**019**

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

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Fibrosis is an abnormal wound healing process characterized by collagen deposition, myofibroblast accumulation, and extracellular matrix remodeling. Fibrosis can also be seen in irreversible organ damage from lupus, including lungs and kidneys. Skin and organ fibrosis is often associated with high morbidity and even mortality, and there is no effective treatment. Recent work from our laboratory has shown that epidermal Vgll3 overexpression in murine Vgll3 causes severe lupus-like skin lesions reminiscent of discoid lupus erythematous (DLE), as well as systemic autoimmune disease with end-organ damage. Given the apparent fibrotic nature of the skin lesions in transgenic (Tg) TG vs. Vgll3 mice, we wanted to determine whether Vgll3 induced fibrosis. We analyzed male and female TG and wild-type (WT) mice aged 2-3 months, comparing fibrotic biomarkers of human DLE and scleroderma. Here, we demonstrate that epidermal Vgll3 overexpression causes development of not only cutaneous inflammation but also severe fibrosis. Changes include increased infiltration of granulocytes/monocytes accompanied by significant expression of fibrotic biomarkers (Acta2, Col1, Tgbf1, and Ccn2), as well as connective tissue growth factor (Ctgf) and pro-fibrotic cytokines (Il6 and Il13) in TG mice. The detection of high expression of Ccn2 and Tgbf1 as well as Coll mRNA and protein in the skin of TG mice, as seen in skin of human scleroderma and DLE patients, suggests that skin-directed overexpression of Vgll3 may implicate fibrosis development, and there may be a role for targeting Vgll3 in cutaneous fibrosis.

Further studies will need to elucidate the specific mechanisms that may be at play.

**021**

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

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Cutaneous lupus erythematosus (CLE) can be subdivided into acute cutaneous (ACLE), subacute cutaneous (SCCLE), and chronic cutaneous (DLE) CLE, and the predominant subtype depends on the inflammatory microenvironment. Previous studies using RNA extracts or traditional immunostaining have demonstrated subtle differences between the subtypes; however, no multiplexed, single-cell approaches have been conducted. We profiled the immune infiltrate in DLE and SCLE using Immunomass (I-Mas), a patient-sourced fluidigm technique for cellular level analysis. 19 SCLE and 18 DLE, treatment-naive FFPE biopsies were stained with 37 metal-conjugated antibodies and analyzed on the Hyperion Imaging System (Fluidigm). Cells were segmented using a nuclear based algorithm on Visiopharm and imported into histoCAT where cell mean pixel intensity data was obtained to cluster cells using the Phenograph algorithm based on cell markers. Significance was determined by the Mann-Whitney test, and bar charts were created in R. 9 unique populations consisting of dermal CD T, CD B, CD14+CD16+ macrophages, CD68+ macrophages, B cells, CD56+ Cells, Treg, conventional dendritic cells (cDC), and plasmacytoid dendritic cells (pDC) with similar percentages between DLE and SCLE (p>0.05). 16 cytokines and phosphorylated inflammatory signaling pathways were included and the data revealed higher pActa2 in DLE compared to SCLE (p<0.05). At the cell type level, the data showed increased pIl13 in DLE pDC compared to SCLE (p<0.05). Overall, these results suggest substantial overlap between DLE and SCLE, with a potential role for pAkt2 and pIl13 in DLE. Future studies are needed to investigate the potential suitability of these pathways as targeted therapies for DLE.

**022**

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

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The transcription factor UHRF1 (UHRF1) is a master regulator of T follicular helper (Tfh) cells, which play a crucial role in the pathogenesis of systemic lupus erythematosus (SLE). However, the mechanisms by which BCL6 expression is regulated are poorly understood. UHRF1 is a known repressor of the BCL6 promoter, and there may be an additional role for BCL6 in the pathogenesis of SLE. In this study, we investigated the role of BCL6 in the differentiation of Tfh cells from SLE patients.

**023**

Single-cell composition and architecture of cutaneous lupus

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Lupus erythematosus (LE) is a systemic autoimmune disease with a variety of cutaneous manifestations. Antimalariae are first-line systemic therapy, yet not all patients respond to hydroxychloroquine (HCQ), quinacrine (QC), or either (NR). Our group has previously shown that QC responders demonstrate increased conventional dendritic cells (cDC) and TNFα relative to HCQ responders. Here, we investigated the differences between these patients using Imaging Mass Cytometry (I-Mas), an unbiased multiplexed technique. 12 HCQ, 11 QC, and 10 NR patients were included in this study. FFPE samples were stained with 37 metal-conjugated antibodies and analyzed on the Hyperion Imaging System (Fluidigm). Images were segmented using a nuclear app-based algorithm in Visiopharm and imported into histoCAT where single cell mean pixel intensity data was obtained to cluster cells using the Phenograph algorithm. One-way ANOVA, Kruskal-Wallis, and post-hoc Tukey/Dunn’s test (p = 0.05) were performed. Correlations were determined by Pearson’s r.

**024**

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

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Lupus erythematosus is a systemic autoimmune disease that can occur in isolation or in the context of systemic lupus erythematosus (SLE). The disease can be divided into acute cutaneous (ACLE), subacute cutaneous (SCCLE), and chronic cutaneous (DLE) CLE, and the predominant subtype is determined by the skin microenvironment. We performed correlative analysis of FFPE biopsies from 37 patients grouped by their response to antimalarial therapy. We performed single-cell RNA sequencing (scRNA-seq) and spatial sequencing to investigate the transcriptional and architectural differences between healthy, nonlesional CLE, and lesional CLE keratinocytes, fibroblasts, and immune cell subsets. Additionally, subclustering of skin biopsy-derived immune cells and PBMCs identified potential circulating precursors to the immune cells that infiltrate the skin and give rise to CLE lesions. Finally, integration of the scRNA-seq data with spatial sequencing data enabled us to explore the spatial organization of immune cells. Our findings reveal the role of UHRF1 in regulating Tfh cell differentiation and provide a potential target for SLE therapy.

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