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ORIGINAL RESEARCH

The Single-Cell Triaptosis Regulatory Pattern in the Immune Microenvironment of Keloids

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Introduction: Keloids are a complex pathological condition of the skin characterized by abnormal proliferation of fibrous tissue and excessive accumulation of extracellular matrix, typically following inflammation after skin injury. Understanding the regulatory mechanisms of immune cells involved in keloid formation is essential for the development of effective treatments.

Methods: This study integrated publicly available single-cell RNA sequencing (scRNA-seq) data with our own keloid scRNA-seq samples to investigate the role of triaptosis in shaping the immune microenvironment of keloids. We analyzed the composition and functional status of fibroblast and immune cell subpopulations.

Results: Immune cells in keloids, especially $CD8^+$ T cells and macrophages, showed significant heterogeneity under the influence of triaptosis regulatory patterns. These triaptosis-associated immune cell clusters exhibited distinct signaling interference compared to mesenchymal fibroblasts and contributed to keloid development. Furthermore, ELMO2 was identified as a key gene with a potential causal relationship to keloids using Summary-data-based Mendelian Randomization and validated through immunofluorescence staining.

Conclusion: Our findings reveal the complexity of cell–cell interactions in the keloid immune microenvironment and highlight triaptosis as a potential regulatory mechanism in keloid pathogenesis. The identification of ELMO2 as a key factor offers a promising therapeutic target. This study lays a foundation for developing novel therapeutic strategies and encourages future investigations into the clinical application of triaptosis-related interventions for keloid treatment.

Keywords: keloids, single-cell RNA sequencing, immune microenvironment, triaptosis, ELMO2

Introduction

Keloids are a complex dermatological disorder marked by an imbalance in fibrous tissue growth and abnormal extracellular matrix (ECM) buildup, usually following skin injury or inflammation caused by infection.[1] These lesions often grow beyond the boundaries of the original wound, invading surrounding healthy tissue and exhibiting uncontrolled proliferation.[2] Even minor skin trauma, such as acne, can trigger their formation, with the keloids appearing as raised, firm patches on the skin.[3] Patients may suffer from symptoms like itching and pain, and the visible nature of these scars can lead to significant emotional and psychological distress, thereby diminishing their quality of life. Although numerous treatment options are currently available—such as surgical removal, silicone-based therapies, hydrogel dressings, laser treatments, intralesional steroid administration, cryotherapy, and topical chemotherapeutic agents—the recurrence rate remains notably high.[4–7] Hence, further investigation into the underlying mechanisms of keloid formation is essential, both to deepen the understanding of their pathogenesis and to explore more effective therapeutic solutions that can improve patient outcomes and well-being.

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Triaptosis is a recently identified form of cell death that is dependent on endosomal function. This pathway is activated by menadione (vitamin K3), a synthetic precursor of vitamin K, which induces oxidative activation of the phosphatidylinositol 3-kinase PIK3C3/VPS34.[8] The resulting oxidative stress impairs endosomal activity, emphasizing how excessive ROS can disrupt intracellular structures and initiate specific death pathways.[9] Interestingly, menadione has demonstrated therapeutic potential in the treatment of diseases such as prostate cancer and X-linked myotubular myopathy—an inherited disorder stemming from mutations in the MTM1 gene located on the X chromosome. Unlike traditional antioxidants that combat reactive oxygen species to prevent DNA damage and mutation, menadione functions as a pro-oxidant and may offer therapeutic advantages through this mechanism.[10] One cytotoxic effect of menadione involves intracellular glutathione (GSH) depletion, though the detailed biochemical processes behind this effect remain to be clarified.[11] As a novel oxidative stress-mediated cell death mechanism, triaptosis's role in human pathology is still under investigation; future research is necessary to determine its utility in cancer therapy and its role in immune activation through antigen presentation.

In the context of the keloid immune microenvironment, analyzing triaptosis-related regulatory mechanisms at the single-cell level reveals promising insights into disease progression and treatment possibilities. Single-cell RNA sequencing provides detailed profiling of immune cell subpopulations within keloids, offering critical information about their immunological behavior.[12] Given that triaptosis depends on endosomal function, its involvement in keloid formation—especially concerning endosomal dysfunction and ECM dysregulation—warrants attention. Since endosomal processing plays a key role in immune cell activation and antigen presentation, understanding triaptosis's immunogenic potential could inform the development of novel immunotherapies. Therefore, exploring triaptosis regulation in individual immune cells not only deepens our understanding of keloid pathophysiology but also holds substantial promise for identifying therapeutic targets and evaluating its role in future immunotherapeutic approaches.

Materials and Methods

Sources of Raw Data

Tissue samples were obtained from three individuals diagnosed with keloids and three healthy donors for single-cell RNA sequencing analysis. This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Approval No: 2024-SR-383). Biopsies were specifically harvested from the central region of the keloid lesions, deliberately excluding the surrounding non-lesional skin. The detailed personal information of these patients can be found in our previous published research.[12] Importantly, none of the keloid patients had undergone chemotherapy, radiotherapy, or intra-lesional steroid treatment prior to surgical excision. Additionally, we incorporated publicly available single-cell RNA-seq data from three keloid dermal tissue samples retrieved from the GSE163973 dataset.[13] To ensure consistency, sample preparation was carried out using protocols identical to those applied in the GSE163973 study. In alignment with established procedures,[14] single-cell suspensions were prepared and labeled using the Chromium Single Cell Library, Gel Bead & Multiplex Kit (10x Genomics). Within the Chromium Controller, individual cells were encapsulated with barcoded gel beads, followed by cell lysis and reverse transcription of barcodes. The resulting libraries were sequenced using the Illumina HiSeq X platform with 150 base pair paired-end reads. Raw sequencing data were processed through Cell Ranger v3.0.2 to align reads to the GRCh38 human genome reference and generate gene expression matrices. Gene and UMI counts were further quantified using GenuageCounts software. In a recently published research, we curated a set of 21 triaptosis-related genes (TRGs), which are detailed in the Supplementary Table 1.[8]

Processing of Single-Cell Sequencing Data

Raw gene expression matrices were imported into R using the "Seurat" package (version 4.2.0) for downstream single-cell analysis.[15] To eliminate potential doublets from the dataset, we applied the "DoubletFinder" package.[16] After initializing a Seurat object, we applied quality control filters, retaining cells with detected gene counts between 300 and 7000, and excluding those exhibiting over 20% mitochondrial gene content or more than 10% hemoglobin gene expression. To capture the most informative features, the top 2000 highly variable genes were identified using the

"FindVariableFeatures" function. The dataset was then normalized via the "ScaleData" function. Integration of the remaining expression data across samples was performed using the "harmony" package to correct for batch effects.[17] Subsequently, principal component analysis (PCA) was conducted using high-variance genes, with the top 30 principal components selected for dimensionality reduction through Uniform Manifold Approximation and Projection (UMAP). Differentially expressed genes (DEGs) across cell clusters were identified using the "FindAllMarkers" function. Cell types and subtypes were annotated based on canonical marker gene expression profiles associated with known immune and stromal cell identities.

