**1**

**ABERNATHY-CLOSE**

**Activation of dermal cGAS/STING signaling in cutaneous lupus erythematosus lesions is associated with skin damage and scarring**

**Lisa Abernathy-Close1**, Mehrnaz Gharaee-Kermani1,2, Annie Lu3, Amanda Victory1, Amy Hurst1, Feiyang Ma1,2, Allison Billi2, J. Michelle Kahlenberg1,2

*1Department of Internal Medicine, Division of Rheumatology, University of Michigan, Ann Arbor, MI, 2Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI, 3Department of Dermatology, University of Michigan, Ann Arbor, MI*

Cutaneous lupus erythematosus (CLE) is a prevalent, disfiguring skin manifestation of lupus. CLE is a heterogeneous skin disease; discoid lupus erythematosus (DLE) lesions often result in alopecia, dyspigmentation, and skin scarring, whereas subacute CLE (SCLE) lesions cause significantly less skin damage and resolve with scarless healing. Dermal fibroblasts are important responders to type I interferons produced in lupus skin, primarily by keratinocytes, and thus may be critical mediators of skin inflammation and resolution of CLE lesions. Here, we aimed to identify dysregulated pathways involved in cutaneous type I interferon responses among scarring versus nonscarring CLE subtypes through single-cell RNA sequencing of fibroblasts and keratinocytes isolated from DLE (n = 5) and SCLE (n = 8) lesions. Pathway analysis revealed that the cyclic GMP–AMP Synthase (cGAS)/Stimulator of Type I Interferon Genes (STING) signaling is specifically activated in fibroblasts from DLE lesions, compared to SCLE, yet the cGAS/STING pathway was not significantly altered in keratinocytes. Immunohistochemistry confirmed that STING is more abundant in non-lesional lupus skin when compared to healthy controls, indicating this is a relevant pathway involved in lupus-specific skin disease pathogenesis. This study identifies a novel role for cGAS/STING signaling in modifying skin damage and scarring in lupus.

**2**

**AKINJIYAN**

**Exploring DDR2’s role in CAF extracellular matrix production and tumor progression**

Favour A. Akinjiyan1, Zainab Ibitoye1 Gregory D. Longmore1, Katherine C. Fuh 1,2

*1Washington University School of Medicine, St. Louis, MO, 63110, USA*

*2University of California San Francisco, CA, 94143, USA*

Tumor metastasis in various cancers is promoted by interactions of stromal cells, such as cancer-associated fibroblasts (CAFs) in the tumor microenvironment (TME), with tumor cells.  CAFs play a key role in tumor progression by remodeling the TME and extracellular matrix (ECM) to result in a more permissive environment for tumor progression. It has been shown that fibroblasts, in particular myofibroblasts, utilize metabolism to support ECM remodeling. However, the intricate mechanisms by which CAFs support collagen production and tumor progression are poorly understood. In this study, we show that the fibrillar collagen receptor, Discoidin Domain Receptor 2 (DDR2), promotes collagen production in human and mouse CAFs through arginase activity (3-fold decrease upon DDR2-depletion, (p<0.001). CAFs with high DDR2 or arginase promote tumor colonization of metastatic sites. In addition, DDR2-depleted CAFs had decreased ornithine levels leading to decreased collagen production and polyamine levels compared to WT control CAFs (5-fold decrease, p<0.001). Tumor cell invasion was decreased in the presence CAF conditioned media (CM) depleted of DDR2 or arginase-1, and this invasion defect was rescued in the presence of CM from DDR2-depleted CAFs that constitutively overexpressed arginase-1. Similarly, the addition of exogenous polyamines to CM from DDR2-depleted CAFs led to increased tumor cell invasion. We detected SNAI1 protein at the promoter region of the arginase-1 gene, and DDR2-depleted CAFs had decreased levels of SNAI1 protein at the arginase-1 promoter region. Furthermore, high stromal arginase-1 expression is correlated with poor survival in ovarian cancer patients (28 months vs 64 months median overall survival). Additionally, high DDR2 and arginase levels are correlated with worse outcomes in various skin cancers.  These findings highlight how DDR2 regulates collagen production by CAFs in the tumor microenvironment by controlling the transcription of arginase 1, and CAFs are a major source of arginase activity and L-arginine metabolites in cancer models.

**3**

**ALPHONSE**

**Inflammasome-mediated host defense against *Staphylococcus aureus* skin infections is mediated by neutrophil-intrinsic NLRP12 and caspase-8 signaling.**

Martin P. Alphonse1, Haiyun Liu1,Dustin Dikeman1, Roger V. Ortines1, Yu Wang1, Qi Liu1, Christine Youn1, Gaofeng Wang1, Emily Cahill1, Denion Prifti1, Andrea Cox2, Luis Garza1, Lloyd S. Miller1,3, Nathan K. Archer1

*1Dermatology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA, 2Molecular Microbiology and Immunology, Johns Hopkins University School of    Medicine, Baltimore, Maryland, USA, 3Janssen Research and Development, Spring House, Pennsylvania, USA*

With the emergence of antibiotic-resistant bacteria, there is an unmet clinical need to develop novel therapies to treat skin infections. The inflammasome components involved in response to *S. aureus* stimulation are well established *in vitro,* however the inflammasome signaling in immune cells during *in vivo S. aureus* skin infections are not entirely understood. Therefore, we used an *in vivo* mouse model of *S. aureus* intradermal infection to define the immune cells with inflammasome formation during *S. aureus* skin infections. Using an ASC-Citrine reporter mouse, we observed neutrophils as the predominant inflammasome-forming population in the infected skin. Interestingly, NLRP3, AIM2, or NLRC4-deficient mice, had no host defense defect compared to WT mice. Therefore, by performing co-immunoprecipitations from infected skin biopsies, we discovered that the inflammasome sensor, NLRP12, associated with the ASC adaptor protein. Furthermore caspase-1, -11, and caspase-1/11 double-deficient mice had no significant difference in host defense compared to WT mice. Thus, we generated S100A8Cre-Casp8fl/fl mice) and discovered a significant host defense defect compared to WT mice. Collectively, our data indicated that NLRP12 and caspase-8 inflammasome signaling in neutrophils was critical for protection against *S. aureus* skin infections, providing potential therapeutic targets.

**4**

**ASSAN**

**Pathogenic *ADAR1* mutations in severe psoriasis vulgaris**

**Assan F**1, McGrath M1, Frémond ML2, Izmiryan A1, Crow Y2, Bachelez H1

***1:*** *Genetic Skin Diseases, INSERM U1163, Institut Imagine, Paris, France*

***2:*** *Neurogenetics and Neuroinflammation, INSERM U1163, Institut Imagine, Paris, France*

Monogenic models of psoriasis vulgaris (PV) remain rare to-date. Adenosine-Deaminase-Acting-on-RNA1 (ADAR1) is a dsRNA editing enzyme which loss-of-function accounts for interferonopathies. We report *ADAR1* variants in early onset severe PV.

We identified by whole exome sequencing (WES) in a familial autosomal dominant early onset PV and interferonopathy phenotype in the proband, a heterozygous (htz) missense mutation in the deaminase domain of *ADAR1*, co-segregating with PV. Transcriptomic analyses showed a type I IFN signature in whole blood of all mutated siblings, and in lesional skin. WES screening identified 10 other *ADAR1* htz variants in PV patients. ADAR1 protein expression was reduced in HK and/or peripheral blood mononuclear cells from patients. *ADAR1* siRNA KD was associated in qRT-PCR with an upregulation of *IFNB1* and other PV cytokines *(IL8/17C/23A, IFI27, TNFA*) in WB with an upregulation of inflammatory activities (LL37, STAT1, NFκB, IRF7), supernatant proteome studies revealed a PV cytokines enrichment. Lentiviral transductions of mutated alleles in HK were associated in 2D and 3D cultures with an upregulation of PV-related genes (*IFNB, IFNK, IFI27, IL23A).*

Altogether, this study establishes a new single gene model of PV due to *ADAR1* loss-of-function mutations, leading to IFN signature, opening new avenues for precision medicine in PV.

**5**

**BODE**

**Ann M. Bode and Eunmiri Roh. The Hormel Institute, University of Minnesota**

Sun exposure is a major risk factor in the etiology of cutaneous squamous cell carcinoma (cSCC). People use sunscreens to prevent sun-induced skin damage and cancer. Nonetheless, the prevalence of cSCC increases every year, suggesting that sunscreens might not be used appropriately or are not completely effective. Here, a solar simulated light (SSL)-induced cSCC mouse model was used to investigate the efficacy of 8 commonly used FDA-approved sunscreen components against skin carcinogenesis. We tested sunscreen components for their ability to block UVA or UVB irradiation by using VITRO-SKIN (mimics human skin properties), and then the efficacy of sunscreen components was investigated in a cSCC mouse model. Results identified the most effective sunscreen components in preventing cSCC development. Unsurprisingly, the results indicated that sunscreen combinations that block both UVA and UVB significantly suppressed cSCC development and decreased the activation and expression of several oncoproteins in SSL-exposed SKH-1 hairless mouse skin. Notably, several sunscreen components that were individually purported to block both UVA and UVB were ineffective. One component even had toxic effects leading to a high mortality rate in mice exposed to SSL. Our findings provide new insights into the development of the best sunscreen to prevent chronic sun-induced cSCC development.

**6**

**BOLLAM**

**Unique roles of Ras homologs in directing immune responses during skin squamous cell carcinoma evolution**

Saumya Bollam1, 2, Reyno del Rosario1, Di Wu1, Chris Hsiung1,3,4,5,6, Diana Cristea1, Rosemary Akhurst1, 7, Charles Voliva8, Luke Gilbert1,4,5,6, Allan Balmain1,9

*1. Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA 94158, USA*

*2. Biomedical Sciences Graduate Program, University of California, San Francisco, San Francisco, CA 94158, USA*

*3. Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305, USA*

*4. Department of Urology, University of California, San Francisco, CA 94158, USA*

*5. Innovative Genomics Institute, University of California, San Francisco, San Francisco, CA 94158, USA*

*6. Arc Institute, Palo Alto, CA 94304, USA*

*7. Department of Anatomy, University of California, San Francisco, San Francisco, CA 94158, USA.*

*8. Oncology Discovery, Bristol-Myers Squibb, Redwood City, CA 94063, United States of America.*

*9. Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, CA 94158, USA.*

Skin squamous cell carcinomas (sSCC) commonly arise from oncogenic Hras mutations, though mouse models have shown that a subtype can also be activated by oncogenic Kras mutations. To deconvolute the possibly distinct roles of these Ras homologs, we investigated their roles in sSCC promotion and maintenance. Through gene expression profiling of over 100 HrasMUT sSCC, we found that *Hras* expression positively correlated with immunological responses which may be necessary for DMBA+TPA induced papillomas. We thus assessed the acute response to TPA in *HrasKO* and WT mice, identifying differences in epithelial cells and the immune cell dynamics. In KrasMUT sSCC, *Kras* expression correlated positively with cell cycle maintenance and negatively with immunological responses. To further explore this, we created a targeted sgRNA library to screen for genes correlated with oncogenic *Kras* that might confer resistance to aPD1 therapy. This *in vivo* CRISPRi screen identified *Kras*-co-expressed genes responsible for mRNA maintenance, previously unrecognized as tumor-specific immunotherapy sensitizers. This study highlights the distinct contributions of *Hras* and *Kras* in different stages of sSCC tumorigenesis. During promotion, *Hras* coordinates a pro-tumorigenic, pro-inflammatory environment, while later, *Kras* facilitates a pro-tumorigenic, anti-inflammatory environment. This distinction has important implications for advancing the detection and treatment of sSCC.

**7**

**BOWMAN**

**Patient-Specific Targeting of the T-Cell Receptor Variable Region as a Therapeutic Strategy in Clonal T-Cell Diseases**

Olivia M. Lucero1,2, Ji-Ann Lee3, **Jenna L. Bowman2,4**, Kara Johnson2,4, Gopal Sapparapu3, John K. Thomas3, Guang Fan5, Bill H. Chang2,6, Karina Thiel-Klare6, Christopher A. Eide2,4, Craig Okada4, Mike Palazzolo3, Evan Lind2,7,8, Yoko Kosaka2,6, Brian J. Druker2,4,9, Nicholas Lydon9, and Peter M. Bowers1

*1Department of Dermatology, Oregon Health & Science University, Portland, Oregon. 2 Knight Cancer Institute, Oregon Health & Science University, Portland, Oregon. 3 Clinical and Translational Science Institute, David Geffen School of Medicine, University of California, Los Angeles, California. 4Division of Hematology and Medical Oncology, Oregon Health & Science University, Portland, Oregon. 5 Department of Pathology and Clinical Laboratory Medicine, Oregon Health & Science University, Portland, Oregon. 6Division of Pediatric Hematology and Oncology, Oregon Health & Science University, Portland, Oregon. 7Department of Molecular Microbiology and Immunology, Oregon Health & Science University, Portland, Oregon. 8 Department of Cell, Developmental, and Cancer Biology, Oregon Health & Science University, Portland, Oregon. 9 VB Therapeutics LLC, Jackson, Wyoming. 10Therapeutic Antibody Laboratory, Department of Pulmonology and Critical Care, David Geffen School of Medicine, Los Angeles, California.*

Targeted therapeutics are a goal of medicine. Methods for targeting T-cell lymphoma lack specificity for the malignant cell, leading to elimination of healthy cells. The T-cell receptor (TCR) is designed for antigen recognition. T-cell malignancies expand from a single clone that expresses one of 48 TCR variable beta (Vb) genes, providing a distinct therapeutic target. We hypothesized that an antibody, exclusive to a specific Vb, would eliminate the malignant clone with minimal effects on healthy T cells. We identified a patient with large granular T-cell leukemia, sequenced his circulating T-cell population, and found that 95% expressed Vb13.3. We developed a panel of anti-Vb13.3 antibodies to test for binding and elimination of the malignant T-cell clone. Therapeutic antibody candidates bound the malignant clone with high affinity, killed engineered cell lines expressing the patient TCR Vb13.3 by antibody-dependent cellular cytotoxicity and TCR-mediated activation-induced cell death, and exhibited specific killing of patient malignant T cells in combination with exogenous natural killer cells. EL4 cells expressing TCR Vb13.3 were also killed by antibody administration in an in vivo murine model. This approach serves as an outline for development of therapeutics that can treat clonal T-cell–based malignancies and potentially other T-cell–mediated diseases.

