Senescent Fibroblasts Drive Chondrogenic ECM Pathology in Keloid Scars: Insights from Single-Cell RNA-Seq

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Background

Keloid scars are fibroproliferative lesions characterized by excessive extracellular matrix (ECM) deposition and persistent inflammation extending beyond the original wound boundaries (1). These scars do not regress spontaneously and are frequently symptomatic and recurrent.

Cellular senescence is a stress-induced state of irreversible growth arrest. While senescent cells contribute to normal wound repair, accumulating evidence suggests that persistent or dysregulated senescent fibroblasts may promote pathological fibrosis and abnormal ECM remodeling in keloid formation (2,3).

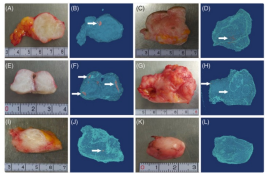


Figure 1: Gross and micro-cT images of keloids (6).

Keloid scars also demonstrate features of a **pseudo-cartilaginous ECM phenotype**, including increased proteoglycan and glycosaminoglycan-rich matrix deposition (4,5). However, **the specific cellular populations responsible for driving this chondrogenic matrix program remain poorly defined.**

Methods

Figure 2. Single-cell RNA-seq workflow
Schematic overview of single-cell RNA-sequencing preprocessing, quality control, integration, clustering, and downstream analyses performed on normal skin and keloid tissue samples.

Nine human keloid single-cell RNA-seq datasets were integrated in **R** using **Seurat v4**. Fibroblasts were identified by **COL1A1** and **PDGFRA** expression and classified as senescence-positive (**CDKN1A/CDKN2A^{+}, MKI67^{-}**) or senescence-negative. Differential gene expression and pathway enrichment analyses were performed in R comparing senescent versus non-senescent fibroblasts. Chondrogenic ECM programs were quantified using a 19-gene chondrocyte module score. Statistical comparisons used Wilcoxon rank-sum tests with multiple-testing correction.

Results

A)

Cluster	Cell Type	Top Markers
0	Fibroblast - chondro-like	ACAN, COL11A1, ASPN, COMP, DPT, POSTN
1	Endothelium-venous	SELE, ICAM1, ACKR1, VWF, FLT1, ERG
2	Fibroblast-reticular	C3, CXCL12, PDGFRA, FBLN2, TWIST2, DKK2
3	Fibroblast-remodeling	COL1A1, COL3A1, FAP, ADAM12, DPT
4	Pericyte	MYH11, ACTA2, RGS5, NOTCH3, PDE5A
5	Endothelium-vascular	PECAM1, CDH5, CLDN5, EMCN, FLT1
6	Keratinocyte - differentiated	DSC1, DSG1, KRT1, KRT2, LYPD3, LGALS7
7	Macrophage	C1QA/B/C, CD163, MRC1, IL1B, HLA-DRA
8	Mast-cell	TPSAB1, CPA3, MS4A2, KIT, HDC
9	Endothelium-lymphatic	PROX1, LYVE1, PDPN, FLT4, MRC1
10	Keratinocyte - basal	KRT14, KRT15, KRT5, TP63, COL17A1
11	T-cel	CD3D/E/G, GZMA/K, CCL5, IL7R
12	Keratinocyte-cornified	SPRR1/2, LCE1/2/3, SERPINB3/B4, IL36G
13	Schwann-cell	SOX10, NGFR, PMP2, PLP1, CDH19
14	Erythrocyte	HBA1, HBA2, HBB
15	Cycling-G2M	MKI67, TOP2A, CDK1, CCNB1/2, BIRC5
16	Endothelium-vascular	VWF, AQP1, ADAM15, RAMP3, APLNR
17	Cycling-Sphase	MKI67, MCM3-7, PCNA, CHEK1, BRCA1/2

B)

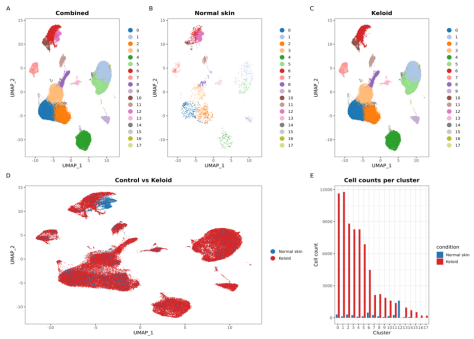
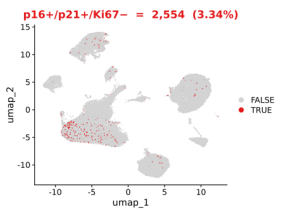


