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Xenium spatial transcriptomics identifies mesenchymal fibroblast expansion and conserved fibrotic ECM programs in keloid

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Keloids are fibroproliferative scars characterized by excessive extracellular matrix (ECM) deposition and progressive growth beyond the boundaries of the original wound. They can develop following surgery, burns, acne, ear piercing, vaccination, or minor trauma, and commonly affect the chest, shoulders, earlobes, and face [1]. Clinically, keloids can cause pain, pruritus, restricted mobility, cosmetic concern, and psychosocial burden. They disproportionately affect individuals with highly pigmented skin, with prevalence reaching an estimated 16% among people of African descent [2]. Keloid pathogenesis involves sustained fibroblast activation and chronic inflammation, particularly through the TGF-β signaling [3]. Despite treatments such as intralesional corticosteroids, surgical excision, and silicone-based dressings, clinical outcomes remain variable and recurrences are common, underscoring the need to better define the mechanisms underlying keloid disease. Bulk and single-cell transcriptomic studies have identified fibroblast-associated fibrotic programs, but cannot resolve their spatial organization within lesions. Spatial transcriptomics addresses this limitation by linking gene expression to the cellular architecture of native tissue [4]. Here, we used targeted Xenium spatial transcriptomics (Xenium ST) at single-cell resolution to define keloid-associated cellular and molecular programs within intact tissue, focusing on fibroblast heterogeneity and conserved profibrotic programs linked to ECM remodeling across diverse ancestry.

This study was conducted according to the principles of the Declaration of Helsinki and was approved by the University of California, Los Angeles Institutional Review Board (IRB #23-0510). Written informed consent was obtained from all participants prior to sample collection and analysis. Written informed consent was obtained from all participants prior to sample collection and analysis. Normal skin from one Asian (AN) donor and keloid tissue from AN, White non-Hispanic (WNH), and Hispanic/Latino (H/L) donors were collected and processed as Formalin-Fixed Paraffin-Embedded sections. Xenium ST was performed using the 10× Genomics 260-gene Human Skin Panel supplemented with a custom 100-gene panel enriched for fibrosis, ECM remodeling, growth factor signaling, and inflammatory pathways. After quality control, 8749 cells from normal skin and 41 264, 54 831, and 22 489 cells from AN, WNH, and H/L keloids, respectively, were retained, with mean and median transcript counts of 167 and 125 per cell. Cells with nCount_RNA $>$ 5 were analyzed using Seurat v5.3.0 and Xenium Explorer (Figure 1a; Supplementary Figure S1a–c; Supplementary Tables S1 and S2). Since normal skin was available only from the AN donor, direct normal–keloid comparisons were restricted to the AN samples, whereas comparisons among keloid samples were interpreted as exploratory lesion-level observations. Detailed methods are provided in Supplementary Detailed Materials and Methods.

Using established skin lineage markers, we annotated 11 major cell populations, including keratinocytes, fibroblasts, endothelial cells, pericytes, lymphocytes, macrophages, dendritic cells, Langerhans cells, mast cells, melanocytes, and cycling cells [5]. Most cycling cells co-expressed fibroblast markers and were detected exclusively in keloid samples, suggesting that this population largely represents proliferating fibroblasts within keloids. H&E images and Xenium-derived cell annotation maps from matched sections showed preserved tissue architecture and broad concordance between histology and spatial cell-type distributions (Figure 1b and c; Supplementary Figure S1d