**8**

**BOYLE**

**Differences in DNA damage from solar UV between a low risk and high-risk phenotype**

Jennifer Boyle1, Stephanie Holtorf1, Teri Johnson1, Derek Gordon2, Tianshun Zhang1, Ann Bode1, Rebecca J. Morris1

*The Hormel Institute/University of Minnesota1 and the Department of Genetics, Rutgers University2*

Improved diagnosis and treatment of nonmelanoma skin cancers (NMSCs) caused by solar ultraviolet radiation (sUV) comprise a critical need. The increased incidence of NMSCs is an enormous public health concern fraught with clinical challenges due to the lack of appropriate animal models designed to identify benign lesions having a high risk of progressing to malignancy. Therefore, we designed a novel mouse model for sUV radiation-induced benign lesions with either low or high risk of conversion to malignancy. The low risk cohort received our conventional protocol of chronic sUV radiation 3 times per week for 15 weeks and the high risk cohort received a brief 5-week exposure. Both cohorts received the same accumulated dose of sUV radiation. We visualized cyclobutane pyrimidine dimers (CPD) by immunohistochemistry. We found that the number of CPD positive cells in each cohort was not statistically significant, but the percentage of CPD positive cells was statistically significant. The low risk mice developed a greater percentage of CPDs (low risk=53.01% vs high risk=37.33%, p=0.0013). We conclude that the exposure to sUV has a significant influence on the development of CPDs, and these dimers may have a downstream effect on the development of skin tumors.

**9**

**BRANCH**

**The epigenetic control of re-epithelialization during adult skin wound healing**

Meagan C. Branch & Elena Ezhkova

*Black Family Stem Cell Institute, Department of Cell, Developmental, and Regenerative Biology, Icahn School of Medicine at Mount Sinai, New York, NY*

The skin acts as a barrier to our external environment. When this barrier is compromised, epidermal stem cells (EpSCs) undergo a transient transcriptional switch to initiate repair function. Wounded EpSCs increase their rate of proliferation and gain the ability to migrate. Any impairment to these processes can lead to the development of chronic wounds. However, the regulation of this shift is unknown. Preliminary data from our lab shows that 53% of dynamically regulated genes during repair are targets of Polycomb Repressive Complex 1 (PRC1), a key chromatin remodeler. To test PRC1 function in wounded EpSCs, we generated epithelial-specific PRC1 knockout mice and induced wounds on the dorsal skin. In PRC1-null mice, wound healing was delayed due to a failure of EpSCs to migrate. Furthermore, we found that E-cadherin transcripts were still expressed in the PRC1-null wound, suggesting a role for PRC1 in epithelial-mesenchymal plasticity (EMP). In conclusion, PRC1 regulates EpSCs’ ability to migrate and potentially plays a significant role in regulating EMP.

**10**

**BUI**

**Characterizing HMGB1 function in human keratinocytes**

Kacey Guenther Bui1, Hai Dang Nguyen PhD2, Anja-Katrin Bielinsky PhD3, Jakub Tolar MD, PhD1,4

*1Department of Genetics, Cell Biology & Development, University of Minnesota, Minneapolis, MN, USA*

*2Department of Pharmacology, Masonic Cancer Center, University of Minnesota, Minneapolis, MN, USA*

*3Department of Biochemistry & Molecular Genetics, University of Virginia, Charlottesville, VA, USA*

*4Department of Pediatrics, Blood and Marrow Transplantation, University of Minnesota, Minneapolis, MN, USA*

Recessive dystrophic epidermolysis bullosa (RDEB) is a rare skin disease caused by loss of function mutations in *COL7A1*. It is characterized by skin fragility, chronic wounds, and metastatic squamous cell carcinoma. Previous reports identified an increase in high mobility group box 1 (HMGB1) protein in serum, which correlates with RDEB disease severity. However, the contribution of HMGB1 to RDEB pathogenesis is not well understood. HMGB1 is a chromatin-binding protein with dual functions in DNA replication/repair and activation of innate immunity. Using human keratinocytes as an *in vitro* model of the skin, we treated cells with lipopolysaccharide (LPS) and monitored IL-6 secretion, a pro-inflammatory cytokine downstream of HMGB1 that is similarly elevated in RDEB patients. LPS treatment induced IL-6 secretion in human keratinocytes. Inflachromene, an inhibitor of HMGB1 secretion, significantly reduced LPS-induced IL-6 secretion by twelve-fold (p<0.0001, one-way ANOVA), suggesting that HMGB1 secretion contributes to the pro-inflammatory response. To determine the molecular function of HMGB1 in pro-inflammation, we used CRISPR-Cas9 to knockout *HMGB1* in a human keratinocyte cell line. Successful knockouts were confirmed by western blot and immunofluorescence. Future work will use these HMGB1KO cell lines to characterize the molecular mechanisms and therapeutic potential of HMGB1 in keratinocyte-specific inflammatory signaling.

**11**

**CHU**

**Unraveling the role of IL-17-experssing mast cells in hidradenitis suppurativa pathogenesis**

Chia-Bao Chu1,2, Chao-Chun Yang1,3, Yuan-Yu Hsueh3,4,5, Shaw-Jenq Tsai2,6

*1Department of Dermatology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan*

*2Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan*

*3International Center for Wound Repair and Regeneration, National Cheng Kung University, Tainan, Taiwan*

*4Department of Surgery, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan*

*5Center of Cell Therapy, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan, Taiwan*

*6Department of Physiology, College of Medicine, National Cheng Kung University, Tainan, Taiwan*

**Abstract**

Hidradenitis suppurativa (HS) is a debilitating skin disorder that severely reduces the patient’s quality of life. This study aimed to identify and characterize the key mediator in HS pathogenesis, focusing on IL-17-positive cells.

Skin samples from healthy, HS, and psoriasis individuals were used to evaluate the expression of interleukin (IL)-17. RNA-seq analysis and *in-vitro* experiments were conducted to validate the expression of IL-17 and its pathogenic effect.

Transcriptomic database analyses revealed IL-17 signaling as a potential contributor to HS. Notably, mast cells were identified as the predominant IL-17-positive cell population in HS tissues, with significantly elevated density, particularly in advanced Hurley stages, compared to normal skin and psoriasis samples. IL-17-expressing mast cells were found to closely interact with IL-17 receptor A (IL-17RA)-expressing keratinocytes. Treatment with IL-17 promotes keratinocyte cell proliferation and the expression of pathogenic genes in HS. Patients received biologics targeting the IL-17 pathway showed significant improvement in disease severity and marked reduction in the number of IL-17-expressing mast cells in affected tissues.

In conclusion, the density of IL-17+ mast cells serve as a valuable diagnostic and prognostic marker for HS and targeting IL-17+ mast cells may be a novel approach for the treatment of HS.

**12**

**DELLAMBRA**

**TrkB is a potential therapeutic target for invasive cutaneous squamous cell carcinoma.**

R. Monetta, V. Bartolocci, D. Campagna, F. Delle Monache, C. Valente, M. Teson, Y. A. Minafò, F. Ricci, L. Fania, I. Bondanza, M. Mancini, G. Scaglione, E. Candi, D. Abeni, S. Mastroeni, and E. Dellambra.

*Istituto Dermopatico dell’Immacolata, IDI-IRCCS, Roma*

A subset of cutaneous squamous cell carcinomas (SCCs) displays a higher likelihood of recurrence and metastasis causing death. Nowadays, no satisfactory diagnostic biomarkers for high risk SCCs has been proposed.

Based on our previous results, the aim of the study was to assess whether TrkB and/or specific downstream proteins (E-cadherin, Yap1, Notch1) could be considered as high-risk biomarkers and therapeutic targets.

The correlation between the expression levels of these four proteins, and histological characteristics of SCCs was investigated. ROC analysis indicated that the combination of these proteins is an effective diagnostic signature discriminating both the SCC types and high-risk invasive SCC.

To investigate if TrkB may be a therapeutic target, two SCC cultures were treated with a TrkB-specific inhibitor (ANA-12). ANA-12 treatment inhibited the STAT3 pathway, reduced the expression of EMT-transcription factors, induced E-cadherin expression and, in turn, recovered cell-cell adhesion properties. Furthermore, ANA-12 treatment induced p21 translocation in the nucleus and, in turn, reduced proliferation, migration and IL-6 secretion. Finally, ANA-12 administration significantly inhibited SCC invasion in 3D SCC *in vitro* models.

Therefore, our data indicate that the signature "TrkB-E-cadherin-Yap1-Notch1" may be a diagnostic biomarker for invasive SCC risk, and TrkB may be an attractive target for SCC therapies.

**13**

**DICKINSON**

**PD-L1 as a novel target in epidermal photodamage: Topical pharmacological PD-L1 inhibition suppresses solar UV-induced inflammatory signaling in SKH-1 mouse skin**

Sally E. Dickinson\*, Prajakta Vaishampayan, Jana Jandova, Ella Yuchen Ai, Viktoria Kirschnerova, Valerie Calvert, Emanuel Petricoin III, Chengcheng Hu, Denise Roe, Clara Curiel-Lewandrowski, Georg T. Wondrak

The immune checkpoint ligand PD-L1 has emerged as a molecular target for skin cancer therapy and might also hold promise for preventive interventions. Here we have explored the role of PD-L1 in acute keratinocytic photodamage testing the effects of small molecule pharmacological inhibition. First, epidermal PD-L1 upregulation in response to chronic photodamage was established using IHC and proteomic analyses of a human skin cohort, consistent with our earlier observation that PD-L1 is upregulated in cSCC. Therefore, the impact of pharmacological PD-L1 inhibition on photodamage was examined. Topical application of the small molecule inhibitor BMS-202 significantly attenuated UV-induced AP-1 transcriptional activity in SKH-1 bioluminescent reporter mouse skin, also confirmed in human HaCaT reporter keratinocytes. RT-qPCR analysis revealed that BMS-202 downregulated UV-induction of inflammatory mediators such as *Ptgs2*, *Tlr4*, *Il-1β*, *Il-6*, *Il-10* and, surprisingly, *Pd-l1* itself. Likewise, UV-induced cleavage of procaspase-3 was strongly attenuated by topical BMS-202. NanoString nCounterTM transcriptomic analysis (‘Mouse Inflammation V2’ panel) confirmed downregulation of innate immunity-, inflammation-, chemokine-, and angiogenesis-related responses. Further mechanistic analysis confirms that BMS-202 antagonizes UV-induced PD-L1 expression both at the mRNA and protein levels in SKH-1 epidermis. These data suggest that topical pharmacological PD-L1 antagonism might be relevant to skin photoprotection and cancer prevention.

**14**

**DIEHL**

**Seronegative atypical diffuse systemic sclerosis**

Kyra Diehl, BS, Western University of Health Sciences, College of Osteopathic Medicine, Pomona, CA

Oliver J Wisco, DO, FAAD, FACMS, Department of Dermatology, Warren Alpert Medical School of Brown University, Providence, RI

Cara Barber, MD, Silver Falls Dermatology, Salem, OR

Daniel E Furst, MD, Division of Rheumatology, University of California Los Angeles, CA

A 54-year-old man with a 16-year history of diffuse scleroderma presented for a follow up visit. On physical examination, the patient has pink-white ulcerated plaques on the lower extremities, diffuse calcified nodules and telangiectasias, sclerodactyly, and taut, thickened skin. Serology was negative for the following antibodies: anti-centromere, anti-double stranded-DNA, anti-La/SSB, antinuclear, anti-ribonucleoprotein, anti-RNA polymerase III, anti-Ro/SSA, anti-Scleroderma-70, and anti-Smith.

The purpose of this article is to demonstrate a case of atypical seronegative diffuse scleroderma.

Antinuclear antibodies (ANA) are positive in over 90% of patients diagnosed with scleroderma. The scleroderma criteria do not include the presence of ANA; therefore, ANA negativity doesn’t exclude diagnosis. A EUSTAR study found only 7.7% of patients with scleroderma had a negative ANA. 0.2% of patients had neither Raynaud’s phenomenon nor a positive ANA. Of those patients, 66.7% had a phenotype consistent with diffuse scleroderma. ANA-negative scleroderma has less vasculopathy, more lower gastrointestinal involvement, a more severe disease course, and is more common in men.

Seronegative scleroderma is rare, and few cases have been reported. The lack of a positive ANA in a patient presenting with scleroderma-like symptoms warrants further investigation but doesn’t exclude the diagnosis of scleroderma.

**15**

**DIMITRIOU**

**Florentia Dimitriou**1, 2, Phil F. Cheng1, 2, 3, Annalisa Saltari1, 2, Katrin Schaper-Gerhardt4, 5, Ramon Staeger1, 2, Veronika Haunerdinger1, 2, Federica Sella1, 2, Aizhan Tastanova1, 2, Christian Urban6, Susanne Dettwiler7, Daniela Mihic-Probst7, 2, Olivier Michielin3, Ralf Gutzmer4, Georgina V. Long8, 9, 10, Burkhard Becher11, Reinhard Dummer1, 2,Mitchell P. Levesque1, 2

1. Department of Dermatology, University Hospital of Zurich, Zurich, Switzerland
2. Faculty of Medicine, University of Zurich, Zurich, Switzerland
3. Department of Oncology, Geneva University Hospital, Geneva, Switzerland
4. Department of Dermatology, Johannes Wesling Medical Center, Ruhr University Bochum Campus Minden, Minden, Germany
5. Department of Dermatology, Medical School Hannover, Hannover, Germany
6. Functional Genomics Center Zurich, University of Zurich/ETH Zürich, Zürich, Switzerland
7. Institute for Pathology and Molecular Pathology, University Hospital Zurich, Zurich, Switzerland
8. Melanoma Institute Australia, The University of Sydney, Sydney, New South Wales, Australia
9. Department of Medical Oncology, Royal North Shore and Mater Hospitals, Sydney, NSW, Australia
10. Faculty of Medicine and Health, The University of Sydney, Sydney, New South Wales, Australia
11. Institute of Experimental Immunology, University of Zurich (UZH), Zurich, Switzerland

Immune checkpoint inhibitors (ICIs) are standard-of-care for the treatment of advanced melanoma, but their use is limited by immune-related adverse events (irAEs). We performed multiomics analysis to unravel the underlying immunobiology. 82 patients treated with anti-PD1/anti-CTLA4 (84%) or anti-PD1 (16%) were retrospectively analyzed (discovery cohort; n=9) or prospectively collected in a main (n=54) and an internal validation set (n=19). The results were confirmed in an external validation cohort (n=30). Proteomic analysis and multiplex cytokine/chemokine assay from serum at baseline and at irAEs onset indicated aberrant T-cell activity with differential expression of Type I and III immune signatures. This was in line with an increase in the proportions of monocytes and decrease of IL-17A producing CD4+ T-cells in the peripheral blood in single cell RNA sequencing. Multiplex immunohistochemistry and spatial transcriptomics on ICI-induced skin rash and colitis tissue showed increase in the proportion of CD4+ T-cells with IL-17A expression. Based on these findings, anti-IL17A mAbs were administered in two patients with myocarditis, colitis and skin rash with resolution of the irAE. This study demonstrates the potential role of Type III CD4+ T-cells in the irAEs development and provides proof-of-principle evidence to support a clinical trial examining anti-IL17A in their management.

