Annual Symposium

Thursday, March 3, 2022
Michigan Medicine
Ann Arbor, MI

Event Program

Our annual symposium is sponsored by:

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MEDICAL SCHOOL UNIVERSITY OF MICHIGAN

UNIVERSITY OF MICHIGAN DEPARTMENT OF DERMATOLOGY

3rd Annual University of Michigan Skin Biology & Diseases Resourced-Based Center Symposium
Zoom & in Danto Auditorium at the Frankel Cardiovascular Center, University of Michigan
Thursday March 3, 2022
8:00 am – 12 noon
Agenda

8:00 - 8:20 am **Welcome & UM-SBDRC Overview**

Research Updates: UM-SBDRC PILOT AWARDS

8:20 – 8:30 am. Mrinal Sarkar, Ph.D.
"*Role of each member of the IL-36 cytokines in keratinocyte immune responses*"

8:30 – 8:40 am. Carole Parent, Ph.D. & Pierre Coulombe, Ph.D.
"*Live imaging of neutrophil infiltration in stressed skin in real time in vivo*"

8:40 - 8:50 am. Gary Fisher, Ph.D. & Taihao Quan, M.D., Ph.D.
"*The Role YAP/TAZ in Dermal Extracellular Matrix Homeostatus and Aging*"

8:50 - 9:00 am. Gargi Ghosh, Ph.D.
"Multi-functional hybrid hydrogel for wound healing"

9:00 - 9:10 am. Olesya Plazyo, Ph.D. & Allison Billi, M.D., Ph.D. (Mentor)
"VGLL3-driven mechanism of sexual dimorphism in immune responses"

9:10 - 9:20 am James Elder, M.D., Ph.D.
"Single-Cell RNA-Seq analysis of the effect of NETs in TH17 polarization"

Research Updates: UM-SBDRC INNOVATION AWARDS

9:20 - 9:25 am Zhaolin Zhang, Ph.D. (Post-Doc) & James T. Elder, M.D., Ph.D. (Mentor)
"Increased expression of AP-1 transcription factors and increased chromatin accessibility of their binding sites after CD3/CD28 activation of human memory T-cells"

9:25 - 9:30 am | Matthew Patrick, Ph.D. & Lam C. Tsoi, Ph.D.
"Nascent RNA profiling to understand early inflammatory response in keratinocytes"

Present & Future Research

9:30 - 9:35 am | Sunny Wong, Ph.D.
"A new mouse model of basal cell carcinoma to investigate novel therapies."

9:35 - 9:45 am | Grace Hile, M.D.

"Hippo signaling in in keratinocytes"

9:45 - 10:45 am **Break & Poster Viewing (on Zoom)**

10:45 - 11:45 am **Keynote Address**

Robert L. Modlin, M.D., Klein Professor of Dermatology in the Division of Dermatology, Professor of Microbiology, Immunology and Molecular Genetics, at the David Geffen School of Medicine at the University of California, Los Angeles

"Acne: a disease of lipid metabolism"

11:45 - 11:55 am Alex Tsoi, PhD and Rachael Wasikowski
"UM-SBDRC Genomic Database Demonstration"

11:55 - 12 noon **Closing Remarks & Poster Awards**

Poster #1

Interferon alpha promotes ultraviolet light-mediated keratinocyte apoptosis in a caspase-8 dependent manner

Shannon N. Estadt, Mehrnaz Gharaee-Kermani, Bin Xu, Tyson M. Moore, Andrew Hannoudi, J. Michelle Kahlenberg

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by sensitivity to ultraviolet radiation (UVR). UVR triggers cutaneous and systemic disease flares, yet mechanisms driving this are not fully characterized. Type I interferons (IFNs) are highly expressed in non-lesional SLE skin and promote increased death of SLE keratinocytes (KCs) after UVR, but the manner in which they do so is currently unknown. This study explores activation and regulation of cell death pathways in KCs exposed to type I IFNs and UVR. We treated immortalized human KCs (N/TERTs) overnight with IFN-alpha (IFN-a) prior to a 50 mJ/cm^2 UVB exposure. Four hours post UVB, IFN-a priming significantly increased the percentage of cleaved-caspase-3$^+$ (CC3$^+$) and Annexin V$^+$propidium iodide$^-$ (AV$^+$PI$^-$) cells compared to treatment with UVB alone. Inhibiting RIPK1, RIPK3, or caspase-1 had no effect on death suggesting this enhanced death does not involve necroptosis or pyroptosis. Use of a pan-caspase inhibitor or a caspase-8 inhibitor significantly reduced cell death following IFN-a and UVB treatment while caspase-9 inhibition did not, suggesting enhanced activation of the extrinsic apoptosis pathway. RNA-sequencing identified pro-apoptotic genes *XAF1*, *TNFSF10* (encodes TRAIL), and *IRF1* as highly upregulated by IFN-a treatment, and more so in SLE compared to healthy control KCs. Intriguingly, neither knockdown of *XAF1* nor neutralization of TRAIL blocked the IFN-mediated increase in UVB-induced apoptosis. Future studies will determine if IFN-induced upregulation of IRF-1 is responsible for UVB-enhanced keratinocyte apoptosis. Together, these data suggest that photosensitive responses exhibited by SLE patients may be due to type I IFN priming of KCs that sensitizes cells to undergo increased extrinsic apoptosis after minimal exposures to UVB. Continued investigation into mechanisms by which this occurs will provide important prophylactic options to prevent SLE flares.