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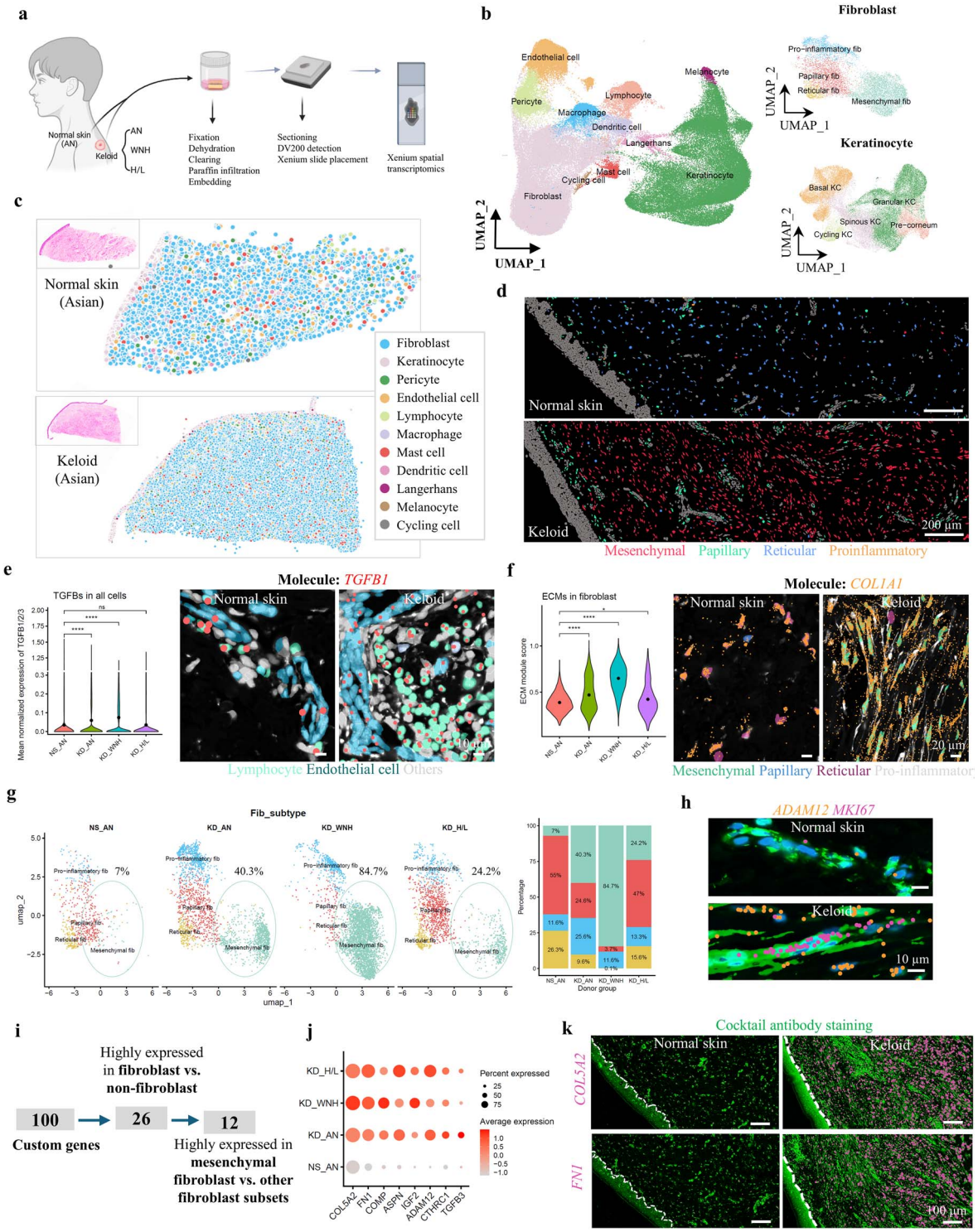


Figure 1 Targeted Xenium spatial transcriptomics reveals conserved fibroblast and extracellular matrix programs in keloid skin. (**a**) Schematic overview of the experimental workflow for Xenium spatial transcriptomics profiling of one normal human skin and three keloid tissues. (**b**) UMAP visualization of normal and keloid skin identifying 11 major cell populations. Fibroblasts and keratinocytes were further resolved by secondary clustering to define transcriptionally distinct subpopulations. (**c**) Representative H&E staining of normal and keloid skin, alongside corresponding Xenium-derived centroid cell segmentation and cell-type annotation maps demonstrating preservation of tissue architecture and spatial organization of major skin cell populations. (**d**) Representative Xenium images of normal skin and keloid tissue from AN, showing mesenchymal, papillary, reticular, and pro-inflammatory fibroblast populations. Scale bar: 200 μm. (**e**) Module score of TGFB ligands (TGFB1–3) across all cell populations (left), showing increased TGF-β signaling in keloid compared with normal skin. Spatial mapping of TGFB1 expression (right), showing enrichment primarily in endothelial cells and lymphocyte populations. Scale bar: 10 μm. (**f**) Fibroblast-specific ECM module score showing elevated ECM transcriptional activity in keloid fibroblasts.

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and e). Multimodal segmentation incorporating membrane markers, intracellular RNA and protein markers, and DAPI staining produced tissue organization patterns consistent with nuclear-expansion-based segmentation. Across samples, fibroblasts were the predominant dermal population, followed by endothelial cells and pericytes, and lymphocytes (Supplementary Figure S1f and g).