**16**

**ERDEN**

**Non-cell-autonomous Regulation of the p53-mediated DNA Damage Response in Skin** **Homeostasis and Carcinogenesis**

Nihan Erden1, Björn Schumacher1

1Institute for Genome Stability in Ageing and Disease, Cologne Excellence Cluster for Cellular Stress Responses in Aging‐Associated Diseases (CECAD), University of Cologne

DNA damage is a causal factor of both cancer development and the aging process. The tumor suppressor p53 is a central mediator of the DNA damage response (DDR) and the single most frequently mutated gene in human cancer. Many studies showed that cell-cycle and apoptosis functions of p53 are important for preventing tumor development and the activation of p53 was regulated cell-autonomously depending on the type and severity of the DNA damage. It was recently discovered that the p53-mediated DDR in stem cells is not regulated only cell-autonomously but also regulated through signaling via the niche cells. The translation initiation factor IFE-4 in *C. elegans* is activated in somatic gonad precursor niche cells that surround the primordial germ cells, when the latter carry DNA damage. Moreover, it was demonstrated that the IFE-4 ortholog eIF4E2 in mammals is induced in niche cells upon UV-induced DNA damage and is required for the induction of p53 in hair follicle stem cells (HFSCs).These data thus indicate a highly conserved mechanism of non-cell-autonomous regulation of the p53-mediated DDR in stem cells.

We are currently employing *in vivo* and *ex vivo* experimental systems with eIF4E2 epidermis specific knockout (eIF4E2epi KO) mice to dissect the mechanisms of the interactions between niche and stem cells, and to understand the role of eIF4E2 in skin homeostasis and carcinogenesis. Our study using eIF4E2epi KO mice in 3C HFSC-niche culture demonstrates the essential role of niche based eIF4E2 in activating p53 upon UV-induced DNA damage in the stem cells of murine skin. Moreover, eIF4E2epi KO mice exhibit two distinct phenotypes concerning UV-dependent erosive skin lesion formation and hair follicle regeneration when compared to the control mice. To further assess the clinical relevance of the eIF4E2 function, we will characterize eIF4E2 in human squamous cell carcinoma.

**17**

**ERICKSON**

**IgE shapes the niche of tumor-initiating cells in squamous cell carcinoma**

Hannah Erickson1, Sachiko Taniguchi1, Naoki Oshimori1,2,3,4

*1 Department of Cell, Developmental & Cancer Biology*

*2 Department of Dermatology*

*3 Department of Otolaryngology, Head & Neck Surgery*

*4 Knight Cancer Institute*

*Oregon Health & Science University, Portland, OR 97239, USA*

**Abstract:** The tumor microenvironment utilizes many methods of communication, including those between tumor-initiating cells (TICs) and immune cells. TICs are critically regulated by extrinsic factors from their unique microenvironment, the TIC niche. Because of the stem cell-like and treatment-resistant nature of TICs, targeting the TIC niche may provide better therapeutic options. Using a mouse model of squamous cell carcinoma, we previously identified an IL-33–TGF-b signaling loop activated between TICs and alternatively activated macrophages expressing the high-affinity receptor for immunoglobulin E (IgE), FcεRI. We aimed to determine if IgE was crucial for immunosuppressive function of niche macrophages, and if so, how B cells, a source of IgE, are involved in shaping the niche. Here we show that FcεRI is important for maintenance of the niche macrophage phenotype, and that B cells play an indispensable role in the development of this phenotype *in vitro.* Additionally, we discovered IgE+ cells in the bone marrow of tumor-bearing mice, as well as increased IgE+ B-cells in the lymph nodes when compared to wild-type counterparts. These results suggest that FcεRI and IgE may play a crucial role in maintaining and reinforcing the TIC-supporting functions of niche macrophages, and therefore may be a good target for therapeutics.

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**FAN**

**Single-Cell RNA Sequencing Defines the Immune Cell Landscape during Cancer Immunoediting in Epidermal Neoplasms**

 Xiying Fan, Dennis R. Roop

*Department of Dermatology and Gates Institute, University of Colorado Anschutz Medical Campus, Aurora, CO 80045, USA*

ABSTRACT

Cancer immunoediting is a dynamic process whereby the immune system can both constrain and promote tumor development, which proceeds through three phases termed elimination, equilibrium, and escape. Although the cancer immunoediting process has set the foundation for understanding the immune system’s dual roles on cancer cells and establishing the basis for revolutionary cancer immunotherapies, very few studies have captured cancer immunoediting in its early stages due to lack of proper mouse models. To address this deficiency, we developed a novel mouse model for fluorescent tracing of somatic, epithelial cell transformation to study the role of immunoediting in skin cancer development. For the first time, our model provides the direct visualization of epithelial cancer development *in vivo* and allows the observation of all three phases of cancer immunoediting. In addition, our model also allows tumor-infiltrating immune cells to be traced during this process. Here, we used single-cell RNA sequencing to map the immune composition of control skin and skin tumors. By focusing our analysis on CD45+ cells, we obtained high resolution identification of the immune cell subsets in control skin, and during the early stages of tumor development. We found that most major immune cell subpopulations including macrophages, T cell and gamma delta T cells, regulatory T cells, dendritic cells, natural killer cells, neutrophils, and mast cells are present in both normal skin and during tumor development. Notably, immune subsets such as macrophages and natural killer cells expanded, while dendritic cells and gamma delta T cells contracted as tumors progressed to the escape stage. Altogether, our work defines the immune cell landscape during epithelial cancer progression and provides insights into the mechanisms underlying cancer immunoediting.

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**FUJITA**

**The role of genotoxic acetaldehyde in melanocyte activation and unique DNA damage in melanocytes.**

Takeshi Yamauchi1, Kimtrang Nguyen1, Zili Zhai1, Akiko Matsumoto2, Bin Gao3, Byoung J Song3, Douglas E. Brash4, Mayumi Fujita1, 5, 6

1. *Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*

*2. Department of Social Medicine, School of Medicine, Saga University, Saga 849-8501, Japan.*

*3. NIAAA, NIH, Bethesda, Maryland*

*4. Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, Connecticut*

*5. Department of Immunology and Microbiology, University of Colorado Anschutz Medical Campus, Aurora, USA*

*6. Department of Veterans Affairs Medical Center, VA Eastern Colorado Health Care System, Aurora, CO, USA*

Humans are exposed to ethanol (EtOH) and its genotoxic metabolite, acetaldehyde (AcAH), through various means, even without drinking alcohol. We demonstrated that the skin is equipped to metabolize EtOH and AcAH; however, melanocytes express lower aldehyde dehydrogenase 2 (ALDH2), a critical enzyme in AcAH metabolism, compared to keratinocytes. Excessive oxidative stress induces mitochondrial dysfunction, and dysfunctional mitochondrial ALDH2 further contributes to oxidative damage and activation of signaling pathways such as MAPK. Therefore, we hypothesized that AcAH induces cellular stress responses leading to melanocyte activation and DNA damage. Using primary human epidermal melanocytes and *Aldh2* KO mice, we demonstrated that AcAH (administration of EtOH in the presence of ALDH2 inhibition) activated adenylyl cyclase and phospho-ERK and induced reactive oxygen species (ROS). AcAH also increased intracellular and extracellular melanin production and secretion in melanocytes and promoted melanocyte proliferation. Furthermore, we detected not only γH2AX, a marker of DNA double-strand breaks, but also a DNA photo-lesion, cyclobutane pyrimidine dimer, in melanocytes by AcAH. In summary, we observed melanocyte proliferation, melanin production, and UV-specific DNA damage in melanocytes by EtOH in the presence of ALDH2 blockade *in vitro* and *in vivo*, suggesting the involvement of the genotoxic AcAH and impaired ALDH2 in melanocyte transformation.

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**FUJITA**

**Translational application of IL-37-expressing Treg cells in inflammation and immunity**

Douglas G Osborne,1 Maki Nakayama,2,3 Mingxia Huang,1 and Mayumi Fujita1,3,4

*1Department of Dermatology, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*

*2Department of Pediatrics, University of Colorado Anschutz Medical Campus, Aurora, CO, USA*

*3Department of Immunology & Microbiology, Anschutz Medical Campus, Aurora, CO, USA*

*4Department of Veterans Affairs Medical Center, VA Eastern Colorado Health Care System, Aurora, CO, USA*

Maintaining immune homeostasis is critical in controlling human diseases with inappropriate immune responses, such as autoimmunity, chronic inflammatory diseases, and transplant rejection. Immunosuppressive drugs may be used to control these conditions. However, they must be administered for a long time and often induce adverse effects, impairing patients’ quality of life. Regulatory T (Treg) cells are a specialized T-cell subpopulation that suppresses inappropriate immune responses and thus has been used to restore immune tolerance in autoimmunity and transplantation. However, despite its minimal side effects, clinical trials using an adoptive Treg cell therapy have shown limited effects, suggesting that these Treg cells may not be robust, stable, or persistent *in vivo*. We have shown that IL-37 upregulates and stabilizes FOXP3 (a master transcriptional regulator of Treg cells), enhances their suppressive function, and prevents Treg cells’ conversion to non-Treg CD4+ T cells, even in inflammatory conditions *in vitro* and *in vivo*. Therefore, we hypothesized that we could enhance the therapeutic efficacy by genetically engineering Treg cells to express IL-37. We demonstrate that the adoptive transfer of mouse or human Treg cells expressing IL-37 can significantly improve inflammatory and immune conditions in mouse models of skin contact hypersensitivity, psoriasis, and graft-versus-host disease.

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**GORKUN**

**Skin Organoid as a Modeling Platform for Skin Development, Physiology and Injury *In Vitro***

Anastasiya Gorkun1, Naresh Mahajan1, Adam Jorgensen1, Gemma Nomdedeu Sancho1, Minsong Wu1,2, Shay Soker1, Anthony Atala1

1. *Wake Forest Institute for Regenerative Medicine, Winston Salem, USA*
2. *Higher Education Institution of Guizhou province, Zunyi Medical university, Zunyi, Guizhou, China*

Due to high demand, 3D skin models are fully replacing animal and traditional 2D models. In this study, we show that skin organoids (SOs) can be utilized as a universal platform for different applications, including skin-like physiology modeling, *in vitro* chemical skin irritation, and UV radiation injury model.

Different key human skin cells were aggregated in SOs and then organoids anatomy and physiology were characterized at different timepoints. For injury modeling, SOs were exposed to irritant chemicals and UVB. Methods included Live/Dead assay, histology, IHC, Photometry, qPCR, and statistics by Prism.

Up to day 21, SOs demonstrated a skin-like layered microstructure, with the surface formed by epidermal cells and the central core composed of dermal and hypodermal cells. SO showed skin-like functionality within epidermal barrier integrity, vasculogenesis, and active melanogenesis.

After UVB radiation SOs developed the ER stress and apoptosis that had been blocked by sunscreen compounds. Also, SOs showed an irritation response matching the Primary irritation index of tested chemicals for skin *in vivo*.

Therefore, this study shows that novel multicellular SO are capable of recapitulating skin structure, functionality and provides an *in-vitro* skin model that could be used as a platform for dermatopathology research.

**22**

**GURRI**

**NRF3 suppresses squamous carcinogenesis through the unfolded protein response regulator HSPA5**

Selina Gurri1, Beat Siegenthaler1, Michael Cangkrama1, Gaetana Restivo2, Marcel Huber3, James Saliba4, Reinhard Dummer2, Volker Blank4, Daniel Hohl3, and Sabine Werner1

*1Institute of Molecular Health Sciences, Department of Biology, ETH Zurich, CH-8093 Zurich, Switzerland*

*2Department of Dermatology, University Hospital Zurich, CH-8091 Zurich, Switzerland*

*3Service of Dermatology, Lausanne University Hospital and University of Lausanne, CH-1011 Lausanne, Switzerland*

*4Lady Davis Institute for Medical Research, McGill University, Montreal, Canada*

Basal and squamous cell carcinomas (BCC and SCC) of the skin are the most common types of cancer in humans. Therefore, the identification of novel therapeutic targets is highly relevant. We discovered an unexpected tumor-suppressive function of the transcription factor nuclear factor-erythroid 2-related factor 3 (NRF3). NRF3 protein expression is strongly down-regulated in invasively growing cancer cells of BCCs and SCCs. This is functionally relevant, because NRF3 deficiency increased the malignant transformation of chemically-induced skin tumors in mice, enhanced clonogenic growth and migration of human skin cancer cells, increased invasiveness in 3D cultures, and promoted xenograft tumor formation. We show that NRF3 promotes tumor suppression through a previously unknown function in the ER and not at the level of transcriptional regulation. Proximity dependent biotinylation identified HSPA5, a crucial regulator of the unfolded protein response, as an NRF3 binding partner. In the absence of NRF3, HSPA5 levels increased, promoting cancer cell survival and migration. Pharmacological inhibition or knock-down of HSPA5 rescued the malignant features of NRF3-deficient SCC cells *in vitro* and in preclinical mouse models. Together with the strong expression of HSPA5 in NRF3-deficient cancer cells of SCC patients, these results suggest HSPA5 inhibition as a promising therapeutic strategy for NMSC.

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**HOLTORF**

**Identification of bone marrow-derived epithelial cells that may contribute to developing chronic cutaneous neoplasms in mice**

Heuijoon Park1,2, Sonali Lad1, Kelsey Boland1, Kelly Johnson1, Nyssa Readio1, Guangchun Jin2, Samuel Asfaha2, Kelly S. Patterson2, Ashok Singh1, Xiangdong Yang2, Douglas Londono3, Anupama Singh1, Carol Trempus4, Stephanie M. Holtorf1, Jennifer Boyle1, Derek Gordon3, Timothy C. Wang2 and Rebecca J. Morris1

*The Hormel Institute, University of Minnesota, Austin, MN1; Columbia University, New York, NY2, Rutgers University, Piscataway, NJ3; National Institute of Environmental Health Sciences, Research Triangle Park, NC4.*

Non-melanoma skin cancers (NMSCs) are a serious and growing public health problem despite a plethora of sunscreens and repeated calls for sun avoidance. In some patients, NMSCs can become intractable and ultimately deadly, leading us to wonder whether some of the cancer cells might originate in the bone marrow, an incubator for numerous stem and progenitor cells. The purpose of our study was to explore the role of bone marrow derived cells (BMDCs) in cutaneous malignancy.