Identification of Marker Genes in Different Cell Clusters by NMF

Building upon findings from our earlier research,[18] we sought to minimize data redundancy and background noise to more accurately investigate the influence of triaptosis-associated gene expression on immune cell populations within keloids. Leveraging previously annotated cell identities, we performed clustering optimization using the "NMF" package in R, thereby generating NMF-defined cell clusters. Differential gene expression analysis within these clusters was conducted using the "FindAllMarkers" function, applying stringent criteria: a log fold change (logFC) greater than 1 and an adjusted p-value below 0.05. NMF clusters were then categorized based on their expression profiles. Specifically, clusters that significantly expressed triaptosis-related genes (TRGs)—meeting both statistical thresholds (adjusted p-value < 0.05 and logFC > 1)—were labeled as TRG-enriched clusters. In contrast, clusters that fulfilled the statistical criteria but lacked TRG expression were defined as non-triaptosis clusters.

Pseudotime Trajectory and Cell-Cell Communication Analysis

To investigate the dynamic expression patterns of triaptosis-related genes (TRGs) within immune cells from keloid single-cell RNA-seq data, we conducted pseudotime trajectory analysis using the Monocle2 algorithm.[19] The process began with the construction of a mycds object via the newCellDataSet function, followed by rigorous filtering to select highly variable genes for downstream analysis. Dimensionality reduction was then performed using the reduceDimension function, applying the DDRTree algorithm to arrange cells along a developmental trajectory.

To further explore intercellular communication within the immune microenvironment, we employed the "CellChat" R package, a comprehensive framework designed for inferring and visualizing cell–cell interactions in single-cell transcriptomic data.[20] Using this tool, we generated a CellChat object, identified significantly overexpressed ligands and receptors, estimated the probability of interactions, and refined communication networks. The package also offers multiple intuitive visualization options, allowing for a clearer interpretation of signaling pathways and interaction dynamics among distinct cell populations.

SCENIC and Metabolism Analysis

To further elucidate the biological roles of differentially expressed genes (DEGs), we conducted Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses using the "clusterProfiler" R package.[21] In parallel, metabolic activity across different cell populations was evaluated through the "scMetabolism" package, which enables metabolism pathway scoring at the single-cell level.[22] For a more integrative assessment of pathway activity, we applied the "irGSEA" package, which incorporates multiple single-cell-based enrichment algorithms, including AUCell, UCell, singscore, ssGSEA, JASMINE, and Viper. By leveraging the robust rank aggregation (RRA) algorithm, irGSEA synthesizes enrichment outcomes across these methods to identify gene sets consistently enriched across analytical platforms.[23] To uncover underlying gene regulatory mechanisms, we employed the SCENIC (Single-Cell ENrichment of INteractive gene Clusters) framework. SCENIC facilitates the reconstruction of gene regulatory networks (GRNs) by integrating single-cell transcriptomic data with transcription factor (TF) motif analysis and expression correlations, allowing for the identification of cell type–specific regulatory programs.[24]

Immunofluorescence Staining

Immunofluorescence staining was performed on both keloid and normal skin tissues to evaluate the expression of ELMO2. The tissue samples were first fixed with 4% paraformaldehyde for 15 minutes at room temperature, followed by permeabilization using 0.1% Triton X-100 for 10 minutes. After blocking non-specific binding with 5% bovine serum albumin (BSA) for one hour, the tissues were incubated overnight at 4°C with a primary antibody targeting ELMO2 (green) at an appropriate dilution. Following primary antibody incubation, cells were washed with PBS and then incubated for one hour at room temperature with a secondary antibody conjugated to a fluorescent dye. The nuclei were stained with DAPI (blue) to visualize them. Images were then captured with a fluorescence microscope, using a 200 µm scale bar for both individual channels (ELMO2 and DAPI) and the merged images.

Statistical Analysis

Statistical analyses were performed using R software (version 4.2.1). The data were analyzed using either the Student's *t*-test or Wilcoxon test, and results were presented as mean ± standard deviation. P-values were adjusted using the false discovery rate (FDR) method. Heatmaps and volcano plots were generated and enhanced with the "ClusterGVis" and "scRNAtoolVis" R packages (https://github.com/junjunlab/ClusterGVis, https://github.com/junjunlab/scRNAtoolVis). The "SCP" R package, which provides an integrated approach for single-cell data analysis, was used to assist with bioinformatics visualization in this study (https://github.com/zhanghao-njmu/SCP). All statistical tests were two-tailed, with significance set at $p < 0.05$.

Results

Microenvironment Characteristics in Keloids

Figure 1 presents the design and outcomes of our single-cell RNA sequencing analysis. We integrated three publicly available keloid datasets (GSE163973) with three additional self-collected samples to achieve a comprehensive characterization of the keloid microenvironment. To investigate cellular heterogeneity and explore mechanisms underlying mesenchymal transition, we analyzed a total of six single-cell RNA sequencing datasets. After rigorous quality control, doublet removal, normalization, and unsupervised clustering, transcriptomes from 45,911 individual cells were retained for downstream analyses.

Following methodologies established in previous studies,[13,25] we identified distinct cell populations by validating clusters with canonical marker genes: fibroblasts (COL1A1, DCN, CFD), endothelial cells (CDH5, CLDN5, PECAM1), smooth muscle cells (TAGLN, ACTA2), mast cells (TPSAB1), keratinocytes (KRT5, KRT14), lymphatic endothelial cells (CCL21, LYVE1), sweat gland cells (SCGB1B2P, AQP5), T lymphocytes (CD3D, CD3E), myeloid cells (LYZ, CD68), neural cells (NRXN1), and melanocytes (TYRP1, PMEL).

