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Application and Perspectives of Single-Cell Transcriptomics in Unraveling the Pathogenic Mechanisms of Keloids

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Abstract: Keloid is a pathological fibrous proliferation disease characterized by tumor-like growth features. Its occurrence is closely related to the abnormal activation of fibroblasts and excessive deposition of the extracellular matrix. Due to the unclear pathogenesis, clinical treatment faces high recurrence rates, and there is a lack of safe and effective preventive and intervention methods. Single-Cell RNA Sequencing, scRNA-seq provides a new tool for revealing complex microenvironmental changes by systematically analyzing the transcriptional characteristics, heterogeneity, and interactions of various cells in Keloid tissues at the single-cell resolution. This review focuses on the application progress of scRNA-seq in Keloid research, systematically summarizing exploratory findings in characterizing key cell types such as fibroblasts, endothelial cells, Schwann cells, and immune cells, constructing cell communication networks, and screening potential therapeutic targets. The aim is to provide theoretical support for a deeper understanding of its pathogenesis and development of precise treatment strategies.

1. Introduction

Keloid is a pathological fibrous hyperplasia disease characterized by abnormal proliferation of fibroblasts after skin tissue injury, leading to abnormal accumulation of Extracellular Matrix (ECM) and invasion into surrounding tissues. This proliferative pattern resembles benign tumors but does not involve distant metastasis. Keloids often protrude above the skin lesions, affecting appearance and causing discomfort such as pain and itching. Long-term presence can severely impact patients' quality of life and mental health, even leading to functional impairments in limbs. Keloids commonly occur on the anterior chest, upper back, shoulders, chin, earlobes, and auricles. Among these, chest and back keloids have the highest incidence and a genetic predisposition. According to the Chinese Keloid Epidemiology Study based on the HQMS database, from 2013 to 2018, a total of 21,777 cases of keloid hospitalizations were reported, accounting for 12.26% of all keloid hospitalizations, with this proportion showing an annual upward trend. Among these, 93.38% were Han Chinese patients, and 59.25% were female patients. Treatment outcomes are less favorable, with a high recurrence rate and higher treatment costs[1].

The formation of keloids is the result of multiple complex factors working together, involving genetic susceptibility, chronic inflammatory responses, sustained mechanical stress, tissue hypoxia, autoimmune abnormalities, and metabolic dysfunction, among other internal and external

environmental factors. Pathologically, keloids exhibit abnormal activation and proliferation of fibroblasts, excessive deposition of collagen fibers, particularly type I and III collagen, persistent high expression of fibrogenic cytokines such as transforming growth factor β (TGF-β), and dysregulation of gene and epigenetic regulatory mechanisms related to wound repair. Additionally, there is a significant increase in neovascularization and abnormal proliferation of nerve fibers in scar tissue, further exacerbating local microenvironment imbalance and driving the continuous progression of keloids. The distribution of different cell types, the randomness of gene expression, and the communication network between cells in keloids have not been fully elucidated. Coupled with changes in transcriptomics and epigenetics, this makes it difficult to precisely analyze the cellular composition of keloids, which is one of the main obstacles to achieving precision medicine. Although high-throughput sequencing provides information on gene expression levels for understanding keloids, traditional sequencing techniques like Bulk RNA Sequencing (Bulk RNA-seq) can only analyze mRNA in whole cells or tissues, overlooking certain unique transcription profiles and obscuring the discovery of specific cell subpopulations. Therefore, higher-resolution methods are needed to reveal the cellular composition and individual cell-specific transcriptomes in keloids.

Emerging single-cell sequencing technologies have demonstrated outstanding application potential in high-throughput sequencing of genomes, transcriptomes, and epigenomes. Among these, Single-Cell RNA Sequencing (scRNA-seq) is the most widely used, capable of reconstructing cell transcriptomes at single-cell resolution. This provides a powerful tool for in-depth studies on intercellular heterogeneity, cell expression profiles, and cell interactions within complex tissues.

This paper systematically reviews the pathogenesis of keloids and the progress of scRNA-seq technology in the study of the microenvironment of keloids, focusing on the heterogeneity of fibroblasts, endothelial cells, Schwann cells, and immune cells, as well as their gene expression patterns. It summarizes key cell subpopulations and their regulatory genes identified by multiple scholars in recent years, and proposes potential molecular therapeutic targets.

