Fibroblast And Endothelial Cells Display CXCL12 Dysregulation In Systemic Sclerosis Skin

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Abstract

There are no cures and limited treatments for systemic scleroderma (SSc). SSc is an often-fatal autoimmune disease where excessive collagen deposition (fibrosis) in the skin and internal organs causes significant loss of mobility, pain, and tissue dysfunction. SSc pathology is characterized by three main processes: autoimmunity, restricted blood flow to tissues, and fibroblast dysfunction leading to fibrosis of ranging organs. In this study, previously published single-cell transcriptomics data from normal skin (NS) and SSc skin was reanalyzed using Seurat and CellChat packages to examine these three core pathologies. CellChat analysis of signaling between fibroblast, immune, and endothelial cell populations identified the CXCL signaling pathway as perturbed in SSc skin. Differential expression testing corroborated this observation, revealing dysregulation in the receptor-ligand pair CXCR4-CXCL12 and primarily implicates endothelial-to-mesenchymal transitioning cells (EndoMT), as well as fibroblasts expressing DMKN as contributors to this dysregulation.

Methods

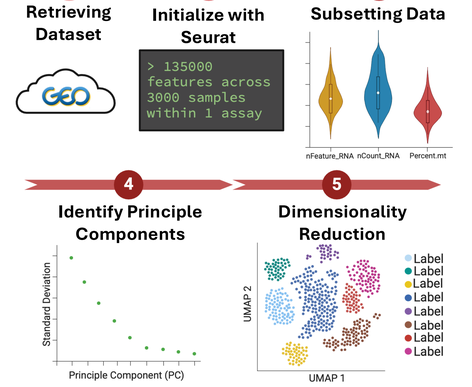

Figure 1. Summary computational workflow used to perform scRNA-seq analysis. scRNA-seq datasets were retrieved from NCBI's GEO database and the Seurat package was used for analysis. Analysis begins removing cells from the dataset with characteristics of failed or dead status. Normalization and PCA tests are run to identify the ideal dimensionality for UMAP clustering. UMAP dimensional reduction test is run, yielding unbiased clustering of cells.

