examining complication risk in patients undergoing Mohs surgery with a history of anticoagulant use

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The use of anticoagulants has been associated with impaired wound healing. Anticoagulants decrease the number of inflammatory cells, fibroblast contents, and growth factors at the wound site, as well as promote hemorrhage via inhibition of the clotting cascade. Delayed healing of surgical wounds can result in critical complications such as infection, persistent bleeding, wound seepage, prolonged hospitalization, and need for additional surgery. The risk of post-operative complications after Mohs Micrographic Surgery (MMS) in patients with a history of anti-coagulant use compared to those with no history of use is unknown. This study aims to elucidate this risk. A retrospective cohort study was carried out using TriNetX, a national federated real time database of over 61 million electronic medical records from 2006-2020. Patients were queried by ICD 10 and CPT to find variables of interest. A 1:1 matched propensity score analysis was conducted, adjusting for comorbidities and demographics. Adjusted Risk Ratios with 95% confidence intervals were generated. A matched cohort of 13,365 patients revealed that patients with a recent history of anticoagulants use were at a significantly higher risk for developing 9 post-operative complications. These include Cellulitis/Lymphangitis (Adjusted Relative Risk [95% CI])= (2.56[1.69-3.90]), Any Cutaneous Infection (1.89[1.48-2.41]), Hematoma (2.42[1.16-5.05]), Wound Dehiscence (2.11[1.32-3.36]), Hemorrhage (2.10[1.31-3.35]), Pain (2.81[1.88-4.20), Pruritus (2.76[1.34-5.70], Muscle weakness (2.55[1.27-5.14]), and Rash (2.63[1.38-5.00]). Patients with a history of anti-coagulant use have a statistically significant increased risk of developing post-operative complication after MMS. It is imperative that patients with a history of anti-coagulant use be identified prior to surgery and greater precautions be taken for such patients to reduce poor health outcomes.

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The infodemiology of impetigo: Examining trends and seasonality of public interest

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Impetigo is a bacterial skin infection that commonly presents in children with yellowish crust. However, the current knowledge on public interest and the seasonality of impetigo is limited. Therefore, the aim was to evaluate the infodemiology of impetigo. Google Trends was searched between January 2004- December 2019 for the term "impetigo" in the USA and worldwide to gain information on public interest. Relative search volume (RSV) data from the USA (a Northern Hemisphere country) and Australia (a Southern Hemisphere Country) was also examined to assess trends in seasonality. A cosinor analysis was used to calculate amplitude {A}, phase month {P}, low point month {L}, and trend significance. Impetigo had an overall increasing trend in search volume in USA and worldwide overall. The 5 countries with the highest RSV was Puerto Rico, United Kingdom, Ireland, Canada, the Czech Republic. Within the USA, the 5 states highest RSV was South Dakota, Louisiana, Mississippi, North Dakota, and Iowa. The cosinor analysis revealed a statistically significant seasonal variation in RSV of impetigo in the USA ({A}=5.7, {P}=8.6, {L}=2.6, $p<0.001$) and in Australia ({A}= 7.7, {P}=2.6, {L}=8.6, $p<0.001$). A pattern out of phase by 6 months was observed between the USA and Australia with peaks around late summer/early fall and troughs around late winter/early spring, confirming that the pattern is truly seasonal as opposed to being calendar-driven. Public interest in seeking information impetigo in the USA and worldwide has increased in recent years while also displaying a true seasonal pattern.

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Prevalence of psoriasis and perceived association with hormone therapy in transgender adults

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Limited evidence suggests that psoriasis risk and severity may be influenced by endogenous and exogenous sex hormones. The role of gender-affirming hormone therapy in the etiology and progression of psoriasis is unclear. We examined the prevalence of psoriasis in transmasculine (TM) and transfeminine (TF) adults and the perceived links between psoriasis and gender-affirming hormone therapy using a cross-sectional survey. The survey was nested within a multicenter validated cohort study of transgender persons enrolled in Kaiser Permanente plans in Northern California, Southern California, and Georgia. Survey eligibility included age 18+ years, chart review-confirmed transgender diagnostic codes, physician consent for survey contact, and self-reported gender identity differing from sex assigned at birth. The prevalence of psoriasis as diagnosed by a doctor and its perceived link to hormone therapy were compared between TM and TF persons using Fisher's exact tests. Among 2,136 eligible transgender persons. 696 (33%) completed the survey. and 595 (85%, including 305 TM and 290 TF) provided a response on psoriasis diagnosis. Ever diagnosis of psoriasis was reported by 17 (5.6%) TM and 13 (4.5%) TF (P=0.58). Prevalence of current psoriasis was 3.0% among TM and 1.4% among TF (P=0.26). Among 29 respondents ever diagnosed with psoriasis, 5 (17%) perceived a link between their psoriasis and gender-affirming hormone therapy. Limitations include small number of cases, self-report, and potential for recall bias. The prevalence estimates of psoriasis did not differ between TM and TF persons and were comparable to prevalence estimates in the general population. Larger longitudinal studies may be warranted to test the perceived association between gender-affirming hormone therapy and psoriasis. Psoriasis is a common skin condition in transgender populations and warrants comprehensive and culturally responsive dermatological care.

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Ensuring the content validity of a quality-of-life measure for patients with chronic itch

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Clinicians routinely ask patients, "*How are you doing?*" but patients—especially those with chronic conditions that affect many aspects of their lives—may feel unable to respond comprehensively. Accurate and complete data about a disease's impacts is critical to personalize health care, and quantitative disease-specific assessments that are reliable and valid can generate useful data for treatment and research. However, the content validity of measurement scales is too often ignored, with more emphasis placed on construct and predictive validity. Our goal was to enhance the content validity of the Skindex-16 for use as an outcomes measure for patients with chronic itch. We conducted one focus group (n=5) and 12 individual interviews with patients with chronic itch. Participants were asked to describe their itching and its impact on their quality of life. Transcripts were coded and analyzed using an iterative inductive/deductive methodology. Deductively, codes, and themes were guided by an explicit biopsychosocial framework. Qualitative analysis was used to identify major thematic categories, and quotes from the transcripts were used to generate potential measurement items. Nine thematic domains and 47 potential items were identified: 1) Self-management practices; 2) Relationship impacts; 3) Activities of daily living; 4) Work; 5) Concentration; 6) Sleep/fatigue; 7) Pain/irritation; 8) Unpredictability; and 9) All-encompassing. Items were compared to the Skindex-16 item set, and 17 items—called the Itch Module– were retained for further testing. These qualitative analyses of responses from patients with chronic itching showed that the Skindex-16 plus the Itch Module had enhanced content validity compared to the parent Skindex-16 alone.

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The impact of inflammatory dermatoses on cancer patients undergoing inpatient chemotherapy administration

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Currently, it is unclear how inflammatory skin diseases impact hospital outcomes for cancer patients receiving chemotherapy. Our objective was to assess the impact of inflammatory dermatoses on hospitalization outcomes for cancer patients undergoing inpatient chemotherapy treatment using national discharge data from the 2014 U.S. Nationwide Readmission Database. We utilized patient discharge diagnoses for chemotherapy administration (ICD 9 CM code V58.1) to identify our primary study population. The subset of patients with inflammatory dermatoses were identified by diagnoses codes for other inflammatory conditions of skin and subcutaneous tissue, urticaria, and symptoms involving skin and other integumentary tissue (ICD 9 CM 690-698, 708, and 782, respectively). In total, 20,848 patient discharges met inclusion criteria. We performed multiple stepwise linear regression to predict adjusted hospital length of stay from inflammatory dermatoses diagnosis, while controlling for other covariates of interest. After adjusting for age, sex, operating room procedures, elective procedures, number of yearly admissions, number of chronic conditions, hospital admittance, and whether the patient had a solid organ or hematologic cancer, we determined an inflammatory dermatitis diagnosis was positively associated with adjusted hospital length of stay ($P<.001$). Adjusted hospital length of stay increased 3.91 days for inflammatory dermatitis diagnosis when controlling for all other covariates ($B = 3.91$, 95% CI [3.57-4.26]). Limitations of the study include evaluating a range of ICD-9 codes, including non-specific skin disease (ICD 9 CM 782) diagnoses, in only a single year. Based on this data, Inflammatory dermatoses negatively impact hospital outcomes for cancer patients receiving inpatient chemotherapy. Further research is needed to evaluate skin morphologies and diseases most responsible for these effects.

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Geographic distribution of non-melanoma skin cancer in the Russian Federation

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Non-melanoma skin cancer (NMSC) incidence has been increasing steadily around the world. The aim of the study is to describe geographic trends in incidence and mortality of NMSC in Russia between 2007 and 2017 and compare findings to other European countries. Oncological data from the Moscow Oncology Research Institute, Ministry of Health of the Russian Federation, for the years 2007—2017 was gathered, geographic information system (GIS) was used to map incident cases, and descriptive analyses were performed. International Classification of Diseases (ICD) C44 code (comprising C44.0-C.44.9) was used to identify NMSC cases. Additionally, we assessed the relationship between ethnicity, geographic latitude/longitude, and NMSC incidence/mortality rates. 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). In total, 733,723 patients were diagnosed with NMSC in Russia over the period 2007—2017, of whom 63% were women. The overall age-standardized incidence and mortality rates were 29.64/100,000 and 0.70/100,000, respectively. There was a consistent increase in age-standardized incidence rates over the study period, with a decreasing mortality rate. Geographic mapping revealed a north-to-south gradient corresponding to increasing UV exposure and east-to-west gradients due to darker skin phenotype and colder climates in the east. This study demonstrated the burden of NMSC in Russia as well as the longitudinal trends for NMSC incidence. Skin phenotype, latitude/longitude, climate zones, and cultural practices remain dominant risk factors defining the epidemiology of NMSC. Moreover, this work identified several regions in the country (i.e., Republic of Adygea, Samara, Krasnodar Krai, etc.), where patient education/sun awareness campaigns will be useful to help reduce the risk of this malignancy.

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Physicians' attitudes towards active surveillance for basal cell carcinoma

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Basal cell carcinomas (BCCs) are typically slow growing, and 30-50% remain stable or shrink in size over time. Most BCCs are treated regardless of life-expectancy and over 100,000 BCCs per year are treated in patients' final year of life. Active surveillance has been proposed as a method for managing patients with limited life-expectancy. Limited data is available on physician comfort and practice in regard to active surveillance of BCCs. The objectives of this study were to determine physicians' comfort level with active surveillance of BCC, and understand which factors and concerns influence their decisions. We conducted a cross-sectional survey study of physician members of the Association of Professors of Dermatology in August/September 2019 to evaluate physicians' attitudes regarding active surveillance of BCC. The main outcomes were percent of physicians comfortable with active surveillance of BCC, factors influencing their decision to monitor BCC, and feared complications. Seventy out of 528 members (13%) responded to the survey. Eighty-three percent of respondents felt comfortable monitoring nodular and/or superficial BCCs. Factors such as medical comorbidities (90%), functional status (84%), age (82%), anatomic location (77%), size (71%), and histologic subtype (66%) determined the level of comfort with monitoring BCC. Over 70% of physicians would feel comfortable monitoring BCC in patients with level 4 functional ECOG status and age older than 85. The top feared complications were larger surgical site defects (84%), bleeding (83%), ulceration (80%), local destruction to adjacent vital organs (64%), and pain (51%). Metastasis (6%) and death (6%) were uncommon concerns. There were no significant differences in responses between general dermatologists and Mohs micrographic surgeons. Majority of physicians were comfortable with active surveillance of BCCs in elderly adults with low functional status, taking into consideration, size, anatomic location and histologic subtype.

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Characterizing silicone granulomas: A multicenter cohort of 21 patients

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Limited data exist on patients with granulomatous reactions to soft tissue silicone injections and ruptured implants, termed silicone granulomas (SG). This study aims to characterize the clinical features and treatment outcomes for patients with SG from two large academic medical centers. A multicenter database was queried using SG-related ICD-9/10 codes and the search term 'silicone.' 21 patients with SG were identified. Data were collected on demographics, symptoms, type and site of silicone administration and treatment outcomes. Statistical analysis was done using Fisher's exact. Median age was 61 years. 80% were cisgender women, 10% cisgender men and 10% transgender women. 48% were White, 43% Latinx, 5% Asian and 5% Middle Eastern. Symptoms were pain (57%), nodule formation (38%), edema (29%), induration (29%), hyperpigmentation (14%) and pruritus (14%). Median time from silicone administration to symptom onset was 7 years. 14 patients had SG from silicone injected to sites including the buttocks (50%), lower extremities (29%), breasts (21%) and face (14%). 7 patients had SG from ruptured silicone breast implants. 43% had silicone migration; migration to distant body parts was limited to 2 injection patients including 1 with life-threatening lung involvement. Treatments included prednisone, doxycycline, minocycline, hydroxychloroquine, methotrexate, mycophenolate mofetil, adalimumab and surgery. 38% had complete treatment response, 52% partial response, 5% no response, and 5% await follow-up. This study reports the largest SG cohort to date. Pain was the most common symptom. SG developed almost a decade after silicone administration, reflecting the delayed nature of this complication. Although migration occured in almost half of patients, distant, life-threatening migration was limited to one injection patient. While surgical excision provided benefit in the majority of patients with ruptured implants, surgery did not result in improvement following silicone injection.

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Most influential authors in dermatology: Standardized citation indicators from updated databases

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Peer-reviewed literature informs evidence-based dermatology clinical decision making. Ioannidis and colleagues created a comprehensive database utilizing standardized citation indicator data from Scopus. Authors were systematically assessed for career-long citation impact and impact during 2019. A composite score integrating six citation metrics, notably considering authorship position, was calculated and reported with common metrics, such as author h-index and number of citations. The top 25, "Dermatology & Venereal Diseases" authors in each dataset were identified according to three metrics, excluding self-citations: 1) total number of citations from 1996-2019 ("career-long") or 2019 alone; 2) h-index as of 2019; and 3) composite score. Last-known institutional affiliation and country were provided by the database; author gender was compiled via Google. Career-long metrics showed a predominance of top authors from the United States: 12/25 (48%) by total citations, 13/25 (52%) by h-index, and 17/25 (68%) by composite score. The University of California, San Francisco was the most common institutional affiliation ($\geq$3 top authors per metric). Women were consistently underrepresented (average $\sim$10%). Single-year data from 2019 revealed marked increases in international and institutional representation. Gender-proportional representation improved to 20% (5/25) female top authors in 2019. Inclusion of self-citations displayed similar trends (increasing diversity in gender, institution, and country). Given that citations accumulate over time, inclusion of citations outside 1996-2019 is warranted, along with resource inclusion beyond Scopus (e.g. Google Scholar). Subsequent database releases will reveal future trends, with women and non-American authors potentially gaining prominence.

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Dermatology research with the Observational Health Data Sciences and Informatics (OHDSI) network

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The Observational Health Data Sciences and Informatics (OHDSI) network enables access to billions of de-identified, standardized health records and built-in analytics software for observational health research. We review dermatology uses of OHDSI. The OHDSI collaborative is an international volunteer network of researchers, emerging as a successor of the Observational Medical Outcomes Partnership, a public-private partnership between the FDA, pharmaceutical entities, and healthcare providers. Instrumental to OHDSI is the Common Data Model, which establishes transformation conventions into a single standardized data format, supporting large scale analytics across heterogeneous data partners. Similarly, a standard vocabulary exists, enabling interoperability between systems, facilitating homogeneity and data transparency, and supporting high-quality research. OHDSI studies may be conducted by writing custom code or using built-in software. OHDSI has dramatically enhanced the ease and speed of observational studies. Its scale lends increased power and reproducibility and characterizes the generalizability of clinical trials to real-world populations; it improves accuracy of estimations and predictions, facilitating the study of rare exposures, diseases, and outcomes. Various applications of OHDSI are represented in the literature, particularly in adverse event reporting, heritability estimation, adherence to treatment guidelines, and characterization of prescribing patterns. Together, these illustrate the potential of OHDSI in dermatology: its adoption would facilitate examination of treatment patterns that lack best practice guidelines, improve the dermatologic knowledge base, and ultimately, patient outcomes. Bibliometric analysis revealed increasing numbers of dermatology-related OHDSI papers in PubMed — from 2 papers in 2014 to 25 papers in 2020, with topics including prediction modeling, pharmacovigilance, and prognostic studies.

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Geographic variations in cutaneous melanoma in the Russian Federation

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Cutaneous melanoma (CM) incidence has been increasing around the world. The goal of this study is to describe geographic trends in incidence and mortality of CM in Russia between 2001 and 2017. Oncological data from the Moscow Oncology Research Institute was gathered for the years 2001-2017, geographic information system (GIS) was used to map incident cases, and descriptive analyses were performed. International Classification of Diseases (ICD) C43 code (comprising C43.0-C.43.9) was used to identify CM cases. Associations between ethnicity, geographic latitude/longitude, and CM incidence/mortality rates were studied. 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 whom 62% were women. The overall age-standardized incidence and mortality rates were 4.27/100,000 and 1.62/100,000, respectively. A consistent annual increase in both age-standardized incidence and mortality was observed for CM. Geographic mapping revealed north-to-south gradient corresponding with increasing UV exposure and east-to-west gradients due to darker skin phenotype in the east and generally colder climates. As the study was fully descriptive, retrospective, and based on official statistical reports, detailed characteristics of clinical forms, anatomic sites, Breslow depth, and treatments could not be analyzed. This study outlined the burden of melanoma in the Russian Federation, and the trends were similar to those observed in countries with similar latitudes and skin phenotype. The importance of the skin color gradient and recreational/cultural practices were some of the most important risk factors highlighted in this study for the development of melanoma in Russia.

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The problematic use of change scores in dermatology clinical trials

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Change scores are common, but important underlying assumptions, such as stable disease at baseline and outcomes changing linearly with an approximate slope of one, are necessary for use and interpretation. Likewise, transformations like dichotomized endpoints and percentage change from baseline present unique problems. To assess change score use, we queried all 2015-2019 clinical trials from JAMA Dermatology, BJD, JID, JAAD, and JEADV, summarized change score use, and evaluated underlying assumptions. Seventy-four trials used pre-post baseline scores, 25 used percentage change from baseline, 93 used dichotomized cut points, and 17 used baseline-adjusted scores. Only one study discussed outcome linearity (0.40%). Eighty-two trials used outcomes for patient selection (32.3%). Seven (8.54%) used a post-selection score for baseline and 16 (19.5%) had a run-in time prior to baseline scoring. Twenty-two studies (15.4%) plotted mean outcome values over time and an additional 39 studies (32.2%) plotted various change scores. Forty-four (38.9%) scores appeared linear, but only 5 (4.40%) had a slope of approximately 1. The FDA often mandates change score outcomes for medication approval. Accordingly, industry-funded trials were more likely to use change scores (OR from logistic regression=2.90, 95% CI 1.75-4.82), especially dichotomized (OR=3.32, 1.89-5.83). Use of change scores is common. Model assumptions are infrequently met or discussed. Many robust approaches to pre-post outcomes are available. Dichotomization is particularly troublesome and leads to loss of data and bias. We recommend the elimination of change score usage when possible, as more robust and easily interpretable models exit. Also, we recommend discussion of FDA endpoint requirements with policymakers.

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Significant disparities in prognosis and survival in Black cutaneous lymphoma patients emphasize the need for more focused study and care

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Cutaneous lymphomas (CLs) are a rare type of non-Hodgkin lymphoma that consist of a diverse group of B- and T-cell subtypes; the most common of which are mycosis fungoides (MF) and Sézary syndrome (SS). While some CL subtypes are indolent, others may be aggressive and associated with decreased survival. Previous studies have shown worse outcomes and poorer survival for Black patients with MF/SS; however, this data is sparse, and racial/ethnic disparities in prognosis across CL subtypes have not been well elucidated. We present a single-center study of 357 patients examining racial/ethnic variance in prognostic features and survival among all subtypes of CL. Our population was comprised of 10.4% Asian, 8.1% Black, 20.4% Hispanic, 59.7% white, and 1.4% of unknown race/ethnicity; 46.2% female and 53.7% male; and 16 distinct subtypes of CL. We found that Black CL patients had worse overall survival ($p<0.0001$) when compared to all other racial/ethnic groups. We affirmed that Black MF/SS patients had worse outcomes and demonstrated that this held true regardless of stage ($p<0.0001$). Additionally, we showed that, in the MF/SS population, Black patients had a higher rate of development of folliculotropism and/or large cell transformation, which are aggressive features that may portend a poor prognosis. Racial/ethnic disparities in CL have a tangible impact on the lives of Black patients with increased morbidity and mortality. Further studies are requisite to investigate the mechanisms, whether intrinsic and/or extrinsic, behind these inequities as to better guide treatment and ancillary care for the improvement of outcomes for the Black CL population.

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Polypoid melanoma is associated with aggressive histopathological characteristics and poor clinical prognosis

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Polypoid melanoma is a rare subtype of melanoma characterized by pedunculated exophytic growth. These tumors tend to have a thick Breslow depth, but it is unknown if the prognosis of this subtype is worse compared to other variants of melanoma. A retrospective review was performed of 37 polypoid melanomas and compared to 264 non-polypoid nodular melanomas. Each case was independently re-evaluated by board-certified dermatopathologists for the following histopathologic parameters including Breslow depth, mitotic rate, ulceration, and angiolymphatic invasion. Basic demographic data and clinical characteristics were collected from electronic medical record data and compared, including clinical stage at diagnosis, rates of recurrence, and survival, between polypoid and nodular melanoma subtypes. Patients with polypoid melanoma had a younger average age than patients with nodular melanoma. Histopathologic review revealed that polypoid tumors had a significantly higher average Breslow depth, and had a higher frequency of ulceration and angiolymphatic invasion than nodular melanomas. Analysis of clinical outcomes by log-rank test showed a higher risk of distant recurrence and worse overall survival in polypoid tumors compared to nodular melanomas. Multivariate analysis showed an association of polypoid subtype with higher distant recurrence and worse survival. This association was independent of other prognostic factors including Breslow depth and ulceration. This study shows that polypoid melanoma is associated with a higher frequency of aggressive histopathological characteristics and poor clinical prognosis compared to non-polypoid nodular melanoma.

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Qualitative study of pain experiences among patients with hidradenitis suppurativa

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Hidradenitis suppurativa (HS) is an inflammatory skin disease with recurrent painful, malodorous abscesses at intertriginous sites. Pain, which is the most burdensome symptom of HS, is more highly correlated with reduction in quality of life (QoL) than is disease severity. Evidence guiding HS pain management is lacking, and individuals living with HS are at increased risk of chronic opioid use. This study employed a grounded theory approach to elucidate pain experiences as well as attitudes regarding opioid use among patients with HS. We gathered quantitative data from patient reported outcomes and disease characteristics and qualitative data from semi-structured interviews. Interviews were conducted with English-speaking patients $\geq$18 years of age with confirmed HS diagnosis and average Numeric Rating Scale (NRS) pain score of $\geq$1 over the preceding week. Data collection continued until thematic saturation was reached, requiring a total of 21 interviews. Mean age was 36.9 years (SD: 12.6); 76% of participants were female and 71% were African American. Almost all (96%) participants had Hurley Stage II or III disease. Mean NRS score for pain over the preceding week was 5.24 (SD: 3.2), and 62% of patients had Dermatology Life Quality Index (DLQI) scores $\geq$11, indicating a very to extremely large impact of HS on QoL. Thematic qualitative data analysis yielded four preliminary domains: pain character, pain impact, pain management, and exacerbating/alleviating factors. Participants described their pain using terms associated with both nociceptive and neuropathic pain character. Within the pain impact theme, participants reported negative impact on constructs such as mental health, relationships, and activities. Characterizing pain experiences in HS is a critical next step to informing the development of interventions that will improve QoL, reduce opioid use, and strengthen the patient-physician relationship.

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The association of patient income levels and prescribing patterns of psoriasis therapies in the medical expenditure panel survey

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Biologic therapy is associated with better outcomes for moderate to severe psoriasis compared to systemic or topical therapies. Do prescription patterns in a national expenditure database identify gaps in financial assistance and equity in optimal care delivery? To determine the degree to which adults below the federal poverty level (FPL) are prescribed biologics, we performed a retrospective cross-sectional study using pooled data from the Medical Expenditure Panel Survey (MEPS). A prescription was defined by a biologic Multum Lexicon code linked to a psoriasis ICD code. Demographics and biologic prescriptions were compared between psoriasis patients below versus at or above the FPL using weighted-subject designs. We identified 1,437,792 yearly-weighted patients with active psoriasis (1,356 total unweighted) between 2007 and 2018, of whom 123,392 were below the FPL (166 total unweighted). Psoriasis patients below the FPL were less likely to be prescribed biologics for psoriasis than psoriasis patients at or above the FPL (5% vs 12%, p=0.01). In addition, they were more likely to be black (p=0.02), be formerly or never married (p<0.001), have public or no insurance (p<0.001), have no degree (p=0.02), and self-report poor/fair physical or mental health (p<0.001). Finally, psoriasis patients below the FPL taking biologics had lower median biologic medication expenditures per person than psoriasis patients at or above the FPL taking biologics ($6,511 vs. $12,152, p=0.003). Biologics are not prescribed equally among all socioeconomic cohorts, notably across racial and socioeconomic strata such as black patients and patients with no degree. Further studies are needed to identify the financial assistance programs that best address the persistent hurdles to equity in the delivery of holistic care in the United States.

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Boundary-aware convolutional neural network for skin lesion segmentation in clinical images

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Accurate segmentation of skin lesions is a key step in automatic skin lesion analysis. In recent years, the methods based on convolutional neural networks (CNNs) have achieved great success in automatic skin lesion segmentation, but they still face some problems, such as poor generalization performance, blur boundary and so on. In addition, most of these methods mainly focus on dermoscopy images, and lack of the attention to clinical images. In this paper, we propose a novel boundary-aware convolutional neural network (BACNN) for skin lesion segmentation in clinical images. Compared with the existing skin lesion segmentation methods that only have the segmentation branch, we create an additional boundary prediction branch and use the boundary prediction map to assist the segmentation task to obtain more refined segmentation results. To validate the effectiveness of our method, we have constructed a skin lesion segmentation dataset containing 1768 clinical images of six skin diseases (including basal cell carcinoma, melanoma, nevus, seborrheic keratosis, squamous cell carcinoma and Bowen's disease). These clinical images are collected from Xiangya Hospital of Central South University, and the segmentation truth of each image is labeled and verified by three expert dermatologists. On this dataset, our method can achieve the Dice coefficient and Jaccard index of 92.68% and 86.88%, respectively, which is higher than the existing skin lesion segmentation methods. We hope that our method can improve the overall performance of the existing clinical image analysis system and be verified in a wider range of clinical practice.

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Factors in topical steroid selection: A qualitative study

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Rising topical steroid (TS) costs place increasing financial burdens on patients. In this study, we evaluate factors dermatologists consider when choosing a prescription TS. We conducted a qualitative study using semi-structured interviews (6/2020-11/2020) of board-certified dermatologists until thematic saturation was reached. Each interview was independently coded by 2 researchers. Code frequency and interrater reliability (IRR) were determined using NVIVO software. 16 dermatologists were interviewed and divided evenly among each practice setting (academic, non-academic, and private practice). IRR ranged from κ=0.86 to κ=0.98, indicating excellent agreement. The most important physician factor when choosing a steroid was patient access to medication (63%). Most (81%) thought about cost regularly with 62% noting concern of patients being unable to afford prescriptions. All physicians had patients unable to pick up their prescription due to cost. Physicians reported not knowing medication costs due to variability of insurance coverage (94%), fluctuating drug prices (75%) and lack of transparency (75%), with 75% of physicians learning about drug costs from patients during follow up. 87.5% of physicians were willing to use a system in which TS are automatically substituted for a cheaper alternative of the same vehicle and class. Physicians reported removing barriers to knowledge of drug costs may result in greater ability to prescribe affordable drugs (75%), less patient costs (69%) and improved patient care (43%). Drug costs are a major barrier to access for patients, a problem exacerbated lack of cost transparency before placing a prescription. In turn, physicians rely on patients for feedback on prescription costs. Scarce access to drug cost information may prevent patients from receiving appropriate and timely treatment. Creative solutions are needed to improve price transparency and assist physicians in prescribing affordable medications.

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Prevalence of non-validated disease scores in dermatology clinical trials

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The nature of dermatologic disease sometimes necessitates non-interval or measured outcomes. To address the complex, and often multifactorial, impact of disease presentation and patient impact, clinical scores are often used. These can pose difficulties as varying outcomes from clinical trials make later comparisons difficult and can hinder systemic reviews that form the backbone of clinical guidelines. Thus, ideally, a well-validated and generally-used outcome is superior to a trial-specific or institution-specific score. To query the prevalence of non-validated score use, we identified all clinical trials published in BJD, JAMA Dermatology, JAAD, JEADV, and JID from 2015-2019. Results are descriptive. Of 265 total trials, 154 used scores as their primary outcome (58.1%). Most were validated (n=111, 72.1%), but 18.9% were non-validated (n=29) and 7 used both (4.55%). Logistic regression demonstrated no statistically significant change in non-validated score use over time, nor with funding source or use of a biostatistician. The majority of the non-validated scores were used in rare diseases such as Sturge-Weber syndrome, but for 13/29 trials (44.8%) (psoriasis, actinic keratosis, scarring, urticaria, and vitiligo), existing validated scores had been previously published. These demonstrate an area for improvement. Where possible investigators should use validated score outcomes or include validated scores for comparison if testing a non-validated score. Reviewers and editors should question non-validated scores, especially for conditions where well-validated, commonly-used, scores are available.

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Sociodemographic factors associated with scabies in the inpatient setting

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Background: Scabies is a highly contagious skin infestation most often spread by skin-to-skin contact. Information regarding the hospital burden of scabies is limited. Our aim was to investigate sociodemographic factors associated with presentation of scabies in the inpatient setting. Methods: We performed a retrospective analysis of the National Inpatient Sample from 2012 to 2016. Our primary outcome was either a primary or secondary diagnosis of scabies at any point during the inpatient admission (*ICD-9-CM* 133.0 and *ICD-10-CM* B86). Exposures were age, sex, race/ethnicity, insurance type, housing status, and median household income by home ZIP code. Generalized estimating equations were used to account for endemic differences in the prevalence of scabies, and sample-weighted multivariate logistic regression was used to analyze the population-averaged probability of a scabies diagnosis present by the end of the hospital stay. Results: Among a total of 32,931,148 inpatient admissions, 9660 patients were diagnosed with scabies (prevalence = 29.3/100,000 inpatients). Homelessness (aOR 12.44, 95%CI 11.45-13.52) and age >19 years (aOR 1.64, 95%CI 1.51-1.78) were the sociodemographic factors most strongly associated with diagnosis of scabies. Compared to White inpatients, Native American (aOR 1.49, 95%CI 1.21-1.84) inpatients were more likely, and Black inpatients were less likely (aOR 0.65, 95%CI 0.61-0.69), to have a diagnosis of scabies by the end of their hospital admission. Compared to patients with Medicare, those with Medicaid (aOR 1.36, 95%CI 1.28-1.44), self-pay hospital stays (aOR 1.20, 95%CI 1.10-1.31), and no charge hospital stays (aOR 1.39, 95%CI 1.10-1.75) were more likely to be diagnosed with scabies. Conclusions: We identified several sociodemographic factors associated with an increased likelihood of scabies diagnosis in the inpatient setting. These findings may assist in developing targeted interventions toward decreasing the incidence and burden of scabies in United States hospital systems.

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The impact of atopic dermatitis on caregivers of patients of all ages

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Atopic dermatitis (AD) carries a substantial burden. Prior research has focused on patients and caregivers of children separately; therefore, we sought to examine the relative impact on patients and caregivers of patients with AD of all ages. Data are from 1,508 respondents to the 'More than Skin Deep' survey fielded online as part of the US FDA's patient-focused drug development initiative. Among 399 caregiver respondents, 43% cared for young children ages 0-5, 47% cared for children ages 6-17, and 10% cared for adults. The primary outcome, a 5-category measure of overall impact, was similar among patients and caregivers (51% vs 53% of respondents reported high or significant impact, p=0.436), and the impact was greater among caregivers of adults as compared to caregivers of children (73% vs 51% of respondents reported high or significant impact, p=0.008). AD severity, mood symptoms, symptom control, topical and adjunctive treatment usage, and time spent managing AD were independently predictive of overall impact in a multivariate ordinal regression model. Among domain-specific impact scores, sleep, family responsibilities, family dynamics, and life decisions were most impacted for caregivers of all ages; diet was more impacted for caregivers of young children ages 0-5, and leisure activities were more impacted for caregivers of older children and adults. In conclusion, AD is impactful for both patients and caregivers, including caregivers of adults, across multiple life domains.

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Cardiovascular risk in atopic eczema is only associated with active disease in adulthood

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Multiple studies suggest an increased risk of cardiovascular disease among patients with atopic eczema (AE) as compared to healthy controls, but there are limited data on which AE patients might be at highest risk. The objective of this study was to examine whether subtypes of AE based on patterns of disease activity from birth are associated with cardiovascular risk in mid-adulthood. We used data from the 1958 National Child Development Study and 1970 British Cohort Study, which are longitudinal cohort studies nationally representative of the United Kingdom (UK) population that follow over 17,000 individuals from birth. We assessed cardiovascular risk at age 46-50 using the QRISK3 score, which is a well-established cardiovascular disease risk score similar to the Framingham risk score developed for use in the UK. We compared cardiovascular risk (both a continuous score and a binary outcome of >5% risk) among 3 previously identified AE subtypes based on the course of self-reported symptoms at 5-8 time points since birth using regression models adjusted for sex, ethnicity, social class in childhood, and cohort. We found that individuals with AE were at increased cardiovascular risk compared to individuals with no AE, and the risk varied by AE subtype. There was no difference in cardiovascular risk relative to individuals with no AE for those in the 'decreasing' probability of AE over time subtype (OR 0.97, 95% CI 0.80-1.19), but the 'high' probability of AE over time (OR 1.62, 95% CI 1.27-2.06) and 'increasing' probability of AE over time (OR 1.94, 95%CI 1.66-2.26) subtypes were associated with an increased likelihood of ≥5% risk of heart attack or stroke over the next 10 years. In conclusion, these data suggest cardiovascular risk is highest among those with increasingly active AE in adulthood, a newly identified AE subtype that warrants more attention in future research.

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Adult atopic dermatitis is associated with lymphopenia in two large cohorts

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Laboratory abnormalities in atopic dermatitis (AD) are poorly understood and lymphopenia could occur due to skin homing of lymphocytes. We aimed to determine the extent to which adult AD is associated with lymphopenia in two large population-based cohorts from the UK and US: CPRD and the 2005-06 NHANES. Using logistic regression models adjusted for age, sex, ethnicity, socioeconomic status, we found an increased risk of lymphopenia in those with AD in both cohorts: OR 1.15, 95%CI 1.09-1.23 in CPRD (168,256 people with AD), and OR 2.77, 95% CI 1.04-7.39 among NHANES participants attending a Mobile examination center (4,607 people with AD in the previous year). In CPRD, we found that the magnitude of association increased with increasing AD severity (OR 1.89, 95%CI: 1.54-2.32). The association was attenuated after adjusting for immunosuppressive drug use (including systemic corticosteroids) in both cohorts, but persisted in the UK data (OR severe AD without immunosuppressive drugs: 1.45, 95%CI: 1.04-2.02). In both cohorts there was no association between AD and total white blood cell count which was used as a negative control. In conclusion, people with AD may be at risk of lymphopenia, which may not be fully explained by immunosuppressive drug use. These findings are important because lymphopenia may be a marker for immunosenescence and/or increased risk of infection and has been associated with an increased risk of mortality.

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Adverse reproductive outcomes among women with hidradenitis suppurativa

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Hidradenitis suppurativa (HS) is a chronic, stigmatizing disease characterized by recurrent, painful abscesses involving intertriginous skin that disproportionally affects African-Americans and women of child-bearing age. While a substantial and debilitating burden of comorbidities among people with HS has been established, there have been few studies that evaluate the effect of HS on female reproductive health and pregnancy. The aim of the present study was to investigate HS comorbidities in the electronic health records of participants in the eMERGE biorepository (project NT227). We used billing diagnosis codes to create an HS cohort and tested for association of phecodes with multivariate logistic regression, controlling for gender, ancestry and enrollment site. We identified 668 cases, of which 38.5% comprised African-American patients and 27,004 controls. Our results confirm previous studies that establish metabolic, cardiac, endocrine, gastrointestinal, rheumatologic and psychiatric comorbidities. Interestingly, we also discovered that HS cases were at a greater risk of endometriosis (OR=2.7; $P<.0001$), irregular menstrual cycles (OR=4.7; $P<.0001$), female infertility associated with anovulation (OR=3.5; $P<.0001$), miscarriage (OR, 2.5; $P < .0001$), ectopic pregnancy (OR=2.5; $P<.0001$), threatened premature labor (OR=2.2; $P<.0001$) and early onset of delivery (OR=2.8; $P < .0001$). These results expose the need for a multidisciplinary approach to the treatment of young women and patients of color with HS, pinpointing that the relationship between HS and adverse reproductive outcomes could represent hitherto unexplored comorbidities.

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Entropion repair outcomes in ocular cicatricial pemphigoid versus other cicatricial etiologies: A retrospective study

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Mucous membrane pemphigoid with ocular involvement or ocular cicatricial pemphigoid (OCP) can lead to severe eyelid disease, including entropion. Surgical repair outcomes of OCP-related entropion compared to other cicatricial entropion has yet to be elucidated. This study aims to understand the association between entropion recurrence and diagnosis of OCP. Entropion repair of OCP patients were compared to patients with entropion of other cicatrizing etiology from January 2010 to June 2020. A generalized estimating equation with binomial likelihood and logit link was used to analyze the association between a diagnosis of OCP and failure of the first entropion repair, determined by recurrence or repair noted. Patient ID was used as the clustering variable with exchangeable correlation structure.19 OCP patients and 60 patients with entropion secondary to other etiologies received 38 and 75 entropion repairs, respectively. The unadjusted odds of failure did not significantly differ between groups (odds ratio (OR) 2.66, 95% confidence interval (CI) 0.92-7.69). No significant effect of OCP diagnosis was found (OR: 2.43, 95% CI: 0.75-7.87) when adjusting for age at repair, sex, repair approach, ophthalmologic steroids, and non-steroidal ophthalmologic immunosuppressants (systemic steroids and non-steroidal immunosuppressive agents were highly correlated with OCP diagnosis and removed from the model). Age at first repair was positively associated with failure (p=0.026). Ophthalmic steroids use decreased the odds offailure (OR: 0.27 95% CI: 0.07-0.96). This study provides evidence that in a population of OCP patients where the majority were systemically immunosuppressed, the likelihood of entropion failure was no different than that for repair of entropion secondary to other cicatrizing etiologies.

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WITHDRAWN

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Large scale epidemiological analysis of common inflammatory skin diseases to identify shared and unique comorbidities and demographical factors

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Inflammatory skin diseases are amongst the most common but yet inherently heterogeneous group of diseases. In this study, we utilized a claim-based EHR dataset, Optum, of >3 million patients who had at least one common skin disease (atopic dermatitis, psoriasis, alopecia areata, vitiligo, and acne) or skin-related disorder (psoriatic arthritis [PsA] and systemic lupus [SLE]) to carry out an epidemiological analysis to identify shared and unique comorbidities of the different skin conditions and evaluate the trend and the strength of the associations. We modeled disease associated variables using 41 comorbidities (type 2 diabetes [T2D], cardiovascular diseases, inflammatory bowel disease, etc.), and demographic and socioeconomic status. We identified on average 5 consistent comorbidities across all five skin diseases, and 6 and 19 for PsA and SLE, respectively. Ankylosing spondylitis, Crohn's disease, and hypothyroidism were the three most common comorbidities for skin diseases. SLE had prominent association with cardiovascular diseases (ORs=1.34 to 3.26), while PsA and psoriasis had the strongest associations with T2D. The strongest comorbidity association was between SLE and scleroderma (OR=9.59; $p<10^{-16}$) among skin-related disorders, and between vitiligo and melanoma (OR= 2.14; $p<10^{-16}$) among skin diseases. Furthermore, socioeconomic status was found to have disease specific effect, and demographic information was a significant factor across multiple skin conditions. Thus, income levels were positively associated with atopic dermatitis, alopecia areata and acne (ORs up to 1.16 -1.43), while there was no association with PsA. These data on skin disease comorbidities will increase the prediction performance of patients' future medical conditions, facilitate development of individualized health care, and optimize clinical management.

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TikTok: An emerging social media platform for dermatologist influencers

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Dermatologists are noted to have a strong presence on many social media platforms. TikTok has recently emerged as a new platform which allows users to share short videos. Unique to TikTok is the "duet" feature which allows users to react to other trending videos. Minimal prior analysis of dermatologic information on TikTok has been performed. We have examined the 10 dermatologists with the highest number of followers, the content of their 3 most recent posts, and user engagement of these posts. Their three most recent posts were identified as educational, advertisement or personal. Engagement was evaluated by recording the number of likes, comments, views, and shares per post. 70% of total posts were classified as educational, 23.33% advertisement, and 6.67% personal. Educational posts had the highest mean user engagement overall. 23.33% of posts were "duets" in response to other TikTok users' videos. Duet videos were all educational and had an average of 340,000 views; they were in response to highly viewed posts. This study highlights TikTok as an effective educational tool for dermatologists, with increased user engagement when compared to other social media platforms. Although underrepresented on TikTok, dermatologists can use the "duet" feature as a way to correct misinformation and answer questions pertaining to widely viewed videos. We propose TikTok as an additional social media platform for dermatologists to engage the general public and disseminate dermatologic information.

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Scarcity of the LGBTQ community in dermatology literature

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Members of the lesbian, gay, bisexual, transgender, queer (LGBTQ) community face many health disparities that include conditions relevant to dermatology. The LGBTQ self-identified community composes 2.7-5.3% of the United States (US) population. These patients are at a particularly high risk of infectious diseases, acne, alopecia, postoperative scars, and skin cancer. Dermatologic care for LGBTQ patients has not been widely discussed within the literature. Herein, we aim to examine the availability of dermatologic literature focused on this community and trends in these publications over the ten-year period of 2010-2020, noting publications before and after 2015, when the Defense of Marriage Act (DOMA) legalized marriage equality in the US. The top ten US and top two international dermatology journals, as well as top five medicine journals ranked by h-index [Scimago.com], were surveyed October 23, 2020 for PubMed publications using Medical Subject Headings (MeSH) = "Sexual Minorities and Gender." With the exception of *Sexually Transmitted Diseases*, none of the top ten American or international dermatology journals published LGBTQ articles from 2010-2014. Starting in 2015, post DOMA, JAAD and JAMA Derm ranged from 1-7 LGBTQ publications per year. *Dermatologic Surgery* published one article regarding this community between 2015-2020. The remaining journals did not publish any articles on this topic after 2015. As this community grows, dermatologists will undoubtedly treat LGBTQ patients within their practice. Therefore, an increase in evidence based medicine will be of benefit. Singer et al. demonstrated that this community is higher risk for skin cancer, making low publications in surgical dermatology an area of focus by researchers. Acne, scars, tanning behavior, and alopecia are complications for homosexual and transitioning patients that clinical dermatology literature can address.

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Gender representation in academic dermatology: A necessary shift to the current paradigm

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In an effort to further illuminate gender representation in dermatology publications, we have completed this analysis to address the gap in dermatologic literature. Clarivate Analytic's Web of Science was used to isolate the names of all first and last authors of published works in the Journal of Investigative Dermatology (JID), the Journal of the American Academy of Dermatology (JAAD), and Journal of the American Medical Association Dermatology (JAMA Derm) from 2009 to 2019. Gender API predicted binary gender for each name. We demonstrated that female first authorship (FFA) percentages has been roughly equal to their male counterparts for the past 10 years, with average FFA percentages from 2009 to 2019 at 50.91% (JID), 49.73% (JAAD), and 52.55% (JAMA Derm). In contrast, the average percentages of female senior or last authors (FSA) have remained substantially below that of their male colleagues at 38.55% (JID), 38.55% (JAAD) and 37.45% (JAMA Derm). No significant differences between journals were found for both FFA and FSA. Since senior authors are often those that are further and more advanced in their academic medical careers, the discrepancy between the percentage of female FFA and FSA can be attributed to unequal gender representation in university professorships. However, the closing gap in first authorship inequality suggests that time may also alter the disjunctional proportions of FSA. Limitations include restricting our search to the top three dermatologic journals and determination of gender based on the GenderAPI algorithm. In an effort to more fully represent the community in which dermatologists serve, additional studies are required to reduce inequalities among dermatologic research.

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The value of an anonymous online interactive forum: What questions are applicants asking?

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In July 2020 the National Dermatology Interest Group Association (DIGA) co-hosted the first national dermatology webinar with the Association of Professors of Dermatology to address the residency application process during COVID-19, with over 996 students in attendance. Prior to the webinar, students anonymously submitted 54 questions through a google survey. Program directors addressed these questions during the webinar. Attendees could ask questions (99) during the webinar anonymously via Zoom's "Chat" function. Following the webinar, attendees completed a survey. The topics of the anonymous questions submitted included application logistics (41.12%, 63/153), USMLE board scores & grades (21.57%, 33/153), demonstrating interest (14.38%, 22/153), away rotations (12.42%, 19/153), special applicant groups (11.11%, 17/153), research (5.88%, 9/153), and letters of recommendation (4.58%, 7/153). 96% percent of survey-responders rated the webinar as "helpful," 93% were satisfied with the content, and 84% found the webinar to be stress-relieving. Application logistics as the top category is pertinent given this unique application cycle. Students responded well to the webinar as indicated by the positive post-webinar survey results. This could be related to the opportunity to anonymously query program directors without fear of retribution. Furthermore, this qualitative analysis may guide programs to increase transparency on their websites. Regardless of the landscape of future application cycles, we advocate for continued use of online platforms annually to assist applicants.

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Skin melanoma and subsequent risk of prostate cancer: A national cancer institute surveillance, epidemiology, and end results study

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Introduction: Prostate cancer and melanoma rank as the first and fifth most common cancers, respectively, among men in the United States. Existing studies have reported prostate and melanoma cancer links due to a shared androgen-dependence hypothesis. However, the relationship between prior melanoma history and subsequent prostate cancer is largely unexplored. We aimed to elucidate the relationship between a history of malignant melanoma (MM) and subsequent risk of prostate cancer (PC) in the National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) database. Methods: The SEER database (2000-2016) was used to determine the overall risk for subsequent PC among patients diagnosed with an initial primary cutaneous MM who survived for at least 2 months after diagnosis of MM. Standardized Incidence Ratios (SIRs), defined as the ratio of the observed number of PC among MM survivors to the expected number among the general population (O:E ratios), and 95% confidence intervals (CIs), were calculated. MM and PC was detected by using Site recode B ICD-O-3/WHO 2008. Results: There were a total of 126,561 men diagnosed with MM and subsequent PC within a 5-year study period. Men ages 45-54 years, with a prior MM diagnosis were at an increased risk of PC development (O:E 1.39, 95% CI 1.19-1.62) compared to the general population. Localized melanoma increased risk of PC among this age group compared to non-localized or unknown disease (O:E 1.48, 95% CI 1.25-1.73).While men ages 45-54 years, with a prior history of non- MM cancer had lower risk of PC development compared to the general population (O:E 0.69, 95% CI 0.65-0.74). Conclusions: The findings from this national database demonstrate a prior MM diagnosis increased the risk of PC diagnosis within 5 years. Thus, it may be important for MM patients to be screened for a history of PC, in particular, men ages 45-84 years old. More studies are recommended to explore the associations among these two cancers.

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Consumer preferences of top-rated over-the-counter acne treatment products: A cohort study

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Over 70% of patients with acne vulgaris wait at least one year before seeing a dermatologist and instead use over-the-counter (OTC) acne treatments. This study aims to use information from online product reviews to identify key product characteristics (ingredients, vehicles, marketing claims) and determine their association with consumer-reported positive and negative features of top-rated acne OTCs. This cohort study evaluated the top 1% of acne OTCs across the five largest online retailers of acne products in June 2019. Products were analyzed for product characteristics and consumer-reported features. Artificial intelligence data scraper software was utilized to collect reviews. A natural language processing algorithm was used to tag key phrases within reviews and categorize them based on characteristics and sentiment (positive or negative). An inter-rater reliability test compared reliability of results between a human rater and the software. In this cohort of 149 products, the most frequent ingredients were salicylic acid (33.6%) and benzoyl peroxide (19.5%). Over one-third of top-selling products contained solely natural ingredients. Product ingredients, over product vehicle, price, marketing claims, or packaging, were most associated with consumer-reported product effects. Products with active ingredients were reported more frequently as effective in treating acne ($p<0.001$) and with side effects such as erythema ($p=0.054$) and hypersensitivity reactions ($p=0.0016$). Products with natural ingredients were associated with improving skin texture ($p=0.008$) and application ease ($p=0.04$). Product ingredients, over vehicle, price, marketing claims, and packaging, were the greatest indicator of a consumer's experience with acne OTCs. Given the wide array of and heavy reliance on OTCs to treat acne, information on product experience informs dermatologists on consumer preferences, which may be beneficial in treatment recommendations and overall outcome for patients.

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Suitability of clinical workflows for automation

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There is increasing interest in using information technology to support healthcare workflows; however not all workflows are suitable for automation. We developed two metrics, enacted complexity (EC) and contextual dependence (CD), to identify and rank clinic workflows on their amenability for automation. EC is a function of the number of paths in a workflow; more paths indicate greater complexity. CD is a function of how much a workflow is influenced by contextual specifics. High CD indicates that a workflow greatly depends on when, where, and by whom it is performed. EC and CD are indicators that a workflow may be difficult to map, monitor, and control, suggesting that the workflow is a less feasible target for automation. In this study, we computed EC and CD using clinical documentation data from the electronic medical record (EMR) for 143,347 visits from 24 different outpatient clinics (Dermatology, Orthopedic Surgery, and Pediatric Oncology). Surgical Pathology data were included as a simple workflow comparator. We used EC and CD to rank order the clinics for automation suitability. EC and CD showed strong correlation (Spearman $r = 0.55$, $p<0.05$). Surgical Pathology workflow consisted of a handful of paths and is very nearly context dependent. In contrast, Dermatology clinics had over 167,000 paths and Orthopedic Surgery clinic had millions of paths. Both Dermatology and Orthopedic Surgery workflows were highly context dependent. Although Dermatology clinics were extremely complex, they appeared more amenable to automation than the other outpatient clinics. We conclude that the two metrics, EC and CD, can identify healthcare workflows that are suitable for and may benefit from automation. This research was supported by NSF (SES-1734237), University of Rochester CTSA (UL1 TR002001).

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Using artificial intelligence (AI) to compare patient perspective of PD-1 and BRAF inhibitors for melanoma treatment

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Due to side effects and adverse psychosocial factors, there can often be a disconnect between clinical impression and the patient perspective of treatment. Melanoma patients frequently use social media to discuss their disease sentiments and outcomes. We analyzed social media on melanoma treatments, PD-1 inhibitors (pembrolizumab/Keytruda, nivolumab) and BRAF inhibitors (dabrafenib, vemurafenib), associated with Patient Global Impression of Change (PGIC) terms to compare and identify patient burden. 12,599,313 publicly available online social media text data were extracted and run through Brandwatch Artificial Intelligence-powered database to categorize treatment-specific posts with PGIC terms associated with sentiment. Out of 52,962 posts related to a select list of melanoma treatments, we identified Keytruda (6,080), nivolumab (1,614), dabrafenib (529), and vemurafenib (329) posts. The top ten types of posts by volume for each treatment were predominantly positive for patient impression of change of treatment (improving, well) in contrast with associated negative emotions (fear and sadness). Patient-perceived better treatments were associated with decrease fear. Keytruda at a higher positive PGIC (92.3%) had markedly less fear (56.9%) compared to nivolumab positive PGIC (78.0%) and fear (81.6%). Similarly, dabrafenib positive PGIC (86.4%) had less fear (71.9%) compared to vemurafenib positive PGIC (78.0%) and fear (81.6%). Our initial results provide an indication for greater understanding of patient perspective and translation into more effective clinical and pharmaceutical response.

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Impact of pregnancy on hidradenitis suppurativa: A systematic review and meta-analysis

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Introduction: Data regarding changes in hidradenitis suppurativa (HS) disease course during pregnancy is mixed. We performed a systematic review and meta-analysis to examine whether HS improves, worsens, or remains unchanged during pregnancy. Methods: A systematic review was performed using the PubMed and Embase databases with the search terms: *hidradenitis suppurativa, hidradenitis, acne inversa, velpeau disease, verneuil disease and pregnant, pregnancy, gestation, conception, childbirth, delivery, woman, women, worsen, deterioration, exacerbation, flare, trigger, amelioration, improvement, remission, postpartum*. A total of 2253 articles were identified. Inclusion criteria were as follows: English language, human studies, original research, more than 5 study patients, and relevant to topic of HS and pregnancy. Two random effects meta-analyses were performed to assess (1) HS improvement and (2) HS worsening during pregnancy; heterogeneity was assessed using the I^2 index. Results: Eight articles (6 cross-sectional, 1 case-control, and 1 retrospective cohort study) met inclusion criteria. Of the 672 total cases, HS improved in 185 (28% overall); across studies, this varied from 0% to 83%. Meta-analysis pooling data showed HS improvement rate as 0.24 (95% CI, 0.13-0.40). HS worsened in 205 cases (31% overall); this varied from 0% to 62%. Meta-analysis pooling data showed HS disease worsening rate as 0.20 (95% CI, 0.11-0.34). A significant amount of heterogeneity between studies was noted in both meta-analyses (I^2=92% and I^2=91%, respectively). Discussion/Conclusion: While a quarter of women with HS may experience improvement during pregnancy, the majority of women have stable or worsened disease course. HS patients should maintain close dermatology follow-up during pregnancy, and strong collaboration between dermatologists and obstetricians is needed.

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Epidemiology and risk factors for the development of cutaneous toxicities in patients treated with immune checkpoint inhibitors: A United States population-level analysis

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A variety of dermatoses have been reported in patients treated with immune checkpoint inhibitors (ICIs), but current understanding of cutaneous immune related adverse events (cirAEs) is limited. The objective of this study was to determine the incidence, distribution, and risk factors of cirAEs using population-level data from the US. Using a national insurance claims database, cancer patients receiving ICI therapy were matched to non-ICI cancer patients on demographics, primary cancer type, and Charlson Comorbidity Index (CCI) via 1:1 exact matching. Multivariable logistic regressions were performed to analyze predictors of cirAEs, after adjusting for ICI target, cancer type, age, gender, CCI grade, and measures of socioeconomic status. All analyses were conducted in R version 3.6.3. 8,637 ICI patients and 8,637 matched controls were included in the study. The overall incidence of cirAEs was 25.1%, with the median onset time of 113 days (IQR 42.0-254.0). Only 10 (23.3%) of the diagnoses previously associated with ICIs had significantly higher incidence in the ICI group, with nonspecific rashes and pruritus most commonly diagnosed. Notably, ICI use was protective of cutaneous squamous cell carcinoma (OR 0.72, 95%CI 0.60-0.86, $p<0.01$) and actinic keratosis (OR 0.45, 95%CI 0.40-0.51, $p<0.001$). Increased incidence of cirAEs occurred in patients with melanoma (OR 2.47, 95%CI 2.11-2.89, $p<0.001$) and renal cell carcinoma (OR 1.65, 95%CI 1.36-2.00, $p<0.001$), and in patients treated with combination therapies (OR 1.53, 95%CI 1.25-1.88, $p<0.001$). The is the first population-level study to characterize the incidence and distribution of cirAEs. Only 10 of the 42 previously literature-reported dermatoses were significantly associated with ICI use.

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The role of illness perception in patients with cutaneous t-cell lymphoma

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Illness perception describes patient's internal beliefs about their illness and their resulting psychosocial impact. This concept is important because it can give healthcare providers a tool to identify issues that may need to be addressed with their patients. However, very few studies have looked into illness perception in cutaneous T-cell lymphomas (CTCLs). CTCL is a chronic, and at times debilitating group of malignancies that can have an indolent but remitting course. Treatment options can also be burdensome to the patient. It is therefore important to gain an understanding of not only what CTCL patients believe about their disease but also how those beliefs impact their quality of life (QOL). Moreover, the current COVID-19 pandemic offers a unique opportunity to investigate how significant disruptions in access to healthcare have impacted illness perception and QOL. The objectives of this study are to identify disease understanding in patients with CTCL, to investigate the impact additional education modalities has on disease understanding, and whether disparities exist between specific groups of patients. We also hope to determine how the COVID-19 pandemic impacted healthcare-related QOL. CTCL patients, above the age of 18, are recruited for this study. Patients are given an electronic survey containing the Illness Perception Questionnaire-Revised (IPQ-R), Skindex-29, FACT-G7, and selected questions based on the Household Pulse survey to assess COVID impact on QOL. Patients are then randomly selected to view an educational CTCL PowerPoint, in addition to verbal education routinely given during their visit. Follow-up responses to these questions will be collected at 2 and 6 months after the initial survey. In this ongoing study, we anticipate a sample size of 100 patients. The outcome of this study will provide insight into the use of additional educational modalities to better patient understanding of CTCL, with the goal of clearing common patient misconceptions about the disease, improve educational resources, and identify actionable paths to diminish obstacles to their access to care.

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Perspective of psoriatic disease patients on novel COVID-19 vaccines

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The National Psoriasis Foundation surveyed a stratified sample of 1,405 individuals with psoriatic disease in the United States. Participants were asked questions about the likelihood of receiving a vaccine for COVID-19, history of receiving vaccination for the flu in the last 12 months, current therapies used to treat their psoriasis and demographic questions. A total of 1,405 participants completed the survey. Of these, 642 (45.7%) had PsO only, 86 (6.1%) had PsA only and 677 (48.2%) had PsA and PsO, 690 (52.3%). Overall, 336 (23.9%) were somewhat to very unlikely to receive a COVID-19 vaccine when it becomes available, 167 (11.9%) were neither likely nor unlikely to receive a COVID-19 vaccine and 900 (64.2%) were somewhat to very likely to receive a vaccine. Results for receiving the flu vaccine in the last 12 month resembled likelihood of receiving a COVID-19 vaccine, 911 (65.0%) had received a flu vaccine in the last 12 months and 491 (35.0%) had not. Chi-square tests for independence were conducted to assess if likelihood of receiving COVID-19 vaccination was associated with race, income, gender, age, disease type, vaccination for flu in last 12 months and biologic therapy use. Results from these tests suggest that likelihood of receiving a COVID-19 vaccination was not associated with disease type (PsO only or has PsA) ($p=.108$) or using a biologic therapy ($p=.817$). Likelihood of receiving a COVID-19 vaccination was associated with race ($p<.05$), income ($p<.001$), gender ($p.001$), age ($p<.001$) and having received the flu vaccine in the last 12 months ($p<.001$).

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Utilization of dermatologic care by patients with advanced melanoma after initiation of immunotherapy and targeted therapy: A retrospective cohort analysis

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Patients with Stage III and IV melanoma are living longer with the introduction of immunotherapy and targeted therapy. Despite National Comprehensive Cancer Network (NCCN) guidelines recommending regular patient follow up with dermatology for skin checks and nodal assessments, little is known about actual health service use in survivors. This study aims to evaluate health care utilization by advanced melanoma patients focusing on employment of dermatologic services in order to determine areas of improvement. A retrospective cohort analysis of Stage III and IV melanoma patients with age greater than 18 at the start of follow-up (first immunotherapy / targeted therapy usage) who were seen at Dermatology clinic at The Emory Clinic or the Winship Cancer Institute from January 1st, 2011 to September 14, 2020 was done. Data was collected from the Emory Healthcare Clinical Data Warehouse and then validated using manual chart review. Primary outcome is the number of visits to Dermatology clinic per year. Descriptive statistics were done in SPSS. Entries were collected from 77 patients who met study criteria. The majority of patients exclusively received immunotherapy (58) while the minority were exclusively treated with targeted therapy (9) or both (10). The mean age at first dermatology follow-up visit was 57.8 years old. The study population included 54.5% males and 45.5% females. The vast majority (90.9%) of patients were Caucasian or White. The mean number of dermatology visits per person-year was 1.9 visits. This did not statistically significantly differ (p=0.107) between patients treated exclusively with immunotherapy (1.8) and targeted therapy (2.4). Limitations include the fact that many patients obtained their dermatologic care at an outside clinic. Future research should examine optimal dermatologic follow up frequency for patients with advanced stage melanoma after immunotherapy and targeted therapy initiation.

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Rate of hidradenitis suppurativa readmission has increased in the united states: A 9- year longitudinal study of the national readmission database

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There is a scarcity of studies on hidradenitis suppurative (HS) readmission trends in the United States (US). This study aims to study longitudinal trends of 30-day readmissions of HS patients over time in the US using national population data. Data were obtained from the National readmission database (NRD). We performed a retrospective 9-year longitudinal trend analysis of NRD 2010 (year of inception)-2018 databases. We searched for index hospitalizations for patients aged≥ 18 years with a principal or secondary diagnosis of HS using ICD codes for the corresponding year. We excluded elective and traumatic readmissions. The trend in the 30-day readmission rate was our primary outcome. Multivariate logistic and linear regression was used to calculate adjusted p-trend for categorical and continuous outcomes, respectively. STATA, version 16 was used for analysis. 30-day readmission rate increased over time, from 11.0% in 2010 to 11.5% in 2018, with a peak of 13.6% in 2016 (adjusted p-trend=0.028). The proportion of readmitted patients with Charleston co-morbidity index (CCI) score ≥3 increased from 0% in 2010 to 37.4% in 2018 (adjusted p-trend<0.0001). Inpatient mortality of HS readmissions increased from 1.1% in 2010 to 1.2% in 2018, with a peak of 1.9% in 2014 (adjusted p-trend=0.086). HS was the most common reason for readmissions across all years. Sepsis was the 2nd most common reason for readmission in all years except in 2010 where cellulitis was the 2nd most common reason for readmission. Rates of 30-day readmissions and co-morbidity burden of hospitalized HS patients has increased over time in the US. HS itself remained the most common reason for readmission of HS patients during the study period. There was no trend in inpatient mortality of readmitted HS patients after adjusting for co-morbidities. Strategies targeted at improving access to urgent outpatient dermatologic care are essential in preventing unplanned readmissions of HS patients.

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Differences in musculoskeletal impact on health among patients with psoriasis based on disease type, disease severity and undiagnosed psoriatic arthritis (PsA)

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The National Psoriasis Foundation conducted a survey within a stratified sample of 1,405 individuals with psoriatic disease in the United States. Participants provided demographics and were asked about a provider diagnosis of psoriasis, PsA, or both. All participants completed the IDEOM Psoriasis Musculoskeletal (MSK) Symptoms Impact of Disease Questionnaire. PsO severity was assessed using the Patient Reported Extent of Psoriasis Involvement (PREPI). Participants were screened for undiagnosed PsA using the Psoriasis Epidemiological Screening Tool (PEST). Analysis of variance was used to assess differences in MSK impact on health based on disease diagnosed (PsO or PsA) among all participants, and undiagnosed PsA and severity of PsO among individuals with PsO only. Post-hoc tests were conducted to assess difference in MSK impact on health between individuals with Mild PsO (BSA < 3%), Moderate PsO (BSA 3 − 10%) and Severe PsO (BSA >10%). Among the 1,405 respondents, 642 (45.7%) had PsO only, 86 (6.1%) had PsA only and 677 (48.2%) had PsA and PsO. Of those with PsO only, 326 (50.8%) reported having Mild PsO (BSA < 3%), 215 (33.5%) reported having Moderate PsO (BSA 3 − 10%) and 101 (15.7%) reported having Severe PsO (BSA >10%); 201 (31.3%) of the PSO only patients had a PEST score ≥ 3, indicating the presence of undiagnosed PsA. Among participants with PsO only, psoriasis severity was not associated with having a PEST score ≥ 3 (p=.381), based on results from a chi-square test for independence. Analysis of variance revealed that great MSK impact was associated with having PsA, having more severe PsO and having undiagnosed PsA.

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Incidence, racial profile, and co-morbidity burden of hidradenitis suppurativa hospitalization has changed in the last decade: A longitudinal study of the national inpatient sample

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This study aims to study longitudinal trends of HS hospitalizations over time in the United States (US) using national population data. Data were obtained from the National Inpatient Sample (NIS) database. We performed a retrospective 11-year longitudinal trend analysis of NIS 2008-2018 databases. We searched for hospitalizations for patients aged≥ 18 years with a principal or secondary diagnosis of HS using ICD codes for the corresponding year. Multivariate logistic and linear regression was used to calculate adjusted p-trend for categorical and continuous outcomes, respectively. The incidence of adult HS hospitalizations in the U.S increased from 3.5 per 100,000 persons in 2008 to 6.9 per 100,000 persons in 2018. The proportion of whites decreased from 46.6% in 2008 to 38.5 % in 2018 (adjusted p-trend<0.0001), while that of blacks increased from 42.6% in 2008 to 47.7% in 2018 (adjusted p-trend=0.014). The proportion of Hispanics and Asians also increased from 7.1% & 0.5% in 2008 to 9.9% & 1.1% in 2018 (adjusted p-trend <0.0001 & 0.004) respectively. The proportion of patients with Charleston co-morbidity index (CCI) score of 0-2 decreased from 88.9% in 2008 to 76.2% in 2018, while those with CCI score of ≥3 increased from 11.1% in 2008 to 23.8% in 2018 (adjusted p-trend<0.0001). Inpatient mortality ranged from 0.3% to 0.6% across the years (adjusted p-trend=0.614). The incidence and co-morbidity burden of hospitalizations of HS patients in the US has increased in the last decade. The proportion of hospitalized whites has reduced, with an increase in minorities such as blacks, Hispanics, and Asians. This may be due to minorities having less access to outpatient specialist care, hence increasing their rate of hospitalization. An interdisciplinary approach is essential in managing HS patients with increased co-morbidity burden.

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Incidence, co-morbidity burden and resource utilization of psoriasis hospitalization has increased in the last decade: A 11-year longitudinal study of the national inpatient sample

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This study aims to study longitudinal trends of psoriasis hospitalizations over time in the United States (US) using national population data. Data were obtained from the National Inpatient Sample database (NIS). We performed a retrospective 11-year longitudinal trend analysis of NIS 2008-2018 databases. We searched for hospitalizations for patients aged≥ 18 years with a principal or secondary diagnosis of psoriasis using ICD codes for the corresponding year. Multivariate logistic and linear regression was used to calculate adjusted p-trend for categorical and continuous outcomes, respectively. The incidence of adult psoriasis hospitalizations in the U.S increased from 34 per 100,000 persons in 2008 to 52 per 100,000 persons in 2018. The mean age increased from 59.9 years in 2008 to 61.2 years in 2018 (adjusted p-trend=0.021). The proportion of whites decreased from 82.7% in 2008 to 81.5 % in 2018 (adjusted p-trend<0.0001), while that of Hispanic increased from 6.4% in 2008 to 7.8% in 2018 (adjusted p-trend=0.017). The proportion of patients with Charleston co-morbidity index (CCI) score of 0-2 decreased from 78.7% in 2008 to 63.9% in 2018, while those with CCI score of ≥3 increased from 21.3% in 2008 to 36.1% in 2018 (adjusted p-trend<0.0001). Mean hospital length of stay (LOS) increased from 4.9 days in 2008 to 5.0 days in 2018, with a peak of 5.2 days in 2014 & 2016 (adjusted p-trend=0.001). Mean adjusted total hospital cost (TOTcost) increased from 12,909 US dollars in 2008 to 14,739 US dollars in 2018 (adjusted p-trend=0.017). Incidence, age, co-morbidity burden, and resource utilization in terms of LOS and TOTcost of psoriasis hospitalizations have increased in the last decade. The racial profile has changed, with a decreased proportion of Caucasians and increased Hispanic hospitalization. Interdisciplinary collaboration is needed to optimize outcomes of hospitalized psoriasis patients with increased co-morbidity burden.

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Biologic and nonbiologic systemic treatment of psoriasis are protective against solid organ, hematologic, and cutaneous cancer in a large multi-institution cohort

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Treatment for psoriasis has been augmented in recent decades by the development of biologic agents targeting specific inflammatory regulators. Given their recent introduction compared to older systemic immunosuppressants, their impact on patients' long-term cancer risk has yet to be fully elucidated. We compared the incidence of cutaneous as well as solid organ and hematologic cancer in a large, multi-institutional cohort using an electronic health record database. Data analysis was conducted using R Studio version 1.3.1093. We identified 69,391 psoriatic patients treated between 1/1/1990 and 10/1/2020. Patients with prior cancer history or a competing autoimmune indication for immunosuppression were excluded. Remaining patients were separated into treatment categories depending on whether they used biologic therapy only (TNF-a, IL-12,23, or IL-17 inhibitors, n=1,427), nonbiologic systemic therapy only (n=2,739), or any combination of the two (n=1,859). A Cox Proportional Hazard Model was used to account for variation in cancer incidence related to age, sex, race, and select comorbidities relative to patients not receiving systemic treatment (n=51,022). Treatment with only biologic therapy resulted in a significant reduction in non-cutaneous cancer (HR 0.41 [0.32-0.53], p<0.001). A protective effect was also observed with exclusively nonbiologic (HR 0.64, CI 0.57-0.73, p<0.001) or mixed regimens (HR 0.60 [0.51-0.70], p<0.001). A similar pattern was observed with cutaneous malignancies, with greater protection observed in patients receiving biologic (HR 0.56 [0.43-0.73], p<0.001) vs. nonbiologic (HR 0.76 [0.66-0.88], p < 0.001) or mixed regimens (HR 0.63 [0.52-0.76], p<0.001). Overall, our study suggests that systemic immunomodulation may reduce cancer incidence in psoriatic patients, particularly in the biologics only group.

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Rate of psoriasis readmission has decreased in the united states: A 9- year longitudinal nationwide study

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This study aims to study longitudinal trends of 30-day readmissions of psoriasis patients over time in the United States (US) using national population data. Data were obtained from the National readmission database (NRD). We performed a retrospective 9-year longitudinal trend analysis of NRD 2010 (year of inception)-2018 databases. We searched for index hospitalizations for patients aged≥ 18 years with a principal or secondary diagnosis of psoriasis using ICD codes for the corresponding year. We excluded elective and traumatic readmissions. The trend in the 30-day readmission rate was our primary outcome. Multivariate logistic and linear regression was used to calculate adjusted p-trend for categorical and continuous outcomes, respectively. The rate of decrease in 30-day readmission rate was steeper for patients admitted with a principal diagnosis of psoriasis (16.7% in 2010 to 10.2% in 2018, adjusted p-trend=0.002) compared to patients admitted with any diagnosis of psoriasis (12.2% in 2010 to 10.4% in 2018, adjusted p-trend<0.0001). Inpatient mortality of readmissions decreased from 4.4% in 2010 to 4.2% in 2018, with a nadir of 3.8% in 2016 (adjusted p-trend<0.0001). The mean length of stay (LOS) decreased from 6.3 days in 2010 to 5.8 days in 2018 (adjusted p-trend<0.0001). Proportion of readmitted patients with Charleston index score ≥3 increased from 0% in 2010 to 51.6% in 2018 (adjusted p-trend<0.0001). Adjusted total hospital cost increased from 13,636 to 14,112 US dollars (adjusted p-trend=0.010). Sepsis was the most common reason for readmission across all years. The rate of readmission has decreased for patients admitted principally because of psoriasis and all admissions of psoriasis patients; however, this decrease is more precipitous for the former. Although hospital cost and comorbidity burden of psoriasis readmissions has increased over time, inpatient mortality and LOS have decreased. These may be due to better outpatient management and more effective treatment options available in recent times.

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Association of multiparity and venous insufficiency in Hispanic women

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Dermatologists commonly manage patients with chronic venous insufficiency (CVI). CVI affects about a third of adults in the USA and causes significant morbidity in diverse populations worldwide. Risk factors associated with CVI include age, female gender, multiparity, family history, and obesity. However, factors such as ethnicity and race may also influence the development and progression of CVI. Information about ethnic differences in Americans with CVI is scarce. South Florida is a melting pot of racially diverse backgrounds with Miami-Dade County reporting 26.2 percent of its residents a minority. Through a cohort of patients with CVI in South Florida, we sought to further characterize the racial and ethnic disparities of CVI. We designed a longitudinal, rolling enrollment cohort study with retrospective chart review components. Subjects who were previously diagnosed with CVI on duplex ultrasound underwent a phone survey and data on specific variables of interest were captured. Of the 538 subjects enrolled, the average age was 62.81 years, average body mass index was 30.42 kg/m2, and 66.6% were females. Most participants identified as White (77.7%), 64.5% identified being of Hispanic or Latin origin. Compared to non-Hispanic women, Hispanic women with CVI were more likely to report higher frequency of pregnancy ($z = -3.3026$, p value < 0.01). Because number of pregnancies can influence the CVI severity, understanding differences in pregnancy rates between ethnic groups can help guide clinicians to intervene earlier in the disease process with simple interventions such as compression therapy. Additional analyses of data on CVI and lifestyle modifications in various racial groups is forthcoming.

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Geographical and environmental factors associated with melanoma incidence in Canada

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Background: We sought to examine the relationship between environmental factors and Canada's distribution of melanoma incidence between 1992 and 2010. Methods: Data was obtained from the Canadian Cancer Registry, Canadian Vital Statistics, and the Canadian Urban Environmental Health Research Consortium. Crude melanoma incidence rates were obtained for forward sortation areas (FSAs). Enviromental variables included: normalized difference vegetation index (NDVI) as a proxy for green space, precipitation, yearly temperature, and number of weather events between 1992-2010. Environmental exposures were modeled as tertiles via a two-level random-effect generalized linear model to evaluate a dose-response relationship. Results: Across Canada, average annual temperature increased significantly over time ($p < 0.0001$). Other significant increases included: absolute annual temperature, average amount of precipitation, as well as the average number of weather events of heat and rain. Greatest increases were observed in Newfoundland, PEI and Manitoba. A positive significant relationship between annual average temperature and melanoma incidence rate was confirmed (Beta: 6.23, 95%CI: 5.2, 9.93). With each increase in NDVI, the odds of melanoma doubled in high-risk FSAs compared to those with low-risk (OR: 2.72, 95%CI: 2.49, 2.97; & OR = 4.31, 95%CI: 3.91, 4.76; for tertiles 2 and 3 respectively). Discussion: Consistent strong positive relationships between the changes in enviromental exposures and melanoma incidence were observed in this study. High ambient temperature leads to more time outdoors, less protective clothing, and greater number of sunburns. The presence of parks with an abundance of foliage may encourage the public to spend more time outdoors increasing UVR exposure. Public health advice may be improved by taking account of both temperature and green space accesibility and their implications for behaviour.