2. Introduction to single cell transcriptome sequencing methods and technical steps

The workflow of single-cell transcriptome sequencing technology includes sample preprocessing, single-cell isolation, cell lysis, nucleic acid amplification, library construction, data standardization, and dimensionality reduction visualization. First, single-cell isolation can be achieved using microplate methods (such as Smart-seq2, CEL-seq2) or microfluidic droplet methods (such as 10x Genomics, inDrop). The former offers high sensitivity but limited throughput, while the latter is suitable for large-scale, high-throughput analysis, especially in rare cell type studies. Next, cell lysis is performed through physical, chemical, or enzymatic methods to extract high-quality RNA, whose integrity directly affects the accuracy of subsequent amplification and sequencing. During the amplification phase, novel quasi-linear amplification techniques like MALBAC-RNA are employed, effectively reducing bias and error rates in traditional PCR amplification, enhancing gene coverage and expression reliability. Following this, the sequencing library is constructed and data standardized, generating a single-cell expression matrix with specific molecular labels. On platforms such as 10x Genomics or BD Rhapsody, systematic noise reduction and normalization processing are conducted using tools like Seurat and Scran. Finally, principal component analysis (PCA) is used for initial dimensionality reduction, complemented by nonlinear methods such as t-SNE or UMAP to visualize high-dimensional data in two-dimensional space. Among these, UMAP has become the mainstream method for single-cell data visualization due to its ability to balance local and global structures, high computational efficiency, and strong reproducibility. This process provides a strong technical support for the in-depth analysis of heterogeneous cell populations.

3. Application of single cell transcriptome sequencing technology in keloid

The main process of scRNA-seq technology in keloid research includes: obtaining biopsy tissue samples from keloid patients, normal skin around keloids, and healthy controls, preparing single-cell suspensions, constructing sequencing libraries, and performing in vitro sequencing and data analysis. The pathological characteristics, sampling sites, and sample processing methods of clinical specimens significantly influence the composition of cell subpopulations within the tissues, so these factors must be strictly controlled to ensure the reliability of the research results. Through scRNA-seq technology, it is possible to deeply reveal the compositional features, cellular heterogeneity, dynamic changes, and regulatory mechanisms of the cellular microenvironment in keloid tissues; identify specific cell subpopulations and key regulatory pathways involved in keloid formation and pathological characteristics; analyze the functional status, differentiation trajectories, gene expression changes at the transcriptome level, and interactions between cells, thereby identifying potential cell subpopulations and therapeutic targets. Additionally, scRNA-seq technology can facilitate the exploration of immune cell infiltration mechanisms and provide deeper insights into the functions of various biomolecules in the pathogenesis of keloids. This is crucial for a more comprehensive understanding of the pathological mechanisms of keloids and the development of more effective treatment strategies.

3.1 ScRNA-seq technology reveals the heterogeneity of scar fibroblasts

It is widely believed that the abnormal proliferation of fibroblasts and excessive deposition of ECM are important mechanisms in pathological scar formation. Under the stimulation of long-term chronic inflammation, the production of various cytokines (such as TGF-β1, IL-1, IL-13, TNF, CTGF, PDGF, VEGF, FGF, IGF), chemokines (such as CCL2, CCL3, CXCL1), and proteolytic enzymes (such as MMP2) increases, especially in areas with high mechanical tension in the skin and individuals with genetic susceptibility. These factors promote the abnormal proliferation of fibroblasts and their differentiation into myofibroblasts (mFB) that secrete collagen, leading to excessive secretion of ECM and ultimately the formation of over-proliferative scar tissue, which may even develop into keloids.