Unsupervised clustering visualized by Uniform Manifold Approximation and Projection (UMAP) revealed 11 distinct cell types (Figure 2A). The distribution of these cell types across the six samples is shown in Figure 2B, with fibroblasts and endothelial cells being the predominant populations in all samples (Figure 2C). The expression of representative marker genes for each cell type is highlighted in Figure 2E, while a bubble plot (Figure 2F) further delineates the top marker genes associated with each cluster.

Additionally, we quantified cell type-specific differential gene expression, observing that endothelial cells exhibit the highest numbers of both upregulated and downregulated genes, whereas fibroblasts show the most pronounced upregulation (Figure 2G).

Importantly, we examined the expression patterns of 21 triaptosis-related genes (TRGs) across the identified cell types (Figure 2D). Among these, NFE2L2 and RAB7A displayed relatively high expression across multiple cell populations, suggesting their potential functional involvement in keloid pathology.

To demonstrate the effectiveness of batch correction and integration, Supplemental Figure 1A shows a UMAP plot with all cells color-coded by their respective datasets (self-sequencing or GSE163973), illustrating good mixing of major cell types post-Harmony integration. Supplemental Figure 1B provides a summary of the cell counts for each identified

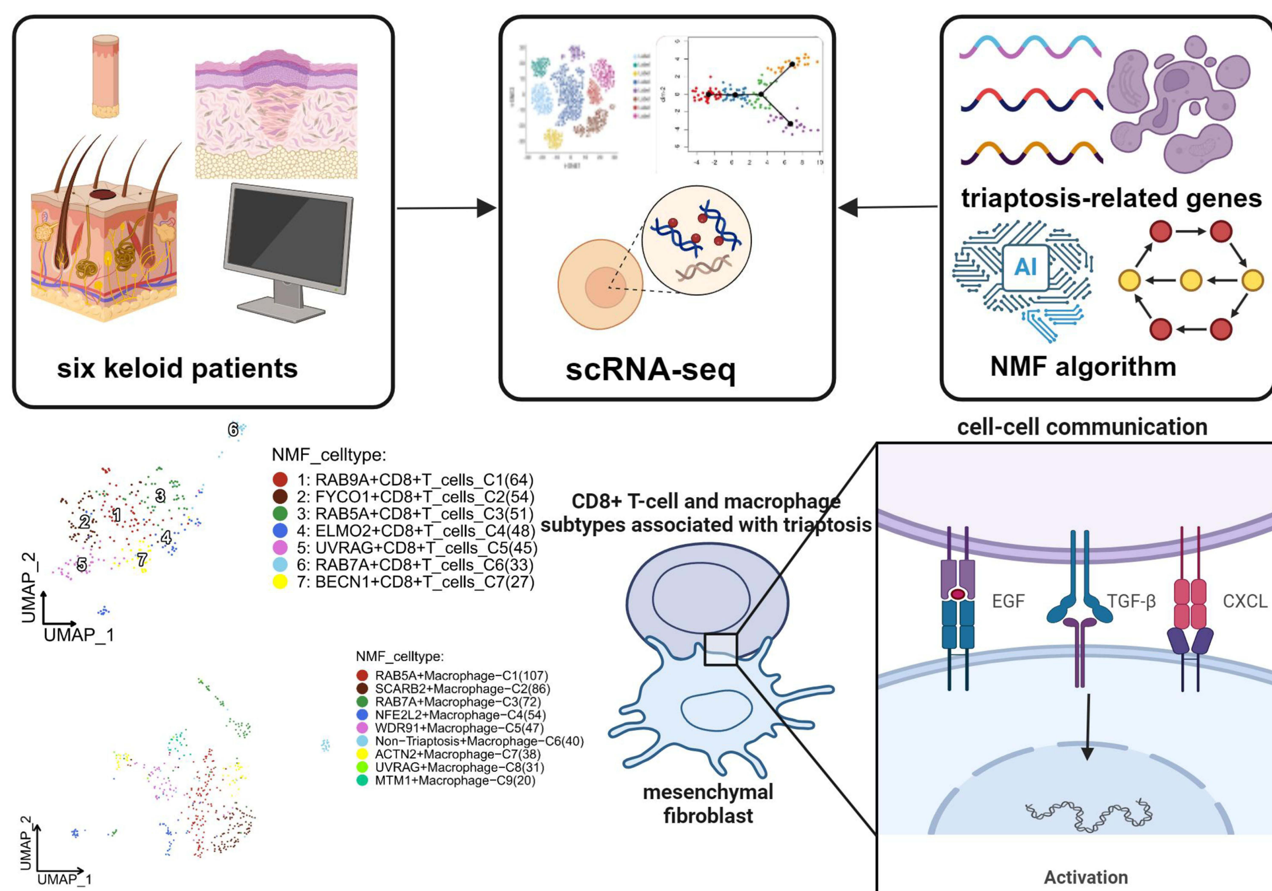

Figure 1 Schematic diagram of the single-cell sequencing workflow and results of this study.

cell type across datasets. This supplementary figure supports the reliability of our data integration and clustering procedures.

Together, these results provide a detailed landscape of cell composition and transcriptional changes within keloids, laying the groundwork for understanding the role of triaptosis in keloid development and progression.