Poster #2

Overexpression of Vgll3 Induces Cutaneous Fibrosis in a Mouse Model of Lupus-Like Autoimmunity Using Single-Cell RNA-seq Analyses

Mehrnaz Gharaee-Kermani[1,2], Allison C. Billi[1], Marisa C. Hildebrandt[1], Jacob Martens[2,3,] Rachael Wasikowski[1], Alex Tsoi[1], J. Michelle Kahlenberg[1,2], Johann E. Gudjonsson[1].

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Fibrosis is characterized by collagen deposition, fibro/myofibroblast accumulation, and extracellular matrix remodeling. Fibrosis can also be seen in autoimmune diseases, where it may be widespread and affect organs beyond the skin, with high morbidity and mortality, and no effective treatment. In cutaneous lupus, scar formation after discoid lesion eruption may evolve from enhanced fibrotic phenotypes. Our research has shown that epidermal-directed overexpression of murine *Vgll3* causes severe lupus-like skin lesions suggestive of discoid lupus erythematosus (DLE). Given the apparent fibrotic nature of the skin lesions in transgenic (TG) *Vgll3* mice, we wanted to determine whether *Vgll3* induces fibrosis. We analyzed male and female TG and wild-type (WT) mice aged 2-3 months. Fibrotic biomarkers of human DLE and scleroderma were analyzed. Epidermal *Vgll3* overexpression resulted in development of not only cutaneous inflammation, but also severe fibrosis characterized by significant expression of fibrotic biomarkers found in human DLE and scleroderma lesions. Overall, lesional *Vgll3* TG skin exhibited higher expression of *Col1a1, Col1a2, Tgfb1 and Ctgf.* ScRNA-seq of *Vgll3* TG lesional skin vs. WT skin demonstrated that the increased expression of these collagen genes was localized to fibroblast (FB) and myofibroblast populations. Four FB subclusters were identified across all samples, with one detected almost exclusively in *Vgll3* TG skin. This subcluster was distinguished by higher expression of *Col1a1, Col1a2, Tgfb1, Ly6c1 and Eln*. The presence of this unique FB subcluster in the skin of *Vgll3* TG mice suggests that epidermal overexpression of *Vgll3* impacts fibrosis development, and there may be a role for this unique FB subcluster in early fibrosis.

Poster #3

Lupus Fibroblasts exhibit exaggerated responses to inflammatory cytokines and upregulate pro-fibrotic collagens in patients who scar

Suzanne Shoffner-Beck*[1], Stephanie Lazar[2] , Lisa Abernathy-Close[2], Amy Hurst[2], Craig Dobry[3], Rachael Wasikowski[3], Kelly Arnold[1], Johann Gudjonsson[3], Lam C. Tsoi[3,4], J. Michelle Kahlenberg[2]
[1] Graduate Program in Biomedical Engineering, Department of Internal Medicine, Division of Rheumatology, [3] Department of Dermatology, [4] Department of Computational Medicine

Introduction

Cutaneous lupus erythematosus (CLE) is a dermatologic manifestation of systemic lupus erythematosus (SLE) that can cause significant patient distress and disfiguration secondary to scar. Scarring mechanisms remain poorly understood and interventions for preventing or treating scarring are lacking in SLE/CLE. Fibroblasts are connective tissue cells that are involved in the regulation of immune responses, inflammation, and scarring. However, the role of inflammatory mediators on fibroblast function in CLE is unclear. In this study, we examined the inflammatory phenotype in fibroblasts isolated from non-lesional skin from healthy controls (HC), and patients with and without scarring CLE, to explore differences in gene expression that occur indisease,and those that are associated with scarring. The goal is to further elucidate potential pathways and mechanisms involved in scarring that could be potential targets for therapy.