To deeply analyze the heterogeneity of fibroblasts in keloids and their pathogenic effects, Deng et al. [2] Applied scRNA-seq technology to compare the fibroblast heterogeneity between normal scar tissue and keloid tissue. Based on the expression characteristics of specific marker genes, they divided the fibroblasts in keloids into four major subpopulations: secretory-papillary fibroblasts (SPF), secretory-reticular fibroblasts (SRF), mesenchymal fibroblasts (MF), and pro-inflammatory fibroblasts (PF). The study found that the proportion of the MF subpopulation was significantly increased in keloid tissue, and its expression profile indicates that the MF subpopulation is involved in collagen synthesis and degradation, wound healing, skeletal system development, and osteoblastic differentiation, accompanied by significant upregulation of related genes such as POSTN, COL1α1, and COL12α1. The SPF and SRF subpopulations mainly participate in ECM remodeling and protease activity regulation, while the PF subpopulation primarily exerts chemotactic factor activity, participating in inflammatory responses. In addition, through the analysis of receptor-ligand interactions on cell surfaces, the study revealed extensive and close signaling exchanges between the MF subpopulation and other cells within keloids, particularly the interaction of the POSTN-ITGAV/ITGB5 fibrosis signaling axis. Further functional validation experiments showed that blocking the interaction between POSTN and its integrin receptor (ITGAV/ITGB5) significantly reduced the activity of fibroblasts in keloids and markedly inhibited the expression of collagen types I and III. These results highlight the critical role of the MF subpopulation and the POSTN-mediated fibrosis pathway in the pathogenesis of keloids, providing new potential therapeutic targets for clinical treatment.

To further explore the functional relationships among different subpopulations of mesenchymal fibroblasts in keloids, Deng et al. [2] also applied Diffusion Pseudotime (DPT) analysis methods and conducted gene ontology (GO) analysis and gene set enrichment analysis (GSEA). They found that the sC4 subpopulation of mesenchymal fibroblasts was significantly enriched in collagen fiber tissue, bone development, and ossification-related pathways, with higher expression levels of mesenchymal precursor-related genes (such as POSTN, ADAM12, COL11A1). This suggests that the sC4 subpopulation exhibits more pronounced mesenchymal characteristics compared to other subpopulations. Similarly, Shim et al. [3] used scRNA-seq and quasi-time series analysis to reveal that fibroblasts gradually differentiate from the FB4 subpopulation in normal skin into the predominant FB1 and FB2 subpopulations in keloids, with the latter also enriched in bone development and ECM remodeling pathways, demonstrating significant mesenchymal features. Both studies highlight the high expression of key genes such as POSTN and ADAM12 in keloids, emphasizing the central role of mesenchymal-related pathways in their pathogenesis and providing important clues for the development of potential therapeutic targets.

Myofibroblasts (mFB) are key effector cells in the fibrotic process, bridging smooth muscle cells and fibroblasts in terms of ultrastructure and physiological function. Shim et al. [3] 's study identified mFB subpopulations in keloids by detecting the expression of known marker genes (such as ACTA2 and TAGLN) in mFB, revealing that multiple key genes associated with keloid fibroblasts are highly specifically expressed in this subpopulation. This suggests that resident fibroblasts in tissues may be the primary source of abundant ECM-secreted mFB, further supporting the critical role of continuously activated mFB in the pathological formation of keloids. Additionally, Liu et al. [4]' s research team used quasi-time series analysis to reveal the dynamic process of keloid fibroblast transformation into mFB phenotype and elucidated the key regulatory mechanisms involved. The study found that the pro-fibrotic transcription factor TWIST1 is highly expressed early in differentiation, potentially playing a central role in promoting the conversion of fibroblasts to mFB. This effect was confirmed through experiments using TWIST1 inhibitor harmine, suggesting that TWIST1 is a potential therapeutic target. Deng et al. [2]'s study also confirmed that mFB labeled with ACTA2 are mainly distributed in the MF subpopulation, indicating that MF plays a crucial role in the excessive collagen synthesis in keloids. Overall, these studies collectively emphasize the importance of fibroblast transformation into mFB phenotype in the formation of pathological scars and reveal the central role of mFB in the fibrotic process of keloids. However, at present, there is still a lack of in-depth functional experiments, in vitro experiments and animal model verification. In the future, these experimental data should be further supplemented to strengthen the clinical value and reliability of the research conclusions.