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Public sunscreen dispensers and consumer sunscreen trends during the COVID-19 pandemic

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Coronavirus Disease 2019 (COVID-19) has impacted societal and public behaviors as prevention efforts restrict activities and socialization. Many citizens are finding themselves turning to outdoor activities to properly social distance and stay physically, emotionally, and mentally healthy. IMPACT Melanoma, a non-profit aimed to reduce skin cancer and conduct skin cancer prevention outreach, provides sunscreen dispensers and sunscreen to many park and recreational institutions. We hypothesize that IMPACT's distribution, along with sunscreen purchases by consumers, will be decreased during the COVID-19 pandemic. Findings of the study demonstrate a 61% reduction of sunscreen dispensers in 2020, when compared to 2019. In parallel, there was a 50.8% decrease in purchases of cases of sunscreen. Sector sponsorship by public health departments and parks/recreational facilities (the largest 2019 sponsors) decreased by 49.7% and 27.9%, respectively. Trends in the general public's purchase of sunscreen reflected similar pandemic-related declines. Consumers in the US purchased less sunscreen starting in March 2020 (-3% for the week ending 3/7/2020, followed by -17% for the week ending 3/14/2020), with the largest decreases in April and May (-31% to -65%). Some tapering in declining sunscreen sales was observed in early June despite the outdoor activity-oriented Memorial Day holiday weekend (-11% for the week ending 6/6/2020). As illustrated by these findings, the increase in outdoor activity among the general public combined with a reduction in sunscreen sales and distribution poses an increased exposure to UV light, which inevitably increases the risk of skin cancer. Additional studies are necessary to further explore the impact of COVID-19 on skin cancer prevention efforts.

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HIV's potential effect on hidradenitis suppurativa disease onset, progression, and diagnosis

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Much interest has recently developed in defining the relationship between HIV and the increased rates and severity of chronic inflammatory disorders but the relationship between HIV and hidradenitis suppurativa (HS) remains poorly understood. Although it is an immunodeficiency syndrome, HIV patients experience more severe psoriasis and higher rates of psoriatic arthritis than patients without HIV, leading us to hypothesize that HIV may have a potential effect on HS disease onset and progression. Through this study we aim to further characterize the population of patients with comorbid HS and HIV and assess the rates of HS misdiagnosis in the HIV community. Data from 63 adults from the University of North Carolina Hospitals were identified who met the criteria for diagnoses of HS and HIV through ICD-9 and ICD-10 codes. This also included subjects with common ICD codes for diagnoses often confused for HS such as abscess, nodule, furuncle, and carbuncle for which chart review was performed and confirmed a clinical diagnosis of HS based on recurrent nodules and abscesses in intertriginous locations. The data revealed features unique to concomitant disease compared to either condition alone. The age of onset for HS among those living with HS and HIV, 31.1 years old, was later than patients living with HS only (2^{nd}-3^{rd} decade of life). HIV was diagnosed prior to HS onset in 63.8% of subjects. Both the late age of HS onset and high rate of HS onset after initial HIV diagnosis may suggest that people with HIV are particularly susceptible to developing HS. Only 37.3% of our cohort have an ICD code for HS. Additionally, only 32.8% of patients were referred to a dermatologist, revealing a knowledge discrepancy regarding HS clinical presentation and management. Overall, our data show unique characteristics of a population with HIV and HS, suggesting a possible association. Additionally, the high rate of misdiagnosis of HS in the population living with HIV necessitates efforts to increase physician knowledge of HS diagnosis and management.

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Are we systematically excluding pediatric hidradenitis suppurativa patients from clinical trials?

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Hidradenitis Suppuritiva (HS) a chronic inflammatory skin disorder that often develops after puberty. Standard guidelines addressing therapeutic drugs as well as clinical trial studies for pediatric HS patients are limited. The large majority of medication trials have excluded patients <18 years, which hinders FDA-approval for pediatric patients. Frequently used inclusion and exclusion criteria in adult trials may discriminate and create major barriers to inclusion for pediatric patients. Using a registry of prospectively collected data, a cohort of about 60 pediatric patients and 700 adult patients from a subspecialty HS clinic at the University of North Carolina Chapel Hill was identified. Descriptive statistics and regression analysis were conducted using STATA statistical software. Mean abscess/nodule (AN) counts in the pediatric cohort was 2.00 compared to 4.94 in adults, with the large majority of pediatric patients having Hurley stage I or II disease. Only 5/60 (8.33%) and 16/60 (26.67%) pediatric patients had AN count of ≥5 and ≥3, respectively. Furthermore, only 4/60 (6.67%) pediatric patients were both Hurley stage II/III with an Count ≥3 (a typical inclusion criteria). An analysis of inclusion criteria for 23 clinical trials for HS that have completed recruitment or are currently recruiting revealed only two trials included patients ≥16 years old, 6 requiring AN count ≥5, 1 requiring AN count ≥ 4, 6 requiring AN count ≥ 3, 1 requiring AN count ≥ 1, and a majority requiring subjects with Hurley Stage II or III. In the context of developing future clinical trials for pediatric HS patients, it is imperative to consider how their baseline characteristics will impact recruitment and disease assessment. Frequently used current trial inclusion criteria would severely limit the ability of adolescent patients to participate. Early intervention and cessation of progression is immensely important and clinical trials should aim to include younger patients with lower disease severity.

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Intramuscular triamcinolone for acute hidradenitis suppurativa flares

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Hidradenitis suppurativa (HS) is a chronic, recurrent, immune-mediated follicular disease managed by wide-ranging therapies, including anti-inflammatory drugs. Among these modalities, intralesional triamcinolone (ILTAC), a standard of care for acute flares, is often impractical in extensive disease. By contrast, intramuscular triamcinolone (IMTAC) has not been studied as an alternative treatment for severe, widespread HS flares. We evaluated the efficacy and patient experience associated with IMTAC therapy. A retrospective analysis and telephone survey focused on 35 patients who received both IMTAC and ILTAC at the Albert Einstein/Montefiore HS Center from January to November 2020. Mean age was 39.1±15.0 years, and approximately half were female (54.3%). Mean disease severity, using a 5-point scale of HS-Physician Global Assessment (HS-PGA), was 4.00±0.91, indicating IMTAC was reserved for advanced disease. Telephone interviews revealed that most patients experienced no pain or discomfort during IMTAC injections (n=26 [74.3%]). Moderate to significant improvement in HS lesional activity was reported by 71.4% (n=25), as reflected by reduced pain (n=25 [71.4%]) drainage (n=17 [48.6%]), and improved mobility (n=18 [51.4%]) and quality of life (n=22 [62.9%]). No adverse effects were reported. Of 29 patients (82.8%) with follow-up visits (mean 7-week interval), a significant decrease was seen in both disease severity (mean initial HS-PGA 4.07 declined to 3.31, p=.009) and pain (mean initial 10-point numerical rating scale of 5.76 declined to 2.81, p=.001). Most preferred or were neutral about receiving IMTAC over ILTAC (33 [94.3%]). Overall, most (25 [71.4%]) reported moderate to substantial satisfaction and almost all (33 [94.3%]) would receive IMTAC injections again if clinically indicated. Our findings affirm an overwhelming positive response to IMTAC therapy for severe, multifocal, and anatomically extensive HS flares. This is the first report of IMTAC as a safe and favorable adjunct for acute HS management.

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Clinical utility and reliability of buccal mucosal biopsies for the diagnosis of ocular cicatricial pemphigoid in patients with isolated ocular disease

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Ocular Cicatricial Pemphigoid (OCP) is a subset of the disease Mucous Membrane Pemphigoid, specifically with involvement of the ocular mucosa. OCP is characterized by chronic conjunctivitis, progressive subepithelial fibrosis, ocular keratinization, and if left untreated, blindness. The current diagnostic gold standard is conjunctival biopsy with direct immunofluorescence demonstrating linear deposition of one or more immunoreactants (IgA, IgG, or C3) at the epithelial basement membrane. However, sensitivity of conjunctival biopsy is variable and reported in the range of 50-80%. Buccal mucosal biopsy has been shown to be positive in patients with OCP, however its use has not been studied in patients with isolated ocular disease. This study aims to identify the utility and reliability of buccal mucosal biopsy for OCP diagnosis in a cohort of 35 patients presenting with cicatrizing conjunctivitis where OCP with isolated ocular involvement is suspected. We observed 40% (14/35) positivity on the first buccal biopsy, with an increase to 60% (21/35) when 2 biopsies were performed, notably at different time points (mean difference = 299 days). Additionally, 50% (7/13) of patients with a negative first buccal biopsy had a positive second biopsy. For patients with persistently negative (≥2) buccal biopsies, conjunctival biopsies were positive in 75% (3/4). Serologic studies were largely negative, with less than 5 patients demonstrating autoantibodies via indirect immunofluorescence or ELISA. Therefore, multiple buccal mucosal biopsies done at separate points in time may be an effective alternative to conjunctival biopsy, offering a safer route for diagnosis of OCP in patients with isolated ocular involvement.

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Dermatology consent form readability: A barrier to comprehension and inclusivity

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While consent forms fulfill legal requirements by reviewing risks and benefits of treatments, their use in educating patients has been questioned. Determining the readability of consent forms is the first step in examining their utility for patient decision making. This study aims to evaluate readability levels of dermatology consent forms. Consent forms were requested from 27 academic dermatology programs. 11 programs declined to participate, did not respond, used verbal consent, or sent multiple, identical, or incorrect consent forms. 16 consent forms were ultimately analyzed. Formatting was standardized and readability was assessed with Flesch Reading Ease Formula (FREF, range 1-100, high scores indicate easier readability) and Flesch-Kincaid Grade Level (FKGL, correlates to educational grade reading level) through Microsoft Word. Average FREF was 34.4±8.9 and FKGL was 13.8±2.0, indicating that consent forms were very difficult to read. FKGL scores ranged from 9th to 17th grade reading levels. 100% of consent forms were over the American Medical Association's (AMA) recommended 6th grade reading level. Our results demonstrate that consent forms require a high degree of literacy, beyond that of the average American and the AMA's recommendations. Consent forms may be difficult to understand, especially for patients with limited health literacy such as those who are elderly, social or ethnic minorities, or those of a low socioeconomic status. Improved understanding improves patient adherence and outcomes, and there is a need for consent forms that promote inclusivity and understanding. More research is needed to develop clearer solutions for creating accessible consent forms for all patients.

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Dupilumab improves health-related quality of life (HRQoL) in children aged ≥6−<12 years with severe atopic dermatitis (AD)

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Severe AD negatively impacts patients' and caregivers' HRQoL in diverse domains such as social functioning, activities, and relationships. The Children's Dermatology Life Quality Index (CDLQI; range 0−30) is a 10-item questionnaire that assesses patient/caregiver-reported impact of AD on HRQoL. In the multicenter phase 3 LIBERTY AD PEDS trial (NCT03345914), 367 severe AD patients aged ≥6−<12 years were randomized 1:1:1 to receive subcutaneous dupilumab every 2 weeks (q2w), every 4 weeks (q4w), or placebo, for 16 weeks with concomitant medium-potency topical corticosteroids (TCS). We report baseline CDLQI ± standard deviation (CDLQI categories: 0−1=no/2−6=small/7−12=moderate/13−18=very large/19−30=extremely large effect on HRQoL) and least squares mean change from baseline in total CDLQI ± standard error for children receiving 300mg q4w if weight<30kg (n=61); 300mg q4w if weight≥30kg (n=61); 200mg q2w if weight≥30kg (n=59); and weight-matched placebo (<30kg/≥30kg, n=61/62) with nominal *P* values (**P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001) vs corresponding placebo. At baseline, mean CDLQI indicated a "very large effect" of AD on patients' lives (<30kg: q4w 16.9±8.1, placebo 16.1±6.9; ≥30kg: q2w 13.0±6.3, q4w 15.5±7.7, placebo 13.2±7.7). Dupilumab+TCS significantly reduced total CDLQI as early as Week 2 (<30kg: q4w −6.9±0.6***, placebo −3.7±0.6; ≥30kg: q2w −5.9±0.6*, q4w −6.4±0.6**, placebo −3.9±0.6) and improved total CDLQI further at Week 16 (<30kg: q4w −10.7±0.7****, placebo −6.3±0.7; ≥30kg: q2w −10.5±0.7****, q4w −10.5±0.6****, placebo −6.4±0.7). The safety profile in this study was consistent with the known dupilumab safety profile. Dupilumab+TCS treatment resulted in highly significant HRQoL improvements in children with severe AD. Improvements were seen after first dose and were sustained over 16 weeks.

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Comparison of patient satisfaction after the laser treatment of female hirsutism: Low fluence or high fluence?

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Excessive hair growth manifests as hirsutism and hypertrichosis. Different types of laser methods have been applied to treat hirsutism. This study aimed to evaluate and compare the satisfaction and side effects of patients who have been treated with two laser therapy techniques: high fluence (BLEND) and low fluence (FDP+BLEND). In this cross-sectional study, the medical records of 182 patients referred to Yazd Laser center were reviewed. Various side effects of laser therapy were assessed, and the satisfaction rate of the patients was evaluated in three phases: after the first session, at the end of the treatment course, and six months after the end of the treatment. Moreover, three types of skin complications were investigated: burn blisters, skin bruises, and folliculitis. The burn blisters were detected in 10 patients vs. 3 patients for BLEND and FDP+BLEND respectively (P value=0.017). The frequency of skin bruise was 4 patients and 1 patient for BLEND and FDP+BLEND respectively (P value=0.16). The frequency of folliculitis was 48 patients vs. 11 patients for BLEND and FDP+BLEND respectively (P-value <0.001). The satisfaction level after the first session of the laser treatment was higher in the FDP+BLEND technique (P-value <0.001). The satisfaction level at the end of treatment was the same in the two groups (P-value = 0.394). However, six months after the end of the treatment satisfaction level was significantly higher in the FDP+BLEND technique (P-value = 0.005). The number of treatment sessions was lower in the BLEND technique. The low fluence (FDP+BLEND) method of laser therapy has fewer complications and greater patient satisfaction.

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Interim analysis of a real-world retrospective multicenter study: Evaluation of effectiveness of secukinumab in adult patients with moderate-to-severe plaque psoriasis in China

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There is a growing body of real-world evidence globally indicating secukinumab is effective in treating patients with moderate-to-severe plaque psoriasis. However, there is a paucity of real-world evidence on treatment effectiveness in China. We present results of an interim analysis of a study on a cohort of adult patients with moderate-to-severe plaque psoriasis, who initiated secukinumab treatment between May 2019 and March 2020. Real-world data collected by retrospective medical charts review of patients initiated secukinumab (baseline) and at 24-week time period. Treatment outcomes were measured by Psoriasis Area and Severity Index (PASI), body surface area (BSA), Investigator Global Assessment (IGA mod 2011) and Dermatology Life Quality Index (DLQI), Changes of severity indicators between baseline, week 12, and week 24 were assessed. Of the 82 eligible patients, mean age at treatment initiation was 38.0±11.0 years old and 78.1% were male. Of all patients at baseline, mean PASI score were 13.5±8.4; 97.1% had PASI>3; 6.3% had IGA score 0-1; 91.5% had BSA>3; only 1.2% reported DLQI 0-1; At week 12, 94.3%, 77.1%, and 40.0% of patients had achieved 75% improvement in the PASI (PASI75), PASI90, and PASI100 respectively; 94.1% patients with IGA 0-1 and 23.5% were BSA > 3. The mean absolute reduction in PASI score from baseline was 14.7±8.9, whilst 75% patients with DLQI 0-1. Among 25 patients who followed up regularly up to week 24, the mean absolute reduction in PASI score from baseline were 17.7±9.0, with 100%, 84.6% and 69.2% of patients achieved PASI75, PASI90, and PASI100 respectively. This interim analysis demonstrated that secukinumab is highly effective in improving PASI, IGA, BSA and DLQI among moderate-to-severe plaque psoriasis in real world settings. The sustainable effect was observed with continuous secukinumab treatment for 24 weeks.

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BOTE (Beginning Of The End) inflammation can be enhanced with SB206, a nitric oxide-releasing topical medication for molluscum contagiosum

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The beginning-of-the-end ("BOTE") sign names inflammation that predicts imminent resolution of molluscum contagiosum (MC), but has never been prospectively studied. Integrated data from two phase 3, multicenter, randomized, double-blind, vehicle-controlled 12-week clinical trials of topical nitric oxide—releasing SB206 gel was used to evaluate an association between BOTE inflammation and MC lesion reduction among 707 randomized patients ≥6 months old. Investigators received training to evaluate BOTE components (erythema, edema, crusting, bullous reaction, erosion) using a 5-grade scoring system, and BOTE condition was scored prospectively during the study. Approximately 80% of patients exhibited BOTE inflammation at any time, regardless of treatment group. At week 12, initial MC lesion counts among vehicle-treated patients decreased by 50.7% from baseline for baseline BOTE+ vs 29.1% for baseline BOTE− patients (P =0 .0015). Among SB206-treated patients, MC lesion counts decreased by 63.3% from baseline for baseline BOTE+ vs 51.7% for baseline BOTE− (P =0 .0194). Among vehicle-treated patients, 48 (22.8%) who never developed BOTE inflammation during the 12-week study had an 18.5% reduction from baseline MC lesion counts vs a 34.0% reduction in 165 patients (76.7%) who experienced BOTE at any time during the study. This suggests a projected duration of 15 months until lesion clearance for BOTE− vs 6 months for BOTE+ patients. Those who were both BOTE+ and treated with SB206 had the greatest reduction in MC lesion count. SB206 may trigger BOTE inflammation and shorten the duration of MC infection.

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Using electronic health records to evaluate factors associated with treatment escalation in psoriasis

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EHRs offer the prospect of utilizing clinical data for health services research (HSR). The University of Utah psoriasis (PsO) clinic uses custom-designed forms in Epic that record PsO-specific measures during clinical care, including patient-reported global assessment (PtGA); 0−10), body surface area (BSA; 0−100%), and overall physician global assessment (PGA; 0−5). These data and other structured variables were extracted to compare PsO severity between patients with and without meaningful therapy escalations. We identified 475 PsO patients with data for ≥3 consecutive visits between Oct 2015−Oct 2018, the first of which is defined as Visit 1. Meaningful therapy escalation was defined as a change expected to improve symptoms (e.g., switch from topicals to phototherapy, switching systemic or biologic therapies, adding phototherapy/systemic to existing biologic therapy). We compared changes from Visits 1−3 for patients with (N=241) and without (N=234) therapy escalations. Female sex (p=0.005), cardiovascular disease (p=0.019), anxiety (p=0.002), obesity (p=0.019), hypertension (p=0.001), and palmoplantar psoriasis (p=0.028) were significantly associated with treatment escalation. Patients on topicals only at Visit 1 were more likely to undergo treatment escalation (80 v 20%), whereas those on systemics and biologics were less likely (39 v 61% and 23 v 77%). Those who underwent treatment escalation had more severe disease (PtGA 6.1 v 2.9; BSA 6.9% v 1.9%; PGA 2.5 v 1.3) and were more likely to have involvement of challenging body sites such as the groin (7.5 v 1.7%), genitals (9.6 v 2.6%), and gluteal cleft (16.3 v 6.4%) at Visit 1. As expected, those who escalated therapy saw larger improvements by Visit 3 (PtGA -2.5 v -0.2; BSA -3.4 v -0.2; PGA -0.9 v -0.1) than those who did not. This study highlights the feasibility and clinical relevance of using EHRs plus condition-specific data collected during clinical practice for conducting HSR studies.

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Non-invasively stratifying atopic dermatitis patients based on inflammatory genes

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Atopic dermatitis (AD) is a chronic inflammatory disease characterized by significant barrier disruption and intense pruritus. In recent years, there has been a growing number of targeted therapies in clinical development with a predominant focus on antagonizing Th2-mediated inflammation; however, these therapies are effective (IGA 0/1) in less than 50% of AD patients. We hypothesized that baseline expression of key inflammatory genes would identify potential subsets of AD patients for a more targeted therapeutic intervention with monoclonal antibody-based therapies. Epidermal skin samples were non-invasively collected from the lesional skin of 31 patients with moderate to severe AD using the 'smart sticker' adhesive skin collection kit . RNA was subsequently isolated and analyzed by RT-PCR for pre-identified genes important to AD disease pathogenesis. IL-4Ra and IL-13Ra1 genes were expressed in 100% of AD patients. Similarly, CCL17/TARC, a biomarker of AD disease severity, was expressed in 96.8% (30/31) of AD patients. Interestingly, the Th2 cytokine IL-13 was expressed in 54.8% (17/31) while IL-31 was expressed in 29.0% (9/31). Additionally, the Th17 associated genes IL-22 and IL-23 were expressed in 51.6% (16/31) and 58.1% (18/31), respectively. Overall, this study demonstrates the potential utility of non-invasive skin sampling to stratify AD patients based on their dominant inflammatory signature and suggests the incorporation of this clinically valuable technique in the personalized treatment of AD patients with targeted therapies.

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Discoid lupus and positive smoking history are negative predictors of disease activity remission in cutaneous lupus

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Little is known about disease remission and recurrence in patients with cutaneous lupus erythematosus (CLE). In a retrospective cohort study of 97 CLE patients, we assessed frequency of and factors associated with remission and recurrence in CLE. The primary outcomes were remission and recurrence of activity which were defined as reaching Cutaneous Lupus Erythematosus Activity and Severity Index activity (CLASI-A) equal to 0, and >1 (after remission) respectively. Time to remission and recurrence of activity was calculated by survival curve analyses. Variables that had a significant impact on time to remission were then used to perform a Cox proportional hazards model to identify variables that are predictive of shorter time to remission. Forty six patients (48%) reached remission of CLE activity within a median of 18 months (IQR: 11-32 months) from the initial visit. Patients who achieved remission were more likely to be lifetime non-smokers (65% v. 33%, p=0.002) and less likely to have discoid lupus erythematosus (DLE) (63% v. 88%, p=0.004). Cox proportional hazards regression model showed that both the absence of DLE (HR:4.20, 95% CI: 1.98-8.92) and lifetime non-smoker history (HR:2.57, 95% CI: 1.22-5.43) were independent predictors of remission; however, these factors did not significantly predict a shorter time to remission. Twenty nine patients (63%) of participants experienced disease recurrence within a median of 13 months (IQR: 7-19 months) from their remission date. Patients with recurrence of CLE activity had a longer disease duration prior to their baseline visit (p=0.002), and were more likely to have DLE (72% v. 29%, p=0.005). These findings support the notion that discoid lupus patients and smokers can be more refractory to standard-of-care treatments in CLE, and may be helpful for clinicians to guide CLE patients on their potential disease course.

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Anti-phosphatidylserine/prothrombin complex antibodies in patients with cutaneous vasculitis: Possible involvement in the pathogenesis

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Objective. It was previously demonstrated that cutaneous vasculitis, including IgA vasculitis and cutaneous arteritis (CA), is associated with the presence of IgM antibodies (Abs) against the phosphatidylserine/prothrombin complex (PS/PT). Recently, novel enzyme-linked immunosorbent assay kits for the detection of IgG and IgM anti-PS/PT (aPS/PT) Abs have become commercially available. Methods. The prevalence of serum IgG and IgM aPS/PT Abs in both cutaneous and systemic vasculitis was determined using these kits. In addition, to examine whether aPS/PT Abs were involved in the pathogenesis of cutaneous vasculitis, inbred wild-type rats were intravenously administered with a rat IgM class aPS/PT monoclonal Ab established previously or with rat immunoglobulins as controls. To express PS on the surface of vascular endothelium, these rats were given a subcutaneous injection of cell-free histones (250 μg/ml, 300 μl/site) 2 hours in advance. Results. Serum IgM aPS/PT Ab levels were elevated in patients with systemic vasculitis with skin involvement and CA compared to those in patients with systemic vasculitis without skin involvement and healthy controls. There was no significant difference in the serum levels of IgG aPS/PT Abs between the patients and healthy controls. Correspondingly, inbred wild-type rats intravenously administered with the aPS/PT monoclonal IgM Ab after appropriate priming—subcutaneous histone injection—developed cutaneous vasculitis. Some rats given rat IgM instead of the aPS/PT monoclonal Ab also developed cutaneous vasculitis, whereas vasculitis did not occur in rats given IgG or only priming by histones. Conclusion. IgM aPS/PT Abs could be involved in the pathogenesis of cutaneous vasculitis.

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Dupilumab normalizes expression of type 2 inflammatory genes

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Background: In a double-blind, placebo-controlled, phase 2 study (NCT02379052), adults with active eosinophilic esophagitis (EoE) were randomized 1:1 to receive 12 weeks of subcutaneous dupilumab 300 mg weekly (qw) or placebo. We analyzed the effect of dupilumab on the expression of type 2 inflammatory genes in the esophagus. Methods: Biopsies were collected from the proximal, mid, and distal esophagus at baseline and Week 12 (n = 41). Mean esophageal gene expression for each patient was compared with the published EoE and healthy transcriptomes. A relative ≥ 2-fold change from baseline, q ≤ 0.05, was considered significant. To test the effects of dupilumab on type 2 inflammatory genes, normalized enrichment scores (NES) based on curated gene sets were generated from this study and published studies of other atopic indications (atopic dermatitis [AD], asthma, chronic sinusitis with nasal polyps [CRSwNP]). Results: The type 2 inflammatory genes' NES were significantly increased in tissues from patients with AD/asthma/CRSwNP/EoE compared with healthy controls. Dupilumab 300 mg qw modulated 1,302 genes and significantly normalized the expression of genes associated with type 2 inflammation, eosinophils, and mast cells in EoE patients. The post-dupilumab transcriptome more closely resembled that of healthy controls. Conclusions: Dupilumab reversed the EoE disease transcriptional signature and normalized the expression of multiple genes upregulated across many type 2 inflammatory diseases, confirming the congruence of the mechanism of action of dupilumab and the mechanism of disease.

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Dermatologist and patient perspectives on implementing cardiovascular risk prevention in the management of psoriasis: A qualitative study

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Background: Psoriasis is an immune-mediated disease associated with excess risk for cardiovascular disease (CVD). Guidelines recognize psoriasis as a CVD risk enhancer; however, psoriasis patients often do not have CVD risk factors identified nor managed. This study examines strategies to improve CVD prevention care from the perspective of dermatologists and patients with psoriasis. Methods: Qualitative interviews were conducted using the Consolidated Framework for Implementation Research to examine the perspectives of dermatologists (N = 8) and patients with psoriasis (N = 8) on barriers/facilitators to CVD prevention. Interviews were transcribed and coded using an integrated approach designed to enhance reliability and validity using NVivo software. Findings: Most dermatologists confirmed that they were not regularly engaging in CVD prevention care with psoriasis patients. Reasons included a lack of familiarity or comfort with guidelines, concern about working outside of their scope of practice, confusing boundaries between other clinicians, and time constraints. Patients confirmed that it was uncommon for their dermatologists to engage them in CVD prevention care but expressed desire for their dermatologists inform them of the risk, and were open to CVD prevention care from them. Implications: These findings will inform the design of a clinical trial comparing the effectiveness of dermatologist implementation of CVD guideline-based counseling, screening and prescribing statins when appropriate in patients with psoriasis. Ultimately, this study aims to increase the lifespan and health of patients living with psoriatic disease by decreasing barriers to their receiving appropriate CVD prevention care.

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Acceptable delay between diagnosis and treatment of melanoma, cutaneous squamous cell carcinoma, and basal cell carcinoma

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BACKGROUND: There is a paucity of literature regarding the acceptable duration of delaying treatment after diagnosing various skin cancers. OBJECTIVE: To gather expert opinion on the number of days acceptable to delay melanoma and non-melanoma treatment after biopsy-proven diagnosis. METHODS: American College of Mohs Surgery (ACMS) members were surveyed about the number of days that they would delay treatment for melanoma, basal cell carcinoma, squamous cell carcinoma, and in-person skin checks, based on the following circumstances: (1) what they would want for a family member or close friend; (2) standard institution or office policy; (3) standard national policy; (4) in a pandemic when PPE is available; and (5) in a pandemic when PPE is unavailable. RESULTS: 389 participants completed the survey. For all skin cancer types, there was a multimodal distribution of responses in the number of days acceptable to delay treatment, and responses ranged from several days to nearly a year. Shorter delays were observed in response to more aggressive cancer types. CONCLUSIONS: There is significant heterogeneity in skin cancer experts' opinions of the acceptable number of days to delay treatment for melanoma and non-melanoma skin cancers. The results of this survey illustrate current perceptions on the *relative* acceptable delay in treating various skin cancer types.

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Sunscreen use and photosensitivity in lupus patients

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Ultraviolet radiation (UVR) exacerbates cutaneous lupus (CLE) and systemic lupus (SLE) symptoms. Patients are instructed to avoid UVR exposure and to use sunscreen daily. Most sunscreens contain organic UVR filters that can penetrate skin, enter systemic circulation and cause unintended biological effects. There is little known about sunscreen use by patients or the determinants that drive its use. A survey was conducted to determine if the frequency of sunscreen use differed between patient types and heathy controls (HC) and if the type of sunscreen (organic or mineral) used correlated with experiencing adverse effects (AE) from sun exposure (rash, flares, feeling sick). Subjects were recruited from clinics (RSRB# 35516) and database mailings (RSRB# 35516). We received 132 surveys between August 2018 and June 2019; 32.1% were HC and 67.9% were patients (29.2% CLE, 70.8% SLE). The survey elicited information on race, Fitzpatrick skin type, sunscreen type, SPF value, whether subjects experienced AE and their frequency of sunscreen use; where frequent use was defined as daily or at least 1x per week. Results find that the frequency of use differed between CLE and SLE patients and HC and whether or not patients reported suffering AE from the sun exposure. 71.8% of all patients suffer AE but frequent sunscreen use varied by race suggesting an opportunity to improve clinical care. HC that report using sunscreen frequently do so to prevent skin cancer whereas patients report usage based on physician advice as well as to prevent skin cancer. Frequent sunscreen users prefer products with SPF >30. Patients with AE are 2x more likely to use organic-based sunscreen. Patients without AE are 3x more likely to use mineral-based sunscreen. Since systemic levels of organic filters rise over time with daily use, it is plausible that sunscreen may contribute to disease pathology. Our on-going studies seek to examine if and how UVR filters contribute to immune cell abnormalities in lupus.

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Outcomes reported in clinical trials of facial aging: A systematic review

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BACKGROUND: It is difficult to compare interventions for facial aging due to heterogeneity in the outcomes used. Understanding the range of outcomes that are commonly reported in trials of facial aging interventions can help clarify the degree of heterogeneity that may exist. OBJECTIVE: To identify outcomes that have been measured in clinical trials of facial aging interventions. METHODS: A systematic review was performed for English-language randomized controlled trials and controlled clinical trials, using terms related to the appearance of facial aging treatments, between 2005-2015. Outcomes were extracted from included studies and categorized into domains based on shared themes. RESULTS: 216 articles were included in the systematic review. 179 outcomes were identified and categorized into 8 different domains: acceptance of care, adverse events/effects, clinical assessment, direct costs, patient satisfaction, patient perception of health, physiologic assessment, and quality of life. 193 studies (89.4%) reported outcomes in the clinical assessment domain, and 171 (79.2%) measured adverse event-related outcomes. The most commonly measured outcomes were "overall appearance of skin" (N=87, 40.3%), and "satisfaction with appearance" (N=71, 32.9%). Quality of life was measured in 22 studies (10.2%). CONCLUSIONS: There is significant heterogeneity in the outcomes measured in facial aging intervention studies. There is need for the selection of a core set of outcomes that would at minimum be reported in all future studies of facial aging.

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Tape-strips capture gene-expression changes in moderate-to-severe atopic dermatitis patients treated with dupilumab

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Although skin biopsy studies utilizing transcriptomic profiling have helped elucidate the immune and barrier dysregulation underlying atopic dermatitis (AD), the invasive nature of biopsies limits their use in larger and longitudinal studies. Tape-strips are emerging as a minimally invasive alternative for the study of AD skin, but they have not yet been used for tracking gene-expression changes with systemic treatment. In this real-life study, we evaluated transcriptomic changes and therapeutic-response biomarkers in AD patients treated with dupilumab (IL-4Rα antibody) using tape-strips, by performing RNA-seq on lesional and non-lesional tape-stripped skin from 18 AD patients before and after 16 weeks of dupilumab therapy as well as from 17 healthy controls. At baseline, we detected 6,745 and 4,859 differentially expressed genes (DEGs) between lesional and nonlesional skin versus normal respectively, while after treatment, we detected 841 and 977 DEGs respectively (fold-change/|FCH|>1.5 and false-discovery-rate/FDR<0.05). Tape-strips captured significant changes in important AD immune (e.g. CCL13, CCL17, CCL18) and barrier (e.g. PPL, FA2H, PSORS1C2) biomarkers with dupilumab therapy. Changes in biomarkers (CCL20, IL-34, FABP7) were also significantly correlated with clinical disease improvements (EASI) (R>0.5 or R<-0.4, p<0.05). Overall, this study is the first comprehensive RNA-seq molecular profiling of tape-strips from moderate-to-severe AD patients treated with dupilumab, revealing that tape-strips capture significant transcriptomic modulations in key AD biomarkers with dupilumab and may provide a helpful approach for tracking therapeutic responses in AD.

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Demographic and clinical factors associated with patient-reported remission in psoriasis

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Achievement of remission in psoriasis is a key goal for patients and clinicians, yet definitions of remission vary. Some treat-to-target initiatives in psoriasis have focused on degree of skin involvement, while others have also incorporated quality of life (QoL) measures. The goal of this study is to identify factors associated with patient-reported psoriasis remission. The National Psoriasis Foundation conducted a survey within a random stratified sample of 1,570 individuals with psoriatic disease in the United States. Participants provided demographics and were asked about a provider diagnosis of psoriasis, psoriatic arthritis, or both. Psoriasis severity was assessed using the Patient Report of Extent of Psoriasis Involvement (PREPI), a validated self-reported measure of body surface area (BSA). Individuals reporting BSA ≤ 3% were asked if they felt their psoriasis was in remission and provided information on comorbidities and QoL. Multivariate logistic regression was used to identify factors associated with remission. Of 929 participants reporting BSA ≤ 3%, 479 (51.6%) felt their psoriasis was in remission, with an average remission duration of 31 months. Of those in remission, 79.1% reported current treatment. Multivariate regression revealed that psoriasis remission was independently associated with female sex, lower BSA, less impairment in the Dermatology Life Quality Index and Global QoL, biologic use, and concomitant diagnosis of psoriatic arthritis. There was no association with age, race, body mass index, or number of comorbidities. Overall, patient perception of psoriasis remission was not solely associated with BSA, but also with sex, quality of life, and treatment type.

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Immune and barrier characterization of atopic dermatitis skin phenotype in Tanzanian patients

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Atopic dermatitis/AD is a common disease, with high prevalence seen in Africa. It is increasingly recognized that AD patients of different ethnic backgrounds have unique molecular signatures in skin, potentially accounting for treatment response variations. However, the skin profile of AD patients from Africa is unknown, hindering development of treatments targeted to this population. The goal of this study was to characterize the skin of AD patients from Africa. We used gene-expression studies including RNA-sequencing (using threshold of fold-change/FCH>2 and false discovery rate/FDR<0.05) and real-time PCR on skin biopsies of Tanzanian moderate-to-severe AD patients and controls. Our results found that Tanzanian AD skin had robust upregulations of multiple key mediators of both Th2 (IL13/IL10/IL4R/CCL13/CCL17/CCL18/CCL26) and Th22 (IL22/S100As) pathways. Markers related to Th17/IL23 (IL17A/IL23p19/IL23A/IL23p40/IL12/PI3/DEFB4B) and Th1 (IFNγ/CXCL9/CXCL10/CXCL11) were also significantly overexpressed in AD tissues, but to a lesser extent. IL36 isoforms showed upregulations in African skin. The barrier fingerprint of Tanzanian AD showed no suppression of hallmark epidermal barrier differentiation genes, such as filaggrin, loricrin, and periplakin, with robust attenuation of lipid metabolism genes (i.e AWAT1). The skin phenotype of Tanzanian AD patients is consistent with that of African-Americans, exhibiting Th2/Th22-skewing, minimal dysregulation of terminal differentiation, and even broader attenuation of lipid metabolism-related products. These data highlight the unique characteristic of AD in black individuals, as well as the need to develop unique treatments targeting AD patients in underrepresented populations.

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Association between atopic dermatitis and headaches throughout childhood and adolescence – A longitudinal study

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Atopic Dermatitis (AD) is associated with sleep disturbance, psychosocial distress, anxiety, depression, and atopic comorbidities, which may be associated with increased headaches. We aimed to understand the association of AD and comorbid asthma, sleep and mental health disturbances with headaches throughout childhood and adolescence. Data were analyzed from The Fragile Families and Child Wellbeing Study, a longitudinal birth cohort study of 4898 urban children born in 1998-2000. AD was associated with headaches at age five (adjusted odds ratio [95% confidence interval]: 2.14 [1.27-3.59]), nine (1.69 [1.27-2.27]) and fifteen years (1.71 [1.37-2.14]). AD at age 9 was associated with higher odds of subsequent headaches at age 15 (1.36 [1.05-1.76]). Children with AD at two (1.60 [1.12-2.29]) or all three (1.79 [1.16-2.75]) study-waves had higher odds of headaches at age 15. In multivariable repeated measures logistic regression models, significant two-way interactions were found for AD with sleep disturbance (4.59 [3.15-6.69]), attention deficit (hyperactivity) disorder (2.85 [1.87-4.35]), asthma (2.87 [2.18-3.76]), anxiety (2.47 [1.76-3.48]) or depression (2.86 [1.89-4.34]) as predictors of headaches. In conclusion, children and adolescents with AD, particularly those with sleep disturbances, atopic and mental health comorbidities, had increased headaches. Persistent childhood AD was associated with headaches in adolescence.

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Treatment of refractory cutaneous Crohn's disease with ustekinumab

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Standardized treatment guidelines for the management of cutaneous Crohn's disease (CD) are limited. Ustekinumab is a biologic agent targeting the shared IL-12/IL-23 receptor and is approved for treatment of moderate to severe CD and plaque psoriasis. Although ustekinumab has been shown to have higher efficacy than anti-TNF biologic agents in the treatment of psoriasis, there is limited information regarding efficacy in the treatment of cutaneous CD. In a prospective cohort of 4215 patients with inflammatory bowel disease at a tertiary center, three patients with biopsy-proven cutaneous CD were identified. We sought to characterize the clinical course and management of cutaneous CD. Patients were young (mean age 31.2 years), two patients were female and one patient had tobacco exposure. The anatomic location of CD was perianal in all three patients, with two patients exhibiting perineal cutaneous CD involvement and a female patient with metastatic CD of the vulva as well. All three cutaneous CD patients had extensive exposure to anti-TNF therapy, including trials of multiple agents with clinical failure. Two of the three patients with cutaneous CD received ustekinumab and both achieved clinical remission, despite prior failure of anti-TNF therapy. The remaining patient who had failed to achieve remission with anti-TNF therapy suffered premature death linked to opioid analgesics prior to attempting ustekinumab therapy. In our institutional experience, anti-TNF therapies were not effective as a first-line treatment approach for biopsy-proven cutaneous CD. Improved clinical outcomes with ustekinumab suggest that this biologic may be better positioned as a first-line agent for cutaneous CD. Further comparative effectiveness studies are needed to better determine an optimal therapeutic approach to cutaneous CD at the time of diagnosis.

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Evaluation of the toxicity of glucocorticoids in patients with autoimmune blistering disease (AIBD) using the Glucocorticoid Toxicity Index (GTI)

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Background: Glucocorticoids(GC) are the mainstay of treatment for autoimmune blistering diseases(AIBD), which are associated with a myriad of adverse effects(GCAE). There is no standardised scoring system used in trials and clinical settings to directly quantify and monitor GCAE. The Glucocorticoid Toxicity Index(GTI) is a newly developed, outcome-based GCAE monitoring instrument. However, the GTI has not been applied to real patients with AIBD in the clinical setting. Objectives: To apply the GTI to patients with AIBD for the first time and to investigate if the GTI score was able to quantify the GC-induced toxicity accurately and specifically in this patient group. Methods: This cohort study included patients with confirmed diagnoses of AIBD and history of GC exposure. The parameters required for GTI calculation were collected at two visits with a minimum interval of three months. Patients were classified into two groups for statistical analysis based on the treatment: currently receiving GC(Group1) or had GC ceased earlier(Group2). Results: Sixteen and eleven Patients were included in Group 1 and Group 2, respectively. The GTI scores were linearly correlated with both cumulative and average daily PRED doses (P<0.05). One-way ANOVA and Kruskal-Wallis H analysis showed a significant difference in GTI scores between the two groups was found (p<0.05). No significant correlation was found between the GTI scores and patients' quality of life scores. Conclusion: The GTI sensitively and specifically captured changes in GC toxicity over time among AIBD patients, both improvement and worsening, while not being confounded by other factors. The GTI could be a feasible tool to be used in future clinical trials as a GC-induced toxicity outcome measure.

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Outcomes in hospitalized patients with cutaneous T-cell lymphomas

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Introduction: Studies suggest that hospitalization of patients with cutaneous T-cell lymphomas (CTCL) is common. However, reasons for hospitalization are unknown, bacteremia/sepsis and pneumonia were identified in the nineties as infectious causes for hospitalization, and it has been described that patients with CTCL are at higher risk of having concomitant diseases known to lead to hospitalizations, such as cardiovascular disease. But there is a gap in knowledge ascertaining hospitalization characteristics and outcomes. Methods: Differences in demographics, clinical findings, and hospitalization course by in-hospital mortality and one-year post-discharge mortality among CTCL patients admitted to Moffitt Cancer Center and Tampa General Hospital between June 2016 to June 2020 were analyzed. Results: In our CTCL cohort of 27 patients, we observed 11.1% inpatient mortality and 37.5% mortality at one-year post-discharge. Most hospitalized patients were male (80.8%), Caucasian (73.1%), had advanced disease stage (96.2%), had low ECOG performance scores, and required intravenous antibiotics to treat serious skin as well as systemic infection. Median hospital length of stay was 8 days, with IQR 1-29. Higher body mass index (BMI) was associated with inpatient mortality (median=36, IQR=28-53.5). History of hematologic malignancies (33.3%), anxiety or depression (44.4%), and longer duration between time from CTCL diagnosis to inpatient admission (median years=5.7, IQR=1.8-14.3) were associated with mortality at one-year post-discharge. We also observed poorer one-year post-discharge survival duration by solid cancer history, respiratory comorbidity history, and presence of large cell transformation. Discussion: In this cohort study of patients with CTCL, CTCL was associated with high in-hospital and one-year post-discharge mortality. A high BMI, comorbid malignancies and psychiatric disease were associated with poorer outcomes. Efforts should focus on addressing these comorbidities to improve risk stratification, guide work-ups and therapeutic modalities, and ultimately improve survival outcomes.

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The molecular features of normal and atopic dermatitis skin in infants, children, adolescents and adults

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Although atopic dermatitis/AD often presents in infancy and persists into adulthood, comparative characterization of AD skin among different pediatric age-groups is lacking. This study aimed to define skin biopsy profiles of lesional and nonlesional AD across different age-groups (0-5 y/o infants with disease duration <6 months, 6-11 y/o children, 12-17 y/o adolescents, ≥18 y/o adults) versus age-appropriate controls. We performed gene expression analyses by RNA-sequencing and real-time polymerase chain reaction and protein expression analysis using immunohistochemistry. Our results found that Th2/Th22-skewing, including IL13, CCL17/TARC, IL22 and S100As characterized the common AD signature, with a global pathway-level enrichment across ages. Specific cytokines IL33, IL1RL1/IL33R and IL9, often associated with early atopic sensitization, showed greatest upregulations in infants. Th17 inflammation presented a two-peak curve, with highest increases in infants, followed by adults. Th1 polarization was uniquely detected in adults, even when compared to adolescents, with significant upregulation in adults of IFNγ and CXCL9/CXCL10/CXCL11. While all AD age groups had barrier abnormalities, only adults had significant decreases in filaggrin expression. Despite the short duration of the disease, infant AD presented robust downregulations of many barrier genes in both lesional and nonlesional skin. Clinical severity scores significantly correlated with Th2/Th22-related markers in all pediatric age-groups. The shared signature of AD across ages is Th2/Th22-skewed, yet differential expression of specific Th2/Th22-related genes, other Th-pathways, and barrier-related genes portray heterogenetic, age-specific molecular fingerprints.

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Treatment of patients experiencing dupilumab facial redness with itraconazole and fluconazole: A single institutional retrospective medical record review

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Intro: Dupilumab facial redness (DFR) is a recently described adverse event of dupilumab use. In this retrospective study, we investigated the management of DFR with itraconazole or fluconazole as a potential treatment for DFR. Methods: Inclusion criteria were: patients on dupilumab for at least 16 weeks, had a diagnosis of DFR, and completed at least two weeks of fluconazole or itraconazole. Investigator global assessment (IGA) before and after azole use was recorded, and self-reported improvement (%) was determined via a follow-up call two weeks after azole initiation. Results: Out of 413 patients prescribed dupilumab, 22 (5.3%) patients were diagnosed with DFR and prescribed an azole. Of 16 patients completing a course of itraconazole, 11 (69%) had a post-treatment IGA of clear or almost clear (0 or 1), and the average self-reported improvement was 52%. Of the four patients treated with fluconazole, none (0%) had an IGA of clear or almost clear, and self-reported improvement of patients on fluconazole was 0%. Of particular note is that one of the patients who did not improve on fluconazole did note improvement on itraconazole. Discussion: The treatment mechanism of azoles for DFR is unknown. Study limitations include the retrospective nature, small number of patients, and the subjective reporting of improvement by patients. Our study provides further, preliminary evidence that itraconazole may improve DFR.

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Effects of anti-calcitonin gene related peptide migraine medications on psoriasis and atopic dermatitis severity

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Calcitonin gene-related peptide (CGRP), a neuropeptide on endothelial and immune cells, is found at elevated levels in individuals with migraines, psoriasis (PS), and atopic dermatitis (AD). Recently, three anti-CGRP medications were approved for migraine prophylaxis. Few studies have investigated the effects of these medications on other diseases. We hypothesized that anti-CGRP medications may improve AD or PS. Using retrospective analysis of electronic medical records (EMR), we identified patients diagnosed with migraines and AD or PS (ICD-10) who were prescribed anti-CGRP medications. Subjects were then interviewed by phone to gather subjective assessments of changes in their migraines and skin disease with anti-CGRP medications. Nineteen subjects were identified (10 (52.6%) = AD; 9 (47.4%) = PS), of which 13 consented (8 (61.5%) = AD; 5 (38.5%) = PS) to the phone interview. Three subjects (2 = AD; 1 = PS) reported no duration of anti-CGRP treatment and did not complete the interview. Average duration of anti-CGRP treatment was 7.2 ± 6.2 months, with 72.7% of subjects reporting improvement in migraines and only AD subjects reporting improvement in their skin disease (2/6 (33%) = AD; 0/4 (0%) = PS). No PS subjects reported improvement in their skin disease. One AD subject reported 40% improvement in itch; another reported 100% improvement in pain. Half of AD subjects reported fewer physician visits for their skin disease. Overall, we observed a modest reduction in AD severity with the use of anti-CGRP medications in subjects with migraines and AD. These results encourage further investigation of anti-CGRP medications for AD patients, especially those who primarily experience pain as a symptom of their disease. We are optimistic that as more patients are prescribed anti-CGRP medications for migraines, more robust data will become available to verify our findings.

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Topical hypericin ointment photodynamic therapy is effective and safe in CTCL (FLASH study)

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Additional cutaneous T-cell lymphoma (CTCL) therapies with better short/long term side effects are needed. Topical synthetic hypericin ointment 0.25% (SGX301) activated with external cool-white visible light is a novel, non-mutagenic photodynamic therapy. We conducted a randomized, placebo-controlled, observer-blinded multicenter Phase 3 trial evaluating its efficacy/safety in early stage IA-IIA CTCL across 37 U.S. sites. SGX301 was applied to 3 index lesions twice weekly, 18-24 hours prior to light therapy for a 6-week cycle for 3 treatment cycles. Cycle 1 (169 patients randomized 2:1 SGX301:placebo) and Cycle 2 (all received SGX301) were required; Cycle 3 (index and additional lesions treated with SGX301) was optional. Index lesion response rate (ILRR) and adverse events (AEs) were assessed 2 weeks after each cycle then monthly for 6 months. The trial primary endpoint was ILRR based on the Composite Assessment Index for Lesion Severity (CAILS) score with improvement $\geq$ 50% over baseline. After Cycle 1, ILRR for SGX301 vs placebo were 16% vs 4% (p=0.04). ILRR for subjects who received 2 cycles of SGX301 was 40% (p<0.0001) and 49% (P<0.0001) after Cycle 3. SGX301 was effective for both patch (42%, p<0.0001 after 2 cycles) and plaque (37%, p=0.0009) lesions. The most common AEs were Grade 1-2 local application site skin reactions (15% of subjects) and only 1% of subjects discontinued the study due to AEs. No drug-related serious AEs occurred and SGX310 was not found systemically. SGX301 is effective in early stage CTCL with a highly favorable safety profile.

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Defining flares in cutaneous lupus erythematosus using the cutaneous lupus erythematosus disease area and severity index (CLASI)

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Flare has been defined for systemic lupus erythematosus (SLE) and used as an endpoint in clinical trials, but has not been defined for cutaneous lupus erythematosus (CLE). Definitions of improvement have been established in CLE using the Cutaneous Lupus Erythematosus Disease Area and Severity Index score (CLASI) (scored 0-70), but definitions of worsening have not. We sought to determine the change in CLASI activity (CLASI-A) score that corresponds to a meaningful flare in disease activity. In this retrospective study of our longitudinal database, we correlated change in CLASI-A with change in physician assessments of skin activity (PGA-A) (scored 0-10) and quality-of-life measure Skindex29+3 (scored 0-100). Twenty-five patients across 118 study visits were included, and each contributed 3-8 visits with documented instances of worsening of PGA-A $\geq$ -2 between visits as well as 2 visits with no change in PGA-A. Using a linear mixed effects model adjusted for time effect and within-subject correlation, each graded worsening in the patients' PGA-A score corresponded to a CLASI-A score increase (worsening) of 3.08 ($p = 8x10^{-13}$). Additionally, for each unit decrease in PGA-A, Skindex29+3 Symptoms (S) and Emotions (E) subset scores increased 4.09 (p = 0.0001) and 3.9 (p = 0.0003), respectively. Using a gold standard flare definition of PGA-A decrease of 2, a change in CLASI-A of $\geq$ 7 represents a flare. This definition is strengthened by the associated change of Skindex-S and -E of 8.18 and 7.8 points respectively, which is in agreement with previous definitions of meaningful change in these measures. This CLE flares definition will be a useful endpoint for measuring response to or failure of drugs in clinical trials, indicating need for medication change, and charting disease progression.

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The Ichthyosis Scoring System (ISS): Development and validation of a novel ichthyosis severity assessment instrument

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Background: Ichthyosis clinical trials require reliable, validated severity assessments to identify appropriate subjects and quantify treatment outcomes. There is no validated scale to measure ichthyosis severity across the entire body. *Objective:* To create and validate a comprehensive and user-friendly instrument to measure total body ichthyosis severity in adults and children. *Methods:* We divided the body into 10 regions to score special regions of interest. Likert scales (0-4) were established to quantify scale and erythema, with descriptors and photographic standards. An 83-image teaching set was created from photographs of ichthyosis patients, Six dermatologists scored all test photographs twice to evaluate intra-rater reliability. Intra-class correlation coefficients (ICCs) determined the overall reliability of our instrument. To examine the impact of training on reliability, six new dermatologists are being trained on proper ISS usage before beginning testing using test photographs administered to the initial six dermatologists. Reliabilities with and without training will be compared. *Results:* Based on the first 6 dermatologists, the ICC for combined scale and erythema scores across the entire body is 0.903 (95% CI, 0.77-0.974). ICCs for scale and erythema subscores are 0.911 (95% CI, 0.789-0.976) and 0.882 (95% CI, 0.723-0.968), respectively. Body sites exhibited moderate-good inter-rater reliabilities for scale, except elbows and lower extremities. Intra-rater reliabilities were excellent (ICC >0.9). *Limitations:* Unable to conduct live testing. *Conclusions:* The ISS is validated as a comprehensive tool for assessing ichthyosis severity across the entire body. The next step is determining the impact of rater training on improving reliability and consistency.

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Factors impacting likelihood of discontinuing immunosuppression in dermatomyositis: A single-center study

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Dermatomyositis (DM) is a chronic idiopathic inflammatory myopathy typically requiring chronic immunosuppressive therapy, but little is known regarding factors related to the likelihood of discontinuing these medications. We conducted a retrospective cohort study of our Stanford cohort of 257 patients with a median follow-up time from disease onset of 4.9 years and a median time for medication discontinuation of 5.1 years. Log rank analysis indicated that patients with a clinically amyopathic course (p=0.034) or DM-specific autoantibodies (p= 0.010) got off of medications significantly earlier than their counterpart populations. Within the latter group, those with anti-NXP2 or anti-SAE1 autoantibodies discontinued medication more rapidly than those with anti-TIF1-γ autoantibodies (p=0.039 and p=0.038, respectively). In addition, non-Hispanic patients trended towards discontinuing medications earlier than Hispanic patients (p=0.077). Cox proportional hazards regression modeling demonstrated hazard ratios of 0.35 (0.17-0.72), 3.41 (1.37-8.46), and 3.62 (1.45-9.06) for relative risk of discontinuing medication for the clinically amyopathic, anti-NXP2, and anti-SAE1 groups, respectively. Our data demonstrate DM patients placed on immunosuppressive therapy take many years to discontinue medications (median 5.1 years) and suggest that demographic, serologic, and clinical factors are associated with medication cessation.

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Health disparities in clinical trials for mycosis fungoides/Sézary syndrome: A systematic review

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Despite the growing evidence of health disparities in cancer clinical trials, there is a dearth of similar research in clinical trials for mycosis fungoides (MF)/Sézary syndrome (SS). We sought to determine whether participants in clinical trials for MF/SS would differ demographically, either by age, gender, or race, compared to the United States (US) population of MF/SS patients. Per the Surveillance, Epidemiology and End Results (SEER) database, the incidence of MF/SS in the US is highest between ages 70-79. Incidence is also higher in males, with a male: female (M:F) ratio of 1.57, and in black patients, with a black: white (B:W) ratio of 1.55. A systematic review was performed of all studies in MEDLINE via PubMed, EMBASE, Scopus, and Cochrane Controlled Register of Trials of phase II, III, and IV clinical trials for MF/SS in the US from 1984 – 2020. Two reviewers performed title/abstract review and data extraction. Forty studies met inclusion criteria, comprising 1,496 participants with a median age of 61. There were 839 (56%) males and 632 (42%) females, with a M:F of 1.32. All trials included participants' age, 39 (98%) included gender, but only 26 (63%) included race. There was no significant difference in the reporting of race between articles published before and after 2000 (p=0.07) when calculated using chi-square. In trials in which race was specified, there were 205 (21%) black and 863 (77%) white participants, with a B:W ratio of 0.23. In a sub-analysis of clinical trials that led to FDA-approved medications, eight trials qualified. The median age of participants was 61. The M:F ratio was 1.1. Of the six (75%) trials that included data on race, the B:W ratio was 0.1. In conclusion, although the gender of MF/SS patients is adequately exemplified in clinical trials, there is a discrepancy in age, with trials favoring younger patients. More prominently, there remains a great disparity in race, particularly among black patients. The limited reporting of race in clinical trials highlights the need to specify racial backgrounds to ensure equitable representation.

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A superfacial ulcer on the scrotum

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A 54-year-old man presented to the dermatology clinic with an itchy red papule on the right scrotum for 2 years, which gradually expanded to a yellow central ulcerated plaque and the crust was not easy to be removed. Topical mupirocin and corticosteroids were not effective. Fluorescent microscopy for fungus was negative, as well as antibodies to syphilis and HIV. There was no similar history in his family, and he had hypertension for 14 years, type 2 diabetes for a year with normal renal function. Biopsy revealed epidermal necrosis and crust with transepidermal penetration and elimination of collagen. Therefore, the diagnosis was acquired reactive penetrating collagenosis (ARPC). Follow-up for 3 months after resection without recurrence. Reactive perforating collagenosis is a rare skin disease that includes both inherited and acquired forms. ARPC is associated with diabetes, chronic renal failure, hyperuricemia and hypertension. It usually occurs on the trunk and limbs, characterized by multiple umbilicated hyperkeratotic papules or nodules with Koebner's phenomenon clinically, but no scrotal lesions have been reported.

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Organ-specific toxicity of Romidepsin in patients with pre-existing cardiac, renal, and hepatic disease: A retrospective analysis

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Background: Romidepsin is a histone deacetylase inhibitor (HDACi) used in the treatment of non-Hodgkin's and Hodgkin's lymphomas. Initial clinical trials of romidepsin excluded patients with pre-existing cardiovascular, hepatic, and renal disease (1,2). The aim of this study was to investigate the incidence of adverse cardiovascular, hepatic, and renal events in patients with relevant pre-existing conditions receiving romidepsin. Methods: The medical records of patients at Columbia University Irving Medical Center from 2010-2020 who received >1 dose of romidepsin with 6 months of follow-up data were retrospectively reviewed. Data collected included demographics, clinical data, and adverse events both while receiving romidepsin and 6 months following the end of therapy. Results: There were 43 patients who met the study criteria. Mean patient age was 57.39 (range: 23-83); the cohort was 37.2% female (n=16). The mean number of cycles of romidepsin therapy received by study patients was 6.02 (range: 1 to 21); dosages ranged from 10mg/m2 to 14mg/m2. Pre-existing cardiovascular disease was observed in 17/43 (39.5%) patients. In total, 4/43 (9.3%) patients experienced cardiovascular adverse events during the review period. Of these patients, 2/4 had pre-existing cardiovascular disease. No significant difference was observed in the relationship between cardiovascular adverse events while receiving romidepsin and pre-existing cardiovascular disease (p= 1.0). Furthermore, no patients with pre-existing hepatic or renal disease experienced relevant adverse events during the observation period. Adjustment for combination therapies and number of cycles received did not affect significance. Conclusions: Our results suggest that patients on romidepsin therapy with pre-existing cardiovascular, hepatic, and renal disease may not have a greater risk of adverse cardiac, hepatic, renal events than patients without pre-existing cardiovascular disease.

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Discerning patient perspectives towards specific treatments of alopecia areata using artificial intelligence

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Despite numerous treatments for alopecia areata (AA), clinical efficacy may not always match patient perceived efficacy due to cost, side effects, etc., creating a potential disconnect between patient and provider. Due to the significant emotional and psychosocial burden associated with AA, patients frequently use social media to discuss their disease. We analyzed social media posts about AA treatment and identified treatment-specific associations between Patient Global Impression of Change (PGIC) and Patient Global Impression of Treatment Satisfaction (PGITS). Delving into the background of these patient sentiments should provide insight into disease burden and perceived efficacy of treatment for AA. Using the Brandwatch Artificial Intelligence-powered database, we identified publicly available social media posts about AA. EmoLex was used to categorize the underlying emotion of our identified posts. Natural language processing was used to analyze emotional response in relationship to PGIC following treatment. We identified 688,992 AA-related posts. Of these, 23,342 were full-text posts related to treatment of AA. As an example, 5172 full-text posts were identified for minoxidil of which 1793 indicated hair growth despite "disgust" as an underlying emotion. Analysis of these posts showed that AA returned within 1 week of stopping minoxidil, perhaps contributing to "disgust." For several other AA treatments, we identified discrepancies in which PGIC did not match expected emotional response to treatment. Further research will identify terms used in these posts that may reveal the basis for the emotion linked to a specific treatment response. Insight from our analysis could help providers and pharmaceutical companies address patient needs.

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Randomized, double-blind, placebo-controlled study of efficacy and safety of secukinumab to treat adults with ichthyoses

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Therapy for congenital ichthyoses remains unsatisfactory. Ichthyoses, characterized by barrier impairment with cutaneous erythema and scaling, share Th17 immune skewing, as in psoriasis, leading us to hypothesize that targeting IL-17A could reduce ichthyosis severity. Adults with ichthyosis were randomized 1:1 to receive 300 mg of secukinumab, an IL-17A inhibitor, or placebo every 4 wks in a 16-wk dual-center, double-blind trial, followed by 16-wk open-label and 20-wk extension phases for safety. Co-primary endpoints were: i) difference in Ichthyosis Area Severity Index (IASI) (p<0.05) in secukinumab- vs. placebo-treated subjects at Wk16 (efficacy); and ii) lack of increased mucocutaneous bacterial and/or fungal infections (safety). Key secondary efficacy endpoints included reduction in other severity and patient-reported outcome scores at Wk16 and Wk32. Of 20 subjects ≥18yo with genotype-confirmed epidermolytic ichthyosis (n=4), Netherton syndrome (n=5), lamellar ichthyosis (n=6), or congenital ichthyosiform erythroderma (n=5) and at least moderate erythroderma, 18 completed the double-blind phase. The safety endpoint was met. No significant differences in severity or patient-reported outcomes measures were noted at Wk16 overall. The secukinumab-first group had a reduced IASI erythema subscore (p=0.04) and Visual Index for Ichthyosis Severity (VIIS) (p=0.01) at Wk32 (16 wks on secukinumab), correlating with a significant diminution in Th17-related biomarkers in biopsied skin. The subset of subjects who reported improvement and continued secukinumab post-study (28%) had decreased total IASI (median, -36%), IASI-E (-37%), and IASI-S (-37%) by Wk32. These data suggest that only a subset with ichthyosis respond to Th17 inhibition, requiring further predictive analyses.

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Spirulina use and its temporal association with dermatomyositis exacerbation

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The immunostimulatory effects of complementary and alternative medicine (CAM) may lead to the exacerbation of autoimmunity. As a result, there is a need to characterize the temporal association between immunostimulatory CAM use and autoimmune skin diseases. We performed a nested retrospective study of prospectively-collected CAM usage data at UPenn. Patients with dermatomyositis (DM), cutaneous lupus erythematosus (CLE), autoimmune blistering disease (AIBD), and healthy controls (HC) without autoimmune disease were surveyed for history of immunostimulatory CAM usage (Spirulina, Chlorella, Alfalfa, Green Algae, Echinacea) and dates of autoimmune disease onset/flare. Analysis of herbal supplement use in autoimmune patients compared to HC was performed using Fisher exact tests at a significance level of 0.05. Temporal analysis from CAM use to disease onset/flare was performed with a Kaplan-Meier survival analysis and log-rank test, censored at 2 years, at a significance level of 0.05. 450 patients were enrolled, including 158 DM, 122 CLE, 31 AIBD, and 139 HC. CAM use was significantly higher among patients with DM compared to HC (19.6% vs. 5.0%, p=0.0002), driven by the increased use of spirulina among DM patients (14.6% vs. 4.3%, p=0.0031). In contrast, CAM use was not significantly greater in CLE or AIBD (p > 0.05). Among the patients who were able to recall duration of Spirulina use, 36.8% of DM patients (7/19) had onset/flare of disease within 2 months of starting Spirulina. 52.6% of DM patients (10/19) had onset/flare within 1 year. This temporal relationship was statistically significant compared to the HC cohort (p=0.009). These results suggest that the increased prevalence of Spirulina in DM is temporally associated with the onset/flare of disease. Patients with DM should be made aware of the risk of Spirulina consumption.

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Atopic dermatitis is not associated with maternal alcohol use or alcohol use during adolescence

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Multiple environmental risk factors contribute towards atopic dermatitis (AD) prevalence and persistence. Maternal alcohol consumption during pregnancy may have pro-inflammatory effects leading to AD in their offspring. Moreover, AD is associated with chronic sleep disturbance, psychosocial distress, stigma, social isolation, anxiety and depression, which might lead to increased alcohol consumption in children and adolescents. We sought to understand the association between 1. maternal alcohol consumption during pregnancy and childhood AD; 2. AD and alcohol use in adolescents. We used data from the Fragile Families and Child Wellbeing Study, a longitudinal US birth cohort study of 4898 urban children. Maternal alcohol use during pregnancy was not associated with the development of AD in offspring at ages 5 (logistic regression; adjusted OR [95% CI]: 1.01 [0.72-1.41], P=0.95) or 9 (0.92 [0.68-1.25], P=0.70). There was a cross-sectional association between maternal alcohol use in the past year and AD at ages 5 (1.30 [1.06-1.60], P=0.04) and 9 (1.50 [1.23-1.82], P=0.0007). There were no associations between paternal alcohol use in the past year and AD at ages 5 (0.80 [0.63-1.02], P=0.12) or 9 (0.79 [0.62-1.00], P=0.12). At age 15 years, AD was not associated with increased alcohol use (1.64 [0.83-3.23], P=0.22). In conclusion, there was no association between the alcohol use during pregnancy and development of childhood AD. Childhood AD was not associated with increased alcohol use in adolescence but was associated with increased maternal alcohol consumption in childhood.

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Correlation of the peripheral blood CD4/CD8 ratio with the disease stage and overall survival in mycosis fungoides

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Mycosis fungoides (MF) is the most common type of cutaneous T-cell lymphoma. The cutaneous manifestation of MF ranges from a patch stage to a more severe plaque or tumor stage. A hallmark of MF is an increased CD4+ T cell population, which results in a high CD4/CD8 ratio. Previous studies have shown the CD4/CD8 ratio is a prognosticator of response to radiation therapy, but the prognostic value of the CD4/CD8 ratio for disease progression or overall survival in MF patients remains unclear. Here, we investigated correlation of the peripheral blood CD4/CD8 ratio with the disease stage (patch, plaque or tumor) and overall survival in 18 MF patients. We monitored disease progression clinically and performed serial peripheral blood flow cytometry over an average follow up time of 79 months. A total of 85 data points of CD4/CD8 ratio were collected (12 in patch, 59 in plaque and 14 in tumor stage). A Student's t-test showed no difference in the CD4/CD8 ratios between patch and plaque stage MF (P-value=0.3) or between patch and tumor stage MF (P-value=0.6). When the CD4/CD8 ratio was categorized to high or low using a cutoff of 5, there was no difference in CD4/CD8 ratios in the three MF stages based on a Fisher's test. Four of the 18 patients progressed to tumor stage; all of them had an initial CD4/CD8 value lower than 5. When patients were stratified by the CD4/CD8 ratio at the time of MF diagnosis or the earliest time after diagnosis, no significant difference in Kaplan-Meier overall survival was overserved between the two groups (P-value=0.4). To conclude, a high CD4/CD8 ratio did not correlate with poorer outcome in MF. The CD4/CD8 ratio did not correlate with the disease stage. The clinical significance of a high CD4/CD8 ratio needs further investigation, but our study is limited by a small cohort.

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Calcipotriene 0.005%/betamethasone dipropionate 0.064% foam as a treatment for nail psoriasis: A case series

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Combination topical corticosteroids and vitamin D analog treatments for nail psoriasis are widely used in cream and ointment vehicles, but patients may prefer a foam vehicle due to its ease of application and favorable cosmetic appearance. Calcipotriene 0.005%/betamethasone dipropionate 0.064% foam (Cal/BD) is an FDA approved therapy for plaque psoriasis, but may also be an effective treatment for nail psoriasis in a novel aerosol foam. We assessed the clinical response of mild to moderate nail psoriasis to treatment with Cal/BD in a case series of three patients in a single-center, secondary care clinic. Patients applied Cal/BD 1-2 times daily to affected nails for at least 4 months. All 3 patients (1 male and 2 female patients; mean age, 49.7 years [range, 42-60 years]) responded positively to treatment with Cal/BD. Remarkable reduction of nail plate surface abnormalities and a decrease in inflammation of the nail folds were assessed with clinical evaluation and dermoscopy, and documented with serial photography. The treatment was well tolerated and no adverse effects were noted for any of the patients. While further research on the efficacy and safety of Cal/BD as a treatment for nail psoriasis is needed, this case series suggests its potential as a combination topical vitamin D analogue and high potency steroid in a foam vehicle.

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Increased risk of hospital acquired sacral pressure injuries in COVID-19 patients

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Background: We aimed to compare risk of hospital-acquired sacral pressure injuries (HASPI) in COVID-19+ and COVID-19- patients. Method: Single-institution, multi-hospital, retrospective cohort review of all hospitalizations from March 1st, 2020 to September 1st, 2020. Patients with new-onset HASPI (stage II or greater) were included in our study. Patients with chronic history of sacral ulcerations or patients with ulceration present on admission were excluded. Patient demographics, baseline ulceration risk (based on Braden risk assessment), HASPI characteristics, laboratory parameters, and ulcer-associated morbidity were collected. Results: During our study period, 36 of 59,208 COVID-19- and 13 of 3,488 COVID-19+ hospitalized patients developed a HASPI. COVID-19+ patients had a 5.5x higher relative risk of developing a HASPI compared to COVID-19- patients (46.5 per 100,000 hospitalization days versus 8.4 per 100,000 hospitalization days, 95% CI 3.3-11.5, p<0.0001). Of patients that developed a HASPI: median age, gender distribution, baseline ulceration risk, nursing skin checks per day, and time from admission to HASPI were similar between COVID-19+ and COVID-19- patients. COVID-19+ patients had larger (median ulcer size 85 cm^2 vs. 7.5 cm^2, p=0.04) and more severe (46.2% Stage 4/Unstageable vs. 19.4%, p=0.03) HASPIs compared to COVID-19- patients. All COVID-19+ patients with HASPI had elevated D-dimer concentrations, with a median peak D-dimer of 6,755 ng/mL that occurred on average 3.5 days before ulcer formation. HASPI led to severe morbidity in 6 of 11 COVID-19+ patients who survived initial hospitalization , including need for debridement or surgery (n=5) and ulcer infection/sepsis (n=5). Conclusions: Hospitalized COVID-19+ patients are at increased risk for developing large and severe HASPI. The etiology of increased HASPI risk is unclear, but may due to poor tissue perfusion secondary to microvasculature occlusion, decreased staffing, and inability to appropriately position hemodynamically unstable patients.

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The study design of two trials of dupilumab in patients with prurigo nodularis inadequately controlled with topical therapies: LIBERTY PN PRIME and PRIME 2

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Multiple case series suggest dupilumab should be studied further in patients with prurigo nodularis (PN). We describe the study design of two phase 3 trials to assess if dupilumab can improve itch and resolve PN lesions. PRIME (NCT04183335) and PRIME2 (NCT04202679) are 2 double-blind, placebo-controlled, multicenter, parallel-group studies consisting of 2−4 weeks screening, 24 weeks randomized treatment with dupilumab or placebo, and 12 weeks follow-up. Low-/medium-potency topical corticosteroids (TCS)/topical calcineurin inhibitors are permitted. Patients are included if they are adults with PN defined by: dermatologist diagnosis ≥ 3 months before screening; 7-day average Worst Itch Numerical Rating Scale (WI-NRS) score ≥ 7 (scale 0−10) prior to baseline (BL); ≥ 20 bilaterally symmetrical PN lesions on ≥ 2 body surface areas; history of failing 2 weeks medium-to-superpotent TCS, or when TCS not medically advisable. Main exclusion criteria include skin comorbidities interfering with PN assessment; PN secondary to medications or to neuropathy/psychiatric disease. The primary endpoint is reduction in WI-NRS score by ≥ 4 from BL to Week 12; key secondary endpoints are reduction in WI-NRS score by ≥ 4 from BL to Week 24 and Investigator's Global Assessment (IGA) score 0 or 1 for PN-Stage (PN-S) at Week 24. Other secondary endpoints include: time to reduction in WI-NRS score at time points as early as Week 2; proportion of responders who reach IGA PN-S at time points as early as Week 4; and change from BL in Dermatology Life Quality Index at Weeks 12 and 24.

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Topological surface mapping with computer vision to measure cutaneous tissue deformation from digital images

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The risks of recurrence and postoperative complications in Mohs micrographic surgery (MMS) increase with increasing lesion size. Clinical errors may be introduced by biomechanical forces that deform tissue during routine MMS manipulations. The purpose of this study is to empirically determine tissue deformation using only before and after digital images and computer vision. We introduce a correlation algorithm that tracks features on tissue before and after strain for quantitation of biomechanical stress. Multichromatic acrylic microdots were painted onto porcine skin and tracked before and after hypodermal tissue bending (n=10) and epidermal flap reconstruction simulations (n=6). Painting resulted in irregularly shaped microdots. Two-dimensional microdot center coordinates were estimated by the correlation algorithm from digital images and compared to a consensus of two expert-raters using two-tailed Welch's t-tests. The correlation algorithm detected 83% of microdots overall. Detection of microdots on epidermal flaps was higher before reconstruction than after, though not significantly (91% vs 84%, p=0.16). Detection of microdots was higher on the epidermis than hypodermis (88% vs 80%, p=0.01). The correlation algorithm detected microdot coordinates within an average error of 11 pixels overall. Accuracy was better on the epidermis than on the hypodermis (6 vs 15 px, p<0.001). This correlation algorithm is an important step towards practically measuring biomechanical forces in the clinic using only digital images of irregular microdot fiducials. It has been optimized for specificity over sensitivity, with sufficient pixel accuracy to estimate deformation in exchange for a modest loss of resolution. Improving these techniques and introducing additional data such as three-dimensional point clouds will expand the applications of optical mapping in clinical research.