Methods

Study design: In this study, punch biopsies were obtained from University of Michigan patients enrolled in the Taubman Institute Innovative Program PerMIPA cohort with SLE (n=22), divided into patients with scarring skin lesions (n=8) and non-scarring skin lesions (n=13), as well as healthy controls (n=34). Scarring disease status was determined using the damage score of the Cutaneous Lupus Erythematosus Disease Area and Severity Index (CLASI).[1] Fibroblasts were isolated from punch biopsies, cultured, and stimulated with cytokines, including IFN-γ, IFN-α, TNF-α, TGF-**β**, IL-1**β**, and ultraviolet light.

Gene Expression Analysis: RNA sequencing was performed on the unstimulated and stimulated fibroblasts. Differentially expressed genes were determined using DEseq2 with fold change of stimulated versus unstimulated >

0.6 and Benjamini–Hochberg adjusted p-value with FDR < 0.05. Pathway analysis was performed by filtering the genes with the largest effect size (log_2FC_{CLE} - log_2FC_{HC}) to identify pathways shared across all stimulations and unique to individual stimulations. All analyses were performed in R.[2]

Results and Discussion

We found differential gene expression between healthy controls and SLE patients with the largest

effect size differences for TGF-β, TNF-α, IFN-γ, and IFN-α stimulations. Cytokine-cytokine receptor signaling pathway genes were upregulated across all conditions, with relatively more up-regulation in SLE in comparison to healthy controls. Genes differentially expressed included genes in the chemokine (C-X-C motif ligand) CXCL family, chemokine (C-C motif) ligand (CCL) family, interleukins, and tumor necrosis factor (TNF). These results indicate that while exposure to inflammatory cytokines is able to successfully up-regulates inflammatory pathways in both healthy and SLE fibroblasts, the effect is magnified in SLE.

Pathway analysis revealed that genes in the interleukin-10 (IL-10) pathway were significantly differentially expressed between the scarring and non-scarring states, especially specific to stimulation with TGF-β, a known fibrotic factor involved in myofibroblast activation. Interestingly, non-scarring patients have stronger upregulation of proinflammatory signatures, including genes in the chemokine (C-X-C motif ligand) CXCL family, chemokine (C-C motif) ligand (CCL) family, and interleukins. We also found that genes in the collagen trimer pathway were significantly differentially expressed between the scarring and non-scarring states. In particular, COL17A1 was up-regulated in the scarring state, especially after inflammatory signals like TGF-β, TNF-α, and IL-1β, while COLQ, COL21A1, and COL4A3 were down-regulated in the non-scarring states across most inflammatory stimulations. This suggests that while all SLE fibroblasts are associated with elevated inflammation, pathological scarring involves upregulation of collagen pathways. Together, these findings provide important insight into the mechanisms of scarring in CLE and the involved pathways with potential targets for intervention.

References

1. Albrecht J, Taylor L, Berlin JA, et al. The CLASI (Cutaneous Lupus Erythematosus Disease Area and Severity Index): an outcome instrument for cutaneous lupus erythematosus. *J Invest Dermatol*. 2005;125(5):889-894. doi:10.1111/j.0022-202X.2005.23889.x
2. R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Poster #4

Biologic Differences Between Preterm and Term Preeclampsia Revealed By Transcriptomic Analyses

Olesya Plazyo[1], Ashley Hesson[2], Elizabeth Langen[2], Joseph Kirma[1], Santhi Ganesh[2], Johann Gudjonsson[1]

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Introduction

Preeclampsia (PE) is an incompletely understood vascular endothelial disorder characterized by gestational-induced hypertension that affects 1 in 25 pregnancies in the United States and can lead to serious perinatal complications and death as well as impose life-long health risks. Although immune dysregulation at the maternal-fetal interface and inadequate remodeling of spiral arteries are known to contribute to PE, better understanding of the molecular mechanisms in PE pathogenesis is urgently needed, as highlighted by the current lack in FDA-approved therapies for this disease.

Methods

To investigate the PE-specific transcriptomes, we performed RNA-sequencing on formalin-fixed paraffin-embedded placenta samples obtained from PE patients (n=43) and gestational age-matched normal controls (n=41) using the Illumina NovaSeq S4-150 platform.