3.2 ScRNA-seq technology reveals the heterogeneity of scar endothelial cells

The structure of the skin vascular system is extremely intricate, with arteries, veins, and capillaries differing in both structure and function. The inner layer of all blood vessels is composed of vascular endothelial cells (VECs). VECs play a crucial role in regulating metabolic pathways, recruiting immune cells, and inducing various inflammatory skin diseases. Angiogenesis driven by these cells is an essential step in the development of fibrotic diseases. Li et al. [5] analyzed 10 normal human skin tissues using scRNA-seq and classified skin endothelial cells into five subtypes based on the expression characteristics of classic marker genes: arterial endothelial cells, capillary endothelial cells, post-capillary venous endothelial cells, venous endothelial cells, and lymphatic endothelial cells. Further analysis using KGEE enrichment analysis and GSEA revealed significant heterogeneity among different subtypes of skin endothelial cells in terms of metabolic pathways, immune responses, and adhesion infiltration capabilities.

The description of the characteristics of Keloid vascular endothelial cells (KVECs) in keloid lesions reveals similar subtypes. Shim et al. [3] identified multiple subpopulations of keloid vascular endothelial cells (KECs) using scRNA-seq technology, with KEC3 being predominant in the lesion and characteristically overexpressing genes such as POSTN, FN1, and TGFBR2, which are closely associated with wound repair. Multiple immunofluorescence staining confirmed the co-expression of POSTN with vascular endothelial marker CD31, suggesting that KECs may have undergone a mesenchymal phenotype transformation. Spatial transcriptomics (ST) further revealed that key molecules of the TGF-β pathway, TGFB1 and TGFB3, and their receptor TGFBR2 are significantly co-expressed in the perivascular region of the dermis, indicating that TGF-β signaling has distinct spatial specificity in the keloid microenvironment. This signaling may contribute to the disease process by promoting mesenchymal activation of endothelial cells and interactions between blood vessels and fibroblasts.

In addition, Liu et al. [6] used scRNA-seq technology to perform unbiased clustering analysis on scar keloid vascular endothelial cells, identifying four subpopulations with unique marker gene expression profiles, further confirming the high heterogeneity of scar keloid vascular endothelial cells in lesions. Analysis of intercellular communication revealed that the VEGFR signaling pathway is significantly activated in scar keloid vascular endothelial cells, suggesting its critical role in pathological angiogenesis. Moreover, the Eph-ephrin signaling pathway, closely associated with myocardial fibrosis, also undergoes significant changes in scar keloid vascular endothelial cells, particularly the EFNB2-EPHA4 pair, which may regulate the transcriptional state of fibroblasts and smooth muscle cells, promoting angiogenesis and exacerbating tissue fibrosis. These findings highlight the complex regulatory network of scar keloid vascular endothelial cells in the development and progression of scar keloids and provide potential targets and theoretical basis for the development of targeted therapeutic strategies.

3.3 ScRNA-seq technology reveals the heterogeneity of scar schwann cells

ScRNA-seq studies on keloids have traditionally focused on fibroblasts and vascular endothelial cells. However, itching and pain, as the primary clinical symptoms of keloids, suggest that Schwann cell (SC) cells play a potential role in keloid formation and should gradually become a new focus of research. Schwann cell cells exist in both myelinated and non-myelinated states, both originating from immature Schwann cell cells and participating together in neural development and injury repair. Schwann cell cells can secrete various cytokines and growth factors, promoting neuronal repair and maintaining the integrity of neural function.

To gain a deeper understanding of the heterogeneity of Schwann cells in keloids, Direder et al. [7] found that the proportion of Schwann cells in keloids was significantly increased, exhibiting a typical spindle shape, with morphological characteristics similar to those of Schwann cells in the repair state. Further subcluster analysis identified four keloid-specific Schwann cell subtypes: SC-Keloid, SC-EC, SC-FB, and SC-Prolif. The SC-Keloid subtype significantly expressed genes related to ECM synthesis (such as NES, IGFBP3, IGFBP5, TGFBI, TNFAIP6), with functional enrichment in pathways such as ECM synthesis, wound repair, and connective tissue development. The SC-EC and SC-FB subtypes co-expressed markers of vascular endothelial cells and fibroblasts, indicating the potential for Schwann cells to differentiate into other lineages. The SC-Prolif subtype is characterized by high expression of proliferation-related genes such as Ki-67 and TOP2A, and double staining with Ki-67 and NGFR confirmed their in situ proliferative behavior in keloid tissue. Additionally, compared to mature Schwann cells in normal skin that express myelin genes like MBP and PLP1, Schwann cells in keloids generally exhibit demyelination and immaturity, suggesting they may be in a continuous repair or activation process. The study also validated the expression of characteristic

genes of each subtype at the protein level through immunofluorescence, enhancing the reliability of the transcriptomic results.