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Identifying locations of Merkel cell carcinoma associated with higher disease-specific mortality

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Merkel cell carcinoma (MCC) can occur anywhere on the skin surface, yet an understanding of whether tumor primary site impacts prognosis is currently incomplete within the literature. To best address this knowledge gap, we designed a study to analyze disease-specific mortality, rather than overall survival. MCC patients are often of advanced age and therefore may die of other causes prior to dying from MCC. Therefore, a death from any other cause represents a competing risk outcome. As such, we applied a competing risk analysis using the Fine-Gray model to investigate disease-specific mortality among patients within the Survival, Epidemiology, and End Results (SEER) database (1973-2016), with MCC tumor site as the primary variable of interest. With the results from this model, we calculated the 5-year cumulative mortality incidence (i.e. probability of mortality), for tumors at nine primary sites (ear, eyelid, lip, scalp/neck, other skin of the face, trunk, upper limbs, lower limbs and unknown primary site), while stratifying by stage at diagnosis. Of the 9407 MCC patients identified, 6305 (67%) had localized disease, 2397 (25.5%) had regional metastasis, and 705 (7.5%) had distant metastasis. Primary tumor site was predictive of cumulative mortality incidence ($p<0.0001$), which varied by stage at diagnosis. MCC involving the scalp/neck carried the highest cumulative mortality among localized tumors (24.3%), and regionally metastasized tumors (48.8%). For MCC with distant metastasis, the lip had the highest cumulative mortality (89.5%). Further, an unknown primary site was found to have a lower cumulative mortality incidence than some, but not all cutaneous tumor sites. Implications of these findings largely pertain to the prognostication of MCC outcomes. AJCC staging guidelines may incorporate tumor site as a means of prognostic stratification. Consideration of treatment escalation may be warranted for tumor sites with worse prognosis.

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SMASH: Perceived stigma and social health in patients with chronic skin disease

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Patients with visible skin conditions frequently endure perceived stigma and social rejection that significantly impacts their well-being. This two phase study evaluates the validity and utility of a new social health patient-reported outcome measure, StigMA & Social Health (SMASH), across five chronic skin diseases to understand its impact on patient's well-being. SMASH is a 12-item survey with two subscales created from the validated Perceived Stigma Questionnaire (PSQ) and Social Comfort Questionnaire (SCQ). Scores ranged from 1 to 5 for each SMASH subscale, PSQ, and SCQ. Subjects (ages ≥13 years) diagnosed with atopic dermatitis (AD), psoriasis (PS), acne, cutaneous lupus (CL), or alopecia areata (AA) consented to the study. In Phase 1, subjects completed SMASH, PSQ, and SCQ at one visit. In Phase 2, subjects completed SMASH, Dermatology Life Quality Index (DLQI), and Patient Global Impression of Severity (PGIS) at two visits. All statistical analyses (correlations, Cronbach's α, intraclass coefficient correlation (ICC)) were performed using JMP14. Phase 1 was completed by 50 subjects (AD=12; PS=12; acne=12; CL=7; AA=7). SMASH subscales exhibited strong internal consistency (Stigma $\alpha = 0.815$; Social Health $\alpha = 0.855$), similar to the PSQ and SCQ ($\alpha = 0.934$ and 0.899). SMASH subscales strongly correlated with PSQ and SCQ across all diagnoses ($r = 0.902$ and 0.858; $p<0.0001$) and within diagnoses ($r \geq 0.681$; $p<0.050$). Acne subjects had the highest mean perceived stigma (2.51 ± 0.73) and the lowest mean social comfort (3.41 ± 0.85). A total of 92 subjects consented to Phase 2 (AD=20; PS=26; acne=26; CL=9; AA=11). We expect good test-retest reliability (ICC>0.700) for SMASH and strong correlation between patient-reported disease severity ($r>0.700$; $p<0.05$). We conclude that SMASH is a valid, reliable measure that captures the emotional burden of chronic skin disease. Utilization of SMASH in future studies would elucidate how social health is modified by treatment and disease course.

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A phase 1/2 trial of PTR-01, a collagen 7 (C7) protein replacement therapy, in patients with recessive dystrophic epidermolysis bullosa (RDEB)

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RDEB is a multisystem disorder affecting the skin, GI and GU tracts, eyes and immune system. Treatments in development primarily target cutaneous manifestations. PTR-01 is human recombinant C7 intended to address both cutaneous and systemic manifestations of RDEB. In a multicenter Phase 1/2 study, we treated 10 adults with confirmed RDEB in 4 cohorts receiving 3 IV infusions of PTR-01 (0.1, 0.3, 1.0 or 3.0 mg/kg) or placebo every other week in a cross-over fashion. Safety (adverse events, tolerability, immune responses) was the primary outcome. Secondary outcomes included pharmacokinetics (PK) and demonstration of C7 at the DEJ. Pharmacodynamic measures and wound healing were also assessed. All patients completed the study. There were no unexpected or drug-related serious adverse events. Mild/moderate infusion-associated reactions occurred in four patients (2 each in Cohorts 3 and 4) and were managed with standard of care treatments (diphenhydramine, acetaminophen/ibuprofen, glucocorticoids). Only two patients (1 each in Cohorts 2 and 3) developed anti-drug antibodies (transient/low titer). PK showed dose-dependent increases. All Cohort 3 and 4 patients had increased C7 NC1 at the DEJ by direct immunofluorescence; largely absent baseline NC2 staining increased in all but one patient. Three patients had modest improvement in suction blister times, one of whom also showed a transient increase in anchoring fibrils. No consistent changes in wounds were seen. In this short-term study, PTR-01 has shown encouraging findings warranting further clinical trials. A longer Phase 2 study to assess efficacy is now enrolling.

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Oral 25-hydroxyvitamin D_3 reduces chemical-induced skin inflammation in humans

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Human skin exposure to chemical irritants such as alkylating agents can induce blisters and skin irritation due to both direct cellular damage and a pro-inflammatory response. In murine models, 25-hydroxyvitamin D3 (D3) mitigates the inflammatory effects of topical alkylating agent nitrogen mustard (NM) and similarly reduces inflammation from experimental sunburns in humans. In this double-blinded, placebo-controlled interventional trial, we set to investigate whether high dose D3 can mitigate skin irritation resulting from topical NM. 28 healthy adults had 4mm2 of skin exposed to NM (FDA-approved 0.016% gel) under occlusion on one arm with repeat exposure on the contralateral arm 2 weeks later. Subjects were randomized to receive either 200,000 IU oral D3 or placebo at the second NM exposure. Skin biopsies from both groups demonstrated brisk infiltration with mixed immune cells. However, proximity extension assay using a panel of 92 inflammatory protein markers revealed 37 differentially expressed proteins (DEPs) in the placebo group and 24 in the D3 group(FCH >2.0 and FDR< .05). 6 weeks from second exposure the number of DEPs in the placebo group was unchanged whereas the D3 group demonstrated a 79% reduction to 5 DEP. The D3 group had reduced skin redness by chromameter assessment at one week after the second exposure compared to the first exposure ($p = 0.02$). In the D3 group, 10 out of 14 subjects (71.4%) were confirmed D3 responders with sustained increase in serum 1,25-dihydroxyvitamin D3 over one week. The placebo group had more redness on the second exposure compared to the first exposure at every time point ($p = 0.002$-0.04) while D3 responders did not ($p = 0.13$-0.32). These results suggest that D3 mitigates skin inflammation from chemical injury and may be useful as an adjunctive treatment to reduce side-effects in patients receiving NM for cutaneous T cell lymphoma.

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Risk factors associated with detection of deep vein thrombosis on ultrasonography in patients with lower extremity cellulitis

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Background: Cellulitis is a common skin infection whose presentation can involve mimickers such as deep vein thrombosis (DVT). Guidelines for radiologic examination of cellulitis are limited. Furthermore, ultrasonography (US) is overused for detection of potential DVT in cellulitis cases. *Objective*: To assess clinical factors associated with detection of DVT on US in cases of lower extremity cellulitis. *Methods*: Single-center, retrospective chart review of adult patients treated for lower extremity cellulitis between 2017 and 2018 was conducted. Patients undergoing US for evaluation of lower extremity cellulitis were identified as the study population. A set of possible risk factors were evaluated for association with detection of DVT via univariate chi-square analysis. Factors meeting a significance threshold ($p<0.1$) were used in a model for detection of DVT via multivariate logistic regression. *Results*: Of 395 patients with lower extremity cellulitis reviewed, 157 patients (39.7%) received 186 USs. 7 patients (4.5%) had 8 USs demonstrating DVT. Multivariate analysis demonstrated history of DVT (OR 9.90; CI 2.13, 46.0) and human immunodeficiency virus (HIV) infection (OR 22.2; CI 3.04, 162) as significant predictors of DVT detection on US. Patients presenting with clinical suspicion for lower extremity cellulitis are unlikely (0.7%; CI 0.0%, 2.5%) to have DVT detected on US. *Conclusions*: DVT is detected by US very rarely in the evaluation of lower extremity cellulitis. Patient history of DVT and HIV emerged as significant predictors of DVT on US. More research is necessary to establish indications for judicious acquisition of US in cases of cellulitis for detection of possible DVT.

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Cutaneous findings in COVID-19 patients hospitalized at a large urban academic medical center

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Background: Cutaneous manifestations have been associated with COVID-19 infection and their clinical significance in hospitalized patients remains unclear. Methods: A retrospective chart review of 1216 patients older than 18 years of age hospitalized with laboratory-confirmed SARS-CoV-2 infection from March 12, 2020 to May 31, 2020 at a large urban academic medical center. A keyword search query of patient records combined with manual chart review by at least two dermatologists identified a study group having cutaneous manifestations concurrent with COVID-19 infection, specifically between 14 days prior to admission and up to discharge. Results: 122 patients with 195 skin lesions concurrent with COVID-19 hospitalization were identified. Dermatology reviewers evaluated clinical photographs for 116 lesions (59.5%) and inpatient dermatology consultations for 42 lesions (21.5%). The most common cutaneous findings in patients with COVID-19 hospitalization were pressure injuries (n=118; 60.5%) and morbilliform eruptions (n=33; 16.9%). A very small number of patients (0.6%; n=7/1216) had exanthems occurring within 2 weeks of COVID-19 symptom onset. The majority of exanthems developed within 14 days of exposure to possible culprit drugs and beyond the 14-day window of COVID-19 symptom onset, making viral association unlikely. Conclusion: Skin lesions concurrent with COVID-19 hospitalization were most frequently linked to hospitalization-related factors, such as pressure injuries or drug-related exanthems, rather than due to novel pathologies related to SARS-CoV-2 itself.

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Outcomes reported in clinical trials of postinflammatory hyperpigmentation: A systematic review

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BACKGROUND: Due to heterogeneity in outcomes measured in clinical trials of post-inflammatory hyperpigmentation (PIH), it is difficult to compare different treatment modalities. Reviewing the diversity in reported outcomes is a necessary step in developing a core outcome set (COS), or a minimal set of outcomes that should be reported in all clinical trials of PIH treatment. OBJECTIVE: To identify outcomes that have been measured in clinical trials of PIH as part of the development of a COS for future clinical studies. METHODS: A systematic review of the literature was conducted to identify clinical trials of PIH treatment published in the English language between January 2010 to August 2020. Reported outcomes were extracted from individual studies and categorized based on similar themes. RESULTS: 36 studies were included, and 101 outcomes were identified and grouped into 6 domains: adverse events/effects, clinical assessment, clinical recurrence, perception of health, patient satisfaction, and quality of life. The most commonly reported outcome was "Erythema" as an adverse event (reported in 16 of 36 studies, 44.4%). Additional common outcomes were "Darkness/intensity of pigmentation" (15 of 36 studies, 41.7%) and "Global improvement of pigmentation" (14 of 36 studies, 38.9%). CONCLUSION: There is considerable heterogeneity in outcomes reported in clinical trials of PIH. Development of a COS is necessary to standardize outcomes reporting in future clinical trials, which will help to facilitate later comparison of outcomes across multiple studies.

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Clinical risk factors associated with MRSA incidence in inpatient pediatric cellulitis

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Background: Methicillin-resistant Staphylococcus aureus (MRSA) infection in pediatric cellulitis can be difficult to diagnose and treat with appropriate antibiotic coverage. Detection of methicillin resistance can be challenging as it requires isolation of the causative organism by microbial culture, and clinical characteristics alone have limited ability to distinguish MRSA infection. Objective: To identify predictive risk factors for MRSA infection in hospitalized pediatric patients. Methods: Single-center, retrospective chart review of 893 pediatric inpatients from 2007 through 2019. Patients were excluded if they had intensive care unit stay or had complicated infections such as at a surgical site. Multivariate logistic regression analysis was conducted using all independent variables that reached statistical significance in univariate testing. In this model, the outcome variable was growth of MRSA from a wound culture. Results: 559 patients (63.3%) met inclusion criteria. Multivariate analysis for prediction of MRSA growth was performed for all patients receiving a wound culture (n=290; 51.9%). Univariate analysis revealed a positive association between MRSA growth on wound culture and infection of the groin and buttocks region, fever at presentation, leukocytosis at presentation, MRSA nasal carriage, and abscess formation ($p<.1$). Multivariate analysis resulted infection of the groin and buttocks region (OR 5.86; 95% CI 1.00, 34.4) and MRSA nasal carriage (OR 29.3; 95% CI 12.9, 66.5) as independent predictors of MRSA growth on wound culture. Conclusion: Both MRSA nasal carriage and infections of the groin and buttocks are strong and independent predictors of MRSA cellulitis. These factors should be considered in antibiotic selection for inpatient pediatric cellulitis.

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Circulating tumor DNA as a biomarker for treatment response in an advanced Merkel cell carcinoma patient

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Notwithstanding recent advances, Merkel cell carcinoma (MCC) persists as an often-lethal cancer for the majority of patients failing out of immunotherapy. There are unmet clinical demands for effective alternative therapies and novel sensitive methods for monitoring therapeutic response of immunotherapy and beyond. Recently, circulating tumor DNA (ctDNA) analysis using a next generation sequencing (NGS) platform has shown to be sensitive and effective in postoperative management, early detection of relapse, and predicting treatment response and prognosis in several human cancers. This is the first case to report the potentially transformative utility of ctDNA analyses to guide treatment decisions and surveil disease in MCC, and the first to demonstrate combinatorial talimogene laherparepvec (T-VEC) and hypofractionated radiation (HRT) as an effective alternative treatment for MCC patients who progress on immunotherapy. Our patient is a 70-year-old female whose MCC initially recurred after surgery and progressed on pembrolizumab, with debilitating side effects. Six weeks after her second surgery and adjuvant radiation, she developed in-transit metastases, leaving with her limited treatment options. Over the course of combinatorial T-VEC and HRT treatment, we found that ctDNA analysis performed using a personalized and tumor-informed (bespoke) NGS assay correlated with increased tumor burden, treatment response, and imaging findings. At three-month follow-up, ctDNA remains undetectable. As her MCC was extremely aggressive and had recurred shortly after every prior treatment modality, this is a promising outcome. Our pioneer observation underpins future study to ascertain the transformative role of ctDNA in MCC management, including post-operative risk stratification, early detection of relapse, biomarkers for treatment response and prognosis.

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Mycophenolate mofetil and methotrexate in dermatomyositis treatment

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While dermatomyositis (DM) treatment often follows a stepwise sequence, data is lacking regarding the true efficacy of methotrexate (MTX) and mycophenolate mofetil (MMF). A cohort of 31 patients with currently skin-predominant DM taking MTX or MMF with $\geq$ two study visits within a 500 day retrospective observation period was seen at The University of Pennsylvania. The Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI) was used to assess severity and outcomes. Patients with mild disease activity defined as a CDASI activity score <14 (maximum sub-score of 100) were excluded from the analysis as were any patients on any other medications used to treat DM besides MTX or MMF, with the exception of chronic antimalarials or topical medications. Responders were defined as those that had an improvement in their CDASI activity score of $\geq$ 40% between their first and last visits post onset of immunosuppressive use. For both MMF (n=19) and MTX (n=12), there was no baseline difference in CDASI activity scores between responders and non-responders at medication initiation. For MTX, 33.3% of patients responded to therapy while 47.4% of patients taking MMF responded. There was no significant difference in the degree of improvement on either medication, with a mean difference in daily CDASI activity change between MTX and MMF of -0.015 $\pm$ 0.018 (p=0.4152). There was a significant difference in daily CDASI activity change between responders and non-responders, -0.043 $\pm$ 0.011 ($p<0.001$) for MMF and -0.046 $\pm$ 0.014 ($p<0.01$) for MTX. Either MMF or MTX may be added to treatment plans for patients with DM who have not responded to antimalarial therapy. Moreover, our data suggest that responders continued to improve over many months while most non-responders showed little improvement at first follow-up (ranging from 2-6 months) during the observation period.

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Altered gene expression following targeted therapy for vascular malformation

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Somatic mutations including *MAP2K1, Tie2 and PIK3CA* were recently identified in endothelial cells of various vascular malformations. These small populations of genetically altered cells are believed to be crucial in promoting angiogenesis and tissue growth that leads to vascular malformations. There is no approved treatment for vascular malformation currently. Therefore, we sought to investigate alteration of gene signature in peripheral blood of patients treated with off label therapy. We performed RNA-Seq on blood samples of 9 patients with vascular malformations. Gene expression were analyzed before and after sirolimus treatment for an average of six months. Raw counts were aligned using STAR and differential expression analyzed using deseq2. Differential gene expression was analyzed using a wald chi-squared test. We have identified n=498 (FC Δ1.3 $p<0.05$, n=10 adjusted p value <0.05) upregulated and n=350 (n=59) down-regulated genes post treatment. The variation of clinical phenotypes of patients analyzed were reflected in their gene expression profiles in peripheral blood. Interestingly, the gene expression profiles became more clustered and exhibited resemblance post treatment. Our results suggest that exhibit similar regulatory effects for different vascular malformations. Gene ontology pathway analysis demonstrated changes in addition to PI3K/mTOR, β- catenin and WNT signaling, cell cycle regulation as well indicated renin angiotensin and cell adhesion molecule signaling. Computational drug repositioning analysis using these gene signatures predicted other therapeutic agents with similar effects as sirolimus such as vincristine, sunitinib and glucocorticoids. Some of these agents have previously shown efficacy in treating vascular anomalies, further validating our results. This pilot study has demonstrated the feasibility to study systemic drug effects on vascular malformation in a simple yet comprehensive way that may enable noninvasive diagnosis and therapeutic discovery in the future.

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Quality appraisal of recent guidelines for adult atopic dermatitis

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Introduction: In 2017, Dupilumab was approved for adults with moderate-to-severe atopic dermatitis (AD). Clinical practice guidelines (CPGs) have since incorporated this option into treatment algorithms. Studies on CPGs quality are needed. Objective: Assess quality of methods and development processes of adult AD CPGs reported since approval of Dupilumab. Methods: A literature search was conducted in June 2020 on MEDLINE, EMBASE, SCOPUS and CINAHL (2017-current). Two reviewers independently screened reports with management recommendations for adults. Quality was independently assessed by 3 reviewers using validated Appraisal of Guidelines for Research & Evaluation II (23 criteria grouped into 6 domains), scored 1 (strongly disagree) to 7 (strongly agree);>70% was considered good quality. Results: Twelve CPGs were retrieved. Median scores per domain were (in %): scope/purpose (r=range), 78 [r=50-96]; stakeholder involvement, 54 [r=28-85] rigor of development, 39 [r=21-63]; clarity of presentation, 85 [r=69-100]; applicability, 27 [r=6-51], and editorial independence, 76 [r=42-100]. In the domains of rigor of development and applicability, none of the guidelines met quality criteria. Quality metrics were lowest for items incorporating procedures for updates, facilitators/barriers, resource implications, evidence-selection, and external review. Only 2 guidelines met criteria for stakeholder involvement. Conclusion:CPGs for adult AD have now incorporated Dupilumab and present options ranging from behavioral change to biologics. The majority of CPGs had high quality scope/purpose, clarity and editorial independence. None of the CPGs met criteria for methodological rigor nor applicability; mechanisms for incorporation of updates, facilitators/barriers to guideline application, resource implications, and stakeholder involvement were lacking. Nevertheless, based on domain scores and reviewers' judgement, most CPGs were recommended for use with modification.

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Evaluation of psoriasis severity using AI

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In recent years, artificial intelligence (AI) plays a major role in modern society. In the medical field, AI research is progressing in the fields of radiology, ophthalmology, gastrointestinal endoscopy, pathology, etc., and AI is already being used in clinical settings. In the Japanese dermatology field, since 2018, the Japanese Dermatological Association (JDA) has been developing a reliable National Skin Disease Database (NSDD) evaluated by dermatologists and developing an AI diagnosis support system using NSDD. Recently, we started AI research to evaluate the disease activity of psoriasis by using Deep convolutional neural networks (CNNs). We trained a CNN using a dataset of 750 psoriatic clinical images scored the rash state of psoriasis from erythema, infiltration, desquamation, and skin lesion area. This is not an AI for diagnosis, but an AI as a medical assistance tool aimed at reducing the burden on medical doctor. Our research goal is "Development of AI that can instantly evaluate the disease activity of psoriasis from one clinical picture." In this presentation, we will show the progress of our AI research and consider the perspective of AI in the evaluation of psoriasis rash.

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Periodontitis as a risk factor for psoriasis

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Patients with psoriasis have an increased risk for inflammatory periodontal disease, however, data are sparse. Inflammatory disorders are well known to trigger psoriasis. We examined the periodontal status in 50 patients with psoriasis compared to 25 healthy control individuals. We examined whether there is (1) an enhanced periodontal risk, (2) increased IL-1 mediated inflammation, (3) increased bacterial load, (4) sufficient oral hygiene, and (5) an enhanced risk for tooth decay in psoriasis. Patients with psoriasis (19 female, 31 male) had a median age of 56 years, controls (17 female, 8 male) of 40 years. Individuals were given a questionnaire about general and dental health. Tooth and periodontal status was ascertained (perio-tools®, CA Ramseier, Univ. Berne, Switzerland). The DMFT-index (decayed, missing, filled teeth), BOP (bleeding on probing), PSI-Index (periodontal screening) were measured. Bacterial analyses from gingival pockets were done by micro-IDent ®/Hain Lifescience measuring 5 parodontal species by PCR. IL-1 gene variants were determined from buccal swabs/Hain Lifescience. Statistical analyses, t-test, U-test, Chi-square, Fisher's exact test were done by SPSS 23 software. We found the following results: Psoriasis patients were smokers in 40% vs. 12% in controls ($p<0.03$). Interdental tooth cleaning was done in 38% of patients but in 80% of controls ($p<0.001$). There was no difference in cariogenic nutrition. Regular dentist visits were less frequent in psoriasis (64 vs. 96%). Gingivitis, tooth migration, tooth loss after loosening, gingival treatment did not differ. Missing, decayed or carious teeth were more frequent in psoriasis (10.3 vs. 6.4, $p<0.013$). DMFT-Index did not differ. Gingival pockets (>3.5 mm) were more frequent in psoriasis (85.4 vs. 60%, $p<0.021$). BOP did not differ. Bacterial growth occurred in 95.8% in psoriasis vs 72% in controls ($p<0.006$), specifically of the Tannerella forsythia-species ($p<0.05$). IL-1 genotype did not differ. Periodontal risk was significantly higher in psoriasis (48% vs. 25%, $p<0.029$). Our data indicate that periodontitis can be considered as a risk factor for psoriasis.

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The use of apremilast in the treatment of patients with lichen planus

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Intro: Lichen planus (LP) is a relatively common dermatologic disease without an approved FDA indication. There is a need for treatments with minimal adverse effects and contraindications to comorbidities seen in LP patients. Apremilast is a phosphodiesterase 4 inhibitor FDA approved for plaque psoriasis, psoriatic arthritis, and Behcet's disease. Methods: A retrospective analysis of medical records for 11 patients who had been prescribed apremilast 30 mg twice daily for LP was conducted. Inclusion criteria included patients who had documented use of apremilast, at least one follow-up and post-treatment documentation. Results: Seven patients met inclusion criteria, while four were excluded due to lack of insurance coverage. Patients' ages ranged 31-67, with an average age of 54.4 and six (85.7%) were male. All patients were previously treated with other agents including topical, intralesional and oral corticosteroids, acitretin, methotrexate, cyclosporine and metronidazole. Three (57.1%) were treated simultaneously with topical agents. Two patients had cutaneous only, two had mucosal only and three had both papular and mucosal LP. Six (85.7%) had clinical improvement in symptoms after treatment with apremilast. Two (28.6%) had significant improvement and three (42.9%) were clear. Four (57.1%) had no loss in response after being treated with apremilast for more than one year. Three (42.9%) experienced mild side effects such as headache, diarrhea, and insomnia. Discussion: Apremilast, an oral phosphodiesterase-4 (PDE-4) inhibitor, has promise to be an efficacious treatment option for LP. In this study, the use of apremilast at 30 mg twice a day was associated with a response for a majority of patients and was well tolerated. Further studies are warranted to better explore the use of apremilast in lichen planus.

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Spironolactone for treatment of concomitant female pattern hair loss in scarring alopecia patients

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Background: Lichen planopilaris (LPP), frontal fibrosing alopecia (FFA), and central centrifugal cicatricial alopecia (CCCA) are primary scarring alopecias that occur most commonly in adult females. Female pattern hair loss (FPHL), a non-scarring alopecia, is the most common form of alopecia in females. Due to overlapping patient populations, concomitant FPHL would be expected in many scarring alopecia patients. Prior studies have demonstrated the efficacy of spironolactone in FPHL. Herein, we evaluated the efficacy and safety of spironolactone in females with scarring alopecia and FPHL. Methods: We performed a retrospective review of female LPP/FFA/CCCA patients presenting to a specialized hair loss clinic in 2018 who were treated with oral spironolactone for patterned hair loss. Results: We identified 18 females with scarring alopecia and patterned hair loss who were treated with oral spironolactone. 66.7% had LPP, 16.7% FFA, 16.7% LPP/FFA, and 11.1% CCCA. 72.2% were diagnosed with concomitant FPHL. Mean age at spironolactone start was 57.6 years (range 21.6-76.3). All patients were simultaneously treating scarring alopecia with other therapies. Spironolactone was started at 100mg (56%), 50mg (11%), or 25mg (33%) daily. 83% of patients maintained or increased dose without issue. 2 patients reported side effects including hyperkalemia and leg cramping. 5.6% decreased dose and 11.1% discontinued due to side effect or personal preference. 11 patients used spironolactone for at least 6 months, with an average of 23.3 months. All patients with at least 6 months of spironolactone use maintained or improved Sinclair score (SS) with an average improvement of 0.4. SS improved by 0.16 and 0.7 in patients with initial SS of 1.5-2.0 and 2.0-2.5 respectively. Conclusion: Our preliminary study demonstrates the safety and efficacy of spironolactone in female scarring alopecia patients with concomitant pattern hair loss.

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Use of technology for the objective evaluation of scratch: A systematic review

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Introduction: Pruritus is a common symptom across a wide range of dermatological conditions, with a negative impact on quality of life and sleep quality. Devices to objectively quantify itch primarily use scratch detection as a proxy. The purpose of this review is to compare and evaluate the performance of technological modalities aimed at objectively measuring scratch behavior. Methods: Articles were resulted in October 2020 from literature searches. Those that did not report a primary statistical performance measure were excluded. The articles were independently reviewed by at least 2 reviewers. Results: The literature search resulted in 6230 articles, of which 26 met eligibility criteria. While validated against video recording of scratch, wrist actigraphy's performance is poorer in pruritic patients compared to healthy subjects and is inherently limited in detection of finger-dominant scratching. Additionally, while there are moderate correlations between actigraphy and objective itch surveys (r_s=0.42-0.64), the correlations with subjective measures of itch are very poor (r^2=0.06, r_s=0.18-0.40 for VAS itch in children and adults). This may be due to varied subjective perception of itch or actigraphy's underestimation of scratch. Comparison between subjective measures and finger motion-sensitive technologies may better discern these discrepancies. Clinically, incorporation of these devices will depend on development of more precise devices and data analysis algorithms. Conclusion: Actigraphy is the most studied modality to assess itch/scratch with large variability in performance. Most studies are limited to nocturnal measurement. Larger studies looking at validation of data analysis algorithms and device performance for actigraphy and other technologies, particularly within target patient populations and during daytime, are needed. There remains a need for objective measurements of itch to support surrogate endpoints for drug development, assess disease severity, and monitor treatment response.

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Mogamulizumab associated rash (MAR) frequently mimics CTCL recurrence: A case series

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Mogamulizumab is a humanized antibody targeting CCR4 for relapsed/refractory mycosis fungoides (MF)/Sezary syndrome (SS). MARs are the most common adverse events reported; however, the clinical and molecular nature of these eruptions remains unclear. To inform future management and recognition of MAR, we report a retrospective case series of MF/SS patients treated with mogamulizumab since FDA approval in 2018 as standard of care. Among 24 CTCL (15 SS, 8 MF, 1 ATLL) patients, sixteen developed MAR (66.7%). Median MAR time to onset (TTO) was 2.4 mo. (0.7-9.0). Median time to resolution (TTR) was 6.6 mo. (3.5-9.3). Five distinct but frequently concurrent clinical patterns were identified: lichenoid (8/16), photodistribution (7/16), psoriasiform (7/16), MF-like lesions (6/16), and erythroderma (3/16). Histopathological patterns included spongiotic (n=21), lichenoid (n=14), psoriasiform (n=9), and interface features across 25 skin biopsies. Eosinophils (n=14), epithelioid granulomas (n=4), and bizarre bi- and multinucleated cells (n=6) that may represent highly activated macrophages were also noted. Concomitant flow cytometry analysis for circulating SS cells and TCR rearrangement studies in skin and peripheral blood were negative during evaluation for MAR, highlighting the importance of ancillary studies to distinguish MAR from CTCL features. ORR was higher in patients with MAR (14/15 93% vs 2/8 25%); 8 patients achieved CR and 6 PR, responses are ongoing. Disease free survival is median 5.1 mo. (3.5-9.3) among these patients. In conclusion, MAR may be characterized by distinct clinical and histopathological patterns. Notably, MAR can clinically mimic CTCL lesions and may occur more often in patients with durable clinical responses, highlighting the need for clinicians to rule out progressive disease vs. drug eruption in treating a condition that historically suffers from poor outcomes and treatment response.

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Efficacy of ultrasound and MR in diagnosis of subungual glomus tumors and subungual myxoid cysts: A case series

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BACKGROUND: The literature on comparison of ultrasound (US) vs. MRI in the evaluation of benign subungual masses, including glomus tumors and myxoid cysts, is emerging. While MRI can characterize the nail apparatus in great detail, it is an expensive and time-consuming modality. Ultrasound has the potential to increase accessibility and speed of diagnosis of subungual masses at a lower cost. The purpose of this study was to compare US and MRI of 6 subjects with histopathologically-confirmed subungual glomus tumors and subungual myxoid cysts. METHODS: Participants with clinical suspicion for subungual glomus tumor or myxoid cysts on exam were recruited. After clinical evaluation, participants underwent XRAY, MRI (one underwent CT in lieu of MR), and US. Differential diagnoses were revised after review of radiology reads and imaging findings were compared to definitive diagnosis by pathology. RESULTS: Six participants were recruited with ages ranging from 29-76 years old (33% female). Of the 5 participants that underwent excision and pathology, 3 had diagnosis of subungual glomus tumor and 2 had suggested diagnosis of subungual myxoid cyst. The remaining participant had spontaneous resolution of the lesion. Of the 5 total participants with definitive pathology diagnosis of glomus tumor or myxoid cyst, 100% had at least one US finding that was characteristic of the final diagnosis. 100% of MRIs aligned with final histopathologic diagnosis of myxoid cysts and glomus tumors. Estimate of size and location agreed quite well between the two modalities. CONCLUSIONS: US and MR were equally efficacious in estimating the shape, size and location of lesions. The presence of contrast enhancement on MR and hypervascularity on Doppler US were key in distinguishing the subungual glomus tumors from the subungual mucoid cysts.

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Correlation between objective measures of sleep and nocturnal scratch in children with atopic dermatitis

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Introduction: Nocturnal itch is thought to contribute to poor sleep quality and disease exacerbation in children with atopic dermatitis (AD). While prior evidence suggests that objective measurements of scratch do not correlate well to self-reported itch, there is limited understanding how well nocturnal scratch correlates to sleep parameters. The purpose of this study is to evaluate whether objective measures of nocturnal scratch captured by a novel acoustomechanic sensor correlate to wrist actigraphy-derived sleep parameters. Methods: Six pediatric AD subjects were recruited for a home study lasting up to 1 week. Sleep parameters were measured using a wrist actigraph (GENEActiv), whereas scratch was measured by the ADvanced AcoustoMechanic (ADAM) sensor; both were compared to infrared video recording. A total of 14 nights of data were analyzable. Actigraphy data were analyzed using Activinsights' R Markdown tools. Scratch time was calculated by visual comparison between ADAM device outputs and video recording. Results: The mean wake after sleep onset (WASO), total sleep time (TST), and median activity duration (in minutes) were 184.3 (standard error of the mean (SEM) 10.2), 468.5 (SEM 13.8), and 1.38 (SEM 0.03), respectively. The mean nocturnal scratch time was 11.4 minutes (SEM 3.2). Scratch time correlated with median activity duration (Spearman's correlation r_s=-0.60, p=0.02), which is the median time of nightly active periods. Scratch time did not correlate with WASO (r_s=-0.10, p=0.73), TST (r_s=-0.015, p=0.95), or sleep efficiency (r_s=0.05, p=0.86). Conclusion: In this small sample size, there was limited correlation between nocturnal scratch and sleep parameters. The negative correlation between scratch time and median activity duration may suggest that severe itch may result in shorter but more frequent bouts of scratching. While self-reported itch, objective scratch, and sleep disturbance are all sequelae of AD, the heterogeneity of effect across subjects reduces the correlation between each of these variables. Measurement of all may be necessary to comprehensively assess AD.

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Mapping recommendations of recent guidelines for adult atopic dermatitis

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Introduction: In 2017, Dupilumab was approved for adults with moderate-to-severe atopic dermatitis (AD). Clinical practice guidelines (CPGs) have since incorporated this option into treatment algorithms. Although Dupilumab is the only approved systemic in N. America, Cyclosporin is approved in Europe and other conventional systemic therapies (CST) are still used. The approach to use and sequencing of CSTs prior to Dupilumab initiation remains unclear. Objective: Evaluate variations in the position of Dupilumab in the treatment algorithms proposed in recent CPGs for the management of adult AD. Methods: A literature search was conducted in June 2020 in MEDLINE, EMBASE, SCOPUS and CINAHL (2017-current). Two reviewers independently selected reports with management recommendations for adults. Recommendations regarding initiation of Dupilumab were extracted and compared. Variations in management approaches were analyzed. Results: Twelve CPGs were retrieved. A variety of approaches were described for management of patients with moderate-to-severe AD who failed topical treatment. With respect to Dupilumab initiation, recommendations fell into the following categories: 1) 2nd line, equivalent to CST (n=2/12) or nbUVB (n=1/12) (n=3/12 total); 2) 2nd line, preferred over CST and nbUVB (n=1/12); 3) 3rd line, after nbUVB (n=2/12) or CST (n=3/12) (n=5/12 total); 4) 4th line after nbUVB and CST (n=2/12); 5) No consensus reached (n=1/12). Conclusion:CPGs for adult AD have now incorporated Dupilumab but with divergent recommendations with regards to its position versus CST. The number of CPGs found to recommend initiation of Dupilumab as 2nd line management option (n=4/12), was slightly lower than those recommending its use as 3rd line agent (n=5/12). Our findings demonstrate a lack of consensus on systemic use in international CPGs for adult AD, reflect a nascent therapeutic landscape, and highlight a need for head-to-head studies and real-world evidence.

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Outcomes reported in clinical trials of basal cell carcinoma: A systematic review

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BACKGROUND: It is difficult to properly compare the different treatment modalities for basal cell carcinoma (BCC) due to a lack of standardization in outcome reporting in clinical studies. Examining the range of reported outcomes can help clarify the need to have a standardized set of outcomes to be measured at minimum in future clinical studies. OBJECTIVE: To identify outcomes that have been reported in clinical studies of BCC treatments. METHODS: A systematic review was performed for English-language human studies of basal cell carcinoma treatments conducted between January 1980 and July 2016. Prospective studies that reported at least one clinical outcome were included for review. Outcomes were then categorized into domains based on shared similarities. RESULTS: 70 studies were included, and 235 outcomes were identified and grouped into 8 domains: clinical assessment; safety and tolerability of treatment; treatment effectiveness; recurrence, progression, and remission; histologic assessment; procedural factors; pharmacokinetic considerations; and patient satisfaction. The most commonly reported outcome was "adverse events" (reported in 22 of 70 studies, 31.4%). Other common outcomes were "overall cosmetic/aesthetic outcome" (10 articles; 14.3%), and "incidence of new BCCs" (9 articles; 12.9%). CONCLUSIONS: There exists wide variability in the outcomes used to measure treatment success for BCC, and there is a need to have a standardized set of outcomes that are reported in BCC studies.

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Hidradenitis suppurativa: Delays in care and misdiagnoses

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Diagnostic delay and health care barriers are commonly faced by hidradenitis suppurativa (HS) patients. We sought to (1) identify reasons HS patients delay seeking care after symptom onset, and (2) identify common HS misdiagnoses by physicians. Between 2017 and 2019, 873 individuals with HS responded to an anonymous survey offered at several HS specialty clinics in the U.S. and online via Facebook HS support groups. Data regarding demographics, personal reasons for delay in presenting to a health care provider, and previously received misdiagnoses were collected. A majority of respondents (85%) reported a delay in seeking care, with a mean delay of 5.1 (STD 7.3) years. The top reason for delay was the belief that lesions would resolve (59.7%), while other reasons included embarrassment (42.4%) or lack of knowledge that treatments are available (32.3%). Younger HS patients were significantly more likely to report embarrassment ($p<0.0001$) and not knowing where to seek care ($p<0.05$). Individuals who reported at least one prior misdiagnosis of HS symptoms (66% of total survey respondents) had a mean delay in HS diagnosis of 10.4 years. The most common misdiagnoses were abscesses (45.4%), cysts (43.7%), and acne (31.2%). Compared to Hurley Stage 1 and 2, Hurley Stage 3 respondents were significantly more likely to have been misdiagnosed with MRSA ($p=0.0021$), abscesses ($p<0.0001$), and sexually transmitted infections ($p<0.0001$). HS patients have significant delays in care and are frequently misdiagnosed when they first present to a healthcare provider. Reasons for barriers to care are multifactorial but all contribute to increased disease burden. Outreach to younger individuals and public awareness programs aimed at normalizing this disorder, along with improved front-line provider education supporting early specialist referral may be especially valuable.

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Screening for hearing loss in pediatric alopecia areata

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Hearing loss has been reported for adults with alopecia areata at higher frequencies than controls. We performed a prospective study of pediatric patients with alopecia to assess risk for hearing loss with emphasis on sensorineural hearing loss. We included 50 children ages 4-12 years of age with alopecia areata with severity greater than 50% and with at least 6 months of disease and tested hearing using standard pure tone audiometry and otoacoustic emission testing. Testing results suggest that hearing screens should be part of clinical care for children with alopecia areata with greater than 50% severity. We hypothesize immune mediated targeting of melanocytes in the hair follicles and inner ear plays a role in this type of hearing loss. Melanocytes play a key role in auditory stimuli transduction and modulation in the inner ear. Since this type of hearing loss is permanent is it important to consider whether we should be more aggressive with systemic therapies in children with severe forms of alopecia areata to prevent these changes. This study once again highlights that alopecia areata is a medical disease and the effects of having this disease are more than cosmetic.

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The role of data augmentation on the performance of automated lesion classification in the presence of imaging artifacts: An evaluation of the 2019 ISIC Challenge

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Convolutional Neural Networks (CNNs) have been shown to achieve dermatologist level accuracy for skin lesion classification, but models have been limited by size and diversity of training datasets. Data augmentation adds alterations (e.g., blur, flipping, etc.) to images to increase classifier robustness to real-world perturbations and artifacts. We identified the augmentation techniques used in 123 submissions to the International Skin Imaging Collaboration (ISIC) 2019 Grand Challenge and compared each classifier's performance on images with and without artifacts. We developed a multiclass-multilabel CNN trained to detect artifacts seen in clinic: hair, blur, rulers, and pen markings, and achieved Area Under the Curve (AUC) of >90% for all artifacts except blur (83%). We assessed ISIC 2019 submissions' performances using balanced multiclass accuracy. Hair and pen markings resulted in a decreased accuracy in 72% and 66% of algorithms respectively, while ruler markings increased the accuracy in 70%. No specific augmentation technique was tied to improved or diminished performance on images with hair, ruler, or pen. 16/123 algorithms used artificial blur and had a 26% better performance than those that did not (p=0.0014). The top 5 algorithms had an average of 8.2 augmentation techniques (59% accuracy) compared to 1.4 in the bottom 5 (18% accuracy), supporting that data augmentation is vital for performance. Our work will shape the development of classifiers for melanoma diagnosis. We introduce a novel artifact classifier useful for quality assurance of dermoscopic images. We show that the diagnostic performance of algorithms on images with hair, ruler, and pen is unaffected by augmentation techniques used, whereas blur is. Machine learning in dermatology may require data augmentation mirroring artifacts seen in clinic, such as artificial hair and pen marker generation. These results will improve the real-world applicability of automated dermatologic classifiers.

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Clinical improvement in primary cicatricial alopecias following mast cell stabilizer treatment

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Primary cicatricial alopecia (PCA), often referred as scarring alopecia, is a group of disorders that result in permanent hair loss, and for which no FDA approved treatments exist. Common forms of PCA include Lichen Planopilaris (LPP), Frontal Fibrosing Alopecia (FFA) and Central Centrifugal Cicatricial Alopecia (CCCA), with LPP predominating in middle aged white women, FFA in the postmenopausal group, and CCCA in women of African descent. FFA is considered to be a variant of LPP, and CCCA shares several pathological features with advanced LPP, however, whether PCAs are separate diseases or share common pathomechanisms remains an unresolved question. To determine if PCAs are molecularly distinct, we performed RNAseq of scalp biopsies from 28 LPP, 30 FFA, and 9 CCCA patients compared to 12 normal controls. This revealed a core set of dysregulated pathways shared among all PCA subtypes: 1) downregulated cholesterogenic genes (CYP51A1, NSDHL), 2) upregulated fibrosis and scarring genes (COL1A1, BMP10, CLDN5), and 3) a striking enrichment of mast cell genes (TPSAB1, MS4A2, CMA1). Notably, positive staining for mast cells (MC tryptase) was detected near the sebaceous gland of PCAs and not in controls. We also found that each subtype was associated with unique pathways, such as JAK/STAT signaling in CCCA, indicating potential molecular signatures within each subtype. To investigate the clinical significance of mast cells in PCAs, we treated 37 patients with the oral mast cell stabilizer drug, cromolyn. Five patients reported improvement within 6 weeks and 3 continued to report transient improvement of symptoms after 3 months. While clinical improvement was transient, optimization in formulation and delivery may improve durability, and many additional drugs to target mast cells are available for investigation. Our findings revealed common gene expression pathways among PCAs, and demonstrated targeting of mast cells as a novel treatment strategy.

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3D head visualization for mapping and tracking dermatological conditions

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Tracking or quantifying changes in hair and skin conditions over time is a task usually left up to the clinician: the affected areas (or images thereof) are either compared to the photos taken during past visits or a standard reference scale is used to compare the condition to a limited set of images or drawings to determine the severity level of the current state. While automated skin detection algorithms are gaining traction for binary diagnostic tasks or to segment areas of interest at a particular time-point, tracking changes automatically remains challenging due to the lack of easily accessible and affordable systems that can be used to quantitatively compare the changes in a standardized way. Focusing on tracking conditions that occur on the head, we present a new method to recover a clinically relevant model of a person's head, defined as the complete 3D head surface in the absence of hair volume, starting only with a video taken from a single hand-held camera. Using techniques from computer vision, more specifically structure-from-motion and multi-view stereo, we determine first the shape of each person's head and then the alignment of the fitted 3D head for all video frames to recover texture mapping information, irrespective of the person's pose. This alignment is then used to map and visualize hair or skin information, for example disease quantifications, onto the head model for tracking changes in the condition over time. We demonstrate that our approach recovers a consistent geometry for varying head shapes, from videos taken by different people, with different smartphones, and in a variety of uncontrolled environments such as outdoors, living rooms, and hallways, and hence, can be applied to the clinical setting as well. Furthermore, we show how, once the head geometry for the person has been recovered, it can also be used in augmented reality compatible smartphones to guide image capture and map dermatological quantifications for integration in the clinic.

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A review of cutaneous lymphoma outcomes during COVID-19 pandemic at Columbia University

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New York-Presbyterian Hospital and Columbia University Irving Medical Center were heavily impacted by the COVID-19 pandemic. Various measures were taken in an effort to ensure patient and staff safety. The management of patients with complex dermatological oncologic conditions, such as cutaneous lymphomas was especially challenging. We retrospectively reviewed the charts of the patients with cutaneous lymphomas who had COVID-19 (n=7) as well as those who did not have COVID-19 (n=26) from March to September 2020. Due to safety protocols, 4/7 (57%) patients who contracted COVID-19 experienced a treatment interruption. Three patients had no treatment interruptions because the timing of their COVID-related illness and scheduled treatments did not overlap. Treatment was delayed for a mean 2.1 months (range: 10 days - 4 months). Two out of four (50%) patients with treatment delays experienced disease relapse. Of the patients who did not have COVID-19, 12 patients experienced treatment delays, and ten (83.3%) of those patients experienced disease progression or relapse. Fourteen patients continued in hospital treatments with no delay, and 2 (14.3%) patients experienced disease progression or relapse. Of the total patients included in this review, 16 (48.5%) experienced a treatment. Delay. Twelve patients (12/16 or 75%) had disease relapse or progression following treatment delays. In contrast, among the 17 patients who did not experience treatment delay, 4 (23.5%) patients had relapse or progression of disease. Treatment delay was associated with a significant risk of disease relapse or progression (p=0.0053). No hospital-related cases of COVID-19 were recorded during the six-month capture period. Treatment interruptions are associated with negative clinical outcomes. Established safety protocols are effective in preventing infections during therapy for cutaneous lymphomas. We do not recommend altering treatment regimens for patients with cutaneous lymphomas if safety protocols can be assured.

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Automated atopic dermatitis severity assessment based on convolutional neural networks

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Convolutional neural networks (CNNs) is artificial intelligence (AI) neural network that can provide a binary or multiclass classification for the diagnosis of various skin disorders. However, there has been a challenge to predict the severity of skin disorders with CNNs. Here we investigated the optimal preprocessing conditions and performance of trained CNN models to grade the severity of atopic dermatitis (AD). Five board-certified dermatologists independently graded the severity of 9192 cropped AD images (8189 from Seoul National University Hospital (SNUH) and 1003 from Seoul National University Bundang Hospital (SNUBH)) based on the 4-scale Investigator Global Assessment (IGA). The dataset from SNUH was divided into a training/validation set (6623 images) and a testing set (1566 images), while that from SNUBH was set as an external validation set. For training, the Inception-Resnet-V2 CNN architecture was employed using three distinct approaches; (1) combination of five gradings (integrated model) vs. training with each grading and combining the results (sum of individual models), (2) median values (one-hot encoding) vs. distributions from five gradings (softmax), and (3) all training dataset vs. dataset with agreement among three and more dermatologists (exclusion of noisy data). Ground truth was determined as the median value of five gradings. The CNN model using an integrated, one-hot encoding approach without noisy data outperformed other models both for a testing set (accuracy: 73%, macro-averaged area under the curve (AUC): 0.93, and macro-averaged f1-score: 0.71) and external validation set (accuracy: 72%, macro-averaged AUC: 0.93, and macro-averaged f1-score: 0.69). The CNN model can help to evaluate the severity of AD more objectively. Proper preprocessing can improve the performance of medical AI algorithms.

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Tirbanibulin, a novel anti-proliferative and pro-apoptotic agent for the treatment of actinic keratosis

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In two Phase 3 trials, tirbanibulin, a new synthetic chemical entity, has been shown to effectively treat actinic keratosis (AK), a precancerous skin condition characterized by uncontrolled proliferation of atypical keratinocytes. Tirbanibulin inhibits tubulin polymerization, which is essential in the assembly of microtubules in the mitotic spindle. *In-vitro* competitive binding assays with purified tubulin demonstrate tirbanibulin binds directly to α- and β-tubulins. Immunofluorescence and flow cytometry assays showed tirbanibulin disrupts the microtubule network, arrest the cell cycle in the G2/M phase and ultimately induces apoptosis in human immortalized keratinocytes (CCD-1106 KERTr). These anti-mitotic and pro-apoptotic effects were observed in the growth inhibition of immortalized keratinocytes ($[GI_{50}] = 40$ nM), and multiple tumor cell lines, including multi-drug resistant cell lines, in cell-based growth assays (MTT). Histologically, features of aborted mitosis and apoptosis of basal keratinocytes were confirmed in the skin of minipigs topically treated with tirbanibulin ointment 0.1%. Lastly, following incubation with tirbanibulin *in-vitro*, immortalized keratinocytes released lower pro-inflammatory cytokines associated with adverse cutaneous reactions (IL-8 and TNF-α) compared to those treated with 5-fluorouracil. Together these studies demonstrate tirbanibulin inhibits tubulin polymerization and exerts potent anti-proliferative and pro-apoptotic effects in actively dividing cells, and thereby support the effective clearance of AK lesions and good tolerance observed in clinical trials.

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A calpain inhibitor ALLN alleviates bleomycin-induced skin fibrosis via antagonizing TGF-β/Smad signaling pathway

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Systemic sclerosis (SSc) is a connective tissue disorder representing fibrosis and vascular damage in the skin and internal organs. An activated differentiation of local progenitor cells to myofibroblasts is likely a key mechanism underlying overproduction of extracellular matrix and resultant tissue fibrosis in SSc. Calpains are family members of Ca^{2+}-dependent cysteine proteases for which the biological action may contribute to fibrosis in various organs. However, the precise mechanism of calpain-dependent fibrosis and the potential utility of their inhibitors in SSc remain unclear. This study aimed to investigate if one of calpain inhibitors ALLN could possess the antifibrotic effects on a bleomycin-induced SSc model mice and cultured human dermal fibroblasts, offering an innovative therapeutic approach for skin sclerosis in SSc. Intraperitoneal administration of ALLN was well tolerated throughout the in vivo experiments. Intraperitoneal ALLN (3mg/kg/day, three times a week) remarkably suppressed the excess dermal fibrosis by 72% in the bleomycin-injected mouse skin. Infiltrating $F4/80^+$ macrophages and $CD3^+$ T cells tended to decrease in number in the ALLN-treated mouse skin compared to the control. On the other hand, in vitro ALLN treatment significantly inhibited over-phosphorylation and nuclear transport of Smad3 in TGF-β1-stimulated fibroblasts. TGF-β1-dependent increase of collage type 1, fibronectin 1, α-smooth muscle actin, and epithelial-mesenchymal transition markers including SLUG and ZEB1, was attenuated in mRNA and protein expression by ALLN. Our data provide evidence that calpain may be a primary contributor and novel therapeutic target for skin fibrosis in SSc, with a treatment perspective of its inhibitor ALLN.

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Novel selective phosphodiesterase inhibitors promote the adipogenic function of dermal fibroblasts: Implication to treat hair loss

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Recent studies have recognized several important non-metabolic functions of dermal adipocytes, including regulation of hair cycling and wound healing, but the underlying mechanism is still unclear. Adipogenesis is partially controlled by the cAMP-PKA signaling pathway, which is negatively regulated by the cyclic nucleotide phosphodiesterases (PDEs). In this study, we aimed to clarify the mechanism by which dermal adipocytes regulate hair growth, and to screen PDE inhibitors with pro-adipogenic function to promote hair growth. Here, we screened several novel PDE inhibitors and tested their in vitro effects in modulating the adipogenic and fibrogenic function of primary murine dermal fibroblasts.Among the PDE inhibitors tested, PDE inhibitor C5 was identified as a potent PDE inhibitor that can promote a spontaneous differentiation of primary dermal fibroblasts into adipocytes. Single cell RNAseq analysis of total skin cells and immunostaining analysis revealed that this PDE isoform was specifically expressed in a group of dermal adipocyte stem cells. In vivo, we found that intradermal injection of adipocytes conditioned medium promoted hair growth in a mouse model of hair depilation. In addition, administration of PDE inhibitor C5 promoted adipogenesis and hair growth in depilated mice. Furthermore, administration of PDE inhibitor to aged mice promoted pigmentation of the regenerated hair and significantly increased the length of the regenerated hair compared to control mice ($p<0.0001$). Together, our results show that dermal adipocytes promote hair regeneration, and PDE inhibitor potently stimulate adipogenesis and is effective to promote hair growth in vivo. These results suggest PDE may be potential selective therapeutic target to treat alopecia/hair loss by targeting adipocytes.

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Is topical remetinostat gel an effective and safe treatment for basal cell carcinoma? Results of a phase 2, open label, single arm trial

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Surgical excision remains the gold standard treatment for basal cell carcinoma (BCC). However, it is associated with potential morbidity, particularly for patients with multiple or recurrent tumors. To identify novel treatment option for BCC, we previously used molecular data from human BCCs to conduct a drug repositioning screen. Our results identified histone deacetylase (HDAC) inhibitors as the leading predicted therapeutic, strongly opposing the BCC gene-expression signature. We conducted a phase 2, open label, single arm and institution trial of the topical pan-HDAC inhibitor, remetinostat, as the first proof-of-principle study of this medication class for BCC. Participants with at least one BCC referred for excision at Stanford were recruited and instructed to apply 1% remetinostat gel three times daily for six weeks. Measurements of tumor diameter and photography were conducted at baseline and week 8, and remaining tumor was then excised for histological examination, with a subset also sent for immunohistochemistry (IHC). 25 participants with 33 BCCs were included in the per-protocol analysis. The objective response rate (the proportion of tumors achieving at least a 30% decrease in the longest diameter from baseline to week 8) was 69.7% (90% confidence interval 54-82.5%), and 54.8% of the tumors demonstrated complete resolution on histological examination. IHC staining supported the pharmacodynamic effect of the remetinostat, demonstrating increased acetylated histone H3 in the excised biopsies compared to baseline. No systemic adverse events were reported, and the medication was well tolerated. Remetinostat, a topical pan-HDAC inhibitor, is a safe and effective topical treatment for BCC, reducing disease burden in a clinically significant manner. Our results provide the first in-human validation of HDAC inhibitors as a potential therapeutic.

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Preclinical evaluation of the role of protease activated receptor-2 (PAR-2) shows its limitations as a target for atopic dermatitis

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PAR2 is a G-protein-coupled receptor that acts as a sensor of extracellular proteases particularly abundant in skin inflammatory conditions. PAR-2 has been implicated in epidermal homeostasis, barrier formation, immune responses and itch, linking it with a potential role in atopic dermatitis (AD). Transgenic mice overexpressing PAR-2 in the skin develop a dermatitis-like phenotype that support this link. However, so far, there is no confirmation that PAR-2 blockade reduces skin inflammation which is a key critical feature of AD. The aim of our study has been to assess the relevance of PAR-2 in this disease. We identified a selective human PAR-2 antagonist from patent review and assessed that it also blocked mouse PAR-2 in vitro. Activity of the compound was assayed in a mouse model of inflammatory pruritus by topical route. The compound completely inhibited pruritus in a therapeutic protocol at 0.3mg/ml. The effect of the compound was then evaluated in two murine models that recapitulate different aspects of AD; the Th2-dependent FITC-induced delayed-type hypersensitivity (FITC-DTH), and a model of skin inflammation induced by repeated applications of oxazolone in the ear of mice (OXA). Tacrolimus was used as a positive control in both models. Topical application of the compound showed not significant inhibition of ear edema in the FITC-DTH model and only a small inhibition of inflammatory signs and a modest increase in gene expression of skin barrier markers in the OXA model. To test whether systemic PAR-2 blockade rather than local inhibition was needed for a significant anti-inflammatory effect, we used PAR-2 deficient mice and induced them dermatitis using OXA and calcipotriol-induced dermatitis as AD models. In neither case the lack of PAR-2 translated into a reduction of skin inflammation. Using the described approaches, our findings confirmed the role of PAR-2 in some aspects of the disease but showed no role in skin inflammation, demonstrating the limitations of PAR-2 role in AD.

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Engineering chimeric antigen receptor (CAR) T cells for treatment of γδ T cell lymphomas

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γδ T cell lymphomas (γδTCLs) have 3 major subtypes, hepatosplenic, mucosal, and cutaneous, reflecting the tissue tropism of γδ T cells. A common feature of γδTCLs is their poor prognosis. Primary cutaneous γδTCLs are highly resistant to treatment, with a median survival of 15 months. Chimeric antigen receptors (CARs) redirect T cells to specifically kill cancer cells if they express the antigen targeted by the CAR. γδ T cells are uniquely identified by expression of a γδ T cell receptor (TCR), and γδ T cell-deficient mice have no baseline phenotypic defects, thus identifying an ideal CAR target for γδTCLs, as well as the 10% of T cell leukemias that express γδ TCRs. Here we show that primary human T cells can be engineered to express CARs that specifically target the TCRδ constant region. In vitro, anti-TCRδ CAR T cells demonstrate robust cytotoxicity toward benign and leukemic γδ T cells while sparing αβ T and B cells. Anti-TCRδ CAR T cells specifically eliminated primary human γδ, but not αβ T cells or B cells, in a human stem cell xenograft model (n=4 per group, p=0.0047). In a tumor xenograft model, a single injection of anti-TCRδ CAR T cells, but not control T cells, eradicated an established human γδ T cell leukemia in a dose-dependent fashion (n=35, p=0.0012) by in vivo imaging, confirmed by flow-cytometric analysis of peripheral blood. Comprehensive histopathological analysis of 19 different tissues from CAR or control treated mice demonstrated absence of acute CAR T cell mediated toxicity (n=16), which was additionally supported by an absence of serum chemistry and blood count differences (n=35) compared to controls. In conclusion, we provide evidence of potent in vitro and in vivo safety and efficacy of anti-TCRδ CAR T cells and establish a novel treatment modality for γδTCL that offers the potential for durable remissions of previously incurable cancers.

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Evaluation of a first in class proteasome inhibitor in patients with moderate to severe rosacea

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Background: Novel, effective, affordable therapies for rosacea are needed. Innovative methods of assessing response for rosacea treatments are needed as well. Objective: This trial was designed to evaluate efficacy and safety of ACU-D1, a novel inhibitor of the 26S proteasome for the treatment of moderate to severe rosacea in a first in human pilot study. In addition, this is the first trial to our knowledge to use Canfield imaging to quantitatively assess responses. ClinicalTrials.gov identifier NCT03064438 Methods: This was a 14-week, randomized, double-blinded, placebo-controlled study, performed at two well established rosacea clinical trial sites, randomized 40 adult subjects with moderate to severe rosacea (Investigator's Global Assessment [IGA]=3/4) to either ACU-D1 (27) or comparator vehicle (13) twice daily. In addition, Canfield imaging was used to assess responses both qualitatively and quantitatively. Results: A total of 39 subjects participated. ACU-D1 displayed efficacy in 92% (25 of 27) of patients in reducing inflammatory lesions and a 2 plus grade IGA reduction of clear to near clear in 27% of patients. There was a trend toward improvement in erythema as well in the active arm over that of the vehicle arm. Limitations: This study was not powered to demonstrate statistical significance between active and vehicle as was an efficacy and tolerability 2a study per FDA recommendations for this first in human study

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Telazorlimab in atopic dermatitis: Phase 2b study shows improvement at 16 weeks

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Telazorlimab, a monoclonal antibody to human OX40 costimulatory receptor on activated T cells, is being developed to treat autoimmune diseases. Herein, we describe topline results of a randomized, multinational, double-blind, placebo-controlled, phase 2b study that investigated the efficacy, safety, pharmacokinetics, and pharmacodynamics of different subcutaneous regimens of telazorlimab for patients with moderate-to-severe atopic dermatitis (AD). Part 1 randomized 313 adults (1:1:1:1): telazorlimab 300 mg every 2 weeks (q2w), 300 mg q4w, 75 mg q4w, or placebo. Part 2 randomized 149 adults (1:1): telazorlimab 600 mg q2w or placebo. All subjects received a loading dose of blinded telazorlimab or placebo at baseline, per their treatment group. The primary endpoint, percent change from baseline in Eczema Area and Severity Index (EASI) score at Week 16, was met by the highest dose of telazorlimab in Part 1 and in Part 2 ($p=0.008$ vs placebo for both). A numerical trend for improvements in key secondary endpoints (Investigator's Global Assessment 0-1, EASI-75) was observed. There were no safety signals in Part 1 or 2. Treatment-emergent adverse events (TEAEs) were comparable for telazorlimab vs placebo in Part 1 (65.4% vs 72.5%) and Part 2 (65.3% vs 50.0%). The most commonly reported TEAEs were AD, nasopharyngitis, upper respiratory tract infections, and headache. Serious AE rates were comparable for telazorlimab vs placebo (Part 1: 3.0% vs 1.3%; Part 2: 1.3% vs 0%, respectively). In conclusion, telazorlimab improved clinical signs and symptoms of moderate-to-severe AD, with a favorable safety and tolerability profile. Further evaluation of telazorlimab in the treatment of autoimmune disease is warranted.

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Investigation of cell death patterns of SJS/TEN model cells harboring formyl peptide receptor 1

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Stevens Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare severe adverse drug eruption. The mortality is up to 25% for TEN. The patients suffer from acute inflammation on their skins, buccal and ocular mucosa, resulting in vast skin detachment and loss of eyesight. No specific pharmaceuticals have been developed yet due to the lack of knowledge about the molecular mechanism of SJS/TEN. Previously, we have revealed an involvement of one of GPCRs, formyl peptide receptor 1 (FPR1), in a unique cell death, necroptosis, observed in lesional skins. Here, we reconstituted in vitro cell death model cells by introducing FPR1 into cultured skin cells, HaCaT cells, to analyze the mechanism of keratinocyte death in SJS/TEN. The expression level of FPR1 was measured based on the amount of cell-bound fluorescent ligands for FPR1 with using FACS. The expression of FPR1 was also confirmed by mRNA level with using qRT-PCR. The cell death was quantified by staining live cells and dead cells respectively with specific fluorescent dyes, calcein-AM and EthD-III. As a result, ATP stimulation induced an increase in the expression level of FPR1 in HaCaT cells. Ligand stimulation to FPR1 triggered transient phosphorylation of ERK, demonstrating the functionality of endogenous FPR1. Interestingly, the increase in the expression level of FPR1 was inhibited by fetal bovine serum. Next, the FPR1-expressing cells were stimulated with FPR1 specific ligand. From four to ten hours after ligand stimulation, 23% of the cells showed spontaneous cell deaths. The cell death was almost completely inhibited by an apoptosis inhibitor, suggesting that FPR1 stimulation induced apoptosis for HaCaT cells, instead of necroptosis. We also exogenously introduced FPR1 into HaCaT cells by lipofection. Ligand stimulation to the exogenous FPR1 also triggered apoptosis. Collectively, expression and activation of FPR1 in HaCaT cells triggered apoptosis instead of necroptosis, implying that additional signal networks might be modulated in SJS/TEN keratinocytes.

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IL-25 induces skin inflammation in mice: Effects of a selective JAK inhibitor

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Interleukin (IL)-25 (IL-17E) is an alarmin produced by multiple cell types, including keratinocytes, macrophages, mast cells, eosinophils, and basophils. Expression of IL-25 (and/or its receptor, a heterodimer of IL-17RB and IL-17RA) has been reported to increase in patients with atopic dermatitis (AD), asthma, and psoriasis. IL-25 increases type 2 cytokine production by Th2 and ILC2 cells and reduces expression of barrier proteins (filaggrin, loricrin) in primary keratinocytes. In the current study, we examined whether direct intradermal injection of mouse IL-25 could cause ear skin inflammation in mice (female BALBc) and if this could be modulated by a selective JAK inhibitor. The IL-25 protein was confirmed active by inducing CXCL1 release from HT-29 cells and suppressing barrier protein (filaggrin, loricrin) expression in keratinocytes. Intradermal injection of mouse IL-25 (0.1, 0.3, 1 ug/per injection, once every other day for two weeks) caused dose-dependent ear swelling, increase in trans-epidermal water loss (TEWL), and increased expression of 14 genes (CCL17, CXCL1, CCL3, IL-4, IL-13, IL-5, IL-22, IL-1b, IL-23, INF-γ, S100A8, Defb4, IL-31, IL-6) in the skin. The selective JAK inhibitor, ABT-317 (3, 10, 30 mg/kg, orally dosed), reduced ear thickening and expression of cytokines/chemokines (mRNA and protein) in a dose-dependent manner. In a separate study, ABT-317 (30 mg/kg, orally dosed) also effectively abrogated the increase of TEWL (a marker of barrier function) and an increase of dermal and epidermal area in IL-25 injected mice. These data suggest that IL-25 is an important proinflammatory factor that contributes to the pathophysiology of skin inflammatory diseases and can be modulated by JAK inhibitors. Further studies are also warranted to better understand the immune responses triggered by IL-25.

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A curcumin-derivative LG283 that inhibits TGF-β/Smad/Snail-dependent mesenchymal transition ameliorates bleomycin-induced skin fibrosis and vascular injury

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Systemic sclerosis (SSc) is a collagen disease characterized by fibrosis and vascular injury in the skin and internal organs. Transforming growth factor-β (TGF-β)/Smad signaling has been considered to play an important role in the fibrotic process in the disease via inducing extracellular matrix (ECM) production, epithelial-to-mesenchymal transition (EMT), and endothelial-to-mesenchymal transition (EndMT). We found a curcumin derivative LG283 that has a potent antifibrotic activity from more than 1,200 compounds by a high-throughput screening. This study aimed to analyze the effects of LG283 on a bleomycin-induced skin fibrosis mouse model, and also inhibitory function for mesenchymal differentiation in vivo and vitro. Oral administration of LG283 was well tolerated throughout the in vivo experiments. Oral LG283 significantly attenuated the dermal fibrosis and vascular injury with reduced expression of phosphorylated Smad3 and EMT-inducing transcription factor Snails in bleomycin-injected skin. However, it did not significantly alter the inflammatory cell infiltration and the expression levels of profibrotic cytokines and other transcription factors. LG283 suppressed the TGF-β-induced expression of ECMs, such as fibronectin 1 and type I collagen, phosphoryled Smad3, and Snails in cultured human dermal fibroblasts. Regarding the effect for EMT and EndMT, LG283 attenuated the TGF-β-induced morphologic change from the round to fibroblastic spindle shape in cultured human epithelial line A541 cells with decrease of cell adhesion, whereas it exhibited the suppressive activity in increased mesenchymal markers and decreased vascular markers in HUVEC cells stimulated with TGF-β. Our data suggest that LG283 inhibits skin fibrosis and vascular injury by antagonizing TGF-β/Smad/Snail-mediated differentiation of epithelial cells, endothelial cells in addition to fibroblasts into myofibroblasts, and can be a novel therapeutic candidate for SSc.

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Evaluation of the *in vitro* percutaneous absorption of progesterone, testosterone, estriol and estradiol topical compounded formulations

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The safety, effectiveness and use of compounded bioidentical hormone replacement therapy has been questioned by the US Food and Drug Administration in the light of the recently published study report by The National Academies of Sciences, Engineering and Medicine. An in vitro study was conducted to evaluate the human skin percutaneous absorption of commonly prescribed bioidentical hormones using the Franz Skin Finite Dose Model. A topical compounded cream (VersaBase Cream) and gel (VersaBase Anhydrous HRT) were prepared for progesterone 100 mg/g, testosterone 1 mg/g, and estriol/estradiol [50%/50%] 2 mg/g. The 6 formulations were applied to the outer surface of ex vivo skin mounted in Franz diffusion chambers (9 tissues/formulation) to evaluate the total absorption, rate of absorption and skin content of the bioidentical hormones. The topical creams and gels for progesterone and testosterone exhibited similar mean flux profiles, characterized by a rise in skin percutaneous absorption to a peak at approximately 7 hrs (progesterone) and 5 hrs (testosterone) after dose application, followed by a slow decline in flux over time (24 hrs). The estrogens did not show a peak rise but instead a steady mean flux that increased slightly over the study time. Overall, the total absorption of the bioidentical hormones was higher for the topical gels in comparison to the creams. The total recovery (receptor medium, skin content and surface wash) of the bioidentical hormones varied from 87-97%. This study demonstrates that the bioidentical hormones penetrate through the skin (stratum corneum, epidermis and dermis) upon application of the topical compounded creams and gels. These results are important evidence to support the effectiveness of compounded bioidentical hormone replacement therapy since the in vitro model used has proven to accurately predict in vivo permeation kinetics.

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Topical treatment of human skin and cultured keratinocytes with high-dose spironolactone reduces XPB expression and induces genotoxicity

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The mineralocorticoid and androgen receptor antagonist spironolactone (SP) is used to treat a variety of disparate disease states ranging from heart failure to acne. Though SP is usually taken as an oral medication, several recent studies have explored the administration of SP topically on the skin to avoid systemic effects in the body. Because SP induces the proteolytic degradation of the XPB (xeroderma pigmentosum group B) DNA translocase protein, which is involved in both DNA repair and transcription, SP may have genotoxic effects in the skin. Using skin explants, we show here that the topical application of a high concentration of either SP or its metabolite canrenone onto human skin induces the loss of the XPB protein and the phosphorylation of the histone variant H2AX, a common marker of genotoxicity. Though high concentrations of SP and canrenone both inhibit cell proliferation, induce H2AX phosphorylation, and stimulate apoptotic signaling in cultured keratinocytes in vitro, canrenone is much less potent at inducing the loss of XPB. Thus, high concentrations of SP and canrenone likely inhibit cell proliferation and induce genotoxicity via additional mechanisms besides XPB proteolytic degradation. Together, this work suggests that care may need to be taken when using high concentrations of SP directly on human skin.

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Development of a tape-stripping/LC-MS method for evaluating deposition of topical tazarotene in skin

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Historically, ex vivo and in vitro techniques have been used to evaluate skin deposition of topical acne treatments. This study was designed to explore whether tape-stripping and liquid chromatography-mass spectrometry (LC-MS) methods could be used to assess deposition in living human subjects. The study included 10 healthy volunteers who had 0.1 g of tazarotene (TAZ) 0.045% lotion and 0.1% cream applied to 1.5-square inch areas on their forearms. After 3 and 6 hours, 21 consecutive D-Squame 7/8" circular tape strips were applied to test areas and held for 10 sec with a controlled pressure plunger (225 g/cm^2). Tape strips were removed and frozen (-20°C), and odd-numbered samples were stored for back-up. Even-numbered strips were dissolved in acetone for LC-MS analysis of TAZ levels, with each strip representing a deeper skin layer from stratum corneum into lower epidermis and superficial dermis. The percent of drug recovery was higher with lotion versus cream (15.5% vs 13.8% for all even-numbered strips at 6 hours), possibly due to the polymeric emulsion technology used to develop the lotion. Most TAZ remained on the skin surface, with 2-fold higher TAZ levels on strip 2 with cream versus lotion, which may contribute to more irritation with higher drug concentrations. Lower TAZ levels were present in deeper skin layers (strip 20), but these levels (which were similar between formulations based on absolute difference in drug recovery) are sufficient for clinical effect since TAZ operates via retinoid-receptor activation. Drug recovery from the 6-hour strips was as follows: strip 2 (1.62 vs 0.82 µg [cream vs lotion]; difference=0.80 µg); strip 20 (0.18 vs 0.09 µg; difference=0.09 µg). Similar trends were observed with the 3-hour strips. These findings were also consistent with clinical trial results for TAZ 0.045% lotion (comparable efficacy to cream but with fewer side effects), which along with the relatively high percent drug recovery, suggests that this tape-stripping/LC-MS method may be used to assess the deposition of other topical medications. Support: Ortho Dermatologics

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High-density lipoprotein-nanoparticles (HDL NPs): A novel therapy for inflammatory skin

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Nanoparticles can be used to synthesize higher order supramolecular materials that seamlessly interface with biological systems to modulate fundamental biologic processes such as cell proliferation, migration, inflammation, autophagy and metabolism. One such example is the synthetic high-density lipoprotein-nanoparticle (HDL-NPs), which are a size, shape, and surface chemical mimic of native, spherical HDLs, enabling binding to the high-affinity and lipid-raft associated native HDL receptor, scavenger receptor type B-1 (SR-B1). Once bound to SR-B1, HDL-NPs can modulate target cell lipid metabolism and exert biological effects. We have demonstrated that an HDL NP topical eye drop has: (i) stability; (ii) minimal adverse side effects; (iii) tissue regenerative capabilities; and, (iv) anti-inflammatory properties. We now report that, when topically applied to imiquimod (IMQ)-treated mouse skin, HDL-NPs can: (i) penetrate through the mouse epidermis; (ii) reduce the thickness of the hyperproliferative mouse epidermis by 15%; (iii) attenuate the inflammatory CD3+ cell infiltration by 66%; (iv) decrease the expression of cytokines and chemokines with a reduction in expression of *Tlr7* and *Il22r* by 40%; (v) improve keratinocyte differentiation with increased loricrin expression; and (vi) inhibit keratinocyte proliferation (20% reduction). In vitro, we show that HDL-NPs reduce LPS-induced NF-κB signaling as demonstrated by SEAP-reporter assay in macrophages. These novel observations on the therapeutic activity of HDLs in the context of skin diseases associated with abnormal inflammation have vast translational significance, particularly when such particles do not display significant adverse side effects.

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Cannabigerol: The mother of cannabinoids demonstrates a broad spectrum of anti-inflammatory and anti-microbial properties important for skin

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Cannabigerol (CBG) is a minor cannabinoid present in cannabis plant that serves as the direct precursor to cannabidiol (CBD), a building block for Tetrahydrocannabinol (THC) and is thus referred to as the "mother of cannabinoids". In most cannabis strains, CBG is present at <1%, therefore it is considered a minor cannabinoid. Producing CBG from cannabis is both challenging and expensive and due to this hurdle, research for its activity on skin is lacking unlike CBD, which has been well studied and demonstrates several benefits to skin when applied topically. Utilizing our novel yeast fermentation technology platform, we can now produce cannabinoids such as CBG identical to what is produced in plants, faster, purer and more affordable than plant-based production. Thus, we sought to begin to characterize CBG's activity and safety profile for skin. *In vitro* results utilizing Normal Human Epidermal Keratinocytes (NHEKs) and Human Dermal Fibroblasts (HDFs) demonstrates that CBG possesses potent anti-inflammatory properties versus different environmental stressors inhibiting ultraviolet (UV), *C. acnes* and *S. aureus* induced pro-inflammatory cytokine release (e.g. IL-6, IL-8). In addition to its anti-inflammatory activity, CBG also shows strong anti-microbial properties towards *C. acnes* and *S. aureus* growth, two bacteria strains shown to play a key role in the pathogenesis of acne and atopic dermatitis, respectively. Moreover, CBG also demonstrates a robust safety profile, showing no signs of skin irritation, eye irritation or phototoxicity, paving the way for its use in skin care. Additional studies including gene array analysis utilizing 3D human skin compare the similarities and differences between CBG and CBD when applied to skin as well as dermal penetration studies. Altogether, the data presented here is the first to demonstrate CBG's strong potential for use in skin care and show the similarities and differences between it and its more well-known predecessor CBD.