Results

The comparison revealed 259 up- and 90 down-regulated genes with statistical significance of $p \leq 0.05$, including those that have been previously linked to PE (*IGFBP1*, *PRL*, *DKK1*, *MAP3K7CL*) as well as those that were less known (*DDX20*, *OPTN*, *ZNF716*, *VWA8*). PANTHER analysis of molecular functions showed enrichment in MHC class II receptor activity, SMAD binding, TGF-β receptor binding, and Wnt-protein binding. Ingenuity Pathway Analysis revealed NFAT5, α-Catenin, and PTCH1 among upstream regulators. When comparing differentially expressed genes (DEGs) in preterm PE (n=20 patients) to those in term PE (n=23 patients), enriched biological processes included cellular response to IFN-γ, cytochrome complex assembly, and antigen processing and presentation via MHC class II in preterm PE. Enriched processes in term PE included metabolic processes, cellular component biogenesis, and extracellular matrix organization. Upstream regulators included KLF7, PDIA4, and LMTK3 in preterm PE and HOXA10, BTG2, and KITLG in term PE.

Conclusion

Collectively, these data provide transcriptome characterization of placenta alterations in term and preterm PE and serve as a gateway for further interrogation of signaling in PE pathogenesis. Currently other transcriptomic approached including single-cell RNA-Seq and spatial Seq are being employed by our group to further characterize the expression signatures identified by these data.

Poster #5

Investigating OX40L and its role in mediating cutaneous and systemic autoimmune disease

Enze Xing, Mentors: Johann Gudjonsson, Allison Billi

Systemic lupus erythematosus (SLE) is a devastating systemic inflammatory disease, with prominent female bias. Recent literature suggests the OX40/OX40L costimulatory pathway may play an important role in development of SLE. GWAS have identified *TNFSF4 (OX40L)* as an SLE susceptibility locus. OX40L stimulation of T cells through the OX40 receptor has been shown to promote a T follicular helper (Tfh) phenotype, and *Ox40l* knockout has been demonstrated to ameliorate the SLE phenotype in transgenic and induced lupus mouse models.

We recently reported a mouse model (*K5-Vgll3*) where epidermal overexpression of the female-biased transcription cofactor *Vgll3* results in upregulation of *Ox40l* in epidermis as well as development of SLE. To address the role of OX40L in this model, we demonstrate that *Ox40l KO* results in decreased disease severity, with later onset of inflammatory skin lesions, and decreased systemic manifestations including splenomegaly and lymphadenopathy, consistent with this pathway being important for propagating VGLL3-mediated inflammation. To investigate the link between VGLL3 and OX40L expression in SLE skin, we created an N/TERT VGLL3-Myc-DDK overexpression cell system. IP-mass spectrometry of VGLL3 complexes identified transcription factor TEAD1, previously reported to bind upstream of *OX40L*, as a top VGLL3 interactor. Assessing OX40L and OX40 expression via immunofluorescence and single-cell RNA-seq (scRNA-seq), we found increased expression of OX40L on KCs and OX40 on CD4+ T cells in both *K5-Vgll3* and SLE skin compared to controls. ScRNA-seq also showed that OX40+ T cells in *K5-Vgll3* and lupus skin were skewed towards a Tfh/T peripheral helper (Tph) phenotype. Similarly, spatial-seq identified a population of Tfh/Tph-like population in SLE skin not present in healthy controls.

Overall, our data indicates cutaneous VGLL3 controls transcriptional regulation of *OX40L* through TEAD1, and OX40L expression is required to mediate downstream SLE-like inflammation, possibly through promoting Tfh/Tph-like cells in SLE compared to healthy skin.

Poster #6

Keratinocytes sense and eliminate CRISPR DNA through STING induced expression of IFN-κ and induction of the cytidine deaminase APOBEC3G

Mrinal K. Sarkar, Ranjitha Uppala, Chang Zeng, Allison C. Billi, Lam C. Tsoi, Austin Kidder, Xianying Xing, Bethany E. Perez White, Shuai Shao, Olesya Plazyo, Sirisha Sirobhushanam, Enze Xing, Yanyun Jiang, Katherine A. Gallagher, John J. Voorhees, J. Michelle Kahlenberg, Johann E. Gudjonsson.

CRISPR-Cas9 has been proposed as a treatment for genetically inherited skin disorders. Here we report that CRISPR transfection activates STING-dependent antiviral responses in keratinocytes, resulting in heightened endogenous interferon (IFN) responses through induction of IFN-κ leading to decreased plasmid stability secondary to induction of the cytidine deaminase *APOBEC3G*. Notably, CRISPR-generated KO keratinocytes had permanent suppression of IFN-κ and IFN-stimulated gene (ISG) expression, secondary to hypermethylation of the *IFNK* promoter region by the DNA methyltransferase DNMT3B. JAK inhibition via baricitinib prior to CRISPR transfection increased transfection efficiency, prevented *IFNK* promoter hypermethylation and restored normal IFN-κ activity and ISG responses. This work shows that CRISPR-mediated gene correction alters antiviral responses in keratinocytes and has implications for future gene therapies of inherited skin diseases using CRISPR technology and suggests pharmacologic JAK inhibition as a tool for facilitating and attenuating inadvertent selection effects in CRISPR-Cas9 therapeutic approaches.