Based on this study, Direder et al. [8] further integrated multiple scRNA-seq datasets from normal skin, normal scars, keloids, and adjacent skin to reduce bias caused by differences in sample sources and processing. They systematically validated the characteristics and distribution of Schwann cells in keloids. The results showed that Schwann cells were significantly enriched in both keloids and adjacent skin, while no clear clusters of Schwann cells were observed in normal scars, suggesting their possible specific involvement in the pathological process of keloids. Through time-series trajectory analysis, it was found that Schwann cells may differentiate from mature myelinated and non-myelinated states into a proliferative, fibroblast-like, and endothelial-like cell population, forming subtypes with fibrotic potential. Particularly at the branching points of the trajectory, key transcription factors such as c-JUN, STAT3, JUNB, KLF4, and EGR1 were upregulated and confirmed to be nuclear-localized in NGFR-positive Schwann cells, indicating their important regulatory role in the dedifferentiation and functional reprogramming of Schwann cells. Among these, the activation of AP-1 family and KLF family transcription factors may be closely related to cellular stress, inflammatory responses, differentiation maintenance, and regeneration processes. These findings further support the high plasticity of Schwann cells in keloids and suggest that they may mediate functional transitions in tissue repair and fibrosis through the restructuring of transcription programs.

The most prominent pathological feature of keloids is the abnormally active fibroblast proliferation and ECM deposition, leading current research to focus on the intercellular communication between Schwann cells and fibroblasts. A scRNA-seq study of ear keloids specifically analyzed the relationship between Schwann cells, fibroblasts, and vascular endothelial cells that promote ECM formation[9]. The intercellular communication network revealed that the number of interactions among cells in keloid samples is richer than in normal skin, and Schwann cells strongly interact with mesenchymal fibroblasts and vascular endothelial cells through SEMA3C signaling pathways, which primarily regulate cell proliferation and migration. Additionally, Schwann cells influence vascular endothelial cells via the VEGF signaling pathway, further promoting their proliferation. Conversely, fibroblasts and vascular endothelial cells promote Schwann cell proliferation and migration through Midkine/PTN family-related signaling pathways. This study also compared data from ear and chest-back keloids to investigate whether the pathological mechanisms of keloids in different sites are site-specific. Preliminary results indicate that interactions between Schwann cells and subpopulations of ECM-promoting cells are site-specific, and this difference may be related to anatomical variations in neural tissues at different sites. However, further in vitro and in vivo experiments, animal model studies, and more comprehensive molecular biological validation are needed to confirm this site specificity.

3.4 Structure of immune microenvironment of keloid

Scar tissue is histologically characterized by irregular collagen fibers and infiltration of inflammatory cells. Many studies have shown that inflammation plays a crucial role in regulating collagen synthesis, with the intensity of inflammation positively correlated with the final size of scars. Some scholars define keloids as pathological scars with significant inflammatory responses, while hypertrophic scar (HS) are defined as scars with milder inflammatory responses. Therefore, the inflammatory response, as a key factor in the scar formation process, can further trigger subsequent immune response cascades, ultimately leading to the formation of pathological scars.

3.4.1 Heterogeneity of macrophages

The heterogeneity of macrophages plays a crucial role in the development and progression of keloids. The Mononuclear Phagocyte System (MPs), composed of monocytes, macrophages, and dendritic cells, is key in tissue remodeling and inflammatory responses. After skin injury, monocytes are recruited to the site of damage and differentiate into various types of macrophages under the regulation of local inflammatory conditions and growth factors, cytokines, and other factors, thereby participating in injury repair and the modulation of inflammatory responses.