We used allogeneic bone marrow transplantation (BMT) along with a multistage cutaneous carcinogenesis model to look for the recruitment of BMDCs into skin tumors that were initiated with the carcinogen, dimethylbenz[*a*]anthracene (DMBA), and promoted with 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Naïve female mice receiving BMTs from DMBA-treated donors developed benign and malignant cutaneous lesions after TPA promotion alone. The donor BMDCs clustered in the recipient lesional epithelium, expressed cytokeratins, proliferated, and stratified.

We wanted to investigate further what bone marrow cells might be responsible for initiating and promoting skin tumors. We hypothesized that there might be a progenitor population of epithelial cells in the bone marrow responsible. Using flow cytometry, immunofluorescence microscopy, and mining of single cell RNA sequencing datasets, we identified a potential population of bone marrow stem cells with classic epithelial markers such as EpCAM (epithelial cell adhesion molecule), keratins, and E-cadherin. We conclude that a subset of squamous lesions long thought to be solely local in origin have a distinct systemic component. These findings may suggest new targets for diagnosis and surveillance of NMSCs.

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**INDRA**

**Biocompatible Nanofiber Mediated Encapsulation of Secretory Factors Facilitates Their Sustained Release and Promotes Efficient Cutaneous Healing *In Vivo***

Arup Kumar Indra1, 2, 3,4and Gitali Indra1,4

 1. *Dept of Pharmaceutical Sciences, OHSU-OSU, Corvallis, OR, USA, 2. Linus Pauling Institute, OSU, Corvallis, OR, USA, 3. Dept of Dermatology, OHSU, Portland, OR, USA, 4. Knight Cancer Institute, OHSU, Portland, OR, USA.*

Healing-delayed or lingering skin wounds present substantial challenges, leading to poor quality of life for millions of people in US and worldwide. Follow-up treatments to correct these wounds imparts an enormous financial burden on society. Delay or failure to skin tissue repair can further lead to infection, dehydration, and death. Hence, clinicians desperately need novel strategies for promoting efficient wound healing and prevent infections through targeting different mechanisms of action than current mode of treatment.

Using a differential gene expression approach, we have recently identified two such factors that activate molecular signals related to enhanced cell proliferation, migration, and survival. Encapsulation of those bioactive compounds in a biocompatible nanofiber-based dressing promoted their sustained release and activated cell-signaling *in vitro* in primary human keratinocytes.  Further, topical delivery of both the bioactive molecules in a controlled and sustained manner accelerated cutaneous wound healing in a delayed wound healing model. Surprisingly, efficient wound closure was accompanied with increased angiogenesis and enhanced myofibroblast differentiation.

Our current results provide a strong foundation for developing the next generation of biocompatible novel therapeutic wound dressings that could greatly speed wound healing, reduce frequency of surgical site infections, and possibly mitigate development of antibiotic resistance.

**25**

**JACKOW-MALINOWSKA**

**Lipid nanoparticles efficiently deliver the base editor ABE8e for *COL7A1* correction in dystrophic epidermolysis bullosa fibroblasts *in vitro*.**

Ina Guri1, Yara Alrokh1, Stehpan Hart2, John A. McGrath1 and Joanna Jacków-Malinowska1

*1 St John’s Institute of Dermatology, King’s College London, London, UK*

*2 Great Ormond Street Institute of Child Health, UCL, London, UK*

Adenine base editor (ABE) converts A•T base pairs to G•C base pairs without requiring double-stranded DNA breaks or donor DNA templates. It represents a new concept of gene therapy for the inherited blistering disease dystrophic epidermolysis bullosa (DEB), which results from pathogenic variants in *COL7A1*, leading to dysfunctional or absent of type VII collagen (C7) in the skin basement membrane. Our goal is to develop therapeutic topical base editing formulation for DEB and therefore we explore the use of a lipid nanoparticle (LNP) system to deliver ABE8e in mRNA format for *COL7A1* correction. Here, we tested six different LNP formulations, all of which use the phospholipid DOPE, microfluidically mixed with one of the following cationic lipids: DTDTMA (C14), DHDTMA (C16), or DOTMA (C18). These liposomes are then either mixed with ABE8e mRNA and sgRNA alone, or with ABE8e mRNA, sgRNA and a peptide containing an epithelial targeting motif (K16GACYGLPHKFCG) to create the final library of six lipid-peptide nanocomplexes. Using Sanger Sequencing, we showed comparably efficient correction of the pathogenic variants (up to 100%) using formulations containing C14 and C16 lipids, both with and without the peptide. Western blot analysis revealed that the correction of the pathogenic variant in *COL7A1* corresponds to an increase in C7 production and secretion in cell lysates and supernatant, respectively. Lactate dehydrogenase (LDH) assay showed that our most efficient formulations induce significantly less cell death compared to lipofectamine. While further safety testing is carried out, these formulations show great potential for clinical translation and *in vivo* application to treat DEB.

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**JOHNSON**

**A novel mouse model for solar ultraviolet radiation-induced skin tumors with** **a high risk of conversion to malignancy**

Teri L. Johnson, Stephanie M. Holtorf, Jennifer N. Boyle, Tianshun Zhang,

Ann M. Bode, and Rebecca J. Morris

*The Hormel Institute/ The University of Minnesota*

The increased incidence of nonmelanoma skin cancers is an enormous public health problem fraught with clinical challenges. This is likely due to the lack of appropriate animal models that are designed to identify benign lesions having a high-risk of progressing to malignancy. We designed a novel mouse model for solar ultraviolet (sUV) radiation-induced benign lesions with either low or high risk of conversion to malignancy. We hypothesized that an acute exposure to sUV would produce benign lesions that are at greater risk of progression to carcinomas compared to chronically sUV-exposed mice.

Results showed that high-risk mice had a tumor latency of 84 days compared to a tumor latency of 98 days in the low-risk mice. The incidence of mice developing tumors was 67% in the low-risk group compared to 75% of mice in the high-risk group. Finally, a multiplicity of 2 tumors per mouse was observed in the high-risk group compared to 6 tumors per mouse in the low-risk group. Importantly, the percentage of suspected squamous cell carcinomas was significantly greater in the high-risk mice with 31.6% of all tumors becoming suspected SCCs versus the low-risk mice with 16.31% of all tumors becoming suspected SCCs.

**27**

**KEENE**

**The Dermal-Epidermal Junction – Ultrastructure and Development of Adherence Assemblies**

Douglas R. Keene and Sara Tufa

*Shriners Children’s, Micro-Imaging Center, Portland, Oregon*

Our focus is on the ultrastructure and function of complexes within connective tissue matrices. Here we compare the spatial/temporal positions of two macromolecular complexes important in dermal-epidermal adhesion. The Anchoring Fibril complex (1) includes keratins 5 and 14 within the cytoplasm of basal cells, BP 230 and BP180/colXVII at hemidesmosomes, laminin 5/6 spanning the lamina lucida and colVII forming anchoring fibrils within the dermis.

Anchoring cords (2) are composed of the Fraser Complex members, with FREM1 spanning the lamina lucida into the lamina densa, FRAS1 adjacent to the LD, and AMACO with FREM2 extending well into the dermis. Anchoring chords are well developed at 13 weeks fetal gestation whereas anchoring fibrils are rudimentary at this age. At 2 years, anchoring cords are lacking and anchoring fibrils are robust. Mutations in both complexes may lead to blistering disorders.

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**28**

**KLEIN**

**Mitochondrial Z-DNA and ZBP1 Drive Autoimmune Photosensitivity**

Benjamin Klein1, Mack Reynolds2, Bin Xu1, Mehrnaz Gharaee-Kermani1,3, Amanda Victory1, Shannon Estadt1, Mary X. O’Riordan2, J. Michelle Kahlenberg1,3

*1Division of Rheumatology, Department of Internal Medicine, 2Department of Microbiology and Immunology, 3Department of Dermatology; University of Michigan, Ann Arbor, Michigan*

Autoimmune photosensitivity is observed in type I Interferon (IFN-I) mediated diseases such as systemic lupus erythematosus (SLE) and dermatomyositis. We previously identified an IFN-I-rich environment even in non-lesional lupus skin, but how this environment drives photosensitivity is unknown. We investigated how UV light and IFN-I impact mitochondrial stress and Z-DNA formation, a left-handed mitochondrial dsDNA that can activate cGAS.

Confocal microscopy of primary keratinocytes (KCs) from controls, SLE patients and immortalized KCs was performed to assess mitochondrial dynamics and cytosolic Z-DNA formation after UV and IFN-α. qPCR, single cell RNA sequencing and immunofluorescence was used for ZBP1 expression.

After UV light, KCs showed upregulated expression of IFN-I-regulated genes which was inhibited by mitoTEMPO. Mitochondrial fragmentation was significantly enhanced after UV and associated with cytosolic Z-DNA accumulation. Strikingly, IFN-I and UV led to large cytosolic Z-DNA puncta. Importantly, ZBP1, the sensor of Z-DNA is upregulated in SLE and dermatomyositis skin but not detectable in controls. SLE KCs showed strong cytosolic Z-DNA accumulation after UV exposure and Z-DNA colocalized with ZBP1 and cGAS. Knockdown of ZBP1 attenuated ISG expression after UVB in an IFN-high environment.

Collectively, we describe a new pathway of Z-DNA sensing by ZBP1 sustaining IFN responses in autoimmune photosensitivity.

**29**

**KONIECZNY**

**An IL-17A-HIF1a axis-controlled dysfunctional epithelial state fuels inflammatory skin disease**

Piotr Konieczny1, \*, Ipsita Subudhi1, \*, Aleksandr Prystupa1,2, Rochelle L. Castillo3,4, Ikjot Sidhu1,2, Erica Sze-Tu1, Yue Xing1, Daniel Rosenblum1, Ilana Reznikov1 and Shruti Naik1,5

*1Department of Pathology, NYU Langone Health, NY, NY 10016*

*2Applied Bioinformatics Laboratories, NYU Langone Health, NY, NY 10016*

*3Division of Rheumatology, Department of Medicine, NYU Langone Health, NY, NY 10016*

*4Psoriatic Arthritis Center, NYU Langone Health, NY, NY 10016*

*5Ronald O. Perelman Department of Dermatology, Department of Medicine, Perlmutter Cancer Center, NYU Langone Health, NY, NY 10016*

Inflammatory diseases are spurred by immune activation, epithelial hyperproliferation, and aberrant differentiation. Yet, the molecular programs that govern dysfunctional epithelial differentiation and their role in potentiating disease remain ill-defined. Recently, we identified the IL-17A-HIF1a axis that controls the optimal program of glucose metabolism, which is necessary for wound re-epithelialization. Whether this axis is similarly hijacked to drive pathology in skin disease is unknown. Using single-cell and spatial transcriptomics we identified a dysfunctional epithelial state in human Psoriasis, Atopic Dermatitis, and Hidradenitis Suppurativa lesions that was marked by HIF1a. In PsO lesions, epidermal HIF1a activation correlated with Type 17 immunity and colocalized with an expression of IL-17RC. HIF1a was abrogated in ⍺IL-17A or ⍺TNFa therapy-responsive patients and in IL17RA-deficient mouse model of psoriasis supporting the notion that epithelial HIF1a activation is a barometer of IL-17 signaling and downstream pathology. Epidermal-specific loss of HIF1a or glucose transporter 1 curtails pathology in both the IL-23 and imiquimod PsO models by curbing both epithelial dysfunction and the Type 17 response. Mechanistically, HIF1a concomitantly promoted transcription of differentiation genes, cytokines, and a metabolic switch from mitochondrial respiration to glycolysis. This aberrant epithelial remodeling state was necessary for basal cell hyperproliferation and inflammation. Taken together, our data identify the IL-17A-HIF1a axis as a master regulator of aberrant epidermal differentiation and underscore the utility of therapeutically targeting dysfunctional epithelial states to disrupt feedforward immune-epithelial inflammatory circuits.

**30**

**KOSEMANI**

**RASSF9 regulates the Cell Death Machinery in Melanoma**

Samson Kosemani1, Ronaldo Rodrigues Ribeiro1, Julia Costa1, Carolina Previdi Mesquita Barroso1, and Gustavo P. Amarante-Mendes1,2

*1Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo 05508-000, Brazil*

*2Instituto de Investigação em Imunologia, Instituto Nacional de Ciência e Tecnologia (INCT), São Paulo, Brazil.*

*Email: samsonkosemani@icb.usp.br*

The Ras-Association Domain Family (RASSF) is composed of Ras effector proteins that share a Ras-association (RA) domain. The family comprises ten members (RASSF1-10) that are divided into two groups depending on the location of the RA domain. RASSF9 is a member of the N-terminal group and is mostly expressed in the stratified epithelium, where it plays an important role in maintaining epidermal homeostasis. RASSF9 has also been identified as a gene whose expression is induced upon continuous exposure to UV radiation. Importantly, RASSF9 has been suggested to act as a tumor suppressor in some cancers by interfering with cellular proliferation. The role of RASSF9 in melanoma remains obscure. To address this point, we developed several RASSF9-deficient murine melanoma cell lines using the CRISPR/Cas9 system. Using these cell lines, we evaluated the role of RASSF9 on both proliferation and resistance to cisplatin, a chemotherapeutic drug used in the clinic, as well as to other cell death inducing drugs. RASSF9 elimination did not significantly interfere with proliferation but resulted in an increased resistance to cell death. Taken together, our results suggest that RASSF9 acts as a tumor suppressor in melanoma by contributing to the activation of the cell death machinery.