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Secukinumab rapidly alleviates fever and skin lesions in an erythrodermic psoriasis patient - A case report

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Erythrodermic psoriasis (EP) is an intractable type of psoriasis. Here we report a case of successfully treatment of lesions and persistent fever in a EP patient transformed from vulgaris psoriasis using secukinumab. This case indicate the anti-inflammatory effect of secukinumab.

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Defining drugs that are high-risk associations for drug reactions within the hospital setting

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Cutaneous drug eruptions are the most common inpatient dermatologic diagnosis and are associated with large mortality and economic impacts. When treating drug eruptions, it is often challenging to identify the offending medication, resulting in patients unnecessarily having multiple medications removed from their treatment regimen. We sought to evaluate medication exposures during an entire hospitalization, with the goal of describing medications and demographic conditions that are associated with developing a drug eruption during hospitalization to assist clinicians in evaluating risks in the context of polypharmacy during a drug eruption. 468 patients that developed a cutaneous drug eruption were identified from a cohort of 18,140 unique inpatients; medication lists and demographic information were assimilated, and drug eruption frequency tables were created. The agents most commonly associated with drug eruptions included many antineoplastic, antifungal, and antibiotic therapeutics: idarubicin (27.78% reaction rate), daunorubicin (26.43%), sorafenib (25.00%), lenalidomide (23.53%), all-trans-retinoic acid (22.58%), decitabine (21.57%), aztreonam (15.15%), posaconazole (14.29%), and voriconazole (13.78%) among many others. Patients diagnosed with drug eruptions were more likely to have private insurance (3.29% vs. 2.58% reaction rate) and were on average older (56.7 vs. 52.6 years), had longer inpatient stay (14.2 vs. 7.9 days), and higher inpatient mortality (5.95% vs. 2.58%) than controls. This study affirmed that hospitalizations in which patients receive medications common to malignancies, such as cytotoxic and antifungal therapies, represent the highest risk hospitalizations for the development of drug eruptions. When diagnosing and treating drug eruptions, clinicians should consider these medication classes with a high index of suspicion.

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Antimicrobials from a feline skin commensal bacterium inhibits drug-resistant *S. pseudintermedius* skin colonization and infection

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S. pseudintermedius is an important emerging zoonotic pathogen that can cause skin and soft tissue infections in dogs and humans. *S. pseudintermedius* contains virulence and antimicrobial resistance mechanisms similar to *S. aureus*. To combat the increasing incidence of bacterial drug resistance, the application and transplantation of commensal antimicrobial bacteria as a bacteriotherapeutic has shown clinical promise. Here we screened a selection of staphylococci isolates collected from the skin, nasal and perineum of domestic dogs and cats for antimicrobial activity against methicillin-resistant *S. pseudintermedius* (MRSP). We identified a *S. felis* C4 isolate from feline skin that inhibited growth of several important gram-positive pathogens. *S. felis* C4 was suitable as a bacteriotherapy, showing sensitivity to common antibiotics and was well tolerated on mouse skin. Competition experiments in mice showed that a *S. felis* C4 significantly reduced MRSP colonization on mouse skin ($p>0.0263$). Furthermore, an antimicrobial extract from *S. felis* C4 supernatant significantly reduced dermonecrotic skin injury during MRSP intradermal infection ($p>0.0145$). Through fluorescence and electron microscopy, *S. felis* C4 extract disrupted bacterial cell membranes but not eukaryotic cells. LC/MS identified several phenol soluble modulin beta (PSMβ1-3) peptides made by *S. felis* that exhibited antimicrobial activity against MRSP. RNA-Seq analysis demonstrated that the extract and PSMβ peptides also exhibited anti-inflammatory activity, suppressing TLR-mediated cytokine release from keratinocytes. Overall, these findings report antimicrobial and anti-inflammatory activity from a commensal bacterium residing on cats that could be utilized in bacteriotherapy against difficult-to-treat animal skin infections.

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Dupilumab provides clinically meaningful improvement in atopic dermatitis (AD) signs and symptoms and quality of life (QoL) in children with severe AD: Results from the LIBERTY AD PEDS phase 3 clinical trial

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Background: Patients with AD suffer from a multidimensional disease burden. We report clinically meaningful improvements in AD signs & symptoms and QoL in dupilumab-treated children. Methods: In the LIBERTY AD PEDS phase 3 trial (NCT03345914), children aged 6–11 years were randomized 1:1:1 to dupilumab 300 mg every 4 weeks (q4w; loading dose 600 mg), 100 mg/200 mg q2w (loading dose 200 mg/400 mg), or placebo, all with concomitant medium-potency topical corticosteroids (TCS). We evaluated the proportion of patients reaching a composite endpoint at Week (Wk) 16, defined as: ≥ 50% improvement in Eczema Area and Severity Index, ≥ 3-point improvement in worst itch score, and ≥ 6-point improvement in Children's Dermatology Life Quality Index from baseline. In this analysis, the 100 mg q2w dosing regimen was not included (not approved for use in AD). Results: This analysis included 304 patients (< 30kg: 300 mg q4w/placebo; ≥ 30kg: 200 mg q2w/300 mg q4w/placebo; n = 61/61/59/61/62). At Wk16, significantly more dupilumab-treated patients achieved all 3 clinically meaningful endpoints compared with patients who received placebo + TCS (< 30kg: 49.2% 300 mg q4w/9.8% placebo; ≥ 30kg: 47.5% 200 mg q2w/39.3% 300 mg q4w/8.1% placebo; $P < 0.0001$ for all). Significantly more patients treated with dupilumab achieved ≥ 1 endpoint compared with placebo at Wk16 (< 30kg: 95.1% 300 mg q4w/62.3% placebo; ≥ 30kg: 94.9% 200 mg q2w/95.1% 300 mg q4w/59.7% placebo; $P < 0.0001$ for all). The safety profile was consistent with the known dupilumab safety profile. Conclusions: Dupilumab + TCS provide clinically meaningful improvements in AD signs and symptoms and QoL in children aged 6–11 years with severe AD.

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Repurposing disulfiram for the treatment of Merkel cell carcinoma

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Merkel cell carcinoma (MCC) is an aggressive neuroendocrine skin cancer with limited treatment options. Although two immune checkpoint inhibitors are approved for the treatment of advanced stage MCC, less than half of patients achieve durable benefit. Therefore, new and effective treatments are needed for MCC. To address this need, we conducted a high-throughput drug screen of approximately 4,000 small molecules and identified disulfiram (DSF), an aldehyde dehydrogenase inhibitor used in the treatment of alcoholism, as an agent that selectively reduced MCC cell viability. We found that complexing disulfiram with copper increased its potency against MCC cells. Treatment with disulfiram plus copper was cytostatic, induced autophagy, and caused non-apoptotic cell death in MCC cells. Interestingly it also increased the expression of immunogenic cell death markers and increased PD-L1 expression at the membrane. We also observed that disulfiram plus copper synergized with the topoisomerase II inhibitor etoposide to reduce MCC cell viability by enhancing DNA damage as evidenced by gamma H2A.X foci in the nucleus. Taken together, our data suggest that disulfiram plus copper can be repurposed for the treatment of advanced stage MCC, either in combination with etoposide or as a way to enhance responses to immunotherapies.

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Systemic collagen VII protein therapy for treatment of advanced RDEB

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Recessive dystrophic epidermolysis bullosa (RDEB) is a genetic skin blistering disease associated with progressive multi-organ fibrosis. RDEB is caused by biallelic *COL7A1* mutations leading to reduced production or functionality of the extracellular matrix protein, collagen VII (C7), which is necessary for epidermal-dermal adherence. C7 is not simply a structural protein but also has multiple functions, including regulation of TGFβ bioavailability and the inhibition of skin scarring after wounding. The multi-organ involvement of RDEB calls for systemic treatment. Intriguingly, intravenous (IV) administration of recombinant C7 (rC7) improves the clinical phenotype of newborn C7 deficient mice and rescues them from neonatal lethality. The effect on established RDEB in adult animals has not been determined. Here, we used small and large adult RDEB animal models to investigate the disease-modulating abilities of rC7 on established RDEB. In adult RDEB mice, IV-injected rC7 accumulated dose-dependently at the dermal-epidermal junction of wounded skin and oral mucosa. Bi-weekly IV injections of rC7 for 7 weeks in adult RDEB mice reduced new skin blisters and effectively reduced progression of the fibrotic mitten deformities of their paws. The inhibition of fibrosis was mediated through reduction of TGFβ signaling. IV infusion of rC7 in adult dogs with RDEB also improved disease by promoting the healing of blister-induced skin erosions without excessive scarring. In both models, IV C7 was well tolerated. These preclinical studies suggest that repeated IV administration of rC7 is an efficacious systemic treatment for established adult RDEB.

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Topical MEK inhibition as precision targeted chemoprevention

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Cutaneous squamous cell carcinoma (cuSCC) comprises at least 20% of all non-melanoma skin cancers. Effective chemoprevention for cuSCC is lacking and high-risk cuSCC causes significant surgical morbidity. Previously, we conducted RNA-seq analysis of human cuSCC, which identified ETS2, a transcription factor in the ERK pathway, as an upstream regulator of cuSCC development. Consistent with this, our computational drug repositioning screen predicted the FDA-approved MEK1, 2 inhibitor, selumetinib, as a therapeutic for cuSCC based on its ability to reverse transcriptional signatures associated with cuSCC development. As a regulator of ERK activity, we reasoned MEK would be a viable chemopreventative target. Although systemic MEK inhibition suppresses tumor formation, systemic MEKi administration causes significant adverse effects. Here, we report the development of a topically formulated, metabolically labile MEK inhibitor, NFX-179, designed to potently and selectively suppress the ERK pathway in the skin prior to rapid degradation in the circulation. In our UV-induced model of cuSCC, topical application of NFX-179 gel reduced the formation of new cuSCCs by an average of 60% at doses of 0.1% and greater at 28 days. No systemic or skin toxicities were observed. Furthermore, we conducted a split-mouse randomized controlled study in 5 mice. NFX-179 0.5% gel was applied to half of the back and vehicle was applied to the other half of each of the UV-irradiated mice. Near complete suppression of cuSCC was observed only in the drug-treated area, demonstrating the targeted and local effect of the intervention. NFX-179 inhibits the growth of human cSCC cell lines in a dose-dependent manner and topical NFX-179 application penetrates human skin and inhibits ERK signaling in human cuSCC explants. Our data provide compelling evidence that topical application of NFX-179 is a safe, effective strategy for SCC chemoprevention in high risk individuals.

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Synthetic melanin nanoparticles as a potential topical therapy for treating injured skin

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Eumelanin is a natural polymer of dopamine found in human skin. In addition to photoprotection, another important property of melanin is its ability to adsorb various organic molecules and scavenge reactive oxygen species (ROS). Based on these properties, we hypothesized that synthetic eumelanin mimics termed polydopamine nanoparticles (PDA NPs) can ameliorate the inflammatory response after chemical skin injury. In our work we utilized mouse nitrogen mustard (NM)–induced injury model. PDA NPs were topically applied two hours after the injury induction, and then at 24 and 48 hours post injury. This treatment resulted in significant reduction of initial wound area in PDA NP-treated mice compared to the vehicle-treated group at days 2 and 3 post injury ($n\geq11$ mice per group, $p<0.05$; $p<0.001$). To confirm that the observed effect was not due to the potential adsorption of residual NM, we utilized UV-induced injury model in which animals were treated with PDA NPs after exposure to 100 mJ/cm2 UVR. Similarly, the PDA NP-treated animals showed significantly decreased inflammatory response as evidenced by the bi-fold skin thickness measurements at days 1-4 post injury ($n\geq4$ mice per group, p values vary between $p<0.05$ and $p<0.0005$). Mechanistically, the PDA NP treatment downregulated expression of oxidative stress-induced genes, most notably Gsr1 ($n\geq4$ mice per group, $p<0.05$) compared to the vehicle-treated group. In addition, in NM-injured skin PDA NPs also downregulated p53 pathway, known to be activated by ROS. Overall, our data suggest that the anti-inflammatory effects of PDA NPs can be attributed to the reduction of overall ROS burden and demonstrate that topical application of PDA NPs post injury improves skin wound healing and has therapeutic potential.

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Sphingosine 1-phosphate receptors are expressed in human scalp hair follicles and their modulation by etrasimod warrants further investigation for management of alopecia areata

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Etrasimod is a selective sphingosine 1-phosphate receptor 1, 4, 5 ($S1P_{1,4,5}$) modulator in development for alopecia areata (AA). $S1P_1$ is a cell surface receptor that regulates T cell trafficking from lymphoid organs to the periphery. In skin, the S1P receptor pathway has been shown to be involved in epidermal keratinocyte proliferation and differentiation. Hair follicles (HFs) also harbor keratinocytes and are the target of autoimmune responses in AA. In AA, HFs lose their immune privilege (IP), are attacked by immune cells, and prematurely enter catagen. In this study, we evaluated the expression of $S1P_{1,5}$ in human HFs and investigated whether etrasimod beneficially impacts HF physiology and pathology. By RNAseq, in situ hybridization (ISH), and immunostaining (IS), we confirmed $S1P_{1,5}$ expression in the HF epithelium and mesenchyme in healthy human scalp skin. Interestingly, the intrafollicular epithelial expression of these receptors was increased in patients suffering from AA. AA HFs also had more perifollicular and intrafollicular $CD8^+$ cells expressing $S1P_1$. To evaluate etrasimod effects, HFs from healthy human scalp were microdissected, treated *ex vivo,* and analyzed by IS and RNAseq. Etrasimod treatment resulted in tendentially more HFs remaining in anagen and preservation of HF IP, revealed by no change in MHC class I expression. RNAseq data revealed a significant up-regulation of genes involved in hair keratinization. To mimic AA-like inflammation, human scalp skin was treated *ex vivo* with 100IU/ml IFNg and analyzed by IS. Etrasimod prevented IFNg-mediated MHC class I up-regulation and inhibited the increase of $CD8^+$, $CD8^+S1P_1{}^+$, and $CD8^-S1P_1{}^+$ cells showing lymphocytic morphology. Taken together, these initial results suggest a novel role for the $S1P_{1,5}$ pathway in HF physiology and pathology. Further investigation of etrasimod as a new oral therapeutic option for AA is warranted.

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A novel plant-derived compound induces apoptosis and cell death in mycosis fungoides

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GZ17-6.02 (6.02) is a novel investigational agent composed of three plant-derived compounds: curcumin, harmine, and isovanillin. 6.02 inhibits the growth of several tumors and is currently in clinical trials to treat solid tumors and lymphoma (NCT03775525). In this study, we have assessed the inhibitory effects of 6.02 on the growth of mycosis fungoides (MF). 6.02 reduced MF cell lines' viability, Myla and HH, with IC50 values of 14.56 μM and 14.37 μM, respectively. Compared to 6.02, the standard-of-care agent comparator bexarotene inhibited Myla and HH at IC_{50} values of 802 nM and 4.46 μM, respectively. To understand the mechanism underlying 6.02's effect on MF cells, Annexin-V/PI apoptosis assay was performed by Flow cytometry. With increasing 6.02 concentration, the mean percentage of Myla cells in early apoptosis (Annexin-V^+PI^-) increased from 3.1 to 8.4 ($p=0.009$), while those in late apoptosis (Annexin-V^+PI^+) increased from 17.4 to 44.4 ($p=0.007$). The percentage of HH cells in early apoptosis increased from 15.3 to 18.4 ($p=0.36$), while those in late apoptosis increased from 26.3 to 65.6 ($p=0.015$). In Myla cells, treatment with 6.02 led to decreased full-length PARP and an increase in cleaved PARP. Furthermore, 6.02-treatment resulted in reduced Bcl-2 expression. In contrast, treatment of HH cells with 6.02 did not produce changes in expression levels of Bcl-2, PARP, and cleaved PARP, suggesting that 6.02-mediated apoptosis in Myla and HH cells occurs via distinct mechanisms. In a subcutaneous HH tumor mouse model, mean (SD) tumor volumes for 6.02-, bexarotene-, and vehicle-treated mice at 31 days of treatment were 1718 (650) mm^3, 1682 (419) mm^3, and 2335 (1096) mm^3 in, respectively. These results suggest that 6.02 is effective in inhibiting tumor growth, similar to bexarotene treatment.

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Functional genomics screen identifies alternative targetable pathways for Merkel cell carcinoma

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Merkel cell carcinoma (MCC) is a rare, aggressive neuroendocrine skin cancer. Most MCC tumors have Merkel cell polyomavirus integrated into the host genome (virus-positive MCC; VP-MCC), whereas virus-negative MCC (VN-MCC) tumors bear UV-induced mutations. Immune checkpoint inhibitors (ICI) are the first-line treatment for metastatic MCC, but the majority of patients are either not candidates for ICI or have disease progression while on treatment. In order to identify alternative therapeutic targets for these patients, we performed a druggable genome RNAi screen in MKL-2 (VP-MCC) and MCC26 (VN-MCC) cell lines. We screened >25,000 siRNA for their ability to reduce MCC viability at 72 hours. The main gene functions identified as essential to MCC viability included cell proliferation (VP- and VN-MCC) and apoptosis (VP-MCC). These results were corroborated by a follow-up RNAi screen including the two original cell lines, WaGa (VP-MCC), MCC13 (VN-MCC), and UISO (VN-MCC). Interestingly, many essential gene products identified in our screen were targets of compounds that selectively reduced MCC viability in an independent high-throughput MCC viability screen of ~4000 small molecules. High-priority lead compounds supported by both screens showed efficacy in preclinical xenograft mouse models of MCC. Overall, our discovery and validation of novel targetable pathways have identified alternative treatment approaches for metastatic MCC.

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Safety profile of topical cannabidiol and palmitoylethanolamide: A compilation of clinical and *in vitro* studies

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While an increasing number of studies have investigated the side effect profile of oral cannabinoids, few studies have provided sufficient data on the safety of topical cannabinoids in humans. We assessed the irritation and sensitization profile of several commercial topical formulations containing cannabidiol (CBD) and palmitoylethanolamide (PEA) on the skin of healthy human subjects. Three human clinical trials and one *in vitro* study were conducted. Skin irritation and sensitization of two formulations containing CBD and PEA, one containing hemp seed oil, and four concentrations of CBD alone (in a grapeseed oil vehicle) were assessed using a patch testing protocol. Phototoxicity was assessed with UV irradiation. Ocular toxicity was tested using a hen's egg chorioallantoic membrane model, with three of the formulations used in the clinical studies. There was no irritation or sensitization of the products evident via patch testing on healthy subjects. Additionally, low phototoxic potential was noted at the 48-hour time point when exposed to UVA and full spectrum irradiation, compared to negative control. The *in vitro* experiment demonstrated comparable effects of cannabinoid products to historically non-irritating products. External validity may be a limitation of this study given that formulations from a single manufacturer were assessed – there is vast heterogeneity across commercial CBD products on the market, along with a lack of FDA regulation. Also, product safety was assessed only on normal, healthy human skin; therefore, extrapolation to those with dermatologic diseases cannot be assumed. However, these specific formulations of CBD and PEA containing products are demonstrably non-irritating and non-sensitizing in healthy adults and further encourage similar research assessing safety and efficacy among those with dermatologic diseases.

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Determination of the critical sources of cAMP for Crisaborole activity in human keratinocytes

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Crisaborole is an FDA approved topical PDE4 inhibitor used to treat inflammatory diseases of the skin. The proposed mechanism of action of Crisaborole is the elevation of cyclic adenosine monophosphate (cAMP), which inhibits inflammatory pathways. Currently, the sources of cAMP responsible and cells affected by Crisaborole are not known. cAMP is produced by various adenylyl cyclases (ACs) and is degraded to AMP by phosphodiesterase (PDE) enzymes such as PDE4. Thus, for Crisaborole to increase cAMP levels, one or more adenylyl cyclases much be active. We have performed a comprehensive analysis using primary human keratinocytes to determine which adenylyl cyclases are required for the actions of Crisaborole in these cells. In addition to using pharmacological inhibitors to adenylyl cyclases, we generated adenylyl cyclase knockout human keratinocytes using the CRISPR/Cas9 system. We have learned that Crisaborole is able to inhibit the induction of inflammatory pathways in human keratinocytes and it appears that sAC, and not tmAC, is the primary source of Crisaborole-induced cAMP generation in human keratinocytes.

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Mechanisms underlying MOR-DOR analgesic synergy in epidermal nerve fibers

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We recently published electrophysiological data showing that the combination of the mu-opioid (MOR) agonist loperamide and the delta-opioid (DOR) agonist oxymorphindole (Lo-OMI), applied topically to mouse toe pads, prevents activation of nociceptive epidermal nerve fibers with high potency and efficacy (Uhelski et al., 2020). The present study tested the hypotheeis that i) heterodimeric opioid receptors mediate this analgesic action, and ii) inhibitory G proteins mediate this action via activation of G protein-coupled inwardly rectifying potassium (GIRK) channels. Recent studies have demonstrated that G protein-biased ligands demonstrate reduced side effects such as respiratory depression and tolerance. Using the complete Freund's adjuvant model of inflammatory pain and the Hargreaves assay for thermal nociception, the anti-hyperalgesic effect of Lo-OMI in male and female mice was challenged by co-administration of a recently developed antagonist against MOR-DOR heteromers, D24M (Olson, Streicher et al., 2018). Then, mice were given an injection of Lo, OMI, or their combination with and without pertussis toxin in order to assess the involvement of $Ga_{i/o}$ signaling on the behavioral anti-hyperalgesia. Finally, in naïve mice, the role of GIRK channels, a downstream target of G proteins, was investigated using the peptide inhibitor tertiapin-Q, as well as GIRK KO mice. The data demonstrate that D24M was significantly more potent in antagonizing the Lo-OMI combination ($p<0.01$) than either drug alone, supporting the heterodimeric opioid receptor hypothesis, and that pertussis toxin significantly reduces the anti-hyperalgesic effect of the combination ($p<0.05$), supporting the $Ga_{i/o}$ signaling hypothesis. Finally, both pharmacologic and genomic inhibition of GIRK2 function results in a significant reduction of the combination's anti-nociceptive effect ($p<0.0001$), supporting the requirement for GIRK channels.

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From transcriptomes to drugs: Psoriasis as a model

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Background: To date, numerous gene expression profiling (GEP) datasets have been accumulated, documenting transcriptomic patterns across a spectrum of disorders. Using psoriasis as a test case, we explored whether such existing data can be harnessed for identifying disease-relevant regulatory genes and corresponding drugs. Methods: We accessed publicly available psoriasis GEP data from 92 psoriasis samples and 82 controls (*PMID: 24441097*), which included 21,510 genes, of which 2528 were down- and 1049 up-regulated. The psoriasis data was interrogated against three independent directional gene interaction databases including CausalR, OmnipathR, and Signor2.0 using the CausalR algorithm (cutoff $p < 0.05$). Meta-analysis of identified upstream regulators using Fisher's combined probability test yielded the final upstream regulator list ($p<0.01$). This gene list was used to query the Target Central Resource Database (TCRD) and identify corresponding drugs. Results: Using only GEP data, we identified 271 unique upstream regulators, 49 of which were successfully mapped to a total of 162 unique drugs in the TCRD database. Among all available 1642 unique drugs in the TCRD database 31 are common psoriasis drugs. Our analysis correctly picked up 12/29 (38.7%) of these drugs, indicating significant enrichment for this class of drugs ($p< 0.00165$). A series of additional drugs, some of which have emerging evidence in psoriasis were identified (e.g., JAK1/2 inhibitors). Conclusion: Using the available GEP data from a well characterized disease we demonstrate the utility of emerging relational databases to identify relevant interaction networks and upstream regulators. Such candidate regulatory genes can be mapped against the available gene-drug data to identify both drugs with known clinical efficacy as well as novel therapeutic leads. Deployment of this approach across a spectrum of skin diseases merits further investigation.

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Solar simulated light induces cutaneous squamous cell carcinoma in inbred mice: A clinically relevant model to investigate T cell responses

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Over one million cases of cutaneous squamous cell carcinoma (cSCC) are diagnosed annually in the United States, and approximately 4% of patients develop metastases and 2% die. Yet, there is a paucity of clinically relevant cSCC murine models. Outbred SKH-1 mice are highly susceptible to solar simulated light (SSL)-induced cSCC. However, an outbred strain limits the ability to evaluate MHC-restricted, antigen-specific T cell responses and perform studies with genetically engineered mice. To address this need, inbred FVB/N, BALB/c, and C57BL/6J mice were exposed to 42 weeks of SSL (cumulative dose: 856 kJ/m^2 UVB and 9,884 kJ/m^2 UVA). SKH-1 mice were exposed to 22 weeks of SSL (cumulative dose: 257 kJ/m^2 UVB and 2,967 kJ/m^2 UVA). SSL reliably induced tumors in FVB/N and BALB/c mice with a median onset of 38 and 41 weeks, respectively, with a delayed onset compared to SKH-1 mice. In contrast, only 15% of C57BL/6J mice developed clinically apparent tumors. Most tumors were histologically diagnosed as actinic keratosis, cSCC in situ, or invasive cSCC. Invasive tumors had significantly decreased percentages of tumor-infiltrating dendritic cells, T cells, CD4+ T cells, CD4+ IFN-γ+ T cells, CD8+ T cells, CD8+ granzyme B+ T cells, CD8+ IFN-γ+ T cells, and regulatory T cells compared to in situ tumors. Progression from early to late cSCC tumors corresponded with a transition from an immune-activating to an immunosuppressive microenvironment. This SSL-induced cSCC model in inbred mice will facilitate future studies investigating anti-tumor T cell responses and genes influencing tumor formation and response to therapy.

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A *Dunaliella salina* extract counteracts skin aging under intense solar irradiation thanks to its anti-glycation and anti-inflammatory properties

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The glycation process is involved in both intrinsic (individual, genetic) and extrinsic (ultraviolet light, pollution and lifestyle) skin aging. UV-induced intracellular buildup of Advanced Glycation End products (AGEs) can damage skin proteins, cause the generation of reactive oxygen species, and trigger inflammatory responses — and is considered a major factor in skin aging. *Dunaliella salina* is a halophile green unicellular microalga, notable for its adaptation to intense solar radiation through its ability to produce large amounts of carotenoids. IFF/Lucas Meyer Cosmetics has developed a natural, supercritical CO_2 hydrophobic extract of *Dunaliella salina*, rich in phytoene and phytofluene, the colorless carotenoids. In *ex-vivo* testing, the extract exhibited anti-glycation and anti-inflammatory activities: human skin explants exposed to methylglyoxal showed strongly reduced formation of N-ε-carboxymethyl-lysine with concurrent treatment with the extract; while explants treated with the extract showed significant reductions in production of key interleukins IL6 and IL8. The above data were borne out in a 56-day double-blind, placebo-controlled clinical study on volunteers submitted to intense and prolonged solar exposure, with 1% *Dunaliella salina* extract in formulation. In this trial, the active significantly reduced the skin's glycation scores, as well as its reaction to histamine; and, significantly improved key skin aging parameters in comparison to the placebo product, including: wrinkle counts and evenness of texture; spots; skin elasticity; and more (all with $p<0.05$). These results demonstrate the value of this *Dunaliella salina* extract, rich in colorless carotenoids, as an antiglycative, anti-inflammatory and anti-aging active ingredient, including in high-irradiation contexts.

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Efficacy of photobiomodulation therapy for the off-label treatment of alopecia in skin types V-VI

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Photobiomodulation (PBM) therapy is an emerging treatment for androgenetic alopecia (AGA). Although various devices are FDA-cleared for treatment of AGA in Fitzpatrick (FST) skin types I-IV, these devices are not FDA-cleared for use in FST skin types V-VI. We initiated this study to investigate the efficacy of PBM therapy for off-label treatment of AGA on darker skin types. Subjects meeting inclusion/exclusion criteria were randomized to one of four PBM devices including laser diodes and/or light emitting diodes and received treatments in-office per manufacturer's recommendations. Standardized global photographs of the scalp were obtained at baseline and monthly for 4 months. Hamilton-Norwood and Savin scales were used to score AGA in males and females respectively. Scalp health was assessed for erythema, scale and folliculitis. Adverse events were tracked at each treatment visit. Five subjects, 3 females/2 males, ages 34-68, with FST skin types V and VI were enrolled. One female and two males were diagnosed with AGA. Baseline Savin score of the female subject was 5 and 4 at the final visit. Baseline Hamilton-Norwood scale of the two males were rated at 5 with one male remaining stable and the other improving to a score of 4. The other two female subjects were diagnosed with central centrifugal cicatricial alopecia (CCCA) and showed improvement in their baseline to final scores. No device related adverse events were reported and no erythema, scale, or folliculitis were noted. Our data suggests that use of PBM therapy for hair growth in FST skin types V-VI is associated with disease stability or some improvement. No worsening of condition was noted. Additionally, no adverse events were reported. However, this data is limited by a small sample size. Further enrollment and research is required to understand the off-label efficacy of these devices in treating hair loss in darker skin types.

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Extracellular vesicles from UVB-irradiated keratinocytes contain cyclobutane pyrimidine dimers

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Exposure to UVB wavelengths of sunlight induces the formation of photoproducts in DNA that are potentially mutagenic or lethal if not repaired properly. UVB photoproducts such as cyclobutane pyrimidine dimers (CPDs) can be removed from the genome via nucleotide excision repair (NER) and can lead to apoptosis and other forms of cell death. However, the ultimate fate of the UVB-damaged DNA is not well understood. Interestingly, recent work in the field of extracellular particles has indicated the presence of DNA and chromosomal proteins associated with small extracellular vesicles (SEVs). Furthermore, we have discovered CPDs in DNA associated with SEVs secreted from UVB-irradiated keratinocytes. I will present our work showing stimulus dependent release of CPDs in keratinocyte-derived extracellular vesicles that is modulated by pharmacologic treatment with drugs such as caspase inhibitors. Uptake of extracellular vesicle-associated DNA has also been shown to elicit various stress responses in bystander cells. We have found that SEV-associated CPDs are taken up by adjacent cells and will present on results of how this damaged DNA may impact stress responses in recipient cells. Lastly, parallel analyses were performed in cultured keratinocytes and in skin explants. Blister fluid-derived and biopsy-derived EVs also indicate that extracellular CPD content is enriched in SEV fractions following UVB treatment. This work to characterize extracellular DNA may improve understanding of cellular processes that export damaged DNA from UVB-irradiated cells. Mechanistic insights could lead to improved clinical tools for understanding DNA damage-response status after exposure to environmental or therapeutic genotoxin treatments.

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Association of development of solar elastosis with increased expression of fibrillin-1, LTBP-2 and fibulin-4 in combination with decreased expression of LTBP-4

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Solar elastosis is the accumulation of disorganized and non-functional elastotic material in photo-aged skin. Solar elastosis occurs through a cycle of elastic fiber degradation followed by extracellular matrix production and reassembly into an organization that differs from the original structure. However, the exact pathomechanism remains unclear. To clarify the pathomechanism underlying solar elastosis, we examined the expression of elastogenic factors by immunofluorescence using eight samples of skin tissue with solar elastosis. Sun-exposed aged skin without solar elastosis (n=7), sun-protected aged skin (n=11) and young skin (n=5) were used as controls. In solar elastotic skin, intense staining of elastin was observed in a thick structure in the dermis, whereas elastin deposition was decreased in aged skin samples without solar elastosis. In solar elastotic skin, intense staining of fibrillin-1, LTBP-2, and fibulin-4 was colocalized with a thick dermal structure that was positive for elastin. The expression of these proteins was decreased in the dermal elastic fibers of aged skin samples without solar elastosis. Notably, the LTBP-4 expression was largely decreased in both solar elastotic skin and control aged skin samples. Five of the eight solar elastotic skin samples showed intense fibulin-5 signals, while the rest of the samples showed decreased fibulin-5 staining, although all samples of aged skin tissue without solar elastosis showed a decreased expression of fibulin-5. We therefore hypothesized that the increased expression of fibrillin-1, LTBP-2, and fibulin-4 in combination with the decreased expression of LTBP-4 might be associated with the development of solar elastosis.

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Administration of nicotinamide riboside (NR) or pterostilbene (PT) to mice inhibits suppression of contact hypersensitivity (CHS) by mid-range ultraviolet radiation (UVR)

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Topical administration of PT inhibits some acute effects of mid-range UVR (UVBR, 280-320 nm) in mouse skin and oral nicotinamide reduces the rate of new non-melanoma skin cancers in high-risk patients. NR+PT inhibits UVBR-induced tissue swelling in mice and does so more effectively than either agent at the dose tested. We have now asked if NR, PT or the combination can inhibit UVBR suppression of CHS in 2 models. In the low-dose model, mice receive a UVBR dose too low to cause gross changes in skin, followed by immunization at the irradiated site, resulting in suppressed CHS. In the high-dose model, mice receive a dose sufficient to cause gross skin changes followed by immunization at an unirradiated site, also with suppressed CHS. C3H mice were placed in groups that were fed standard diet/water or chow containing 0.08% PT, water with 0.4% NR or both for 4 wks. In low-dose experiments, mice in each group had their dorsa shaved and depilated. Half in each group were exposed to 1,700 J/m^2 of UVBR daily for 4 d while the other half were mock-irradiated. Mice were immunized on the exposed dorsum with 25 µl of 0.5% dinitrofluorobenzene (DNFB) 4 h after the last irradiation. Mice were challenged 7 d later with 10 µl of 0.2% DNFB to each ear and 24-h ear swelling assessed. CHS in mice not given PT, NR or both was significantly reduced by UVBR exposure compared to mock-irradiated mice. In mice fed NR, PT, or both the UVBR effect was significantly less pronounced. In a preliminary high-dose experiment, groups of mice were treated similarly except that a single dose of 10,000 J/m^2 of radiation was administered and immunization was performed on the unirradiated shaved abdomen 3 d later. In this experiment, the UVBR effect was also significantly less pronounced in the NR, PT or NR+PT groups. NR and PT both inhibited UVB-induced immune suppression at the doses administered as did the combination.

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Expression, distribution and subcellular location of RGR in human skin

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Background: Retinal G-protein—coupled receptor opsin (RGR) functions as a photoisomerase that convert the all-trans-retinal to 11-cis-retinal in human retinal pigmented epithelium. Our research and others have demonstrated that opsin-based UVR phototransduction system existed in human skin. However, whether RGR is expressed in the human skin has not yet been identified. Objective: To determine the expression, distribution and subcellular location of RGR in human skin. Method: The expression characteristics of RGR in human normal skin sections of UV-exposed and non-exposed skin sites were investigated with immunohistochemistry staining and immunofluorescent staining. The mRNA and protein levels of RGR in human melanocytes, keratinocytes and fibroblasts from the foreskin of children were detected by real-time fluorescent quantitative PCR or western blot analysis, respectively. The expression and subcellular localization of RGR in these cells were detected with the immunofluorescent staining under fluorescence microscopy and laser scanning confocal microscopy. Results: Immunohistochemistry staining of the sections showed RGR was highly expressed in the epithelial layer of UV exposure sites, especially in the basal cell layer, spinous cell layer of the epidermis and the cells of the skin appendages. Co-immunostaining of RGR with melanocyte marker, keratinocyte markers and fibroblast markers further confirmed the RGR is mainly expressed in the nucleus of the suprabasal layers and the cells of the skin appendages. The mRNA and protein levels of RGR in keratinocytes were higher than that in other types of cells. RGR was expressed and localized to the nucleus of keratinocytes while in the cell membrane of melanocytes and fibroblasts under fluorescence microscopy and laser scanning confocal microscopy. Conclusion: Our results suggest that higher expression of RGR in the nucleus of keratinocytes in the basal cell layer, spinous cell layer of the epidermis and the cells of the skin appendages. RGR might contribute to the proliferation and differentiation of human keratinocytes.

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***Ex vivo* preclinical testing of a wearable UVA phototherapy device**

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Phototherapy is used to treat several skin disorders, with certain UV wavelengths benefiting different diseases. However, the limitations of phototherapy, including limited availability, restricted light penetration, unaffected tissue exposure, and long latency to treatment response, prevent its widespread use. As such, we fabricated a wearable, depth-modulated UVA (360 nm) phototherapy device to address these deficits. We previously demonstrated the curvilinear conformability and capability of the device to deliver UVA light up to 250% deeper than a standard UVA lamp. Presently, we report results from *ex vivo* preclinical testing in human skin explants. Potential adverse side effects of phototherapy using conventional methods are sun-burning and DNA damage in keratinocytes. We exposed excised human skin to clinically-relevant low (13.5 J/cm^2) and moderate (67.5 J/cm^2) doses of UVA light using a lamp or the device. Morphological assessment of tissue sections by hematoxylin and eosin staining indicated signs of photoxicity in the lamp groups apparent by tissue acidification, pyknotic nuclei, and vacuolated keratinocytes throughout all epidermal layers. These changes were absent in the device groups. As the keratinocyte morphology suggested apoptosis, we detected *in situ* expression of cleaved caspase 3 (CC3). Indirect immunofluorescence revealed a significant decrease in the number of CC3+ basal keratinocytes in the device groups compared to the lamp groups ($P < 0.0001$). Use of the device as a therapeutic modality is particularly applicable in the treatment of morphea, a deep tissue sclerosing skin condition, which can be managed with UVA phototherapy. In morphea, UVA treatment can downregulate disease-associated genes, including TGFβ. Transcript analysis of full-thickness skin samples by qPCR showed a downregulation of TGFβ with the device ($P = 0.0336$). Together, these data indicate an improved safety profile for the device in delivering UVA phototherapy and an enhanced ability to modulate pathogenic gene expression.

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Nanoparticle encapsulation enhances stability and efficacy of sunscreen actives

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The pre-clinical criteria for the development of an effective sunscreen include chemical and photostability of actives, water resistance, skin penetration, and effectiveness as assessed by SPF and inhibition of ultraviolet radiation damage. We hypothesized that encapsulation of sunscreen filters within biodegradable bioadhesive nanoparticles (BNP) would improve their stability and effectiveness, while decreasing their skin penetration. To assess photostability, free and BNP-encapsulated sunscreen actives were exposed to solar-simulator UVR. BNP-encapsulation of AVO showed greater protection (80±1.1%) from degradation relative to free AVO (60±2.1%, $p<0.001$). Furthermore, AVO was equally stabilized by either mixing single encapsulated avobenzone with single encapsulated octocrylene, or by co-encapsulating these two agents. To elucidate the persistence of BNP particle adhesion to human skin equivalents, non-adherent control nanoparticles (NNP) and BNP (incorporating AVO, AVO/OCR, or Uvinul-A plus) were applied to Vitro-Skin and porcine skin. In a rotating waterbath at 450 rpm, 95-100% of the BNP (0.01mg/cm^2) adhered to the surface of Vitro-Skin even after 3 hr of washing, whereas 50-55% of the NNP readily detached after only 5 min of washing. On porcine skin, BNP at maximum concentration (2 mg/cm^2) remained adherent (89±5.3%) relative to NNP (27.2±8.5%). Also, addition of 25% titanium to encapsulated sunscreen actives does not affect bioadhesion. To quantify the penetration of sunscreen actives into viable skin, free AVO in DMSO and BNP-AVO in water were applied on the surface of an ex vivo human skin equivalent (NativeSkin). After 24 hrs, ~2.3x greater free AVO penetrated into the skin as compared to BNP-encapsulated AVO ($p<0.005$). These results indicate that BNP encapsulation of sunscreen actives provides enhanced safety and performance in an optimized sunscreen formulation.

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Potential involvement of microvesicle particles in the synergistic effects of Ultraviolet-B radiation and Platelet -Activating Factor receptor agonists on cytokine production

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Inflammation plays a major role in different pathological and physiological conditions. Ultraviolet B radiation (UVB) is known to trigger the generation and release of multiple cytokines in the keratinocyte, which play a role in UVB-mediated pathology. Previously, we and others reported that UVB + other agents such as IL-1 or the lipid mediator Platelet-activating Factor (PAF) results in the synergistic production of cytokines such as TNFa. Subcellular microvesicle particles (MVP) which are released from cells in response to cellular stress have been implicated in multiple pathologic processes through their abilities to carry bioactive agents such as cytokines. Of importance, MVP have been demonstrated to be released from keratinocytes following multiple stimuli such as UVB in a PAF-dependent manner. The current studies sought to define the role of MVP in the synergistic cytokine generating effects of PAF + UVB. Studies using the human HaCaT keratinocyte cell line in vitro and human skin explants ex vivo demonstrate that combining UVB + PAF agonist carbamoyl PAF (CPAF) results in augmented production of cytokines including TNFa as well as increased MVP generation. Blocking MVP generation/release via the acid sphingomyelinase inhibitor imipramine totally blocked MVP yet only partially inhibited cytokine production. These studies suggest that MVP could be responsible for a part of the cytokine production in response to combining PAF agonist + UVB.

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Xeroderma Pigmentosum A deficiency results in increased generation of microvesicle particles in response to Ultraviolet B Radiation

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Xeroderma Pigmentosum is a family of genetic disorders in which the ability to repair UVB damaged DNA is decreased. The Xeroderma Pigmentosum Group A (XPA) protein is known to recognize UVB photoproducts in DNA and facilitate damage removal by nucleotide excision repair. XPA deficiency therefore decreases UVB photoproduct repair efficiency and is linked to photosensitivity. Our group has previously demonstrated that the augmented acute inflammation associated with UVB in XPA-deficient mice is due to the lipid mediator Platelet-activating factor (PAF). Moreover, the ability of UVB to generate subcellular microvesicle particles (MVP) is also due to PAF receptor signaling. Hence, the present studies were designed to test if XPA deficiency results in augmented UVB-induced MVPs. Studies testing the HaCaT keratinocyte-derived cell line in which XPA was knocked down revealed increased MVP release in response to UVB. Similarly, XPA-deficient mice generated increased MVPs in both skin and plasma in response to UVB as compared to wild-type counterparts. However, the absence of XPA did not affect MVP release in response to PAF receptor agonist or phorbol ester TPA. As MVPs have been implicated in UVB signaling, these studies suggest that these subcellular bodies could play a role in the augmented UVB responses associated with XPA-deficiency.

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A high throughput method of identifying naturally-occurring sunscreen agents

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Ultraviolet radiation (UVR) is the major environmental risk factor for the development of skin cancer. Sunscreen agents mitigate this risk by preventing UVR from inducing direct and indirect mutagenic effects within skin cells, though current sunscreen formulations may have safety or aesthetic concerns. Natural products (i.e. derived from plants, algae, and other organisms that have evolved systems for photoprotection), may provide alternative compounds to the currently used synthetic sunscreen agents. In addition to their capacity to absorb UVR, selected natural compounds may also exhibit antioxidant and anti-inflammatory properties and provide a lower environmental impact. Prior investigation of photoprotective natural products has focused on characterizing individual compounds or classes of compounds known to be of interest. To more fully explore this potential, we developed a high-throughput assay for the efficient screening of natural products. We obtained 915 natural products from the NCI Natural Products Set and MicroSource Pure Natural Products libraries. The products were plated on 384-well plates and screened using spectrophotometry for absorbance in the UV spectrum (280-400nm) before and after exposure to UV light. We identified 220 products that showed both significant UVR absorbing capacity and photostability after UVR exposure. Follow-on screening of candidate agents includes a series of cytotoxicity, phototoxicity, and free radical scavenging assays to identify the most promising safe and effective natural products for their potential use as standalone photoprotective agents, or in combination with other sunscreens or cosmetic products to boost protection against UVR-induced damage.

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UVB-irradiated keratinocytes-derived extracellular vesicles: Mediator of proinflammatory responses in macrophages

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Ultraviolet B irradiation (UVB) contributes to skin inflammation. As UVB mostly affects the epidermis, the crosstalk between the epidermis and dermis in response to UVB warrants investigation. Extracellular vesicles (EVs), lipid bilayer membrane vesicles secreted by many cells, can carry lipids, proteins and nucleic acids to mediate signal transduction. As a critical sensor and adaptor for the host immune response to cytosolic DNA and cyclic dinucleotides, stimulator of interferon genes (STING) plays a critical role in immunity and inflammation. We hypothesized that EVs derived from UVB-irradiated keratinocytes (KCs) might trigger STING-mediated proinflammatory responses in dermal cells. KCs (HaCaT cells) were irradiated with UVB and cultured for 24 hours. EVs were isolated by ultracentrifugation and used to stimulate fibroblasts or macrophages with/without STING signaling inhibitors. The supernatant was harvested for ELISA and the lysed cells were collected for Western blot. UVB irradiation increased caspase-3 cleavage and autophagic LC3-II expression, along with increasing p-STING and its downstream p-IRF3 in KCs ($P<0.05$, n=3). UVB irradiated HaCaT cells also released more extracellular vesicles than unirradiated cells ($1.78x10^9$/mL *vs* $3.31x10^8$/mL), with a similar mean size (74.7nm *vs* 73.5nm). STING antagonist H-151 pretreatment decreased UVB-triggered caspase-3 cleavage and LC3-II expression. UVB-irradiated-keratinocyte-derived EVs (KEV-UVB) expressed more exosome surface markers than non-irradiated-KEVs. KEV-UVB triggered more interferonb (IFNβ) release from macrophages than fibroblasts (111.1±21.45 vs. 4.85±0.72 pg/mL $P<0.05$ n=3). H-151 attenuated KEV-UVB triggered IFNβ production in macrophages (13.18±6.38 vs. 111.1±21.45 pg/mL $P<0.05$). TBK1 inhibitor MRT67307 also showed similar effect (12.6±0.71 vs. 304.6±94.4 pg/mL $P<0.05$). KEV-UVB were mediators of inflammation, and triggered STING-mediated cytokine release. Targeting the STING signaling pathway may provide insight into a potential therapeutic approach for UVB-induced skin inflammation.

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Ultrasmall prussian blue nanoparticles protect human skin fibroblasts from ultraviolet A stress induced premature senescence

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Background: Skin aging is one of the major challenges our society faces, and the generation of reactive oxygen species(ROS)seems to play a major role in age-related skin modifications.Thus, ROS scavengers that can block excessive production of ROS have great therapeutic potential. Ultrasmall prussian blue nanoparticles(USPBNPs),which belong to the iron-based metal-organic frameworks, exhibited an intensive ability of scavenging free radicals as nanozyme and showed great application potential in the treatment of free radical-related diseases. Herein, we propose an efficient treatment strategy in which an artificial nanozyme with multienzyme activity drives photoprotection against ultraviolet A stress induced premature senescence(UVA-SIPS) primarily by scavenging ROS. Methods: In the present study, UVA-SIPS model was established by UVA radiation on human skin fibroblasts .Cell viability was determined using the Cell Counting Kit-8 (CCK-8). Senescent cells were detected by senescence β-galactosidase staining.Fluorescence microscopy and flow cytometry were used to assay the intracellular ROS concentration.Cell cycle was measured by flow cytometry.The expression of cellular γH2A.X,p16,p21 and p53 were also measured. Results: The results revealed that UVA radiation could cause premature senescence in human skin fibroblasts. Interestingly, the pretreatment of USPBNPs significantly reduced senescent cells and attenuated several features of cellular senescence, including morphological and metabolic changes,senescence related DNA damage, cell cycle arrest and more senescence-associated secretory phenotypes (SASPs). Conclusions: This study provides a proof of concept for a novel class of protective nanoagents that might be beneficial for prevention of skin photoaging and other age-related disorders.

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Complex phototoxic properties of a cigarette smoke extract on human keratinocytes

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Sun exposure and cigarette smoke, studied separately, are two well-known environmental factors that accelerate skin aging. However, their combined effects has been poorly characterized. Some studies have determined that cigarette smoke components can accumulate in the skin by contact or by systemic effect after inhalation. Moreover, it is well-established that solar rays can penetrate the epidermis and dermis. Those factors could thus interact with one another in the skin. The aim of this study is to assess the phototoxic effect of cigarette smoke on the skin, more precisely on skin aging. A cigarette smoke extract (CSE) was obtained by capturing the soluble fraction of cigarette smoke. 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 an MTS assay, establishing the cellular metabolic activity. The CSE photo-oxidation was studied using fluorescent markers measuring ROS levels. The CSE total antioxidant capacity (TAC) was also assessed. CSE and solar irradiation alone showed no cytotoxicity up to the maximal doses tested. However, CSE was highly phototoxic when irradiated. Indeed, when exposed to 5% CSE at 14.5 kJ/m^2 and 29 kJ/m^2 UVA, keratinocytes showed respectively a 39±1% and 2±17% cellular viability (p-value<0.001). Photo-oxidation results indicate a decrease in type I ROS and a slight increase in type II ROS. The TAC test revealed that CSE have antioxidant capacities. Our results showed a synergistic toxicity between cigarette smoke and sun exposure on skin cells, caused in part by a photo-oxidation reaction. Our work is still focussing on deciphering the mechanisms involved in this synergy.

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Skin protection in extreme conditions: A multiparametric approach for a new SPF50$^+$ suncare product containing TriAsorB

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Photoprotection is a major issue in public health to prevent the harmful effects of sunlight radiations. Recently, we developed a new sunfilter called Phenylene Bis-Diphenyltriazine (or TriAsorB) that was recently approved in Europe (CAS N°55514-22-2). We combined TriAsorB in an innovative photoprotective system and we investigated by multiparametric approach the new SPF50$^+$ suncare product photoprotection and maintenance in conditions of use, but also in real life extreme conditions. Photoprotection was assessed *ex vivo* by using human skin model exposed to solar-simulated radiation (SSR, UV+VIS light, 3 and 10 DEM) and quantification of sunburn cells as readout. Then, *in vitro* and *in vivo* approaches (55 adults, 44 children) were used to study the resistance of the sunscreen to high ultraviolet index, temperature, humidity but also water-resistance and use in tropical and sea/mountain environments. Perceived efficacy was also studied by 65 consumers. Sunburn analysis showed that the suncare prevented keratinocyte apoptosis under an acute SSR exposure (100% protection at 3DEM and 93.8% at 10DEM). Sun protection resistance evaluated *in vitro* performed in 4 different extreme conditions (continental, dry, polar, tropical) showed resistance > 75% to each condition (from 76.3% to 107.6%). Clinical studies confirmed the high performance (tolerance and efficacy) of this sunscreen under real sun exposure extreme conditions in tropical (i.e.: mean T° > 29°, mean humidity > 87% and mean UV index > 13). Indoor testing showed a very high resistance to water even after 6 baths of 20 minutes. The consumer test confirmed high satisfactory evaluations related to perceived efficacy and resistance (94% satisfied / mean score = 8,1/10) in extreme conditions. Altogether, the multiparametric approach demonstrated the high photoprotective performance of a new SPF50$^+$ suncare product containing TriAsorB in different extreme conditions, suggesting it might be used in various climatic environment by consumers.

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Phenylene Bis-Diphenyltriazine (TriAsorB), a new full-spectrum photoprotector against sunlight radiation induced skin damage

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Introduction: Photoprotection is a major issue in public health to prevent the harmful effects of sunlight radiations (actinic keratosis, skin cancers and photoaging). Thus, we have developed a new generation of non-soluble organic sunfilter called Phenylene Bis-Diphenyltriazine (or TriAsorB) that was recently approved in Europe (CAS N°55514-22-2). Objective: To assess the photoprotective efficacy of TriAsorB to prevent skin damage across the entire spectrum of sunlight from ultraviolet (UV) to visible/infrared (VIS/IR light) including blue light. Materials and Methods: Photoprotection was assessed by spectrophotometric assays: absorption and reflectance from UV (290-400nm) to visible/infrared (VIS/IR 400-2500 nm). DNA damage was also evaluated *in vitro* by using reconstructed human epidermis: cyclobutane pyrimidine dimer (CPD) following solar-simulated radiation (SSR, UV+VIS light), and 8-hydroxy-2'deoxyguanosine, (8OHdG) in response to high energy visible (HEV)/blue light exposure (400-500 nm). The specificity of TriAsorB efficiency was evaluated *versus* placebo formulation. Results: TriAsorB has a broad spectrum UVB+UVA filter including long UVA. Interestingly, it also absorbs VIS radiations, especially in the HEV or blue light area. HEV radiations were also reflected. Protection in the IR spectral range was detectable. Furthermore, the sunfilter prevented SSR-induced CPD formation, but it also specifically protected the skin against the generation of the oxidative lesions 8OHdG induced by exposure to HEV radiations. Placebo was unable to protect the skin along the sunlight spectrum. Conclusion: TriAsorB is an innovative full spectrum sunfilter that might be used in suncare products to provide skin photoprotection from UV to VIS/HEV/IR radiations. Finally, it prevents sunlight genotoxicity and protected the skin against the harmful effects of solar radiations on DNA, especially in the blue light spectral range.

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The toll like receptor-4 antagonist, TAK-242, enhances repair of ultraviolet radiation-induced DNA damage and inhibits UVB-induced tumor development in mice

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Ultraviolet (UV) irradiation of the skin induces acute inflammation, as characterized by erythema, edema, immunosuppression, and development of skin cancer. In addition, UVB induced DNA damage in the form of cyclobutane pyrimidine dimers (CPDs), can result in stable mutations. Toll-like receptor 4 (TLR4), a component of innate immunity, plays an important role in cancer. Previous studies from our laboratory indicate that UVB-induced DNA damage was greatly reduced in TLR4 deficient mice, indicated by significantly fewer CPD lesions in the skin of these mice. TLR4 deficient mice were also found to be resistant to UVB-induced immune suppression and carcinogenesis. In this study, we determined the efficacy of the TLR4 antagonist TAK-242 in regulation of UVB-induced DNA damage, inflammation and tumor development. Our results indicate that TAK-242 treatment increased the expression of the xeroderma pigmentosum group A (XPA) gene, resulting in repair of UVB-induced CPDs in the skin of SKH-1 mice. Treatment with TAK-242 also inhibited the activation of NLRP3 ($p<0.05$) in UVB-exposed skin of SKH-1 mice. When SKH-1 mice were exposed to multiple doses of UVB radiation (180 mJ/cm^2) for 30 weeks, cutaneous carcinogenesis was significantly retarded ($p<0.05$) in mice treated with TAK-242 in comparison to vehicle treated mice. Pro-inflammatory cytokines like IL-1b, IL-6, and TNF-a were also found to be significantly upregulated ($p<0.05$) in vehicle treated mice than TAK-242 treated mice. Together, our data indicate that TLR4 inhibitor TAK-242 inhibits UVB induced DNA damage and inflammation, and prevents the development of UVB induced skin cancers in mice.

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Uvb-induced necrosis in human epidermal keratinocytes

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Ultraviolet B radiation (UVB) is responsible for approximately 5.5 million annual skin cancer diagnoses in the United States in part due to its ability to cause direct DNA damage resulting in mutations. UVB also induces inflammation through multiple mechanisms which contribute to photocarcinogenesis. However, UVB is well known to induce apoptosis, a non-inflammatory and tumor suppressor cell death mechanism. Necrosis is a type of cell death which results in the release of cellular contents into the extracellular environment and elicits an immune response. The release of damage associated molecular patterns (DAMPs) and activation of toll-like receptors (TLRs) characteristic of necrosis is also observed with UVB-induced inflammation. TLR activation has been shown to induce a necrotic, inflammatory type of cell death, termed necroptosis. Despite UVB promoting DAMP release, TLR activation and inflammation, it is unclear if UVB induces keratinocyte (KC) necroptosis. To examine if UVB induces necrosis in a sub-population of epidermal KCs which may contribute to UVB-induced inflammation, we utilized live cell imaging of HaCaT KCs. HaCaT cells were exposed to 30 mJ/cm^2 UVB and cultured with propidium iodide (PI), a membrane impermeable nucleic acid stain, and FITC-conjugated Annexin-V, to detect early apoptotic cells. Cell morphology and death kinetics were monitored over 20 hours following UVB exposure. Heterogenic morphological types of cell death were observed, including apoptosis, secondary necrosis and primary necrosis. Addition of Dabrafenib, a multi-kinase and necroptotic RIPK3 inhibitor, or zVAD, a pan caspase inhibitor, significantly inhibited UVB-induced necrotic cell death (determined by PI-exclusion) in normal human epidermal keratinocytes (NHEKs). Dabrafenib also inhibited the UVB-induced necroptosis effector phospho-MLKL in NHEKs. Addition of Dabrafenib up to 4 hours post-UVB also inhibited necrotic cell death. Together, these data indicate that UVB induces a necrotic type of cell death, possibly necroptosis, which can be inhibited hours after UVB, potentially reducing UVB-induced skin inflammation and photocarcinogenesis.

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p63 regulates XPC binding dynamics and global nucleotide excision repair in keratinocytes

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The p63 transcription factor, critical for epidermal development, also regulates the response to DNA damage, including global nucleotide excision repair (NER) efficiency. We have reported that vitamin D receptor (VDR) is also required for efficient global NER of ultraviolet radiation (UV)-induced 6-4 pyrimidine-pyrimidone photoproducts and for releasing XPC—the initial ultraviolet radiation (UV)-induced DNA damage recognition sensor—from DNA damage. Since p63 and VDR have been reported to regulate each other, we assessed p63's effect on XPC binding and dissociation. TERT-immortalized keratinocytes expressing shRNA targeting all isoforms of p63 exhibited reduced levels of VDR relative to non-targeting controls. Upon UV irradiation through 3 μm pores in otherwise opaque filters to create sub-nuclear focal spots of DNA damage, keratinocytes depleted of p63 exhibited slower removal of 6-4 photoproducts than control cells over 90 minutes. Co-staining with antibodies to XPC revealed that XPC rapidly accumulated at DNA damage foci, peaking within 15 minutes and gradually fading over 90 minutes as NER proceeded in control keratinocytes. In p63-depleted keratinocytes, XPC associated with DNA damage with comparable efficiency, but the dissociation of XPC was delayed so that substantially more XPC was retained at 30 minutes than in control cells. These results support a model where XPC dissociation is necessary for normal completion of subsequent NER steps and confirm our previous observations that p63 is important for efficient removal of UV-induced DNA damage. The results recapitulate those in keratinocytes depleted of VDR and are consistent with crosstalk between p63 and VDR in promoting normal dissociation of XPC from damaged DNA such that absence of either p63 or VDR can lead to non-productive binding of XPC and reduced NER. These results further link two important regulators of epidermal growth and differentiation to DNA repair.

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Quantitation of blue light irradiation dose emitted by electronic communication devices and its potential impact on human skin

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The dose of UV and infrared irradiation required for generation of free radicals and its potentially damaging effect on human skin is well studied. However, the information specifically on the dose and duration of the blue light irradiation spectrum and its capacity to induce free radicals and thus affect human skin is unclear. Review of the literature showed that blue light irradiation at 50 J/cm2 or higher decreased carotenoid levels 24 hours following exposure indicating the formation of free radicals during light exposure. We set out to investigate the dose and duration of blue light irradiation and fluency emitted by common electronic communication devices such as computer monitors, laptops and smart phones. The experiment involved measuring the absolute irradiance (W/cm^2/nm) of blue light (430nm-480nm) emitted from a computer monitor[a], a laptop screen[b] and a smart phone[c] using a calibrated spectrometer. Light was collected with a cosine corrector which was placed one foot away from the screens and delivered to the spectrometer using a fiber. The results showed that it would take 32, 46 and 241 days of constant exposure to reach doe equivalence of 50 J/cm^2 blue light dose for the monitor, laptop screen and phone with 100% brightness. We further tested a topical cream containing carotenoid actives, capsanthin and capsanthin esters for its capacity to shield against 50 J/cm2 dose of blue light. The 50 J/cm^2 of blue light dose was reduced by 25%, 39% and 54% when the topical application amount was 2, 4, 8 mg/cm^2 respectively. a: Dell U2412M, b: Microsoft surface book, c: Samsung S10

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20-hydroxytachysterol: Synthesis and biological activity

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Tachysterol (T3), a phototransformation product of 7-dehydrocholesterol (7DHC), is formed when exposed to the UVB energy. Previously, we have detected 20-hydroxy-7DHC (20(OH) 7DHC) in the human epidermis and serum. We have produced 20(OH)7DHC by a chemical route, which is then exposed to UVB to open the B-ring. Upon equilibration of the reaction mixture, 20-hydroxytachysterol (20(OH)T3) was then purified by the RP-HPLC. The purity and chemical structure were confirmed by RP-HPLC with dioarray monitoring, NMR, and mass spectrometry. 20(OH)T3 was submitted for biological testing. It inhibited the proliferation of epidermal keratinocytes and dermal fibroblasts in a manner that is comparable to calcitriol. The incubation of 20(OH)T3 with melanoma cells that contain genetically engineered vitamin D receptor (VDR) coupled to GFP (VDR-GFP) has shown VDR-GFP translocation to the nucleus from the cytoplasm. Similar incubation of T3 precursor had no effect. Studies using a human aryl hydrocarbon (AhR) reporter assay system revealed marked activation of AhR by 20(OH)T3 with only minimal effect seen for the T3 precursor. Molecular docking using crystal structures of the ligand binding domains (LBDs) of genomic binding site of VDR and AhR revealed high docking scores for 20(OH)T3 and other theoretically predicted T3-hydroxyderivatives. The scores for AhR were even better than those of its natural ligands, induribin and indole acetic acid, predicting tight binding of 20(OH)T3 and other derivatives to the receptor. The scores for non-genomic binding site of the VDR were very low indicating poor or lack of interaction with T3 ligands. In summary, we have synthesized 20(OH)T3, identified its anti-proliferative properties in skin cells, and identified VDR and AhR as its genomic receptor targets. We believe that these findings open new areas for studies of the role for active forms of T3 in skin physiology and pathology.

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Chronic UV exposure decreases sun sensitivity by a tanning independent mechanism

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Keratinocytes protect themselves from UV-induced damage and death by signalling to melanocytes to produce and transfer melanin to protect their nuclei from further UV damage. The keratinocyte-melanocyte communication axis defines the tanning response, which is integral to epidermal homeostasis. We studied the effects of occasional, moderate and chronic UVA/UVB exposure on keratinocytes *in vitro* using immortalised human keratinocytes in monoculture. We show that UV exposure led to UV signature 7 mutations with predominant C>T nucleotide substitutions across the genome. We observed that keratinocytes with any UV treatment history were better able to withstand further UV exposure, showing decreased UV sensitivity, despite the absence of melanin transfer from melanocytes. We explored our findings *in vivo* using immunocompetent wild type mice, and observed a striking decrease in sunburn cells (apoptotic keratinocytes), TUNEL, p53 and TT-dimers 24 hours after UV exposure in skin that had a prior history of significant UV damage. This *in vivo* response is independent of melanin protection, as mouse melanocytes are intradermal and do not transfer melanin to epidermal keratinocytes. These data show that chronic UV confers protection to further UV exposure independently of the tanning response. We are investigating transcriptional and immune changes that may infer a mechanism.

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Intrinsic heterogeneity in human keratinocyte sensitivity to ultraviolet radiation

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Variable sensitivity to ultraviolet B radiation (UVB) in humans is apparent from individual responses to UVB-induced sunburn, tanning and skin cancer. This variation is largely credited to variability in skin pigmentation; however we have observed heterogenic UVB sensitivity of cultured normal human epidermal keratinocytes (NHEK). Different strains of NHEKs exposed to 20 mJ/cm^2 UVB and cultured for 18 hours exhibited cellular viability ranging from 5.8% to 73% (mean = 51.5% ± 22.0%, N=9). Given that these NHEKs lack melanin producing cells, this indicates that there are factors affecting UVB sensitivity independent of pigmentation. To study adaptive changes in NHEKs which may alter their sensitivity to UVB, we developed a HaCaT cell line (HaCaT UVR) by repeatedly exposing the human keratinocyte cell line HaCaT cells to chronic low dose UV (CLUV) radiation. CLUV treatment consisting of 10-20 mJ/cm^2 UVB exposure for 7 treatments over a 9-month period. Sensitivity of HaCaT parental and HaCaT UVR cells was determined every other CLUV treatment using the Sulforhodamine B cell viability assay and LC50s were determined. The LC50 of HaCaT parental cells was 21.2 mJ/cm^2. Subsequently, LC50s were determined following HaCaT UVR CLUV treatments 1, 3, 5, and 7 for HaCaT parental and UVR cells. HaCaT Parental:UVR LC50s ratios were 16.0:19.2, 20.6:24.7, 22.6:23.4, and 21.8:21.2 mJ/cm^2 for CLUV treatments 1, 3, 5 and 7, respectively. Thus we observed a transient resistance in the HaCaT UVR cells at treatments 1 and 3, but this resistance was not sustained. To explore intrinsic differences in NHEK sensitivity to UVB, we determined UVB LC50 values for four NHEK strains and assayed UVB-induced changes in inflammation-associated gene expression by RT-qPCR. The four NHEKs had LC50s of 41.7, 34.0, 32.3 and 25.0 mJ/cm^2, with the more resistant strain demonstrating higher induction of all three genes examined (PTGS2, R^2=0.94; IL1B, R^2=0.91 and TNFA, R^2=0.82). Thus, significant variability in human keratinocyte sensitivity to UVR exists with resistance to UVR correlating with robust induction of pro-inflammatory genes.

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Exploring the melanoma survivorship experience: A qualitative study

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US survivorship programs and guidelines for melanoma are in their infancy. We used in-depth interviews to explore experiences and challenges of patients diagnosed with melanoma in a tertiary cancer center. We interviewed 30 patients equally distributed between Stage I-II and Stage III-IV melanoma. Patients were prospectively enrolled from pigmented lesion and medical oncology clinics. Eligible patients had a histologic diagnosis of cutaneous melanoma and completed either surgical treatment with no plans of initiating systemic treatment or at least 12 months of systemic treatment with no evidence of disease. Thirteen (43%) patients had Stage IA, 1 (3%) had Stage IB, 1 (3%) had Stage IIA, 5 (17%) had Stage III, and 10 (33%) had Stage IV melanoma. Major themes on physical, psychosocial, spiritual, and information challenges were identified. Limitations in physical activity appeared particularly impactful. Participants restricted or completely stopped outdoor activities they enjoyed to avoid ultraviolet radiation. Others noted surgical complications or immunotherapy side-effects limited their day-to-day function. Their reliance on others eroded their sense of independence. Patients also noted uncertainty surrounding medical appointments as a large source of stress but ultimately found providers as a significant source of reassurance. Quality of life for melanoma patients can be most affected at time of initial diagnosis, highlighting the importance of early intervention. Focus on personalized physical therapy and photoprotection were noted as important resources in survivorship programs to preserve or regain function. We found that the unknowns surrounding upcoming appointments are a source of significant stress and anxiety for patients. This should be accounted for in psychoeducation interventions in survivorship programs. This study is limited by its generalizability due to its small single city sample. Deeper understanding of melanoma survivors' experiences can inform the development of survivorship programs and patient care.

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Validation of the Skindex-mini in patients with advanced melanoma receiving immunotherapy

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Checkpoint inhibitor immunotherapy has improved survival among patients with advanced melanoma, but health-related quality of life (HRQOL) impact of common cutaneous adverse effects are not well studied. Skin-related HRQOL is not routinely measured because validated surveys like Skindex-16 are long and burdensome to administer. Shorter surveys may be more feasible in clinical settings. We aimed to correlate skin-related HRQOL measurements using Skindex-16 and Skindex-mini in a cohort of patients with Stage III-IV melanoma. Participants were identified prior to receiving immunotherapy; Skindex-16 and Skindex-mini were measured at weeks 1, 2, 3, 4, 8, and 12 after immunotherapy initiation and at onset of cutaneous adverse effects. Pearson correlations between Skindex-16 and Skindex-mini were calculated during and after onset of cutaneous adverse effects. 164 measurements were collected from 26 participants, of whom 11 developed cutaneous adverse effects. Mean (SD) Skindex-16 symptom, emotion, and functional scores were 8.7 (13.4), 1.4 (4.5), and 5.2 (9.4), respectively. At onset of cutaneous adverse effects, correlations between Skindex-16 and Skindex-mini were 0.82 (0.36-0.95) for symptom, 0.93 (0.70-0.98) for emotion, and 0.89 (0.54-0.97) for functional domains. After onset of cutaneous adverse effects, correlations were 0.71 (0.43-0.86) for symptom, 0.34 (-0.07-0.65) for emotion, and 0.40 (0.01-0.68) for functional domains. All 3 domains almost perfectly correlated at onset of cutaneous adverse effects, but correlations between emotion and functional domains were attenuated after onset of cutaneous adverse effects. Study limitations included small sample size and mild severity of cutaneous adverse effects. Skindex-mini accurately characterizes symptomatic, functional, and emotional burden at onset of cutaneous adverse effects from immunotherapy but may less accurately capture milder emotional and functional burden after dermatological treatment of cutaneous adverse effects.

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Associations between influenza vaccine and immunotherapy outcomes in metastatic melanoma patients

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Immune checkpoint inhibitors (ICI) elicit antitumor response in 30-50% of metastatic melanoma (MM) patients. Prior studies have implicated a decreased melanoma risk and enhanced immune response with influenza vaccination. However, associations between this vaccine and patient outcomes on ICI, including immune-related adverse events (irAEs), progression-free survival (PFS), and overall survival (OS) are unclear. We performed a single institution, retrospective cohort analysis characterizing influenza vaccination patterns in MM patients receiving ICI. Inclusion criteria included newly diagnosed stage IV or unresectable stage III MM patients who received ICI as first-line systemic treatment from 2013-2018 and had vaccination records. With a vaccinated cohort of 90 patients and unvaccinated cohort of 86 patients, median age of vaccinated patients was significantly higher compared to unvaccinated at the initiation of ICI (70 vs 59 years; $p<0.0001$). Vaccinated patients were more likely to receive single agent anti-PD1 therapy (68% vs 38%; p=0.0003). IrAEs were more prevalent in the vaccinated cohort compared to the unvaccinated cohort (67% vs 40%; p=0.0005), predominantly due to cutaneous irAEs (61% vs 34%; p=0.0002). Both cohorts stopped ICI most often due to disease progression, but this was less common for the vaccinated cohort (46% vs 64%; p=0.03). Vaccinated patients had longer PFS than unvaccinated (HR=0.67; 95% CI, 0.47-0.97), but not OS (HR=0.95; 95% CI, 0.62-1.46). Overall, 51% of patients received the recommended flu vaccination prior to ICI. Vaccinated patients experienced a higher rate of irAEs yet had a higher PFS compared to unvaccinated patients. Larger cohorts are necessary to explore further associations between this simple, universally recommended preventative health adjunct and potentially improved ICI outcomes in MM patients.

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***ANRIL* inherited variants associated with primary melanoma TIL grade**

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Genome-wide association studies have reported an association between genetic variants in or near the *ANRIL (CDKN2B-AS1)* gene and age-related diseases. Despite these variants' proximity to *CDKN2A*, which encodes melanoma tumor suppressor genes, their associations with melanoma prognostic factors are unknown. Here we investigated the associations of these variants with primary melanoma Breslow thickness, ulceration, and tumor-infiltrating lymphocyte (TIL) grade. Our analysis included 3,285 European-origin participants in the Genes, Environment and Melanoma (GEM) Study with incident invasive primary melanoma. Their germline DNA was genotyped for 10 age-related disease risk variants. We used linear regression models to estimate the mean change in log of Breslow thickness and 95% confidence interval (CI) per age-related disease risk variant. Logistic regression models estimated the odds ratio (OR) and 95% CI for presence versus absence of ulceration and TILs per age-related disease risk variant. Models were adjusted for age, sex, study center, and lesion status as first- or higher-order primary. The rs518394*C (OR=1.22, 95% CI=1.06−1.39), rs10965215*A (OR=1.22, 95% CI=1.06−1.39), and rs564398*A (OR=1.28, 95% CI=1.11−1.47) alleles were positively associated with presence of TILs, passing the false discovery threshold ($P < 0.005$), but not with Breslow thickness or ulceration. Our findings indicate genetic variants in *ANRIL* influence TIL presence in primary melanoma. These variants should be further explored, especially given the impact of TILs on responses to immunotherapy.

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BMP signaling is active in early melanoma lesions and promotes melanoma development

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Neural crest identity and neural crest factors have previously been shown to be important in the initiation and progression of melanoma. However, it is unclear what pathways or mechanisms regulate the acquisition of this neural crest identity. Recently, the melanoma oncogene *GDF6* was shown to promote the expression of neural crest factors and repress melanocyte differentiation factors in both melanocyte development and melanoma through activation of canonical BMP signaling. Given this potent regulation of neural crest and melanocyte factors, we hypothesized BMP signaling may be important in the acquisition of neural crest identity during melanoma initiation. Here, we show that BMP signaling is active in 65% of primary melanomas in humans and 90% of melanoma initiating lesions in a zebrafish melanoma model. We further show that activation of BMP signaling within our zebrafish model increases the development of melanoma initiating lesions by 2-fold compared to controls and causes an acceleration in median melanoma onset to 12 weeks compared to 17 weeks in controls. Additionally, we found suppression of BMP signaling results in the opposite effect, with fewer melanoma initiating lesions and delayed median onset of melanoma. These results suggest a role for BMP during melanoma development and indicate the potential of BMP signaling as a therapeutic target for patients with melanoma.

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Genipin contained in gardenia fruit induced skin pigmentation

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Gardenia fruit is widely used in herbal medicine. An ingredient of gardenia fruit is attracting attention as a possible cause of mesenteric phlebosclerosis, which is characterized by fibrotic changes or calcification of the mesenteric vein and the bronze coloration of the colonic membrane. It is suggested that genipin, a metabolite of geniposide (the major ingredient of gardenia fruit) is involved in the bronze coloration. We previously described a patient who had consumed extract of gardenia fruit for seven years and developed skin pigmentation complicated by mesenteric phlebosclerosis (Mizawa M et al. JAMA Dermatol., 2020). Histological examinations of her pigmented skin showed hyperpigmentation of the basal layer and brown pigment granules in the macrophages and spindle cells around the vessels and interstitium in the reticular dermis. The brown pigment was revealed to be melanin on Fontana-Masson staining. The present study investigated whether or not genipin was involved in skin pigmentation through *in vitro* experiments. Time-of-flight secondary ion mass spectrometry of a skin section was used to detect genipin. This analysis revealed a peak with the molecular weight of genipin. In human melanocytes treated with genipin, an increased melanin production was shown by Fontana-Masson staining and the measurement of absorbance values at 405 nm for detecting intracellular melanin. The exact cause of the skin pigmentation is not known, but we hypothesized that the underlying cause of the hyperpigmentation was melanin deposition that was enhanced by genipin.