The prevailing view is that M1 macrophages primarily play pro-inflammatory and anti-fibrotic roles in the early stages of wound repair. They mediate cell clearance and collagen degradation by secreting cytokines such as IL-1β, IL-6, CCL2, CCL7, TNF-α, and MMPs. In contrast, M2 macrophages promote collagen deposition and inhibit inflammatory responses during the later stages of wound healing. They also participate in tissue remodeling and fibrosis by secreting factors like TGF-β, PDGF, and VEGF. During keloid formation, insufficient activation of M1 macrophages in the early stage leads to low levels of pro-inflammatory factor expression, potentially weakening the initial inflammatory response. In the late stage, the delayed but continuously enhanced activation of M2 macrophages, along with their ongoing anti-inflammatory and pro-fibrotic functions, promotes excessive collagen deposition and abnormal scar proliferation. Additionally, M2 macrophages are highly concentrated at the edges and superficial regions of keloids, a distribution characteristic consistent with their invasive behavior of expanding into surrounding normal tissues. These features indicate that the spatial distribution and cellular state of macrophages play a crucial role in keloid formation.

To explore the immunological characteristics of keloids and determine the heterogeneity of immune cells, Feng et al. [10] conducted scRNA-seq analysis on 12 keloid samples and adjacent normal skin samples, finding a significant increase in the proportion of macrophages in keloid tissue, suggesting their potential dominant role in inflammatory responses and fibrosis. Further analysis revealed that M2 macrophage subpopulations were predominant in keloids, exhibiting characteristic transcriptional features such as upregulation of mitochondrial oxidative phosphorylation-related genes (MT-CO1, MT-CYB, MT-ND5, MT-CO3) and ECM synthesis-related genes (COL1A1, SPARC), while the expression of MMP9, an ECM degradation gene, was relatively lower. Through time-series trajectory analysis and hotspot module identification, the authors divided macrophages into 13 functional modules. Among these, Module 2 is located at the edge of the scar, enriched with anti-inflammatory and tissue repair-related genes such as RNASE1, C1QA, CD163, CD14, C1QC, FCGRT, MS4A7; Module 10 is concentrated within the scar, significantly upregulating genes related to lipid metabolism and proteolysis such as APOC1, CTSB, CTSL, TYROBP. Both modules exhibit transcriptional features similar to those of tumor-associated macrophages (TAMs), suggesting that they may play an immune regulatory role similar to that of the tumor microenvironment by promoting M2 polarization, regulating lipid metabolism, and matrix protein degradation in the formation and maintenance of keloids. Researchers further utilized CellPhoneDB for cell communication analysis and found significantly enhanced ligand-receptor interactions between macrophages and various subtypes of fibroblasts in keloid tissue. These interactions primarily include the TGFB2-TGFBR2, TGFB3-TGFBR3, CCL2-CCR1, and CCL2-CCR5 pathways. These specific signaling axes not only regulate the polarization state of macrophages but also modulate their transcription programs by activating key transcription factors (such as RB1 and ATF4). This establishes a fibrosis signaling network centered around macrophages, which collaboratively promotes fibroblast activation and collagen deposition, driving the continuous proliferation and remodeling of scar tissue.

However, there are still shortcomings in the current research. First, existing scRNA-seq studies lack systematic validation through functional experiments and require further confirmation of the reliability of single-cell data using multidimensional methods such as in vitro cell function assays and

animal models. Second, the dynamic changes of macrophage subpopulations during the formation and development of keloids remain unclear, and their specific mechanisms need to be further elucidated. Additionally, the synergistic interactions between macrophages and other immune cells in the immune microenvironment of keloids require deeper exploration to better understand the pathological mechanisms of keloids and provide theoretical foundations for the development of new therapeutic strategies.

3.4.2 Heterogeneity of T cells

T cells exhibit high heterogeneity in the development and progression of keloids. They play both cytotoxic and immunosuppressive roles in the immune microenvironment, demonstrating dual functional characteristics. Therefore, systematically analyzing the composition and functional status of T cell subpopulations in keloids is crucial for gaining a deeper understanding of their immune regulatory mechanisms and identifying potential therapeutic targets.