Results

Figure 2A. Human NS and SSc Skin scRNA-seq Immune Cells

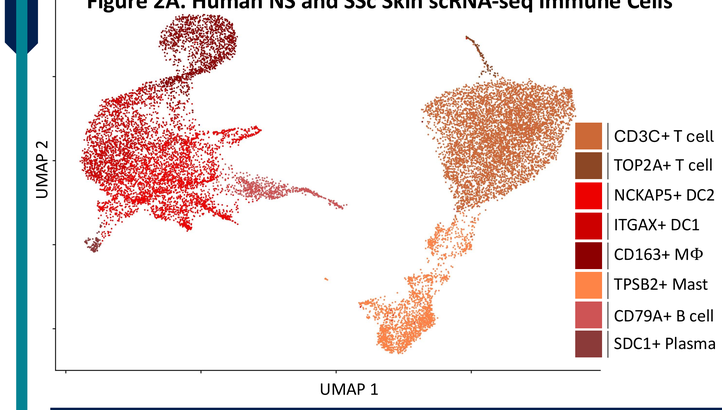

Figure 2B. Human NS and SSc Skin scRNA-seq Fibroblast Cells

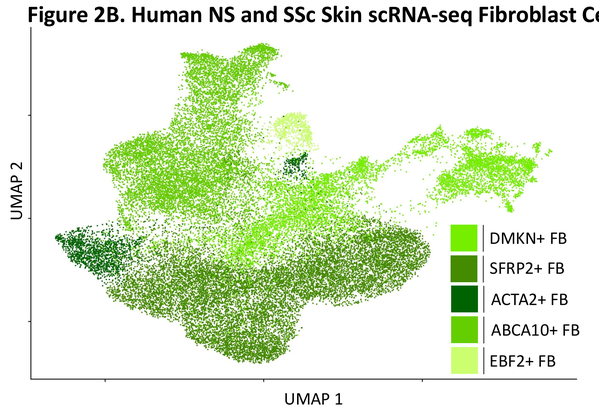

Figure 2C. Human NS and SSc Skin ScRNA-Seq Vascular Endothelial Cells

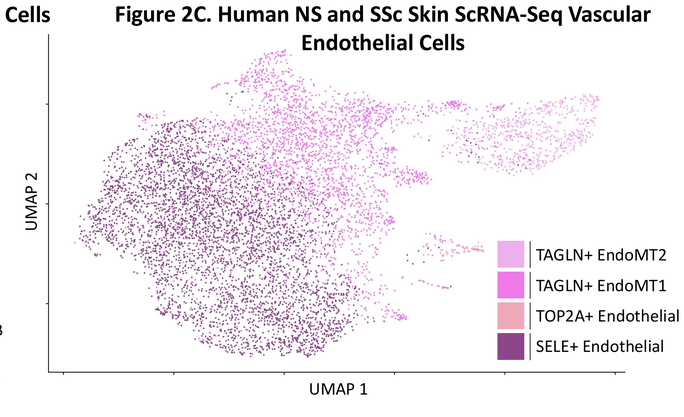

Figure 2D. Information Flow of Signaling Pathways

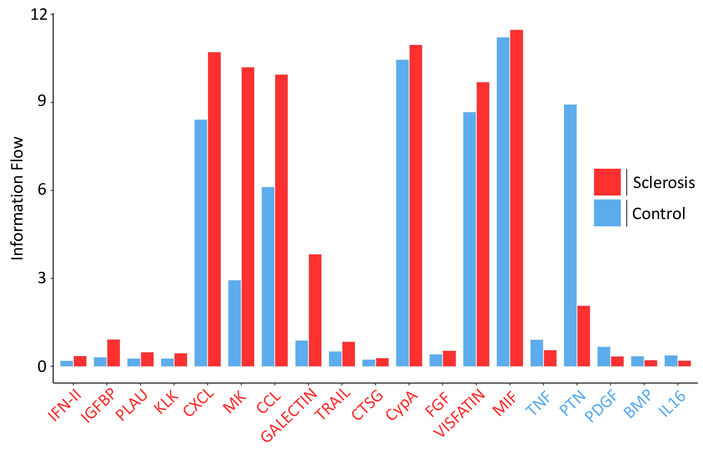

Figure 2E. CXCL Signaling Between Immune, Fibroblast, and Endothelial Populations

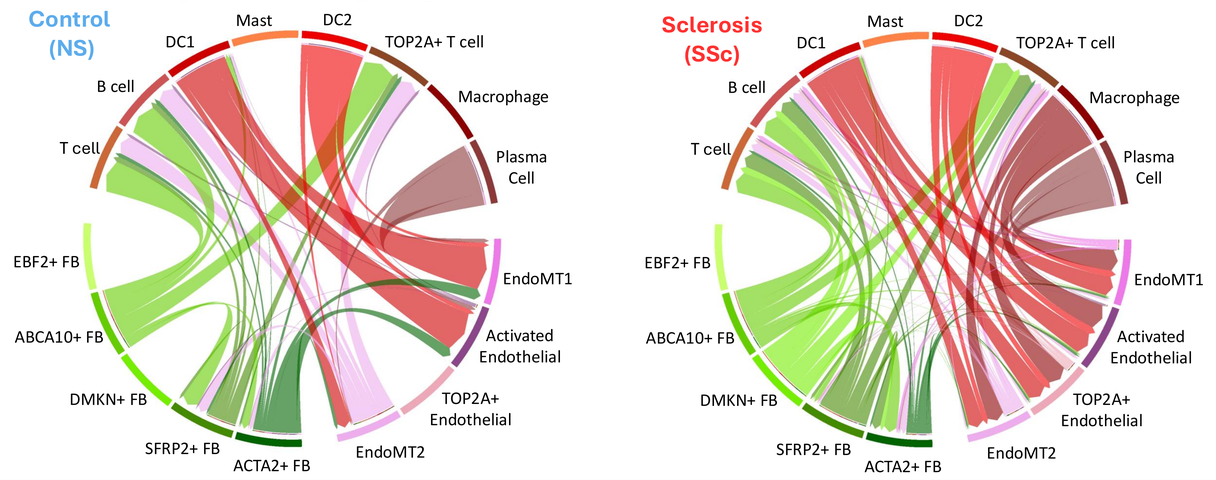

Figure 2F. CXCL12 / CXCR4 Signaling Between Immune, Fibroblast, and Endothelial Populations