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Functional, inherited vitamin D-binding protein variants associated with mortality among melanoma patients

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Vitamin D regulates several pathways involved in cancer progression including cell proliferation, apoptosis, angiogenesis, and metastasis; and vitamin D blood concentrations are associated with survival outcomes of several cancers including melanoma. Two functional missense variants (rs7041 and rs4588) in the vitamin D-binding protein gene (*GC*) encode for three common protein isoforms—Gc1s, Gc1f, Gc2—that are associated with differences in vitamin D blood concentrations and binding affinity. We investigated the association of the genotypes encoding the Gc1s, Gc1f, and Gc2 isoforms with melanoma-specific and all-cause mortality among 3,995 incident, primary melanoma patients in a pooled analysis of two population-based studies using multivariable Cox-proportional hazards regression. Individuals carrying the rs7041 and rs4588 alleles encoding Gc1f had a 41% lower risk of melanoma-specific mortality relative to those without the Gc1f-encoding alleles (HR 0.59; 95% CI 0.44−0.80; P=4.05x10^{-4}) after adjusting for age, sex, whether a first or higher-order primary melanoma, study center, site, pigmentary phenotypes, and Breslow thickness. Our findings suggest that melanoma patients with the Gc1f-encoding genotype may have a lower risk of dying from melanoma compared to those with genotypes encoding Gc1s or Gc2.

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Expression of OPN3 correlating to tumor initiation and development in cutaneous melanoma

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The function of OPN3 in melanoma remains largely unknown. To assess the biological role of OPN3 in the initiation and development of cutaneous melanoma, we first systematically investigated the expression patterns of OPN3 gene from human melanoma based on the available three independent gene expression datasets (GEO GSE3189, GSE8401 and GSE4587). We found that the OPN3 mRNA was upregulated in human melanoma when compared to normal skin and nevus tissue samples, especially in vertical growth phase melanoma. Additionally, no statistically significant difference was evident between the primary melanoma and metastasis of melanoma tissues. By the weighted correlation network and protein-protein association networks analysis, it suggested that OPN3 might play a role in the initiation and development of cutaneous melanoma. Next, to further illustrate the possible mechanisms by which OPN3 mediates melanoma cell tumorigenicity, RNA-seq analysis was performed on low expression of OPN3 and untreated M14 human skin melanoma cells. In this present study, our heatmap revealed that 5029 differentially expressed genes (DEGs) were upregulated, while 4495 DEGs were downregulated. Notably, in GO (Gene Ontology) term enrichment analysis our results indicated that the DEGs were evidently enriched in post-transcriptional gene regulation and cell cycle regulation, suggesting that OPN3 was mainly involved in proliferation and cell cycle regulation. The KEGG pathway analysis, OPN3 was dramatically enriched in cell cycle regulation process, neurotrophin signaling pathway and mRNA surveillance pathway. Taken together, we here showed that high expression of OPN3 can promote melanoma initiation and development by regulating cell cycle progression and tumor-associated signaling pathway.

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Opsin3 expression in human nevus and reconstructed nevus model *in vitro*

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Background Opsin3 is a non-visual opsin and is a member of the opsin family. Recent researchers found that opsin3 is highly expressed in human epidermal keratinocytes, melanocytes, hair follicles and fibroblast. However, it is rarely reported in human nevus cells. Objective To investigate whether opsin3 express in human nevus cells, nevus tissues, and 3D model of reconstructed nevus in vitro. Methods The expression of opsin3 in nevus tissues, paired adjacent normal skin tissues were analyzed by quantitative real-time PCR and western blotting. In addition, primary nevus cells were isolated in vitro and a reconstructed nevus model was to reconstructedrmis. Cell suspension(2×10^5cells) were applied onto the dermal surface of the De-epidermized dermis (DED) in 150μl culture medium. After 4h of incubation, the system was immersed in a new aliquot of the same medium. After 3days, the system was raised to the air-liquid interface and left in culture for 14days. The marker of Melan-A, NSE, S100, MITF and TYR were used to identify the nevus cells and reconstructed nevus model with the immunofluorescence. The expression of opsin3 in nevus cells and reconstructed nevus model were analyzed by the immunofluorescence too. Results The expression of opsin3 in nevus, cultured nevus cells and reconstructed nevus model in vivo and vitro was observed, followed by Quantitative Real-Time PCR, Western Blotting and Immunofluorescence. The expression of melanocyte related marker of Melan-A, NSE, S100, MITF and TYR could be detected in cultured nevus cells and the reconstructed nevus model. In the reconstructed nevus model, nevus cells grow and distribute in the basement membrane on the surface of DED, and form clusters like nevus nests in the pit. In the place of residual cutaneous appendages tube wall, they grow in a ring and band shape. Conclusions the expression of opsin3 in nevus, nevus cells and reconstructed nevus model in vitro,the function of OPN3 in the nevus deserves further study.

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HHLA2 modulates MMP/NF-kappa B recycling to drive malignant melanoma metastasis

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Background: Malignant melanoma (MM) is a highly aggressive tumor. Its treatment has been revolutionized by the development of immune-checkpoint inhibitors which targeted at B7 protein or their putative receptors. HHLA2 was a newly identified member of B7 family, but its expression and function in MM was unknown. Herein, we aimed to study its clinical implications and potential regulatory role in MM. Methods: In this study,we examined HHLA2 expression in tissue-arrays containing nevus, malignant melanoma and metastatic malignant melanoma by immunohistochemistry staining. The intervention of HHLA2 in A375 cell line was performed and its effect on the cellular function was also analyzed. Then we identifed the diferentially expressed genes upon HHLA2 knockdown in A375 cell lines by using KEGG and GO analysis. Results: We found that HHLA2 was overexpressed in mucosal melanoma tissues and metastatic melanoma tissues but absent in nevus and cutaneous melanoma tissues. In vitro analysis, knockdown of HHLA2 in human MM cell line inhibited its proliferation, migration and invasion ability. According to the microarray data, MMP3 and NF-kappa B signaling pathway were involved in HHLA2 mediated progression in A375 cells. We confirmed the down-regulated expression of MMPs and core genes of NF-κB signaling pathway upon the HHLA2 knock-down. In addition, we checked the expression of epithelial-mesenchymal transition (EMT) related markers, and found that HHLA2 knock-down induced a MET process in A375 cells. Conclusion: Our findings indicated a novel role for HHLA2 in regulation of the metastatic capacity, which might be a potential therapeutic target for MM.

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Analysis of BRAF mutation and expression of NGFR and P16 in nevus and melanoma

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Association between tumor initiation and development and $BRAF^{V600E}$ mutation in melanocytic nevi (MN) and malignant melanoma (MM) remains controversial. Most of the data regarding $BRAF^{V600E}$ mutation is limited in Caucasian population. Herein, we detected the $BRAF^{V600E}$ mutation of MN and MM in a Chinese population. In all, 50.6% cases of MN (n=160) harbored $BRAF^{V600E}$ mutation by sanger sequencing. The highest mutation frequency is 92.5% of intradermal nevus, while junctional nevus was 10.7%. The mutation rate of different types is statistically significant difference (P<0.001). $BRAF^{V600E}$ mutations are more likely to occur in exposed parts and higher in younger age (P<0.05). In all, only 10.7% cases of MM (n=67) harbored the $BRAF^{V600E}$ mutation. There is a difference in age between the mutation group and the non-mutant group (P=0.009). In addition, the expression of NGFR, p16 in MM and MN tissues was detected by immunohistochemistry. Both 50% of nevi and 80% of melanomas samples displayed NGFR positive expression. Interestingly, the expression of p16 protein in the $BRAF^{V600E}$ mutant group was higher than that in the non-$BRAF^{V600E}$ mutant group, but it was not obvious in the malignant tumor. These results suggested that the mutation frequency might be not associated with malignant transformation. And $BRAF^{V600E}$ linked to high-expression of NGFR might play crucial role in transforming pigmentation nevus into melanoma. P16 might have an inhibitory effect in the transformation of nevi into melanoma.

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Opsin 3 promotes invasion of melanoma cells in an artificial melanoma model

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Opsin is family of G protein-coupled receptors (GPCRs) and serve a variety of nonvisual functions. Our recent study found that high opsin 3 expression is significantly associated with ALM and metastatic phenotype. The artificial melanoma model can simulate the physiological state of the skin and avoid harm to animals which is a good model for studying the pathogenesis of melanoma invasion. Whether opsin 3 can promote the invasion of melanoma cells and replicate clinically observed phenomena in artificial melanoma models is not yet clear. Objective: To investigate whether Opsin 3 promote invasion of melanoma cells in artificial melanoma model. Method: Used the GFP-opsin3 plasmid to constructed an MV3 melanoma cell line stably overexpressed opsin 3 and vector group as a control. Transfection efficiency observed by fluorescence microscope and opsin3 stably overexpression in MV3 detected by western blot. Compared the invasion and migration ability of MV3 cells in the with or without overexpression opsin 3 through Transwell. Overexpression GFP-opsin3 MV3 cells and control cells were seeded onto the DED and maintained at the air-liquid interface after submerged culture about8,12,15 days. Then HE was used to detect the distribution of MV3 cells on the reconstructed melanoma tissue in different group. Result: Opsin 3 was stably overexpressed in MV3 cells. Transwell showed that MV3 cells in the opsin 3 overexpression group had stronger invasion and migration capabilities. The artificial melanoma model was successfully constructed, and the HE results showed that MV3 cells disturbed at the surface of DED and gathered circularly at lumina of the cutaneous appendages.MV3 cell which are with or without overexpression of opsin 3 could invade down the porosity on the surface of the DED, but more obvious performance in overexpression opsin 3 group. Conclusion: Opsin 3 could promote invasion of MV3 melanoma cells in an artificial melanoma model.

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Keratinocyte desmoglein 1 as a target and mediator of paracrine signaling in the melanoma niche

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The melanoma microenvironment is important for tumor progression, but the role of neighboring keratinocytes (KC) in the tumor niche remains poorly understood. We previously showed that loss of the KC-specific desmosomal cadherin, desmoglein 1 (Dsg1), results in release of KC cytokines to alter melanocyte pigmentation and dendricity. This led us to address whether KC Dsg1 plays a role in the melanoma microenvironment. Here, we show that Dsg1 is reduced in regions surrounding melanoma lesions but is unchanged in KCs adjacent to benign nevi. To address whether melanoma cells contribute to Dsg1 loss, we cultured KCs in melanoma cell conditioned media and measured Dsg1 protein and mRNA levels. Melanoma cell, but not melanocyte, conditioned media reduced KC Dsg1 expression downstream of decreased Grhl1, a transcriptional activator of Dsg1. As we showed that Dsg1 loss stimulates expression of pro-migratory cytokines, we carried out transwell assays to compare behavior of melanoma cells treated with conditioned media from control, Dsg1-deficient and Dsg1-deficient KCs rescued with exogenous Dsg1. Media from Dsg1-deficient KCs increased melanoma cell migration compared to media from control KCs or KCs with Dsg1 rescue. Consistent with these data, staining of melanoma tumors revealed a negative correlation between KC Dsg1 expression and melanoma cell movement in the tumor niche. ERK1/2-dependent expression of CXCL1 increased in KCs exposed to melanoma cell conditioned media downstream of Dsg1 loss, and inhibition of the CXCL1 receptor CXCR2 abrogated Dsg1 loss driven melanoma cell migration, confirming the contribution of KC CXCL1 to the observed cell migration. Taken together, these data point to Dsg1 loss as a key regulator KC-melanoma cell cross talk in the tumor niche and a possible contributor to melanoma progression.

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WITHDRAWN

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The impact of particulate matter on melanogenesis

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Ambient particulate matter (PM), one of the main component of air pollutants, is a major concern for human health, because it is closely associated with mortality and morbidity from respiratory and cardio- and neuro-vascular diseases. Recently, it has been reported that PM can aggravate allergic skin diseases by affecting skin barrier or inducing inflammation. However, it is unclear if PM can induce melanogenesis and which processes are involved in PM-affected melanocyte biology. This study investigated the effect of PM on pigmentation and searched for involved factors that affect PM-induced melanogenesis. Methods: After PM was treated to primary human epidermal melanocytes, melanogenesis related molecules and their signaling pathways were evaluated. Pigmentation changes after PM treatment were observed through ex vivo skin culture and in vivo experiment. Candidate target molecules, which were screened out through RNA sequencing, were verified using target inhibitors and siRNA techniques. Results: Melanocytes exposed by PM showed increased melanin production along with upregulation of tyrosinase. Phosphorylation of CREB and CaMKII was increased by treatment with PM. Ex vivo and in vivo skins showed increased melanin production following PM treatment. RNA-sequencing data revealed that PM treatment to melanocytes induced the expression of endoplasmic reticulum (ER) stress-related genes among several candidate genes. We evaluated the association between ER stress-related molecules and PM-induced melanogenesis. Conclusion: These data suggest that PM might induce melanogenesis by affecting ER stress-related genes.

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Focus on a new esterified lipoaminoacid skin tanning booster

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Human skin pigmentation is a complex biological process responsible for the color and tanning of the skin. It is triggered by a signaling cascade leading to melanin synthesis by melanosomes in melanocytes, followed up by their transfer in surrounding keratinocytes. Our aim was to investigate the effect of a newly-developed esterified lipoamino acid ELA derivate on skin tanning. First, ELA was evaluated on the production of melanin by human and murine melanocytes. ELA was able to boost melanin synthesis in both models. This result was explained by the overexpression of mapk1, usf1, mitf genes regulating melanogenic enzymes production and *aim1, ap1m1, pmel17* and *stx6* genes regulating melanogenic enzymes transport, which were studied in 3D human pigmented reconstructed epidermis. Then, its capacity to regulate melanosome transfer was investigated on a melanocyte/keratinocyte co-culture model by flow cytometry and immunofluorescence. We found that ELA was able to stimulate melanosome transfer and the amount of melanosome transferred from melanocyte to keratinocytes. This could be explained at the gene expression level by the stimulation of melanosome transfer related genes such as *rac1, f2rl1, ktn1* and *myo5* in human pigmented reconstructed epidermis. Finally, a clinical evaluation was performed on 20 caucasian women with a phototype III to demonstrate the efficacy of the ingredient on tanning. Volunteers applied formula twice a day and were exposed to their minimum pigmentation dose of UVA radiations to mimic a normal incidental daily sun exposure. We could observe that ELA was able to provide a natural healthy glow from day 4, and a long-lasting tan up to 15 days after the last application. In conclusion, our new esterified lipoaminoacid boosts and prolongs skin tanning allowing to reduce sun exposure time.

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Impact of electrical impedance spectroscopy on diagnostic accuracy and clinician confidence in a survey-based evaluation of melanocytic skin lesions suspicious for melanoma

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Nevisense is an FDA-cleared device to aid in diagnosing melanoma. Using a non-invasive probe, the device measures electrical impedance spectroscopy (EIS) of target skin lesions. While EIS has demonstrated high sensitivity in diagnosing melanoma, its impact on a clinician's diagnostic confidence remains unknown. We conducted a pilot study evaluating whether the addition of EIS scores to clinical and dermoscopic images increases diagnostic confidence, accuracy, sensitivity, and specificity for students and dermatologists when evaluating lesions clinically suspicious for melanoma. Three pigmented lesions specialists and three 4th year medical students completed an online survey to evaluate 34 melanocytic lesions suspicious for melanoma. For each lesion, participants provided their diagnosis, biopsy recommendation, and confidence in diagnosing a lesion as benign or malignant based on history and clinical and dermoscopic images, and again after receiving an EIS score. Addition of EIS scores increased mean biopsy sensitivity for melanoma/severe dysplastic nevi (DN) from 70% to 84% (p =.014) and mean diagnostic accuracy from 74% to 86% (p =.005). Mean diagnostic confidence increased for 29/34 lesions, of which 26 were accurately diagnosed by $\geq$4 evaluators. Increases in diagnostic confidence were significant for common melanocytic nevi, DN, and melanoma, for both students and dermatologists (all p <.05). Use of EIS may increase clinicians' confidence to provide greater reassurance regarding dermoscopically equivocal lesions such as DN. EIS thus has the potential to help clinicians better alleviate patients' anxieties during skin exams. EIS may also improve management of melanocytic lesions suspicious for melanoma among novice and expert diagnosticians, though further investigation is needed to determine if these findings translate to clinical settings. NB: All authors except for Ms. Fried are team members for a separate study utilizing a Nevisense device, loaned to NYU by Scibase.

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Functional melanoma cell heterogeneity is regulated by MITF-dependent cell-matrix interactions

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Functional phenotypic cancer cell heterogeneity limits the efficacy of targeted and immunotherapies. The transcription factor MITF is known to regulate melanoma cell plasticity and, consequently, response to drugs. However, the underlying mechanisms of this phenomenon remain incompletely understood. Here, we show that MITF negatively regulates peroxidasin and fine-tunes the ability to contract the extracellular matrix, the maturation of focal adhesions and ROCK-mediated melanoma cell contractility. This, in turn, results in control of functional melanoma cell heterogeneity through spatio-temporal downregulation of p27^{kip}. Modulation of MITF expression alters extracellular matrix organization, melanoma cell morphology and solid stress in three-dimensional melanoma spheroids, thereby accounting for spatial differences in cell cycle dynamics. Together, our data identify MITF as a master regulator of the melanoma micro-architecture and point towards novel targeting strategies for cancer cell heterogeneity. Significance: Development of drug resistance is a major cause of melanoma therapy failure. The role of MITF in melanoma response to therapy has been discussed controversially, which can be explained, at least in part, through the rheostat model linking MITF activity to cell proliferation. Heterogeneity is widely associated with therapy resistance, however, whether cell phenotype switching, mediated by MITF, is responsible for treatment resistance is not known. Our findings provide an in-depth mechanistic understanding of the MITF-mediated regulation of cell cycle behavior and physical regulation of the tumor architecture. As MITF is not amenable to direct drug targeting, the identification of mediators of MITF-triggered functional heterogeneity reveals novel targets that can be deployed to control this phenomenon.

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Sox9 knockout in the endothelium decreases melanoma tumour vascularisation, metastasis, and alters melanoma gene expression

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The development of new vascular structures is a pre-requisite for melanoma growth and spread. Endovascular progenitor cells (EVPs), residing in vessel walls give rise to mature endothelial cells and contribute to a new blood vessel network formation in the tumour. Sox9 is a transcription factor that is playing an important role in stem cell self-renewal and quiescence and is highly upregulated in EVPs. In this study, we aimed to explore its role in tumour vascularisation and metastasis. An endothelial-specific knock out mouse model, Sox9fl/fl/Cdh5CreERt2/Rosa-YFP was utilised to delete Sox9 conditionally in the endothelium. In B16-F0 or HcMel12 (hgf transgenic) melanoma tumours inoculated subcutaneously, a significant reduction in tumour EVPs was observed upon Sox9 deletion in the endothelium. Immunofluorescence on tumour sections confirmed a significant reduction in the number and area of CD31+ vessels. HcMel12 melanoma tumours produced much less lung metastases in mice with Sox9 conditional deletion. This reduction in metastasis was at least in part related to alteration of the metastatic niche as B16-F10 cells administrated intravenously in mice with conditional Sox9 deletion resulted in reduced number and area of nodules in the lungs. Upon RNA sequencing of HcMel12 melanoma cells, 237 differentially expressed genes were identified. Sirt1, Tgfb3, Tgfbr1, Igf1r, and Notch1 were among the downregulated genes in tumours inoculated in Sox9 deleted group. In summary, Sox9 deletion in the endothelium resulted in the depletion of EVPs, prompting fewer de novo vessels in the centre of the tumour. Furthermore, Sox9 deletion in the endothelium reduced metastases. This new knowledge strongly suggests EVPs as a new target in the field of anti-vascularisation treatment in cancer and metastasis.

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The role of autophagy in IFN-γ effects on global gene expression in keratinocytes

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Background: Vitiligo is a common acquired skin disease with characteristic pigment disorder and there is no effective treatment. IFN-γ has been regarded as a crucial factor in the progression of vitiligo. Autophagy also plays an important role in maintaining the function of skin cells and defects in autophagy cause defective pigmentation; however, the relationship between autophagy and IFN-gamma effects in keratinocytes remains unclear. Objective: The aim of the study was to explore whether autophagy could regulate the IFN-gamma effects in keratinocytes. Methods: The induction and activity of autophagy in keratinocytes was determined by western blotting, immunofluorescence and electron microscopy after IFN-γ stimulation.n RNA-Seq analysis was performed to investigate IFN-γ effects on global RNA expression in keratinocytes from autophagy competent (Atg7 f/f, "WT") and autophagy incompetent (Atg7 f/f K14: Cre mice, "KO") mice of young and old age. Expression of specific genes was confirmed by real-time q-PCR. Results: Western-blotting showed that the ratio of LC3II/LC3I increased after the stimulation of IFN-γ in keratinocytes. Immunofluorescence showed an increase in the number of autophagy puncta with IFN-γ stimulation. Electron microscopy showed an increase in the number of autolysosomes with IFN-γ stimulation. They suggested that IFN-γ could induce autophagy in keratinocytes. Ingenuity pathway analysis of differentially expressed genes (DEGs) revealed pathways and functions activated by IFN-γ under different genotypes. Pathways like "Dendritic Cell Maturation" and "p38 MAPK Signaling" showed significant differences in z-scores between WT and KO groups. Conclusions: Our results suggested that IFN-γ could induce autophagy in keratinocytes; The ability to perform autophagy can influence the interferon gamma response; Activation of autophagy by its agonist rapamycin can modify the interferon gamma response depending on the age of the cell donor.

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Human skin organ culture permits to preclinically assess the effects of "UV-free tanning" and "skin lightening" agents on epidermal melanogenesis

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Clinically relevant pre-clinical assay systems that permit one to predict the efficacy and potential adverse effects of candidate "skin lightening" or "UV-free tanning" agents in human skin are much-needed, not the least since candidate agents with strong *in vitro* potency often fail in clinical trials. Even 3D skin "equivalent" models for studying hypo- or hyperpigmentation-inducing agents inadequately capture the complex biology of human epidermal melanogenesis. Therefore, we have explored whether the serum-free organ culture of full-thickness human over 3-6 days provides a suitable *ex vivo* assay for testing the melanogenesis-modulating effects of candidate pigmentation-modulatory agents, despite the relatively short culture window. Here, we report that melanogenesis can be significantly promoted within only 3 days by treatment with [Nle4, DPhe7]-alpha-melanocyte stimulating hormone (1 μM) or forskolin (25 μM) as positive controls, using quantitative (immune-) histomorphometry (increased: melanin production by Warthin-Starry staining, *in situ* activity of tyrosinase, and (pre-) melanosome formation Gp100 labeling intensity). Conversely, hydroquinone, an established anti-melanogenetic agent, significantly suppressed melanogenesis, as assessed by the above read-outs. This confirms the robustness, sensitivity and instructiveness of human skin organ culture for testing both hyper- and hypopigmentation-inducing agents under clinically relevant *ex vivo* conditions and on multiple levels of epidermal melanogenesis. This assay can be customized to test candidate skin lightening/tanning agents in the presence of hyperpigmentation-inducing stressors (e.g., UV irradiation, ROS, histamine, estrogens, pro-inflammatory cytokines).

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Inhibition of soluble adenylyl cyclase (sAC) rescues defective melanosomal pH and pigmentation in oculocutaneous albinism type 2 (OCA2)

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Oculocutaneous albinism type 2 (OCA2) results from a defective melanosomal anion channel leading to an acidic melanosomal pH, reduced tyrosinase activity and decreased melanin production. Reduced melanin production in OCA2 affected people leads to the development of skin cancers and other forms of damage due to ultraviolet radiation. Currently, pharmacological approaches to repair melanosomal pH and pigmentation do not exist. We have shown that inhibition of soluble adenylyl cyclase (sAC) in melanocytes alkalinizes melanosomal pH and enhances tyrosinase (TYR) activity. Therefore, we asked whether inhibition of sAC would restore wild type melanosomal pH and melanin production in OCA2 melanocytes and mice. OCA2 melanocytes had a more acidic melanosomal pH and sAC inhibitors restored wild type melanosomal pH and increased tyrosinase activity. We generated $Oca2^{-/-}$, sAC floxed (f/f), Tyr-CRE-ERT2 mice and induced sAC knock out via post-natal (P2-4), topical application of 4-hydroxytamoxifen. The $Oca2^{-/-}$; $sAC^{-/-}$ mice (n=23) had noticeably darker hair color at P28 as compared to $OCA2^{-/-}$; $sAC^{f/f}$ (n=14) littermates. HPLC analysis of the hair collected on P28 showed a significant increase in total melanin in $Oca2^{-/-}$; $sAC^{-/-}$ mice corresponding to increased amounts of both eumelanin (PTCA) and pheomelanin (AHP). Individual hair strands of $Oca2^{-/-}$; $sAC^{-/-}$ mice revealed distinctive banding patterns as compared to $Oca2^{-/-}$; $sAC^{f/f}$ littermates, and histology showed that the $Oca2^{-/-}$; $sAC^{-/-}$ mice also had less overall hair follicles. Thus, loss of sAC in melanocytes may affect hair morphogenesis. In conclusion, our studies show that restoring melanosomal pH by inhibiting the sAC signaling pathway is a potential therapeutic approach for the treatment of OCA2 and related diseases.

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$BRAF^{V600E}$-inhibition drives EMT gene expression enhancing invasiveness and metastasis in a bioluminescent murine model of $BRAF^{V600E}$/$NRAS^{Q61K}$ melanoma

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BRAF inhibitor (BRAFi) resistance compromises long term survivorship of malignant melanoma patients. Mutant $NRAS^{Q61K}$ with constitutive activation of PI3K/AKT/mTOR signaling is a major mediator of BRAFi resistance, and BRAFi therapy can accelerate pre-existing RAS-mutant malignancies including NRAS-mutant leukemias in melanoma patients. Here, employing NanoString transcriptomic analysis of isogenic (A375-$BRAF^{V600E}$ versus A375-$BRAF^{V600E}$/$NRAS^{Q61K}$) malignant melanoma cells we demonstrate that BRAFi treatment selectively targets $BRAF^{V600E}$/$NRAS^{Q61K}$ cells with induction of Epithelial to Mesenchymal Transition (EMT) gene expression, paradoxically promoting invasiveness and metastasis *in vivo*. Cancer progression nCounter™ pathway analysis identified 'EMT' and 'Proliferative Control' gene expression networks specific to $BRAF^{V600E}$/$NRAS^{Q61K}$ status. Strikingly, in contrast to BRAFi (vemurafenib, VEM)-induced antiproliferative and antiinvasive effects in $BRAF^{V600E}$ cells, VEM enhanced proliferation and invasiveness of $BRAF^{V600E}$/$NRAS^{Q61K}$ cells. RT^2Profiler PCR array analysis confirmed VEM-upregulation of genes promoting EMT and proliferation [*AKT1*, *MMP3*, *PDGFRB*, *RAC1*, *SPARC*, *ZEB1*, *ZEB2* ($\leq$ 350-fold; $p<0.05$)] detectable only in $BRAF^{V600E}$/$NRAS^{Q61K}$ cells, while causing the expected downregulation of EMT-driver genes [*CDH2*, *FN1*, *FOXC2*, *IGFBP4*, *MMP9*, *VIM*, *WNT5A* ($\leq$ 40-fold; $p<0.05$)] only in the $BRAF^{V600E}$ isogenic variant. Phenotypic transwell migration assays confirmed the seemingly opposing effects of VEM treatment on melanoma cell invasiveness [achieving blockade ($BRAF^{V600E}$) or enhancement ($BRAF^{V600E}$/$NRAS^{Q61K}$)]. In a bioluminescent SCID mouse metastasis model using A375-luc isogenic variants, VEM treatment (50 mg/kg; p.o., q.d.) enhanced lung tumor burden imposed by $BRAF^{V600E}$/$NRAS^{Q61K}$ cells, while blocking metastasis of $BRAF^{V600E}$ cells. Our data provide preclinical evidence that identifies a BRAFi-driven upregulation of EMT-related gene expression potentially enhancing invasiveness and metastasis in human $BRAF^{V600E}$/$NRAS^{Q61K}$ melanoma.

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The high expression of pro-apoptotic BCL2 family members in uveal melanomas contribute to their sensitivity to MCL1 inhibitors

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Uveal Melanoma (UM), a rare melanoma of the eye, has a poor survival prognosis and lacks any FDA approved drug treatments; thus, there is an urgent need for treatments. UM does not have BRAF-V600 mutations commonly found in cutaneous melanoma (CM). BH3 mimetics are a novel class of drugs that mimic pro-apoptotic BCL2 family members. In hematological cancers, venetoclax (BCL2 inhibitor) is approved and S64315 (MCL1 inhibitor/MCL1i) is in clinical trials. This study aims to examine the efficacy of various BH3 mimetics in UM and to determine the underlining mechanism for the differing sensitivity we found between UM and CM. We used *in vitro* assays (viability, IncuCyte live cell imaging, immunoblot, sphere formation, shRNA techniques), *in vivo* mouse models, and bioinformatics analyses of the TCGA database. We found that treatments of MCL1i alone at nM doses were highly effective in reducing viability, inhibiting cell proliferation, and activating caspase 3/7 in human UM cell lines ($p<0.01$), but not CM lines. MCL1i also significantly reduced tumor growth *in vivo* ($p<0.05$). In addition, UM lines displayed higher endogenous expression of pro-apoptotic proteins BMF, PUMA and BAD, compared to CM lines, and blocking BRAF/MAPK signaling in CM with MEKi increased their expression. Further, knock down of BMF, PUMA, and BAD partially protected UM against MCL1i ($p<0.05$). Results indicated that the lack of activating mutations in BRAF/MAPK signaling in UM was the cause for greater pro-apoptotic protein expression. Moreover, TCGA data also showed a higher expression in UM vs. CM, indicating the clinical relevance of our finding. However, MCL1i alone was ineffective at killing the UM stem-like cells, but a combination with venetoclax synergistically killed UM stem-like cells, inhibited their capacity to self-renew, and reduced tumor growth *in vivo* ($p<0.01$). In summary, our data indicated MCL1i, alone or in combination with venetoclax, are promising treatments for UM.

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Melanoma in pregnancy: Profiling the tumor microenvironment

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Immunosurveillance is thought to play a critical role in eliminating nascent transformed melanoma, which is the most common malignancy occurring during pregnancy. Since pregnancy induces a state of immunosuppression, we sought to identify pregnancy-associated alterations to the melanoma tumor microenvironment. Our specific objective was to evaluate the immunophenotype of pregnancy-associated melanoma (PAM). We identified 7 cases of PAM from the electronic medical records of the University of California, Davis. PAMs were compared with melanomas from age-matched, non-pregnant females (N=8) and males (N=8). We recorded the presence, distribution, and density of lymphocytes and lymphocyte subsets (CD3, CD4, CD8, CD20, FOXP3, and PD-L1) and calculated a score for tumor infiltrating lymphocytes (TIL) and stroma infiltrating lymphocytes (SIL) and lymphocyte subsets. Fisher exact tests were utilized to compare TIL and SIL scores between the groups. In our preliminary analysis, no significant differences were found in the TIL and SIL scores between PAM and the control groups. Additional studies are necessary to fully understand the impact of pregnancy-related changes of the immune system on melanoma development.

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Long-term outcome of pigmented lesions clinically suspicious for melanoma previously tested with the Pigmented Lesion Assay (PLA): Results from the TRUST Study

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The assessment of pigmented lesions suspicious for melanoma remains a challenge. The non-invasive Pigmented Lesion Assay (PLA) guides biopsy decisions and detects melanoma at its earliest stages based on genomic atypia. The TRUST Study presented here is a long-term 12-24-month re-test follow-up study of lesions that initially tested negative for melanoma with the PLA. The study was designed to determine the proportion of true negative lesions among those that tested negative. Of the 1,781 lesions in the long-term follow-up screening cohort, there were no melanoma deaths or late-stage melanoma detected from these lesions. Ten lesions from the full cohort had received a melanoma diagnosis after initial testing, with four (0.3%) at Stage 0 (*in situ*) and six (0.5%) at Stage 1a. The negative predictive value (NPV) in a subset of 1,233 lesions with confirmed follow-up evaluations was 99.2% ($CI_{95\%}$= 98.5 - 99.6). Of the 302 lesions assessed by means of repeat testing with the PLA, none (0%) were found to have clinically obvious melanoma upon the subject's return to the clinic, confirming the results of the initial chart review. Of these 302 lesions, 88.7% percent (268 lesions) were negative on repeat testing with the PLA and 34 (11.3%) were positive. All 34 lesions (100%) were surgically biopsied, with 3 (1%) diagnosed as Stage 0 (*in situ*), identified 13, 14 and 19 months after the initial PLA (NPV = 99.0% [$CI_{95\%}$ = 97.1 - 99.8]). This long-term repeat-testing study confirmed the NPV of the PLA and found no adverse outcomes related to the test's routine use.

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Measurement of melanin metabolism in live cells by [U-^{13}C]-tyrosine fate tracing using LC-MS

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Melanin synthesis occurs within a specialized organelle called the melanosome. Traditional methods for measuring melanin levels rely on the detection of chemical degradation products of melanin by high-performance liquid chromatography (HPLC). Although these methods are robust, they are unable to distinguish between melanin synthesis and degradation, and are best suited to measure melanin changes over long periods of time. We developed a new method that actively measures both eumelanin and pheomelanin synthesis by fate tracing [U-^{13}C] tyrosine using liquid chromatography-mass spectrometry (LC-MS). Using this method, we confirmed previous reports of melanin synthesis differences between melanocytes derived from individuals with different skin color or MC1R genotype, and uncovered new information regarding the differential *de novo* synthesis of eumelanin and pheomelanin, also called mixed melanogenesis. We also revealed that distinct mechanisms that alter melanosomal pH differentially induce new eumelanin and pheomelanin synthesis. Finally, we revealed that the synthesis of L-DOPA, an important metabolite of tyrosine, is differentially controlled by multiple factors. Because tyrosine fate tracing is compatible with untargeted LC-MS based metabolomics, this novel approach enables the broad measurement of cellular metabolism in combination with melanin metabolism, and we anticipate that this approach will shed new light on multiple mechanisms of melanogenesis.

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Regulatory T cell production of IFN-γ in vitiligo

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Vitiligo is a common autoimmune skin disease that affects 1-2% of the population. In vitiligo, CD8+ effector T cells (Teffs) target melanocytes, the pigment producing cells. Over time, this targeted destruction leads to patchy skin depigmentation, which can result in social stigma and emotional distress to affected individuals. Regulatory T cells (Tregs) help maintain tolerance in peripheral tissues by suppressing Teffs that target self-antigens and thus limit vitiligo disease severity. We performed single cell RNA sequencing on cells isolated from vitiligo patients and determined that Tregs adopt a type 1 cytokine expression profile in lesions, but not nonlesional skin. Consistent with this profile, human lesional Tregs produce IFN-γ, which we confirmed in our mouse model of vitiligo. IFN-γ production by Tregs could indicate reduced function with conversion to effector status, or in contrast, improved function by adopting a similar profile to their effector targets. Additional studies will determine what role IFN-γ plays in Treg function during the progression, maintenance, and stabilization of vitiligo.

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p38 kinases in cutaneous melanoma: Insights from *in vitro* studies and database mining

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Skin cutaneous melanoma (SKCM) accounts for 75% of skin cancer deaths, with incidence rates continuing to rise alarmingly. Therapeutic strategies for advanced melanoma, such as targeted therapies and immunotherapies, are rapidly emerging, but drug resistance and toxicity remain challenges for many patients. The p38 protein kinases coordinate adaptive cellular responses to extracellular stimuli and modulate important processes dysregulated in tumorigenesis, such as proliferation, differentiation and survival. Although p38 signaling is of potential importance in melanoma, the isoform-specific functions of the p38s in SKCM remain unclear. We studied the effects of pharmacologic and RNAi-mediated inhibition of p38 isoforms in human melanoma cell lines A375 and WM164 using colony formation assay. We report that p38α/p38β inhibition with SB203580, pan-p38 inhibition with Compound 62, or a concurrent p38α and p38δ knockdown enhanced colony formation ability in both cell lines, indicating both specific and redundant roles for p38 isoforms in negative regulation of human melanoma cell survival. We also analyzed the gene expression, prognostic value, and clinical correlations of the p38 isoforms in The Cancer Genome Atlas SKCM sample datasets, utilizing GEPIA, LinkedOmics, TIMER, and GSCALite bioinformatic tools. We report that *p38γ* expression was upregulated, while *p38δ* was downregulated in SKCM. In addition, a low level of *p38δ* correlated with worse disease-free survival. These findings support a novel tumor-promoting role for p38γ and confirm a tumor-suppressing role for p38δ. Furthermore, higher p38δ was correlated with increased immune cell infiltration, including $CD8^+$ T cells and dendritic cells, and increased T cell exhaustion, suggesting that targeting p38δ in the SKCM TME may stimulate antitumor immunity. Our study highlights the potential paths for translational research efforts.

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Design and *in vitro* efficacy of TRP-1 CAR T cells to target melanoma

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Chimeric antigen receptor (CAR) expressing T cells offer a promising advance in cancer immunotherapy. To become a lead immunotherapeutic strategy for melanoma, a melanoma-specific surface antigen should be identified that can be targeted without putting healthy cells at risk. The 2nd generation CAR we have designed can address this need. The new CAR targets the melanoma-associated antigen TRP-1 (tyrosinase-related protein 1). TRP-1 is the most abundant protein in melanoma cells, and is not normally recognized by T cells. TRP-1 is reportedly highly expressed on the melanoma cell surface. It is however not expressed by other cell types, and expression is not found on the surface of normal melanocytes. We performed FACS analysis of B16 mouse melanoma cells, 624.38 human melanoma cells, and normal human melanocytes. Cells were lifted in EDTA, and incubated with anti-TRP-1 antibody at 4°C to prevent internalization. We observed a full population shift for both melanoma cells lines, with a >4-fold increase in fluorescence over background for both melanoma cell lines. No surface expression was found on melanocytes. We then cloned the gene encoding the variable regions of an antibody to surface TRP-1, fused to a CD8 hinge region, a CD28 signaling domain, and CD3ζ into a Moloney Murine Leukemia virus (MMLV)-based plasmid. The fusion protein was followed by a viral slippage sequence, and a truncated CD34 segment to sort CAR-expressing T cells. Normal human T cells from PBMC were transduced and expanded in the presence of IL-2 and CD3/CD28 coated beads, then sorted to obtain >95% TRP-1 CAR transduced T cells. We next incubated TRP-1 CAR T cells and 624.38 melanoma cells and observed that CAR T cells effectively eliminated 80% of 624.38 melanoma cells in culture within 24 hours even at 1:1 E:T ratio. IFN-γ release was 35-fold greater in 1:1 cocultures with CAR T cells compared to untransduced T cells. TRP-1 CAR T cells show great promise for translation to an effective melanoma treatment.

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Inpatient burden of Lyme Disease in Skin of Color

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Lyme disease is a multi-system disease caused by Borrelia burgdorferi (Bb) bacterium, which typically affects the skin, heart, nervous, and musculoskeletal system. Most individuals characteristically develop erythema migrans after exposure to Lyme disease, which presents differently in Skin of Color populations. Previous studies have noted there is an increased need for racial diversity in Lyme-related dermatological education in order to provide patients with timely care. We sought to compare the baseline characteristics and inpatient financial burden of Lyme disease between White and Non-White patients. We investigated adult hospitalizations with Lyme disease (ICD-10-CM CodeA69. 2) as a diagnosis from the National Inpatient Sample (NIS) in 2016, yielding 9,540 hospitalizations. Patients admitted with Lyme disease were stratified into two groups: White and Non-White (Black, Hispanic, Asian, Pacific Islander, Native American, other). We compared groups by age on admission, gender, hospital region, season at admission, bed-size, median household income, and death during hospitalization by performing a chi-squared test. Using a one-way ANOVA test on charges per day and length of hospital stay (LOS), we found that White patients were charged a mean of $11,736 ± 746 per day, whereas Non-White patients were charged $12,490 ± 1,540 per day. The LOS was significantly greater for Non-White patients (6.9 days) in comparison to White patients (4.9 days, $p<0.001$). We further performed a multivariate linear regression and found that Non-White patients were positively correlated with longer hospital stays (95 % CI: 0.57 to 3.02, $p<0.005$).Therefore, significant healthcare disparities exist in Lyme disease. Delayed diagnosis of treatment due to low clinical suspicion potentially contributes to increased financial burden and LOS. Future studies should explore racial differences in severity of presentation in Lyme disease as well as time to diagnosis across various skin types.

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No racial differences in mental health comorbidities in psoriasis patients

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Little data exist on the impact of psoriasis on mental health among different races. This study sought to evaluate the relationship between psoriasis and mental health in patients from different racial backgrounds. We performed a nationwide, cross-sectional study evaluating 7,519,662 (weighted) patients, comparing white patients versus patients with skin of color (SOC), using 2004-2017 Medical Expenditure Panel Survey (MEPS). Overall, no significant differences in mental health outcomes existed between white and SOC patients with psoriasis. Psychological distress, as measured by Kessler 6-Item Psychological Distress Scale (K6), was similar between white and SOC patients [4.132 (95% CI, 3.679-4.586) and 3.710 (95% CI, 2.932-4.488), p=0.407]. Depression, as measured by Patient Health Questionnaire 2 (PHQ2), was similar between white and SOC patients [0.886 (95% CI, 0.744-1.027) and 0.748 (95% CI, 0.506-0.989), p=0.385]. Overall mental health, as measured by Mental Component Summary (MCS), was similar between white and SOC patients [49.959 (95% CI, 48.979-50.939) and 50.257 (95% CI, 48.449-52.065), p=0.789]. Perceived mental health state, as measured by Perceived Mental Health Status (MNHLTH), was similar between white and SOC [2.159 (95% CI, 2.065-2.253) and 2.103 (95% CI, 1.911-2.294), p=0.603]. Clinicians should consider screening for and managing mental health comorbidities in psoriasis patients of all racial backgrounds. Because treating psoriasis effectively is associated with mental health improvement, it is important to advocate for better access for many patients with SOC and those from disadvantaged socio-economic backgrounds.

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Fitzpatrick skin type and photographic skin color assessment in a diverse population

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The Fitzpatrick scale was initially developed to categorize response to UV light, but in practice, Fitzpatrick skin types (FSTs) are often used as a proxy for skin color. Research has examined the relationship between spectrophotometry and FST, but there are few data on the relation of FST to skin color in photographs, which are increasingly available for research. We used data on a diverse group of 208 participants from the Baltimore Longitudinal Study of Aging (BLSA) which included the participant's self-reported FST from response to a validated FST questionnaire on burning tendency and standardized photographs of the forearm. Investigators coded the photographs using a published FST six-color categorization scale. Color was also assessed using the sum of red, green, and blue (RGB) color values extracted by Adobe Photoshop 2021 from a standardized 50x50 pixel area. The RGB sum represents a quantitative measure of color on a spectrum from white to black. Investigator-assigned color categories only agreed with self-reported FST 14% of the time. And while there was no trend between self-reported FST and the continuous RGB sum, there was a clear linear trend between investigator assigned color categorization and RGB sum (p-value = 0.00). In conclusion, the RBG sum, a continuous measure of skin color, can be reliably calculated from standardized photographs, and self-reported FST should not be used interchangeably with skin color in research.

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Herpes simplex virus type 1 infects melanocytes

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Herpes simplex virus type 1 (HSV-1) is a normal pathogen that can infect human through skin or mucous regions. HSV-1 infects murine melanocytes Melan-A cell line, thus inhibits proliferation and induces apoptosis. Our study was sought to investigate the effects and underlying mechanism of HSV-1 on human epidermal melanocytes. The melanin synthesis and tyrosinase activity were measured by melanin content measurement and tyrosinase activity assay. Immunofluorescent assay, real-time PCR and western blot were conducted to measure relative markers, and Isobaric tags for relative and absolute quantitation were used to identify differentially expressed proteins. The results revealed that, after HSV-1 infection, PIG1 cells shrank and rounded, the length of dendrites shortened and the numbers of dendrites decreased. The melanin synthesis and tyrosinase activity were inhibited in HSV-1 infection PIG1 cells and PIG3V cells. Meanwhile, the expressions of microphthalmia-associated transcription factor (MITF), tyrosinase, tyrosinase related protein-1, gp100 all significantly decreased. In conclusion, our study firstly confirms HSV-1 can infect human epidermal melanocytes and inhibit melanogenesis.

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Cluster analysis of circulating plasma biomarkers in prurigo nodularis reveals a distinct systemic inflammatory signature in African Americans

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Prurigo nodularis (PN) is a chronic inflammatory skin disease associated with intense pruritus and a significant reduction in quality of life. Emerging evidence suggests that African American (AA) PN patients have a greater comorbidity burden, unique clinical disease phenotype, and worse prognosis than other racial groups. However, there are limited data on the pathogenesis to explain this disparity. To characterize the systemic immune dysregulation in PN, plasma levels of 12 cytokine biomarkers, representing a variety of immune functions, were measured in 20 PN (12 AA, 8 Caucasian) and 15 healthy control (10 AA, 5 Caucasian) patients. PN patients were clustered using the unsupervised machine learning algorithm Partitioning Around Medoids, resulting in two clusters of PN patients with non-inflammatory (cluster 1) and inflammatory (cluster 2) plasma profiles. Continuous and categorical variables were compared with Mann-Whitney *U* and Pearson's χ^2, respectively. Cluster 2 had more African Americans (67% vs. 18%, P=0.027), higher itch numeric scores (9.5 ± 0.9 vs. 8.3 ± 1.2, P=0.036), and a more significant reduction in quality of life as reflected by higher Dermatology Life Quality Index scores (21.9 ± 6.4 vs. 13.0 ± 4.1, P=0.015). Compared to controls, cluster 2 had higher IL-4 (P=0.019), IL-5 (P=0.017), IL-10 (P=0.010), IL-12 (P=0.010), IL-17A (P=0.023), and IFN-α (P=0.036). Compared to cluster 1, cluster 2 had higher IL-1a (P=0.001), IL-4 (P=0.002), IL-5 (P=0.037), IL-6 (P=0.028), IL-10 (P=0.003), IL-17A (P=0.037), IL-22 (P=0.002), IL-25 (P=0.002) and IFN-α (P=0.036). These findings suggest that African American PN patients have a novel inflammatory signature characterized by greater systemic immune dysregulation resulting in higher itch intensity and lower quality of life.

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Targeted immunotherapy response in acral melanoma patients—A retrospective review from a tertiary care center

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Acral lentiginous melanoma (ALM) is the most common form of melanoma in ethnic populations and is associated with a poor response to treatment in advanced stages. BRAF V600E mutations are common in other cutanaeous melanomas but less common in ALM, and are associated with greater response to newer targeted therapies. ALM can be associated with a variety of genetic mutations for which successful targeted treatments remain elusive. Response of targeted therapies in ALM patients with BRAF mutations, however, is unclear. A retrospective pathology and medical chart review was conducted at a Johns Hopkins to identify a total of 93 patients (51 male and 42 female) with a pathology confirmed diagnosis of ALM between 1990 and 2019. Nineteen patients (20%) with a pathology confirmed diagnosis of ALM received systemic therapy with a racial makeup of 63% (12/19) Caucasian, 21% (4/19) Black, 11% (2/19) Asian, and 5% (1/19) Hispanic. Immunologic or targeted therapy was used as a primary or adjuvant treatment in 17 patients. The majority (79%) of patients receiving systemic therapy underwent genetic mutation panel testing. Overall, a BRAF mutation was identified in 33% (5/15), NRAS mutation in 13% (2/15), and c-KIT mutation in 7% (1/15). All patients with the BRAF V600E mutation experienced disease progression on targeted therapy with an average overall survival of 22.5 months compared to 63.2 months in patients who underwent other treatments such as high dose interferon, or other chemotherapy. In all, 42% of patients undergoing systemic therapy eventually succumbed to their disease. Overall, response to systemic therapies in ALM patients was overwhelmingly poor. Unlike the positive prognosis associated with BRAF V600E mutations in other melanomas, our findings raise concern for a decreased response to targeted therapies in BRAF V600E mutated ALM. Further investigation of BRAF V600E mutated ALM-specific response to targeted therapies in a larger cohort may be of benefit in guiding future treatment recommendations.

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Impact of ethnicity and socioeconomic status on acral lentiginous melanoma incidence and survival: A SEER analysis

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Acral lentiginous melanoma (ALM) is a rare type of melanoma that forms on the palms, soles, or beneath the nail. It is the most common type of melanoma among people with darker skin. However, little is known about how incidence and survival trends of acral melanoma have changed over time across different ethnicities. As such, the goal of this study was to examine the epidemiology of ALM among ethnic and racial minorities incorporating the effect of socioeconomic (SES) status. To do this, we performed a retrospective analysis in the Surveillance, Epidemiology, and End Results (SEER) 18 Registry for patients diagnosed with ALM between 2000-2016. 2676 patients were identified: 380 (14.2%) Hispanic, 195 (7.2%) Asian/Pacific Islander (API), 219 (8.2%) Non-Hispanic Black (NHB), 1882 (70.3%) Non-Hispanic White (NHW). The overall incidence rate of ALM (cases per 100,000) increased by 1.65% (95%CI 0.62% - 2.7%, p<0.01) annually during the study period, which was driven primarily by an increasing incidence among NHW (2.07% annually; 95%CI 1% - 3.15%, p<0.01). The annual incidence rate remained flat across the other ethnicities. A multivariate Cox proportional hazards model, stratified adjusting for demographics and stage at diagnosis, revealed higher hazard ratios (HR) of mortality among NHB (HR 1.26, 95%CI 1.01-1.59) and Hispanics (HR 1.33, 95%CI 1.09-1.61) as compared to NHW. Male gender (HR 1.55, 95% CI 1.36-1.76), older age at diagnosis (HR 1.05, 95%CI 1.05-1.06), and lower SES status were also independent predictors of mortality. Specifically, when compared to the highest SES quintile (5), patients in quintiles 1-4 were at significantly higher risk of mortality: 1.37 (1.09-1.72), 1.53 (1.26-1.86), 1.27 (1.04-1.54) and 1.12 (0.92-1.36), respectively. Our work provides novel insights into the impact of ethnicity and socioeconomic disparities on outcomes in ALM. Future research should focus on identifying disparities in ALM management, including rates of immunotherapy utilization.

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Assessment of skin of color and diversity and inclusion content published in the Journal of Investigative Dermatology: An analysis and call to action

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It is perceived that dermatology is a field of medicine where despite being a field that is supposed to be interested in the skin, there is an underrepresentation of education and publication on skin of color people in our literature. This study develops criteria to assess skin of color related publications in the Journal of Investigative Dermatology. We developed the first prespecified criteria that allows for the assessment of diversity in dermatology literature. Using this criteria, the archives of 52 dermatology journals from January 2018 to October 2020, were analyzed for association with skin and hair of color, diversity and inclusion and socioeconomic/health care disparities that affect under-represented minorities. Out of 52 journals, the Journal of Investigative Dermatology ranked 43rd regarding the percentage of articles from 2018 through 2020 (only 4.42%) relevant to skin of color. Across all journals, the mean percentage of articles relevant to skin of color published from 2018 to 2020 was 15.01%. Our study consisted of 41 (78.85%) clinical journals and 11 (23.08%) scientific journals. The percentage of SOC articles was significantly greater in clinical journals than in scientific journals; $t(41.72) = -2.421$, $p = 0.020$. We hope the results of our study will assist the field of dermatology in its continuous and noteworthy efforts of becoming a more inclusive and diverse specialty. We encourage journal editors to use the criteria we developed to evaluate their issues for skin of color content and to provide guidance for publication invitations that are dedicated to topics relevant to patients of color.

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Scalp cooling therapy for chemotherapy-induced alopecia including skin of color patients: A review of the literature

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Chemotherapy-induced alopecia (CIA) continues to be a challenging side effect of cancer treatment. Scalp cooling therapy has proven its efficacy as a prophylactic remedy for hair loss with its ability to reduce chemotherapeutic agents from reaching the hair follicle. However, most studies in the current literature are in a predominantly straight-haired Caucasian population. Hair type in African descendants has physical properties such as texture and shape that differ from straight hair, potentially altering the effectiveness of scalp cooling. A literature review was conducted to assess the use of scalp cooling in Black/African American breast cancer patients with Afro-type hair between 2011 to 2020. A total of five studies met the inclusion criteria. Of those, only two studies exclusively assessed scalp cooling in a population of Black/African American subjects but contradictory results in reference to hair type were reported. The other three studies included Black/African American subjects as part of their patient demographic; however, no comparisons were made on the efficacy of scalp cooling amongst different hair types or races. The psychological repercussion of hair loss and cultural regard for hair care, compounded with the emotional burden taken on by cancer patients make hair management a crucial part of the treatment plan. In order to better serve a diverse patient population, more studies need to be done on scalp cooling in Afro-type hair to discover potential treatment nuances and to help patients maintain a sense of identity throughout chemotherapy.

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Racial disparities in melanoma: Subtypes, stage at diagnosis, and mortality in the U.S.

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While melanoma is relatively rare among people of color, Black patients with melanoma have higher mortality than White patients. It remained unclear what subtypes of melanoma and stage at diagnosis are associated with increased mortality in Black patients. To investigate racial disparities in melanoma between White and Black populations, we used the Surveillance, Epidemiology, and End Results (SEER 18) database and focused on non-Medicaid insured patients (excluding uninsured and Medicaid patients) to minimize the impact of suboptimal insurance on patient outcomes. Between 2007 and 2016, 46,349 White and 202 Black non-Medicaid insured patients were diagnosed with invasive melanoma. Relative to White patients, Black patients had higher odds of being diagnosed with regional melanoma (odds ratio (OR) 2.88; 95% confidence interval (CI) 2.06−3.95) and distant melanoma (OR 6.82; 95% CI 4.03−10.8). Also, Black patients had higher odds of being diagnosed with nodular melanoma (OR 1.50; 95% CI 1.07−2.06) and acral lentiginous melanoma (OR 35.4; 95% CI 26.5−47.2). Compared to White patients, we found increased risks of mortality in Black patients with localized nodular melanoma (hazard ratio (HR) 3.23; 95% CI 1.21−8.66) and in Black patients with localized acral lentiginous melanoma (HR 2.60; 95% CI 1.25−5.39). These findings suggest that Black patients with melanoma have more advanced stage at time of diagnosis, more aggressive subtypes, and increased risk of mortality in localized nodular melanoma and localized acral lentiginous melanoma. This study revealed that higher risk of mortality in Black patients with melanoma is associated with specific subtypes and stage.

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Racial/ethnic diversity in U.S. clinical trials for acne, atopic dermatitis, and psoriasis

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Participant diversity in clinical trials is inadequate across multiple medical conditions. Literature on the diversity of dermatologic clinical trials remains limited. We aimed to describe the reporting and racial/ethnic distribution of U.S. participants in clinical trials for common chronic inflammatory skin diseases. We performed a review of acne, atopic dermatitis (AD), and psoriasis clinical trials published between 01/01/2014 and 07/03/2019. Literature search of the PubMed, Scopus, EMBASE and Web of Science databases identified 9,272 acne, AD, and psoriasis publications of which 110 publications representing 120 unique trials met our study eligibility criteria. Data analysis was limited to 17 acne, 10 AD, and 34 psoriasis trials for which U.S.-specific participant data were available. Race and ethnicity data were extracted and summarized by skin condition using descriptive statistics. Phase 3 trials represented 24%, 50%, and 71% of the acne, AD, and psoriasis trials, respectively. Race and ethnicity were reported in 82% and 33%, respectively, of all trials. Weighted percentages of non-Hispanic White participants in acne, AD, and psoriasis trials were 76%, 63%, and 85%, respectively. Our data show continued under-reporting of ethnicity, in particular, in publications of dermatologic clinical trials. Additionally, compared to the overall U.S. population, of which 60-62% were non-Hispanic Whites between 2014-2019, we found over-representation of non-Hispanic Whites in acne and psoriasis trials. Even among AD trials, in which the proportion of non-Hispanic Whites approximated that of the U.S. population, absolute numbers (median [interquartile range]) of minority participants remained low (Black 40 [24, 45], Asian 22 [12, 25], and Hispanic 20 [19, 109]) and limit the ability to perform subgroup analyses by race or ethnicity within a single trial. Efforts to increase the reporting of race/ethnicity and ensure adequate representation of racial/ethnic minorities in dermatologic clinical trials are needed.

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Patient-dermatologist racial/ethnic and gender concordance are associated with higher Press Ganey scores

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Racial/ethnic and gender concordance between patients and physicians is suggested to contribute to better patient-physician relationships; this has not been well studied in dermatology. We aimed to evaluate the associations between patient-dermatologist racial/ethnic or gender concordance and the patient experience as measured by the Press Ganey (PG) Outpatient Medical Practice Survey. A cross-sectional analysis of PG surveys returned for adult outpatient dermatology visits within the University of Pennsylvania Health System between 2014 and 2017 was performed. The primary outcome was receipt of the maximum score for the "likelihood of your recommending this care provider to others" question in the PG survey. The primary exposures were patient-dermatologist racial/ethnic or gender concordance. Generalized estimating equations clustering on physicians with exchangeable intracluster correlations and cluster-robust standard errors were used to evaluate the associations between each primary exposure and outcome. We evaluated 16,289 surveys corresponding to 15,172 unique patients (mean [standard deviation, SD] age 57 [16] years, 41% men, 89% White) and 45 unique dermatologists (mean [SD] age 44 [11] years, 54% men, 76% White). Among racially/ethnically concordant vs. discordant visits, mean (SD) PG scores were 4.83 (0.56) vs. 4.76 (0.68), respectively (p<0.001). In adjusted analyses, racial/ethnic discordance was associated with lower odds of dermatologists receiving the maximum score (adjusted odds ratio [aOR] 0.89; 95% confidence interval [CI] 0.69-0.94). Among gender concordant vs. discordant visits, mean (SD) PG scores were 4.82 (0.58) vs. 4.79 (0.63), respectively (p=0.002). In adjusted analyses, male dermatologists were less likely to receive the maximum score from female vs. male patients (aOR 0.55; 95% CI 0.35-0.86). Our results suggest gender differences in the experience of care provided by male dermatologists and support the need for increased racial/ethnic diversity of the dermatology workforce and the delivery of care in a culturally mindful manner.

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Racial/ethnic differences in quality-of-life among adults with atopic dermatitis

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The Dermatology Life Quality Index (DLQI) is commonly used to measure health-related quality-of-life (QoL) in adults with skin diseases. Among patients with psoriasis, racial/ethnic minority patients were found to report higher DLQI scores, indicating greater QoL impact, than Non-Hispanic (NH) White patients. We aimed to determine whether DLQI scores differ by race/ethnicity among adults with atopic dermatitis (AD). We performed a cross-sectional study using data from the Atopic Dermatitis in America online survey. The study included adults who met age-modified United Kingdom Working Party Criteria for AD. The primary outcome was continuous DLQI score. Racial/ethnic categories were NH White [reference], NH Black, Hispanic, and NH other. Multivariable linear regression was performed to evaluate the association between race/ethnicity and logarithmically transformed DLQI scores. Effect modification by level of depression or anxiety symptoms based on the Hospital Anxiety and Depression Scale (HADS) score ($\leq$8 [low] vs. >8 [high]) was identified. Sample weights were applied for all analyses. The study sample included 672 adults. Median (interquartile range [IQR]) age was 43 (30-58) years; 61% were women. Racial/ethnic distribution was 56% NH White, 15% NH Black, 17% Hispanic, and 12% NH other. Median (IQR) Patient-Oriented SCORing Atopic Dermatitis index was 24 (13-35) indicating mild AD severity. Median (IQR) DLQI scores by race/ethnicity were: NH White, 1.2 (0.3-4.4); NH Black, 1.7 (0.2-9.1); Hispanic, 1.6 (0.5-6.2); and NH other, 1.0 (0.3-2.9). In adjusted analyses, among those with HADS score>8, NH Black adults reported higher DLQI scores than NH White adults (β coefficient 0.56; 95% confidence interval, 0.29-0.83). Among U.S. adults with AD and greater symptoms of anxiety or depression, DLQI scores were 75% higher among NH Black vs. NH White adults. Clinicians should recognize racial/ethnic differences in the QoL impact of AD, especially among those with anxiety/depression.

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Progress on the development of a Keloid Area and Severity Index (KASI) to aid in evaluation of keloids in clinical and research settings

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While the field of dermatology has many condition-specific area and severity indices, such as the PASI (Psoriasis Area and Severity Index), the EASI (Eczema Area and Severity Index), and the MASI (Melasma Area and Severity Index), there is no such metric to quantify the disease status of keloids. More commonly occurring in those of African, Asian, and Hispanic backgrounds, keloids result due to an exaggerated wound healing response. Due to a lack of standardized parameters in the literature, it is difficult to objectively compare results of clinical trials involving keloid treatment. It is similarly difficult to document the clinical progression of keloids. Using patient feedback, previous literature, and clinical expertise, the keloid area and severity index (KASI) was developed. It includes both an activity score and a damage score, with contribution from lesion characteristics of erythema, suppuration/crust, elevation, and percent body surface area covered. A group of 6 dermatologists and dermatology resident were trained virtually with a PowerPoint presentation detailing use of the KASI. 20 sets of patient photographs were identified and rated with the proposed index by this group of raters twice, about 1 week apart. To determine the validity of the proposed index, analysis is currently being performed to establish the level of intra-rater and inter-rater reliability.

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Using the CLASI score to categorize and identify risk factors for disease activity and damage in CLE

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Classifying scoring measures for CLE provides a standardized way to categorize disease. Prior studies have been done on small, racially homogenous cohorts to classify CLASI activity scores (CLASI-A) into mild, moderate, and severe categories. However, this classification has not been validated in larger cohorts involving patients of color. Additionally, CLASI damage scores (CLASI-D) have not yet been similarly classified. The objective of our study is twofold: to use a large, racially diverse cohort to classify CLASI-A into severity categories and compare to prior studies, and classify CLASI-D. 270 patients were included in this cross-sectional study. Physician global assessment (PGA) ratings were used to assign patients as having mild, moderate, or severe disease activity and damage, which were then analyzed with CLASI scores. Cutoff points were selected as the last of three consecutive CLASI scores with the highest frequency in the same category. Receiver operating characteristic curves were used to evaluate ranges. Patient characteristics were compared via Chi-Square. The majority of participants had skin of color (69%). Mild activity corresponded with CLASI-A $\leq$ 6 (sensitivity 96%, specificity 68%, correctly classified (CC) 80%) while severe activity corresponded with CLASI-A $\geq$ 15 (sensitivity 72%, specificity 96%, CC 70%). These cutoffs performed better than previously published ones. Mild damage corresponded with CLASI-D $\leq$ 5 (sensitivity 86%, specificity 96%, CC 95%), and severe damage with CLASI-D $\geq$ 17 (sensitivity 65%, specificity 90%, CC 56%). Black race was associated with worse disease damage than non-black patients in all activity levels ($p<0.0001$), and was a risk factor for severe disease damage. Limitations include single center study with one rater. We propose that these new cutoffs for CLASI classification levels be considered for clinical trial use and to provide vital clinical context for patients and providers.

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Epidermal remodeling and immunogenicity within sinus tracts in hidradenitis suppurativa at the single-cell resolution

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Hidradenitis suppurativa (HS) is a severe chronic inflammatory skin disease affecting human apocrine sweat gland-bearing skin regions. The overall prevalence of HS ranges from 0.05-4.1% with higher occurrence among females and African Americans, and strong associations with smoking and obesity. One unique feature of HS is the development of highly immunogenic keratinized sinus tracts that grow deeply in the dermis which further complicate HS pathogenesis and treatment. Using single cell transcriptomic analyses, we finely dissected different epidermal cell types in the HS lesional skin and revealed significant dysregulation of skin barrier function in the sinus tracts. We demonstrated that sinus tract keratinocytes exhibit dual cell fates of surface epidermis and skin appendages, and derived from progenitors in infundibulum of the apocrine-pilosebaceous unit. By analyzing ligand-receptor expressions between different skin appendages and immune cells, we highlighted Th17 and TNF responses at early and late stages during HS progression, respectively. Our work provides unprecedented understanding of pathological epidermal remodeling in human inflammatory diseases and important implications for therapeutics.

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Skin transcriptomic analysis reveals fibroproliferative and microvasculopathic signatures in African Americans with diffuse systemic sclerosis

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Systemic sclerosis (SSc) is an autoimmune disease affecting multiple target organs including the skin. Studies have observed that African American (AA) patients have greater skin disease severity. However, the immune mechanisms that dictate the propensity for SSc severity in AA patient skin are unclear. To explore the racial heterogeneity of early diffuse SSc (dSSc) immune pathology, we analyzed skin transcriptomes obtained in the Prospective Registry for Early System Sclerosis using *in silico* computational methods. We used Gene Set Variation Analysis (GSVA) to find enriched pathways previously implicated in dSSc and xCell to infer the specific cell types involved. Lesional biopsies from 6 white and 6 AA dSSc patients with similar distributions of age, sex, biopsy site modified Rodnan skin score, and disease duration were selected and compared to nonlesional biopsies from 4 white and 4 AA healthy controls, respectively. Using GSVA, dendritic cell cytokine production, interferon receptor activation, CD8+ T cell activation, B cell receptor signaling, and Th1/Th17 responses were upregulated in white patients, whereas TGF-β receptor activity, extracellular matrix assembly, VEGF receptor activity, platelet aggregation, and blood coagulation were upregulated in AA patients ($P<0.05$ for all pathways). Furthermore, xCell analysis revealed increased fibroblasts and microvascular endothelial cells in AA patients ($P<0.05$ for all comparisons), but not in white patients. Together, these results uncovered an inflammatory signature in white patients, and a fibroproliferative and microvasculopathic signature in AA patients, highlighting the racial heterogeneity in SSc. These findings may partially explain the increased disease severity of SSc in AA patients as there are fewer treatments for dSSc that target these processes, underscoring the need to develop such novel therapeutics.

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Differences in discoid lupus distribution and characteristics in black and non-black patients

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Epidemiological studies have shown that discoid lupus erythematosus (DLE) has a higher incidence and prevalence in minorities, particularly Black individuals. Racial differences in clinical features amongst DLE patients are not well understood. The objective of this retrospective cohort study was to examine the differences in DLE lesion distribution and characteristics of Black individuals compared to non-Black individuals. All visit data was analyzed. A total of 183 DLE patients (112 Black and 71 non-Black) who had a reported race/ethnicity and completed Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI) scores were included. Univariate analysis was used to determine significant clinical factors between groups. Blacks had worse overall CLASI damage scores (median: 10.0, interquartile range: 6.0-14.5) v. non-Blacks (median: 6.0, interquartile range: 3.0-10.0, $p=<0.001$). Black DLE patients trended toward more scalp involvement (87% v. 72%, $p=0.013$). Of patients with scalp involvement, black individuals were more likely to have dyspigmentation (95% v. 63%, $p<0.001$). Black DLE patients less frequently had scale in the face, excluding malar area (37% v. 65%, $p=0.006$). Among those with arm involvement, dyspigmentation was more likely in Black individuals (100% v. 80%, $p=0.006$). Black DLE patients have important lesion location and characteristic differences compared to non-Black DLE patients. Specifically, Black DLE patients had greater disease damage and were more likely to have dyspigmentation of the scalp and arms. This study provides important information on clinical differences in Blacks and non-Blacks with DLE and emphasizes that disease damage may be more of a significant issue in Black DLE patients. Larger studies are needed to confirm our findings.

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CK2 inhibition synergizes with MAPK inhibition to overcome resistance in acral melanoma

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Acral melanoma (AM) occurs on the palms, soles and subungual sites and occurs in a much higher proportion in darker-skinned individuals compared to cutaneous melanoma. AM is the most common subtype of melanoma in patients of African and Asian descent. AM is associated with activation mutations in the KIT gene and/or inactivating mutations in NF1 (neurofibromin1). These tumors respond poorly to currently available targeted therapies and immune checkpoint inhibition, underscoring the urgent need for treatment alternatives that improve overall survival of patients with metastatic disease. The protein kinase CK2 (casein kinase II) is upregulated in several types of cancer, including melanoma, and especially in AM. CK2 overexpression contributes to maintenance of the malignant phenotype and is associated with resistance to targeted therapies. Here, we investigated the potential of CK2 inhibition to overcome resistance to MEK inhibitors (MEKi) in preclinical models of AM. We found that *in vitro* treatment of NF1-null human melanoma cells with a small-molecule CK2 inhibitor (CX4945, silmitasertib) resulted in inhibition of cell proliferation. This was accompanied by downregulation of PI3K and ERK-MAPK signaling, as demonstrated by dephosphorylation of AKT (S129 and S473) and ERK (p44/42 MAPK; Thr202/Tyr204). CX4945 also showed a synergistic anti-proliferative effect with the MEKi trametinib in MTS viability assays, and an increase in apoptosis. Further, in colony formation assays, CX4945 significantly reduced the appearance of trametinib resistant colonies. To translate these findings, we treated NF1-null human melanoma cells grown as xenografts in nude mice with CX4945, and noted a significant reduction in tumor growth that resulted in stable disease. Together, our results suggest that the combination of CK2 inhibitors with MEK inhibitors, and potentially other tyrosine kinase inhibitors, may improve therapeutic outcomes in difficult to treat malignant melanomas of acral origin.

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Transcriptome analysis reveals intrinsic pro-inflammatory signaling in healthy African American skin

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Differences in morphology and physiology of darkly pigmented compared to lightly pigmented skin are well recognized. There are also disparities in prevalence and clinical features for many inflammatory skin diseases including atopic dermatitis (AD) and psoriasis; however, the underlying mechanisms are largely unknown. We compared the baseline gene expression in full thickness skin biopsies from healthy individuals with Fitzpatrick skin type V-VI or I-II, and self-reported as either African American (AA) or White Non-Hispanic (WNH). Extensively validated RNA-Seq analysis identified 670 differentially expressed genes (DEG) in AA skin including immunoglobulins (Igs) and their receptors such as FCER1G; pro-inflammatory genes such as TNFα, IL-32; EDC (epidermal differentiation cluster) and keratin genes. DEGs were functionally enriched for inflammatory responses, keratinization/cornified envelope formation. Analysis of putative transcription factors involved in AA DEG regulation revealed pro-inflammatory NF-kB, regulator of Igs OCT1, and KLF4 important for skin barrier development. RNA-Seq analysis of 3D human skin equivalents (3D HSE) made from AA and WNH epidermal keratinocytes revealed more than 300 DEGs enriched by similar functions as in skin. AA 3D HSE were more responsive to TNFα pro-inflammatory effects. Most genes induced by 24h TNFα treatment in AA 3D HSE were functionally enriched for inflammation, whereas a majority of DEGs were linked to GSEA categories related to ECM and collagen fibers organization/degradation and MMP activation in CA samples. Finally, AA-specific DEGs in skin and 3D HSE significantly overlapped with molecular signatures of pre-lesional and even lesional skin in AD and psoriasis patients. Overall, these findings suggest the existence of intrinsic pro-inflammatory circuits in AA keratinocytes/skin that may account for disease disparities and will help to build a foundation for the development of targeted skin disease prevention approaches.

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A genome-wide association study in an African American cohort implicates IL-12A in acne

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African Americans have not been included in any acne genome-wide association study (GWAS), despite a higher risk for disfiguring post-inflammatory sequelae in this population. Using data from the eMERGE network of electronic health record linked biorepositories (project NT227) we identified a case-control cohort with genetically defined African ancestry, tested 6,859,180 genetic variants for association with acne, and identified a novel, significantly associated, ancestry-specific locus at chromosome 3q25.33, indexed by rs2243130 ($P=4.5x10^{-9}$, odds ratio (OR)=2.27, 95%CI=2.00-2.55), that contains a single protein coding gene, *interleukin-12A* (*IL-12A*). Risk alleles are associated with decreased gene expression levels of *IL12-A*. *In silico* functional prediction algorithms identified regulatory mechanisms for two SNPs, (rs2243133 ($P=1.0x10^{-8}$) and rs16830960 ($P=1.9x10^{-8}$). *IL12-A* encodes IL-12p35, a member of the IL-12 cytokine family with potent roles in regulating immune responses within the context of inflammatory disorders, autoimmunity, infection, and cancer. Decreased levels of IL12p35 are associated with increased symptom severity for several immune mediated diseases. Ustekinumab targets the IL-12/IL-23 pathway and has been shown to improve acne. While our results require replication in an independent cohort, they implicate a gene with strong biological plausibility and clinical relevance. Our work supports the existence of differences in the genetic architecture of acne across ancestries and suggests that the inclusion of participants with African genetic ancestry in future acne studies is imperative.

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Morphea in patients of color: A cross sectional study of the morphea in adults and children (MAC) cohort

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Morphea is an autoimmune sclerosing skin condition. While clinical manifestations of morphea are well described in Caucasians, characteristics of morphea in patients of color have been poorly studied. Thus, the aim of this study was to determine the demographic and clinical characteristics of morphea in patients of color. This was an analysis of patients in the Morphea in Adults and Children Registry from 2007 to 2020. Kruskal-Wallis, Chi-square and Fischer's exact tests were performed to investigate differences between racial groups. Post-hoc analysis was performed with Dunn's test to directly compare individual racial groups. Of 772 patients in the registry, the majority had linear morphea (47%), and were white (73%). Overall, patients of color comprised 27% of the entire cohort, with 5% Black, 4% Asian, 14% Hispanic, and 4% Other. Age of morphea onset showed significant differences between racial groups (p=0.004). Black patients were more likely to have a later disease onset compared to non-Black patients and were less likely to have linear morphea (p=0.005). Asian and Hispanic patients were found to have similar subtype distribution to white patients. Years to diagnosis from disease onset also varied between racial groups (p=0.0058). Black and Hispanic morphea patients had a delayed diagnosis compared to White morphea patients [2.85 and 2.73 years in Black (p=0.015) and Hispanic (p=0.011) patients respectively vs 2.08 years in white patients]. Hispanic patients had higher disease damage by PGA-D, when compared to compared to White patients (Median: 30, IQR: 20-50 v. Median: 20, IQR: 10-40, p=<0.001). Our study demonstrates that morphea is infrequent in patients with skin of color, but still occurs in a substantial number of patients. We underscore the need for dermatologists to carefully consider distinct disease characteristics in patients of color, especially given the delay in diagnosis time experienced by Black and Hispanic patients compared to White patients.