Feng et al. [10] performed dimensionality reduction and clustering analysis on scRNA-seq data from keloid tissue, identifying multiple functionally heterogeneous subpopulations of CD4+ and CD8+ T cells. Among these, CD4+ T cells include initial T cells (highly expressed CCR7, LEF1, TCF7), effector memory T cells (GZMK, CCL5, IFNG), and regulatory T cells (Treg, highly expressed FOXP3, IL2RA, CTLA4); CD8+ T cells include effector T cells (highly expressed GZMB, PRF1, IFNG), mucosal-associated invariant T cells (MAIT, highly expressed RB1, SLC4A10, RORC), and proliferative T cells (highly expressed MKI67, TOP2A, STMN1). Functional enrichment analysis revealed that these subpopulations are involved in primary immune responses, maintenance of immune memory, anti-infection and anti-tumor reactions, immune tolerance, and cell proliferation. Notably, CD8+ effector T cells dominate in keloids, and immunohistochemistry further confirmed their extensive infiltration in the tissue, suggesting a strong cellular cytotoxic immune response locally, which may be closely related to persistent chronic inflammation and tissue injury repair.

Shan et al. [11] further explored the mechanisms by which CD8+ T cells function in the immune microenvironment of keloids, finding a significant increase in CD8+ T cells within keloid tissue, accompanied by abnormal upregulation of co-stimulatory molecules such as CD28, CD80, CD86, and CD40L. This suggests that CD8+ T cells may be in an abnormally activated state, thereby enhancing local immune responses. Further co-culture experiments demonstrated that CD8+ T cells can inhibit the proliferation of keloid fibroblasts and induce their apoptosis, indicating that they can regulate scar formation through direct action on fibroblasts. Additionally, the neural network model constructed in the study showed that the expression levels of CD28 and CD8+ T cells can serve as potential predictors of keloid severity, providing new insights for personalized treatment and disease assessment.

CD8+ cytotoxic T lymphocytes (CTLs) are a specific subset of CD8+ T cells that can mediate target cell apoptosis directly by secreting cytotoxic molecules such as granzyme B and perforin, or by activating Fas-FasL and TNF-related signaling pathways. Therefore, they are widely regarded as core effector cells in anti-tumor immune responses. Xu et al. [12] compared CTLs in the peripheral blood and scar tissue of keloid patients with healthy donors using scRNA-seq analysis. They found a significant reduction in the number of CTLs in patients, accompanied by decreased expression levels of key cytotoxic molecules (such as granzyme B, perforin, IFN-γ), suggesting that CTLs exhibit both quantitative reduction and functional inhibition during keloid formation. This characteristic is highly similar to the "T-cell exhaustion" state induced by chronic antigen stimulation, characterized by limited cell proliferation, weakened cytolytic effects, and insufficient production of effector factors (such as IL-2, TNF-α, IFN-γ). It may be part of the mechanism underlying immune escape in keloids.

To explore the potential mechanisms of CTLs functional inhibition, researchers found through differential gene expression analysis that the upregulation of the NKG2A/CD94 complex in keloids

may be a key factor leading to CTLs functional suppression. Multiple immunofluorescence staining further confirmed that NKG2A+CD8+ T cells are significantly enriched in keloids. This complex can specifically bind to HLA-E and limit the cytotoxic activity of CTLs via inhibitory signaling pathways, similar to PD-1 in mechanism. In clinical cohort studies, researchers observed in patients treated with fluorouracil combined with triamcinolone acetonide that the levels of soluble HLA-E (sHLA-E) in peripheral blood significantly decreased after treatment, and this change was closely related to prognosis. Further large-scale validation results showed that sHLA-E exhibits high sensitivity and specificity in distinguishing keloid patients from healthy individuals, hypertrophic scars, and other interfering diseases, confirming its feasibility as a potential biomarker for keloids and its predictive value for recurrence risk. This study not only elucidates the critical role of the NKG2A/CD94–HLA-E axis in CTLs functional exhaustion and immune evasion in keloids but also provides a theoretical basis for its use as a therapeutic target and for sHLA-E as an indicator for diagnosis and prognosis assessment.