Heterogeneity of Fibroblasts in Keloids

Fibroblasts play a central role in keloid formation through excessive proliferation and abundant collagen production, contributing to scar tissue development and nodule growth. To explore fibroblast heterogeneity, we reclustered 8941 high-quality fibroblast cells and identified 11 distinct clusters visualized by UMAP (Figure 3A). These clusters were annotated according to their marker gene expression profiles. Following a previous classification of dermal fibroblasts in healthy skin into four subgroups—secretory-papillary, secretory-reticular, mesenchymal, and pro-inflammatory[26]—we aligned our subpopulations accordingly. Specifically, THBS4$^+$, ELN$^+$, POSTN$^+$, and COL1A1$^+$ fibroblasts were categorized as mesenchymal fibroblasts; APCDD1$^+$ and ID1$^+$ fibroblasts as secretory-papillary fibroblasts; SLPI$^+$ fibroblasts as secretory-reticular fibroblasts; and APOD$^+$ and CCL19$^+$ fibroblasts as pro-inflammatory fibroblasts (Figure 3B). Notably, Figure 3B presents a bubble plot illustrating the expression of these key fibroblast marker genes across all subclusters, facilitating marker distribution comparison. To characterize transcriptional differences, volcano plots depict significantly upregulated and downregulated genes for each fibroblast cluster (Figure 3C). HALLMARK pathway enrichment analysis revealed POSTN$^+$, THBS4$^+$, and ELN$^+$ fibroblasts are positively associated with epithelial-mesenchymal transition and oxidative phosphorylation pathways, whereas COL1A1$^+$ fibroblasts correlate strongly with KRAS signaling but inversely with those pathways (Figure 3D). Further functional enrichment showed APOD$^+$ and POSTN$^+$ fibroblasts enriched in the TGF-β signaling pathway (KEGG), ELN$^+$ fibroblasts linked to neuronal

Figure 2 Single-cell atlas of keloids. (**A**) UMAP plot showing single-cell features colored by 11 major cell types in this study. (**B** and **C**) Bar chart and pie chart displaying the relative proportions of each major cell subtype in each sample. (**D**) Heatmap of TRGs expression in each cell type. (**E**) UMAP feature plots of typical marker genes for fibroblasts (COL1A1), endothelial cells (PECAM1), smooth muscle cells (TAGLN), mast cells (TPSAB1), keratinocytes (KRT5), lymphatic endothelial cells (LYVE1), sweat gland cells (SCGB1B2P), T cells (CD3D), myeloid cells (LYZ), neural cells (NRXN1), and melanocytes (PMEL). (**F**) Bubble plot showing the marker genes for all cell types. (**G**) The number of upregulated and downregulated genes in each cell type, including shared variable genes among different cells.

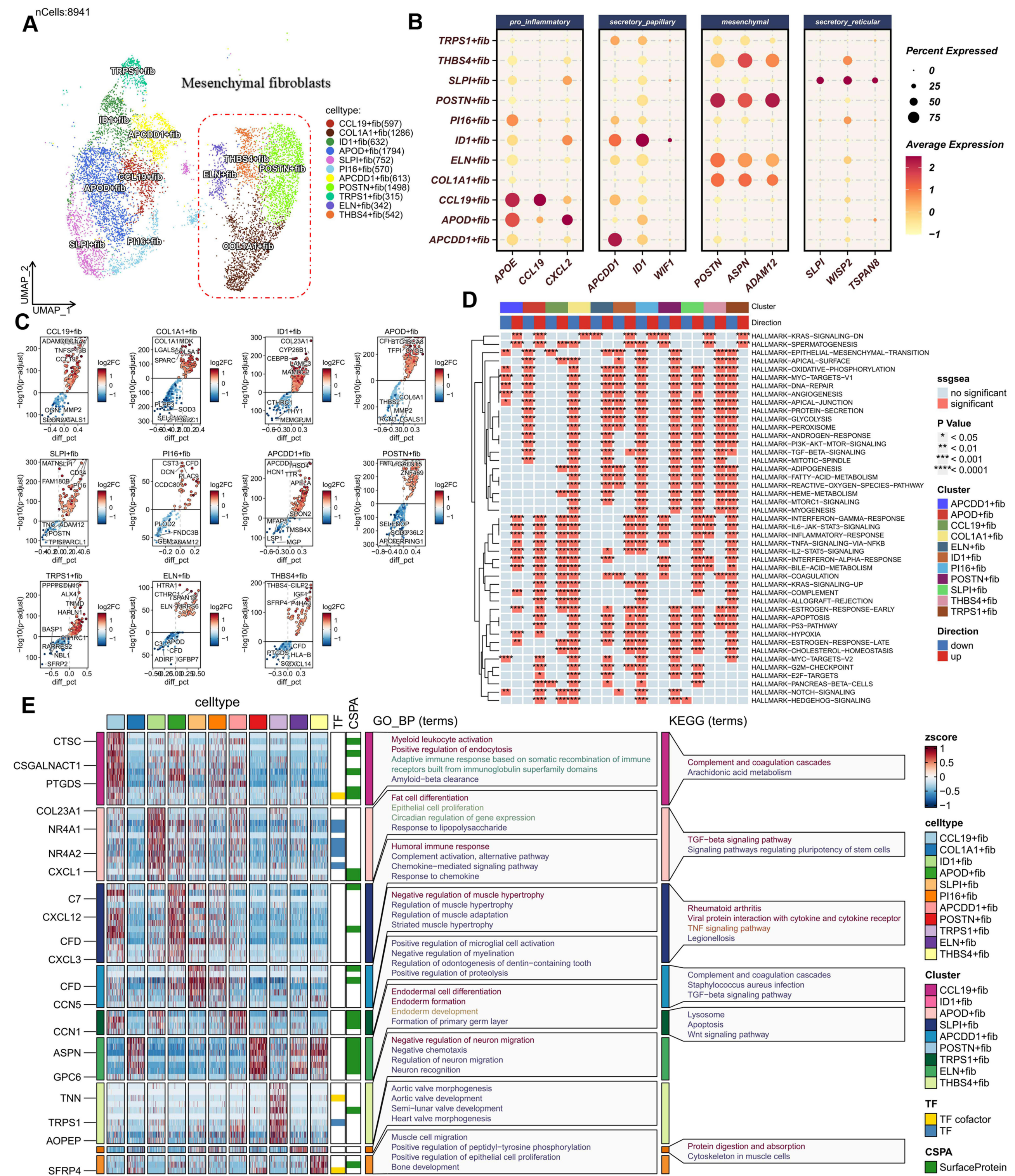

Figure 3 Heterogeneity of fibroblasts. (**A**) UMAP plot of 11 fibroblast subpopulations obtained from further clustering. (**B**) Bubble plot showing the expression of marker genes for the four subtypes: secretory-papillary, secretory-reticular, mesenchymal, and pro-inflammatory in the 11 fibroblast subpopulations. (**C**) Volcano plot displaying all upregulated and downregulated genes in the 11 fibroblast subpopulations. (**D**) HALLMARK enrichment analysis of the 11 fibroblast subpopulations. (**E**) GO-BP/KEGG enrichment analysis results for differential genes and their corresponding transcription factors and surface proteins in the 11 fibroblast subpopulations.

migration regulation (GO_BP), and THBS4^{+} fibroblasts associated with heart valve morphogenesis (Figure 3E). In addition, Supplemental Figure 1C provides UMAP visualization of the expression patterns of triaptosis-related genes (TRGs) across fibroblast subpopulations, highlighting heterogeneity in triaptosis signaling within the fibroblast compartment.