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Ethnic differences in acral melanoma epidemiology: A multi-institutional cohort study

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Acral lentiginous melanoma (ALM) disproportionately affects patients of color and carries a poor prognosis. This retrospective analysis aims to provide an institutional examination of the incidence and clinical patterns of acral melanoma, specifically assessing for ethnic and racial differences. Data was extracted from the Epic electronic medical record using the Registered Patient Data Repository at Mass General Brigham Healthcare. Presence and stage of ALM was validated via an independent manual curation of patient medical records. Patients were evaluated across four sets of variables: (1) demographic information; (2) clinical prognostic measures (tumor location, laterality, stage at diagnosis, length of follow-up); (3) patterns of therapy utilization; and (4) mortality. Racial and ethnic categories were defined by the U.S. Office of Management and Budget standards on race and ethnicity. To assess for the impact on skin of color (SOC), race and ethnicity were further categorized into white and SOC using criteria defined by Taylor et al. Statistical analyses were performed using SAS version 9.4. A total of 374 patients with acral melanoma were diagnosed at Massachusetts General Brigham between 1/1/1980-12/31/2020, of whom 90.4% (n=338) were white and 9.6% (n=36) were SOC. Compared to whites, SOC patients with acral melanoma were likely to present at a similar stage, but were more commonly female and older. All racial groups presented predominantly with left-sided tumors. However, the most frequent location differed by ethnicity (left foot for SOC and right foot for whites). SOC patients had lower overall rates of medical management when compared to white patients (19% vs 23%). This disparity was driven by differences in use of immunotherapy. SOC patients had higher mortality, less follow-up and lower rates of insurance coverage. Our data provides additional information on racial and ethnic differences in acral melanoma. Future research is needed to further assess the implication of these disparities on clinical outcomes.

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Racial and ethnic differences in cutaneous immune-related adverse events and outcomes

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Immune checkpoint inhibitors (ICIs) have revolutionized cancer therapy, but lead to significant toxicities, with cutaneous toxicities (cirAEs) most commonly reported. However, the data on racial and ethnic incidence of cirAEs is limited. Using electronic medical record data from 41 US medical centers, we examined the influence of race and ethnicity on cirAE incidence and outcomes. We identified 33,297 patients receiving ICI treatment (69.1% non-Hispanic white, 7.0% non-Hispanic black, 1.9% Asian, and 4.1% Hispanic). The overall cirAE incidence was 21.5%. Nonspecific rashes (12.2%), drug eruptions (4.6%), and pruritus (6.4%) were the most common diagnoses. Compared to white patients, black patients had a lower risk of cirAEs (RR=0.65, p <0.0001), and Asian patients had a higher risk (RR=1.41, p<0.01), after controlling for age, sex, and cancer type. Asian (RR=0.66) and Hispanic (RR=0.80) patients had a lower risk of mortality following ICI therapy initiation (both p<0.0001). However, despite their overall lower rate of cirAEs, black patients had overall similar mortality to white patients (RR=0.96, p=0.29). A sensitivity analysis exploring the risk of cutaneous diagnoses in the non-ICI cancer setting showed similar rates of dermatoses among Asian (RR=0.96, p=0.07) patients, but lower rates among black and Hispanic patients (RR=0.83 and 0.87, respectively, both p<0.0001) compared to white patients. This finding suggests that the increased incidence of cirAEs among Asian patients is related to ICIs, but that the decreased rate seen among black patients may be due to poorer identification of these conditions in skin of color, requiring investigation into addressable biases. Future studies should also explore specific contributions of genetic ancestry and germline genetic variation to the observed differences in incidence.

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A deep learning classifier for the analysis of tiger tail banding in trichothiodystrophy

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Trichothiodystrophy (TTD) and xeroderma pigmentosum (XP) are both rare, autosomal recessive disorders of DNA repair/ transcription genes. Despite having (different) mutations in the same genes, they have markedly different phenotypes. TTD patients have multisystem developmental abnormalities involving fetal growth, and brain, eyes, bones and immune system with normal cancer risk. XP patients have normal development and a greatly increased risk of sunlight induced skin cancer. The hallmark of TTD hair is an alternating dark and light "tiger tail" banding pattern under polarized light microscopy along with defects of the hair shaft and reduced content of sulfur containing amino acids. XP patients have normal hair. Some XP/TTD complex patients may have features of both disorders making the diagnosis and evaluation of cancer risk difficult. Here we present a U-Net convolutional neural network for segmentation and classification of tiger tail hair and normal hair images from polarized light microscopy. The model was trained with 35 TTD and 35 normal hair images. The dataset was augmented using standard crops, flips, and rotations. Shears were excluded to preserve the banded features. In future studies, a third class consisting of hair images from XP/TTD complex patients will be introduced to the U-Net to evaluate the differences in banding patterns. Use of artificial intelligence deep learning may be able to assist in diagnosis of patients with different types of hair abnormalities.

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An extract of *Leontopodium alpinum* prolongs anagen phase in human hair follicles *ex vivo*

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Aging and hair follicle disorders, such as telogen effluvium or androgenetic alopecia, lead to premature catagen induction and decreased hair density. Hair loss is an aesthetic problem that may cause major psychological distress in affected individuals. While severe types of hair loss are targeted by the pharmaceutical industry, with drugs exhibiting unwanted side effects, the cosmetic industry is looking for milder, and natural treatment options. Bearing this in mind, we focused on an extract of *Leontopodium alpinum* (Edelweiss) and investigated its effects on hair growth *ex vivo*. We treated microdissected anagen VI scalp hair follicles (HFs) from three individuals (two female, one male) with 0.001% edelweiss extract and evaluated hair growth-associated parameters. After 5 days of treatment, we observed significantly more HFs remaining in anagen, and increased hair matrix keratinocyte proliferation. On a molecular level, higher activity of alkaline phosphatase, and up-regulated expression of versican was observed in the dermal papilla of female treated HFs, suggesting improvement of dermal papilla inductivity. In addition, the expression of hair growth-associated growth factors, i.e. IGF-1 or FGF7, was found to be up-regulated in the outer root sheath, and/or dermal papilla of female hair follicles. Our *ex vivo* results provide preliminary evidence for a beneficial effect of the *Leontopodium alpinum* extract in limiting hair shedding *in vivo*.

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A systematic review of autologous adipose-derived stromal vascular fraction (SVF) for the treatment of noncicatricial alopecia

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SIGNIFICANCE: Stromal vascular fraction (SVF) isolated from adipose tissue includes stem cells, growth factors, and other cellular components. Its regenerative potential and ease of procurement make SVF a potentially useful treatment for alopecia. AIM: To evaluate evidence on the efficacy and safety of autologous adipose-derived SVF for treatment of noncicatricial alopecia. METHODS: A systematic review of the literature was conducted utilizing MEDLINE, Embase, and the Cochrane Central Register of Controlled Trials from inception to November 2020 for interventional and observational studies of SVF or similar adipose-derived stem cell (ADSC)-containing derivatives for patients with noncicatricial alopecia. Ongoing clinical trials were identified through ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform. FINDINGS: Of 4131 records, and 14 met inclusion criteria: 8 completed studies, including 3 randomized controlled trials and 5 case series or case reports, and 6 ongoing clinical trials. Collectively, 196 patients were treated in the 8 completed studies, and there were no reports of serious adverse events (AEs). Pain, headache, bruising and edema were the most frequently reported AEs. 7 studies reported increased hair density for patients treated with SVF, with mean change from baseline of 2.78 to 32.4 hairs/cm^2. In 6 studies, this increase in hair density was statistically significant, though in some studies it was dependent on severity of alopecia and time post-treatment, suggesting that individual patient characteristics and treatment frequency may be important considerations. Ongoing clinical trials are assessing outcomes such as safety, hair density, and patient satisfaction. CONCLUSION: While the quantity and quality of evidence is limited, SVF may be an effective and safe treatment for alopecia. More clinical trials evaluating safety as well as optimal patient characteristics for treatment are needed.

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Effects of UVA irradiation on the stemness and Opsin Expression of human amniotic mesenchymal stem cells *in vitro*

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Human amniotic mesenchymal stem cells amplified in vitro can be used for a variety of tissue and organ regeneration , and also has the potential to treat a variety of diseases. However MSCs cultured in vitro for a long time will weaken their self-renewal and multi differentiation ability . After long-term culture, MSCs became larger and flattened, and lost the ability to divide. Therefore, in vitro culture can reduce the pluripotency of MSCs. We chose UVA Irradiation to investigate the effect of OPN on the stemness of hAMSCs. Methods: hAMSCs were seeded onto cell Culture Dishes and irradiated with Ultraviolet A light at 0.25 J/cm2, 0.5 J/cm2, 5J/cm2, 10J/cm2, 20J/cm2 or not irradiated, The cell lysates were collected for further research 0, 12h, 24h , 48h, 72h after UVA irradiation. CCK-8 assay was used to measure cell viability, and apoptosis was evaluated by AnnexinV/PI, senescent cells were stained with a b-galactosidase staining kit. Expression of opsins and Sox2, OCT4, HIF-1α were evaluated by Western blotting.In this study, We found that 5J/cm2 irradiation with UVA induced no marked decrease of live cells and dead cells after 72H, Additionally, no remarkably increase of cell apoptosis and senescent cells were observed in UVA-irradiated cells as compared with non-irradiated control group with 5 J / cm2 . Western Blot to detect the expression of opsins showed that with the prolonged irradiation time, the expression of OPN3 did not increase significantly, The expression of OPN5 gradually increased with time, and the expression was significant at 72h, showing a time-dependent manner. Increased OCT4 and Sox2 that resulted from UVA irradiation at 72H were detected more than in control cultures. These results indicated that UVA irradiation improve OPN5 expression as well as hAMSCs stemness of OCT4 and Sox2.

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Genetic ablation of autoimmune regulator (Aire) results in spontaneous alopecia

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Autoimmune regulator, Aire, is a transcriptional regulator best known for eliminating autoreactive T cells in the thymus. Aire is also expressed in follicular keratinocytes, and patients with loss-of-function mutations in *AIRE* are 15x more likely to develop alopecia areata (AA) than the general population. We report here that adult C57BL/6J female mice genetically null for Aire (*Aire*$^{-/-}$) spontaneously developed patchy non-scarring hair loss as early as three months of age that macroscopically resembled AA (n=35/73; 48%). Our data indicate this is a novel mouse model of AA. Histopathology of alopecic *Aire*$^{-/-}$ skin, relative to age- and body site-matched *Aire*$^{+/+}$ skin, revealed several AA hallmarks including miniaturized hair follicles (HFs), decreased hair shaft diameter (55.1% reduction, p<0.0001), and increased inflammatory infiltrate at the anagen hair bulb (191% increase in perifollicular CD8+ T cell density, p=0.0546; 61% increase in mast cell density, p=0.0004). It is widely accepted that anagen HFs are targeted by autoreactive T cells in AA due to a collapse in HF immune privilege (IP), a phenomenon characterized in part by abnormal overexpression of major histocompatibility complex proteins (MHC class I and II) and upregulation of Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling. Using RT-qPCR we observed a 3-fold and 3.2-fold increase in MHC class I and MHC class II expression, respectively, as well as a 15.6-fold increase in interferon-γ expression and an upregulation of JAK-STAT signaling components in *Aire*$^{-/-}$ lesions (n=7) compared to controls (n=3). Additionally, immunohistology indicated significant upregulation of MHC class I expression along anagen HFs in *Aire*$^{-/-}$ lesions (n=7) relative to controls (n=4). This discovery provides a basis for why AA is more prevalent in patients with *AIRE* mutations, is likely to offer novel insight into AA etiology, and highlights a new role for Aire in HF biology and IP regulation.

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miR-184 represses stemness and behaves as a tumor suppressor in the epidermis

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Cutaneous squamous cell carcinoma (SCC) is the second most common form of cancer. It is 18-20 times more prevalent than melanoma. Most SCC can be treated locally and are curable, but metastatic cases have grim survival rates. Therefore, there still a need for further understanding of the molecular pathways that control SCC and cancer stem-cells. Micro ribonucleic acids (miRNAs) are small non-coding RNAs that regulate gene expression. miRNAs play a role in various physiological processes including cancer. Here we focused on miR-184, a highly evolutionary conserved microRNA that controls corneal epithelial stem cell phenotype. We show that knock-down of miR-184 in mice results in epidermal thickening and that miR-184 induced epidermal stem cells (SC) differentiation through Notch activation. In line, we report that loss of miR-184 enhanced epidermal stemness phenotype and predisposition to skin cancer in a two-stage carcinogenesis (TPA-DMBA) model. Supporting these results, the level of miR-184 was lower in mice papilloma compared to adjacent murine skin and in human SCC cell lines compared to human skin. RNAseq analysis showed that miR-184 inhibited proliferation and cytokine-mediated signaling pathways. Moreover, knockdown of miR-184 in squamous cell carcinoma (SCC) cells and increased SC-related genes (e.g., p63, K14, Sox2) , enhanced colony formation of SCC cells in culture as well as xenograft tumor formation in nude mice. Altogether, this study indicates that miR-184 represses stemness, and behaves as a tumor suppressor in the epidermis.

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Hair growth stimulation effects of b-catenin stimulating peptides through DKK-1 inhibition

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Alopecia is a common skin disorder known to be induced by both extrinsic factors including oxidative stress, exposure to excessive heat and pollution, and intrinsic factors such as inflammation and genetic disposition. Through the hair growth cycles, hair grows in anagen phase and stops growth in catagen phase, then enters telogen phase of resting stage. Based on hair growth cycle, increasing the hair follicles in the anagen stage and decreasing the follicles in catagen and telogen stage is commonly adopted strategy for treating hair loss or alopecia. Wnt/β-catenin signaling pathway is known to be importantly involved in hair follicle morphogenesis, and stimulation of β-catenin signaling is known to induce an anagen phase. Wnt/β-catenin inhibitor Dickkopf (DKK)-1 binds to low-density lipoprotein receptor-related proteins 5 and 6 (LRP5/6), and binding of DKK1 results in phosphorylation of β-catenin and inactivation. In order to develop new anti-hair loss ingredients, new peptide molecules based of LRP5/6 sequence were synthesized and their activity was explored. Using cultured human dermal papillar (hDP) cells, changes of β-catenin signaling molecules and expressions of hair cycle-related growth factors was were measured. As results, several new peptides were identified as having a significant stimulating activity on β-catenin signaling and growth factor expressions as well, at least in part, through the DKK-1 mediated signaling pathway. In hair follicle organ culture model, selected peptide showed a hair shaft elongation activity. In conclusion, these results suggest that Wnt/β-catenin/DKK-1 signaling is important for hair cycle modulation and DKK-1 inhibiting peptide can be used for hair growth through β-catenin signaling stimulation.

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Hair histology and glycosaminoglycans distribution probed by infrared spectral imaging: Focus on heparan sulfate proteoglycan and glypican-1 during hair growth cycle

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Heparan sulfate proteoglycans (HSPGs) distribution in hair follicle (HF) is classically investigated by conventional histology, biochemical analysis, and immunohistochemistry. In this study, a novel approach is proposed to assess hair histology and HSPG distribution changes in the HF at different phases of the hair growth cycle using infrared spectral imaging (IRSI). Particularly, we were interested in the expression of glypican-1 in different HF compartments and their potential roles during hair shaft growth, as their roles are still poorly understood. The distribution of HSPGs in HFs was probed by IRSI using the absorption region relevant to sulfation as a spectral marker. Findings were supported by Western immunoblotting and immunohistochemistry assays focusing on the glypican-1 expression and distribution in HFs. This study demonstrates the capacity of IRSI to identify the different HF tissue structures and to highlight protein, proteoglycan (PG), glycosaminoglycan (GAG), and sulfated GAG distribution in these structures. The comparison between anagen, catagen, and telogen phases shows the qualitative and/or quantitative evolution of GAGs, as supported by Western immunoblotting. Thus, IRSI can simultaneously reveal the location of proteins, PGs, GAGs and sulfated GAGs in HFs in a reagent- and label-free manner. From a dermatological point of view, IRSI shows its potential as a promising technique to study alopecia.

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Dermal EZH2 orchestrates dermal differentiation and epidermal proliferation during murine skin development

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Skin development and patterning is dependent on factors that regulate the stepwise differentiation of dermal fibroblasts concomitant with dermal-epidermal reciprocal signaling, two processes that are poorly understood. Here we show that dermal EZH2, the methyltransferase enzyme of the epigenetic Polycomb Repressive Complex 2 (PRC2), is a new coordinator of both these processes. Dermal EZH2 activity is present during dermal fibroblast differentiation and is required for spatially restricting Wnt/β-catenin signaling to reinforce dermal fibroblast cell fate. Later in development, dermal EZH2 regulates the differentiation to reticular dermal fibroblasts and initiation of secondary hair follicles. Embryos lacking dermal Ezh2 have elevated epidermal proliferation and differentiation that can be rescued by small molecule inhibition of retinoic acid (RA) signaling. Together, our study reveals that dermal EZH2 acts as a rheostat to control the levels of Wnt/β-catenin and RA signaling to impact fibroblast differentiation cell autonomously and epidermal keratinocyte development non-cell autonomously, respectively.

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Hair cycle regulation by a mitochondrially localized protein: Is MPZL3 a central component of the elusive hair cycle clock?

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Hair follicles (HFs) undergo cycles of growth (anagen), regression (catagen) and relative quiescence (telogen). The intrinsic and autonomous molecular oscillator system that drives the hair cycle (HC)—the "hair cycle clock (HCC)"—remains incompletely understood. In this study, we present evidence that Myelin Protein Zero-like 3 (MPZL3), a multifunctional nuclear-encoded protein localized in mitochondria and is primarily known to be involved in epidermal differentiation, also regulates the murine HCC. In the absence of functional MPZL3, *Mpzl3* global knockout mice commence HF cycling with retarded first catagen-telogen transition after normal postnatal HF morphogenesis. However, they then display strikingly accelerated HF cycling, i.e., a precocious telogen-anagen transition during the second HC, compared to controls, suggesting that MPZL3 normally functions as an inhibitor of anagen entry. We also show that intrafollicular MPZL3 protein expression oscillates in an HC-dependent manner. In telogen HFs, MPZL3 is localized to the secondary hair germ, an epicenter of HC regulation, partially co-localizing with P-cadherin, in contrast to the hair matrix localization during early-mid anagen. Intriguingly, keratin 14 promoter-driven Cre-mediated *Mpzl3* knockout mice recapitulate the precocious telogen-anagen transition during the second HC observed in global *Mpzl3* knockout mice, indicating that skin epithelia-derived, MPZL3-dependent signals dictate the HCC. These findings introduce the novel concepts that a) mitochondria are more actively involved in HC control than previously recognized in the context of energy metabolism, b) MPZL3 plays a central role in the elusive HCC, and c) targeting MPZL3 and/or its downstream effectors may constitute an innovative and effective strategy for therapeutic HC manipulation in alopecia treatment.

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Transcriptomic analysis to identify protective dermal papilla signature in occipital scalp

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Androgenetic alopecia (AGA) is defined by the miniaturization of hair follicles (HF) on the frontal (F) scalp, while occipital (O) HF are spared. Miniaturization is characterized by both thinning of the hair fiber and a decrease in cell number within the HF dermal papilla (DP). While previous research has looked at differences in HF stem cells between miniaturizing and growing HF in humans, to our knowledge no groups have looked at differences in the ex vivo DP between the F and O sites. Since the DP in FHF is affected by miniaturization, we hypothesized that a unique transcriptional signature in the ODP protects them from miniaturization. To investigate this, we carried out unbiased transcriptomic profiling of patient-matched DP and dermal sheath (DS) from FHF and OHF from 4 males undergoing hair transplantation surgery. By cross comparing, we identified 16 genes that are expressed at significantly higher levels in the ODP, compared to these 3 other dermal cell types. We believe this ODP signature protects DP from size reduction and cell loss during transition through the hair cycle, and thus protects OHF from miniaturization. Genes, previously identified as core DP genes, such as *HHIP* and *SOSTDC1* were present in the unique ODP signature. Previous research using a murine patch assay to assess inductivity has shown that cultured FDP are significantly less inductive than cultured ODP. To interrogate the roles of ODP signature genes and evaluate their protective role against AGA, we developed an animal-free model of inductivity for high throughput analysis. When ODP cells are combined with epidermal keratinocytes (KC), cells self-organize into organoid structures with an ODP core, surrounded by KC. The KC in this model expresses a hair differentiation marker, K75 at significantly higher levels compared to organoids with a non-follicular fibroblast core, suggesting DP-induced KC reprogramming. We are now using this model to assess how ODP signature genes contribute to the KC reprogramming ability of DP organoids.

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UBE2N is essential for normal epidermal homeostasis and controlling inflammation

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UBE2N, also named Ubc13, is a K63-Ub-specific E2 conjugase. UBE2N plays an essential role in immunity and embryonic tissue development and represents a potential therapeutic target for cancer and immunological disorders. However, its function in adult skin homeostasis is unknown. Here, we demonstrate that topically induced deletion of UBE2N in mouse skin cells resulted in apparent skin abnormalities, including hair growth defects, skin blistering, and subsequent thickening that resembled psoriatic plaques. The thickened and crusty epidermal growth phenotype was especially pronounced in male animals. Immuno-histological analyses showed that UBE2N null skin expressed high levels of K14 and differentiation markers, including involucrin, loricrin, and filaggrin, and had markedly increased Ki-67-positive cells. This was accompanied by increased pMEK1/2, pc-Myc, pc-Jun, and JunB and the loss of β1-integrin and collagen VII. Further immunostaining and flow cytometry analyses showed that UBE2N loss significantly increased skin infiltrations of CD3, CD4, and CD8+ T-cells, as well as F4/80 and CD11b+Gr1+ cells. Lastly, mutant animals developed systemic reactions including splenomegaly. Our findings indicate that UBE2N is essential for normal epidermal homeostasis and suppresses local and systemic inflammation. We are currently assessing the effects of keratinocyte-specific deletion of UBE2N on epidermal homeostasis and using an unbiased proteomic approach to determine UBE2N target proteins in keratinocytes.

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Senotherapeutic strategy to alleviate age related skin damages

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Cellular senescence is a hallmark of tissue's aging including the skin. The age-related accumulation of senescent cells contributes to tissue dysfunction. Senescent cells are characterized by a pro-fibrotic and pro-inflammatory senescence-associated secretory phenotype (SASP). Several therapeutic strategies were explored to target specifically senescent cells (SC) in order to alleviate their damaging effects. These strategies are based on the development of molecules able to either selectively eliminate the SC (senolytics) or suppress SASP secretion (senomorphics). In order to address these biological activities and select an efficient plant extract (AI) performing on both of them, we first developed and validated a relevant replicative- senescent model using dermal fibroblasts and characterized by assessing the b-galactosidase positive cells, p21 and inflammatory secretory phenotype. We demonstrate that the senolytic effect of the AI is mediated through the selective activation of caspase 3/PARP apoptotic pathway in SC without affecting viability of non-senescent cells. In addition, we found that the AI presents also senomorphic properties. Indeed, the AI mitigates the secretory phenotype of SC as confirmed by the dosage of three known SASPs factors IL-6, IL-8 and MMP-1. Interestingly, SC phenotype reversion was observed in presence of AI resulting from a markedly decrease of cell cycle arrest markers. Limiting the paracrine and deleterious effect of these SASPs on human keratinocytes and restoring collagen synthesis in senescent dermal fibroblasts clearly demonstrate the benefit of AI towards skin aging. Besides, AI delays the normal occurrence of skin senescent cells. The beneficial effect of the AI was confirmed at the clinical level on human aged skins. These results demonstrate that a global senotherapeutic approach is an efficient mean to counteract skin senescent cells damages and we propose this AI as a holistic senotherapeutic agent.

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Skin organoids as an *in vitro* skin injury model

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Traditional 2D cell cultures or animal models do not represent well human skin physiology or reaction to skin injury and chemical irritation. A complex structure of 3D organoids can allow for physiological cell-cell/cell-matrix interactions and enable them to serve as a model for drug testing and disease. In this study, we created human skin organoids containing multiple skin cell types as a novel tool to recreate a skin microarchitecture and restore native cell interactions. Seeding a mix of skin cells in Low Attachment 96 Well Plates supported self-organization into spherical long-term viable organoids. The visual analysis showed maturation of the skin organoids by obtaining uniform pigmentation and decreasing their size significantly by day 21. Multi-parametric analysis including histology, SEM, and IHC revealed skin-specific layer organization within the organoids, with the surface layer formed by epidermal cells (keratinocytes, melanocytes) and the central core formed by dermal (fibroblasts and follicle dermal papillae cells) and hypodermal (pre-adipocytes) cells. In addition, the skin organoids capillary-like structures composed of endothelial cells were observed inside the organoids, as well as high production of extracellular matrix proteins (collagen III and laminin). To test the response of the skin organoids to external insults, they were exposed to UVB light and to skin-irritating chemicals. The organoids were able to metabolize retinol into retinoic acid and increase melanin synthesis in response to UVB and showed cell viability and anatomical changes in response to chemicals. Taken together, our novel approach for creating multicellular skin organoids is less technically demanding and is more reproducible compared to other skin organoid models. Ultimately, this technique could provide a reliable *in vitro* model of skin and be used as a platform for an investigation of different dermato-pathologies.

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Regulation of sexual dimorphism in dermal fat by the THY1-TGFBR pathway axis

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Sexual dimorphism exists in human skin and in the regional fat distribution. Dermal fat is a unique layer of adipocytes within the skin dermis. Recently, several nonmetabolic activities have been discovered for dermal fat and its fibroblast precursors. However, sexual difference of dermal fat has not been well characterized, and the underlying mechanism remains poorly understood. We have previously shown that dermal fat is lost in male mice during aging through an age-dependent activation of the TGFβ-TGFBR-SMAD2/3 pathway. Here, by comparing sex-related changes of dermal fat in mice during post-natal period and aging, we found that female skin was more resistant to age-related fat loss compared to male skin. In vitro, primary dermal fibroblasts (dFBs) isolated from male mice lost their adipogenic potential by 2 month of age, while female dFBs retained adipogenic function during adulthood, suggesting that there is an intrinsic sex-related difference in the dermal adipocyte progenitors. Single cell RNA-seq (scRNAseq) and flow cytometry analyses found that the expression of THY1, a marker for adipocyte progenitors (APs), was selectively lost in male dermal APs but not in female APs. Ablation of THY1 in dFBs promoted a spontaneous activation of the TGFBR-SMAD2/3 signaling pathway, promoting a pro-adipogenic to pro-fibrotic switch in dFBs. Furthermore, administration of TGFβ receptor inhibitor prevented fat loss in male mice, suggesting that loss of THY1 in male APs may contribute to fat loss by activating the TGFBR signaling cascade in mice. Together, our results have identified a THY1-TGFBR pathway axis that regulates the sexual dimorphism of dermal fat. These results may provide new insights into mechanisms underlying sex-related skin diseases, such as systemic sclerosis and systemic lupus erythematosus.

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Hair follicle chemosensation: TRPM5 signaling is required for anagen maintenance

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Transient Receptor Potential (TRP) ion channels are considered as central players in chemosensation. Several TRP channels (TRPV1, TRPV3, and TRPV4) are expressed in the human hair follicle (HF), and the pharmacological activation of these TRP channels induces catagen in organ-cultured human HFs. In the current study, we explored the role of another TRP channel, TRPM5, which is also activated by a wide range of extra- and intracellular signals. Marked TRPM5 receptor protein expression was identified in epithelial HF compartments, most prominently in outer root sheath (ORS) keratinocytes. Importantly, siRNA mediated knockdown of TRPM5 significantly enhanced HF regression (catagen) in organ-cultured human HFs, associated with a marked reduction in the number of proliferating Ki-67+ cells in the hair matrix and proximal bulb ORS, whereas the number of apoptotic (TUNEL+) cells increased. Further, transcript levels of the anagen-promoting factors *LEF1* and *IGF1* were significantly down-regulated, while expression of the catagen-promoters, *TGFB2* and *SFRP1*, was significantly upregulated in siTRPM5-transfected HFs. In addition, similar to TRPM5 silencing, inhibition of TRPM5 activity with triphenylphosphine oxide (TPPO) promoted catagen, while the TRPM5 activators 2-heptanone and 2,5-dimethylpyrazine maintained HF anagen. Finally, TPPO treatment of HFs down-regulated transcription of anagen-promoters, *FGF7* and *IGF1*, whereas TRMP5 stimulation by 2-Hep reduced levels of catagen-inducing *TGFB1/2*. Taken together, we demonstrate that TRPM5 signaling represents a novel, therapeutically targetable chemosensory control of human HF cycling whose "tonic" stimulation is required for maintaining hair growth. This function of TRPM5 is quite unique compared to all other previously examined TRP ion channels, which actually inhibit hair matrix keratinocyte proliferation and induce catagen in human HFs *ex vivo*.

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Evaluation of anesthetic-like effect of *Aquaphilus dolomiae extract-G3*: *In vitro* and clinical studies

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Sensitive skin is a condition of hyper-reactivity of the skin with subjective discomfort signs. Skin electrical resistance variation is the result of physiological modifications that are unconscious and due to sympathetic nervous system. Nociceptors are the nerve fibers expressed up to the upper skin level and responsible for the transfer of the nociceptive signal to the central system and therefore could be sensitized by changes in their electrical environment. Electrodermal response(EDR) is the phenomenon that the skin momentarily becomes a better conductor of electricity when stimuli occur. EDR is a good tool to assess emotional response associated to pain or uncomfortable feeling. Our objective was to assess by EDR the skin reactivity after 84-day application of formulations containing *Aquaphilus dolomiae extract-G3*(ADE-G3*)* and to investigate the pharmacological effect of ADE-G3 in a model of human sensory neurons. The open label intra-individual study included 2 groups of 25 healthy subjects with sensitive facial skin who applied products (cream or balm) twice daily for a 3-months duration. EDR measurements were performed in response to 2 different stimulations (heat and friction) before and after the first application and after 28, 56 and 84 days of use. We used a sensory neuron model derived from human IPSC stimulated by veratridine. This toxin induces sodium channel activation and Substance P release. The EDR values, measured at D29, D57 and D85, were significantly decreased after the two stimulations for subjects testing balm and cream($p<0.001$) compared to baseline data. The positive control, lidocaine, inhibited veratridine-stimulated release of SP from human sensory neurons by 86%. ADE-G3 at 0.08 and 0.8% significantly inhibited SP release by 92%($p<0.01$) and 116%($p<0.001$), respectively. Lowered EDR values between D1 and D85 with 2 products as well as a lidocaine-like effect with ADE-G3 reflected a decreased activation of the sympathetic nervous system that led to a reduction of skin sensitivity.

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Chromatin architectural protein CTCF regulates terminal keratinocyte differentiation in the developing epidermis and hair follicles

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Chromatin architectural protein CTCF controls three-dimensional organization of the genome and long-range enhancer/promoter interactions in differentiating cells. In mouse embryonic skin, CTCF protein is expressed in the developing epidermis, dermis and hair follicles. ChiPseq analyses revealed presence of CTCF binding sites at the borders of Topologically-Associating Domains (TADs) harboring Keratin Type I and II gene loci on mouse chromosomes 11 and 15, respectively, as well as within the corresponding TADs. To study the role of CTCF in the control of skin and hair follicle development, K14-Cre/Ctcf fl/fl mice were generated. K14-Cre-mediated Ctcf ablation resulted in marked decrease of CTCF protein in the developing epidermis and hair follicle epithelium, while CTCF expressions in the dermis and hair follicle mesenchyme were not changed compared to the controls. CTCF ablation in keratinocytes resulted in alterations of epidermal and hair follicle development: epidermal thickness and keratinocyte proliferation were decreased, a number of hair follicles was significantly reduced and their development was retarded compared to controls. RNAseq analyses of the primary keratinocytes isolated from newborn K14-Cre/Ctcf fl/fl and wild-type mice revealed alterations in the expression of a number of epidermal keratin genes (Krt6a, Krt6b, Krt10, Krt16, Krt17), hair follicle-specific keratin genes (Krt25, Krt33a, Krt33b, Krt73, Krt75), as well as marked upregulation of embryonic epithelial keratin genes Krt8 and Krt18 upon CTCF ablation. Thus, these data demonstrate that CTCF plays essential roles in the control of terminal keratinocyte differentiation and regulate lineage-specific gene expression in Keratin type I and II loci in the epidermal and hair follicle keratinocytes.

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Characterization of lichen planopilaris in men: A retrospective analysis

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Lichen planopilaris (LPP) and subtype frontal fibrosing alopecia (FFA) are primary lymphocytic cicatricial alopecias distinguished by morphology and distribution. LPP affects Caucasian women from 25 to 70 years with a female to male ratio of 1.8:1. LPP is thought to have an autoimmune component and has significant association with thyroid disease. Due to low male prevalence, a paucity of literature demonstrates the characterization of LPP/FFA in men. This study evaluates the clinical characteristics of a male cohort with LPP and/or FFA at a single tertiary hair center. A retrospective review of male patients diagnosed with LPP and/or FFA at Massachusetts General Hospital Hair Loss Clinic from 2017 to 2020 was performed. We identified 24 men diagnosed with LPP, FFA, or LPP/FFA overlap. The mean age at presentation was 45.5 years (range 15-74 years). 70.8% were Caucasian, 12.5% Asian, 8.3% Black, 8.3% unknown/unreported. Eyebrows (20.8%), arms/legs (16.7%), and beard (16.7%) were the most commonly reported areas of non-scalp involvement. 62.5% reported one or more scalp symptoms or signs including pruritus, burning, tenderness/pain, flaking or redness. 50% reported 1 or more first degree relatives with hair loss and of those, 8.3% had similar hair loss type. Two patients reported a family history of 1 or more autoimmune conditions with 1 report of thyroid disease. No patients reported a personal history of thyroid disease. Most commonly reported treatments tried for hair loss prior to diagnosis included topical minoxidil (25%) and topical clobetasol (16.7%). 20.8% of patients reported regular use of SPF yearround and 12.5% regularly colored their hair. Our study demonstrates the unique characterization of LPP/FFA in men. Most men reported scalp symptoms and 16.7% had beard involvement. Most men reported no history of thyroid disease. Further studies are warranted to characterize the LPP/FFA male population.

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Asynchronous and perturbed catagen regression in C3H/HeJ mice precedes the onset of alopecia areata

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Alopecia areata (AA) is an autoimmune disease driven by an influx of cytotoxic $CD8^+$ T cells into the lower hair follicle (HF) resulting in premature regression. AA is postulated to be an antigen driven disease, however, the specific targets of cytotoxic T cells within the HF and the upstream events that lead to presentation of HF antigens, are not known. To pinpoint the target cells in AA, we first investigated the mechanisms of cell death that occurs in the HF of affected C3H/HeJ mice with active AA. We found that cytotoxicity of the HF epithelium occurs via both the extrinsic and intrinsic caspase pathways. We identified the $K71^+$ inner root sheath (IRS) layer as the cells that are selectively targeted for killing by $CD8^+NKG2D^+$ T cells, suggesting these cells harbor AA antigens. Notably, this IRS layer also co-expresses the NKG2D ligand H60, marking it as a target for killing via $CD8^+NKG2D^+$ T cells. To define the temporal-spatial mechanisms by which HF antigens become exposed to the adaptive immune system, we next examined the hair cycle kinetics of C3H/HeJ mice, prior to the onset of AA. Unexpectedly, we discovered a profoundly asynchronous and perturbed catagen progression with delayed apoptosis in C3H/HeJ mice, resulting in accumulated HF epithelial layers. These HF remnants are cleared by activated $CD11b^+$ immune phagocytes showing an elongated spindle shape and multiple dendrites, characteristic of activated antigen presenting cells. The discovery of intrinsic catagen defects in C3H/HeJ mice prior to the development of AA suggests that HF antigen exposure occurs in the hair cycles that precede disease onset. Our findings invoke a model for AA initiation in which intrinsic catagen defects represent predisposing events for HF antigen presentation, which may trigger AA initiation and subsequent immune responses in genetically susceptible individuals.

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Developmental transcriptomics reveal conservation between mouse Merkel cell differentiation and Merkel cell carcinoma

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Merkel cells (MC) are innervated light touch sensors that comprise <0.3% of the mouse epidermis.Terminally differentiated MC are derived from epithelial progenitors and possess both epidermal and neuronal features.Using FACS-based single-cell RNA sequencing (scRNAseq) of skin from MC-specific GFP reporter mice,we captured full-length transcriptome of single MC from embryonic,neonatal,and postnatal skin. MC-specific genes were identified,including established MC markers such as *Atoh1* and *Krt8*. Cell transcriptome clustering identified MC at different differentiation stages,from epithelial precursor to terminally differentiated MC.Trajectory analysis traced the transcriptional changes occurring during MC differentiation. *Krt79* was expressed in MC precursor,and genetic lineage tracing experiments demonstrated that *Krt79*-expressing cells give rise to MCs.Early and late MC markers were validated via whole-mount RNA in-situ hybridization on neonatal mouse back skin.Gene regulatory network (GRN) analysis identified active transcription factor networks during sequential stages of MC differentiation.Consistent with previous findings, targets of the hedgehog signaling effector Gli1 were activated in embryonic MC precursors.Human Merkel cell carcinoma (MCC) transcriptomes are enriched for early MC gene signatures. Moreover,GRNs activated in MCC cell lines correlated with those seen in early or precursor stages of MC differentiation.Taken together,our FACS-seq approach captured high quality scRNAseq 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,including those active in MCC

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Recapitulating atopic dermatitis *in vitro* with a multi-organ 3D model

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Atopic dermatitis (AD) is an inflammatory skin disease affecting up to 20% of infants and 2% of adults. AD is a multifactorial disease, leading to difficulty in the discovery of treatments that can target various aspects of disease etiology. Prior clinical studies highlighted the pleiotropic nature of AD pathogenesis, underscoring the crosstalk between the skin, eyes and nasal tissues. Additionally, a combination of genetic factors, such as barrier dysfunction and immune system dysregulation, together with environmental factors such as allergens and the skin microbiome, contribute to the development of AD. Current models are insufficient to fully recapitulate AD pathogenesis, since animal models are limited by inter-species differences and current *in vitro* models do not enable the crosstalk between different organs. Here, we aimed to create a multi-organ platform for AD to better understand the interplay of tissues and environmental factors influencing AD pathogenesis, and to test drugs to effectively treat this disease. Our platform simultaneously supports 3D skin, nasal mucosa, and corneal constructs, combined with polarized Th2 T cells and skin microbiota, to replicate the multi-organ responses observed in patients. We found that the introduction of polarized Th2 T cells into 3D skin constructs induced the disruption of epidermal integrity, as detected by the absence of filaggrin and loricrin expression. Additionally, our Th2 T cell infused model recapitulated pathologic features of the AD phenotype including spongiosis, acanthosis and epidermal thickening, increased proliferation of cells, and reduced differentiation. The development of a novel physiologically-relevant *in vitro* multi-organ model of AD will be valuable for recapitulation of the atopic phenotype and future drug screening initiation.

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Application of an artificial intelligence (AI) photographic device to track platelet-rich plasma treatment outcomes in females with alopecia

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Platelet-rich Plasma (PRP) injections are promising cosmetic, self-pay treatments for the treatment of androgenetic alopecia (AGA). Efficacy is typically assessed after two or three treatments spaced 4 weeks apart. Measurement tools include physician and patient impressions and global assessment of pictures. An unmet need has been the absence of an efficient methodology that adds quantitative information to the clinical and subjective evaluations. This unmet need may have now been met with the development of an AI driven, non-invasive photographic/trichoscopy device, HairMetrix® by Canfield Scientific, Inc. This device incorporates trichoscopic evaluation of scalp health and quantitative assessment of several parameters that can also be collected longitudinally without the need to clip or stain hair shafts. Information which can be obtained and monitored longitudinally includes: terminal to vellus hair ratio, average hairs per follicular unit, average hair width, hair count per cm^2, follicle count per cm^2, and inter-follicular mean distance, presence or absence of perifollicular erythema and scale, tufting, diffuse erythema, and telangiectasia. We have used this device to measure PRP treatment outcomes and scalp health in two adult Caucasian female patients with AGA. Six HairMetrix photos were taken at defined scalp locations pre-PRP treatments - frontal anterior, midscalp, vertex, occipital, right and left temporal scalp. Average hair width and follicle count increased after one PRP treatment along with a decrease in mean intrafollicular distance. The use of this AI technology offers both patients and physicians objective information to assess treatment efficacy and scalp health. In the case of PRP treatments, such information also has the potential to help define the treatment interval needed to sustain the desired clinical response between scalp injections. This is the future of alopecia clinical management.

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Incidence of cicatricial alopecia among new alopecia patients undergoing platelet rich plasma

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Platelet rich plasma (PRP) is an effective treatment for pattern hair loss (PHL). However, there is little evidence for PRP as a treatment of cicatricial alopecia (CA). PRP is expensive and carries inherent procedural risk. A thorough history and exam can exclude inflammatory causes of alopecia and optimize patient outcomes. High patient demand for PRP coupled with lenient procedural regulations open the possibility for inadequately trained personnel to miss a diagnosis of CA in patients presenting for PRP. We examined the incidence of new CA diagnosis among our PRP patients to evaluate this potential risk. A retrospective review of new patients presenting for PRP between August 2019 and December 2020 was performed. A total of 82 patients (45 female) with a mean age of 44.5 were included. 76.8% were undergoing alopecia treatment for presumed pattern hair loss at the time of presentation, most were PRP naïve (92.7%) and had never underwent scalp biopsy (91.5%). On clinical and trichoscopic exam, 12 (14.6%) were noted to have mild to moderate perifollicular scale, erythema, or both, 2 of whom had known biopsy proven CA. A scalp biopsy was recommended to 10 (12.2%) patients due to signs of inflammation and 2 (2.4%) declined biopsy due to personal preference. Of the 8 (9.8%) that underwent scalp biopsy, 5 (62.5%) were diagnosed with CA and the remaining 3 (37.5%) were PHL. We report 6.1% of new patients presenting to our clinic for PRP had undiagnosed CA. CAs can present similarly to PHL and early treatment is critical to limit irreversible hair loss. PRP is not an effective monotherapy for CA, is costly, and carries procedural risk. Trichoscopic and clinical evaluation by a trained dermatologist before PRP therapy can help to optimize treatment outcomes by correctly identifying CA when present. This study highlights the potential risk that non-trained personnel providing PRP may pose to patient safety.

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Gibbin toggles CTCF binding and DNA methylation to drive epithelial development

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Proper skin, hair, and the neural tube formation relies on a foundation of chromatin conformation decisions made in the developing ectoderm. While proper ectodermal development requires initiation transcription factors like GATA3 to promote maturation by p63, how these factors act to stably pattern gene expression remains poorly understood. Here we use multi-dimensional genomics to identify Gibbin, encoded by the Xia-Gibbs AT-hook DNA Binding Motif Containing 1 (*AHDC1*) disease gene, as a key non-neural ectoderm regulator. Promoter-bound Gibbin facilitates the initiator function of GATA3 without affecting DNA binding or chromatin accessibility. Using cohesin HiChIP, we find that Gibbin regulates gene expression by maintaining enhancer-promoter chromatin contacts. Proximal proteomics reveal that the Gibbin interactome is enriched in zinc-finger TFs, methyl-CpG binding proteins, and the newly identified ChAHP/CTCF regulatory complex. We further show that Gibbin blocks methylation at lineage-specific CTCF binding sites, promoting CTCF binding, stable genome topology, and transcription at subsequent steps in differentiation. Consistent with its role in regulating the activity of initiator TFs, Gibbin-mutant ES-derived skin organoids maintain p63 levels but exhibit defective keratinocyte differentiation and stratification. Novel *in vivo* CRISPR mouse mutants reveal a spectrum of surface ectoderm defects affecting craniofacial structure, abdominal wall closure, epidermal adhesion, and hair follicle development. We conclude that Xia-Gibbs syndrome derives from a loss of early ectoderm genome topology through abnormal toggling of cell-type and gene-specific CTCF/DNA methylation decisions. These results highlight the spectrum of Xia-Gibbs syndrome with methylation, cohesin, and ChAHP-associated diseases as well as potential use of Gibbin/*AHDC1* as a biomarker in ectodermal dysplasias.

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Single-cell transcriptome profiling reveals vascular endothelial cell heterogeneity in human skin

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Vascular endothelial cells (ECs) are increasingly recognized as active players in intercellular crosstalk more than passive linings of a conduit for nutrition delivery. Yet, their functional roles and heterogeneity in skin remain uncharacterized. To investigate the tissue-specific features and intra-tissue heterogeneity in dermal ECs, human dermal EC atlas of over 23,000 single-cell transcriptomes was obtained and further analyzed. In comparison with ECs from other human tissues, extracted from previously reported data, dermal ECs possess unique characteristics in transcriptional modulation, metabolic and chemotactic functions. We identified five major subtypes of human dermal ECs and their molecular signatures. Within different layers in dermis, ECs are also varied in their biological activities. Metabolic transcriptome analysis revealed a gradual switch from oxidative phosphorylation to glycolysis in dermal EC subtypes along the blood flow. Moreover, dermal capillary ECs abundantly expressed HLA class II molecules, suggesting its immune-surveillance role. ECs in post-capillary venules, with high levels of adhesion molecules, were equipped with possible capacity in immune cell arrest, adhesion, and infiltration. Our study provides a comprehensive characterization of EC features and heterogeneity in human dermis and sets the stage for future research in identifying disease-specific alterations of dermal ECs in various dermatoses.

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Evaluation of anti-hairloss shampoo through *in vitro* activity in human hair follicule dermal papilla cells and sensorial study

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The hair follicle undergoes cycles of growth (anagen), regression (catagen) and resting (telogen) phases during its post-natal life. Hair growth cycle is a highly regulated process during which different compartments of the hair follicle maintain close relationships through many molecular communications. Among these exchanges, Wnt/β-catenin pathway activation in dermal papilla cells plays a central role to promote anagen onset and hair growth. The aim of this study was to evaluate the effect of a 6 active ingredient complex of an Anti-Hair Loss Shampoo (Biotin; Hydrolyzed Wheat Protein; Dexpanthenol; Piridoxine; Tocopheryl Nicotinate; Ruscus Extract) on Wnt/β-catenin pathway activation in Human Hair Follicle Dermal Papilla Cells (HHFDPC). Sensorial properties of the shampoo containing the active complex were also assessed. HHFDPC were incubated with the active complex, Finasteride was used as positive control. Semi-quantification of β-Catenin by immunofluorescence technique was performed. The sensorial study was performed on 24 subjects after one single application of the Shampoo on half-head. The subjects themselves also responded to a questionnaire. After 48h incubation, the active complex significantly increased the production of β-Catenin (+192%*** vs baseline) to a level equivalent to that of Finasteride. The sensorial study revealed that the tested shampoo showed great cleaning efficacy associated with volume and shine. Our results showed that the active ingredient complex of the Anti-Hair Loss Shampoo increased the production of β-Catenin, allowing initiation and prolongation of the anagen phase thus helping to promote hair growth. Moreover, the Shampoo showed good sensorial properties associated with hair fiber quality improvement. Altogether, the final product showed the essential attributes expected from an anti-hair loss shampoo.

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Hair growth properties of *Cinchona succirubra* Extract, *Leontopodium alpinum* Extract and Manganese PCA in human hair follicle dermal papilla cells

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The hair follicle (HF) undergoes cycles of growth (Anagen), regression (Catagen) and rest (Telogen) phases. Both chronic and reactive hair loss are linked to a deregulation of this hair growth cycle leading to premature hair shaft loss. The aim of this study was to evaluate the properties of three active ingredients on the promotion and maintenance of hair growth. Signals and molecular markers that regulate hair growth were studied in Human Hair Follicle Dermal Papilla Cells (HHFDPC). Potential synergistic activities were also investigated. Wnt/b-catenin pathway activation was measured using a gene reporter assay strategy (transfection of HHFDPC with a lentivirus expressing luciferase gene under the control of TCF/LEF promoter). Vascular Endothelial Growth Factor (VEGF) protein expression levels were measured in HHFDPC culture supernatants using ELISA technology. Manganese PCA incubation enhanced VEGF secretion (+234%** vs basal control). The association of *Cinchona succirubra* extract, *Leontopodium alpinum* extract and Manganese PCA activated Wnt/b-catenin pathway (+148%** vs basal control) in HHFDPC. Manganese PCA, *Cinchona succirubra* extract and *Leontopodium alpinum* extract showed interesting hair growth promoting properties in HHFDPC, promoting Anagen onset by activating Wnt/b-catenin pathway, and maintaining hair growth by enhancing VEGF secretion. Thus, these ingredients are good candidate for anti-hair loss treatment.

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mTORC1 activity controls human scalp hair follicle pigmentation and growth

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The mechanistic Target of Rapamycin Complex 1 (mTORC1), whose activity is inhibited by rapamycin (Rapa), is a multiprotein complex that senses nutrients, growth factors and cellular stressors, and regulates multiple cellular processes including proliferation, autophagy, and Wnt signaling. Since loss-of-function mutations of the key endogenous inhibitor of mTORC1 activity, tuberous sclerosis complex (TSC), are associated with hair depigmentation (poliosis), we asked whether mTORC1 also impacts on human hair follicle (HF) pigmentation. Interestingly, pharmacological inhibition of mTORC1 with Rapa in organ-cultured, healthy human scalp anagen VI HFs, lead to anagen prolongation and up-regulation of hair matrix keratinocyte proliferation, while apoptosis was significantly down-regulated (indicating a prolongation of the "window of opportunity" for anti-greying treatment). Furthermore, HF melanogenesis, gp100 expression, and number and dendricity of gp100+ HF melanocytes were significantly up-regulated by Rapa in anagen HFs in a hair cycle-independent manner. Our preliminary data suggest that Rapa treatment can even partially restimulate pigmentation in some greying scalp HFs *ex vivo*. In contrast, TSC2 silencing *ex vivo* induced hyperactivation of mTORC1 and reduced human scalp HF melanogenesis. In summary, we provide the first evidence that mTOR activity is an important, previously unappreciated physiological regulator of human hair growth and pigmentation. Thus, inhibiting intrafollicular mTORC1 activity by Rapa and rapalogs may become an attractive novel intervention strategy in the future management of greying, and alopecia.

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Depilatory laser induces dermal papilla cell necrosis through thermal diffusion and miniaturizes hair

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Depilatory laser that targets melanin has gained popularity for the treatment of hypertrichosis. Permanent hair reduction is obtained when laser is delivered in anagen. How permanent hair reduction is achieved remains unclear. We found that irradiating C57BL/6 mice in anagen with an alexandrite laser (755 nm, pulse duration 3 msec) lead to hair miniaturization in the following cycle. In addition to thermal disruption of melanin-containing cells in the precortex region, unexpectedly, we also detected thermal necrosis of non-pigmented dermal papilla cells abutting them. The dermal papilla cells decreased by 24% after laser injury while the number of bulge stem cells remained unchanged. When laser was delivered to telogen hair follicles where no melanin was present adjacent to dermal papilla, thermal necrosis and cell reduction was not detected in dermal papilla and no hair miniaturization was revealed. Because a larger dermal papilla supports the growth of a thicker hair, the results suggest that depilatory laser miniaturizes hair by inducing thermal necrosis of dermal papilla cells due to thermal diffusion from melanin-containing cells in anagen hair follicles. Therefore, dermal papill is the long-sought target of depilatory laser that mediates the miniaturizing effect. Methods that can enhance thermal diffusion for dermal papilla cell depletion will augment the treatment effect of depilatory laser.

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DNA dioxygenases TET regulate keratin gene expression and enhancer networks within lineage-specific gene loci during epidermal and hair follicle-specific keratinocyte differentiation

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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. Here, we show that 5hmC modified DNA and Tet1/2/3 proteins show dynamic changes in their abundance in the developing epidermis and hair follicles (HFs). To uncover the roles for Tet2/3 in the control of skin development, we used conditional knock-out mouse model with *Krt14-Cre* mediated ablation of *Tet* genes (*Krt14-Cre/Tet2* $^{fl/fl}$*/Tet3* $^{fl/fl}$ or DKO). Consistently with the expression pattern of *Krt14* gene, the level of 5hmC was markedly decreased in the developing epidermis and hair follicle, as well as in hair matrix keratinocytes. *Krt14-Cre* mediated *Tet2/3* ablation resulted in alterations of epidermal differentiation and appearance of wavy hairs followed by hair loss compared to WT and *Tet2* or *Tet3* single knockout controls. Furthermore, RNA-seq analysis of epidermal and hair matrix keratinocytes isolated from DKO mice revealed alterations in expression of epidermal (Krt10, Krt16, Krt17) and hair follicle-specific *(Krt25, Krt26, Krt27, Krt28, Krt31, Krt32, Krt33a, Krt33b, Krt34, Krt35, Krt71, Krt73, Krt81, Krt83, Krt84, Krt86)* genes upon *Tet2/Tet3* ablation. Changes of gene expression in the Keratin Type I and II gene loci were associated with changes in the H3K27ac enrichment within the corresponding Topologically Associating Domains harboring these loci, thus suggesting the re-wiring of the enhancer-promoter interactions in keratinocytes upon Tet2/Tet3 ablation. Taken together, these data demonstrated a complex role of TET enzymes in the control of epidermal and hair matrix keratinocyte differentiation associated with regulation of gene promoters and enhancers in lineage-specific gene loci.

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Connecting signaling dynamics with cell fates in live mice

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Many tissues are composed by multiple cell types, which are derived from and maintained by tissue resident stem cells. How stem cells coordinately differentiate into multiple cell fates is largely unclear. By using a novel ERK/MAPK signaling reporter mouse, our recent study showed that during early mouse embryogenesis, stem cells bifurcate their fates by transiently modulating ERK activation, suggesting that different cell fates can be determined by distinct types of ERK activation dynamics in stem cell differentiation. Here, we are using hair follicle as a multi-cell-type system to investigate the ERK signal-mediated cell fate coordination mechanism, as ERK pathway has been shown to regulate multiple cell fates in the hair follicle. By combining two-photon imaging approach and the ERK reporter, we are able to capture the real-time ERK signal dynamics at the single cell level throughout hair follicle regeneration in live mice, along with the cellular dynamics we have characterized before. Time-lapse analyses show that ERK signal changes its activation modes at different stages of hair follicle growth, which is coupled with the progressive cell fate specification and maintenance. Drug treatment assays uncover that distinct upstream signaling pathways contribute to different types of ERK signal activation. Further genetic manipulations are performed to understand the genesis of these ERK dynamics as well as their specific roles in coordinating cell fates and behaviors. This work bridges the gap between the dynamic activation of the key regeneration regulator and the coordinated cell fate decisions and will provide new sights into the fundamental principles of tissue regeneration. Given ERK signal is dysregulated by many oncogenes, this work will also have implications in cancer etiology and treatment.

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Generation of Dkk4-Cre knock-in mice to study morphogenesis of ectodermal appendages

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How ectodermal appendages are formed and patterned are still open questions in mammalian biology. Ectodermal appendage morphogenesis is initiated during mouse embryonic development through a series of reciprocal interactions between the mesenchyme and the epithelium. The molecular cross-talk is followed by the formation of the histologically visible structures, which further give rise to teeth, mammary glands, sweat glands and hair follicles (HFs). We have previously shown that Dickkopf 4 (Dkk4), the canonical Wnt signalling pathway inhibitor, mRNA is exclusively expressed in ectodermal placodes. In this work, we used CRISPR/Cas9 and the Dkk4 locus to study ectodermal appendage formation by generating Dkk4-Cre knock-in mice. To assess Cre expression, we crossed Dkk4-Cre mice with EGFP Cre-reporter mice. EGFP-positive epithelial appendages were found at different developmental stages confirming that the activity of the Cre recapitulated Dkk4 mRNA expression. Unexpectedly, the posterior half of the embryos harboured EGFP-positive mesenchymal, adipocyte and muscle cells. Because the Dkk4 mRNA was not expressed in these populations, we reasoned that the progenitors of these cells arose earlier during development. In agreement, lineage tracing revealed a few EGFP-positive cells in the epiblast of early gastrulating mouse embryos (embryonic day, E6.5), which correlated with more EGFP-positive cells in the presomitic mesoderm and the posterior somites around midgestation (E9.5). Focusing on HF development, our data showed that not all the HF epithelial cells were EGFP-positive, suggesting that epidermal cells that do not express Dkk4 also contribute to the HF. In summary, the Dkk4-Cre mouse line is a suitable model to study ectodermal appendage morphogenesis using lineage-tracing and time-lapse imaging.

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Cutaneous overexpression of cyclooxygenase-2 models androgenetic alopecia in adult mice

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Androgenetic Alopecia (AGA) is a common form of hair loss affecting both men and women. Recent evidence has revealed that prostaglandin signaling is associated with the development of AGA. Prostaglandin D2 (PGD2) is elevated in the bald scalp of men with AGA and has been shown to slow hair growth in mice through the activation of the DP2 receptor. Currently, the exact mechanism of hair loss in AGA is unclear. One of the barriers to studying AGA on a cellular level is the limited number of animal models that can replicate the progression of the disease in phenotypically normal adults. To address this, we developed a transgenic mouse that conditionally expresses cyclooxygenase-2 (COX2), an enzyme upstream of the PGD2 synthesis pathway, in the skin using a doxycycline inducible system (K5-rtTA; TRE-COX2). Induction of COX2 in adult transgenic mice led to sebaceous gland hyperplasia and reduced hair shaft size, both hallmarks of AGA. Basal sebocytes of the enlarged sebaceous glands showed a slight increase in proliferation. Hair shafts of the transgenic mice were thinner, shorter and, unidentifiable from the characteristic four hair types found in mice (guard, awl, auchene, and zigzag). The hair loss phenotype occurred in a single hair cycle after doxycycline induction and histologically featured miniaturization of the hair follicle. While the transgenic mice showed cell proliferation in the nascent germ of growing follicles, they failed to reach the full size of control mice anagen follicles before prematurely regressing into catagen. Reducing the expression of COX2 to control levels in transgenic mice led to a complete recovery of hair shaft width, length, hair type, and follicle size within a single hair cycle. This mouse model will provide a helpful tool for studying the cellular processes driving the development of AGA.

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Neuroimmune control of adult mammalian scarless skin regeneration

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Adult mammalian wounds, with rare exception, heal with fibrotic scars that severely disrupt tissue architecture and function. Regenerative medicine seeks methods to avoid scar formation and restore the original tissue structures. We show in three adult mouse models that pharmacologic activation of the nociceptor TRPA1 on cutaneous sensory neurons reduces scar formation and can also promote tissue regeneration. Local activation of TRPA1 induces tissue regeneration on distant untreated areas of injury, demonstrating a systemic effect. Activated TRPA1 stimulates local production of interleukin-23 (IL-23) by dermal dendritic cells, leading to activation of circulating dermal IL-17—producing gd T cells. Genetic ablation of TRPA1, IL-23, dermal dendritic cells, or gd T cells prevents TRPA1-mediated tissue regeneration. These results reveal a cutaneous neuroimmune-regeneration cascade triggered by topical TRPA1 activators that promotes adult mammalian tissue regeneration, presenting a new avenue for research and development of therapies for wounds and scars.

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Symmetry breaking of tissue mechanics in wound induced hair follicle regeneration

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Tissue regeneration is a process that recapitulates the molecular and mechanical aspects of development and evolution. We use the wound-induced hair neogenesis (WIHN) model to investigate the mechanical and molecular responses of the laboratory (*Mus*) and African spiny (*Acomys*) mice. Laboratory and spiny mice showed an opposite trend of spatiotemporal morphogenetic field for WIHN during wound healing, and wound stiffness gradient across the whole wound bed predicated pattern of hair formation. Using RNA-seq analysis and K14-Cre-Twist1 transgenic mice, we identified the central role of the Twist1 pathway as the mediator of epidermal-dermal interaction and the emergence of periodic hair primordia. Lastly, we generated a Turing model with an underlying measure of stiffness to support a two-scale tissue mechanic model to explain the setup of a morphogenetic field from a wound bed (mm scale) or periodically arranged hair primordia from a morphogenetic field (μm scale). Delineating the common and distinct chemo-mechanical events during regenerative wound healing between laboratory and African spiny mice reveal its evo-devo advantages, which provide new perspectives for regenerative medicine.

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Deregulated immune signature orchestrated by FOXM1 impairs human diabetic wound healing

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Diabetic foot ulcers (DFUs) are a life-threatening disease that often result in lower limb amputations and a shortened lifespan. However, the transcriptional networks and molecular mechanisms contributing to the pathogenesis of DFUs remain poorly understood. We used next-generation sequencing to compare the pathogenic DFU transcriptional profile to that of human oral acute wounds, a model of "ideal" adult tissue repair due to accelerated closure without scarring. Although we found common signatures between the two, we identified major transcriptional networks deregulated in DFUs that promote activation and survival of immune cells, resulting in decreased neutrophils and macrophages activation and overall poorly controlled inflammatory response. We identified transcription factors *FOXM1* and *STAT3,* which function to activate and promote survival of immune cells, to be inhibited in DFUs. Moreover, inhibition of FOXM1 resulted in delayed wound healing and decreased neutrophil recruitment *in vivo.* Our data emphasize the role of perturbed, ineffective inflammatory response as a major contributor to the pathogenesis of DFUs revealing FOXM1 as a novel target for potential therapeutic reprogramming.

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Oral epithelial regenerative transcription factor Pitx1 reprograms keratinocytes to promote cutaneous wound healing

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Oral wound healing is an ideal system of wound repair due to its rapid healing without scar formation. We previously reported that transcription factors Sox2 and Pitx1 are part of a transcriptional signature that may be critical to the regenerative capacity of oral mucosa. However, it is still unclear if Pitx1 modulates a regenerative transcriptional program in the oral mucosa and if this program can be functionally induced in the skin to promote tissue repair. In this study, we characterized wound healing in control and conditional, basal Pitx1-overexpressing skin (K5-Tet-Pitx1). Pitx1 enhanced full-thickness wound closure by increasing keratinocyte migration and proliferation. ChIP-qPCR analysis of Pitx1 in keratinocytes revealed binding of Pitx1 to at least one Sox2 promoter, correlating with an increase in Sox2 mRNA and protein expression and corroborating our own previous results in oral and cutaneous keratinocytes. RNA-sequencing (RNA-Seq) of healthy and wounded whole skin and subsequent Ingenuity Pathway Analysis (IPA) revealed that K5-Tet-Pitx1 mouse keratinocytes expressed oral keratinocyte markers (keratins 4 & 13 and cornulin) and had a global transcriptional shift towards oral keratinocytes, suggesting that Pitx1 might induce a cutaneous-to-oral lineage change in epidermal keratinocytes. Furthermore, Pitx1 enhances the expression of aldehyde dehydrogenase 1a3 (Aldh1a3), an enzyme critical for production of retinoic acid (RA). IPA analysis of RNA-Seq data from epidermal keratinocytes revealed significant enrichment of pathways related to retinoid biosynthesis. This study illustrates that Pitx1 has a pleiotropic effect on key downstream pathways that may be critical to the establishment of an oral-like regenerative healing program in the skin.

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Wnt signaling induces fibrotic fat loss via DPP4 in skin fibrosis

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Fibrotic diseases involve a loss of fat or lipid-filled cells in several organs including the lungs, liver, and skin in addition to expansion of extracellular matrix (ECM). Dermal fibrosis including excess scarring, scleroderma, and eczema, affects about 40 million people, yet there are no therapies for its reversal. Skin is unique among fibrotic organs due to its accessibility and distinct fat compartment, called dermal white adipose tissue (DWAT). Reduced lipid among mature adipocytes which comprise the DWAT has implications for their many functions. *The mechanisms governing fibrotic DWAT lipid depletion and the effects of lipid depletion are not known.* Wnt signaling is dysregulated among fibrotic tissues and sustained Wnt signaling in mouse dermis is sufficient to cause dermal fibrosis including DWAT lipid depletion through previously unknown mediators. Here we test the following hypothesis: Induced Wnt signaling stimulates lipid breakdown via dipeptidyl peptidase 4 (DPP4) in dermal adipocytes impacting ECM expansion. Using an inducible and reversible genetic mouse model of dermal Wnt activation, we identify cellular mechanisms of lipid depletion and recovery. Wnt activation leads to breakdown of intracellular lipid in DWAT, stimulation of the lipolytic axis, and increased ECM remodeling, all of which recover during reversal. Genetic ablation of candidate Wnt-responsive factor, DPP4, in our model leads to DWAT preservation, attenuated lipid breakdown, diminished ECM remodeling, and reduced dermal thickening. Finally, chemical inhibition of DPP4 in our mouse model leads to accelerated recovery of DWAT and dermis. Together these data demonstrate that Wnt-DPP4 modulates lipid homeostasis in adipocytes, impacting neighboring dermal fibroblasts. Thus, our results suggest that treatment of dermal fibrosis with FDA-approved DPP4 inhibitors may effectively target lipid depletion, a new cellular player in fibrosis.

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Bacteria induce skin regeneration via IL-1β signaling

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Environmental factors that enhance regeneration are largely unknown. We hypothesized that skin bacteria modulate regeneration. Here, we assessed low, medium, and high levels of bacterial burden in wound healing and Wound Induced Hair follicle Neogenesis (WIHN), a rare adult organogenesis model. WIHN levels and stem cell markers indeed correlated with bacterial counts, being lowest in germ free (GF) (fold= -17.9, n=13, p=1.9X10^{-6}), intermediate in conventional specific pathogen free (SPF), and highest even in mice infected with pathogenic *Staphylococcus aureus* (fold= 3.3, n=12, p=7.5X10^{-5}). We identified IL-1β and keratinocyte-dependent IL-1R-MyD88 signaling as necessary and sufficient for bacteria to promote regeneration. Finally, in a small clinical trial, we found that a topical broad-spectrum antibiotic slowed skin wound healing. These results demonstrate a novel role for IL-1β to control morphogenesis and counter conventional notions that infection inhibits regeneration with a need for full sterility of small wounds.

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A systematic review of autologous adipose-derived stromal vascular fraction (SVF) for the treatment of acute cutaneous wounds

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SIGNIFICANCE: Stromal vascular fraction (SVF), derived from adipose tissue via either enzymatic or mechanical processing, is comprised of a heterogenous population of cells and stroma, including adipose-derived stem cells (ADSCs), growth factors, fibroblasts, and pericytes. The regenerative capacity of SVF lends to its broad range of clinical applications, including dermatologic applications such as the healing of acute cutaneous wounds. AIM: To evaluate the available evidence on the efficacy and safety of autologous adipose-derived SVF or similar ADSC-containing derivatives for treatment of acute cutaneous wound in humans. METHODS: In accordance with PRISMA guidelines, a systematic review of the literature was conducted utilizing MEDLINE, Embase, and the Cochrane Central Register of Controlled Trials from inception to September 2020 to identify published clinical trials assessing SVF or ADSC-containing derivatives for patients with acute cutaneous wounds. This was supplemented by searches for ongoing clinical trials through ClinicalTrials.gov and the WHO International Clinical Trials Registry Platform. FINDINGS: The initial 872 records were reduced to 10 studies upon application of inclusion and exclusion criteria: 2 completed non-randomized controlled trials and 8 ongoing clinical trials. The two completed studies reported a statistically significant advantage for the SVF treatment arms in terms of percentage re-epithelialization and time to healing. No information regarding safety was provided. Ongoing clinical trials are evaluating outcomes including safety, patient and observer reported scar appearance, wound healing rate, and wound epithelization. CONCLUSION: Although there are limitations in the quantity and quality of available evidence, it appears both enzymatically and mechanically isolated SVF may speed healing of acute cutaneous wounds. Additional clinical trials with improved outcome measures and safety assessment are required.

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Efficacy and safety of Oleogel-S10 (birch triterpenes) for recessive dystrophic epidermolysis bullosa (RDEB): Results of 3 months double-blind treatment of the phase 3 study 'EASE'

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Background: Epidermolysis bullosa (EB) is a group of rare, inherited diseases affecting the integrity of epithelial tissues. The EASE study was a prospective, randomized, Phase 3, double-blind, controlled study (NCT03068780) to evaluate the efficacy and safety of Oleogel-S10 (birch triterpenes) in EB. Methods: Patients with dystrophic EB (RDEB, DDEB) or junctional EB (JEB) with a partial thickness target wound (10−50cm^2, between 21-days−9-months duration) entered the study. Study gel was applied at dressing change (≤4 days) to all wounds. Primary endpoint was the proportion of patients with first complete target wound closure within 45 days. Efficacy was also evaluated by EB subtype. Results: 223 patients were enrolled. The primary endpoint was met with first complete target wound closure in 41.3% on Oleogel-S10 vs. 28.9% on control gel; RR 1.44 (p=0.013). In the RDEB group (n=175), 44% on Oleogel-S10 vs. 26.2% on control gel achieved the primary endpoint; RR 1.72 (p=0.008). No statistically significant differences were observed in the DDEB or JEB groups. Reduction in procedural pain (Wong-Baker FACES) in RDEB group was observed at each visit (p=0.032 at Day 90). Total wound burden (area, EBDASI skin activity score) at Day 90 was reduced in the RDEB group with no statistically significant difference between treatments. In the overall population, adverse events were comparable in both groups; the majority being mild or moderate in severity. Conclusion: Oleogel-S10 demonstrated evidence of accelerated wound healing in EB, and the primary benefit was observed within the RDEB subgroup. Oleogel-S10 is a potential treatment for patients with this intractable disease.

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Glucosone induces senescence in dermal endothelial cells: Comparison of carnosine and Carcinine, two well-known anti-glycation ingredients

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Diabetes is a disease characterized by high levels of circulating sugar that favours the glycation process. Glycation induces protein reticulation and/or degradation and the generation of glucose self-oxidation molecules such as dicarbonyl glyoxal, methylglyoxal or glucosone which leads to an accumulation of advanced glycation endproducts. In skin, they were reported to cause an alteration of microcirculation. Since blood vessel growth and turnover in the skin are key to tissue homeostasis and wound repair, this accumulation leads to impaired healing and premature aging. Senescence (a process mainly characterized by altered cell morphology and metabolism) of endothelial cells (EC) is linked to several vascular processes involved in skin aging. Since senescence can be induced by various factors such as oxidative stress, we investigated whether glucosone, that is present in high quantity in diabetic patients, could also induce senescence in EC from blood vessels and accelerate aging. We thus developed a new experimental model where human dermal microvascular EC were exposed to glucosone for 3 days. Senescence Associated-b galactosidase (SAßG) activity and cell morphology changes were monitored. Using this novel model, we showed that glucosone is able to induce senescence in EC as it increases SAßG activity, the size and granularity of the cells. Besides, Carcinine (a stable biomimetic peptide used in cosmetics to fight the effects of aging) was found to be more effective than carnosine (an anti-glycation carnosinase-sensitive product) in restoring all the tested parameters. This data clearly shows that our new model of glucosone-induced senescence is robust and reliable. It also suggests that Carcinine could be a better candidate than Carnosine for limiting the senescence of EC observed in different physio-pathological processes such as diabetes, and may therefore reduce associated symptoms such as skin pallor and impaired healing.

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AMP-IBP5 improves diabetic wound healing via activation of EGFR/STAT/MAPK pathways

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Background: Impaired keratinocyte functions are major factors responsible for deficient diabetic wound healing. In addition to its antimicrobial activity, AMP-IBP5 (antimicrobial peptide derived from insulin-like growth factor-binding protein 5) also promotes keratinocyte and fibroblast proliferation and migration. However, its effect on wound healing and the underlying mechanism remains unclear. Objective: To examine the effect of AMP-IBP5 on diabetic wound healing and to clarify its mechanism. Methods: Keratinocytes were cultured in normal or high-glucose milieus. The production of angiogenic factors was evaluated by ELISA. Cell proliferation was assessed by BrdU incorporation, whereas migration was evaluated by chemotaxis chamber and wound scratching assays. EGFR, STAT and MAPK activation was determined by Western blotting. Wounds in normal and streptozotocin-induced diabetic mice were monitored and histologically examined.Angiogenesis was evaluated by qPCR and immunofluorescence. Results: AMP-IBP5 rescued high-glucose-induced impairment of the production of angiogenic factors such as angiogenin and VEGF, cell proliferation and migration in keratinocytes cultured in high-glucose conditions to mimic diabetes. This activity was controlled by the EGFR, STAT, and MAPK pathways, as evidenced by the inhibitory effects of their respective specific inhibitors. Indeed, AMP-IBP-5 induced activation of these pathways. Additionally, AMP-IBP5 accelerated wound healing in both normal and diabetic mice, and reversed the suppressive effect of high-glucose on angiogenesis and re-epithelialization in diabetic mice. Conclusion: The finding that AMP-IBP5 accelerated the wound healing and angiogenesis in diabetic mice suggests thatAMP-IBP5 might be a potential therapeutic target for chronic diabetic wounds.

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Glucose transporter 1 enhances glycolysis, oxidative stress, and fibroblast proliferation in keloid

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Keloid is a skin fibroproliferative tumor. Proliferating keloid fibroblasts (KF) demand active metabolic utilization. How glycolysis and glucose metabolism contribute to the fibroproliferation of keloid remain unclear. This study aims to determine the regulation of the glycolysis and glucose metabolism by glucose transporter 1 (GLUT-1), an essential protein to initiate cellular glucose uptake, in keloid and in KF. Tissues of keloid and healthy skin were explanted for KF and normal fibroblasts (NF), respectively. GLUT-1 expression in tissue, KF, and NF, was measured by immunofluorescence, RT-PCR and immunoblotting. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) in KF and NF were measured with or without WZB117, a GLUT-1 inhibitor. Reactive oxygen species (ROS) were assayed by MitoSOX immunostaining. The result showed that glycolysis (ECAR) but not OCR is enhanced in KF. Profiling of the main enzymes in the glycolytic pathway revealed that GLUT-1 expression is selectively increased in KF. Consistently, GLUT-1 expression is increased in keloid tissue. Treatment with WZB117 abolished the enhanced ECAR, including glycolysis and glycolytic capacity, in KF. Although basic and maximal OCR was similar between NF and KF, only in NF both OCR was enhanced by WZB117. ROS levels were increased in KF than those in NF. GLUT-1 inhibition suppressed not only the ROS levels but also the cell proliferation in KF. In summary, we showed that GLUT1-dependent glycolysis but not oxidative activity is enhanced in KF. Moreover, ROS production is accentuated in KF. The GLUT-1-dependent glycolysis and ROS production mediate fibroblast proliferation in keloid. GLUT1 might be a potential target for metabolic reprogramming to treat keloid.