Keywords: RASSF9; Melanoma; Cell Death

Financial support: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

**31**

**KOUTSOUKOS**

**Concordance in the secretome of recessive dystrophic epidermolysis bullosa and cellular senescence reveals collagen VII dependent perturbations**

Stefanos A. Koutsoukos1, Maryna Pavlova1, Patrick S. McGrath2, Shennea McGarvey1, Jocelyn Castillo Flores1, Anna L. Bruckner3, Dennis R. Roop1, Igor Kogut1, and Ganna Bilousova1

1. *Department of Dermatology, University of Colorado School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado 80045, USA*
2. *Department of Pediatrics, University of Colorado School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado 80045, USA*
3. *Children’s Hospital Colorado, Aurora, Colorado 80045, USA*

Recessive dystrophic epidermolysis bullosa (RDEB) is a congenital skin condition caused by biallelic pathogenic variants in *COL7A1*, which encodes type VII collagen (C7). Deficiencies in C7 cause a dysfunctional dermal-epidermal junction resulting in a clinical presentation that includes severe generalized blistering. Patients living with RDEB have a 90.1% risk of developing aggressive squamous cell carcinoma (SCC) with high metastatic potential at a considerably younger age (by age 55) relative to the general population, suggesting a premature aging phenotype. In the following study, we investigated the role of C7 in cellular senescence, and more specifically the senescence associated secretory phenotype (SASP), in primary patient derived fibroblasts (*COL7A1*-/-huFs) as well as organoid derived fibroblasts (iFBs) generated from primary fibroblasts transitioned through induced pluripotency that were either uncorrected (*COL7A1*-/-iFBs) or genetically corrected using CRISPR/Cas9 (*COL7A1*+/-iFBs). SomaScan proteomic analysis of the *COL7A1*-/-huFs, *COL7A1*-/-iFBs, and *COL7A1*+/-iFBsrevealed numerous C7 dependent perturbations, including significant changes phosphoglycerate 1 mutase (PGAM1) expression. Increased PGAM1 expression has been described to predict poor prognosis of noncutaneous squamous cell carcinomas and is associated with cell migration. Additional proteomics analyses are currently underway to elucidate other C7 dependent perturbations associated with SASP and reveal novel targets causal in aggressive RDEB associated SCC.

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**KOUTSOUKOS**

**Ex vivo genome editing of *ASPRV1* for the treatment of autosomal dominant lamellar ichthyosis**

Stefanos A. Koutsoukos1, Igor Kogut1, and Ganna Bilousova1

1. *Department of Dermatology, University of Colorado School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado 80045, USA*

The ichthyoses are rare genetic conditions commonly characterized by general dry skin, scaling, hyperkeratosis, and erythroderma. Recent advances in human genetics and next-generation sequencing have allowed for the identification of numerous genes causal in their pathogenesis. Autosomal dominant lamellar ichthyosis (ADLI) is a recently characterized subtype caused by pathogenic variants in *ASPRV1* featuring palmoplantar keratoderma and lamellar ichthyosis. Active ASPRV1 enzyme is generated by autocleavage and targets filaggrin resulting in monomers for which their byproducts function as the natural moisturizing factors of the skin. Pathogenic variants in *ASPRV1* result in aberrant perturbations in autocleavage and filaggrin processing. In the following study, we investigate a gene editing strategy for *ASPRV1* and its ability to rescue ADLI pathology. Fibroblasts from three related individuals with ADLI harboring the pathogenic *ASPRV1* c.595A>G mutation were reprogrammed into iPSCs and edited using a validated CRISPR/Cas9 strategy. Clonal uncorrected and genetically corrected iPSCs were used to generate keratinocyte lineages for which functional studies regarding the rescue of aberrant ASPRV1 autocleavage and filaggrin processing are currently underway. Functional validation of our combined gene editing and reprogramming approach provides a newly established basis for the treatment of ADLI and additional subtypes of ichthyosis.

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**KULKARNI**

**Elucidating mechanisms of action of DRQ, a novel anti-cancer biologic agent**

Nabeela Khan1, Rebecca Nichols1, Connor Hall1, Zachary Garrison1, Terri Clister1, Rosalyn Fey1, Khanh Doan1, Rob Meza-Romero3, Arthur Vandenbark3, Renee Shirley4,Rajan P. Kulkarni1,2,3

1 Department of Dermatology, OHSU

2 Knight Cancer Institute, OHSU

3 VA Portland Health Care System

4 Virogenomics, Portland, OR

We have developed the novel third generation DRQ class of biologic compounds that were initially designed as potent inhibitors of MIF binding to CD74 and have shown promise in preclinical models of melanoma. Macrophage migration inhibiting factor (MIF) is expressed by both immune and tumor cells and MIF overexpression is increasingly implicated in worse outcomes in melanoma patients and thus is a novel therapeutic target. The cognate receptor for MIF is CD74 and binding activates multiple pro-growth pathways. Targeting MIF activity may be a promising therapeutic strategy in melanoma. DRQ significantly controlled tumor growth in two localized intradermal melanoma tumor models when compared with vehicle control. While DRQ DRQ decreased pERK and pSTAT3 expression in melanoma as expected, it also appears to reprogram the tumor microenvironment (TME) to decrease immunosuppression. This reprogramming results in increases in immunostimulatory (‘M1’) macrophages and dendritic cells in the TME, with associated increases in infiltrating CD8+ lymphocytes. These results suggest that DRQ could be a novel class of anti-melanoma biologic agents that have multiple complementary immunostimulatory effects to control tumor growth and spread and thus fill a critical unmet therapeutic need.

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**LEE**

Jia Jun Lee1, Claire Higgins1

1. *Department of Bioengineering, Imperial College London*

Human chronic skin wounds have been extensively characterised, but ethical limitations of collecting and studying wounds from healthy individuals means there is a sparsity of data regarding normal wound healing. Here, we characterise a model of normal wound healing in human skin, using *ex vivo* skin explants. We found that partial re-epithelialisation and full healing of 2mm wounds occurred 2-3 days and 4 days post-wounding, respectively. Therefore, we conducted single-cell RNA sequencing (scRNA-seq) from *ex vivo* skin explants 2 days post-wounding to interrogate spatial heterogeneity in keratinocytes relative to the wound edge. This data was compared with scRNA-seq data derived from healthy unwounded human skin. We found an increase in keratinocytes (both basal and suprabasal) expressing *S100A7* in wounded compared to unwounded skin. This finding was consistent in all biological replicates (N=3). *S100A7* was previously reported to be increased at the wound edge in human keratinocytes, highlighting that gene changes in our *ex vivo* human skin explant model reflect those occurring *in vivo*. We are currently analysing the scRNA-seq data to identify genes present in the leading and trailing edges during normal skin wound healing.

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**MA**

**Wnt signaling activation stimulates lipolysis in mature dermal adipocytes in skin fibrosis**

Qiannan Ma1, Ella X Segal1, Rachel H Wyetzner1, Valerie Hosely2, Radhika P Atit1,3,4

*1Department of Biology, College of Arts and Sciences, Case Western Reserve University, Cleveland, Ohio, USA; 2Department of Molecular and Cell Biology, Yale University, New Haven, Connecticut, USA; 3Department of Genetics and Genome Sciences, School of Medicine, Case Western Reserve University, Cleveland, Ohio, USA; 4 Department of Dermatology, School of Medicine, Case Western Reserve University, Cleveland, Ohio, USA*

Skin fibrosis is characterized as accumulation of extracellular matrix (ECM) and dermal fat loss, leading to compromised skin function and elasticity with no existing therapies for reversal. Wnt signaling pathway is profibrotic and anti-adipogenic, fibrotic fat loss was recently shown to be dependent on Wnt activation. However, the mechanism of how the Wnt signaling pathway activates dermal fat loss and its impact on ECM accumulation is unclear. Lipolysis is a homeostatic pathway in adipocytes for lipid catabolism and energy generation. My hypothesis is that **the Wnt signaling pathway stimulates lipolysis in mature dermal adipocytes leading to skin fibrosis**. The Wnt signaling pathway is activated in mouse dermal mature adipocytes in a region of the dorsal skin *in vivo* (Adipo-β-catistab). The size of individual adipocytes and thickness of dermal white adipose tissue significantly decreases after Wnt activation without compromising cell survival. Interestingly, ECM accumulation and collagen remodeling was elevated in the dermis of Adipo-β-catistab mutant. Preceding tissue level changes, we detected elevated expression of phosphorylated hormone-sensitive lipase, an essential enzyme of lipolysis, in Adipo-β-catistab dermal adipocytes. Ongoing experiments are testing the functional role of lipolysis in Wnt induced fibrotic fat loss, to develop a novel therapeutic target in skin fibrosis.

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**MADHAVAN**

**WNT signaling activation causes early decline of *de novo* lipogenesis leading to lipid depleted dermal adipocytes.**

Suneeti R. Madhavan1, Michael C. Rudolph2,3, Radhika P. Atit1,4,5

*1Department of Biology, College of Arts and Sciences, Case Western Reserve University, Cleveland, Ohio, USA; 2Department of Physiology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA; 3Harold Hamm Diabetes Center, The University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA; 4Department of Genetics and Genome Sciences, School of Medicine, Case Western Reserve University, Cleveland, Ohio, USA; and 5Department of Dermatology, School of Medicine, Case Western Reserve University, Cleveland, Ohio, USA.*

Skin fibrosis is characterized by accumulation of extracellular matrix (ECM) and lipodystrophy in the dermal white adipose tissue (DWAT), affecting 1 in 5,400 people globally each year with no therapies for reversal. **Many profibrotic factors contribute to dermal ECM expansion, but the mechanism of lipodystrophy in DWAT remains elusive.** Recently, we showed that skin fibrosis related lipodystrophy is dependent on Wnt signaling activation via DPP4. Gene expression profiling of Wnt-activated dermal adipocytes preceding fibrotic lipodystrophy showed dysregulation in the lipid metabolism gene set, specifically *de novo* lipogenesis (DNL). Using an inducible-reversible mouse model of Wnt activation in the dermal and DWAT compartments, we tested the hypothesis that **Wnt activation induced the downregulation of *de novo* lipogenesis in mature dermal adipocytes.** We found that FASN, a key DNL enzyme, is significantly downregulated after 5 days of Wnt activation and recovers upon 10 days of removing the Wnt stimulus. Remarkably, FASN expression is protected after Wnt activation in a Dpp4-/- background. Thus, DNL is dependent on Wnt/DPP4 activation and ongoing studies are investigating its functional role in fibrotic fat loss that precedes skin fibrosis. Unraveling the molecular mechanisms of DWAT loss will provide new therapeutic targets to treat skin fibrosis.

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**MORRIS**

**Bone marrow-derived epithelial cells contribute to developing**

**chronic cutaneous neoplasms in mice**

Heuijoon Park1,2, Sonali Lad1, Kelsey Boland1, Kelly Johnson1, Nyssa Readio1, Guangchun Jin2, Samuel Asfaha2, Kelly S. Patterson2, Ashok Singh1, Xiangdong Yang2, Douglas Londono3, Anupama Singh1, Carol Trempus4, Jennifer Boyle1, Stephanie M. Holtorf1, Derek Gordon3, Timothy C. Wang2 and Rebecca J. Morris1

*The Hormel Institute, University of Minnesota, Austin, MN1; Columbia University, New York, NY2, Rutgers University, Piscataway, NJ3; National Institute of Environmental Health Sciences, Research Triangle Park, NC4.*

Non-melanoma skin cancers (NMSCs) are a serious and growing public health problem despite a plethora of sunscreens and repeated calls for sun avoidance. In some patients, NMSCs become intractable and ultimately deadly leading us to wonder whether some of the cancer cells might originate in the bone marrow, an incubator for numerous stem and progenitor cells. The purpose of our study was to explore the role of bone marrow derived cells (BMDCs) in cutaneous malignancy. We used allogeneic bone marrow transplantation (BMT) and a mouse multistage cutaneous carcinogenesis model to probe recruitment of BMDCs in skin tumors initiated with the carcinogen, dimethylbenz[*a*]anthracene (DMBA), and promoted with 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Donor BMDCs clustered in the recipient lesional epithelium, expressed cytokeratins, proliferated, and stratified. We detected cytokeratin induction in plastic-adherent bone marrow cells (BMCs) cultured in the presence of filter-separated keratinocytes (KCs) and bone morphogenetic protein 5 (BMP5). Lineage-depleted BMCs migrated towards HMGB1 protein and epidermal KCs in *ex vivo* invasion assays. Naïve female mice receiving BMTs from DMBA-treated donors developed benign and malignant cutaneous lesions after TPA promotion alone. In summary, BMCs contribute to both initiation and promotion of cutaneous carcinogenesis. We conclude that a subset of squamous lesions long thought to be solely local in origin have a distinct systemic component. These findings may suggest new targets for diagnosis and surveillance of NMSCs.

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**NELSON**

**Understanding Melanoma Epidemiology and Prognostic Factors in** Oregon

Jacob Nelson1, Jordan Gillespie1, Jaclyn Roland-Mcgowan1, Kyra Diehl2, Elizabeth Stoos1, and Sancy A. Leachman1

*1Oregon Health & Science University, Department of Dermatology*

*2Western University of Health Sciences, College of Osteopathic Medicine of the Pacific*

Approximately 1,300 people in Oregon are diagnosed with malignant melanoma every year. This study aims to better understand the epidemiology of melanoma in Oregon, including rates of known melanoma prognostic factors within Oregon during the years prior to a statewide public education campaign (War on Melanoma™) aimed at increasing early detection, and decreasing mortality due to melanoma. We obtained deidentified case data from the Oregon State Cancer Registry (OSCaR) for melanoma cases diagnosed from 2014-2018 and performed a descriptive analysis. Cases with missing data were excluded. Within our sample of 6,170 melanoma cases, 98.8% (5,913) were non-Hispanic white, 56.4% (3,482) were male, and mean age at diagnosis was 63 (SD=15.3). Late-stage at diagnosis (regional or distant SEER summary stage) occurred in 11.5% (710/6,170) of cases. The average Breslow depth for early-stage cases was 1.0mm, and 3.9mm for late-stage cases. Ulceration was present in 9.8% (89/910) of early-stage cases and 50.5% (47/93) of late-stage cases. Average mitotic rate in early vs late-stage disease was 3.7 mitoses/mm2 and 7.6 mitoses/mm2 respectively. These data provide a baseline for future analysis of the effect of the War on Melanoma™.

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**NGUYEN**

**Prolonged TGF-β signaling in chromatin-mediated regulation of cancer stem cell phenotypes**

Kayla Nguyen1, Sachiko Taniguchi1, and Naoki Oshimori1,2,3,4

*1Department of Cell, Developmental & Cancer Biology,*

*2Department of Dermatology,*

*3Department of Otolaryngology - Head & Neck Surgery,*

*4Knight Cancer Institute, Oregon Health & Science University, Portland, OR 97239, USA.*

**Keywords**

Cancer, Cancer stem cell, TGF-β, Macc1, epigenetics.

