Meta analysis of hidradenitis suppurativa single-cell studies shows a distinct population of fibroblasts is responsible for the majority of CXCL and CCL signaling in skin

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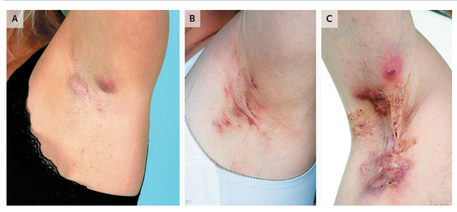
Abstract

Hidradenitis suppurativa (HS) is a chronic inflammatory disease that causes deep, painful lesions that can develop into abscesses, draining sinus tracts, and eventually may form extensive scarring[1]. HS is known to have genetic, environmental, and behavioral associations, although the pathophysiology of disease initiation and progression is still not well understood[1]. Clinical studies show a broad upregulation of proinflammatory cytokines of the CXCL and CCL families, as well as high MMP levels in the skin[2,3]. Despite the significant burden for patients and the increasing prevalence of HS, it remains poorly studied at a molecular level and difficult to treat medically. Single-cell RNA sequencing showed upregulation of immune system processes in both immune cells and in the core component cell populations of the skin, like keratinocytes and fibroblasts. However, to date, no meta analysis has been performed on all scRNAseq datasets. Furthermore, contributions to disease pathogenesis by various cellular players are still poorly understood. Here, we aggregated data from publicly available scRNAseq datasets from HS patients and show that a subpopulation of fibroblasts is responsible for the vast majority of CXCL-, CCL-, and MMP- expression in HS skin, further elucidating complex cellular interactions and roles in the disease.

Introduction



Ref 1

Hidradenitis suppurativa by stage:
A: Hurley stage I
B: Hurley stage II
C: Hurley stage III

- Hidradenitis suppurativa (HS) is a chronic dermatologic condition mostly involving areas of mechanical stress like the axilla, groin, and other intertriginous locations[1].
- HS can include abscesses, skin tunnels, and open, nonhealing wounds[1].
- HS is extremely difficult to medically manage, and only one FDA approved treatment, adalimumab (TNFα inhibitor) is available[1].
- Some HS patients harbor haploinsufficiency in the gamma secretase complex, although most patients do not[4].
- The exact pathogenesis of HS is unclear, although it likely involves a combination of genetic causes, epigenetic changes from environmental shifts, and immunological effects[1,2,3].
- Clinical studies have shown upregulation in many CXCL, CCL, and MMP genes such as[3,4]:
  - CXCL13, B cell chemotactic
  - CXCL8, neutrophil chemotactic
  - CXCL10, chemotactic/stimulant
  - CCL4 & CCL2, immune stimulants
  - MMP2, 8, & 9, pathogenic matrix remodeling
- Several scRNAseq studies have been performed on HS patients, but most analysis has focused on the immune cells in the disease[5,6].
- Here, we perform a meta analysis on all publically available scRNAseq data from HS patients, and analyze the signaling environment.

Methods

scRNAseq datasets from two studies (GSE154775[5] and GSE175990[6]), comprising samples from a total of 12 HS patients and 1 healthy control, were processed using the R package Seurat version 4.3.0.

Pre-analysis quality control and cell selection followed standard Seurat guidelines[7,8]. Briefly, samples were filtered to include cells with RNA counts between 200 and 2,500, and mitochondrial transcripts of less than 5% of total RNA transcripts. The RNA expression data of each cell was then normalized and transformed into a logarithmic scale. Highly variable features were identified to improve processing efficiency of downstream analysis, and all data scaled so the variance across all cells was 1.

To analyze the quality controlled data, an unsupervised PCA analysis was performed to cluster cells. Cell cluster identities were determined by calling the Seurat function FindMarkers and manually determining the cell types using the top 5 genes expressed for each cluster.

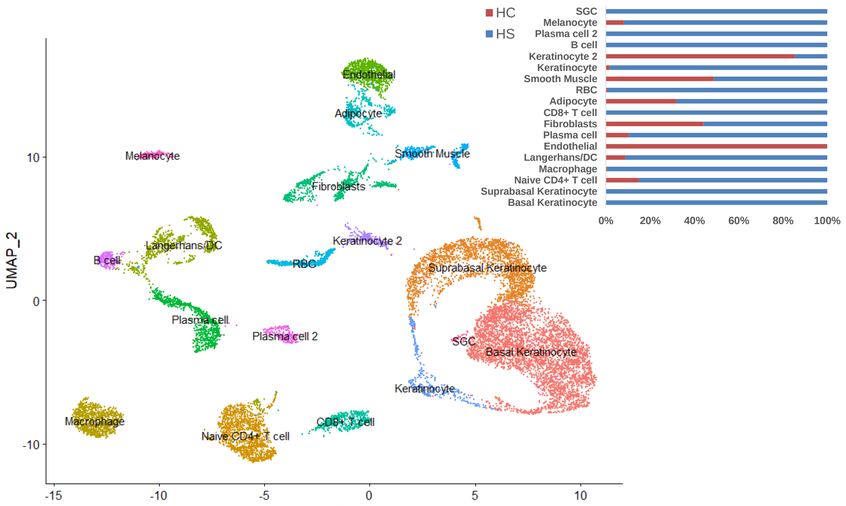
Signaling analysis was performed using the R package CellChat version 1.6.1[9,10]. Standard preprocessing was performed, followed by individual signaling analysis of the CXCL and CCL pathways.