Poster #7

Functional Analysis of lncRNAs within Psoriasis using Single Cell RNAseq

Rachael Wasikowski, Matthew T Patrick, Sutharzan Sreeskandarajan, Haihan Zhang, Qinmengge Li, James T. Elder, Allison C. Billi, Johann E. Gudjonsson, Lam C Tsoi

Long-noncoding RNAs (lncRNAs) have been shown to have regulatory functions in skin, and studies based on bulk RNA-seq data have demonstrated that lncRNA can have disease specificity and cell-type specificity. Psoriasis, a chronic inflammatory disease, is enriched in pro-inflammatory genes as well as numerous lncRNAs of unknown functionality. We studied lncRNA expression profiles in scRNA-seq of lesional (PP)skin and non-lesional (PN) skin samples from 6 psoriasis patients as well as 5 control samples. Differential gene expression analysis yielded cell-type specific up-regulated lncRNA, mainly in keratinocytes, which constitute >50% of all lncRNA expressing cells. We performed an *in silico* analysis to determine DNA binding affinity for 121 differentially expressed lncRNAs in 12 cell types from (FDR<=5%), to understand their potential functional roles of lncRNA. This analysis identified 5,570 potential protein-coding gene targets. For example, LncRNAs *MALAT1* (FC=1.42 and FDR<$1x10^{-16}$) and *NEAT1* (FC=2.88 and FDR<$1x10^{-16}$) were significantly up-regulated within PP keratinocytes, and their predicted downstream targets (only correlated with *MALAT1* and *NEAT1* in PP) include *PPP1R15A, ZFP36*, and *SFN,* which control cell cycle progression in the epidermis. Interestingly, *NEAT1* promotes activation of the inflammasome as well as influencing innate immunity, whereas *MALAT1* has been shown to be involved within the pathology of diseases by mediating alternative splicing, autophagy, and nuclear organization. In addition, *MALAT1* and *NEAT1* were negatively correlated with *DMKN* (pro-inflammatory), and *FABP5* (proliferation inducing) (R<= 0.4, p<2.2e-16), suggesting a regulatory role to control hyperproliferation. These findings allow us to contextualize the role of lncRNAs within psoriasis and infer functionality within a gene regulatory network.

Poster #8

Functions of Long non-coding RNAs Predicted from Large-Scale Transcriptome Data

Matthew T Patrick, Sutharzan Sreeskandarajan, Alanna Shefler, Rachael Wasikowski, Mrinal K Sarkar, Jiahan Chen, Errol Prens, Alain Hovnanian, Stephan Weidinger, James T Elder, Chao-Chung Kuo, Johann E Gudjonsson, Lam C Tsoi

Long non-coding RNAs (lncRNAs) are defined as being over 200 nucleotides in length and have been found to regulate gene expression and splicing. Our previous research showed lncRNAs are differentially expressed in psoriasis and atopic dermatitis lesional skin, however their biological roles are often unclear due to lower expression levels than protein-coding genes. To help elucidate the functions of lncRNAs in skin, we combined GENCODE v29 with Broad' s Human Body Map and our own novel skin-expressing transcripts, to increase the number of cataloged lncRNAs by 31%, then measured their expression levels in over 800 RNA-seq skin samples from psoriasis, atopic dermatitis, hidradenitis suppurativa, Netherton syndrome and keratinocytes under cytokine stimulations. Using a random forest classifier in 10-fold cross validation, and averaging predictions over 100 trials, we identified 918 lncRNAs as belonging to cytokine IFNa, IFNg, IL17, IL4/IL13 and TNFa signaling pathways. We then clustered lncRNAs according to their k-mer profiles and found one cluster to be enriched among lncRNAs predicted to be involved in TNFa and IL4/IL13 signaling, while another was more enriched among IL17 and IFNg signaling lncRNA. These findings can help explain how different lncRNAs can play roles in different skin diseases.