Cancer stem cells (CSCs), a small subset of the tumor cells with long-lived tumorigenic potential, contribute to tumor development and recurrence. CSCs often exist in quiescent and stress-resistant states, which can attribute to drug resistance. We previously identified TGF-β-responding tumor cells as drug-resistant CSCs of invasive squamous cell carcinoma (SCC). Lineage tracing of this CSC population revealed that the progeny exhibited long-lasting phenotypic changes, including poorly-differentiated states and epithelial-to-mesenchymal transition (EMT). Since we often observed that CSCs reside in TGF-β-rich tumor microenvironments, prolonged TGF-β signaling from the CSC niche might cause a distinct gene expression program responsible for the malignant phenotypes. To address this, we examined differentially accessible chromatins in TGF-β-responding tumor cells *in vivo* and identified genes potentially induced by the chromatin structure alteration, including *Macc1* (metastasis-associated in colon-cancer-1). MACC1 is known to be involved in metastasis and drug resistance in colon cancer. We found that a prolonged TGF-β treatment upregulated MACC1 expression in primary keratinocytes *in vitro* but not by short-term treatment, suggesting the potential chromatin-mediated regulation. Moreover, experiments using shRNA-mediated Macc1 knockdown suggested that MACC1 may regulate cellular quiescence. Our study may provide new insight into the epigenetic mechanisms underlying the malignant CSC phenotypes.

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 **NOMDEDEU-SANCHO**

**Development of a Combined Melanoma/Skin Organoid System to Study Tumor-Stroma Interactions and Therapy Resistance**

Gemma Nomdedeu-Sancho1, Anastasiya Gorkun1, Naresh Mahajan1, Daniel Gironda1, Azza El-Derby1, Shay Soker1, Anthony Atala1.

*1Wake Forest Institute for Regenerative Medicine*

Current *in vitro* melanoma models fail to represent the skin's cellular and structural complexity. Cancer interaction with the tumor microenvironment - specifically cancer-associated fibroblasts (CAFs) - can impact growth and immune response, affecting immunotherapy efficacy. Developing physiologically and architecturally accurate melanoma models is crucial to studying the crosstalk between cancerous and healthy cells that impacts the tumor’s response to therapy.

We incorporated an aggressive melanoma cell line into spherical layered skin organoids to generate *in vitro* 3D combined melanoma-skin organoid systems that can be used to simulate melanomagenesis, explore tumor-stroma interactions, test therapies, and identify early melanoma biomarkers.

Melanoma foci quickly proliferated and migrated outside the healthy skin organoids. To force cancer-stroma interactions, we embedded the dermal and melanoma cells in skin extracellular matrix and surrounded it with a mixture of epidermal cells. Confinement of melanoma in the core allowed us to analyze the expression of CAF-specific markers, modeling the melanoma-induced transformation of fibroblasts to support tumor progression.

We created the first spherical layered melanoma-skin organoid system, which can serve as an *in vitro* model to study melanoma progression in a three-dimensional realistic environment. By incorporating patient-derived melanoma cells, we expect to enhance our understanding of therapy resistance and improve personalized melanoma treatment.

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**ODELL**

**IL-6 Trans-Signaling in a Humanized Mouse Model of Scleroderma**

**Ian D. Odell, MD, PhD**1,2, Kriti Agrawal6,7, Esen Sefik, PhD2, Anahi V. Odell, PhD2, Elizabeth Caves3, Nancy C. Kirkiles-Smith2, Valerie Horsley, PhD1,3, Monique Hinchcliff, MD, MS4, Jordan S. Pober, MD, PhD1,2,5, Yuval Kluger, PhD5,6,7, and Richard A. Flavell, PhD, FRS2,8,\*

*1Department of Dermatology, Yale University School of Medicine, New Haven, CT, USA*

*2Department of Immunobiology, Yale University School of Medicine, New Haven, CT, USA*

*3Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06520, USA*

*4Department of Internal Medicine, Section of Rheumatology, Allergy and Immunology, Yale School of Medicine, New Haven, CT, USA*

*5Department of Pathology, Yale University, New Haven, CT 06511*

*6Program in Computational Biology and Bioinformatics, Yale University, New Haven, CT*

*7Program in Applied Mathematics, Yale University, New Haven, CT 06511*

*8Howard Hughes Medical Institute, Chevy Chase, MD, USA*

Scleroderma is an autoimmune disease that causes skin and internal organ fibrosis. However, no mouse model fully recapitulates human disease. We utilized MISTRG6 mice, which develop an immune system like humans when engrafted with human hematopoietic stem cells (HSC), to model scleroderma by transplantation of healthy or scleroderma skin from a patient with pansclerotic morphea to humanized mice engrafted with unmatched allogeneic HSC. We discovered that scleroderma skin grafts contained both skin and bone marrow derived human CD4 and CD8 T cells along with human endothelial cells and pericytes. Unlike healthy skin, fibroblasts in scleroderma skin were depleted and replaced by mouse fibroblasts. Furthermore, HSC engraftment alleviated multiple signatures of fibrosis, including expression of collagen and interferon genes, and proliferation and activation of human T cells. Fibrosis improvement correlated with reduced markers of T cell activation and expression of human IL-6 by mesenchymal cells. Mechanistic studies supported a model whereby IL-6 trans-signaling driven by CD4 T cell derived soluble IL-6 receptor complexed with IL-6 promoted excess ECM gene expression. Thus, MISTRG6 mice transplanted with scleroderma skin demonstrated multiple fibrotic responses centered around human IL-6 signaling, which was improved by the presence of healthy bone marrow derived immune cells.

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**ODORISIO**

**Histone deacetylase inhibition mitigates fibrosis-driven disease progression in recessive dystrophic epidermolysis bullosa**

Emanuela De Domenico\*1, Alessia Primerano\*1, Francesca Cianfarani\*1, Naomi De Luca1, Giovanna Floriddia1, Massimo Teson1, Cristina Cristofoletti2, Silvia Cardarelli3, Giovanni Luca Scaglione1, Davide Cangelosi4, Paolo Uva4, Jörn Dengjel5, Alexander Nyström6, Simona Mastroeni7, Enke Baldini3, Salvatore Ulisse3, Daniele Castiglia1, Teresa Odorisio1

*1. Laboratory of Molecular and Cell Biology, IDI-IRCCS, Rome, Italy*

*2. Laboratory of Experimental Oncology, IDI-IRCCS, Rome, Italy*

*3. Department of Surgical Sciences, Sapienza University, Rome, Italy*

*4. Clinical Bioinformatics Unit, Giannina Gaslini Hospital-IRCCS, Genoa, Italy*

*5. Department of Biology, University of Fribourg, Switzerland*

*6. Department of Dermatology, University of Freibug, Germany*

*7. Epidemiology Unit, IDI-IRCCS, Rome, Italy*

Recessive dystrophic epidermolysis bullosa (RDEB) is a mucocutaneous blistering disease due to type VII collagen gene mutations. Transforming growth factor-β (TGF-β)-dependent fibrosis is responsible for severe RDEB complications. Reduced histone acetylation is a hallmark of fibrosis in pathologies affecting organs other than skin, where inhibition of histone deacetylases (HDACs) proved effective in reverting fibrosis. Here, HDAC inhibitor (HDACi) ability to counteract RDEB progression was investigated. We found that histone acetylation is decreased in RDEB skin and fibroblasts. Treatment with two HDACis, valproic acid (VPA) and Givinostat, counteracts RDEB fibroblast fibrotic traits, including contractility, α-smooth muscle actin expression, TGF-β1 release, and proliferation. Moreover, systemic VPA administration mitigates severe manifestations (corneal opacification and digit loss) in a RDEB mouse model. This effect associates with inhibition of skin fibrosis (presence of subcutaneous fat, thinner collagen bundles, decreased expression of fibrotic markers). Proteomic analysis of mouse skin revealed that VPA treatment decreases expression of genes involved in protein synthesis and increases levels of proteins with role in immune response. These findings highlight epigenetic changes as contributing to RDEB pathogenesis and disclose molecular mechanisms leading to skin fibrosis. Treatment with HDACi may represent a disease modifier therapeutic approach for RDEB and other skin fibrotic disorders.

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**OSHIMORI**

**Tumor-initiating cells establish privileged access to niche precursors to drive cancer progression**

Hannah L. Erickson,1 Sachiko Taniguchi,1 Anish Raman,1 Justin J. Leitenberger,2 Sanjay V. Malhotra,1,4,5 and Naoki Oshimori1,2,3,5\*

*1Department of Cell, Developmental & Cancer Biology, 2Department of Dermatology,*

*3Department of Otolaryngology, Head & Neck Surgery, 4Center for Experimental Therapeutics,*

*5Knight Cancer Institute, Oregon Health and Science University, Portland, OR 97239, USA*

The evolving crosstalk between tumor-initiating cells (TICs) and the niche microenvironment promotes cancer progression and therapeutic resistance. In squamous cell carcinoma (SCC), TICs upregulate interleukin (IL)-33 to induce a subset of anti-inflammatory macrophages and establish an immunosuppressive niche. However, IL-33 exerts pro-tumor and anti-tumor effects depending on cell types responding to this cytokine. It remains unclear how TICs utilize IL-33 to induce niche macrophages to harness their tumor-promoting functions, without causing anti-tumor effects by other pro-inflammatory cells. Using a mouse model of SCC, we show that TICs release plasma membrane-derived extracellular vesicles through the autophagy-related gene ATG9B to deliver IL-33 to niche precursors. Mechanistically, the lipid scramblase activity of ATG9B endows the vesicular surface with Annexin A1 (ANXA1), which facilitates IL-33-induced differentiation of ANXA1 receptor+ immature myeloid cells into niche macrophages. We found that such niche precursors were overproduced in the bone marrow of tumor-bearing mice. Depleting ANXA1 or blocking the ATG9B’s scramblase activity reduced the accumulation of niche macrophages to the proximity of TICs, thus suppressing the invasive progression of SCC. This hitherto unappreciated signaling mechanism advances our understanding of the evolving crosstalk between TICs and the niche, which may help us combat treatment-resistant cancers.

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**PENG**

**Autophagy activation is required for antimicrobial peptide human-β-defensin-3-induced improvement of skin barrier function in atopic dermatitis**

Ge Penga, Rui Zou b, Wanchen Zhaoa, Alafate Abudouwanlia, Quan Suna, Ko Okumuraa, Hideoki Ogawaa, Shigaku Ikedaa, François Niyonsabaa, c

a *Atopy (Allergy) Research Center, and b Department of Data Science, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. c Faculty of International Liberal Arts, Juntendo University, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan.*

Among skin-derived antimicrobial peptides, human β-defensins (hBDs) are the most studied and are involved in various skin diseases, in which they exhibit pleiotropic antimicrobial and immunomodulatory activities, including the regulation of skin barrier function. However, its contribution to autophagy regulation remains unclear, and the role of autophagy in the regulation of the epidermal barrier in atopic dermatitis is poorly understood. Here, keratinocyte autophagy was restrained in the skin lesions of patients with atopic dermatitis and murine models of atopic dermatitis. Interestingly, hBD-3 alleviated the T helper 2 cytokines interleukin-4- and interleukin-13-mediated impairment of the tight junction barrier through keratinocyte autophagy activation, which involved aryl hydrocarbon receptor (AhR) signaling. While autophagy deficiency impaired the epidermal barrier and exacerbated inflammation, hBD-3 attenuated skin inflammation and enhanced the tight junction barrier in atopic dermatitis. Importantly, hBD-3–mediated improvement of the tight junction barrier was abolished in autophagy-deficient atopic dermatitis mice and in AhR-suppressed atopic dermatitis mice, suggesting a role for hBD-3–mediated autophagy in the regulation of the epidermal barrier and inflammation in atopic dermatitis. Thus, autophagy contributes to the pathogenesis of atopic dermatitis, and hBD-3 could be used for therapeutic purposes.

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**RIETSCHER**

**Targeting phosphorylation of the keratin-desmosome complex for the treatment of Epidermolysis Bullosa Simplex**

Katrin Rietscher1, Matthias Rübsam2,3, Nancy Ernst4, [Ryan F. L. O'Shaughnessy](https://pubmed.ncbi.nlm.nih.gov/?term=O%27Shaughnessy+RF&cauthor_id=27913303)5, Cristina Has6, M. Bishr Omary7, Carien M. Niessen2,3, Ralf J. Ludwig4, and Thomas M. Magin1

*1Institute of Biology, Division of Cell and Developmental Biology, Leipzig University, Leipzig, Germany.*

*2Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany.*

*3Department Cell Biology of the Skin, Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany.*

*4Lübeck Institute of Experimental Dermatology and Center for Research on Inflammation of the Skin, University of Lübeck, Lübeck, Germany.*

*5Centre for Cell Biology and Cutaneous Research, Blizard Institute, Faculty of Medicine and Dentistry, Queen Mary University of London, UK.*

*6Department of Dermatology, Medical Center, University of Freiburg, Freiburg, Germany.*

*7Center for Advanced Biotechnology and Medicine, and Robert Wood Johnson Medical School, Rutgers University, Piscataway, New Jersey, USA.*

Mutations in K5 and K14 cause the skin disorder Epidermolysis Bullosa Simplex (EBS), associated with a collapse of keratin filaments into cytoplasmic protein aggregates. Phenotypic consequences comprise fragility of basal keratinocytes and skin blistering upon mild mechanical trauma. Current treatment of EBS is only supportive and consists primarily of wound care and avoidance of mechanical stress.

Numerous post-translational modifications (PTMs) such as phosphorylation and acetylation occur on keratins, controlling the re-organization of keratin networks. We and others have recently found that EBS-associated mutations such as K14.R125C affect keratin phosphorylation and acetylation at distinct sites and thereby can aggravate keratin aggregation and EBS severity. We identified the multi-kinase inhibitor PKC412 as a drug that promoted reformation of filaments from mutated aggregates and stimulated formation of stable desmosomes, thereby strengthening intercellular cohesion. At the molecular level, global phosphoproteomic analysis revealed novel phospho-sites in keratins and desmoplakin that were reduced upon PKC412 treatment. Subsequent PamGene-based kinase profiling of PKC412-treated EBS cells identified a rapid decrease in upstream Tyr kinase activity that reduced several Ser/Thr kinases acting on desmoplakin and keratins. Given that PKC412 is already in clinical use, our data pave the way for a clinical trial using PKC412 for patients with EBS.