Characteristics of Triaptosis-Mediated T Cells in Keloids

Through the analysis of marker genes in T lymphocytes, we identified CD4+ T cells, CD8+ T cells, and NK cells (Figure 4A). Since most CD4+ T cells in keloids are predominantly naïve, we focused on CD8+ T cells for further clustering analysis. Pseudotime trajectory analysis of CD8+ T cell differentiation revealed that triaptosis-related genes (TRGs) such as RAB7A, ACTN2, RAB5A, KXD1, and WDR91 were progressively upregulated, while PIK3C3, KEAP1, and NFE2L2 showed a gradual downregulation over differentiation time (Figure 4B). Using NMF clustering, we identified seven distinct CD8+ T cell subclusters: RAB9A+ CD8+ T cells-C1, FYCO1+ CD8+ T cells-C2, RAB5A+ CD8+ T cells-C3, ELMO2+ CD8+ T cells-C4, UVRAG+ CD8+ T cells-C5, RAB7A+ CD8+ T cells-C6, and BECN1+ CD8+ T cells-C7 (Figure 4C). Intercellular communication analysis revealed that RAB5A+ CD8+ T cells-C3, UVRAG+ CD8+ T cells-C5, and mesenchymal fibroblasts had the most significant number of communication links (Figure 4D). Notably, UVRAG+ CD8+ T cells-C5 exhibited the most prominent TGF-β signaling activity (Figure 4E). We also analyzed transcription factor activity within the triaptosis-related NMF cell clusters and visualized the activity of eight transcription factors using UMAP (Figure 4G). A heatmap of transcription factor activity across the NMF clusters showed that RAB9A+ CD8+ T cells-C1 exhibited high expression of JUND, CREM, and MYBL1 (Figure 4F). Additionally, ELMO2+ CD8+ T cells-C4 had elevated exhaustion and cytotoxicity scores (Figure 4H). GO enrichment analysis revealed that UVRAG+ CD8+ T cells-C5 were associated with protein complex regulation and negative regulation of T cell differentiation, while ELMO2+ CD8+ T cells-C4 were linked to actin regulation (Figure 4I).

Characteristics of Triaptosis-Mediated Macrophages in Keloids

Building on previous research,[12] we categorized myeloid cells in keloids into subtypes such as monocytes, macrophages, Langerhans cells, and several dendritic cells (cDC1, cDC2, cDC3, pDC). Dimensionality reduction using UMAP revealed the distribution of these myeloid cell types (Figure 5A). Given the crucial role of macrophages in keloid formation—where they promote collagen synthesis and scar tissue proliferation through the secretion of cytokines and growth factors—we focused on macrophages for further subtype analysis. Pseudotime trajectory analysis of macrophage differentiation showed that triaptosis-related genes (TRGs) such as RAB5A, NFE2L2, and FYCO1 were predominantly expressed in the early stages, while genes like ELMO2 and NCKAP1 were upregulated in later stages (Figure 5B). Using NMF clustering, we identified nine distinct macrophage subpopulations: RAB5A+ macrophages-C1, SCARB2+ macrophages-C2, RAB7A+ macrophages-C3, NFE2L2+ macrophages-C4, WDR91+ macrophages-C5, non-triaptosis+ macrophages-C6, ACTN2+ macrophages-C7, UVRAG+ macrophages-C8, and MTM1+ macrophages-C9 (Figure 5C). RAB5A+ macrophages-C1 showed notable interactions with mesenchymal fibroblasts (Figure 5D). NFE2L2+ macrophages-C4 exhibited strong TGF-β, TNF, and EGF signaling, suggesting their involvement in keloid fibrosis (Figure 5E). Interestingly, both RAB7A+ macrophages-C3 and NFE2L2+ macrophages-C4 had prominent TGF-β signaling activity (Figure 5G). Further, we analyzed the metabolic activity across the nine triaptosis-related macrophage clusters. SCARB2+ macrophages-C2 displayed significantly enhanced metabolic activity, particularly in processes such as the tricarboxylic acid cycle, oxidative phosphorylation, and lipid metabolism (Figure 5F). Correlating the macrophage clusters with M1/M2 polarization markers revealed that NFE2L2+ macrophages-C4 had a higher M2 score (Figure 5H). A heatmap of transcription factor activity levels highlighted that NFIL3 was more active in NFE2L2+ macrophages-C4 (Figure 5I). GO enrichment analysis suggested that NFE2L2+ macrophages-C4 were linked to stress response and nuclear signaling pathways, while WDR91+ macrophages-C5 were associated with hematopoietic and lymphoid organ development (Figure 5J).

Figure 4 The role of triaptosis-mediated CD8+ T cells in the keloid microenvironment. (**A**) Isolation of CD4+ T cells, CD8+ T cells, and NK cells from T cells using specific marker genes, with UMAP displaying the distribution characteristics of these cells. (**B**) Heatmap of trajectory analysis for TRGs in T cells. (**C**) Further dimensionality reduction clustering of CD8+ T cells using NMF, with UMAP showing the distribution characteristics of the cells. (**D**) The number of communications between CD8+ T cell NMF clusters and mesenchymal fibroblasts. (**E**) Heatmap showing incoming and outgoing interactions between CD8+ T cell NMF clusters and mesenchymal fibroblasts. (**F**) Heatmap displaying significantly different TF activity among the seven CD8+ T cell NMF clusters based on AUCell average scores. (**G**) Expression levels of eight transcription factors in CD8+ T cells. (**H**) Heatmap showing T cell exhaustion and cytotoxicity associated with three CD8+ T cell NMF clusters. (**I**) GO-BP/KEGG enrichment analysis results for differential genes and their corresponding transcription factors and surface proteins in the seven CD8+ T cell NMF clusters.