3.4.3 Heterogeneity of dendritic cells

Dendritic cells (DCs) in keloid tissue have gradually attracted attention due to their cellular heterogeneity and functional characteristics in recent years. Wu [13] et al. used scRNA-seq technology, combined with qRT-PCR and immunohistochemistry, to systematically map the composition and heterogeneity of dendritic cells in keloid lesion areas, non-lesion areas (≥10cm from the lesion), and normal skin. The results showed that DCs were significantly enriched in keloid tissue, particularly classic DCs expressing CD11c and "inflammatory DC subpopulations" such as OX40L+ and FCεR1+ associated with atopic dermatitis. These findings not only revealed the immune involvement of DC subpopulations and their correlation with the Th2 inflammatory axis but also provided theoretical support for immunomodulatory strategies targeting Th2-related signaling pathways like OX40L and TSLP. This study highlights the critical role of scRNA-seq in identifying potential pathogenic cell populations and therapeutic targets in keloids.

4. Summary and outlook

Keloid is a complex benign skin tumor disease that involves multiple cells and molecules. The various pathological mechanisms of keloids have temporal and spatial characteristics, which not only interact with each other but also exhibit dynamic changes, making it difficult to describe them clearly. In recent years, scRNA-seq technology has made significant progress in exploring the mechanisms of keloid formation and development. Currently, scRNA-seq has achieved important results in the field of keloids. Through this technology, researchers have successfully identified multiple subpopulations of fibroblasts, endothelial cells, Schwann cells, and immune cells within keloid tissues, and have deeply analyzed their specific gene expression patterns and functional states. Among these, the diversity of fibroblast subpopulations is closely related to mesenchymal transformation, myofibroblast differentiation, and abnormal collagen fiber deposition; the heterogeneity of endothelial cells reveals their critical role in pathological angiogenesis and fibrosis; studies on Schwann cells have highlighted their significant cellular plasticity, involvement in neural regeneration, and scar fibrosis processes; and the analysis of immune cells, particularly macrophages, T cells, and dendritic cells, has elucidated the immune status and inflammatory response characteristics in the keloid microenvironment. These findings comprehensively depict the intricate communication network between cells in keloids, deeply explaining the synergistic effects and pathological development mechanisms of different cell types, providing a solid theoretical basis for developing specific therapeutic targets. However, current research still has many shortcomings, such as being mostly descriptive, lacking in-depth functional validation, and having limited sample sizes and sources. These limitations restrict the direct translational and application of research findings in

clinical settings. Therefore, there is an urgent need to conduct more comprehensive and in-depth studies to address these deficiencies and further advance the clinical translation of keloid research.

Looking ahead, research needs to strengthen the following areas: The research team should first increase the number of study samples and standardize the tissue acquisition process to reduce heterogeneity's interference with data interpretation. Second, they should systematically integrate multimodal analysis techniques, including spatial transcriptomics (ST), spatial proteomics, and epigenomics, to further uncover functional interaction networks between cell subpopulations at both spatial structure and epigenetic regulation levels. Third, there is an urgent need for broader and deeper basic functional validation experiments, such as using animal models, multi-dimensional culture systems, and drug interventions, to further clarify the clinical and translational potential of the pathological mechanisms indicated by single-cell data. Additionally, expanding the sample size and sources is crucial, covering patients with keloids from different sites and stages of disease, to confirm the generalizability and clinical applicability of the research findings. Furthermore, it is essential to fully integrate scRNA-seq technology with clinical treatment methods, such as triamcinolone acetonide injections, laser therapy, and cryotherapy, to analyze changes in the transcriptome before and after clinical treatments, identify effective biomarkers to predict treatment outcomes and assess recurrence risks, ultimately achieving more precise and personalized clinical treatment strategies.

In summary, single-cell transcriptome sequencing technology has brought new opportunities and profound insights to the study of keloids. However, to truly realize the clinical translation and application of these technological advantages, further efforts are needed in multimodal data integration, dynamic change mechanism analysis, functional experimental validation, and clinical translational research. As technology and research methods continue to improve, scRNA-seq is expected to play a greater role in elucidating the pathogenesis of keloids, identifying key disease-causing cell populations and their molecular pathways, and providing a more solid theoretical foundation and potential therapeutic approaches for precise diagnosis and treatment of keloids.

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