To examine fibroblast subpopulations in detail, the fibroblast cluster identified from all samples was extracted into a new object, and the clustering analysis was repeated. To visualize all CXCL/CLL expression in feature plots, a modular score was calculated by summing expression data for all genes beginning with CXCL-, CCL-, or MMP-, and assigning this value as a separate “gene”.

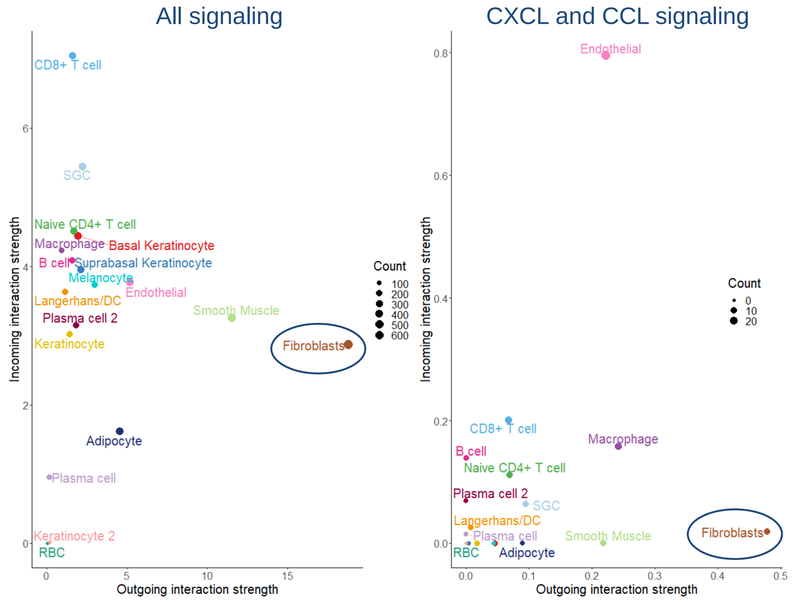
Results

Cells from multiple HS scRNAseq studies aggregate into 18 clusters

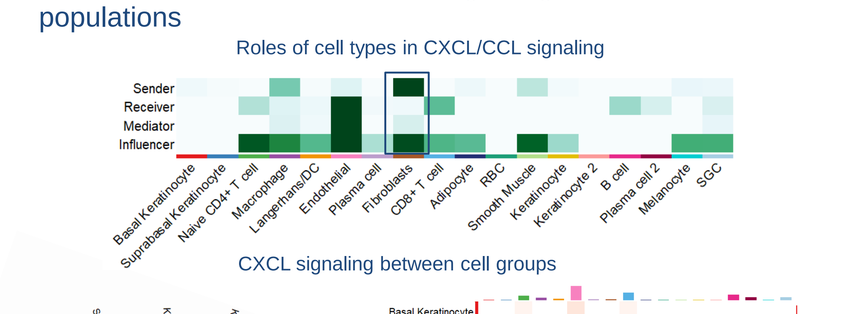
Cells from all samples clustered into the 18 groups shown below. Several cell types (keratinocytes, plasma cells, fibroblasts) are represented by multiple clusters, often separating by representation in healthy vs HS skin, or sometimes from a batch effect between studies.