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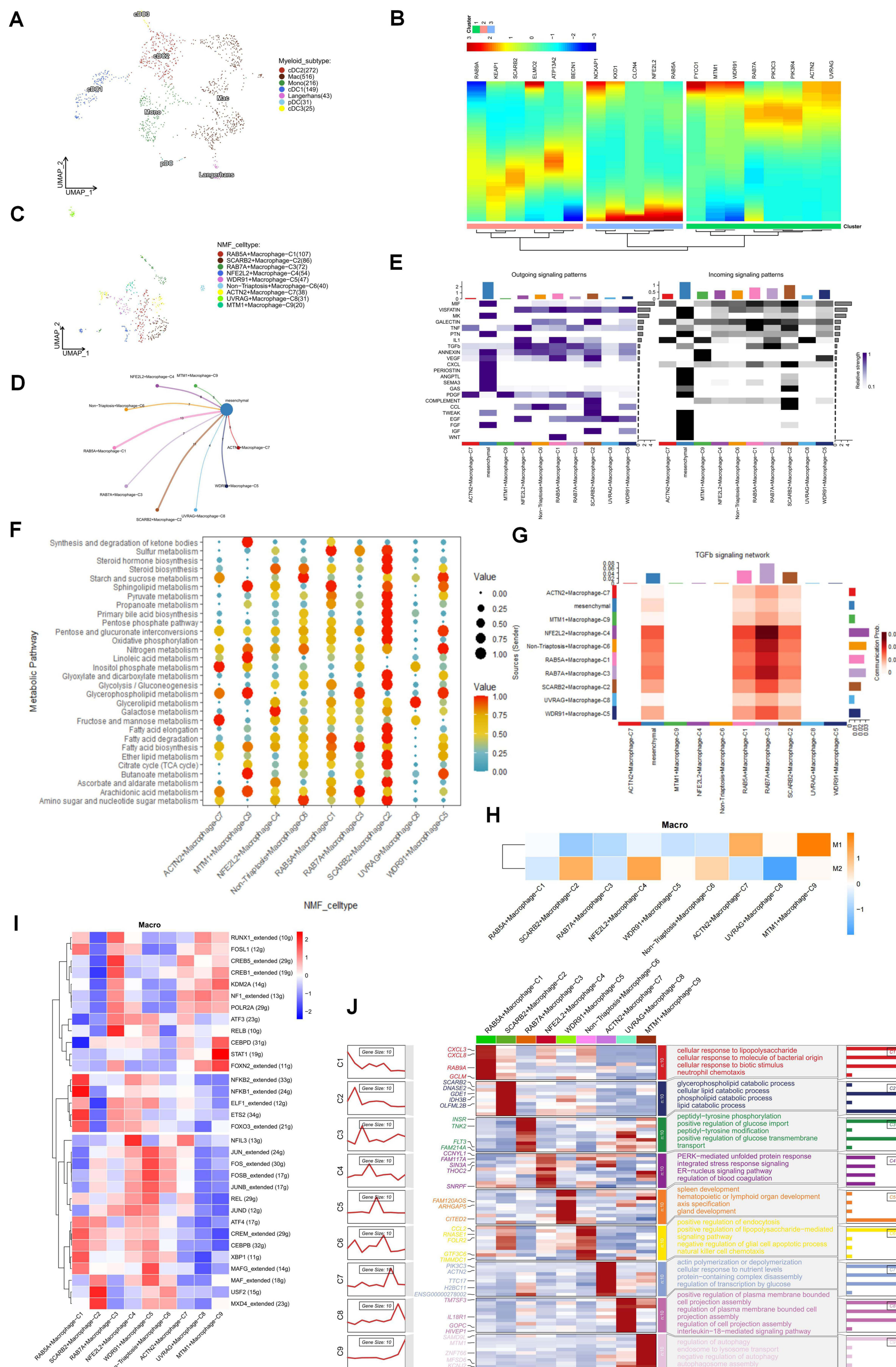

Figure 5 The role of triaptosis-mediated macrophages in the keloid microenvironment. (**A**) Isolation of macrophages, monocytes, and dendritic cells from myeloid cells using specific marker genes, with UMAP displaying the distribution characteristics of these cells. (**B**) Heatmap of trajectory analysis for TRGs in myeloid cells. (**C**) Further dimensionality reduction clustering of macrophages using NMF, with UMAP showing the distribution characteristics of the cells. (**D**) The number of communications between macrophage NMF clusters and mesenchymal fibroblasts. (**E**) Heatmap showing incoming and outgoing interactions between macrophage NMF clusters and mesenchymal fibroblasts. (**F**) Heatmap displaying significant differences in metabolic signaling pathways among macrophage NMF clusters. (**G**) Intensity of TGF-β signaling communication between nine macrophage NMF clusters and mesenchymal fibroblasts. (**H**) Polarization scores for nine macrophage NMF clusters based on M1 and M2 macrophage marker genes. (**I**) Heatmap showing significantly different TF activity among nine macrophage NMF clusters based on AUCell average scores. (**J**) GO-BP/KEGG enrichment analysis results for differential genes and their corresponding transcription factors and surface proteins in the nine macrophage NMF clusters.