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**RUDD**

***hFWE* orchestrates terminal differentiation of epidermal keratinocytes**

Justin C. Rudd1, Greer L. Porter1, Peter O. Halloran1, Patrick T. Kuwong1, Louise Monga1, Rachel E. Johnson1, Mrinal K. Sarkar2, James A. Grunkemeyer1, Johann E. Gudjonsson2, Laura A. Hansen1

*1Department of Biomedical Sciences, Creighton University School of Medicine, Omaha, NE*

*2Department of Dermatology, University of Michigan, Ann Arbor, MI*

Keratinocyte terminal differentiation is essential for establishing epidermal barrier function that prevents external insult and loss of bodily fluids. Impaired differentiation is a hallmark of many epidermal pathologies including psoriasis and cutaneous squamous cell carcinoma (cSCC), but mechanisms governing differentiation are incompletely understood. Here we demonstrate that *Flower* (*hFWE,* also known as *CACFD1*), a gene encoding several alternatively spliced transmembrane proteins with putative calcium channel activity but no known function in skin, is essential for proper execution of keratinocyte differentiation in the epidermis and derivative tumors. Using patient tissue, genetically engineered epidermal raft cultures, and cSCC xenografts we reveal that *hFWE* expression is induced upon terminal differentiation and is most abundant in filaggrin-positive granular keratinocytes. The predominant isoform, hFWE4, undergoes calcium-dependent internalization from the plasma membrane during terminal differentiation, before ultimately localizing to keratohyalin granules in the granular layer. Knockout of *hFWE* produces significant terminal differentiation defects, leading to near complete loss of keratohyalin granules and late differentiation markers loricrin and filaggrin. Finally, we reveal potential involvement of hFWE in the aberrant differentiation observed in psoriasis, showing that *hFWE* expression is both reduced by IL-17A stimulation in differentiated keratinocytes, and mislocalized to the upper stratum spinosum in psoriatic epidermis.

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**SCHUSTER**

**Multi-antigen management by dendritic cells within the melanoma microenvironment**

Victoria Schuster1, Kasidy Brown1, Julia Unsworth1, Tim Nice2, Naoki Oshimori1,3, Antonin Weckel4, Tiffany Scharschmidt4, Megan K. Ruhland1,3

*1Department of Cell, Developmental and Cancer Biology, 2Department of Molecular Microbiology and Immunology, 3Knight Cancer Institute, Oregon Health & Science University, Portland, Oregon, 97201, 4Department of Dermatology, University of California San Francisco, San Francisco, CA 94143*

Dendritic cells (DCs) help maintain skin homeostasis via reinforcement of tolerance to self while concurrently initiating inflammatory responses to threats. It is unclear how an individual DC manages different antigens from various sources simultaneously. Additionally, how this multi-antigen uptake impacts immune responses is not well understood. We leverage an in vivo fluorescent reporter system to track different antigen types, both activating (i.e. pathogen) and tolerizing (i.e. self or commensal) alongside melanoma antigen. We find DCs harboring both self- and pathogen-derived antigen compartmentalize these antigens into distinct endosomes. However, DCs bearing tumor and self antigens co-house both within the same endosomes, suggesting antigen sorting based on source. Further, commensal antigen is similarly co-housed with self, raising the possibility that DCs segregate activating and tolerizing antigen and that tumor may be handled inappropriately as ‘safe’. In vitro, we find dual antigen uptake impacts presentation and co-stimulation as well as endosomal maturation. Together our data suggest DCs phagocytose antigen from multiple sources concurrently, and sort the antigen to achieve differential responses, potentially resulting in blunted activation against tumor antigens. Understanding how DCs navigate the tumor microenvironment where multiple antigen sources are present may enable the design of more effective melanoma immunotherapies.

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**SIMMONS**

**Dermal fibroblasts have a critical role in skin immunity through responses to keratinocyte interleukin-1**

Jared Simmons, Teruaki Nakatsuji, Kellen Cavagnero, Richard L. Gallo

*Department of Dermatology – UC San Diego*

Subsets of fibroblasts can express cytokines and antimicrobial peptides, but their relevance to inflammation and host defense is poorly understood compared to other cell types . Here, we hypothesized that some dermal fibroblasts may execute innate defense mechanisms in response to signals from keratinocytes at the surface. To test this, we cultured different fibroblasts (HPAd, MDFb, 3T3-L1) with keratinocyte (NHEK, HaCaT) conditioned media. Bulk RNA-seq analysis revealed a two-fold increase of >130 genes in HPAd exposed to NHEK supernatant. qPCR analysis confirmed this response (e.g., 57-fold increase in CCL20, p<0.0001) and these fibroblasts showed strong chemotactic activity for neutrophils. scRNA-seq of skin after topical *S. aureus* infection confirmed this response of dermal fibroblasts in mice. We found that the cytokine response to keratinocytes depended on IL-1α, as it was absent in dermal fibroblasts from IL-1R1-/- mice and blocked in HPAd with IL-1Ra (anakinra) or anti-IL-1α, but not anti-IL-1β. The response was also emulated by addition of recombinant IL-1α/β (500 pg/mL). Together, these data strongly suggest that dermal fibroblasts sense keratinocyte-derived IL-1α and mediate a proinflammatory response to cutaneous danger signals. This novel role of fibroblasts in immunity could pave the way to better target treatments for inflammatory skin diseases.

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**SMITH**

**Aging Effects on Skin Biomechanics**

Hayden B. Smith1, Shramana Ghosh1, Katie A. O’Connell1, Madeleine Alder1, Joanna Chen1, Arved Vain2, Eric R. Tkaczyk1

*1VA Tennessee Valley Healthcare System, Nashville, TN, United States*

*2University of Tartu Institute of Physics, Tartu, Estonia*

The biomechanical properties of skin can be used in the assessment of cutaneous manifestations of systemic diseases including chronic graft-vs-host disease and scleroderma. Skin biomechanics can be quantitively characterized using non-invasive technologies including the Myoton, a handheld device that characterizes the skin’s biomechanical properties through indentation. Aging can affect skin biomechanics. The purpose of the study was to identify which of the Myoton parameters best capture the effects of aging on skin biomechanics.

66 healthy subjects were measured using the Myoton. Linear models were used to examine the association of age, sex, and BMI with each parameter. Age had significant positive associations with decrement, creep, and stiffness parameters. Sex was significantly associated with creep, stiffness, relaxation time, and frequency.

The Myoton parameters related to elasticity and the rate of recovery of skin from impact (decrement and creep) best capture aging. These findings support the importance of age-matching controls to subjects in studies assessing skin biomechanics of systemic diseases with cutaneous manifestations.

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**TASHAYYOD**

Tashayyod, Davood

*Lumo Imaging*

Early-stage identification of Suspicious Pigmented Lesions (SPLs) in primary care settings can lead to improved melanoma prognosis and reduction in treatment costs. LumoScreen is designed as a full body SPL detection and classification system. However, for its first clinical studies, it will only be used as a full-body ugly duckling(s) detection system. LumoScreen’s main components are: 1) a blob detection and validation system for finding all lesions, 2) a best view detector for finding the wide-field image that provides the highest magnification and best focused view of each lesion (amongst the many wide-field images that may have a view of the lesion), 3) an SPL classifier, and 4) a classifier for detecting ugly duckling(s) amongst those SPLs.  Some of the future avenues for research and collaboration regarding wide-field lesion classification include: 1) utilizing the Attention and Transformer AI techniques to improve classification accuracy and explainability; 2) performing multi-scale segmentation of the lesion and using Natural Language Processing algorithms; 3) combining real and synthetic lesion to improve training and network generalization, 4) illuminating lesions with other spectra (405nm and 365nm) and 5) most importantly developing a TBP system that is affordable for the majority of dermatologists.

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**TASHAYYOD**

Tashayyod, Davood

*Lumo Imaging*

Although the dermascopic criteria for evaluation of A, B and D scores is consistent with strategies employed by prevalent algorithms, the same cannot be said about the C score.    The existing algorithms do not give special consideration to the colors that correspond to melanin distribution, regression and inflammation/neovascularization (e.g. to areas lighter than surrounding skin).

To provide a more consistent computation of the C score, we proposed using LAB, a color space that covers the entire gamut of human perception and the CIEDE2000 color-difference algorithm. We proposed two approaches: 1) clustering all colors found within a lesion to find the predominant colors, then computing the C score as the sum of the pairwise CIEDE2000 differences between predominant colors; and 2) identifying five segments within the LAB color space for the Reds, Browns, Blues, Whites, and other colors observed in a public lesion image dataset. The algorithm then associates each pixel in the lesion with one of the five segments. It then computes four C scores: CRed, CBrown,CBlue, CWhite based on prevalences of and tonal variances within the corresponding colors.   We will present classification performance results from the existing and the two proposed ABCD classifiers.

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**UBEROI**

**Commensal-derived tryptophan metabolites fortify the skin barrier: Insights from a 50-species gnotobiotic model of human skin microbiome.**

Aayushi Uberoi1, Preeti Bhanap1, Sofia Murga1, Amy Campbell1, Monica Wei, Laurice Flowers, Simon A. B. Knight1, Anya Chan1, Taylor Senay1, Ellen K. White1, Jordan C. Harris1, Carrie Hayes Sutter1, Charles Bradley1, Clementina Mesaros1, Thomas Sutter2, Elizabeth Grice1

*1University of Pennsylvania, Perelman School of Medicine, Department of Dermatology, Philadelphia, PA 19014, USA.*

*2University of Memphis*

The skin epidermis is the body's main defense against dehydration and harmful substances. We used germ-free mice to show that the microbiota is essential for proper differentiation and repair of the epidermal barrier. This effect is mediated by the aryl hydrocarbon receptor (AHR), a regulator of epidermal differentiation. We hypothesized that the skin microbiota activates AHR to promote barrier repair. Tryptophan metabolites, which are potent AHR ligands, are enriched in the skin's outermost layer (stratum corneum) and can be produced by microbial metabolism. We constructed metabolic enzyme profiles and mined them against healthy human skin metagenomes. These analyses revealed motif enrichment for enzymes that metabolize tryptophan to indole and its derivatives. To identify microbially regulated tryptophan metabolites *in vivo*, we established a gnotobiotic model with 50 skin commensals from healthy humans and performed targeted mass spectrometry on murine skin. We found three novel indole-related metabolites that are regulated by microbes and tested therapeutic efficacy in a murine model of atopic dermatitis. We provide a novel ecological framework that demonstrates that microbiota regulates skin barrier formation and repair via tryptophan metabolism. This knowledge can guide the development of precise microbe-based therapies for various skin disorders characterized by impaired epidermal barrier function.

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**VALDEBRAN**

**Effects of exercise and physical activity on gut microbiota: Potential intervention for auto-inflammatory skin conditions.**

Manuel Valdebran1,2, Victoria Palmer3

*1Department of Pediatrics, Medical University of South Carolina, Charleston, SC 29425, USA*

*2Department of Dermatology and Dermatologic Surgery, Medical University of South Carolina, Charleston, SC 29425, USA*

*3Department of Medicine, Richmond University Medical Center, Staten Island, NY 10310, USA*

The influence of physical activity in shifting the gut microbiome has been increasingly studied due to a positive correlation with various metabolic and immunomodulatory functions relevant to linked metabolic and auto-inflammatory skin conditions.

The purpose of this study is to review the relationship between exercise and changes in gut microbiome responsible for anti-inflammatory or biochemical metabolites.

Reduced replication rate of *Prevotella copri* was associated with degradation of branched-chain amino acids (BCAAs). Elevated abundance of *Eubacterium hallii*, *Veillonella* and *Coprococcus* was associated with enhanced capacity for biosynthesis of short-chain fatty acids (SCFAs) and reduction in pro inflammatory cytokines. High-intensity interval training showed increased abundance of *Subdoligranulum* and *Akkermansia muciniphila* (positive correlation with HDL-cholesterol levels and negatively correlated with insulin resistance, leptin, insulin, CRP, and IL-6. Abundance of *Faecalibacterium praunsnitzii* has been associated with reduction of IL-2, interferon-gamma, and increased secretion of IL-10. *Roseburia hominis* has been associated with strengthening gut barrier function and enhancing regulatory T cell (Treg) population.

Exercise may amplify subtle differences of gut microbiota by modifying metabolic and local immunity, though, there may be inter-individual variability. Immunomodulation may therefore be possible through introduction or stimulation of relevant microorganisms via exercise.

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**VALDIVIESO**

**Unraveling the Interplay and Spatiotemporal Dynamics of Cellular Senescence in Skin Healing**

**Karla Valdiviesoa,b,c,d**, Tomaz Rozmarica,b,c, Barbara Schädlb,c,e, Nadja Anneliese Ruth Ring a,b,c, Helene Dworaka,b,c, James Fergusonb,c, Susanne Drechslerb, Johannes Grillarib,c,d, Heinz Redla,b,c and **Mikolaj Ogrodnika,b,c**

*a Ludwig Boltzmann Research Group Senescence and Healing of Wounds, Vienna, Austria*

*b Ludwig Boltzmann Institute for Traumatology in AUVA Research Center, Vienna, Austria*

*c Austrian Cluster for Tissue Regeneration, Vienna, Austria*

*d Institute of Molecular Biotechnology, University of Natural Resources and Life Sciences, Vienna, Austria*

*e University Clinic of Dentistry, Medical University of Vienna, Vienna, Austria*

Cellular senescence is a state of pro-secretory cell cycle arrest that plays crucial roles in development, aging, and tissue regeneration. However, it is unclear what are markers, properties and role of senescent keratinocytes and fibroblasts in injuries. Our aim is to elucidate the properties of senescent cells in wounds and the effects of modulating these cells on the healing process.

In this presentation we will share unpublished results on the time course of senescent cells in acute mechanical wounds of pigs and mice. Using high-resolution molecular histology and RNA-*in situ* hybridization, we were able to determine spatial positioning of senescent cells in wounds and identify the secretory factors related with wounds-associated senescence. Moreover, our research elucidated how induction of cellular senescence of skin cells such as keratinocytes and fibroblasts *in vivo* changes their properties for better execution of the healing-related processes. Finally, we will present the results of pharmacological *in vivo* inhibition of these cells through the application of the p21 inhibitor (UC2288), among others, which has an impact on both wound properties and healing rate. Overall, our findings establish a signature of cellular senescence in wounds and shed light on the role of senescent cells in the healing process.

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**VALENZUELA**

**Long-term Outcomes after Amputation with Sentinel Node Biopsy in a**

**Single-Institutional Subungual Melanoma Series**

Cristian D. Valenzuela, MD, Graham Fowler, BA, Kaiya Kozuma, BS, Sonny Kusaka, BS, John T. Vetto, MD, FACS

**Background**

Subungual melanomas (SMs), represent less than three percent of cutaneous melanomas. Thus, little data exists on optimal treatment and long-term outcomes. We present our longitudinal institutional series of SMs treated operatively with digital amputation and sentinel node biopsy.

**Methods**

We performed a retrospective review of our prospectively-maintained melanoma database at Oregon Health & Science University, including SM patients treated with digital amputation and sentinel lymph node biopsy between January 2020 and January 2022. Median follow-up was 8.9 years. Primary endpoints were overall survival (OS) and recurrence free survival (RFS). Kaplan-Meier analyses with log-rank testing was performed.