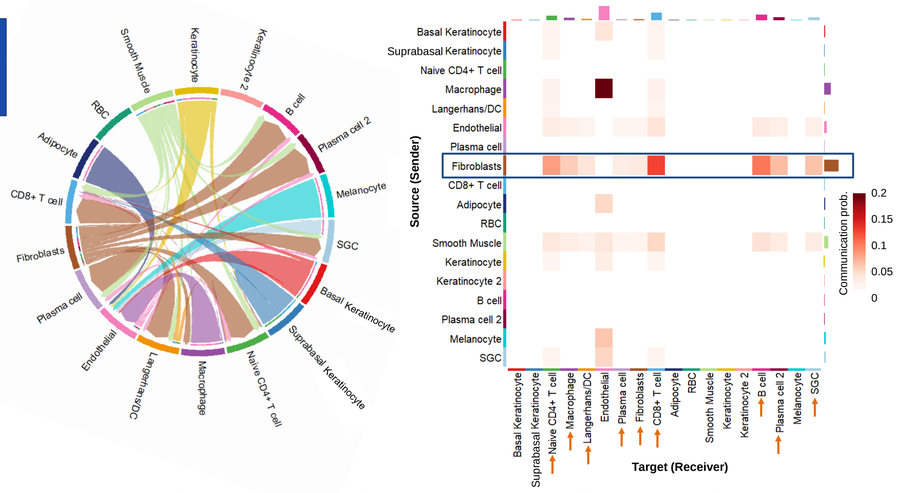


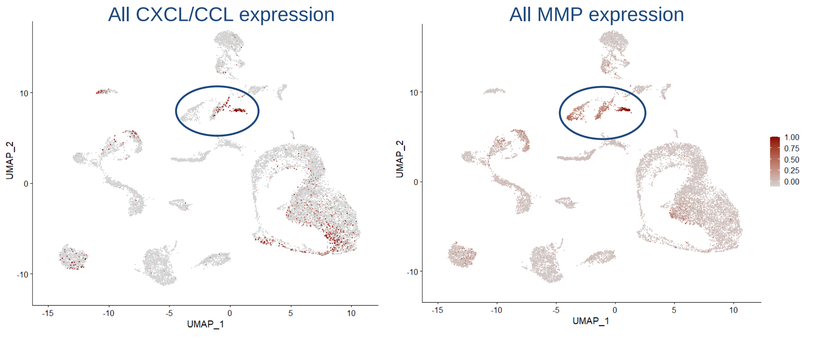
Fibroblasts are the most prolific signaling cell type in the meta analysis of HS skin



Fibroblasts send and coordinate signaling to immune cell populations

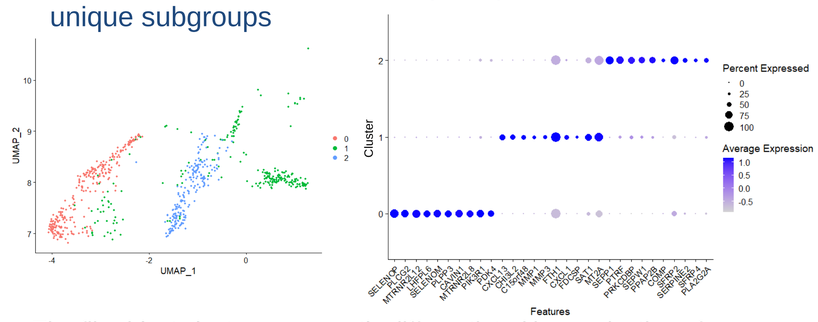




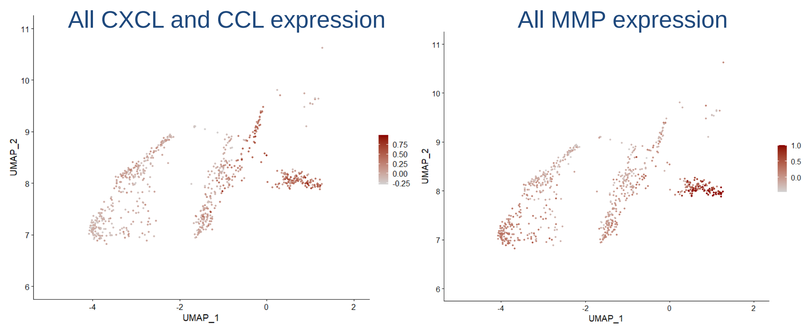


Results cont.

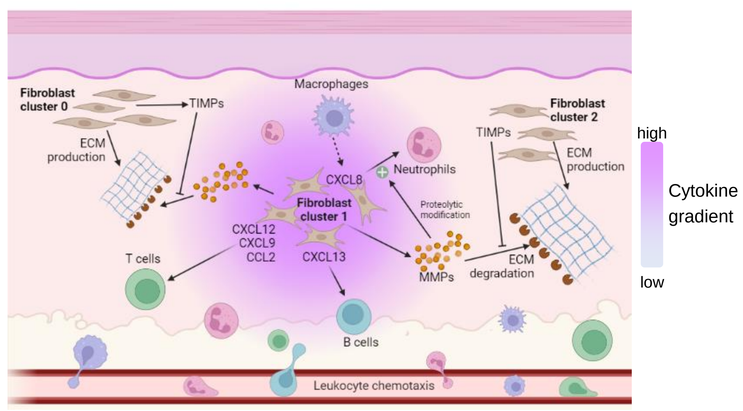
Fibroblasts from HS scRNAseq samples cluster into three unique subgroups



The fibroblast clusters are mostly differentiated by production of proinflammatory cytokines in the CXCL family and MMP production, foremost in cluster 1. Cluster 0 is dominated by fibroblasts from healthy donors, while cluster 2 is comprised of cells from HS samples, but without an inflammatory expression profile.



Graphical representation of summarized fibroblast subcluster signaling



Conclusion

We found fibroblasts were by far the most prolific producers of outgoing signaling in HS skin – both in over all signaling, and specifically in contribution to proinflammatory CXCL and CCL signaling. We also found fibroblasts to be the dominant producers of MMPs, also implicated in HS pathogenesis. We further found fibroblasts could be subclustered into three distinct groups, with cluster 1 being primarily responsible for CXCL, CCL, and MMP expression.

In summary, a distinct population of fibroblasts in HS is responsible for the majority of inflammatory signaling in the skin.

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