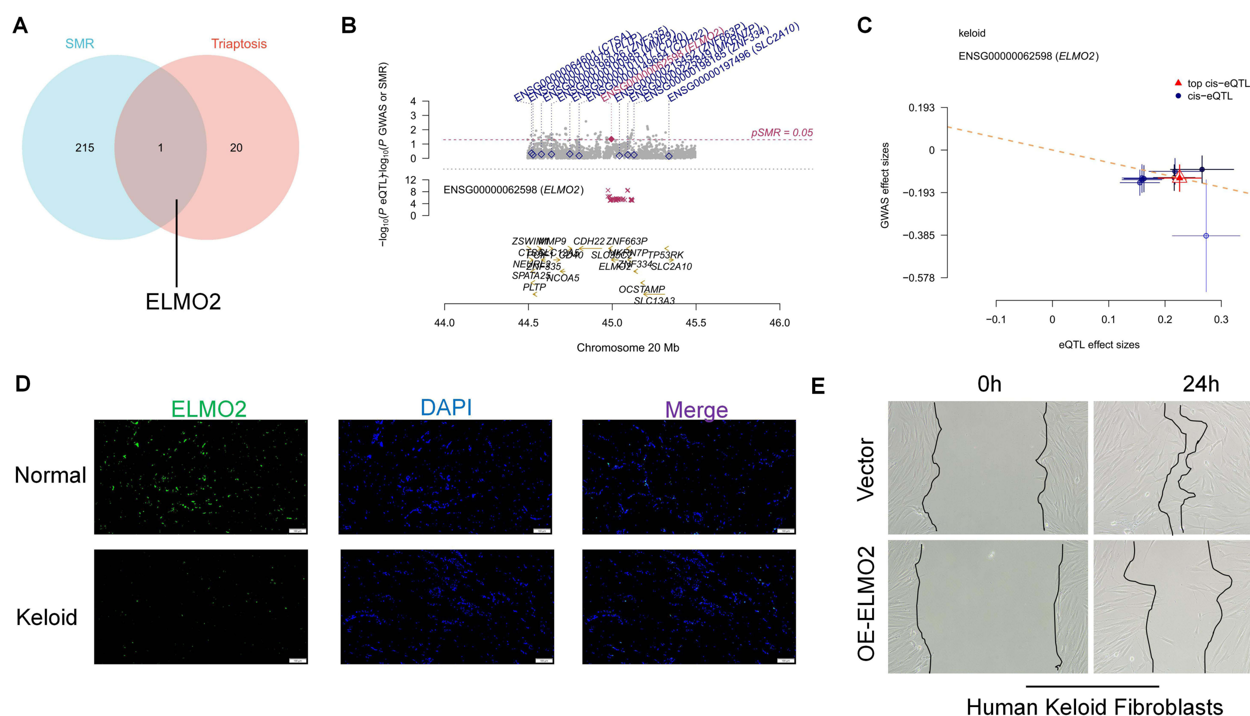

Figure 6 Reduced expression of ELMO2 increases the risk of developing keloids. (**A**) Venn diagram showing the intersection genes from SMR results and TRGs. (**B**) Loci for risk-related genes in keloids. (**C**) Effect sizes corresponding to risk gene ELMO2 from GWAS data used for HEIDI testing against SNP effect sizes. (**D**) Immunofluorescence staining of ELMO2 in normal tissue versus keloid tissue, showing the percentage of ELMO2+ cells in normal versus keloid tissues. (**E**) Scratch assays to examine the changes in cell migration ability. After overexpression of ELMO2, the migration ability of HKF cells significantly decreased.

Identifying ELMO2 as a Target for Triaptosis Research

Using GWAS data from keloid patients, we performed a summary data-based Mendelian randomization (SMR) analysis, which identified 216 susceptibility genes. By integrating these genes with triaptosis-related genes, we pinpointed ELMO2 as a key gene associated with keloid formation (Figure 6A). The SMR analysis also revealed relevant genetic loci for ELMO2, accompanied by corresponding SMR effect plots (Figure 6B and C). Immunofluorescence staining of skin tissue showed that ELMO2 expression was notably lower in keloids (Figure 6D). Furthermore, we overexpressed ELMO2 in human keloid fibroblasts (HKF) and conducted scratch assays to examine the changes in cell migration ability. We found that after overexpression of ELMO2, the migration ability of HKF cells significantly decreased (Figure 6E). These findings suggest that reduced expression of ELMO2 is linked to an increased risk of keloid development.

Discussion

In the field of keloid research, the role of programmed cell death has been a focal point of academic interest.[27–29] Although several studies have revealed the connection between programmed cell death and the pathogenesis of keloids, research on the potential pathogenic function of triaptosis at the single-cell level remains scarce. This study fills this gap by integrating publicly available and self-generated single-cell RNA-seq datasets, and by comprehensively analyzing the major cell types involved in triaptosis within the keloid immune microenvironment, marking our first step in exploring this area. We performed a systematic expression analysis of 21 triaptosis-related genes (TRGs) across 11 major cell types, revealing cell type-specific patterns and highlighting fibroblasts and CD8+ T cells as potential mediators of triaptosis signaling. Furthermore, this research unveils for the first time the complex interactions between triaptosis-related immune microenvironment cell subtypes and mesenchymal fibroblasts in keloids. This novel perspective not only deepens our understanding of how triaptosis affects various cellular components in the immune microenvironment but also provides new insights into its impact on the prognosis of keloid patients. Through these findings, we can more accurately delineate the role of triaptosis in keloid development and provide a scientific basis for developing new therapeutic strategies.

Fibroblasts play a central role in the pathogenesis of keloids, as they are crucial for synthesizing large amounts of extracellular matrix (ECM) and are closely associated with the occurrence and development of keloids.[30] Recent advances have revealed significant differences between the biological characteristics of fibroblasts in keloids and normal fibroblasts, including microstructural, metabolic, and proliferative properties.[31] Additionally, fibroblast heterogeneity is particularly pronounced in keloids. Our reclustering analysis of 8941 high-quality fibroblasts identified 11 transcriptionally distinct subclusters, which were further mapped onto four canonical fibroblast subtypes (mesenchymal, secretory-papillary, secretory-reticular, and pro-inflammatory), in accordance with previous definitions.[13] These subpopulations may play distinct roles in the formation and maintenance of keloids. TRG expression across fibroblast subtypes revealed that mesenchymal fibroblasts (eg, POSTN+ and THBS4+ clusters) had elevated expression of RAB7A, UVRAG, and NFE2L2, suggesting these subtypes may be actively engaged in triaptosis-associated signaling. These findings emphasize the complexity and diversity of fibroblasts in the pathological processes of keloids and provide new perspectives for future therapeutic strategies.[32]