**Results**

Of 25 patients with SM, 56% were female; 36% were toe and 64% were finger. Median age was 60.3 years and mean Breslow thickness was 3.4mm. Mean OS and RFS were 14.4 and 16.8 years respectively; medians were not reached. RFS was significantly longer for patients older than 60 versus younger patients (p=0.044). Sentinel node positivity rate was 8%. Amputations at the distal-most joint (n=20) had significantly better mean OS compared to more extensive proximal amputations (n=5): 16.8  vs 5.2 years (p<0.002), and significantly better mean RFS: 18.1 vs 6.2 years, respectively (p=0.029), despite comparable Breslow thicknesses between these groups (3.6mm vs 2.3mm, p=0.14).

**Conclusions**

SMs were well-treated with distal amputations, and had lower rates of recurrence in older patients. SM can be treated in the same fashion as cutaneous melanoma with good outcomes.

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**VIGNALI**

**Hypoxia within melanoma tumors promotes an unappreciated suppressor function in exhausted T cells and limits antitumor immunity**

Vignali PDA1,2, DePeaux KD1,2,#, Watson MJ1,2,#, Lontos K3, McGaa NK2, Scharping NE1,2, Menk AV2, Delgoffe GM1,2

*1Department of Immunology, University of Pittsburgh, Pittsburgh, PA 15213 USA*

*2Tumor Microenvironment Center, UPMC Hillman Cancer Center, Pittsburgh, PA 15232 USA*

*3Division of Hematology/Oncology, UPMC, Pittsburgh, PA, 15213 USA.*

*#These authors contributed equally*

CD8+ cytotoxic T cells (CTL) are crucial for identification and elimination of malignant cells. However, we now appreciate that features of the tumor microenvironment promote the differentiation of CTL to a poorly functional, short-lived state termed *exhaustion*. T cell exhaustion results in progressive loss of proinflammatory immune functions, and enrichment of so-called terminally exhausted T (tTex) cells within melanoma tumors correlates with poor therapeutic response following checkpoint blockade (*e.g.*, anti-PD-1) and worse clinical outcomes. We propose that the low oxygen tensions within melanoma tumors–a key feature of aggressive disease–is a significant contributor to T cell exhaustion and in fact, also promotes acquisition of a previously unappreciated immune regulatory function within CD8+ tTex cells. Suppression by tTex cells is mediated by the ectonucleotidase, CD39, upregulated in response to hypoxia exposure. Thus, both selective deletion of CD39 in CD8+ T cells and genetic or pharmacologic mitigation of tumor hypoxia limits the suppressive capacity of tTex cells and improves response to checkpoint blockade therapy in preclinical models. These findings suggest tTex cells are not solely dysfunctional but are indeed *directly* deleterious to antitumor immunity. Reprogramming or depletion of this population may be required to improve patient response to immunotherapy.

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**WARSHAUER**

**Secret Sephardic Origins Revealed for Rare Skin Disorder, Recessive Dystrophic Epidermolysis Bullosa, in Individuals Carrying the Unique c.6527insC Mutation**

Emily Mira Warshauer1, Paul A. Maier2, Goran Runfeldt2, Ignacia Fuentes3,4,5, María J. Escámez6, Laura Valinotto7,8, Mónica Natale7, Graciela Manzur9, Nuria Illera6, Marta García6, Marcela del Río6, Ángeles Mencia6, Almudena Holguín6,Fernando Larcher6,10, Garrett Hellenthal11  Adam Brown12, Liliana Consuegra13,Carolina Rivera13,14, Inês Nogueiro15, Jean Tang16, Anthony Oro16,17, Peter Marinkovich17, Francis Palisson4,5, Matthias Titeux18,19, Alain Hovnanian18,19,20, Eli Sprecher21,22, Karl Skorecki23,24, David Norris1,25,Anna Bruckner25, Igor Kogut1,25, Ganna Bilousova1,25, Dennis R. Roop1,25

1Charles C. Gates Center for Regenerative Medicine, University of Colorado School of Medicine, Anschutz Medical Campus, Aurora, CO2FamilyTreeDNA, Gene by Gene, Houston, Texas, USA 3Centro de Genética y Genomica, Facultad de Medicina Clínica Alemana, Universidad del Desarrollo, Santiago, Chile 4Fundacion DEBRA Chile, Santiago, Chile 5Departamento de Biología Celular y Molecular, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile 6Universidad Carlos III de Madrid, Departamento de Bioingeniería (UC3M), Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas (CIEMAT), Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER-ISCIII), Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD), Madrid, Spain 7Center for research in Genodermatosis and Epidermolysis Bullosa (*CEDIGEA*), Argentina 8National Scientific and Technical Research Council (*CONICET*), Argentina 9Head of the Dermatology Unit, Hospital de Clínicas, UBA, Buenos Aires, Argentina 10Epithelial Biomedicine Division, Centre for Energy, Environment and Technology Research (CIEMAT), Madrid, 11UCL Genetics Institute (UGI), Department of Genetics, Evolution and Environment, UCL, London, United Kingdom 12Avotaynu Foundation, Englewood, NJ 13Fundacion DEBRA Colombia, Bogotá, Colombia 14Department of Medical Genetics, Pediatric Hospital, Fundacion Cardioinfantil-Universidad del Rosario, Bogotá, Colombia 15Institute of Molecular Pathology and Immunology of the University of Porto Porto, Portugal ; Faculty of Sciences, University of Porto Porto, Portugal 16Department of Dermatology, Stanford University, Stanford, California 17Program in Epithelial Biology and Department of Dermatology, Stanford University School of Medicine, Stanford, California 18Imagine Institute, Paris Cité University, 75015 Paris, France 19Laboratory of Genetic Skin Diseases, INSERM U1163, 75015 Paris, France 20Department of Genetics, Necker Hospital for Sick Children, AP-HP, 75015 Paris, France 21Department of Dermatology, Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel 22Department of Human Molecular Genetics, Sackler Faculty of Medicine, Tel-Aviv University, Tel Aviv, Israel 23Department of Genetics & Developmental Biology, Rappaport Faculty of Medicine & Research Institute, Rambam Health Care Campus, Technion-Israel Institute of Technology, Haifa, Israel24Department of Nephrology, Rappaport Faculty of Medicine & Research Institute, Rambam Health Care Campus, Technion-Israel Institute of Technology, Haifa, Israel 25Department of Dermatology, University of Colorado School of Medicine, Anschutz Medical Campus, Aurora, CO

**Abstract**

Recessive Dystrophic Epidermolysis Bullosa (RDEB) is a rare and severe blistering skin disorder caused by mutations in the type VII collagen gene (*COL7A1*). The *COL7A1* c.6527insC mutation is curiously prevalent amongst RDEB individuals. Previous research suggested the possibility of a Sephardic Jewish origin of the mutation, however RDEB individuals are not known to have predominant Jewish ancestry. In this study, a global cohort of 126 RDEB individuals with the c.6527insC founder mutation from Spain, France, Argentina, Chile, Colombia, and the USA were investigated. Sephardic ancestry was identified at the haplotype spanning the c.6527insC mutation in 85% of the individuals, despite mixed ancestry elsewhere in the genome and no known recent Sephardic ancestry. An age estimation analysis of the c.6527insC mutation was performed and identity-by-descent matching between this RDEB subpopulation and a known crypto-Jewish community in Belmonte, Portugal was ascertained, providing support for crypto-Jewish ancestry in this unique RDEB subpopulation.

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**WEINBERG**

**ΔNp63α activates NFκB-c-Rel and modulates the tumor microenvironment in a murine model of v-RasHA-initiated squamous cancer pathogenesis**

Nozomi Sakakibara1, Paul E. Clavijo2, Kathryn King1, Veronica Gray1, Andrea George1, Roshini M. Ponnamperuma1, Zhong Chen2, Carter Van Waes2, Clint Allen2 and Wendy C. Weinberg1

*1Center for Drug Evaluation and Research/FDA; 2National Institute on Deafness and Other Communication Disorders/NIH*

Overexpression of the p63 isoform, ΔNp63α, is a distinguishing feature of human squamous cell carcinomas across organ sites. In studies to elucidate the contribution of this feature, we previously established that elevated levels of lentiviral-driven ΔNp63α in primary wildtype murine keratinocytes lead to nuclear accumulation of activated NFκB/c-Rel, override normal calcium-induced growth arrest, and drive 100% malignant conversion of v-rasHA-initiated tumors in an orthograft model.  C-Rel nuclear accumulation is mediated by Syk kinase and is required for ΔNp63α/v-rasHA-mediated carcinogenesis. Given the established role of NF-κB in inflammation, we characterized the immune infiltrates in v-rasHA-initiated papillomas relative to ΔNp63α/v-rasHA carcinomas in orthografts from immune competent hosts. Polymorphonuclear (PMN) myeloid cells, experimentally validated to be immunosuppressive and therefore representing myeloid-derived suppressor cells (PMN-MDSCs), were recruited at significantly higher levels into the tumor microenvironment of ΔNp63α/v-rasHA carcinomas two weeks post-grafting. ΔNp63α/v-rasHA-driven carcinomas expressed higher levels of chemokines implicated in recruitment of MDSCs compared to v-rasHA-initiated tumors. In contrast, in vitro the chemokine profile of keratinocyte cultures appeared to be largely mediated via v-rasHA. Our data suggest that ΔNp63α in cooperation with v-rasHA promotes an immunosuppressive tumor microenvironment through production of chemokines and recruitment of PMN-MDSCs.

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**WONDRAK**

**Melanotransferrin (MELTF) as a determinant of melanomagenesis: *MELTF* expression attenuates melanoma progression in A375-luc murine disease models and human patients**

Jana Jandova and Georg T. Wondrak

*Department of Pharmacology and Toxicology, R.K. Coit College of Pharmacy & UA Cancer Center, University of Arizona, Tucson, AZ, USA*

The cell-surface glycoprotein melanotransferrin (MELTF) is a member of the iron-binding transferrin superfamily expressed in human melanocytes and other cell types. Dysregulated MELTF expression in malignant melanoma has been reported before but its specific role in tumorigenesis has remained elusive. Following our interest in the role of the transferrin receptor (TFRC) in melanomagenesis, our initial TCGA-analysis suggested that increased *MELTF* expression is a positive predictor of melanoma patient survival. In order to inform the ongoing debate on the mechanistic role of MELTF in melanoma we then performed CRISPR/Cas9-based *MELTF* deletion in A375-luc2 human malignant melanoma cells. ICP/MS-analysis indicated that there is no difference in iron-content between *MELTF*\_WT and *MELTF*\_KO cells. In  contrast, phenotypical screening revealed the significant enhancement of proliferative capacity and matrigel invasiveness of *MELTF*\_KO cells. Accordingly, *MELTF*\_KO- outperformed *MELTF*\_WT-xenograft tumor growth in SCID mice, and, likewise, intracardial injection followed by bioluminiscent detection of metastases revealed a decreased survival of the *MELTF*\_KO group. NanoString nCounterTM ('PanCancer-Progression-Panel') gene expression profiling confirmed upregulation of EMT- and proliferation-related pathways in MELTF\_KO cells. These data support a heretofore unrecognized  tumor-suppressive function of *MELTF* expression in melanoma, a mechanistic role consistent with TCGA-based data indicating a survival benefit associated with high *MELTF* expression.

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**WONDRAK**

**Topical hypochlorous acid (HOCl) suppresses solar UV-driven inflammatory gene expression and skin carcinogenesis**

Jeremy A. Snell1,2, Anh B. Hua2, Prajakta Vaishampayan2,3, Jana Jandova1,2, Sally E. Dickinson2,3, Georg T. Wondrak1,2

*1Department of Pharmacology and Toxicology, R.K. Coit College of Pharmacy, University of Arizona, Tucson, AZ, USA*

*2UA Cancer Center, University of Arizona, Tucson, AZ, USA*

*3Department of Medical Pharmacology, University of Arizona, Tucson, AZ, USA*

Hypochlorous acid (HOCl), an innate immune factor and environmental toxicant, is an important component of the skin exposome. HOCl, a weak halogen-based acid and powerful oxidant, serves as a chemical disinfectant used in freshwater processing on a global scale, both in the context of drinking water safety and recreational freshwater use. Our recent studies have explored the interaction between UV photons and HOCl-related environmental co-exposures as relevant to human skin in a ‘swimming pool-exposure’ environment using reconstructed human epidermis and SKH-1 mouse models. The anti-inflammatory activity of topical HOCl was established *in vivo* and attributed mechanistically to blockade of AP-1-driven inflammatory signaling as assessed by bioluminescent imaging of luciferase reporter SKH-1 mice exposed to solar UV with and without topical HOCl. Moreover, HOCl exposure blocked tumorigenic inflammatory progression in UV-induced high-risk (tumor-prone) SKH-1 mouse skin, a finding substantiated by NanoString nCounterTM transcriptomic analysis (‘Mouse Inflammation V2’ panel) that confirmed downregulation of inflammation-related responses. In addition, a novel stratum corneum-specific chloramine-adduct, formed as a consequence of topical HOCl application was identified by LC-MS after tape stripping of human *ex vivo* skin exposed to environmentally relevant chlorination stress, serving as an investigational biomarker of topical cutaneous chlorination stress.

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**ZHANG**

**The mechanistic role TOPK in solar UV-induced skin damage and carcinogenesis**

Asad U. Khan, Qiushi Wang, Eunmiri Roh, Ann M. Bode, Tianshun Zhang

Nonmelanoma skin cancer (NMSC) primarily arises due to exposure to solar ultraviolet (UV) radiation and is one of the most common forms of cancer in the USA. T-LAK cell-originated protein kinase (TOPK), a serine-threonine kinase, is activated by solar UV irradiation and is implicated in skin carcinogenesis. Studies have shown that deletion of TOPK efficiently blocks solar UV-induced carcinogenesis in mouse models. Furthermore, the use of TOPK inhibitors, e.g., HI-032 or ADA-07, effectively reduces the incidence and volume of tumors in a chronic solar UV-induced mouse model. However, the precise mechanisms underlying TOPK's involvement in NMSC development have yet to be fully elucidated. In this study, we focused on determining the mechanistic significance of TOPK in solar UV-induced skin damage and carcinogenesis. Using RNA sequencing (RNAseq) in TOPK knockout (KO) mice compared to wild-type (WT) mice exposed to solar UV, we observed significant alterations in gene expression profiles, highlighting potential downstream targets and pathways influenced by TOPK. Collectively, our findings underscore the crucial role of TOPK in NMSC progression and suggest its potential as a promising therapeutic target. Further mechanistic studies are warranted to uncover the underlying mechanisms by which TOPK contributes to solar UV-induced skin damage and carcinogenesis.