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IL-10 producing CD4+ T-cells mitigate dermal fibrosis

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Background: CD4$^+$ T-cells are essential in regulating dermal fibrosis and are a main source of IL-10. We have shown that IL-10 reduces dermal fibrosis by upregulating high molecular weight hyaluronan (HMW-HA). However, little of known the role of these cells in scarring. We hypothesize that IL-10 producing CD4$^+$ cells attenuate dermal wound fibrosis via mediating inflammation and altering fibroblast extracellular matrix (ECM) production. Methods: C57BL/6J murine(6-10wk) splenocytes were enriched and sort into CD4$^+$ IL-10 producing cells (Treg&Tr1). In vitro, fibroblasts were co-cultured with Treg or Tr1; fibrotic (Col1a1, aSMA) and ECM remodeling (HA synthases (HAS)1-3) markers were analyzed (qRT-PCR). In vivo, we performed bilateral 6mm dorsal full-thickness wounds on SCID mice (female,8-10wk), and adoptively transferred 10^6 CD4$^+$, Treg, or Tr1 cells. Wounds were harvested at days 7/28 and analyzed for closure (H&E), fibrosis (trichrome), cell-cell spatial relationships (imaging mass cytometry) and inflammation panel (Luminex). Results: We confirmed the population (flow cytometry) and functionality (IL-10 ELISA) of Treg and Tr1. In the co-culture, Col1a1 in Tr1/Treg treated fibroblasts was reduced by 52.4% or 43.5%; and aSMA was reduced by 25.2% or 44.7%, respectively, compared to untreated fibroblasts. HAS2, a hyaluronan synthase which primarily produces HMW-HA, increased 3.11 or 2.95-fold with Tr1/Treg respectively. In vivo, Tr1 treated mice wound showed expedited wound closure at d7 and significantly greater aSMA (IHC), though both CD4+ and Tr1 treatments resulted in significantly reduced collagen content at d28 (trichrome). Reduced F4/80 were observed in all wounds treated with T cells (IHC). Luminex assay revealed wounds treated with Tr1 had decreased proinflammatory cytokines IL-6, MCP-1, and GM-CSF. Conclusions: IL-10 producing CD4+ cells regulate fibroblast ECM deposition and inflammatory cytokine balance to attenuate fibrosis. Promoting Tr1 recruitment, IL-10 production, and HMW-HA synthesis in wounds may a therapeutic target to improve wound healing.

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RNase L is a regeneration repressor gene

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Mammalian injury responses are characterized by fibrosis and scarring rather than functional regeneration. Limited regenerative capacity in mammals could reflect a loss of pro-regeneration programs or active suppression by genes functioning akin to tumor suppressors. To uncover programs governing regeneration in mammals, we performed comprehensive transcript screening in human subjects after laser rejuvenation treatment and cross-referenced these transcripts to those found in mice with enhanced Wound Induced Hair Neogenesis (WIHN), a rare example of mammalian organogenesis. We find the anti-viral endoribonuclease RNase L to be a powerful suppressor of regeneration. Rnasel$^{-/-}$ mice exhibit remarkable regenerative capacity, with elevated WIHN (n=10, p<0.0001) through enhanced IL-36α (n=3, p<0.01). Consistent with the known role of RNase L to stimulate caspase-1, we find that pharmacologic inhibition of caspases promotes regeneration (n=3 versus 4, p<0.001) in a novel IL-36-dependent manner (n=4, n.s.= not significant). Additionally, these responses are not limited to skin but extend to other organs, such as the colon (n=4, p<0.05), suggesting that suppression of regeneration is a fundamental characteristic of epithelial wound healing. Taken together, this work suggests that RNaseL functions as a regeneration repressor gene in a functional tradeoff that prioritizes host antiviral abilities and is a target to enhance healing in multiple epithelial organs, perhaps even during viral infection.

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Development of a tissue-engineered immunocompetent psoriatic skin model for the study of communication between T cells and pathological epithelial cells

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Psoriasis is a common immune-mediated skin disease involving a wide range of epithelial and immune cells. Over the years, the efficacy of biological treatments targeting the IL-23/IL-17 axis has highlighted their central role in the psoriasis pathogenesis. The development of an adequate model bringing together the diverse important components of the disease remains a major challenge in the understanding of psoriasis' molecular basis. In this study, we investigated the impact of activated T cells on the inflammatory microenvironment characteristic of psoriasis when included in our tissue-engineered psoriatic skin model. To this end, activated T cells were seeded into healthy (HS) and psoriatic (PS) skin models. PS inflammatory microenvironment exhibited significantly higher expression of various chemokines and cytokines such as CCL2, CXCL1, CXCL10, IL-1ra, IL-6, CXCL8 and IL-17A compared to HS. Moreover, PS showed an increased T cells infiltration into the pathological epidermis. These results highlighted the role of pathological keratinocytes in the initiation of cutaneous inflammation. This study describes the first model that uses primary psoriatic skin cells and enriched T cells capable of long-term production of IL-17A. This new model could stand out as a convincing tool for studying the communication occurring between epithelial and immune cells in psoriasis.

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TNFα in impaired diabetic wound healing: A role for GM3

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Type 2 diabetes (T2D) is associated with chronic exposure to increases in tumor necrosis factor alpha (TNFα), which contributes to insulin resistance and the development of foot ulcers in 25% of affected individuals. The mechanism by which TNFα leads to insulin resistance and poor wound healing is unclear. We hypothesized that glycosphingolipid GM3 is a critical mediator of TNFα-induced pathology in diabetic skin. In undifferentiated, cultured normal human epidermal keratinocytes (NHEKs), chronic TNFα treatment (100 pM x 4 days) increased the expression of both GM3 (8-fold increase, P<0.001) and its synthesizing enzyme GM3 synthase (*GM3S*) (4-fold increase, P<0.01), as shown by lipidomics, flow-cytometry and RT-qPCR. Chronic TNFα prevented insulin-like growth factor 1 receptor (IGF1R) autophosphorylation in NHEKs, and GM3 depletion by either small molecule inhibition of GM3 synthesis or knockdown of *GM3S* expression reversed the inhibitory effect of TNFα on IGF1R activation. Additionally, chronic TNFα slowed keratinocyte migration at all time points beginning at 3 hours in a 2D scratch assay (P<0.01), which was reversed by GM3 depletion. To further test the role of increased GM3/GM3S on wound healing in human T2D, we generated a diabetic 3D human skin equivalent (HSE) model using diabetic foot ulcer fibroblasts (DFUFs) and normal keratinocytes. After wounding the 3D diabetic HSE model, closure was delayed by 65% compared to wounded 3D HSEs with normal foot fibroblasts (NFFs) (P<0.01). Treatment of NFF-embedded HSEs with chronic, low-dose TNFα (100 pM) impeded wound healing by 50% at 2 days post-wounding (P<0.02), while glucosylceramide synthase small molecule inhibitor treatment, which dramatically reduced GM3 levels, improved wound healing in DFUF HSEs by 40% and 80% at 2- and 3-days post-wounding, respectively (P<0.02). These data suggest that GM3 is a key downstream modulator of TNFα in causing cutaneous insulin pathway resistance and further supports reduction in GM3 as a treatment for chronic diabetic wounds.

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Hyperthermia-induced plasma membrane translocation of aryl hydrocarbon receptor promotes phosphorylation of EGFR in human keratinocytes

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Hyperthermia as a proved effective measure to treat HPV infectious skin diseases has been widely explored in our team. Aryl hydrocarbon receptor (AHR) is a classic ligand-dependent transcriptional factor mainly in cytoplasm and nucleus even though recent researches have turned to focus on its 'non-genomic' function. We previously observed that simply overexpression of AHR led to increased phosphorylation of epidermal growth factor receptor (EGFR) at Y1068 site, which indicated some spatial interactions existed between AHR and EGFR. Meanwhile, hyperthermia treatment at 44°C in human HaCaT cell line could also promote p-EGFR levels. Thus, in this study, we treat cell at 44°C with different time points and results showed p-EGFR levels increased while hyperthermia for 20 minutes and 30 minutes with the maximum. Next, we heated cells with knockdown of AHR at the same points and we found p-EGFR levels were decreased compared with negative control (NC). In this way, we hypothesized hyperthermia might induce AHR plasma membrane translocation to interact with EGFR in certain complex form. Immunofluoresence and Western Blot results showed when treated at 44°C for 5 minutes, membrane protein levels of AHR were strikingly elevated and cell membrane AHR staining intensities were stronger than NC group. The spatial and temporal disparities along with the fact AHR was not a kinase to phosphorylate EGFR led us continue to search for complex formed by AHR and EGFR since co-immunoprecipitation results indicated interactions between AHR and EGFR. Since phosphorylation of EGFR is of great significance in cell life decision, mobility and differentiation, we believe our study deserve further exploration. In conclusion, we found hyperthermia at 44°C for 5 minutes could induce AHR plasma membrane translocation to involve in the phosphorylation of EGFR in human keratinocytes.

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Langerhans cells promote revascularization and repair during skin wound healing

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Skin wound healing is a complex and tightly regulated process that is essential for human health. In order to efficiently recognize tissue damage, clear invading pathogens and cellular debris, and then regenerate new, healthy skin, diverse cell types must communicate and coordinate their behaviours. Innate immune cells perform several functions that are essential for proper wound healing, including producing signals to initiate reparative processes within the skin macroenvironment. Langerhans cells (LCs) are a subset of antigen presenting cells that reside in the epidermis and are capable of detecting and responding to skin damage. However, little is currently known about the functional contributions of LCs to wound healing. Here, we show that LCs are poised at the edges of wound beds throughout wound healing, and that multiple repair processes are abrogated in the absence of LCs. Skin wounds in LC-depleted mouse models exhibit pronounced defects in blood vessel regeneration, including reduced endothelial cell proliferation and impaired vessel formation. We also show that during wound healing, LCs express a program of genes involved in blood vessel growth and development, including *Vegfa*, a canonical pro-angiogenic signalling factor. These data suggest a novel role for LCs in directly promoting re-vascularization and tissue regeneration following skin injury.

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Contribution of miRNAs as epigenetic regulators of retinol efficacy in the skin dermis

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Vitamin A and its derivatives, particularly retinol, slow both chronological and photo-induced aging processes. Retinol reduces the appearance of wrinkles and the decrease of the skin firmness and elasticity by protecting against the collagen and elastin fiber changes. In addition to retinol's rejuvenating skin benefits through direct transcriptional activation, recent studies also suggest an epigenetic regulation through micro-RNAs (miRNAs) modulation. As miRNAs play key roles in controlling retinoid-dependent stem cell differentiation, we investigated if collagen and elastin fibers synthesis increased by retinol treatment, could be similarly regulated by miRNA. We investigated how retinol supports the stimulation of type I collagen and elastin through proteomic, transcriptomic and epigenetic miRNA-expression changes in human skin fibroblasts. Five miRNAs, previously described as targeting collagen or elastin gene expression, were particularly studied after validation of their expression in normal human fibroblasts by miRNA sequencing analysis. Retinol reduced the expression of the five studied collagen- or elastin-inhibiting miRNAs and simultaneously stimulated both type I collagen and elastin protein. Thus, characterizing the epigenetic activity of retinol may contribute to increase the knowledge on its mode of action related to rejuvenating skin benefits.

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Early-stage bilayer tissue-engineered skin substitute formed by adult skin progenitor cells produces an improved skin structure *in vivo*

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Significant progress has been made in developing highly complex tissue-engineered skin substitutes (TESSs) for wound healing in recent years. However, the time required, and the lack of skin appendages, such as sweat glands and hair follicles, are two major limitations that hinder its application in the clinic. It is necessary to develop a competent TESS in a short time to meet the needs for clinical applications. Adult scalp dermal progenitor cells and epidermal stem cells together with type I collagen as a scaffold material were used to reconstitute bilayer TESSs in vitro. TESSs at 4 different culture times (5, 9, 14, and 21 days) were grafted onto full-thickness wounds created in the dorsal skin of athymic nude/nude mice. The skin specimens formed from grafted TESSs were collected 4 and 8 weeks later and then evaluated by histological analysis, immunohistochemistry, and immunofluorescent staining. Early-stage bilayer TESSs after transplantation had a better efficiency of grafting. A normal structure of stratified epidermis was formed in all grafts, but higher levels of the proliferation marker Ki-67 and the epidermal progenitor marker p63 were found in the epidermis formed from early-stage TESSs. Interestingly, the transplantation of earlystage TESSs produced a thicker dermis that contained more vimentin- and CD31-positive cells, and hair follicle formation was only observed in the skin grafted from early-stage TESSs. Early-stage TESSs expressed high levels of p63 but had low expression levels of genes involved in the activation of the apoptotic pathway compared to the late-stage TESSs in vitro, they should potentially provide better wound healing when applied in the clinic in the future.

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Effect of titrated extract of *centella asiatica* on skin repair process

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Normal wound repair is a dynamic and complex process involving multiple coordinated interactions between various skin cells, cytokines, chemokines and growth factors. Titrated extract of *Centella asiatica* (TECA) is known to have benefits on skin repair as it acts on different wound healing and protection-related biological processes. Previous in vitro study revealed TECA was able to sooth and limit epidermal cell damages by inhibiting inflammatory cytokine IL-1α and protecting skin against free radicals. It also showed efficacy to accelerate the skin repair process in the dermis by promoting fibroblasts migration and reducing glycation. Furthermore, TECA participates in skin remodelling and maturation by regulating angiogenesis (TSP-1, VEGF) and stimulating contraction (α-SMA) and protection of the ECM against degradation by limiting MMP-1 and MMP-9 secretion. Our aim was to investigate TECA's effects on the skin repair process in vivo. A clinical study was conducted as a randomized double blind trial, to compare the effects of a formula containing 0.2% TECA versus a placebo on damaged skin. Volunteers willing to have a laser intervention were recruited. Respecting the integrity of the epidermis, a non-ablative fractional laser created thermal lesions within the dermis, defined as micro-thermal zones (MTZ), thus generating both inflammation and ECM damages stimulating the repair process. An inflammation was also induced within the epidermis via the laser act. First, using ultrasound imaging analysis, we could observe a repairing and anti-inflammatory effect of the TECA-containing formula. TECA was able to increase the dermis density from day 1 after product application in comparison with placebo and to reduce MTZ height from day 2. Furthermore, using confocal microscopy, we could observe after 4 days of application, a return to baseline of the inflammation signal density in the TECA group in comparison to 14 days for the placebo group. In conclusion these results showed TECA's ability to efficiently repair damaged skin and reduce inflammation.

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The impact of a wound dressings starter kit on hidradenitis suppurativa patient quality of life

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Hidradenitis suppurativa (HS) is a devastating disease leading to inflammatory nodules, abscesses and tunnels that greatly impact the quality of life (QOL) of patients. Lesions are often painful and require multiple wound dressing changes per day due to exudate and odor. Despite the need, a HS-specific wound care protocol has yet to be developed. Our preliminary data suggested that no single dressing can address the heterogeneity of the disease. We therefore hypothesized that giving patients a kit with different classes of dressings will address this gap. We designed a feasibility, prospective cohort pilot study to determine the effect of dressing kit usage on the QOL of people with HS. Adults with HS diagnosis by a dermatologist and at least one draining tunnel or wound were included. After consent, subjects were given a HS wound dressing starter kit with tape and 3 types of dressings: hydrogel, foam and a gelling fiber (Hypafix Gentle Touch®, Cutimed® Sorbact® Hydroactive B, Cutimed® Siltec®, Sorbion® Sana multi-star, Essity, Hamburg, Germany). After 2 weeks, subjects returned to receive only the dressings they liked for an additional 4 weeks. Subjects kept usage logs, and data on disease severity, concomitant treatments and QOL using the Dermatology Quality of Life Index (DQLI) was recorded. Here we report our preliminary data of 11 patients after 2 weeks of kit usage. The cohort consisted of patients with moderate-severe HS, of which 64% were women and the average age was 33. DQLI significantly improved from the initial visit (M=18, SD=9.02) to the second visit (M=10, SD=6.69) after 2 weeks of using the dressings starter kit (p=0.003, CI 3.34, 12.66). This population, although small, is consistent with the known HS demographics and baseline DQLI scores are similar to baseline scores in other studies. Accessibility to quality dressings in the form of a HS wound dressing starter kit may improve quality of life for HS patients.

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PLGA-immune modifying particles as a potential therapy for treating injured skin

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Inflammatory monocytes, especially macrophages, play key roles in cutaneous wound healing. Immune-modifying particles (IMPs) composed of biodegradable poly(lactic-*co*-glycolic acid) (PLGA-IMP), an FDA-approved biopolymer, are taken up by blood-borne inflammatory monocytes via the scavenger receptor MARCO (macrophage receptor with collagenous structure). Circulating monocytes have a propensity to engulf particulate material, and daily dosing of PLGA-IMP induces monocyte apoptosis and their sequestration within the spleen. We have shown that PLGA-IMP has anti-inflammatory properties and can block a cytokine storm resulting in improvement of immune-induced pathology in diverse tissues. Using a nitrogen mustard (NM)-induced skin injury model in C57/BL6 mice we examined whether PLGA-IMP intervention is protective from severe acute skin inflammation, induration, swelling and vesication. Here we show that NM-induced skin injury induces infiltration of MARCO+ macrophages. Daily intravenous administration of PLGA-IMP post NM insult decreased infiltration of inflammatory monocytes into the wound area, and significantly reduced skin edema ($p<0.0001$ days 1 to 5, n=19). PLGA-IMP treatment also delayed eschar formation and significantly promoted skin repair (p=0.047, n=3). *Ex vivo* analysis of the skin demonstrated reduction in numerous chemokines and inflammatory factors. The most notable effects include a 60% reduction of IL-1β, a hallmark biomarker indicative of NM-induced skin damage (p=0.027, n=4), and a 40% increase in CCL2 (p=0.04, n=9), a chemokine that recruits monocytes to inflammation sites. In summary, we describe a novel therapeutic that protects mice from severe injury following toxic exposure to a chemical insult. PLGA-IMP is in advanced clinical trials and may represent a treatment for life-threatening injuries including burns and toxic skin reactions.

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IRF6 is required for directed cell migration in murine primary keratinocytes

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Interferon regulatory factor 6 (IRF6) plays a significant role in wound healing as it regulates keratinocyte migration, proliferation, and differentiation. Patients with *IRF6* mutations display increased post-surgical wound healing complications and previous studies in vitro show that in the absence of IRF6, keratinocytes are unable to efficiently polarize and migrate in the direction of the wound. However, nothing is currently known about the role of IRF6 in the polarization of migrating keratinocytes. To understand the function of IRF6 in directed cell migration, wild-type and *Irf6*-deficient murine primary keratinocytes were cultured in medium without Epidermal Growth Factor (EGF) and with low calcium concentration (baseline cell growth medium) on collagen IV. Characterization of cell migration was performed following live imaging of cells plated at low density on laminin 5 in unstimulated (baseline cell growth medium) or stimulated (with EGF and high calcium) conditions. Wild-type cells in stimulated conditions were shown to travel greater distances, at faster speeds, and were more persistent in maintaining directed cell migration than wild-type cells in non-stimulated conditions. *Irf6*-deficient cells in stimulated conditions, however, were shown to travel shorter distances, travel at slower speeds, and were less persistent in maintaining directed cell migration than wild-type cells in stimulated conditions and showed no change from *Irf6*-deficient cells in unstimulated conditions. Together, these data demonstrate that IRF6 is required for directed cell migration in murine primary keratinocytes and contribute to the understanding of wound healing defects in patients with *IRF6* mutations.

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Lineage tracing at single-cell resolution unveils complex differentiation trajectories of adipocyte precursors in the skin

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White adipose tissue (WAT) plays fundamental roles in the skin, including participating in the immune response, wound healing, and hair growth. The generation of mature adipocytes, which is key for the role of WAT in the skin, relies on the proliferation and differentiation of adipocyte precursors (APCs). However, heterogeneity within the APC population prevents current lineage-tracing tools from tracking and identifying the differentiation process of these cells. Using single-cell RNA sequencing (scRNA-seq) we reveal the extent of APC heterogeneity in the skin, identifying two major populations of APCs (progenitors and preadipocytes) that give rise to mature adipocytes in vitro and in vivo. To identify a possible lineage relationship between progenitors and preadipocytes we have developed a barcode-based cell-tracing assay that is compatible with scRNA-seq (CellTag). Using CellTag, we are able to trace the differentiation paths of APCs. Our results show that our approach is suitable to trace progenitor and preadipocyte cells in their differentiation process. Furthermore, our CellTag approach allows us to identify that progenitor and preadipocyte cells have non-overlapping differentiation trajectories, unveiling a complex process in the generation of mature adipocytes in the skin. Together, our findings suggest that the origin of APCs (progenitor or preadipocyte) determines their differentiation trajectory. Our results are relevant for the understanding of adipogenesis in the skin during homeostasis and disease.

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Adipocyte-derived fatty acids induce metabolic activation of macrophage differentiation in the wound bed

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After injury, a timely inflammatory response that involves the recruitment of blood-derived circulating monocytes is essential for tissue repair. Once in the wound bed, these cells differentiate into macrophages during the inflammatory phase, a process that goes awry during aging in chronic non-healing wounds. Cell differentiation is a highly energy-demanding process, and metabolic substrates present in the wound bed niche are important for the wound healing outcome. We previously demonstrated that dermal adipocytes release fatty acids into wound beds after injury to promote inflammation, yet the function of adipocyte-derived fatty acids in the initiation of the inflammatory response after injury is not known. To unveil the role of adipocytes as providers of fatty acids used to fuel the metabolic requirements of immune cell differentiation, we set out to evaluate their role on monocyte to macrophage differentiation in the skin wound bed. Here, we utilize *in vivo* mouse models of skin injury and metabolic assays to reveal that monocytes utilize fatty acids to fuel metabolic programs that induce macrophage differentiation. We show that extracellular vesicles (EVs) loaded with lipids are actively taken up by monocytes *in vitro* and in mice *in vivo*. Using real-time respirometry, we show that these fatty acids activate the b-oxidation metabolic pathway in monocytes and its inhibition leads to the abrogation of macrophage differentiation. Furthermore, we show that with age, not only adipocyte-derived EV production was impaired, but more importantly, these particles were less effective in rewiring monocyte metabolism towards oxidative phosphorylation. In summary, our findings reveal an essential adipocyte-monocyte metabolic axis that controls inflammation in the wound bed niche.

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Regulation of IFN kappa in keratinocytes of diabetic wounds

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Chronic inflammation in non-healing wounds of Type 2 Diabetes (T2D) patients represents the most common cause of amputation and mortality. Thus, a critical need still exists for understanding the wound healing defects in T2D to develop better targeted therapies. Current data points to a role for keratinocytes in orchestrating appropriate wound healing and demonstrates that wound keratinocytes in T2D are dysfunctional; however, the mechanisms that regulate this dysfunction is unclear. Interferon kappa (IFNk), a type I IFN primarily produced by keratinocytes, has been shown to play an important role in other chronic skin diseases. Thus, this project explores the role of keratinocyte-mediated IFNk in wound repair. To examine IFNk expression, we utilized skin samples from T2D patients and mice, along with their respective controls. We found that IFNk expression is impaired in both human and murine models of T2D wounds. This attenuation is particularly noted in basal keratinocytes. Interestingly, we identified by ChIP PCR the IFNk promoter in T2D keratinocytes has decreased expression of H3K4me3 compared to control in a mixed lineage leukemia (MLL)-dependent manner. To further understand the role of IFNk in wound repair, a wound curve was performed on IFNk KO and WT mice. We show that knockout of IFNk impairs wound healing. Thus, our data suggest IFNk impairs wound healing in T2D patients and this attenuation of IFNk expression in keratinocytes is regulated epigenetically. Continued investigation into the mechanism through which keratinocyte-mediated IFNk impairs wound healing is key to development of novel treatments for T2D patients.

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Single cell transcriptomics identifies a two way conversion program between dermal progenitors and adipocytes during skin development and regeneration

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Dermal white adipose tissue (dWAT) is a unique layer of adipocytes and their fibroblast precursors within the reticular dermis of the skin, and recent studies have found that dWAT plays an essential role in skin development and regeneration. However, little is known how adipocytes are developed from their dermal progenitor and how adipogenesis is controlled during skin regeneration. Here, we performed single-cell RNA-sequencing (scRNA-seq) analyses of dermal fibroblasts (dFBs) isolated from mice at various post-developmental ages. Pseudotime analyses of these dFB clusters identified a $Pdgfra^{+}CD24^{hi}Thy1^{lo}Sca1^{lo}$ population as developmental progenitors for preadipocytes (pAD) and this progenitor population was highly abundant in neonatal skin early in life but declined in adulthood. In contrast, a $Pdgfra^{+}Sca1^{hi}DPP4^{hi}$ adult reticular interstitial (RI) adipocyte progenitor (AP) population that developed postnatally was found in adult skin. ScRNAseq analyses were then performed on dFBs from wounded skin to determine the cell fate and role of these adult RI-APs during skin regeneration. Pseudotime analysis and immunofluorescence analysis of wounded skin showed that RI-APs rapidly infiltrated the wound center and gave rise to pAD/AD and/or myofibroblasts during early stages of wound healing, contributing to dermal regeneration. Pathway analyses revealed that this two way conversion between dermal progenitors and pAD/AD was dynamically regulated by an interplay between the WNT, TGFβ and NFKB pathways. Together, our results have identified and defined the developmental pathways that shape adipogenesis from dermal progenitors during skin development and regeneration. These results provide insights into how defective adipocyte progenitor function and adipogenesis may be associated with defective wound healing associated with aging and diabetes and/or fibrotic diseases such as keloid scarring.

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Mechanical stretch mobilizes Lgr6+ stem cells to drive skin growth

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In mammals, most organs achieve a final size at maturity and vary little thereafter. By contrast, the body's largest organ — skin — is capable of dramatic changes in size in adults in response to physiologic cues like pregnancy or pathophysiologic cues like obesity. This growth capacity is also utilized therapeutically in a process called tissue expansion wherein surgically placed subcutaneous balloons are gradually inflated to create new skin. The mechanisms underlying the striking capacity for adult skin growth in these various circumstances are unclear. Here, we utilize a system of controlled tissue expansion in mice to uncover cellular and molecular determinants of stimulated skin growth. Through machine learning, automated image alignment and annotation, and three-dimensional tissue reconstruction, we capture morphometric changes accompanying skin growth in response to the expansion. While all skin compartments are subjected to stretch in this system, growth is principally driven by proliferation of the epidermis, with more limited changes in dermal and subdermal compartments. Epidermal growth is in turn achieved through preferential mobilization of Lgr6+ epidermal stem cells and their descendants in a manner dependent on the Hippo pathway effector YAP. Forced activation of YAP in epidermal stem cells results in greater epidermal proliferation with more skin growth per expansion stimulus. Through the use of single-cell RNA sequencing, we uncover further changes in Hippo pathway components as well as in transcripts like integrins, focal adhesion kinase, and PI3K-AKT which may transduce the physical stretch stimuli into epidermal growth and proliferation. In aggregate, these studies point to therapeutic strategies to enhance skin growth and establish a platform for understanding organ size dynamics in adult mammals.

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Factors associated with prolonged wound healing

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Background: Wounds have significant impact on patient morbidity and quality of life, and are also associated with substantial economic burden. In Calgary (Canada), patients with challenging wounds are referred to a dedicated multidisciplinary wound clinic facilitated by physicians that specialize in wound care. Determining factors that contribute to prolonged wound healing is key to optimizing patient care. Methods: After ethics approval, a retrospective chart review was performed of new referrals to the wound clinic from January to March 2018. Results: Of the 112 new patients referred to the wound clinic, 72.3% (81/112) experienced complete healing of their wound within one year from their first appointment. The average time to heal chronic wounds after the initial wound clinic visit was 86.7 days, significantly shorter than the average wound duration prior to the initial visit (189.9 days, $P < 0.05$). Unhealed wounds were felt to be secondary to the absence of a surgical option (5.4%, 6/112), non-compliance with compression or off-loading (4.5%, 5/112) and intolerable compression (1.8%, 2/112); some patients were lost to follow-up or had no open wounds at the initial appointment (16.0%, 18/112). The average age of referred patients was 66.1 years old. The average wound healing time in patients 70 years and older was 155.4 days, significantly longer than the wound duration of patients under 70 years old (104.1 days, $P < 0.05$). Wound duration was significantly longer for patients with three or more comorbidities than those with fewer comorbidities (142.1 vs. 83.8 days, respectively, $P < 0.05$). Cigarette smoking was identified in 35.7% (40/112) of patients, and was associated with significantly longer time until healing began than in those that did not smoke (60.4 vs 26.1 days, $P < 0.05$). Conclusions:The expertise from a dedicated wound clinic accelerated wound healing. Factors associated with prolonged wound healing included advance age, multiple comorbidities and cigarette smoking.

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Abrogation of *Sox9* expression in the endothelium blocks aberrant vascular EndMT and fibrosis

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The endothelium possesses a profound regenerative capacity to adapt and reorganise in homeostasis and disease. The capacity to regenerate is increasingly attributed to a population of vessel-resident endovascular progenitor (EVP) cells that governs an endothelial hierarchy and have the ability to form entirely new *de novo* vascular networks. Using fate map analysis, we show that two transcription factors *Sox9* and *Rbpj* specifically demarcate the EVP population and regulates lineage specification; either endothelial or mesenchymal. Conditional knock-out of *Sox9* from the vasculature (*Sox9*$^{fl/fl}$/*Cdh5-Cre*^{ER} *RosaYFP*) drove the depletion of EVP to a mature differentiated endothelial phenotype with a complete loss of self-renewal capacity and enhanced *Rbpj* expression and Notch signalling. Additionally, skin wound analysis from *Sox9* knock-out mice demonstrated a significant reduction in pathological endothelial to mesenchymal transition (EndMT) resulting in reduced scar area. The converse was observed with *Rbpj* conditionally knocked-out from the vasculature (*Rbpj*$^{fl/fl}$/*Cdh5-Cre*^{ER} *RosaYFP*), with enhanced *Sox9* and EndMT-related gene expression. We now report that vascular sonic hedgehog signalling (*Ptch1*$^{fl/fl}$/*Cdh5-Cre*^{ER} *RosaYFP*) upregulates the expression *Sox9* and is key in driving pathological EndMT and vascular fibrosis, resulting in over 3-fold increase in scar area in skin wound healing. Importantly, using topical administration of siRNA against *Sox9* on skin wounds significantly reduced scar area by blocking pathological EndMT. The understanding of how vascular resident EVP function opens exciting new avenues for more effective therapies in blocking vascular fibrotic disease.

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Cellular senescence profiling of chronic wounds

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Chronic wounds, including diabetic, venous, and pressure ulcers, are characterized by multifactorial delay in wound healing secondary to excessive inflammation, fibroblast senescence, and alterations in wound bed flora. Cellular senescence, an irreversible state of cell-cycle arrest, occurs in various aged tissues and at sites of pathogenesis in chronic conditions. Here we sought to delineate cellular senescence profiling potentially related to delayed or poor healing of chronic wounds. Electronic search of our institution's health records from January 2005 to December 2015 included adults diagnosed with chronic wounds biopsied ± 7 days of presentation. Sequencing was performed of RNA extracted from formalin-fixed, paraffin-embedded blocks to detect cellular senescence markers. Time-to-event analysis was performed by fitting Cox proportional hazards models. Mean age of the 79 patients was 62.5 years (range 25.9-80.4). The majority of wounds were located on the lower extremity (73; 92.4%). Patients were evaluated for diabetes (28, 35.4%), infection (22; 27.8%), vascular disease (39; 49.3%), steroid use (27; 34.2%), chemotherapy and/or radiation exposure (3; 3.8%), and smoking status (24; 30.4%). During the follow-up period, 29 healed with a median time between wound care date and evaluation of 4.4 months (IQR, 2.1-6.4 months; range 4 days-32.1 months); the median follow-up for the remainder was 2.8 months (IQR 0.4-10.4). Focusing on the first 9 months of follow-up, quantitative PCR showed those patients with lower expression levels of CDKN1A ($p21^{CIP1}$ senescence marker) were more likely to heal (HR=1.48 per 1 unit increase, 95% CI 1.06-2.07; p=0.022), as were patients with lower expression levels of IL8 (HR=1.11, 95% CI 0.99-1.25; p=0.075) and TNC (HR=1.41, 95% CI 0.99-2.01; p=0.055). Future studies will evaluate if senescent cell clearance enhances kinetics of chronic wound closure.

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Hair follicle grafting therapy to accelerate functional regeneration of chronic non healing wounds

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Cutaneous wound healing is a complex process involving highly regulated cross-talk between multiple cell types that work to heal the injury and restore the barrier function. A perturbed wound healing program in conditions such as diabetes, leads to chronic wounds which do not heal by standard treatment. Autologous hair follicle grafting has recently been discovered as a novel therapy to stimulate closure of such non-healing wounds. We investigated the quality of repair of chronic ulcers in a cohort of Indian patients with chronic leg ulcers, three months after receiving autologous hair follicle graft. Our study showed that hair follicle grafting facilitated regeneration of various undifferentiated and differentiated layers of the epidermis with restoration of E-Cadherin junctions between epidermal keratinocytes renewing barrier function. Impaired blood supply and tissue fluid drainage was resolved via regeneration of blood and lymph vasculature in the dermis. Loss of perception to touch and impaired thermoregulation in the wound area was rescued by regeneration of nerve and sweat gland structures respectively. Interestingly, mRNA levels of commonly upregulated inflammatory cytokines in chronic wounds such as IL1α, IL6, TNFα and IL8 were more than 40% decreased ($p<0.001$) in post-treatment skin with a comparable decrease in inflammatory cell numbers. This raises the interesting possibility of anti-inflammatory soluble factors derived from the grafted cells. Our observation that wounds created on the healed area post-treatment, heals with normal wound healing kinetics suggests that hair follicle grafts which contains multiple stem cell pools have the potential to sustainably regenerate multiple cutaneous compartments in chronic wound beds.

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Single-cell transcriptomics of human pressure ulcers reveals MHCII expressing keratinocytes in patients with a worse healing outcome

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Pressure ulcer (PU) is a chronic non-healing wound caused by continuous pressure of the bodyweight to the skin, which is often seen in spinal cord injury patients and the bedridden elderly population. Despite high mortality, the pathophysiology of PU remains poorly understood. Here we compared single-cell transcriptomic profiles on human epidermal cells from PU wound-edges with uninjured skin and acute wounds (AW) from healthy donors. We identified significant shifts of cellular composition and gene expression pattern in PU wound-edges, which could stratify PU into two groups. Interestingly, our study identified a subset of keratinocytes expressing major histocompatibility complex class II (MHCII), and these cells are enriched in patients with worse healing outcomes. We showed that IFNg in PU-derived wound fluid could induce MHCII expression in keratinocytes, and these wound fluid-treated keratinocytes inhibit autologous T cell activation. In line with this, we found that T cells from PUs enriched with $MHCII^+$ keratinocytes produced less inflammatory cytokines. Together, our study provides a high-resolution molecular map of human PU compared to AW and the skin, which sheds new insights into the understanding of PU pathology and the future development of tailored wound therapy.

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Clinical characteristics and misdiagnosis of pyoderma gangrenosum of the head and neck

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Pyoderma gangrenosum (PG) is a rare, ulcerative neutrophilic dermatosis. PG has a misdiagnosis rate of 10-20%, likely due to the lack of specific markers. The lower limbs and trunk are most commonly affected, but rarely, patients can develop PG on the head and neck (HN). Distinguishing features of this variant have yet to be identified, which further complicates diagnosis and management. We conducted a literature review in addition to 12 previously unreported cases of HN PG to provide a framework for recognition and management of this rare clinical variant. Data collected included clinical features, microbiology and histopathology, treatment, outcome, time from presentation to diagnosis, and initial evaluating provider specialty. A total of 145 cases were reviewed; 118 cases reported outcomes (57% healing within 6 months vs 43% delayed healing). Of those with reported outcomes, the primary location was the face (90 cases); the most reported secondary location was the lower extremity (52 cases). Cases initially evaluated by a non-dermatologist versus dermatologist totaled 74 and 60, respectively. The diagnostic accuracy of non- dermatologists was 36.5%, compared to 65.0% accuracy by dermatologists. PG was most commonly mistaken for infection in both groups. HN presentations represent a unique PG variant, and these patients are often initially evaluated by non-dermatologists. Furthermore, just over one-third (36.5%) of the evaluating non-dermatologists in our review secured the correct diagnosis. While still a rare disease, accurate diagnosis and targeted treatment of HN PG generally lead to favorable clinical outcomes.

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REDD1 (regulated in development and DNA damage 1) is essential for skin wound healing

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The major adverse effects of topical glucocorticoids (GCs) are skin atrophy and impaired wound healing, especially pronounced in older patients. Previously we showed that knockout of REDD1, a negative regulator of mTOR/Akt signaling, protects skin from GCs-induced atrophy. Here we investigated whether REDD1 status affected wound healing delay induced by GCs using REDD1 KO and isogenic C57Blx129 12-15-month-old mice. Wound healing in untreated REDD1 KO mice was slightly delayed compared to WT animals. Glucocorticoid fluocinolone acetonide (FA) significantly delayed wound healing in WT and especially in REDD1 KO mice. As expected, FA inhibited keratinocyte proliferation near the wound edge in WT animals. In contrast, in REDD1 KO, FA increased the proliferation by ~50-70%, resulting in epidermal hyperplasia above the level induced by wounding. The complex process of wound healing involves increased keratinocyte migration and proliferation at the wound edge. Thus, we accessed the effect of REDD1 on proliferation and motility using human HaCaT keratinocytes with CRISPR/Cas9-generated REDD1 KO. In a scratch assay, the wound closed faster in REDD1 KO than in WT/Cas9 cells. However, when proliferation was inhibited by Mitomycin C, WT and REDD1 KO keratinocytes showed similar migration activity. In correlation with in vivo results, REDD1 KO HaCaT cells became resistant to FA anti-growth effects. RNA-seq analysis of the WT and REDD1 KO HaCaT transcriptome revealed significant alteration of FA molecular signature and consistent upregulation of cell cycle regulators CDK1, CCNA2, CCNB1, CCNB2 and proliferation marker PCNA in REDD1 KO keratinocytes. Overall, REDD1 regulated keratinocyte growth but not motility, suggesting that abnormal, non-controlled keratinocyte proliferation during skin wound healing could be an undelaying mechanism of significant wound healing delay induced by GCs in REDD1 KO mice.

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miR193b-3p suppresses wound healing and tumor formation in diabetic foot ulcers

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There are many cellular and molecular parallels between chronic wounds and cancer. Diabetic foot ulcers (DFUs) exhibit a cancer-like cellular microenvironment mirrored by a hyperproliferative epidermis, activation of β-catenin pathway and c-myc overexpression. However, malignant transformation is uncommon in DFUs. To understand molecular mechanisms that contribute to such unique cellular phenotype, we focused on miRNAs (miRs) in DFUs. We found induction of miR193b-3p in the epidermis of non-healing DFUs, but not of healing wounds, venous leg ulcers or cutaneous squamous cell carcinoma (cSCC). To dissect its mechanism in DFUs, we tested ectopic overexpression of miR193b-3p and found that it inhibits migration of human keratinocytes and fibroblasts. Moreover, miR193b-3p showed a dominant negative effect on keratinocyte motility by suppressing wound closure even in the presence of pro-migratory miRs. Overexpression of miR193b-3p in the organotypic model and murine wounds *in vivo* resulted in inhibition of wound healing. Anti-migratory effect of miR193b-3p was driven by disruption of stress fiber formation and inhibition of RhoA activity. Using transcriptomic approach, we further identified suppression of miR193b-3p target network involved in migration in DFUs. In addition, miR193b-3p targets that orchestrate neoplastic transformation were found differently regulated between DFUs and cSCC. Moreover, we identified proto-oncogene KRAS as a central effector of miR193b-3p anti-healing and tumor suppressive activity in DFUs. In summary, we identified miR193b-3p as a master regulator of non-migratory epidermis in DFUs that could serve as therapeutic target for both wound healing and cancer.

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Bioprinted skin integrates into full-thickness porcine wounds and supports healthy skin repair by modulating inflammation and tissue remodeling

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The purpose of this study is to assess the feasibility and efficacy of autologous bioprinted skin in the treatment of full-thickness porcine wounds. We hypothesize that bioprinted skin constructs will improve wound healing by modulating inflammation, skin remodeling, and epidermal maturation. Porcine keratinocytes, fibroblasts, pre-adipocytes, and endothelial cells were isolated, expanded, suspended in bioink, and bioprinted to form a biomimetic trilayer skin construct. 5x5cm excisional full-thickness wounds were then treated with bioprinted skin from autologous or allogeneic cells, skin autograft or allograft, hydrogel, or left without treatment. Digital planimetry of photographs taken over 28 days demonstrated improved wound closure in autologous bioprinted skin treated wounds. Histological analysis confirmed these results and showed improved epidermal maturity, less fibrosis, and more normal collagen organization in the bioprinted autologous skin group. Hydrogel and skin allograft groups had increased total wound serum proteins and proteolytic activity, with an associated increase in inflammatory and proteolytic gene expression compared to the bioprinted autograft. These findings suggest that treatment of full-thickness porcine wounds with hydrogel generates inflammatory wound healing and protease activity. Alternatively, bioprinted constructs composed of autologous skin cells embedded in hydrogel induce healthy, pro-remodeling protease activity, resulting in normal wound healing. Taken together, our results suggest that bioprinted autologous skin rapidly integrates into the wound to support skin regeneration and confirms the feasibility of skin bioprinting technology for clinical treatment of full-thickness wounds in human patients.

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Role of energy metabolism in patient heterogeneity in the degree of fibrosis in dermal wounds

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Similar dermal wounds in different patients can result in varying levels of fibrosis. Proliferation and ECM production by fibroblasts(FB) relies on energy metabolism through oxidative phosphorylation and glycolysis to fuel tissue repair. We hypothesize innate differences in FB bioenergetic metabolism underlie scarring variants. Using paired normal-skin and cesarean scar tissue and FB from abdominoplasty samples in patients with low(LS) and high scar(HS) phenotypes clinically stratified with Vancouver Scar Scale <3 vs. >6 respectively, oxidative phosphorylation(OCR), glycolysis(ECAR), ATP-production at resting and stress/hypoxia(seahorse-assay), mitochondrial membrane potential($\Delta\Psi$m;JC-1) and mitoROS(MitoSOX) were measured. LS/HS FB(10^6 cells/wound) were added to full thickness-stented wounds on SCID mice(female,8-10wk). Wounds were harvested at d7,28. Wound closure(H&E), a-SMA and UPC2-3(RT-PCR), inflammatory profile(Luminex;IHC), collagen expression(trichrome) were analyzed. n=3-4 independent cells/group;p-value by ANOVA. Both normal-skin and scar FB from HS had higher basal OCR and ECAR than LS($p<0.01$), suggesting more bioenergetic metabolism in HS. HS FB responded to stress(FCCP/Oligomycin treatments) with a significant increase in respiratory and glycolytic reserve capacity than LS($p<0.01$). Under hypoxia, OCR decreased in both HS and LS FB, but only HS showed a significant increase in glycolysis. HS normal-skin FB had more depolarized mitochondria and mitoROS than LS($p<0.01$). Proteome-profiler assay showed Hsp-27 phosphorylation(p-Hsp-27Ser82) was significantly lower($p<0.001$) in HS FB. In vivo, murine wounds with HS FB showed expedited wound closure at d7 and reduced IL-10, IL-17, MIP-1a/b, and G-CSF expression via immunoplexing, increased a-SMA(25-fold) and UCP2(18-fold) gene expression, and marked collagen staining at d28. FB of different scarring phenotypes display characteristic bioenergetic metabolism profiles and produces distinct scarring in murine wounds, suggesting a shift to aerobic glycolysis is associated with increased fibrosis in HS.

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Antimicrobial Perforin-2 in chronic wounds correlates with healing outcomes

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Venous leg ulcers (VLU) and diabetic foot ulcers (DFU) affect millions worldwide, with detrimental impact on quality of life and healthcare cost. Persistent inflammation and bacterial colonization are major factors to wound chronicity. Perforin-2 (P-2) is a highly conserved pore-forming antimicrobial protein with an indispensable role in the innate immune response against intracellular bacteria. In chronic wounds, P-2 is downregulated and leads to increased accumulation of intracellular *Staphylococcus aureus*. To further define the innate antimicrobial response in wounds, we analyzed expression of antimicrobial peptides (AMPs) in transcriptomic profiles from human acute wounds (n=6), VLU (n=10), and DFU (n=8). β defensins, S100 alarmins, RNAses and dermcidin were regulated similarly between acute and chronic wounds. P-2 was the only AMP exclusively upregulated in acute wounds while downregulated or no change in DFUs and VLUs, respectively. Moreover, treatment of chronic VLUs with bioengineered bilayered living cell construct (BLCC) modulates P-2. BLCC is an FDA-approved therapy for VLUs that we previously demonstrated shifts nonhealing VLUs (n=30) to a healing phenotype by inducing acute wound-like inflammatory response in the wound edge (NCT01327937). Using a similar genomic approach here, we find BLCC upregulates P-2 expression in VLUs to promote an enhanced antimicrobial response as part of the healing process. Specifically, P-2 upregulation was significant only in the subgroup of VLUs that healed, and not in VLUs that did not heal. This is the first evidence that a cell-based therapy in chronic wounds triggers antimicrobial response of the host. Together, our findings support a specific role for P-2 in the antimicrobial innate immune response necessary for successful wound closure of chronic wounds, and elucidate potential antimicrobial mechanisms of action by cell-based therapy.

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Clinical analysis of Hidroacanthoma simplex

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Objective: To investigate the clinical, histopathological findings, immunohistochemical features and therapeutic methods of hidroacanthoma simplex (HS). Methods: The clinical presentation, histological findings, immunohistochemical features and therapeutic methods of 5 patients with HS diagnosed between January 2015 and August 2020 in the Second Affiliated Hospital of Xi"an Jiaotong University and 17 patients with HS reported in the literature searched in CNKI were retrospectively analyzed. Results: Mean age at onset of the skin eruption was 62 years (range 36-86 years), 14 patients (63.6%) were female, and the skin lesion had been present for a mean of 7.84 years (range 5 months-50 years). Typical clinical findings were keratotic plaques mainly distributed the lower extremities and the trunk. Skin biopsy specimens from 22 patients showed the "Jadassohn phenomenon" and was composed of bland basaloid cells that were smaller than neighboring epidermal keratinocytes. Intracytoplasmic glycogen and occasional ductal structures within the nests of cells were also found. And one case (4.5%) was diagnosed as malignant HS. Immunohistochemically, the cytoplasm of some tumor cells showed a positive reaction to EMA and the normal ductal structure showed a positive reaction of CEA. Most patients (81.8%) chossed surgical resection and no recurrence and malignant transformation in follow-up. Conclusion: HS is a rare intraepidermal benign tumor, but it has risk of malignant transformation. And this disease mostly occurs in female patients, which is different from previous literature reports. For dermatologists, it is extremely important to correctly recognize and diagnose this disease.

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Dermoscopic findings and HPV genotypes of genital keratotic lesions: Bowenoid papulosis, seborrheic keratosis, and condyloma acuminatum

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Dermatologists often encounter keratotic lesions in the genital area. Although making a clear diagnosis can be difficult, it is important for the treatment and prognosis closely related to the patient's quality of life. Dermoscopy has proven to be a useful, non-invasive tool. However, there is still a lack of dermoscopic data comparing bowenoid papulosis (BP), seborrheic keratosis (SK), and condyloma acuminatum (CA). More than 40 human papillomaviruses (HPV) genotypes infect the genital area and manifest as various intraepithelial neoplasms. This study is conducted to find distinctive dermoscopic features and HPV genotype distribution of BP, genital SK, and CA. Dermoscopically, glomerular vessels were predominant in BP that appeared in 7 cases (70.0%). Hairpin vessels were the most common vascular structures that accounted for 12 cases of CA (66.7%). SK was the least vascular-patterned disease as no vessel was observed in eight cases of SK (66.7%). Mucosal pigmentation was observed in 6 cases (60.0%) of BP. Seven cases of BP (70.0%) were classified into 'flat'. SK showed cerebriform appearance in seven cases (58.3%). Most CA cases had knob-like or finger-like appearance and whitish halo. All of BP ad CA presented positive results in HPV DNA detection, while seven cases (58.3%) of SK had positive results. For the high-risk genotype, principally HPV 16, BP showed the highest detection rate with 90.0%. SK and CA showed 58.3% and 44.4%, respectively. For the low-risk genotype, principally HPV 6 followed by HPV 11, CA presented the highest detection rate, with 88.9%. BP and SK showed 40.0% and 8.3% detection rate, respectively. The coexistence of the high-risk and the low-risk HPV was seen in three cases (30.0%) of BP, one case (8.3%) of SK, and six cases (33.3%) of CA. Dermoscopy can be useful for differentiating the entity of genital keratotic lesions ahead of an invasive method and a physician should consider the morphologic plasticity of HPV-related keratosis in the genital area or the genital wart in the expanded concept.

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Validation of the Optimal Psoriasis Assessment Tool (OPAT) as a method of assessing psoriasis severity and impact from physician and patient perspectives

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OPAT is a simple tool to assess psoriasis severity using two measures: one clinical (body surface area (BSA)) and at least one of the following patient reported outcomes (PRO) – itch, skin pain, or patient global assessment of disease severity (PatGA). Previous results show that OPAT provides a straightforward and practical alternative to the Psoriasis Area and Severity Index (PASI) assessment, which is mostly used in research settings but rarely adopted in clinical practice due to its complexity. Furthermore, PASI does not capture patient perspectives. A correlation between OPAT scores and Dermatology Life Quality Index (DLQI) has also been shown. This analysis aimed to validate OPAT scores using PASI and DLQI data from the IXORA-R trial. Patients with moderate-to-severe plaque psoriasis (N=1027) were randomized to receive guselkumab (N=507) or ixekizumab (N=520). Pearson correlations were calculated for BSA and PRO measures versus PASI and DLQI at baseline, weeks 4, 8, and 12. The results from regression analysis for PASI using two measures, BSA and one PRO assessment, at week 12 showed high correlation (0.80 (PatGA), 0.78 (skin pain), 0.78 (itch)). Sensitivity analyses for OPAT versus PASI scores confirmed the results with a PASI75 sensitivity of 88.0% for the BSA and PatGA model and 85.5% for the BSA and itch model. The sensitivity of PASI90 was 87.9% for the BSA and PatGA model and 84.0% for the BSA and itch model. OPAT provides a simple alternative to assess psoriasis severity, utilizes both physician and patient perspectives, and can be easily adopted into routine clinical practice.

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Improving surveillance for merkel cell carcinoma patients: A web-based tool to interpret sequential merkel cell polyomavirus antibody test results

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Merkel cell carcinoma (MCC) is a rare skin cancer with a ~40% recurrence rate. In the US, MCC is causally linked to the Merkel cell polyomavirus (MCPyV) in ~80% of cases, while the remaining 20% are caused by UV-induced mutations. About half of MCC patients produce antibodies to MCPyV oncoproteins at diagnosis. Sero-positive patients can be tracked with a clinically available MCPyV antibody test (titer rises if disease recurs, falls if not) allowing recurrence detection that is both more sensitive and specific than imaging studies. This antibody test has been in clinical use since 2014 and is included in national cancer guidelines for MCC. Although this test is widely used in the MCC community, there are no available tools to facilitate the clinical interpretation of test results which can be challenging. For example, there is enormous patient-to-patient variability in the antibody titer, with some patients' baseline positive titers being below 100 and others being above 100,000. Here, we sought to create a web-based model that uses antibody test results to determine whether or not a patient's MCC has recurred. Our cohort consisted of 268 sero-positive patients with 1,613 antibody tests. Median follow-up was 2.9 years and 82 patients had a recurrence. A Cox model was developed using continuous, time-varying covariates. This model quantifies the absolute risk of recurrence based on the diagnosis date, current test date, previous titer and most current titers. The change in titer was strongly predictive of recurrence (HR: 1.77 for each 2-fold increase of titer, 95% CI: 1.47-2.12, $p<0.001$), independent of the current titer and time from diagnosis. This web-based tool should improve interpretation of antibody test results to guide patient-specific surveillance plans. We are currently assessing whether established baseline risk factors (stage, age, sex, and immune status) could further improve the performance of this antibody test interpretation model.

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Moisturizer prevents skin barrier damage induced by prolonged face mask usage

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Prolonged wearing of face masks, a new daily practice for people due to the COVID-19 pandemic, introduces high levels of humidity locally to facial skin, which may have unexpected skin health consequences. An IRB approved double-blinded, randomized, split-face clinical study was conducted to investigate skin properties after repeated prolonged mask usage by comparing skin inside and outside of the mask-covered areas. Twenty-one healthy female volunteers wore face masks for at least 6 hours every day for one week, with one side of their face treated with a moisturizer three times daily. On day 8, and after 5 hours of wearing the mask, facial skin properties (sebum, hydration and TEWL) were assessed at 15, 60, and 120 min post-mask removal, followed by barrier disruption and recovery evaluations. Mask usage compromised facial skin properties compared to uncovered areas, including significantly larger reduction of skin hydration ($p< 0.02$ at 15 min) and a weakened stratum corneum barrier in response to tape strip challenge ($p < 0.03$ after stripping). Sebum production also increased significantly ($p < 0.01$ at 15 min). Notably, applying a daily moisturizer mitigated these effects by significantly increasing and maintaining two-fold more hydration ($p < 0.01$) and strengthening barrier integrity against barrier challenge. Daily and prolonged usage of a facial mask, which is an essential personal and public health practice due to the Covid-19 pandemic, can create a high-humidity microenvironment, which may negatively impact skin properties. However, facial moisturization can help maintain skin homeostasis under the mask.

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Using implementation science to understand teledermatology during the COVID-19 pandemic

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Implementation science (IS) has been recognized for its potential to improve the integration of evidence-based practices into routine dermatology care. The COVID-19 Pandemic led to rapid telemedicine implementation by dermatologists worldwide. We aimed to use tools from IS to identify factors associated with the successful implementation of telemedicine during the COVID-19 crisis. An anonymous, online survey was distributed to Association of Professors of Dermatology (APD) members. It incorporated sub-scales from the Organizational Readiness to Change Assessment, a validated measure of organizational characteristics that predict implementation success. A total of 35 dermatologists responded with 91.4% in academic practice. All respondents (100%) implemented or scaled-up telemedicine during the pandemic. Most agreed or strongly agreed that they had sufficient training (68.6%), financial resources (57.1%), and facilities (57.2%). However, only 42.8% agreed or strongly agreed that they had adequate staffing support. All providers agreed that telemedicine reduced travel time and expense for patients; additional COVID-19 specific advantages included continued patient care, avoiding risk of infection, and work flexibility (from home). Barriers to telemedicine implementation included technology issues (62.9%) and challenges caring for elderly patients (51.4%). Overall, the hybrid model of synchronous video/audio visits with stored digital photographs was the most favored telemedicine modality (65.7%), and 90.6% of providers reported telemedicine was acceptable for existing patients and medication monitoring. Importantly, 94.3% of respondents plan to use telemedicine after the pandemic. This study revealed that despite the advantages of telemedicine for dermatologists during the pandemic, there were clear limitations. In sum, our survey used IS research methods to identify organizational factors that can be optimized to improve future telemedicine implementation efforts in dermatology.

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Sequencing of cutaneous squamous cell carcinoma primary tumors and patient-matched metastases reveals *ALK* as a driver in metastases and low mutational concordance in immunocompromised patients

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Background: Cutaneous squamous cell carcinoma (SCC) is a common skin cancer that is responsible for 1,000,000 cases and up to 9,000 deaths annually in the United States. Metastases occur in 2-5% of patients and are responsible for significant morbidity and mortality. Objective: The objective of this study is to perform targeted next-generation sequencing on a cohort of SCC primary tumors and patient-matched lymph node metastases. Methods: An oncology 76-gene panel was run from formalin-fixed paraffin-embedded (FFPE) samples of patient-matched primary SCCs (10) and resultant metastases (10) (Vela Diagnostics). Results: *ALK* was discovered to be a driver mutation in metastases using two different algorithms, oncoCLUSTand dNdScv. Mutational concordance between primary tumors and metastases was notably lower in immunosuppressed patients, especially among pathogenic mutations (41.7% versus 83.3%, p=0.01). Conclusions: Sequencing of matched SCC primary tumors and lymph node metastases identified genes and pathways that may have clinical importance, most notably *ALK* as a potential novel driver mutation of metastasis. Given the low mutational concordance observed, especially in immunosuppressed patients, sequencing of both primary tumors and metastases may improve the efficacy of targeted therapies.

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Validated outcome measures and post-surgical scar assessment instruments in eyelid surgery: A systematic review

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Outcome measures are important following dermatologic surgery of the eyelid. Existing dermatologic surgery assessment instruments, most of which are not anatomically specific, fail to adequately cover these outcome measures. To address this, we performed two systematic reviews of 1. validated outcome measures following eyelid surgery and 2. post-surgical scar assessment tools. We hypothesized that a single or combination of two assessment tools would encompass all validated eyelid outcome measures. Included outcome measure papers were sorted into three tiers of evidence based on study design. Then, 26 outcome measures were sorted into eight categories: Patient Subjective, Visual Function, Mechanical Function, Daily Activities, Adverse Effects, Aesthetic Quantitative: Clinical Measurements, Aesthetic Qualitative: Global, and Aesthetic Qualitative: Specific. 25 assessment tools were evaluated based on which outcome measures each covered. Our findings revealed that no combination of two existing assessment instruments cover all validated eyelid outcome measures. Outcome measures related to the subjective patient experience were included in the majority of post-surgical scar assessment scales (n=15), while outcome measures for visual function and eyelid-specific clinical measurements were absent from all existing instruments. 10 of the 26 outcome measures, across four categories, failed to be represented in any instrument. These results direct future dermatologic surgery investigators to select the best assessment instrument for their study, based on their outcome measures of interest. Most importantly, this work enables open disclosure of the limitations of a selected instrument, based on which outcome measures are excluded. This data can also inform the creation of a more comprehensive, anatomically-specific eyelid assessment tool for physicians and investigators.

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Plasma cytokine profiles in atopic dermatitis: Association with itch intensity

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Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by intense pruritus that significantly impairs quality of life. Recent advancements identified key cytokines implicated in AD pathogenesis, allowing the development of novel targeted therapies. Although some serum biomarkers of AD were previously shown to correlate with disease severity, these were limited to small sets of markers and not correlated to itch intensity. We thus explored the correlation of plasma cytokine profiles and itch severity in AD. With IRB approval, plasma samples from 15 patients with moderate-to-severe AD (11 African American [AA] and 4 whites) and 30 controls (18 AA and 12 whites) were collected at the Johns Hopkins Itch Center, and levels of 28 cytokines were measured through Luminex bead-based immunoassays. Group differences were compared with the Mann-Whitney *U* test, and multivariable linear regression models were generated with cytokine concentration as the dependent variable, and age, race, sex, and itch numeric rating scale score as independent variables. All statistical analyses were performed with Stata v.16.1. Compared to controls, AD patients had higher plasma levels of CCL18 (p=0.0476), CCL17 (p=0.0017), and OSM (p=0.0235). Itch severity scores were significantly associated with increased levels of IL-5 (p=0.013), IL-12 (p=0.001), IL-33 (p=0.019), and IFNa2 (p<0.001). Among AA AD patients, itch severity score negatively correlated with IL-10 and positively correlated with IFN-a2, IL-15, TNF, IL-5, IL-4, IL-12. Compared to Caucasians, AA AD patients had increased levels of IL-31 (p=0.0417) and IL-1a (p=0.0404). In conclusion, itch severity correlated with levels of several pro-inflammatory cytokines in AD, and racial differences in cytokine profiles were observed. With several targeted therapies in development for AD, future studies are needed to uncover cytokine profiles of various AD endotypes.

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A computational approach to understanding rosacea, acne, and hidradenitis suppurativa

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Next-generation sequencing has revolutionized the way we study biological systems at a level and a resolution never before possible. Now, clinicians must contend with how best to translate multiomic data into clinical practice. Common inflammatory skin diseases such as rosacea, acne, and hidradenitis suppurativa (HS) are considered distinct clinical entities based on presentations. However, we hypothesize that inflammatory processes share similar mechanisms molecularly and such mechanisms share mutual therapeutic targets for intervention. Using the transcriptomic data from GEO (GSE65914, 6475, and 137141), we performed a protein-to-protein interaction network analysis (https://string-db.org/), identifying six genes, S100A7, S100A8, S100A9, LCN2, CCL19, and GZMB of global dependency in rosacea, acne, and HS. In particular, S100A8 and S100A9 are involved in inflammation-driven carcinogenesis, which raises an important clinical question of if such a systemic burden presents a real risk and how to mitigate the risk preventatively and/or therapeutically. Additionally, we identified eight differentially expressed genes that are common to all three diseases and mapped them to the gene-drug interaction database (https://go.drugbank.com/) and found that zinc derivatives (zinc acetate, zinc chloride, and zinc sulfate) target these shared genes. In the literature, there are limited evidence supporting the use of zinc as anti-inflammatory and anti-carcinogenic agents. The therapeutic efficacy of zinc in rosacea, acne, and HS are still debated in practice. Computationally, we demonstrate that zinc does target the genes shared by all three diseases. Moreover, zinc targets S100A8 and S100A9, two genes of global dependency, that drive inflammation-induced carcinogenesis. Here, we demonstrate an example of how transcriptomic data can be utilized to offer novel insights into disease mechanisms and identify potential therapeutic targets.

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Characteristics and merits of NIH award recipients of dermatology research

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Intro: NIH funding is pivotal for researchers advancing the field of medicine. We aim to explore the gender gaps in NIH award recipient demographics and scholarly merits for dermatology related projects. Methods: The NIH Research Portfolio Online Reporting Tools was used to extract dermatology related projects funded by the National Institutes of Arthritis and Musculoskeletal and Skin Diseases from 2015-2019. Demographic and scholarly merit information for award recipients was collected using the world wide web and the Scopus database. The 2019 Blue Ridge Institute for Medical Research Report was used to determine the top 20 NIH funded dermatology departments/divisions. Results: Compared to females, there were 35% more unique male award recipients. The most prevalent award was the Research Grant (R) award (1264, 790M, 723F), followed by the Career Development (K) award (267, 123M, 153F). Amongst all award recipients, there was an average of 116.7 publications and an average h-index of 37.1. However, on average, males had 53.1% (136.1M vs 88.9F) more publications and 37.2% (41.7M vs 30.4F) higher h-indexes than females. Among the top 20 NIH funded institutes, there were less gender gaps in the number of total awards received, average publication numbers, and average h-index. Discussion/Conclusion: Award recipients excel in research merits however vast gender discrepancies are present. Of note, this gap is decreased amongst the top 20 NIH funded institutes. Quantitative research metrics do not fully encompass the value of the researcher as many female awardees have demonstrated. However, the unequal distribution of awards underscores the need for equal opportunities and award selection refinement.

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Readability of isotretinoin resources from iPLEDGE in English and Spanish

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Decreased health literacy is associated with health disparities, including medication misuse. Patient education materials should be at a 6th grade level for the average American reader. iPLEDGE is a pregnancy-management program with the goal of eliminating severe birth defects caused by fetal exposure to isotretinoin, a drug used to treat nodular acne. Previous work has shown that earlier iterations of English iPLEDGE materials were too difficult to read for the average American. iPLEDGE brochures and consent forms, which differ for males and females and are provided in both English and Spanish, were collected and processed for readability analysis, including converting bullet points to sentence fragments. Flesch Reading Ease Score (FRES) and Fry Reading Graph (FG) was calculated and used to compare English documents. Fernández-Huerta Index (FHI) and Gilliam-Peña-Mountain (GPM) were calculated and used to compare Spanish documents. To compare between languages, FRES was compared to FHI and FG to GPM. The English materials (FRES=52.58, "Fairly Difficult"; FG=12th grade) were more difficult than the Spanish materials (FHI=56.26, "Fairly Difficult", GPM=9th-10th grade). The female materials (FRES/FHI=52.84, "Fairly Difficult"; FG/GPM=10th-11th grade) were more difficult than the male materials (FRES/FHI=55.08, "Fairly Difficult"; FG/GPM=10th-11th grade). The consent forms (FRES/FHI=48.66, "Difficult"; FG/GPM=12th-13th grade) were more difficult than the brochures (FRES/FHI=56.72, "Fairly Difficult"; FG/GPM=10th grade). iPLEDGE implemented many of the NIH's "Clear & Simple" guidelines for developing low-literacy materials, like defining new words, limiting the number of concepts in each piece and balancing white space with words. All of the materials exceeded the recommended 6th grade level for the average American reader. The only way to really see how readers interact with these materials is through a future usability study.

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Persistence of mature dendritic cells, Th2A and Tc2 cells characterize clinically resolved atopic dermatitis under IL-4R-alpha blockade

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Current therapeutic options for atopic dermatitis (AD) consist of either broad or targeted immunosuppressive agents. However, the natural course of AD can hardly be modified, as the disease invariably returns after cessation of treatment. Tissue-resident memory T-cells are hypothesized to be relevant players in mediating disease-specific 'immune memory', but their exact immunopathological phenotype is so far unknown. By using a multi-omics approach involving single-cell RNA sequencing combined with multiplex proteomics of skin samples, we studied AD patients undergoing short (16 weeks) and long-term (one year) treatment with the IL-4Rα blocker dupilumab. IL-4Rα blockade resulted in clearance of disease, decrease in skin immune cell counts, and normalization of transcriptomic dysregulation of keratinocytes. Interestingly, we found distinct populations of dendritic cells (DC) and memory T-cells that were largely absent in healthy control skin to persist in AD up to one year of treatment. These included LAMP3+ CCL22+ mature DC, CRTH2+ CD161+ Th2A cells, and CRTAM+ cytotoxic T-cells, expressing peak levels of CCL17 (DC) and IL13 (T-cells). Th2A cells showed a specific receptor constellation of IL17RB, IL1RL1 (ST2) and CRLF2, possibly rendering them key responders to the AD-typical epidermal alarmins IL25, IL33 and TSLP. We thus identified persisting mature DC and T-cells that maintained an inflammatory phenotype up to one year of treatment,equipped with all receptors to facilitate a keratinocyte-DC-Th2-mediated inflammatory response. These cell populations emerge as central players of a skin-intrinsic disease memory that leads to disease recurrences, and might therefore be promising targets to achieve a more sustained therapeutic response.

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The diagnostic and prognostic utility of known and emerging biomarkers in cervical and vulvar squamous cell carcinomas

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Cervical squamous cell carcinoma (SCC) and vulvar squamous cell carcinoma (VSCC) are epithelial cancers that are frequently the result of infection with high-risk human papillomavirus (HPV). The goal of this project is to determine the utility of an HPV-encoded circular RNA, circE7, as a tissue marker in cervical SCC and VSCC as well as identify novel biomarkers and correlate these with clinical and prognostic factors. We hypothesize that stage 1B cervical SCC with a recurrence are biologically distinct from those without a recurrence, and HPV+ VSCC is biologically distinct from HPV-. A retrospective case-control study was performed. Archived cases of women with cervical SCC and VSCC and available formalin fixed paraffin embedded tissue samples were collected. The samples will be analyzed by RNA-sequencing, PD-L1 immunohistochemistry, HPV in-situ hybridization, and circE7 quantification. A total of 18 cervical SCC samples, 36 VSCC, and 6 controls were identified. Chart review demonstrated improved survival of cervical SCC patients predicted by absence of recurrence (p=<0.0001) and age >36 (p=0.0033). Improved progression-free survival (PFS) was predicted by age >36 (p=.0099) and pre-op conization procedure (p=.0483). VSCC demonstrated improved survival with absence of recurrence (p=.0118), negative nodal status (p=.0180), vulvectomy procedure (p=.0151), undergoing nodal dissection (p=.0003), and infiltrating borders of tumor (p=.0041). VSCC patients had improved PFS for unifocal tumors (p=0.0211) and nodal dissection (p=.0001). Clinical data will be analyzed together with the molecular studies. These results have the potential to identify novel diagnostic tests and insights on the pathogenesis of both cervical SCC and VSCC.

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Cell therapy trial of ectopic fibroblasts to modify skin identity

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Skin identity is controlled by a combination of intrinsic features of the epidermis and dermis, as well as crosstalk between the two compartments. The modification of skin identity might have many clinical uses, such as the conversion of stump (Non-volar) skin of an amputee to pressure-responsive palmoplantar (Volar) skin in an effort to enhance prosthetic use and minimize skin breakdown. To this end, we first tested the effects of injected autologous volar (AVF) and non-volar (NVF) fibroblasts on mismatched (i.e. ectopic) locations in healthy volunteers. We measured histologic endpoints known to be greater in native volar skin to see if these were enhanced in injected non-volar skin. Greater KRT9 expression, higher epidermal thickness, larger keratinocyte cytoplasmic size, and longer collagen length are markers of volar skin. We find that these are ectopically increased in non-volar skin after AVF injection (n=31, p<0.03; n=32, p=0.0003; n=32, p=0.001; n=18 p=0.05 respectively), maintained even after 5 months. RNA seq demonstrates gene ontology categories of extracellular matrix organization and morphogenic pathways, such as FGF, Wnt, Notch, and epidermal growth factor receptor (EGFR), with confirmed immuno-histologic changes in EGFR (Y1058 n=31, p<0.0001) and Notch (RBPJ, n=31, p=0.001). Finally, single cell RNA seq demonstrates that ectopic fibroblasts have the greatest effect on transitional keratinocytes leaving the basal layer that we term Liminal Keratinocytes. The long-term engraftment of these cells and tissue changes of our model create a robust platform to test concepts of stem cell therapy toward the development of new therapeutics.

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Dupilumab associated facial and neck erythema

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Frequently reported adverse events (AE) of dupilumab treatment for atopic dermatitis (AD) in phase 3 clinical trials included conjunctivitis, injection site reactions, and herpes infections. Although not reported in randomized controlled trials, there have been increasing reports of dupilumab-associated facial and/or neck erythema (FNE) in clinical practice. A systematic review of existing literature was conducted in order to identify all reported cases of dupilumab-associated FNE and identify potential etiologies and management strategies. A search was conducted on EMBASE and PubMed databases. Two independent reviewers identified relevant studies for inclusion and performed data extraction. 101 patients from 16 studies were reported to have dupilumab-associated FNE. 52/101 (52%) had baseline involvement of the face and/or neck and 45/101 (45%) reported cutaneous symptoms differing from their pre-existing AD, possibly suggesting another etiology. Suggested etiologies included rosacea, allergic contact dermatitis, and head and neck dermatitis. Most commonly used treatments included topical corticosteroids, topical calcineurin inhibitors, and antifungal agents. 29/57 patients saw improvement, 4/57 had clearance, 16/57 had no response, and 8/57 had worsening symptoms despite treatment. 11/101 patients discontinued dupilumab treatment due to this AE. Some patients on dupilumab have developed FNE which differs from their usual AD symptoms. Educating patients on this AE prior to initiation may allow for prompt identification and early treatment, minimizing potential AE related discontinuations.

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Psoriasis patients with subclinical atherosclerosis parse into distinct endotypes by differential gene expression

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Psoriasis is an independent risk factor for atherosclerotic cardiovascular disease (ASCVD). This study aims to uncover shared molecular targets for psoriasis-ASCVD therapies and reduce diverse heterogeneous presentations into endotypes. We compared PAXgene RNA-seq of psoriasis patients with low (Agatston<100; n=21) versus moderate-to-high (Agatston≥100; n=7) coronary artery calcification scores (CACS), a surrogate for subclinical atherosclerosis. Differentially expressed genes (DEGs; α=0.05; |logFC|≥0.1; e. Bayes w/o adj.) were identified with a linear model controlling for age (56.8±13.8 yo), sex (38% F), and batch (n=3). Females were less likely to have moderate-to-high CACS than males (10% vs. 33%), although the odds ratio did not reach significance (OR=0.22; 95%CI:0.02,2.18; p=0.36). Pearson hierarchical clustering of the top 50 DEGs (|t|>3.3) revealed three distinct transcriptomic endotypes with median CACS values of 111 Agatston (IQR:0,189; 5 of 8 patients ≥100), 21 Agatston (IQR:0,83; 2 of 10 patients ≥100), and 0 Agatston (IQR:0,0; 0 of 10 patients ≥100), respectively (p=0.06, Kruskal-Wallis H test). Enriched pathways (α=0.01; e. Bayes w/o adj.) were identified using gene set variation analysis on MSigDB hallmark gene sets. The top three pathways by log-fold change were interferon-α (e.g., IFI44L), interferon-γ (e.g., IFIT1), and PI3K/AKT/mTOR signaling (e.g., E2F1). Thus, the psoriasis endotype most prone to calcifying ASCVD distinguishes itself by altered prominent inflammation pathways exhibiting interferon signatures. Identifying patients that express these signature pathways may advance personalized prediction and prevention of an ASCVD-prone psoriasis endotype.