Poster #9

The Hippo pathway component WWC1 is a key regulator of apoptosis and photosensitivity in lupus keratinocytes

Grace A. Hile[1], Patrick Coit[2], Bin Xu[2], Amanda M. Victory[2], Shannon N. Estadt[3], Mitra P. Maz[3], Jacob W. S. Martens[3], Rachael Wasikowski[1,4], Craig Dobry[1], Lam C. Tsoi[1,4,5] Ramiro Iglesias-Bartolome[6], Celine C. Berthier[4,5], Allison C. Billi[1], Johann E. Gudjonsson[1], Amr H. Sawalha[7], J. Michelle Kahlenberg[1,2]*

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Background: Skin inflammation and photosensitivity are common manifestations of systemic lupus erythematosus (SLE), yet the pathogenesis is largely unexplained. Non-lesional skin of SLE patients exhibits a propensity for cell death and inflammation that persists in culture, supporting a role for epigenetics to sustain this phenotype.
Methods: We analyzed genome-wide DNA methylation changes in primary SLE vs. control (HC) keratinocytes (KC). We measured protein expression using Western blotting and phosphorylation of YAP *in vivo* by immunofluorescence microscopy of non-lesional frozen biopsies. Hippo target gene expression in non-lesional biopsies was measured via single cell RNA-seq. We used an immortalized cell line with inducible protein (TEADi) that blocks YAP-TEAD signaling and a LATS1/2 kinase inhibitor in primary healthy control and SLE KCs to study effects on UVB-mediated apoptosis using active caspase 3/7 staining.
Results: Hippo signaling is the top differentially methylated pathway in SLE KCs. The key Hippo regulator WWC1 is significantly hypomethylated ($\Delta\beta$ =-0.17, P= 4.36 X 10^{-9}) and is overexpressed in non-lesional KC (p= 0.0059). Increased Hippo signaling was noted by increased ratio of phosphoYAP to YAP in non-lesional epidermis of SLE (p=0.0278). Induction of TEADi resulted in robust increase in UVB-mediated apoptosis. Inhibiting phosphorylation of YAP via TRULI abrogated the enhanced apoptotic response to UVB in SLE KCs.
Conclusion: Our work describes a novel mechanistic paradigm in lupus KCs in which aberrant apoptosis to UVB is driven by dysregulation of the Hippo pathway via promotion of YAP phosphorylation and

restriction of coactivation of TEAD transcriptional activity. Thus, modulation of the Hippo pathway could serve as a novel target for photosensitivity in SLE and CLE.

Poster #10

HERC6 negatively regulates type I interferon activity in keratinocytes through modulation of STING-IRF3 signaling

Ranjitha Uppala[1,2], Mrinal K. Sarkar[2], William R. Swindell[3], Lam C. Tsoi[4], J. Michelle Kahlenberg[5], Allison C. Billi[2], Johann E. Gudjonsson[2]

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Abstract

Canonical STING-DNA sensing pathway activation leads to type I interferon (IFN) production in keratinocytes (KCs). Aberrant STING and IFN signaling is a hallmark of lupus KCs, where amplified type I IFN responses by epidermal KCs lead to increased expression of IFN stimulated genes (ISGs) like *MX1* and *OASL*. While a pathogenic role for type I IFNs and STING signaling in autoimmune skin diseases is well established, the key regulators of IFN signaling and the crosstalk between type I IFN and STING signaling in KCs remain unidentified. To identify regulators of the IFN response, we analyzed *MX1*-correlated genes from 118 human primary KCs microarray dataset. This revealed a striking enrichment of ISGs associated with ubiquitination activity, with *HERC6* exhibiting the highest score (r=0.93). HERC6 is an E3 ubiquitin ligase, constitutively expressed by basal and differentiated KCs of the epidermis with an unidentified role in the skin. We found that human primary KCs stimulated with a type I IFN (IFNα) or STING agonist (cGAMP) have increased *HERC6* expression. KCs lacking HERC6 show enhanced induction of ISGs ($p<0.05$) upon treatment with IFNα or cGAMP but not RNA-sensing TLR3 agonist (Poly(IC)) suggesting that HERC6 is a negative regulator of ISG expression and specific to dsDNA but not exogenous RNA sensing. *HERC6* KO KCs exhibited increased STING activation and STING-IRF3 signaling, resulting in feedback amplification of ISG expression upon cGAMP stimulation. Collectively, these data suggest a role for HERC6 in response to cytosolic DNA and activation of downstream type I IFN responses.