Since fibroblasts and keratinocytes were the most abundant and transcriptionally heterogeneous populations, we further characterized these lineages by secondary clustering. Keratinocytes were organized into basal, spinous, granular, pre-corneum, and cycling states, aligning with the expected epidermal differentiation program [6]. The outermost epidermal layers showed minimal mRNA signal, as expected for terminally differentiated cornified keratinocytes, indicating lower background noise in the Xenium ST data (Figure S2a and b).

Fibroblasts were separated into mesenchymal, papillary, reticular, and pro-inflammatory subsets [7]. Mesenchymal fibroblasts, marked by *ASPN*, *ADAM12*, and *POSTN*, were preferentially localized within the deeper dermis. Papillary fibroblasts were enriched near the dermal–epidermal junction, reticular fibroblasts were broadly distributed throughout the dermis, and pro-inflammatory fibroblasts localized near immune-enriched regions (Figure 1d; Supplementary Figure S2c). These spatial patterns recapitulate dermal compartmentalization and enable single-cell localization of keloid-associated fibroblast states.

Additional lineage markers further supported the accuracy of cell-type annotation. Langerhans cells and melanocytes were localized to the epidermis; endothelial cells and pericytes delineated dermal vascular structures; and lymphocytes, dendritic cells, macrophages, and mast cells formed immune-enriched aggregates within the dermis (Supplementary Figures S2 and S3). Together, these findings demonstrate that Xenium ST captured the major cellular populations and tissue organization of normal and keloid skin.

To characterize key molecular pathways underlying keloid pathogenesis, we next analyzed the custom 100-gene panel, which included genes related to ECM organization and remodeling, profibrotic growth factor signaling, inflammation, and other keloid-associated processes (Supplementary Tables S1–S3).

We first examined the TGF-β–ECM axis, a key regulator of fibroblast activation and matrix deposition. Relative to the AN normal-skin sample, *TGFB1–3* expression was higher in AN and WNH keloids but comparable in the H/L keloid. Increased *TGFB1* expression was mainly observed in lymphocytes, macrophages, mast cells, and endothelial cells (Figure 1e; Supplementary Figure S4a). We next assessed a fibroblast-focused ECM module comprising downstream mediators of TGF-β signaling (*SMAD3*, *CCN2*), structural matrix genes (*COL1A1*, *COL3A1*, and *FN1*), collagen-processing and remodeling genes (*P4HA2*, *CTHRC1*), and additional ECM-associated genes (*ACAN*, *ASPN*, *VCAN*, *COMP*, *ELN*, *LUM*, *TNC*, *SPP1*, and *SDC1*) [8]. Keloid fibroblasts showed increased ECM module activity, and spatial mapping demonstrated marked enrichment of *COL1A1* throughout the keloid dermis (Figure 1f).

Growth factor-related genes (*PDGFB*, *IGF1*, *IGF2*, *HGF*, *EGFR*, *FGF5*, *FGF10*, and *FGF11*) and inflammatory genes (*IL6*, *IL17A*, *TNFRSF1A/B*, *CXCL1–3*, *NFKB1/2*, *RELA*, *NLRP3*, *GRN*, and *SORT1*) were also elevated in the AN and WNH keloids relative to AN normal skin but not in the H/L keloid (Supplementary Figure S4b and c). Together, these findings indicate increased activation of fibrotic ECM programs accompanied by lesion-level signaling heterogeneity in growth factor and inflammatory signaling across individual keloids.

We next focused on mesenchymal fibroblasts because of their central role in keloid fibrosis. Mesenchymal fibroblasts represented $\sim$ 7% of fibroblasts in AN normal skin but were expanded in all three keloids, accounting for 40.3%, 84.7%, and 24.2% of fibroblasts in the AN, WNH, and H/L lesions, respectively (Figure 1g). In addition, *ADAM12*$^{+}$ mesenchymal fibroblasts showed increased *MKI67* expression, consistent with enhanced proliferation within this keloid-associated fibroblast compartment (Figure 1h; Supplementary Figure S1e).