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Pro-energetics creatine and nicotinamide prevent stress-induced senescence in human dermal fibroblasts

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Senescence is the process by which cells irreversibly avoid dividing without undergoing cell death and enter a state of irreversible growth arrest. Fibroblast senescence associated with aging is known to contribute to the increased incidence of non-melanoma skin cancer in the aged population. To that end, agents that can inhibit fibroblast senescence could be protective. Senescence can be induced by various cellular stressors such as DNA damage, oncogenic activation and oxidative stress. Hydrogen peroxide (H_2O_2), ultraviolet light, tert-butyl hydroperoxide, and hyperoxia are pro-oxidative stressors which are used experimentally to induce premature senescence. The present studies were designed to test if the pro-energetics creatine and nicotinamide can block H_2O_2-induced senescence in primary cultures of human fibroblasts in vitro. Short-term exposure of fibroblasts with H_2O_2 followed by a three day incubation resulted in senescence as denoted by increased b-galactosidase (b-gal) staining, increased P21 expression, decreased insulin-like growth factor-1 and increased expression of pro-inflammatory cytokines IL-6, IL-8 and TNFa. Pretreatment with creatine and nicotinamide blocked experimental senescence as measured by normalization of all these parameters associated with experimental senescence. Of interest, post-treatment with creatine or nicotinamide following H_2O_2 had no effect on oxidant-induced senescence. Creatine and nicotinamide pre-treatment also blocked H_2O_2-mediated increased levels of reactive oxygen species, providing a potential mechanism for their protective effects. These studies suggest that creatine and nicotinamide could have clinical use in preventing fibroblast senescence

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Matrix metalloproteinase 2 is a potential mediator of retinoid efficacy in photoaging

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Considered the gold standard in the treatment of photodamaged skin, tretinoin (retinoic acid) is thought to restore collagen in sun-damaged skin through the induction of pro-collagen I and suppression of matrix metalloproteinases (MMPs) which degrade collagen, particularly MMP1, MMP3, and MMP9. The clinical efficacy of tretinoin therapy, however, is often limited by local adverse effects, such as burning, itching, or peeling. Cosmeceutical formulations containing precursors of tretinoin have been marketed to provide similar efficacy as tretinoin while minimizing toxicity. We previously reported that tretinoin precursors indeed resulted in comparable improvements in signs of photodamage with better tolerability compared to tretinoin. It remains unclear, however, which genes are responsible for these therapeutic effects. To address this question, skin biopsies were taken from participants at baseline and after 24-week application of 0.02% tretinoin (RA, n=11 patients) or 1.1% triple tretinoin precursor (TTP, n=9 patients) formulation containing retinol, retinyl acetate, and retinyl citrate to photodamaged facial skin. Analyzing mRNA expression in skin biopsies by RT-qPCR, we found significant induction in cellular retinoic acid-binding protein 2 in both groups (confirming RA signaling), but we failed to detect significant changes in pro-collagen I or MMP1/3/9 in TTP-treated samples. We extended our analysis to MMP2, which is known to be induced by ultraviolet radiation, albeit by an unknown mechanism. In addition to being the most abundantly expressed MMP in the skin, MMP2 mRNA was significantly reduced by 40% with TTP treatment (p=0.04). Furthermore, changes in MMP2 mRNA expression, but not in any other evaluated gene, were tightly correlated with clinical improvements in fine wrinkles, particularly periorbital fine wrinkles (r=0.506, p=0.02). These results not only show that suppression of collagen degradation may be more clinically relevant than induction of pro-collagen I, but also highlight MMP2 as a newly relevant mediator of retinoid efficacy.

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Effect of dupilumab on the host-microbe interface in atopic dermatitis

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Atopic dermatitis (AD) is characterized by *Staphylococcus aureus* (SA) colonization, epithelial defects & type 2 immunity. Dupilumab (DPL), which blocks the biological actions of IL-4 & -13, provides an opportunity to probe the import of type 2 inflammation on microbial, epithelial & immune features of AD. To do this, the Atopic Dermatitis Research Network designed a 6wk, RDBPC trial with high density sampling (Days 0, 3, 7, 14, 21, 28 & 42) to quantify these changes & assess how they relate to disease severity (EASI, NRS, IGA & SCORAD). Seventy-two, moderate-severe adult AD subjects were assigned 2:1 to DPL vs placebo. The primary endpoint was SA abundance (qPCR) on lesional skin at 28 days. Secondary endpoints were: **1)** SA abundance on lesional (L) skin at remaining timepoints & nonlesional (NL) skin at all timepoints, **2)** skin barrier function (Transepidermal Water Loss before & after tape-strips) and **3)** EASI, IGA, SCORAD & NRS. Exploratory endpoints are: **1)** composition & abundance of bacterial taxa at NL & L skin, **2)** skin biopsy transcriptome (NL & L skin), **3)** lipidomics (NL & L skin), **4)** expression of SA virulence factors (NL & L skin), **5)** confocal imaging of tight junctions & SA (NL skin only), **6)** PBMC immunoprofiling & **7)** serum biomarkers. Mechanistic readouts are anticipated by Q2 2021. By integrating this multi-scale, high density data, we will model the complex interactions between the host and microbiome with and without type 2 blockade.

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Quantifying desmoplasia in cutaneous squamous cell carcinomas

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Desmoplastic cutaneous squamous cell carcinomas (cSCC) are a clinically highly aggressive histopathologic variant of cSCC. Desmoplasia is the abnormal deposition of collagen around a tumor. To date, there is no reliable, objective method for quantifying the degree of desmoplasia in cSCC. Herein, we characterize and quantify the differences between desmoplastic collagen and non-desmoplastic collagen in cSCC. These characteristics will be used to develop an objective grading system using machine learning and computer vision. The degree of desmoplasia in cSCC was assessed by a pathologist using a semi-quantitative scoring scale. 15 cSCC with prominent desmoplasia and 15 without desmoplasia were imaged with second-harmonic generation (SHG) microscopy. SHG signals from collagen fibers were analyzed using ImageJ and CurveAlign, a curvelet-based program for fiber tracking, quantification, and bulk assessment of fiber characteristics. SHG images were used as the primary input for CDeep3M, a convolutional neural network (CNN)-based machine learning model. Analysis of SHG images restricted to a 20-micron peritumoral zone revealed a significantly lower signal intensity of desmoplastic collagen fibers compared to non-desmoplastic fibers (34 vs 70, $p < 0.001$). Unrestricted whole-field analysis showed desmoplastic cSCC have a significantly higher fiber alignment coefficient (A_c) than non-desmoplastic tumors (0.41 vs. 0.30, $p < 0.01$). More of the perimeter of desmoplastic cSCC is covered by collagen compared to non-desmoplastic cSCC (51% vs 39%, $p< 0.05$). These findings indicate that desmoplastic collagen fibers are quantifiably different from non-desmoplastic fibers and suggest inherent differences in the structure of desmoplastic collagen vs native collagen. These characteristic differences enable the construction of a machine learning model for objective, quantitative assessment of desmoplasia in cSCC.

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Qualitative assessment of patient values in decision making for alopecia areata: Preliminary results

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Alopecia areata (AA) is a common disease of hair loss with no FDA-approved therapies. While many treatments are available to patients, each have their own benefits and risks. Clarifying patient values is an important step to help patients choose the treatment that best fits their unique needs. A qualitative study using semi-structured interviews was conducted with 10 patients recruited from Brigham and Women's Hospital dermatology clinic. Interviews were split and independently coded among two pairs of researchers. Codes were determined by consensus. Coding reliability and frequency were determined using NVIVO software. Interrater reliability ratings were $\kappa = .77$ (% agreement: 99%) and $\kappa = .69$ (% agreement: 98%) for the two coding teams, indicating good-excellent agreement. Key themes emerged: 100% of patients considered treatment efficacy, safety profile, and convenience of use when choosing an AA treatment. 90% of patients reported barriers to receiving appropriate treatment for various reasons, such as lack of local experts in hair loss, other time commitments, and health insurance coverage. The majority of patients considered physician recommendations (80%) and information found on the internet (70%) when choosing a treatment. 60% of patients sought dermatologists with qualifications such as affiliations with teaching hospitals and specialization in hair loss. Patients preferred physicians that reviewed treatment options comprehensively with good bedside manner (50%). Understanding patient values and barriers to treatment is important in the shared-decision making process. This study demonstrates that patients highly value information on treatment risks and benefits, which they obtain from multiple sources including physicians and online-based materials. These findings are important to discuss to help patients make higher quality decisions that align with their treatment preferences.

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***In vivo* characterization of melanoma metabolism in human patients through intraoperative [U-13C] glucose infusions**

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Reprogrammed metabolism is a hallmark of cancer cells which must meet high demands for energy formation, macromolecular synthesis, and redox homeostasis. In recent years, alterations in glucose metabolism have been implicated in melanoma development, metastatic efficiency, and BRAF-inhibitor treatment resistance. Due to the challenges of performing human in vivo studies, most of this knowledge is derived from mouse models or in vitro data. To characterize melanoma metabolism in a clinically relevant setting, we developed a clinical trial at UTSW to perform perioperative isotope tracing of [U-13C]-labeled glucose in melanoma patients undergoing surgical resection. In this protocol, patients receive a bolus of 8 grams of [U-13C] glucose followed by a continuous infusion of 4 grams/hr for an average of 3 hours prior to tumor acquisition. Peripheral blood and tumor samples are analyzed by GC-MS to study glucose tracing and ^{13}C-incorporation in downstream metabolites such as lactate, lipids, and TCA cycle intermediates. Thus far, we have obtained 14 melanoma samples from 6 patients who received intraoperative infusions. All patients achieved a steady-state of glucose labeling in the peripheral circulation after 30 minutes. Importantly, we have observed that glucose oxidation occurs, to a variable degree, in all melanomas sampled. The extent of melanoma glucose utilization and oxidation differs between patients and even within the same patient across distinct metastatic sites. From 4 patients, we have generated patient-derived xenografts of their melanoma tumors and performed similar infusions to identify aspects of glucose metabolism that are and are not conserved in mouse models. To our knowledge, this is the first study to use stable isotope tracers to examine metabolic pathways in human melanoma. Future work will determine metabolic features of melanoma that are associated with prognosis and treatment response.

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Leukocytes as an objective measure of hidradenitis suppurativa disease severity

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Hidradenitis suppurativa (HS) is a chronic recurrent inflammatory disease of hair follicles. Though the pathogenesis is poorly understood, it has been proposed that the dysfunctional innate and adaptive immune responses affect circulating leukocytes. Leukocytes play a role in the various stages of inflammation, including pro-inflammatory cytokine recruitment, macrophage functioning, and destruction of infectious agents. We sought to investigate the association of leukocyte parameters with HS disease severity. A retrospective chart review targeted 404 patients seen at the Albert Einstein/Montefiore HS Center from March 2019 to November 2020. The mean age was 35±13 and most were female (74%). Disease severity was classified according to the HS-Physician Global Assessment (HS-PGA) scale. Serum samples were analyzed for a complete blood count. When adjusting for age and sex, more severe disease was associated with increased leukocytes (OR:1.216, 95%CI:1.129-1.310), neutrophils (OR:1.327, 95%CI:1.209-1.456), eosinophils (OR:5.694, 95%CI:1.322-26.902), basophils (OR:3.021, 95%CI:1.190-7.668), monocytes (OR:2.974, 95%CI:1.157-7.642), and neutrophil/lymphocyte (NL) ratio (OR:1.731, 95%CI: 1.426-2.101). Our findings align with the expected high inflammatory burden experienced by patients with HS. The clinical benefit of tracking leukocyte parameters cannot be overstated due to the paucity of objective markers associated with disease severity. We urge the incorporation of leukocyte parameters when evaluating patients and developing treatment plans. Future research should focus on longitudinal analyses to correlate leukocyte parameters with fluctuations in disease activity. Reference: 1. Constantinou, C.A., G.E. Fragoulis, and E. Nikiphorou, *Hidradenitis suppurativa: infection, autoimmunity, or both?* Ther Adv Musculoskelet Dis, 2019. **11**: p. 1759720X19895488.

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Morphological and histological effect of emollient application in actinic keratoses

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Evaluation of actinic keratosis (AK) treatment response is based primarily on clinical endpoints. Although inactive ingredients in topical therapeutics are assumed to be inert, effect on photodamaged (PD) human skin and existing AK lesions is largely unknown. The aim of this study is to assess histological, morphological, and clinical features of AKs and PD skin after daily emollient application to understand potential confounding effects of emollient vehicles. 24 patients were enrolled and randomized to either the emollient group (N=12) or control group (N=12). Patients in the emollient group applied twice daily Vanicream™ to study sites for 12 weeks. Three AKs and two areas of nearby PD skin were evaluated per patient. Sites were imaged by reflectance confocal microscopy (RCM), clinically evaluated, and biopsied for immunohistochemical evaluation. Expression of Ki67, a marker of cell proliferation, was significantly higher in AKs (35%, SD 10.2) compared to PD skin (20%, SD 11.4) at baseline. Caspase-14, a marker of differentiation, was significantly lower in AKs (26%, SD 14.9) compared to PD skin (61%, SD 13.7) at baseline. Baseline expression of p53 was not significantly different between AKs (34%, SD 17.3) and PD skin (32%, SD 18.2). There was no observed effect of emollient on biomarker expression over time in AKs or PD skin. However, there was a significant decrease in RCM-measured stratum corneum thickness in emollient treated AKs (-26μm, p=0.035), suggesting that emollient-treated lesions may appear less hyperkeratotic on exam. It is critical to determine emollient effect on AKs to control for potential confounders in clinical trials and to understand how inactive ingredients may modulate evaluation of AK response to treatment.

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JAK inhibitor functional profiling in CTCL

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Cutaneous T cell lymphoma (CTCL) is a skin-homing non-Hodgkin lymphoma, often involving the blood and lymph nodes in advanced stages. Complete responses to advanced CTCL current therapies are rare. Although CTCL genetic heterogeneity poses further therapeutic challenges, simultaneously uncoupling key aberrant pathways in CTCL with combination therapies to minimize toxicity and thwart single-agent resistance holds promise for future CTCL therapies. We previously reported that first-generation JAK1/2 inhibitor ruxolitinib synergistically potentiates venetoclax (BCL2 inhibitor), vorinostat (HDAC inhibitor), and mivebresib (BET inhibitor) in CTCL. Characterizing the broader applicability of JAK inhibitors in CTCL and their potential use in combination therapies is crucial for informing our future analyses of dysregulations in CTCL JAK/STAT signaling and how these interactions with other aberrant networks can be therapeutically exploited. To this end, we performed in vitro CTCL patient-derived cell viability assays against a panel of agents exhibiting different JAK family member selectivity profiles, singly and in combination with BCL2, HDAC, and BET inhibitors. Patient-isolated CTCL cells exhibited greater sensitivity to JAK2-selective over JAK1/2-selective ($p<0.003$) and pan-JAK inhibitors ($p<0.0002$). All tested JAK and BCL2/HDAC/BET inhibitor in vitro viability combination assays revealed varying degrees of synergy using the Chou-Talalay method, with combination indices ranging from 0.0080-0.9168 at 90% fractional inhibition. These functional-level screenings inform exploration of strategic combination formulation and lay the groundwork for analysis of the interplay between key dysregulated pathways in CTCL.

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Health-related quality of life in pyoderma gangrenosum: A qualitative analysis

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Pyoderma gangrenosum (PG) is a rare inflammatory skin disorder characterized by recurrent, rapidly progressive cutaneous ulceration. These ulcerations are painful and disfiguring with the potential to negatively impact quality of life; yet, quality of life has received little attention in this patient population. To meet this need, we conducted a series of interviews to identify factors impacting quality of life in PG. Semi-structured interviews were performed for patients with a diagnosis of PG at an academic dermatology clinic. A total of 10 patients were interviewed, 4 males and 6 females, aged 29-83 years. Interviews were audio-recorded, transcribed verbatim, and analyzed using inductive thematic analysis to yield themes and subthemes. Five main themes were uncovered: pain, physical limitations, self-image, treatment-related frustrations, and mental health. Pain was most significant while sleeping, performing daily tasks, bathing, and with dressing changes. Patients reported difficulties with ambulation, household chores, bathing, and at work. Odor, appearance, and drainage of wounds impacted patient self-image, leading to avoidance of social interactions. Sources of treatment-related frustrations included slow healing of ulcers, disease progression and relapse, and chronic wound care needs. Depressed mood attributed to skin disease was commonly reported, with patient isolation, dependence on others, and fear of disease outcomes often contributing to mental health. The impact of PG on quality of life was found to be multifaceted, influencing physical, psychological, and social functioning of patients. Deeper understanding of the patient experience provides a framework for dermatologists to assess and respond to the complex needs of patients living with PG. This framework may be built upon by future quantitative studies to create a validated PG-specific quality of life instrument for clinical care.

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Single-cell RNA sequencing (scRNA-seq) of sarcoidosis skin biopsies reveals key pathogenic cytokines and potential treatment targets

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Sarcoidosis is an idiopathic inflammatory disorder characterized by granuloma formation in affected tissues. Sarcoidosis commonly affects the skin and can be difficult to treat. An incomplete understanding of the molecular pathogenesis of this disease has impeded the development of targeted therapies. We performed scRNA-seq on dissociated skin biopsies from sarcoidosis patients (n=3) and healthy donors (n=3). Comprehensive receptor-ligand expression analysis among cell types was performed with CellphoneDB. Differential gene expression and pathway analyses were also used. Key receptor-ligand interactions identified with this approach were validated through analysis of bulk gene expression data from additional cases of cutaneous sarcoidosis (n=15). Single cell devolution with CIBERSORTx was also performed on the bulk expression data. A total of 24,034 cells were analyzed and 12 major cell types including T cells and macrophages were identified. We characterized a unique population of Th1-like CD4+ T cells in the sarcoidosis samples that produced high levels of *IFNG*, *CSF2* (GM-CSF), *IL21* and monocyte chemokines. Receptor expression and transcriptional patterns suggested these factors acted on macrophages, fibroblasts, and/or in an autocrine fashion. Sarcoidal macrophages showed an activated phenotype and in turn expressed *IL15*, *IL6*, *TNF*, as well as other novel factors (*CHIT1*, *FBP1*, *CHI3L1*) and both T-cell and monocyte chemokines. We also identified a distinct population of classical dendritic cells in the sarcoidosis samples that produced *IL12B*. In summary, we use scRNA-seq to deconvolute the sarcoidal granuloma microenvironment and to identify key cytokine drivers of inflammation in sarcoidosis. Notably, all cytokines identified (except TNF-alpha) signal via the JAK-STAT pathway. These observations are consistent with recent reports suggesting the potential efficacy of JAK inhibition in sarcoidosis. Further evaluation of JAK inhibitor treatment of sarcoidosis is supported by this molecular immunologic data.

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Utility of circulating tumor DNA testing in Merkel cell carcinoma patients

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Merkel cell carcinoma (MCC) is a skin cancer that recurs frequently (~40%). While the Merkel cell polyomavirus (MCPyV) antibody assay can be used to efficiently detect recurrence in about 50% of patients, the others require frequent imaging surveillance. In this study, we assessed whether circulating tumor DNA (ctDNA) can provide insight into MCC disease burden and detect recurrent disease for both virus-positive (VP) and virus-negative (VN)-MCC. We used the Signatera™ platform in which tumor-specific mutations are identified from archival tumor and blood is serially assessed for tumor-specific DNA. Starting in April 2020, we collected ctDNA samples from 6 MCC patients (13 samples). Baseline patient characteristics included median age of 65, gender (male 3; female 3), stage (pIIIA 1; pIIIB 5), median tumor size of 3.2 cm (range 2.0-4.5), and immune status (immune-suppressed 2; immune-competent 4). All 6 patients had detectable ctDNA in their serum, 3 were also positive for antibodies to the MCPyV oncoprotein. Among the 6 patients, 4 had serial assays. ctDNA levels following the initial treatment decreased in all 4 patients (3 became negative 2 months after treatment; 1 decreased by 80%). Interestingly, a VN-MCC patient who had a negative ctDNA test after treatment became positive again 2 months later, prompting imaging that showed a lesion in a distant site. Biopsy confirmed MCC metastasis and immunotherapy was started. To our knowledge, this study is the first to explore ctDNA testing in MCC patients. We conclude ctDNA tracking can be useful for MCC patients regardless of tumor viral status. In individual patients, serial ctDNA testing helped assess response to treatment and detect early recurrence. Ongoing studies will assess the minimum MCC tumor volume needed for ctDNA detection and determine the relative sensitivity of ctDNA and MCPyV antibody assays.

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Variation in Medicare topical steroid prescription costs

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Rising costs of topical steroids (TS) have doubled out-of-pocket costs for seniors from 2011-2015. Still, variation within and between different drug potencies and regions is unknown. Herein, we analyze differences in Medicare TS costs. TS retail costs for the 2 most common medications in each potency class (low: hydrocortisone valerate 0.02% & desonide 0.05%; mid: fluocinonide 0.05% & triamcinolone acetonide 0.1%; high: betamethasone dipropionate 0.05% & clobetasol propionate 0.05%) were collected from Medicare.gov. Data was collected for the most and least populated zip codes in the most and least populated states in each of the 10 Centers for Medicare & Medicaid Services regions. In each location, 5 pharmacies (2 retail, 1 department store, 1 community and 1 grocery store) closest to each zip code's geographic center were identified, and the cost for each TS was evaluated for the top 5 Medicare Part D plans with the most enrollees in that state. The overall mean, median, and interquartile range (IQR) TS retail costs were $45.83, $30.85 and $57.94, respectively. Costs varied among TS potencies, with low potency having the highest and mid potency having the lowest costs (mean $63.10 vs $19.82, median $59.40 vs $7.36, IQR $52.77 vs $29.64). Within a potency class, the IQR of clobetasol propionate 0.05% was 17 times larger than betamethasone dipropionate 0.05% ($142.59 vs $8.11). Regionally, the mean price of high potency TS was 34% higher in the Midwest than the Northeast ($60.74 vs $45.36). High potency TS had a much higher IQR in the Southeast ($133.10) compared to the Northeast ($19.76). Drug costs remained similar between urban and rural locations and between retailers. Our study demonstrates that TS costs vary by potency and geography. Consequently, 2 Medicare patients receiving the same potency TS may pay highly different costs depending on location and the specific TS prescribed. Our study emphasizes the need for transparency of drug prices through legislation to improve physician prescribing practices and accessibility for patients.

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Personal protective equipment needs during the COVID-19 pandemic: A survey of medical dermatologists

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Background: The World Health Organization recommends healthcare providers wear personal protective equipment (PPE) to prevent COVID-19 infection. There are no universally accepted recommendations for PPE usage by healthcare providers when caring for asymptomatic patients who can transmit infection. Objective: To survey PPE usage across medical dermatology practices and assess provider concern for COVID-19 transmission in the workplace. Methods: An anonymous survey was distributed via e-mail to dermatologists with membership in the Society for Dermatology Hospitalists and the Association of Professors in Dermatology. Questions pertained to use and perception of PPE in outpatient encounters. The survey remained open for 6 weeks and reached a predetermined target response number of 50-100 dermatologists among 429 recipients. Results: 88 dermatologists completed the survey (20.5%) and the majority practice in an outpatient clinic (80.7%). When caring for patients, most practices officially recommended utilizing a surgical mask (95.5%) and face shield (52.3%). Though a minority of practices recommended an N95 respirator (9.1%) or gloves (29.5%), a larger fraction of dermatologists self-reported using an N95 respirator (26.1%) and/or gloves (43.2%). 35.2% of dermatologists supplemented practice-provided PPE by providing personally obtained PPE, and 18.2% of dermatologists felt their institution did not adequately address their perceived risk of contracting COVID-19. 42.0% of physicians perceived their risk of contracting COVID-19 at work to be moderate or high. Conclusion: An appreciable fraction of dermatologists perceived their COVID-19 infection risk to be medium or high, and some dermatologists are taking supplemental safety measures. This study calls attention to the level of provider-perceived risk and highlights opportunities to address provider concerns regarding COVID-19 transmission.

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Multiple gaps in photoprotection behaviors exist in diabetic adults: Results from National Health and Nutrition Examination Survey 2005-2018

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Previous studies showed higher rates of skin cancer in diabetic patients. However, photoprotection behaviors and sunburn risk among diabetics are not known. We conducted a cross-sectional analysis using self-reported data among adults (20-59YO) from the 2005-2018 National Health and Nutrition Examination Survey (NHANES). 17671 adults without diabetes (No Diabetes/ND group) and 1918 adults with diabetes or a glycohemoglobin level at or above 6.5% (Diabetes or High Glycohemoglobin/DHG group) were included. We completed cross-tabulation analyses, then calculated relative risk ratio (RRR), odds ratios (OR) and 95% confidence intervals (95%CI) using unconditional logistical regression and multinomial logistic regression adjusted for age, gender, and race. We also adjusted for photoprotection use when analyzing sunburn risk. [AC1] We found that 79% of DHG group sometimes, mostly, or always stay in the shade. However, majority of DHG group rarely or never use sunscreen (69.5%) or wear long sleeved shirts (65.4%). Compared to ND group, DHG group was associated with a greater use of shade (OR 0.83, CI=0.79-0.87, p=0.00) and less use of sunscreen (OR 1.26, CI= 1.21-1.31, p=0.00). There was no significant difference in odds of long sleeve shirt use (OR 1.04, CI= .99-1.08, p=0.058) between the two groups. Furthermore, higher glycohemoglobin levels were associated with a decreased relative risk of severe sunburn for a few days with peeling (RRR 0.93, CI= 0.87-0.99 p=0.03) and a mild sunburn with some tanning (RRR 0.87, CI= 0.83-0.91, p=0.00). There was no significant difference between the groups in the relative risk of severe sunburn without blisters, turning darker without a burn, or no reaction after half an hour of sun. Our results indicate multiple gaps in photoprotection behaviors in patients with diabetes. This study highlights the need for targeted photoprotection education in this population to prevent dermatologic complications associated with these malignancies.

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Desmoplasia induces T cell exhaustion in cutaneous squamous cell carcinomas

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Desmoplastic cutaneous squamous cell carcinomas (cSCC) are a clinically highly aggressive histopathologic variant of cSCC. Desmoplasia, the deposition of abnormal collagen around a tumor, is an independent adverse prognostic factor for disease-specific death in cSCC patients. However, the mechanisms underlying this association with poor outcomes have not been identified in cSCC. Herein we evaluate whether desmoplasia is associated with the suppression of local T cell immune response. Tissue samples from 69 patients who underwent excision for cSCC were graded for desmoplasia by a pathologist using a semi-quantitative grading scale developed in our laboratory. Samples with prominent desmoplasia and no desmoplasia were analyzed with multispectral immunofluorescence imaging. The effects on T-cell subsets (CD3, CD8, and FoxP3), and immune checkpoint and exhaustion markers (PD-1, PD-L1, and TIM-3) were quantified. Compared with non-desmoplastic tumors, cSCC with prominent desmoplasia showed a 30% reduction in cytotoxic CD8+ T cells at the tumor-stromal interface and in the tumor stroma ($p < 0.01$, respectively). cSCC with prominent desmoplasia also exhibited an increase in the fraction of exhausted CD8 T cells (5-fold increase in the tumor interior, 20-fold at the tumor-stromal interface, and 7-fold in the peritumoral stroma; $P < 0.01$, respectively). Similarly, comparing desmoplastic to non-desmoplastic cSCC, there is a 5-fold increase in the proportion of exhausted CD4+ T cells at the tumor interior, tumor-stromal interface, and the surrounding stroma ($P < 0.01$, respectively). Lastly, there is no significant difference in the distribution of FoxP3+ regulatory T cells between desmoplastic and non-desmoplastic cSCC. These results suggest that desmoplasia mediates tumor immune evasion by inducing T cell exhaustion, and not by recruiting immunosuppressive regulatory T cells. Desmoplasia may be an independent mechanism of tumor immune evasion that can be targeted to improve patient outcomes.

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Novel injectable coolant induced reduction in cutaneous nerve fiber density is comparable to the FDA-approved cryoneurolysis device

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Postherpetic neuralgia (PHN) is a disorder characterized by neuropathic pain stemming from cutaneous nerves. Cryoneurolysis was recently reported a new method of treating refractory PHN. However, the extremely cold temperatures applied for cryoneurolysis limit its use, due to non-selective nature. Herein we introduce a new method of *injectable* and *nerve-selective* cryoneurolysis technology, able to induce long-lasting analgesia for up to 60 days. We compare our method to an FDA-approved cryoneurolysis device (Iovera) used for treatment of PHN. Using the rat sciatic nerve model, we examine the mechanism of reduced nociceptive response after ice-slurry injection. We show that ice-slurry induces Wallerian degeneration in the nerve demonstrated by increase in expression of P75NTR, GFAP, and decrease in MBP genes. Using coherent anti-Stokes Raman scattering (CARS) microscopy and immunofluorescence (IF) staining, we demonstrate myelin degeneration in branches distal to the treatment site consistent with Wallerian degeneration. 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 ($P<0.0001$) in ice-slurry treatment group. Using skin biopsy samples from the rat hindpaw which is innervated by the sciatic nerve, we quantified cutaneous nerve fibers to demonstrate the effect of ice-slurry on skin innervation. IF staining showed decreased density of myelinated dermal nervess from baseline of 56±3/mm^2 to 22±3/mm^2 ($P<0.0001$) in ice-slurry treatment group, and 17±6/mm^2 ($P<0.0001$) in Iovera group. Thus, ice-slurry injection induces Wallerian degeneration and reversible reduction in cutaneous nerve fiber density comparable to the Iovera device. Although more work needs to be done, ice-slurry could become a long-lasting treatment PHN associated neuropathic pain.

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Identifying inflammatory gene expression signatures for skin and soft tissue infections

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Background: Misdiagnosis of skin and soft tissue infections (SSTIs) due to clinical mimics can result in delay of care, unnecessary antibiotic exposure, and inappropriate hospitalization. Comprehensive screening of inflammatory genes in SSTIs could identify biomarkers to distinguish SSTIs from mimics.Methods: We performed a search of the MGH James Homer Wright Pathology Laboratories database from 2010-2019 for diagnoses of necrotizing fasciitis, cellulitis, and stasis dermatitis, yielding 103 samples. Diagnoses were verified by chart review and categorized by discharge diagnosis. Three samples from each category, and three controls from location-matched skin were selected for further study. mRNA isolated from paraffin-embedded skin biopsies was analyzed by Nanostring, with 594 inflammatory genes profiled. Results: We identified differentially expressed genes between necrotizing fasciitis, cellulitis, and infectious cases (necrotizing fasciitis and cellulitis) compared to stasis dermatitis and lower-extremity controls. Differentially upregulated genes in SSTIs included those with known roles in inflammation (*CXCR2*, *IL6*, *IFI16*, *TNFRSF1B*) and transcriptional regulation (*BCL3*, *MBP*). We also identified differential expression of novel genes including the apoptosis regulator *MCL1*, monocyte differentiation antigen *CD14*, and iron-binding protein *LTF*. Conclusions: We characterized transcriptomic signatures of severe and moderate SSTIs compared to stasis dermatitis and normal skin from the lower extremities. Though limited by small sample size, these data support the utility of a prospective study analyzing outcomes of patients diagnosed with SSTIs based on gene expression signatures. Identifying SSTI-specific gene expression signatures could help differentiate true skin infections from non-infectious inflammatory skin conditions, facilitating more accurate diagnoses and improving patient care.

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Lebrikizumab directly reverses IL-13 driven neuronal gene regulation and neuronal excitability

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Atopic dermatitis (AD) is a chronic, inflammatory, relapsing skin disease with a preponderance of type 2 immune cells, which release cytokines (i.e., IL-4, IL-13, and IL-31) that orchestrate the multi-faceted downstream effects of the disease. A clinical hallmark is chronic, persistent, and highly prevalent severe itch impacting the quality of life of AD patients. Our key objective is to understand the mechanistic basis of chronic itch and gain insight into the efficacy of lebrikizumab, a monoclonal investigational anti-IL-13 antibody that is in development for the treatment of moderate-to-severe AD. To ascribe a laboratory surrogate to chronic itch, we employed a primary human dorsal root ganglion (hDRG) tissue culture model and stimulated these sensory neurons with IL-13 along with different pruritic as well as other inflammatory agents (with or without lebrikizumab). Live-cell calcium measurements demonstrate that acute as well as prolonged exposure of sensory neurons to IL-13 amplifies the neuronal responses to a multitude of signals. These arrays of neuronal potentiation elicited by IL-13 were attenuated by lebrikizumab. Additional studies with electric field stimulation suggest that acute and prolonged exposure of IL-13 increases neuronal excitability in the DRG, which is reversed by lebrikizumab underlining a direct neuromodulatory role and may be complementary to potentiation of itch responses. To highlight the possible molecular basis of neuronal activity, we measured the downstream transcriptional targets of IL-13 using RNA Seq. Dominant transcripts that were differentially regulated by IL-13 include immune-regulatory and neuroinflammatory genes. These IL-13 mediated changes were reversed by lebrikizumab highlighting lebrikizumabs' ability to counteract the IL-13 driven neuroactive effects in this hDRG culture model.

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Xerosis cutis and its association with chronic inflammatory illnesses

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Disruption in skin barrier is associated with increased systemic inflammatory markers. It is unknown whether this epidermal disturbance, clinically manifested as xerosis cutis (XC), is linked to systemic illnesses. Using Epic Slicer Dicer, we collected cross-sectional, retrospective data on patients ≥ 50 (N=6384) over a 5-year period (March 2015-March 2020). Patients with ICD-10 diagnosis code of XC were identified and association with chronic illnesses explored in comparison to control group. Stata was used to calculate odds ratios (OR), 95% confidence intervals (CI), and p-values. Caucasian male and female patients with XC were both 1.3 times more likely to develop type II diabetes (OR 1.3,CI 1.1-1.6,P=0.007;OR 1.3,CI 1.0-1.6,P=0.026, respectively) and 1.4 times more likely to develop essential hypertension (OR 1.4,CI 1.2-1.7,P<0.0001;OR 1.4,CI 1.2-1.6,P=0.0001). African American male and female patients with XC were 2.8 and 2.2 times more likely to develop type II diabetes (OR 2.8,CI 1.4-5.5,P=0.001;OR 2.2,CI 1.3-4.0,P=0.003), but only males were 9.4 times more likely to develop essential hypertension (OR 9.4,CI 4.6-20.9,P<0.0001). Caucasian males with XC were 2.2 times more likely to develop atherosclerosis (OR 2.2,CI 1.7-2.8,P<0.0001) and 1.3 times more likely to develop hyperlipidemia (OR 1.3,CI 1.1-1.5,P=0.0004), while African American males were 2.9 times more likely to develop atherosclerosis (OR 2.9,CI 1.3-6.2,P=0.007) with no significant association with hyperlipidemia. Caucasian male and female patients with XC were 1.6 and 1.7 times more likely to develop osteoporosis (OR 1.6,CI 1.1-2.3,P=0.007;OR 1.7,CI 1.4-2.0,P<0.0001), while African American males were 3.5 times more likely to develop depression (OR 3.5,CI 1.5-7.9,P=0.003). Xerosis cutis is associated with specific chronic illnesses. The increased inflammatory cytokines seen may contribute to these diseases. Additional research is needed to further elucidate this link and explore the role of skin barrier restoration in the management of systemic disorders.

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Distinct IL-13 production and accumulation in lesional and non-lesional skin of atopic dermatitis patients

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Background: IL-13 is a central mediator of atopic dermatitis (AD) pathophysiology. While strongly elevated in atopic dermatitis skin, the cellular origins and the localization of this cytokine in human tissue remain poorly defined, largely due to technical limitations. Objective: To use validated methodologies for the detection of IL-13 protein and mRNA in human skin biopsies from atopic dermatitis patients. Results: Using confocal microscopy and flow cytometry, we show that IL-13 protein is highly sensitive to paraformaldehyde (PFA) fixation, which masks key epitopes needed for optimal detection. We demonstrate that PFA-free protocols or heat-induced epitope retrieval lead to successful detection and accurate quantification of this cytokine in the human epidermis and dermis. In order to distinguish between accumulation and production of IL-13 in AD patient skin, we next compared the localization of IL-13 protein with mRNA expression by using confocal *in-situ* hybridization in lesional versus non-lesional skin of AD patients (n=4). Our results demonstrated that IL-13 protein mean fluorescent intensity was higher in the epidermis compared to the dermis in both lesional and non-lesional skin of AD patients. IL-13 protein in lesional skin was also higher than in non-lesional skin. In contrast, a greater number of IL-13$^+$ cells were detected in the papillary dermis compared to the epidermal compartment (n=4), both in lesional and non-lesional AD skin. The majority of IL-13$^+$ cells co-expressed mRNA for T-cell receptor as well as Th2 transcription factor GATA3 (n=4). Our findings also confirm the presence of TCR$^-$ IL-13$^+$ cells. Conclusion: The detection of IL-13 mRNA correlated with some but not all sites of IL-13 protein accumulation, indicating that the target epidermal compartment for IL-13 receptor binding in human skin is distinct from the predominantly dermal production site. These methods and results pave-the-way for more precise identification of the role of IL-13 in human skin during health and disease.

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Reprogramming of tumor-associated macrophages with anti-PD-L1 & lenalidomide in cutaneous T cell lymphoma

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M2-like tumor-associated macrophages (TAMs) promote the growth of cutaneous T cell lymphoma (CTCL) by inducing immunosuppression. Little is known about the underlying mechanisms of PD1+ M2-like TAM phenotypes in CTCL. Understanding the mechanisms of macrophage reprogramming will identify novel therapeutic targets to induce anti-tumor responses. We used multiplex immunofluorescence on CTCL skin samples demonstrating co-localization of PD1 on CD163$^+$ M2 macrophages**.** RNA-seq analysis on tissue sections from same CTCL specimens revealed an up-regulation of the TLR/NF-κB and JAK/STAT signaling pathways. To further confirm PD1 expression on M2 TAMs in CTCL, we cultured CD14$^+$ cells from healthy donor-derived peripheral blood in conditioned media from MyLa cells. We observed macrophage differentiation towards an PD1+ M2 phenotype, with significant CD163, CD206 and PD1 upregulation but not CD80 expression. Our RT-PCR results show significantly elevated IL-10 levels, but decreased IL-1β, CXCL-10 and CXCL-11 compared to control. To determine whether PD1+ M2-like TAMs could be reprogrammed, anti-PD-L1 and lenalidomide were used for treatment of MyLa-conditioned media induced human peripheral blood monocyte-derived PD1+ M2-like TAMs. The results show that anti-PD-L1 and lenalidomide synergistically increased IL-1β, CXCL-10, and CXCL-11 expression, but significantly decreased IL-10 level compared with the untreated control through ablation of the TLR/NF-κB and JAK/STAT signaling pathways, which was linked with functional changes in phagocytic activity and cell migration. In conclusion, TLR/NFκB and JAK/STAT signaling pathways may drive PD1+ M2-like TAMs programming in the CTCL microenvironment, anti-PD-L1 and lenalidomide may polarize PD1+ M2-like to M1-like TAM phenotypes and induce functional changes through ablation of TLR3/4 and downstream signaling pathways.

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A psoriasis mouse model with persistent skin lesions and comorbidities

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A previous psoriasis model using imiquimod applied-wild type mice has major shortcomings, such as lack of chronic disease course and well-known comorbidity of psoriasis. This study aimed to establish a new animal model of psoriasis that properly reflects its pathophysiology. Interleukin-10 deficient (IL-10-/-) and wild type (WT) mice received either imiquimod or vehicle cream for 6 weeks. IL-10-/- mice with imiquimod for 6 weeks showed persistent psoriasis-like inflammation and higher severity index (p=0.001) than did WT mice. They also demonstrated significant body weight loss and shorter colon length (p=0.000 and 0.006) . Histopathologically, they demonstrated significantly thicker epidermis and larger number of CD45+, myeloperoxidase+ cells (p=0.000, 0.000, and 0.004). They also exhibited higher colitis severity score and higher number of CD45+ cells in the colons (p=0.007 and 0.007). Furthermore, quantitative reverse transcription-polymerase chain reaction with regional lymph nodes revealed significantly higher imiquimod-induced IL-17 in IL-10-/- mice (p=0.001). They also showed significantly higher serum levels of IL-17A and cardiac enzymes by enzyme-linked immunosorbent assay (p=0.037 and 0.001). However, IL-10-/- mice with imiquimod showed no significant difference of severity in joint, kidney, or liver inflammation. IL-10-/- mice model with imiquimod may better reflect pathophysiology of severe psoriasis with chronic nature, systemic inflammatory state, and comorbidities such as inflammatory bowel disease or cardiovascular disease.

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Comparing the pediatric and adult plaque psoriasis transcriptomes

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Psoriasis begins in childhood in approximately one-third of cases. While plaques clinically appear quite similar to adult disease, lesions in children may have some differences as a result of early triggers and depending on disease persistence. Children 2 months to ≤18 years of age with plaque psoriasis (n=16, mean age of 16 yr) were compared to age and sex-matched healthy children of similar race (n=16) and to adults age ≥ 40 years with plaque psoriasis starting after 25 years of age (n=12, mean age of 61 yr). Expression of 34,465 genes were compared (RNA-Seq, Hi-Seq 2500) between lesional and unaffected skin. Pathway analysis of the top differentially expressed genes showed a strong role for 1L-17 related genes and those involved with formation of the cornified envelope and anti-microbial humoral response in pediatric psoriasis (fold change >2, p <0.05). The majority of genes involved in pediatric and adult plaque psoriasis were similar, but 3 genes did show difference (CXCL12, NEFH, and FBN2). Findings were validated via RT-PCR performed on 5 genes. Overall, we found that the pediatric plaque psoriasis transcriptome is very similar to the adult transcriptome, but pediatric psoriasis may also be more influenced by neutrophil innate immunity. Looking at treatment-relevant gene expression, children are expected to respond similarly to existing biologic and small molecule therapies.

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How to soothe scalp irritation when your head is covered most of the day?

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The skin of the scalp has several unique features. Amongst them, the follicular density is much higher, creating a dark, warm and moist environment which could conduct to hyperkeratosis (scaling), pruritus, alopecia, and inflammatory signs (erythema, purulence). It is known that the regular wearing of helmets in tropical environment and in polluted cities, or when doing intense sports as well as the scarf wearing (Hijab, Burqa...) increase the moist and the friction of the scalp. These two categories of population are thus prone to more frequent scalp disorders (pruritus, scale apparition and erythema) and may require specific treatment to soothe irritation. In this context, Lucas Meyer Cosmetics developed from by-product of perfume industry a pink berry extract with very efficient antioxidant and anti-inflammatory properties. Its effect at 1% in a leave-on product was analyzed in a double-blind placebo-controlled trial against erythema, pruritus and scalp flakes on 31 volunteers with sensitive scalp wearing helmet or Burqa in New Delhi, a polluted city in a subtropical climate, during the monsoon. Versus placebo, it was observed a decrease in erythema in both group from 7 days (through mexameter measurement or clinical erythema score), a decrease in pruritus score after 14 days on helmet wearer (-27%), a decrease in scalp scaling score at D14 for burqa wearer (-6.1%) and -10.6% decrease in adherent scalp flake scoring at D7 for helmet wearer. The self-assessment confirmed these good results on each group compared to the placebo, attesting to a healthier scalp. It is also interesting to note that the pink berry extract effect is slightly different for each group, showing the different issues observed: greasy scalp and irritation for burqa wearers surely triggered by the friction and for helmet wearers, adherent flakes, burning sensation and pruritus surely triggered by the excess perspiration.

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An integrated scalp and blood biomarker approach suggests the systemic nature of alopecia areata

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Alopecia areata/AA is characterized by immune dysregulation in both scalp and blood, but a largescale approach establishing biomarkers of AA incorporating both scalp tissue and serum compartments is lacking. We aimed to characterize the transcriptomic signature of AA lesional and nonlesional scalp compared to healthy scalp and determine its relationship with the blood proteome in the same individuals, with comparative correlations to clinical AA disease severity. We evaluated lesional and nonlesional scalp tissues and serum from patients with moderate-to-severe AA (n=18) and healthy individuals (n=8). We assessed 33,118 genes in AA scalp tissue using RNAseq transcriptomic evaluation and 340 inflammatory proteins in serum using OLINK high-throughput proteomics. Univariate/multivariate approaches were used to correlate disease biomarkers with Severity of Alopecia Tool/SALT. 608 inflammatory genes were differentially expressed in lesional AA scalp (FCH>1.5, FDR<.05) including Th1 (IFNG/IL12B/CXCL11), Th2 (IL13/CCL18), and T-cell activation-related (ICOS) products. Th1/Th2-related markers were significantly correlated with AA clinical severity in lesional/nonlesional tissue, while keratins (KRT35/KRT83/KRT81) were significantly downregulated in lesional compared to healthy scalp (P<.05). Expression of cardiovascular/atherosclerosis markers (MMP9/CCL2/IL1RL1) in lesional scalp correlated with their corresponding serum expression (P<.05). AA scalp demonstrated significantly greater biomarker dysregulation compared to blood. An integrated multivariate approach combining scalp and serum biomarkers improved correlations with disease severity. This study contributes a unique understanding of the phenotype of moderate-to-severe AA with an integrated scalp and serum biomarker model suggesting the systemic nature of the disease, advocating for immune-based systemic treatment.

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Atopic dermatitis-induced psychological stress-related behavioral changes in NC/Nga mice model

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Previous studies reported that atopic dermatitis (AD) can be triggered or exacerbated by psychological stress, and emotional factors can change the natural course of the disease. Also, AD patients frequently experience depression, anxiety, and anxiousness and showed lower life quality than healthy controls. However, how psychological stress occurs in patients with atopic dermatitis is not fully understood. To understand the interconnection between AD and psychological stress, we evaluated stress-related behavioral changes in the AD mouse model using NC/Nga mouse. In this study, we utilized an open field test, tail suspension test, sociability test, and glucose preference test to evaluate anxiety, stress, social novelty, and anhedonia. In AD-induced mice, anxiety and stress levels were higher than that of the control group, but social novelty and anhedonia were not different than those of the control group. When psychological stress was relieved with mouse refuge, diamond twist, and nesting material, AD-induced anxiety and stressful behavior were recovered. However, the addition of chronic restraint stress in the AD mouse model did not alter stress-related behavior or clinical symptoms. Overall, our results have shown that the development of AD itself can increase anxiety and stress, and the relief of psychological stress can be a potential adjuvant treatment for AD. In the future, further investigation of molecular changes in brain, skin, dorsal root ganglion, and immune cells will be needed to fully understand the relationship between AD and psychological stress and brain-skin connection.

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Neutrophil and C5aR dynamics in hidradenitis suppurativa disease progression

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Hidradenitis suppurativa (HS) is a chronic skin disease characterized by neutrophil-rich inflammatory nodules, abscesses or sinus tracts. The complement 5a receptor 1 (C5aR or CD88) is highly expressed on neutrophils and is a major driver of the pro-inflammatory functions of complement activation. We have previously demonstrated complement activation and elevated C5aR gene expression in HS skin lesions. In this study we examined alterations in neutrophils and C5a receptors in circulation and within the skin lesions of HS patients. HS patients showed higher circulating neutrophils as a % of total leukocytes compared to healthy donors (60% vs. 51%), as well as fewer monocytes (3.1% vs 7.4%) and eosinophils (0.9% vs. 2.8%). Blood neutrophil counts in patients with severe disease (Hurley stage III) were increased compared to patients with moderate stage II disease (6.4 vs. 5.3, $x10^9$/mL). HS patient leukocytes also showed changes in C5a receptor surface levels. C5aR levels were reduced by 15% on HS-derived compared to healthy-derived neutrophils, whereas levels of the immunomodulatory C5a receptor C5L2 (C5aR2 or GPR77) were increased in HS patients by an average of 1.3 fold on neutrophils, 1.7 fold on monocytes, and 1.95 fold on eosinophils. HS neutrophils were more prone to activation as evidenced by higher production of reactive oxygen species upon stimulation. In HS skin lesions, neutrophil infiltration was observed deep in the dermis and adjacent to sinus tracts, and neutrophil extracelluar trap formation was detected. Recently we demonstrated that avacopan, a specific C5aR inhibitor, significantly improved the Hidradenitis Suppurativa Clinical Response (HiSCR) vs. placebo (42.6% vs 22.2%, mean responder rate) in Hurley stage III patients in a phase 2 trial. Together, these findings implicate a critical role of C5aR in disease progression and warrant further development of avacopan for HS.

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Interrogating mechanisms of granuloma annulare pathogenesis through RNA sequencing

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Granuloma annulare (GA) is a cutaneous granulomatous disease with a wide array of clinical presentations but occurs most often as localized, non-scaly, erythematous, annular plaques on the distal extremities. While lesions are typically self-limited, they may recur and become chronic, negatively impacting patient quality of life and leading many to seek therapy due to cosmetic concerns. Although GA is quite common, with a prevalence of 0.1-0.4%, the etiology and pathogenesis of the disease remain unknown. Thus, therapies offered to patients, especially those with recurrent or chronic disease, are unstandardized and rarely evidence based. In one study of 67 GA patients, duration of lesions did not differ significantly between treated and untreated patients, suggesting current treatments are not efficacious. To investigate the transcriptomic differences between GA lesional skin vs. healthy skin, we isolated and sequenced RNA from FFPE blocks of 32 patients with clinically defined GA and 6 healthy controls. Using two-fold changes in expression and false discovery rate of 10% as criteria, we identified 3,512 differentially expressed genes (DEGs) in GA, with 1,583 upregulated. Gene ontology analysis of these upregulated genes revealed significant immune dysregulation, including reactome pathways for innate immunity, adaptive immunity, cytokine signaling, toll like receptor (TLR) cascades, and T cell receptor signaling. The top activated canonical pathways include Th1, Th2, TREM1 signaling, and granulocyte/agranulocyte trafficking. Upstream analyses reveal downstream expression patterns responsive to activation of innate transcriptional regulators, including TLRs, MYD88, NFkB, TNF, IL-1B, and many more. These transcriptional analyses support findings from prior studies which implicate dysregulation in innate and adaptive pathways as central to GA pathogenesis, provide a closer look at the downstream networks, and suggest intriguing new therapeutic avenues.

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Gut dysbiosis plays a role in the development of alopecia areata

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Alopecia Areata (AA) is a highly prevalent autoimmune disease leading to hair loss in affected individuals. We and others have demonstrated a strong genetic component in the development of AA, however, emerging evidence suggests that environmental factors clearly contribute to AA pathogenesis. To determine the role of the microbiome as an environmental factor in AA, we performed 16S sequencing of skin, hair follicle (HF), and feces from 34 AA patients and 12 healthy controls (HCs). We found no significant differences in the composition of the skin or HF microbiome, but we discovered striking gut dysbiosis in AA patients compared with HCs. We detected a pronounced dysbiosis characterized by an increased relative abundance of members of the *Firmicutes* phylum and decreased relative abundance of the *Bacteroides* phylum in the gut microbiome of AA patients compared with HCs. Utilizing metagenomics sequencing, we identified perturbations in the relative abundance of different microorganisms of the *Firmicutes* and *Bacteroides* phyla, such as *Ruminococcus sp.*, *Alistipes shahii*, and some *lactobacillales* in the gut microbiome of AA patients. To investigate the causal role of the gut microbiome in AA development, we conducted antibiotic-mediated depletion of the gut microbiome in C3H/HeJ mice, and found that treated mice were largely protected from hair loss, concomitant with decreased numbers of $CD8^+$ T cells and an increase in the Treg/$CD8^+$ ratio. Taken together with recent reports of reversal of AA in patients following fecal microbiota transplant (FMT), our findings suggest that restoring homeostasis of the gut microbiome may represent a new therapeutic approach for AA. We recently initiated a clinical trial of FMT for treatment of AA, in which we will monitor the normalization of gut microbiome dysbiosis relative to the restoration of hair growth.

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Topical steroids as a noninvasive treatment for pediatric pyogenic granulomas

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Pyogenic granulomas (PG) are benign vascular proliferations of the skin and mucous membranes that are traditionally treated by surgical excision, electrocautery, cryotherapy, or laser therapy.[1] Because procedural treatments can be costly, painful, inconvenient, leave a scar, and require a live patient intervention, alternate options are of investigational interest. We present two children with PGs who were successfully treated with topical clobetasol 0.05% ointment under occlusion. In our first case, a 15-year-old boy with autism spectrum disorder and two facial PGs underwent biopsy and was unable to tolerate further procedural treatment; after applying topical clobetasol nightly for three months, the lesions reduced in erythema and size. The second patient, a 3-year-old girl with a clinically diagnosed facial PG, used topical clobetasol daily for eight weeks, resulting in significant PG regression at 6 weeks and complete involution 10 months later. For both patients, there were no adverse responses noted following consistent topical application between 8 weeks to 3 months. Our cases demonstrate that topical potent corticosteroid applied under occlusion may offer a safe, effective, and noninvasive therapeutic option for intact PGs in children. The vasoconstrictive properties of topical steroids in addition to possible angiogenic factor inhibition may contribute to the pathophysiologic mechanism behind clinical response.[2] In addition to pediatric patients, topical steroids may provide a PG treatment alternative for patients unable to tolerate procedures and/or those seen virtually. 1. Lin RL , Janniger CK. Pyogenic granuloma. Cutis 2004;74:229-33. 2. Greenberger S, Boscolo E, Adini I, Mulliken JB , Bischoff J. Corticosteroid suppression of VEGF-A in infantile hemangioma-derived stem cells. N Engl J Med 2010;362:1005-13.

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Pityriasis rubra pilaris treated with guselkumab: Interim analysis of a single-arm trial

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Pityriasis rubra pilaris (PRP) is a rare inflammatory skin disorder characterized by generalized pruritic erythematous scaly plaques and waxy palmoplantar keratoderma with painful fissuring. PRP is a life-altering condition with severe detriment to patients' quality of life, but as of yet there is no FDA-approved medication for the treatment of PRP. The exact pathogenesis of PRP is unclear, but previous studies have demonstrated the role of the IL-23/Th17 axis in the development of this condition. We are conducting an ongoing investigator-initiated single-arm trial to investigate the use of guselkumab, a monoclonal antibody against IL-23, in the treatment of PRP (clinicaltrials.gov identifier NCT03975153). The study cohort includes patients 18-99 years of age with moderate-to-severe PRP (body surface area $\geq$ 10%) and no major comorbidities. Subjects received guselkumab 100 mg subcutaneous injection according to the FDA-approved dosing schedule for psoriasis (weeks 0, 4, 12, and 20) and progress was tracked via both investigator- and patient- reported outcomes. The primary endpoint of the study was at week-24; to date, six subjects have completed 24 weeks of treatment. Results from this cohort were analyzed using two-tailed paired t tests. An intention-to-treat analysis was conducted. The study cohort is composed of 4 males and 2 females with a median age of 59.5 years. Mean clinician-assessed severity as measured by PASI decreased significantly from week-0 (31.4, SD=13.5) to week-24 (10.8, SD=10.5) (p=0.0159). Five of six patients experienced a greater than 50% improvement in PASI score, and one patient did not complete the trial due to lack of improvement. Patient-reported quality of life as measured by the Dermatology Life Quality Index (DLQI) was also significantly improved from week-0 (21.3, SD=5.7) to week-24 (9.5, SD=9.4) (p=0.0074). Interim results demonstrate the disease burden of untreated PRP and the dramatic improvement in PRP symptomology following a course of guselkumab therapy.

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Interleukin 6 signalling in endovascular progenitors is a driver of melanoma vascularisation and metastasis

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The development of new vascular structures is a pre-requisite for melanoma growth and spread. Previous studies have shown that tumour vessels arise from endovascular progenitor (EVP) forming transit amplifying (TA) and then differentiated (D) endothelial cells in various melanoma models. RNA-seq of EVPs as compared to D cells pointed to *IL-6/JAK/STAT* signalling as significantly upregulated in the EVP. This was validated using P-STAT3 staining on sorted cells. Of importance IL6Ra was upregulated substantially in tumour EVPs but not in other vascular beds such as the aorta. We next sought to specifically target EVP activity. Anti-IL6Rα and Ruxolitinib blocked JAK/STAT signalling, significantly reducing EVP infiltration into the tumour. Importantly, IL6Rα treatment caused a reduction in tumour size. Surprisingly, leading anti-angiogenic, Anti-VEGF-A did not affect EVP function. In HCMel12 metastatic model of melanoma injected subcutaneously, IL6Rα inhibition reduced EVP infiltrate into primary tumours delays metastatic spread. Neoadjuvant treatment with anti-mIL6Rα antibodies significantly reduced the number of metastatic nodules and size of nodules, confirmed by micro-CT imaging and subsequent H&E staining of murine lung tissue. Of importance, IL6Rα inhibition significantly reduced neutrophil and macrophage infiltration but increased the number of infiltrating CD8+ T cells suggesting that part of the observed benefit emanates from anti-tumour immunity. In conclusion, IL6 signalling is a key regulator of endovascular progenitor ability to form vessels and regulate immune infiltration and tumour metastases.

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Rational engineering of recombinant modified vaccinia virus Ankara to enhance both innate and adaptive immunity for cancer immunotherapy

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Intratumoral (IT) delivery of immune-activating viruses can serve as an important strategy to turn "cold" tumors into "hot" tumors resulting in overcoming resistance to immune checkpoint blockade (ICB). Modified vaccinia virus Ankara (MVA) is a highly attenuated, non-replicative vaccinia virus that has a long history of human use. Here we report that IT recombinant MVA (rMVA) lacking the E5R gene, which encodes a cGAS inhibitor, and with the expression of Flt3L, a dendritic cell growth factor, and OX40L, a T cell co-stimulatory molecule, generates strong antitumor immunity. The antitumor effects are dependent on the cGAS/STING-mediated cytosolic DNA-sensing pathway, STAT1/STAT2-dependent type I IFN positive feedback loop, as well as $CD8^+$ T cells. RNA-seq analyses of tumors one day after injection revealed STING-dependent upregulation of Ifnb, proinflammatory cytokines and chemokines, and dendritic cell activation markers. Remarkably, IT rMVA results in the depletion of $OX40^{hi}$ regulatory T cells (Tregs) via OX40L/OX40 interaction. $OX40^{hi}$ Tregs isolated from tumors are more suppressive than $OX40^{low}$ Tregs and exhibit striking transcriptomic differences compared with $OX40^{low}$ Tregs, which includes high expression of genes involved in cell proliferation, DNA repair, oxidative phosphorylation, and low expression of genes involved in IFN responses. Furthermore, *ex vivo* infection of rhMVA which expresses human OX40L in human extramammary paget's disease tumors results in the reduction of Tregs and activation of $CD8^+$ T cells. Taken together, our study provides a proof-of-concept design resulting in the improvement of MVA-based cancer immunotherapeutics through modulation of both innate and adaptive immunity.

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Validation of CXCL9 as a biomarker in morphea

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Morphea**,** also known as localized scleroderma, is a skin condition that has an initial presentation of inflammation followed by sclerosis. Clinical disease state (active versus inactive) is difficult to ascertain in morphea using clinical examination alone. To date, no biomarkers indicative of disease activity and/or severity have been validated in morphea. Biomarkers are needed to help guide clinical assessment of patients to determine 1) disease state, 2) subclinically active disease, and 3) early detection of recurrence of activity. Based on our preliminary studies, we hypothesize that interferon-gamma regulated chemokines, particularly CXCL9, may serve as useful biomarkers of morphea disease state. We analyzed baseline sera samples from 387 patients with morphea using enzyme-linked immunoassays to quantify CXCL9 levels. Analysis revealed morphea patients had elevated CXCL9 levels (median 81.6pg/mL, IQR 45.8-177.0) when compared to 26 age, race, and gender-matched controls (59.7pg/mL, IQR 44.1-79.8) (p=0.05). Importantly, CXCL9 levels were more highly elevated in those with active morphea (defined by a mLoSSI score >3) compared to inactive morphea, where median values were similar to that of controls (median 118.1 vs 59.7, IQR 53.8-278.3 vs 44.1-79.8) (p=0.003). CXCL9 also demonstrated correlation with clinical outcome measures indicative of an active disease state (activity component of the validated LoSCAT) (r=0.36, p<0.0001). Longitudinal analysis of 58 patients followed over a mean of 5 years with a median of 3 follow-up visits corroborated these results and also identified a correlation between elevated CXCL9 levels with increased age and greater functional involvement. Taken together, this study further validates CXCL9 as a biomarker for activity in morphea. Furthermore, it may be useful in monitoring for disease recurrence as elevations may occur prior to clinically evident activity. Consideration should be made to incorporate this measure as a supplement to clinical assessment.

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Pharmacological blockade of the CX3CR1/CX3CL1 fractalkine axis prevents alopecia areata in C3H/HeJ mice

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AA is an autoimmune disease driven by effector cytolytic $CD8^+$T cells (CTLs) that infiltrate the hair follicle (HF). Therefore, targeting T effector migration and function represents a promising strategy for treating AA. The CX3CR1/CX3CL1 chemokine axis, also known as fractalkine, has been implicated in immune cell migration and directing a Th1 inflammatory response. Using RNA seq analysis of whole skin, we found that the expression of the CX3CR1, as well as its ligand CX3CL1, were significantly increased in lesional skin from both patients with AA and C3H/HeJ AA mice. To define the role of the CX3CR/CX3CL1 chemokine axis in AA pathogenesis, we investigated CX3CL1 expression in lesional skin of patients with AA as well as C3H/HeJ mice, and studied the effects of CX3CR1/CX3CL1 axis blockade in the C3H/HeJ mouse model of AA. Using immunostaining, we found that the expression of the ligand CX3CL1 was significantly increased in HFs from human AA lesional skin compared to normal control skin. We found that the immune infiltrates contained several cell types expressing the receptor, including $CX3CR1^+CD4^+$ T cells, $CX3CR1^+CD8^+$ T cells, and $CX3CR1^+CD68^+$ monocytes in AA patient skin compared to controls. In lesional C3H/HeJ mice with AA, we also detected $CX3CR1^+CD8^+$ T cells infiltrating in and around HFs, which also showed increased HF expression of CX3CL1. Lastly, we investigated the effect of CX3CR1/CX3CL1 axis blockade on disease development in C3H/HeJ skin grafted mice. We found that AZD8797, a small molecule inhibitor of the chemokine receptor CX3CR1, prevented AA development in C3H/HeJ skin grafted mice. Furthermore, neutralizing CX3CL1 using an anti-CX3CL1 mAb significantly delayed AA development in C3H/HeJ skin grafted mice. Collectively, our data suggest that the CX3CR1/CX3CL1 axis contributes to the pathogenesis of AA, and introduces a novel therapeutic target for the treatment of AA.

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Vascularization in the deep dermis of hidradenitis suppurativa lesions

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Hidradenitis Suppurativa (HS) is a chronic inflammatory disease with presentation ranging from nodules and abscesses to draining tunnels. Dermal tunnels are unique to HS, and are marked by keratinocyte hyperproliferation and differentiation, raising the question of whether the tunnels are akin to neoplastic structures. Recent data by our lab demonstrated elevated levels of CXCL8 (IL-8), a potent mediator of angiogenesis. CXCL8 staining was especially prominent around HS tunnels. We hypothesized that high levels of CXCL8 and cellular proliferation could lead to neovascularization in the dermis and deep dermis. In this study, we aimed to characterize the angiogenic changes relating to HS and dermal tunnels. Following Institutional Review Board approval, we enrolled untreated Hurley Stage II and III HS patients. Biopsies were taken from lesional, perilesional and nonlesional skin, and control skin was obtained from healthy volunteers. Samples were stratified based on the presence or absence of histologically confirmed dermal tunnels. Neovascularization was assessed via immunohistochemistry. Chemokine levels were analyzed by RT-qPCR from HS biopsies. HS samples showed significantly elevated vascularization compared to control samples. Vessel development was concentrated in the dermis. Neovascularization was extensive but was especially prominent around dermal tunnels and primarily composed of blood vessels. ICAM-1 confirmed widespread inflammatory activation of blood vessels. The association between CXCL8, dermal tunnels and neovascularization supports a model of extensive remodeling in HS tunnels. Positive ICAM-1 staining suggests leukocyte transmigration into HS lesions from new vessels. This suggests vascularization may be instrumental in supporting the continuous immune infiltration in HS and may contribute to the chronicity of lesions. Treatments targeting angiogenetic pathways may be explored to target HS progression.

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Caloric restriction during aging alters expression of dermal extracellular matrix-related genes in mice

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Multiple studies in mice show that long-term caloric restriction (CR) during aging effectively increases lifespan. However little is known regarding the effects of CR on skin aging. The major manifestations of skin aging are related to deleterious alterations of dermal extracellular matrix (ECM) homeostasis. Therefore, we have investigated the impact of CR on dermal ECM-related gene expression. Beginning at four months of age, mice (n=12, both genders) were either fed ad libitum or given 30% fewer calories. Dorsal skin samples were taken at 4, 12 and 24 months of age, total RNA was isolated, and gene expression was quantified using a custom ECM Array by real-time PCR. The array measured expression of 91 genes (plus internal control house-keeping gene) known to have important ECM functions, including structural proteins, ECM-binding integrins, matrix metalloproteinases, growth factors, cytokines, and regulatory mediators. Interestingly, CR altered expression of a relatively small subset of the measured genes. Expression of CCN2 (connective tissue growth factor), CCN3, Smad3, Tenascin C, Aggrecan, Nidogen 1, Collagen 4A1, and Collagen 14A1 were significantly elevated (2-fold or greater). Importantly, the functions of the proteins encoded by these genes are involved in maintenance of the dermal ECM or basement membrane. In addition, three members of the membrane-type matrix metalloproteinase (MT-MMP) family MMP-14, MMP-15, and MMP-16 were elevated in skin of CR mice. Unlike secreted forms of MMPs, these MT-MMPs are thought to function in ECM homeostasis. Expression of a smaller number of genes was significantly reduced (reduced at least 50%) by CR. These genes included Fibulin 1, Tenascin XB, Versican, Lysyl Hydroxylase 1, and Interlukin-6. Notably, elevated levels of interleukin-6 are associated with aging-related physiologic decline. In summary, the above data reveal that CR may improve ECM homeostasis by regulating a specific subset of genes in mouse skin.

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The CDK4/6 inhibitor palbociclib enhances the vulnerability of Merkel cell carcinoma via the HIF2α pathway

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Merkel cell carcinoma (MCC) is a highly aggressive and immunogenic skin cancer. About 80% of MCCs are caused by infection with the Merkel cell polyomavirus (MCPyV), where T-antigens are constantly expressed in host cells and promote tumor formation by altering the regulation of the cell cycle and other cellular pathways involved in cell transformation. CDK4/6 are critical components of the cell cycle and play an essential role in the initiation and progression of tumors including MCC. Recent evidence also implicates CDK4/6 in immune surveillance; preclinical studies indicate that CDK4/6 inhibitors potentiate anti-tumor immunity via PD-L1 posttranslational upregulation when combined with anti-PD-(L)1 therapy. Although the effects on PD-L1 protein stability induced by palbociclib are already known, the possibility that palbociclib regulates PD-L1 transcription has not been investigated. Indeed, we found that PD-L1 mRNA levels are significantly upregulated by palbociclib in MCC. We found that this effect is the result of an increase in Hypoxia-inducible factor 2α (HIF2α), a transcription factor induced by intracellular Reactive oxygen species (ROS) after palbociclib treatment. However, PD-L1 expression is not a reliable biomarker for response to PD-(L)1 inhibitors, as some patients with little to no PD-L1 expression can experience a good response. In addition, HIF2α is the main driver of cellular responses to low oxygen and plays an important role in tumor progression and metastasis. Therefore, we administered TC-S7009, a HIF2α-specific inhibitor, which prevented palbociclib-induced PD-L1 increase and enhanced cell death compared to treatment with palbociclib alone. In this study, we focused on the induction of immunogenic cell death (ICD) by combining the cell cycle inhibition with hypoxia, thereby indicating a mechanism of anti-tumor activity beyond inhibition of cell proliferation by CDK4/6 inhibitor(s).

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Blood analysis uncovers novel inflammatory, oncologic and cardiovascular biomarkers in psoriasis and Hidradenitis Suppurativa

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Hidradenitis Suppurativa (HS) is a chronic inflammatory skin disease with manifestations ranging from nodules and abscesses to draining tunnels. Despite the significant morbidity associated with the disease, there is a lack of effective treatments and biomarkers of disease activity. To evaluate the serum proteomic signature of HS relative to other systemic skin diseases, we analyzed 1536 serum biomarkers using the OLINK high-throughput cardiovascular, neurology, oncology and inflammatory panels in moderate to severe HS (n=11), psoriasis (n=10) and healthy control volunteers (n=10). Overall, HS had >190 unique differentially expressed proteins (DEPs, abs(FCH)≥1.2, p≤0.05) relative to healthy controls whereas psoriasis had >55 DEPs relative to healthy controls. Both psoriasis and HS showed an increase of serum proteins related to multiple inflammatory pathways, however, there was a higher inflammatory burden associated with HS. Compared to age- and BMI-matched healthy controls, psoriasis and HS had an increase of atherosclerotic and cardiovascular biomarkers, with HS having a higher number of these biomarkers relative to psoriasis. Further analysis identified >40 biomarkers significantly (≤0.05) correlated with PASI score and >130 biomarkers significantly correlated with ISH4 scores. In HS, several biomarkers of atherosclerotic disease significantly correlated with ISH4 scores. This study was limited by inclusion of only moderate-severe psoriasis and HS patients. Our data suggests that HS has a distinct immune, cardiovascular/atherosclerotic and oncologic signature, which presents novel therapeutic avenues.

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Transcriptomic immune sensors and endotype modeling in psoriasis via systems biology

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We previously reported that individuals with psoriasis exhibit an increase in inflammatory monocytes which could be used as a predictor of psoriasis severity and which may act as immunologic sensors of systemic alterations in the inflammatory milieu. This current large cohort study confirms critical links between psoriasis-related transcript sensors and associated comorbidities. We present a multifaceted biological analysis incorporating whole blood RNA-seq data from individuals with psoriasis (n=68, 15 PsA; mean PASI=10.0 (range: 0-35.6)) and controls (n=15) along with phenotypic data and GSVA pathway enrichment (p<0.05). Using RNASeq performed on a NextSeq 550 (15M+ paired reads/sample, 75 bp), we identified significantly differentially expressed genes (DEGs; p<0.05) and pathways between psoriasis (including PsA) patients and controls as well as linear regression signatures of age and PASI. Linear regression with PASI revealed a unique set of biomarkers involved in interferon (IRF8), cytokine (CCR6), and T cell activity (IL17RC, CD40LG) in the most severe forms of psoriasis. Linear regression of age identified a signature in older individuals characterized by upregulation of genes associated with antigen-presenting cells (CD86, HLA-DRA) and downregulation of adhesion molecules (SIRPG, NRCAM, ITGA6) and metalloproteinases (ADAM12, MMP28). Application of a comprehensive systems biology approach revealed candidate gene targets within adhesion, interferon, TGF-β, and AKT/mTOR signaling pathways in psoriasis patients. We also identified interesting correlations between clinical outcomes (BMI, hsCRP, CBCs, and patient self-reported such as itch), surface markers via flow cytometry (intermediate monocyte %), and the transcriptome. Combining whole blood-derived RNA-seq data with phenotypic data may allow us to identify a patient-specific and multi-omic endotype that can point to specific biomarkers or targets within circulating immune cells and associated pathways.

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Not 'Just Another Kinase': The therapeutic potential of JAK inhibitors in the treatment of atopic dermatitis

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Background: Atopic dermatitis is a chronic, relapsing and remitting disease that can be difficult to treat despite recently approved biologic therapies targeting IL-4/IL-13 receptors. Oral Janus Kinase inhibitors (JAKi) represent a novel therapeutic class of targeted therapy to treat moderate-severe atopic dermatitis (AD). Objective: To review the efficacy, safety, and pharmacokinetic characteristics of JAKi in the treatment of AD. Methods: A PRISMA systematic review was conducted using MEDLINE, EMBASE (Ovid), and PubMed databases for studies assessing the efficacy, safety, and/or pharmacokinetics of oral forms of JAK inhibitors in the treatment of AD in pediatric or adult populations from inception to December 2020. Given the relatively limited evidence for each JAKi and the differences in patient eligibility criteria between studies, the data was not deemed suitable for a meta-analysis at this time. Results: 365 papers were reviewed. Of 21 articles that underwent full text screening, 8 met our inclusion criteria for final qualitative review. Three studies examined abrocitinib; three studies examined bariticinib; one examined gusacitinib (ASN002), and another upadacitinib. Significant clinical efficacy and a reassuring safety profile was reported for all JAKi agents reviewed. Rapid symptom control, as early as the first and second week of initiation, was reported for abrocitinib and baricitinib. The most common treatment-emergent adverse events included respiratory and gastrointestinal symptoms, which were mild and transient in nature, and amenable to symptomatic treatment. Conclusion: Given its rapid symptom control combined with a reassuring safety profile, we recommend considering the use of JAKi as a second-line systemic therapy for adult patients with moderate-severe AD who are unresponsive to topical treatment or biologic therapies.

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Single cell transcriptomic analysis of the peripheral neutrophil compartment in psoriatic arthritis reveals heterogeneity and novel potential therapeutic targets

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Psoriatic arthritis (PsA) is a debilitating immune-mediated inflammatory disease that affects approximately 20% of patients with plaque psoriasis (PsO). Neutrophil subsets are elevated in psoriatic disease, however their role in chronic inflammation and mechanisms driving synovio-entheseal inflammation has yet to be fully elucidated. We performed single cell RNASeq of peripheral blood neutrophils from age- and sex-matched patients to identify neutrophil specific pathways driving the development of PsA. Machine learning was performed for non-linear dimension reduction analysis and revealed significant heterogeneity within the neutrophil compartment, identified specific clusters enriched in PsA patients, and key molecular genes and networks (extracellular matrix remodeling, adhesome and inflammasome) that are differentially regulated in neutrophils from patients suffering psoriatic arthritis vs. plaque psoriasis. We also generated complex pseudotime trajectories to accurately reconstruct neutrophil biological transition states. We identified key regulatory genes and transcription factors such as MEF2C, shown to promote myeloid progenitor proliferation in a mouse model, and whose deletion suppresses progenitor expansion and corrects neutrophilia in miR-223-/- mice. We identified novel potential therapeutic targets for preventing or reversing neutrophil-mediated mechanisms driving psoriatic arthritis, and significantly expanded our understanding of the complicated disease pathophysiology that differentiates psoriatic arthritis from plaque psoriasis to guide therapeutic approaches.

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Hypoxia induced Multipotent Stem Cell-Secreted Proteins Induce Hair Growth in a Phase 1a/2b trial in Male Pattern Baldness

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A Phase 1b/2a clinical trial was performed to study the safety and efficacy of a bioengineered human cell-derived formulation, termed Hair Stimulating Complex (HSC), in stimulating hair growth and preventing hair loss in subjects with male pattern baldness. HSC contains naturally secreted growth factors known to be important in hair growth, including follistatin, KGF, VEGF, PLGF, HGF, and angiogenin. These growth factors are key to stimulating the hair follicle stem cells, supporting keratinocyte migration into the follicle to result in new hair formation, and promoting angiogenesis to supply increased nutrients to support the highly metabolically active anagen phase. Several ELISA and cell-based assays, including a human outer root sheath assay, are used to release the clinical product. The study was a double-blind, 2:1 randomized, single center trial in 36 subjects with Norwood Hamilton scores of 3V, 4, and 5. All subjects tolerated well the twenty 0.1cc intradermal injections, distributed between the vertex and temporal regions, administered at baseline, 6, and 12 weeks, with no signs of a serious adverse reaction reported. Canfield image analysis of treated sites were taken at baseline and 18 weeks. At the 18-week time point, 75% of the HSC subjects responded to treatment and showed new hair growth and a cessation in hair loss whereas 63% of the control subjects continued to lose hair. Canfield imaging at week 26 will assess total hair count numbers as well as vellus, non-vellus hairs and hair thickness. These results clearly demonstrate the safety and efficacy of intradermal injections of HSC in subjects with androgenetic alopecia and support initiating a larger phase 2 dosing study.

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