Poster #11

Prediction of changes in AP-1 family transcription factor binding during CD3/CD28 activation of human memory T-cells: Relevance to psoriasis

Z Zhang[1], Y Zhao[1], LC Tsoi[1], PE Stuart[1], X Wen[1], X Chen[2], JB Harley[2,3], M Weirauch[2], RP Nair[1], JT Elder[1,4], [1]University of Michigan, [2]Cincinnati Children's Hospital Medical Center, [3]Cincinnati VA Hospital, [4]Ann Arbor VA Hospital

We generated 1,090 ATAC-seq and 1,057 T-cell RNA-seq libraries from 153 subjects, derived from 8 flow-sorted T-cell subsets (defined by CD4/CD8, CLA+/ CLA-, and 0/24h CD3/CD28 activation). Effects of activation and skin-homing were analyzed by DESeq2. We identified 2,795, 3,629, and 10,673 differentially expressed genes (DEGs; FDR < 0.05, |log2 FC|≥ 0.585) for CD8/CD4, CLA+/CLA-, and 0/24h, respectively. Activation-related DEGs were enriched for the KEGG term "Cytokine-cytokine receptor interaction" (FDR = 2.1e-04), with dramatic up- regulation of IL-17 pathway markers IL17A (109-fold), IL17F (1,052-fold), and IL22 (146-fold) at 24 h. After peak calling, 78,234 consensus peaks were present in ≥ 30 ATAC-seq libraries across all conditions. We used the same FDR and FC criteria to identify 9,072, 3,934, and 21,174 consensus peaks as differentially accessible regions (DAR) in CD8/CD4, CLA+/CLA-, and 0/24h, respectively. Volcano plots revealed a strong bias toward increased accessibility at 24h vs. 0 h (20,154 increased vs. 2,020 decreased). Combining the consensus peak sequences, JASPAR human transcription factor binding profiles, and cell specific ATAC-seq bam files, we defined 153 out of 1,202 candidate Jaspar motifs as active motifs using CENTIPEDE, and identified 28, 46, and 128 differentially bound motifs (DBM; Chisq_p < 0.05 and |log2FC| ≥ 0.585) for CD8/CD4, CLA+/CLA-, and 0/24h, respectively. Volcano plots of the 128 DBMs at 24 vs. 0h revealed the most strongly positive FC and the most highly significant FDRs for AP-1 TF members. AP-1 family TF binding sites identified from ChIP-seq datasets using the Regulatory Element Locus Intersection (RELI) algorithm were enriched across 65 psoriasis genetic signals.

These results demonstrate the strong up-regulation of IL-17 pathway genes during CD3/CD28-mediated T-cell activation, the prominence of AP-1 TF family member binding during the activation process, and the relevance of AP-1 TF binding sites to psoriasis via overlap with psoriasis-associated genetic signals.

Poster #12

Rates of Genetic Referral and Outcomes of Cancer Genetic Testing among Patients with Sebaceous Lesions

Authors: Meera Kattapuram BA, Erika Koeppe MPH, Kelly Cha MD PhD, and Tobias Else MD

Background: Sebaceous lesions are associated with Lynch syndrome (LS), an inherited syndrome associated with increased cancer risks. Immunohistochemistry (IHC) staining of these lesions can be used to screen patients for LS, and genetic evaluation is recommended in those with abnormal IHC, normal IHC with personal or family history of other LS-associated neoplasms, or multiple sebaceous neoplasms. Data regarding the frequency at which such lesions and corresponding IHC findings trigger appropriate genetic referral is limited. Here we describe the rates of genetic referral in a large cohort of patients with sebaceous lesions and the utility of IHC in this process.

Methods: Retrospective chart reviews were performed for patients with a pathology-confirmed diagnosis of a sebaceous lesion at Michigan Medicine between January 2009 - December 2019. Demographics, organ transplant status, SL subtype, anatomical location, IHC staining, microsatellite instability testing, recommendation for genetic evaluation, and genetic testing results were extracted from charts.

Results: 447 patients with a total of 473 baseline sebaceous lesions were identified. Excluding 20 patients with previously diagnosed LS, IHC analysis was conducted in 173 (41%) patients. 92 patients had abnormal results, of which only 69 (75%) patients were referred to genetics, although 7 of these patients had IHC as part of initial genetics visit. An additional 32 patients were referred in the absence of IHC testing (n=10) or normal IHC (n=22). Of 101 total patients referred, 65 were ultimately seen and 47 completed genetic testing. Pathogenic/likely pathogenic germline variants associated with LS were found in 7 (15%) patients, 6 of which had abnormal concordant IHC findings and 1 had no IHC performed.

Conclusion: Rates of referral for genetic testing for LS in patients with sebaceous lesions are low (101/427, 24%). Provider education on importance of genetics referral for patients with sebaceous lesions, especially those with abnormal IHC, is warranted.

Poster #13

Dermatology Referral Rates to Cancer Genetics for Birt-Hogg-Dubé Syndrome

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Introduction: Birt-Hogg-Dubé (BHD) syndrome is a genetic condition caused by pathogenic variants in the FLCN gene resulting in benign skin lesions, spontaneous pneumothorax, and increased risk for kidney tumors. As with other hereditary syndromes the rare skin manifestations can serve as an identification of index patients, which can upon positive genetic testing be subjected for life-long surveillance for renal cell cancer. However, it is currently unknown, how many patients with these rare skin manifestations actually get referred for genetic testing.