In the field of pathological research on keloids, the key role of the immune microenvironment is increasingly recognized. Studies indicate that keloid formation is closely linked to abnormal inflammatory responses characterized by immune cell infiltration and complement component deposition, resembling skin manifestations in autoimmune disease patients. Inflammation plays a critical role in collagen synthesis and remodeling processes, with the severity of scarring positively correlated with the intensity of inflammatory responses.[33] T cells, particularly regulatory T cells (Tregs), play an important role in keloid development by promoting excessive collagen production through TGF-β secretion.[34] The high expression of fibrotic cytokines in Th2-type immune responses, along with the roles of IL-6 and IL-17 in maintaining a chronic pro-fibrotic state in keloids, further underscores the central position of inflammation in keloid formation.[35] In our dataset, CD4+ T cells represented a sizable portion of the T cell compartment. Although not deeply analyzed in this study, we observed expression of naïve T cell markers such as LEF1, TCF7, and CCR7 in a subset of CD4+ cells, supporting their identity as predominantly naïve. However, given our focus on TRG-related mechanisms, we prioritized the analysis of CD8+ T cells. Utilizing RNA-seq and immunohistochemical techniques, researchers have found significant increases in gene expression related to T lymphocyte activation and Th2, Th1, Th17/Th22 immune responses in keloid tissues, accompanied by enhanced immune cell infiltration.[36]

In keloids, macrophages exist in both M1 and M2 polarized states; M2 macrophages are closely related to tissue repair and fibrosis processes, with their phenotypic diversity suggesting that multiple macrophage subpopulations may exist within keloids.[37] These research findings not only reveal the multifaceted roles of the immune microenvironment in keloid formation but also provide valuable clues for developing future treatment strategies.

Our study reveals the complexity of CD8+ T cell subpopulations in keloids, particularly their potential roles in keloid development. CD8+ T cells play critical roles in many autoimmune diseases and chronic inflammatory states by influencing tissue damage and repair processes through cytokine secretion and direct cytotoxicity.[38] In keloids, CD8+ T cells may participate in abnormal fibrosis by affecting fibroblast activation and proliferation. We performed pseudotime and NMF clustering analysis of CD8+ T cells and identified seven distinct subpopulations, each with unique TRG expression profiles. Specifically, subclusters such as ELMO2+ CD8+ T cells (C4) and UVRAG+ CD8+ T cells (C5) exhibited elevated exhaustion and cytotoxicity scores, respectively, suggesting functional polarization. The observed changes in TRG expression may indicate specific functional states for T cells within keloids that differ from their roles in other immune-mediated diseases. For example, we found expression changes for genes such as RAB7A and ACTN2 in CD8+ T cells that are closely related to T cell activation, migration, and cytotoxic functions. These findings suggest that modulating T-cell activity could be a potential strategy for treating keloids. The diversity and polarization states of macrophages represent another important discovery from our research. The polarization states (M1/M2) of macrophages are closely linked to inflammation and tissue repair processes. In keloids, M2 macrophages may exacerbate fibrosis by promoting collagen synthesis and tissue repair.[39] We identified nine macrophage subclusters, including NFE2L2+ macrophages (C4), which showed high TGF-β, TNF, and EGF signaling activity. These signaling features suggest that this macrophage subtype may play a key immunoregulatory role in the keloid microenvironment, possibly promoting fibroblast activation and matrix remodeling. Furthermore, interactions between macrophages and fibroblasts may further amplify inflammatory responses and fibrotic signals, potentially driving the development of keloids. Our study also

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identified specific macrophage subpopulations such as NFE2L2+ macrophages-C4 that exhibit pronounced TGF-β, TNF, and EGF signaling output patterns, suggesting their potential role in advancing fibrosis within keloids. These macrophage subpopulations may play immunoregulatory roles within the immune microenvironment of keloids, providing new targets for future therapies.

In summary, the specific functional states and interactions between T cells and macrophages within the immune microenvironment of keloids may jointly drive their development. These cells shape the immune microenvironment through cytokine secretion, direct intercellular interactions, and metabolic activities. Our study emphasizes the need to consider the complexity of the immune microenvironment as well as the multifaceted roles of immune cells in fibrosis when developing treatments for keloids. Future research could further explore the specific mechanisms by which these immune cell subpopulations operate and how they respond to different therapeutic interventions to provide a scientific basis for developing new treatment strategies. Additionally, given the heterogeneity of keloids, future studies should also focus on differences in immune microenvironments across different patients or disease stages to achieve more precise treatments.

This study not only deepens our understanding of cellular interactions within the immune microenvironment of keloids through investigating single-cell triaptosis regulatory patterns but also provides a scientific basis for developing new therapeutic strategies. Future research needs to further explore the specific mechanisms by which triaptosis influences keloid development and its potential applications in immunotherapy. In particular, we identified ELMO2 as a key gene causally associated with keloids through GWAS data analysis; this could provide new biomarkers and therapeutic targets for diagnosing and treating keloids. ELMO2 is involved in regulating RAC1 signaling pathways, which play a central role in cell motility and phagocytosis.[40] In keloids, extracellular matrix remodeling and cell migration are critical factors contributing to their formation. The expression of ELMO2 may regulate these processes by influencing Rac1 signaling pathways, thereby reducing the risk of developing keloids. Given ELMO2's role in cell migration, signal transduction, and vascular integrity, therapeutic interventions targeting ELMO2 may represent a potential strategy for reducing keloid risk.[41] Enhancing ELMO2 function or modulating its downstream signaling pathways may help control the progression of keloids.

Conclusion

In summary, this study delineates the complexity and heterogeneity of triaptosis regulatory patterns across key cellular components in the keloid immune microenvironment. Specifically, we identified that $CD8^+$ T cells undergo distinct transcriptional changes associated with triaptosis, including altered expression of key genes such as RAB7A, ACTN2, and ELMO2, which may influence cytotoxicity and exhaustion. Among macrophage subpopulations, $NFE2L2^+$ macrophages displayed prominent activation of TGF-β, TNF, and EGF signaling, implicating their role in promoting fibrosis. Additionally, mesenchymal fibroblast subsets (eg, $POSTN^+$, $THBS4^+$) showed higher triaptosis-related gene expression, indicating their involvement in fibrotic remodeling. These findings collectively suggest that triaptosis contributes to aberrant immune regulation and fibrosis in keloids via cell-type-specific pathways. By uncovering these distinct cellular responses, our study provides a conceptual framework and scientific rationale for developing targeted therapeutic interventions focused on triaptosis modulation. Future investigations should validate these findings in vivo and explore their clinical translational potential.

Data Sharing Statement

Publicly available datasets were analyzed in this study. The raw data for this study were obtained from GEO (http://www.ncbi.nlm.nih.gov/geo/) databases. Raw sequencing data will be provided upon reasonable request.

Ethics Approval and Consent to Participate

This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (Approval No: 2024-SR-383). This research was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants involved in the study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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