To define transcriptional features associated with mesenchymal fibroblasts, we performed a stepwise analysis of the custom 100-gene panel. Of the 100 genes, 26 were enriched in fibroblasts relative to non-fibroblast populations (Figure 1i; Supplementary Figure S5a). Twelve of these genes were further enriched in mesenchymal fibroblasts compared with the other fibroblast subsets, defining a distinct mesenchymal transcriptional program. Notably, eight genes (*COL5A2*, *FN1*, *COMP*, *ASPN*, *IGF2*, *ADAM12*, *CTHRC1*, and *TGFB3*) were enriched in mesenchymal fibroblasts and showed increased expression across all three keloid samples (Figure 1j and k; Supplementary Figure S5b). The magnitude of expression levels varied across individual lesions, suggesting that mesenchymal fibroblasts share a core profibrotic program while retaining lesion-specific transcriptional features. Functionally, ASPN, ADAM12, COL5A2, COMP, and FN1 are associated with ECM organization and remodeling [7], whereas IGF2, CTHRC1, and TGFB3 have been implicated in fibrosis and ECM

Figure 1 The ECM module includes TGF-β downstream mediators (SMAD3, CCN2), structural matrix genes (COL1A1, COL3A1, FN1), collagen remodeling enzymes (P4HA2, CTHRC1), and additional ECM components (ACAN, ASPN, VCAN, COMP, ELN, LUM, TNC, SPP1, SDC1) (left). Representative spatial images showing increased COL1A1 expression across fibroblast subsets in keloid tissue (right). Scale bar: 20 μm. (**g**) UMAP visualization of fibroblast subpopulations across normal and keloid samples, highlighting mesenchymal fibroblasts (left). Bar plots show the proportion of fibroblast subsets, demonstrating expansion of mesenchymal fibroblasts in keloid tissue and variability across lesions and donor backgrounds (right). (**h**) Representative Xenium images showing ADAM12^{+} MKI67^{+} mesenchymal fibroblasts in normal and keloid tissue. (**i**) Schematic overview of the analytical workflow used to identify genes enriched in mesenchymal fibroblasts in keloid relative to normal skin across multiple donors. (**j**) Dot plot showing the average expression levels and percentage of cells expressing 8 genes enriched in mesenchymal fibroblasts across fibroblast subpopulations (mesenchymal, papillary, reticular, and pro-inflammatory). (**k**) Representative spatial images showing expression of fibrotic genes COL5A2 and FN1 (red) alongside antibody staining (green) in normal and keloid tissue. The dashed line indicates the epidermal–dermal boundary. *NS* normal skin, *KD* keloid, *AN* Asian, *WNH* White non-Hispanic, *H/L* Hispanic/Latino, *ECM* extracellular matrix, *Fib* fibroblast

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synthesis [9, 10]. Collectively, these findings suggest that mesenchymal fibroblasts are expanded in keloid and exhibit a conserved profibrotic program associated with ECM production and remodeling.

We used targeted Xenium ST to resolve keloid-associated cellular programs within intact skin at single-cell resolution. The concordance among histology, cell annotation, and expected tissue organization supports the fidelity of spatial mapping. By including keloid samples from donors with diverse ancestry and pigmentation backgrounds, our study begins to address heterogeneity relevant to keloid risk. Keloids exhibited increased TGF-β–associated ECM activity, expansion and proliferation of mesenchymal fibroblasts, and enrichment of a shared profibrotic program. Growth factors and inflammatory programs varied across lesions, suggesting a shared fibrotic fibroblast state within heterogeneous signaling environments. However, larger studies with matched controls and functional validation are needed to establish the mechanistic significance of these findings.

Xenium ST identified mesenchymal fibroblast expansion and a shared profibrotic ECM program as prominent features of keloid tissue, providing a spatial framework for future mechanistic and therapeutic studies.

Author contributions

Min Deng (Conceptualization, Data curation, Formal analysis, Investigation [lead], Methodology [equal], Writing—original draft [lead]), Gregory M. Brewer (Resources [lead]), Alejandro Espinoza (Data curation [equal], Resources [lead]), Julio Orellana-Montes (Formal analysis, Visualization [equal]), Keshvad Hedayatyanfard (Data curation, Formal analysis [equal]), Chanyue Hu (Data curation, Formal analysis, Visualization [equal]), Md Golam Sarowar Jahan (Data curation, Formal analysis [equal]), Mojan Deriss (Resources [equal]), Jeremy C. Davis (Resources [equal]), Carol E. Cheng (Resources [equal]), Matteo Pellegrini (Conceptualization, Data curation, Formal analysis [equal]), and George W. Agak (Conceptualization [lead], Funding acquisition [lead], Project administration [lead], Writing—review & editing [lead])

Supplementary material

Supplementary material is available at *Burns & Trauma* online.

Study approval

This study was conducted according to the principles of the Declaration of Helsinki and was approved by the University of California, Los Angeles Institutional Review Board (IRB #23-0510). Written informed consent was obtained from all participants prior to sample collection and analysis.

Conflicts of interest

The authors state no conflict of interest.

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