Figure 3. Cellular landscape of normal skin and keloid tissue. This figure shows 76,389 cells in total with 72,009 cells from the keloid samples and 4,380 cells being from normal skin. (A) A table summarizing cell cluster identities derived from single-cell RNA sequencing, annotated using canonical marker genes. Identified populations include multiple fibroblast subtypes, endothelial cells, keratinocytes, immune lineages, perivascular cells, and proliferating cells. (B) UMAP visualizations of integrated single-cell transcriptomes. Top panels display all cells, normal skin only, and keloid tissue only. Fibroblast-enriched clusters (Clusters 0, 2, and 3) are highlighted. Bottom panels show UMAP colored by condition and a bar plot of cell counts per cluster, demonstrating increased fibroblast representation in keloid tissue.

A)



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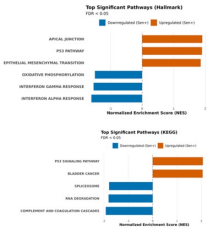
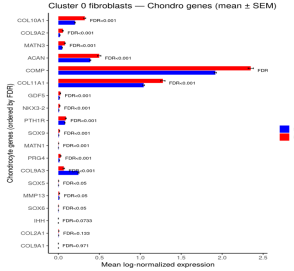


Figure 4. Senescent fibroblasts localize to fibroblast-enriched clusters and exhibit altered pathway activity. (A) UMAP projection highlighting senescence-positive cells, defined by a **p16^{+}/p21^{+}/Ki67^{-}** signature. Senescent cells (red) are overlaid on the full cellular atlas (gray). A total of 2,554 senescent cells (3.34% of all cells) were identified, localizing predominantly to fibroblast clusters with minimal representation in epithelial or immune populations. (B) Gene set enrichment analysis comparing senescent and non-senescent fibroblasts. Hallmark pathway analysis demonstrates enrichment of **p53 signaling**, **epithelial–mesenchymal transition**, and **apical junction** pathways, with concurrent downregulation of **oxidative phosphorylation** and **interferon response** pathways (FDR < 0.05). KEGG analysis similarly identifies enrichment of the p53 signaling pathway. Bars represent normalized enrichment scores.

A)



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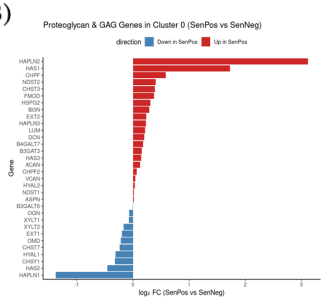

Figure 5. Senescent fibroblasts drive chondrogenic ECM gene expression. (A) Differential expression analysis of Cluster 0 fibroblasts comparing senescence-positive (SenPos) and senescence-negative (SenNeg) cells demonstrates significant upregulation of cartilage-associated genes in senescent fibroblasts, including **ACAN, COMP, COL11A1, GDF5, and SOX9**. Data are shown as mean log-normalized expression ± SEM and ordered by false discovery rate. (B) Log_2 fold-change analysis of proteoglycan and glycosaminoglycan pathway genes reveals upregulation of ECM assembly and GAG synthesis genes (e.g., **BGN, LUM, ACAN**) in senescent fibroblasts, with concurrent downregulation of select matrix-processing genes. Red bars indicate genes upregulated and blue bars indicate genes downregulated in SenPos cells.

Summary

Keloid scars exhibit a marked expansion of fibroblast populations, consistent with their fibroproliferative phenotype. Using single-cell RNA sequencing, we identified a distinct subset of **senescent fibroblasts** within keloid tissue that display a functionally altered transcriptional profile.

Senescent fibroblasts were enriched for **chondrogenic extracellular matrix (ECM) lineage markers** and genes involved in **proteoglycan and glycosaminoglycan synthesis**, distinguishing them from non-senescent fibroblasts. Pathway enrichment analyses reveal a shift toward p53-associated stress responses and matrix remodeling programs, supporting a senescence-driven mechanism underlying the **pseudo-cartilaginous ECM** characteristic of keloid scars.

These findings suggest that senescent keloid fibroblasts actively contribute to pathological matrix deposition rather than representing a passive or terminal cell state.

Future Directions

Future studies will validate the chondrogenic ECM program of senescent fibroblasts using histologic and protein-level analyses in our human keloid tissue samples. Senescent fibroblasts will be isolated and functionally characterized in vitro, including evaluation of ECM production and response to senolytic therapies. These approaches will be extended to human keloid explant cultures to assess senolytics as a potential strategy to modulate pathologic ECM remodeling.

References

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Acknowledgements

Thank you to the members of the Roh Lab and the Medical Student Summer Research Program at Boston University.

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