Methods: We evaluated patients who have had a pathological diagnosis of fibrofolliculoma/trichodiscoma up until October 2020 to evaluate if a genetic evaluation had been recommended at any level of their care (e.g. pathology report, dermatology clinic notes), if they were referred for a genetic evaluation and if they were found to have a disease-causing variant in FLCN, molecularly confirming the diagnosis of Birt-Hogg-Dube syndrome. To do this we utilized the EMERSE system, MiChart, and Microsoft excel to collect and analyze data. Demographic data, pathologic data, personal and family history of BHD-related diagnoses, genetic referral, and genetic evaluation data, genetic testing results and history of other neoplasms were abstracted from the electronic medical record by individual chart review.

Results: A total of 101 patients with a pathology report mentioning the terms trichodiscoma and/or fibrofolliculoma were identified. In 37 pathology reports, the diagnosis was mentioned, but rejected. Of the remaining 64 reports, 43 patients had a confirmed diagnosis of fibrofolliculoma (24), trichodiscoma (13), or fibrofolliculoma and trichodiscoma (6). Twenty-one patients had lesions for which fibrofolliculoma and/or trichodiscoma remained a possible diagnosis in the differential diagnosis, but the final diagnosis was inconclusive.

Sixteen of the 64 patients with a biopsy in the spectrum were referred to cancer genetics, a referral rate of 25%. Of the 43 patients with a confirmed diagnosis of fibrofolliculoma and/or trichodiscoma, 13 (30%) were referred for genetic evaluation after biopsy. Twelve of these 13 patients completed genetic evaluation, with pathogenic variants in FLCN detected in 9 patients. All nine were the first in their family diagnosed with BHD. Of 21 patients with a possible diagnosis, 3 (14%) were referred to cancer genetics. Two completed testing, and in one of these, a variant favored to be benign was detected.

Conclusions: Trichodiscoma and fibrofolliculoma can lead to the identification of patients with a pathogenic variant in *FLCN*, confirming the diagnosis of BHD. Every patient with a confirmed diagnosis of fibrofolliculoma and/or trichodiscoma on biopsy should be referred to cancer genetics for an evaluation for Birt-Hogg-Dubé Syndrome. Patients with biopsies that only mention fibrofolliculoma/ trichodiscoma as a potential differential diagnosis remains on the differential with other diagnoses are less likely to carry a pathogenic variant in *FLCN* and most often do not have a diagnosis of BHD. Potential roadblocks for Cancer Genetics referrals include lack of awareness on fibrofolliculoma/trichodiscoma association with BHD or loss to follow-up.

Poster #14

The Effects of UVA1 on Photoaging in Darker Skin Types

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Solar UV irradiation causes photoaging which is characterized by fragmentation and reduced production of type I collagen fibrils that provide strength to skin. Exposure to both UVA and UVB irradiation contributes to photoaging. UVA irradiation is particularly important to consider because it is 10 to 100 times more abundant in natural sunlight than UVB irradiation and penetrates deeper into the dermis. Previous skin aging research has largely studied tissue samples from lightly pigmented individuals. Historically, it has been thought that darker skin types are less affected by photoaging from chronic UV irradiation. The current study will focus on skin types V and VI to determine the effects of low level UVA1 irradiation on the molecular markers that define skin aging.

To mimic the type of sun exposure that a person might encounter in daily life, two groups were exposed to UVA1 with different exposure intervals. Group 1 received a single exposure and biopsies were performed at three different time points (immediately after, 24 hours after, and 1 week after the single exposure). Group 2 received consecutive exposures on days 0, 1, and 2, and then received biopsies on day 3. Four biopsies were taken from the buttocks of each patient, including 1 control. Using qPCR, skin samples were evaluated for gene expression of procollagen 1 and 3, and MMP 1 and 3.

For Group 1, receiving only 1 exposure of UVA1, there was no significant change in either procollagen or MMPs. For Group 2, receiving 3 consecutive exposures of UVA1, there was no significant change in procollagen or MMP1; however, there was a statistically significant increase in MMP 3 from treatment 1 to treatment 3 (p value =0.019).

These findings suggest that even consecutive low dose exposures to UVA1 induces an increase in MMP 3 in darker skin types. This may have therapeutic implications and also reiterates the importance of photoprotection, even in Fitzpatrick skin types V and VI.