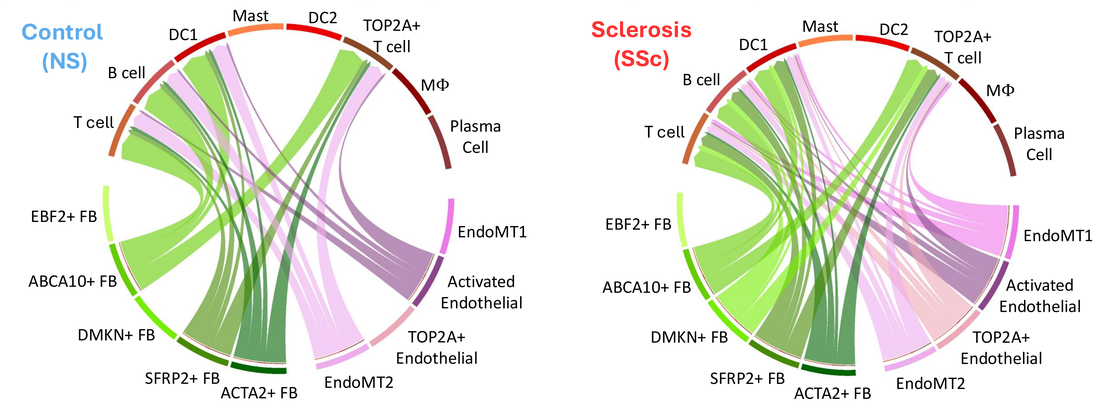

Figure 2G. CXCL12 is Differentially Expressed by Fibroblast and Endothelial Populations

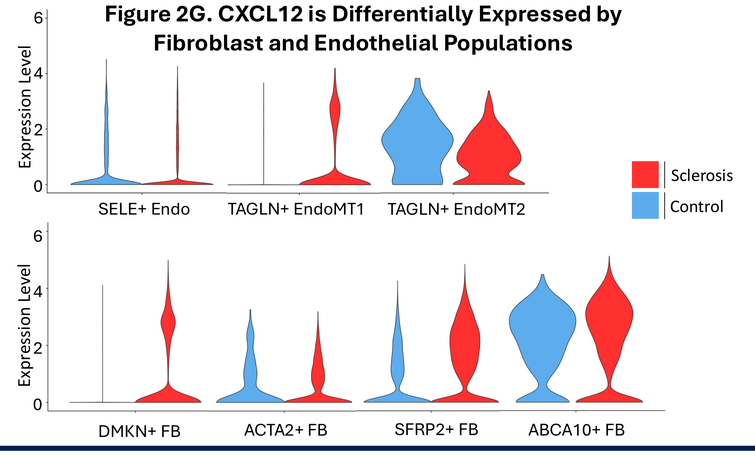

Figure 2. (A-C) UMAP plots showing immune, fibroblast, and vascular endothelial cell populations with 16,457, 35,877, and 7,864 cells respectively. **(D)** Cell signaling pathways with significantly different information flow between SSc and NS identified by CellChat. **(E)** Chord diagrams illustrating CXCL signal senders and receivers within the combined immune, fibroblast, and vascular endothelial populations. **(F)** Chord diagrams showing single ligand-receptor signaling between CXCL12 and its receptor, CXCR4, within combined populations. **(G)** Violin plots showing differential expression of CXCL12 between SSc and NS.

Discussion

Immune, fibroblast, and endothelial cells are highly significant in the pathogenesis of systemic sclerosis (SSc). By preforming a clustering analysis of these cell types we identified distinct cell populations within each subset, revealing unique transcriptional profiles between control and SSc cells. Using the CellChat package to infer cell-to-cell communication based on expression of ligand-receptor pairs between cell populations, we found that the CXCL pathway was perturbed in SSc samples. Further investigation of this observation revealed an increase in signals originating from fibroblast and endothelial populations via the CXCL12-CXCR4 ligand-receptor pair.

Further investigation of these populations showed that the TAGLN+ EndoMT1 population surprisingly expressed DCN, LUM, PDGFRA, and COL1A1 genes. DMKN+ Fibroblasts are upregulated in many inflammatory skin diseases, and in mesenchymal stem cells [1]. Expression of DMKN taken with the dysregulation of CXCL12 and inferred communication with pathologically relevant lymphocytes suggests that future investigation of this population may be valuable.

Acknowledgements & References

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[1] Ma, F., Tsou, PS., Gharaee-Kermani, M. *et al.* Systems-based identification of the Hippo pathway for promoting fibrotic mesenchymal differentiation in systemic sclerosis. *Nat Commun* **15**, 210 (2024). https://doi.org/10.1038/s41467-023-44645-6

[2] Huang, C., et al. (2017). Dermokine contributes to epithelial-mesenchymal transition through increased activation of signal transducer and activator of transcription 3 in pancreatic cancer. *Cancer science*, *108*(11), 2130–2141. https://doi.org/10.1111/cas.13347