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Single-cell sequencing technology in diabetic wound healing: New insights into the progenitors-based repair strategies

Xiang Z *et al.* Single-cell sequencing in diabetic wound healing

Abstract

Diabetes mellitus (DM), an increasingly prevalent chronic metabolic disease, is characterised by prolonged hyperglycaemia, which leads to long-term health consequences. Although much effort has been put into understanding the pathogenesis of diabetic wounds, the underlying mechanisms remain unclear. The advent of single-cell RNA sequencing (scRNAseq) has revolutionised biological research by enabling the identification of novel cell types, the discovery of cellular markers, the analysis of gene expression patterns and the prediction of developmental trajectories. This powerful tool allows for an in-depth exploration of pathogenesis at the cellular and molecular levels. In this editorial, we focus on progenitor-based repair strategies for diabetic wound healing as revealed by scRNAseq and highlight the biological behaviour of various healing-related cells and the alteration of signalling pathways in the process of diabetic wound healing. ScRNAseq could not only deepen our understanding of the complex biology of diabetic wounds but also identify and validate new targets for intervention, offering hope for improved patient outcomes in the management of this challenging complication of DM.

Key Words: Single-cell sequencing; Diabetic wound healing; Cell subpopulations; Heterogeneity; Pathogenesis; Progenitor cells

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Core Tip: Understanding the mechanism of diabetic wound healing is crucial for the development of novel therapeutic strategies. In this editorial, we focus on advances in the biological behaviour of various healing-related cells and the alteration of signalling pathways in the process of diabetic wound healing. Single-cell RNA sequencing (scRNAseq) has emerged as a powerful tool to explore cellular heterogeneity, reveal

new cell subpopulations and predict developmental trajectories. Summarising the current results of scRNAseq in diabetic wounds has provided new insights into progenitor-based repair strategies and possible therapeutic targets.

INTRODUCTION

The prevalence of diabetes mellitus (DM), currently affecting an estimated 550 million individuals, continues to escalate^[1]. Diabetic foot ulcer (DFU) is a common and severe complication in patients with diabetes, characterised by its stubbornness, difficulty in treating, and high recurrence rate. It mainly manifests as peripheral neuropathy, lower limb arterial sclerosis and local infection, which profoundly impact the patient's quality of life and disease prognosis. Without proper treatment or inadequate management, it can easily lead to lower limb paralysis, disability and even amputation. Approximately 19% to 34% of diabetes patients worldwide will develop DFU, and about 20% of DFU cases will require lower limb amputation^[2]. Current treatment approaches for DFU predominantly encompass wound debridement, offloading strategies, glycemic control, and the management of infections^[3]. Recently, several innovative therapeutic modalities have surfaced, including hyperbaric oxygen therapy, application of dressings, negative pressure wound therapy, growth factor therapy, tissue stem cell therapy and application of tissue-engineered skin^[4]. Despite the development of various innovative technologies and drugs to treat DFUs, their therapeutic effects remain unsatisfactory. Progenitor and stem cell therapies, including embryonic stem cells, adult stem cells and mesenchymal stem cells, have been introduced in clinical settings to enhance diabetic wound healing, but the intricacies of stem cell application and tissue interactions are not fully comprehended. The advent of single-cell RNA sequencing (scRNAseq) technology offers a promising avenue for elucidating these complex biological processes, thereby potentially unlocking novel therapeutic targets for DFU.

SINGLE-CELL SEQUENCING TECHNOLOGY

ScRNA-seq represents a technique for amplifying the entire transcriptome at the individual cell level. This process involves the reverse transcription of mRNA into cDNA, subsequent amplification of the cDNA, and high-throughput sequencing^[5]. As a quintessential instrument for single-cell analysis (Figure 1), scRNA-seq facilitates unbiased, high-throughput investigations requiring minimal initial sample volumes. It allows for the detection of cell-specific attributes and intercellular variances through cell mapping, delves into the cooperative functions of cells, and examines the heterogeneity within tissues^[6]. Thus, using scRNAseq to investigate progenitor-based repair strategies may yield novel insights into the mechanisms of diabetic wound healing.

SINGLE-CELL SEQUENCING IN DIABETIC WOUND HEALING

Traditionally, it was believed that endothelial cells, epithelial cells, fibroblasts, keratinocytes, macrophages, inflammatory cells and tissue stem cells were the main contributors to wound healing. ScRNAseq has shown that endothelial cells, fibroblasts, epithelial cells, keratinocytes, monocytes, macrophages, B cells, T cells and tissue stem cells are significantly present in both non-healing and healing DFU wounds. By applying scRNAseq, we can gain a clearer understanding of the biological behaviour of various cell types during diabetic wound healing. The following content presents five applications of scRNAseq in studying diabetic wounds and unveiling cellular behaviour and molecular changes during the healing process (Table 1). The samples, primarily from DFUs, non-DFUs and healthy subjects, exhibited cell quantities ranging from 21819 to 174962, facilitating the identification of cell populations, such as fibroblasts, keratinocytes, macrophages, vascular endothelial cells and the discovery of associated signal pathways.

Through a cluster analysis of the data from the GEO database, Li *et al*^[7] and Wang *et al*^[8] found that the proportions of macrophages, leukocytes and monocytes were higher in patients with DFUs, which indicated a higher level of inflammation; while elevated proportions of pluripotent stem cells and stromal cells were observed in patients with DM, which indicated a higher level of dryness. These findings were in line with

research by Theocharidis *et al*^[9], who noted that DFU healers had a higher presence of naive and early differentiated progenitor T-lymphocytes, while non-healers had more cytotoxic natural killer T cells at the systemic level. Additionally, the proportion of M1 macrophages (classically activated macrophages that promote inflammation) was higher in DFU-healer than in DFU-non-healers, compared to M2 macrophages (alternatively activated macrophages with anti-inflammatory properties)^[9]. These results implied that suppressing systemic immuno-inflammatory responses while activating local responses in the wound environment facilitates diabetic wound healing. Liao *et al*^[10] found highly expressed keratinocyte genes, including SFN, LYPD3, S100A8, KRT1, KRT10, KRT6A, KRT5, and KRT16, underscoring the crucial role of keratinocytes in diabetic wound healing. By analysing the skin specimens of DFU patients and healthy controls using scRNAseq, Du *et al*^[11] found that human dermal microvascular endothelial cells (HDMECs) isolated from DFU patients showed considerably impaired tube formation compared to those from healthy controls; they also found that the significantly underexpressed RAB17 in DFU-HDMECs may be the key factor leading to the impaired angiogenic capacity in DFUs. Moreover, it was proven in the diabetic mouse wound-healing model that the STZ-induced diabetic mice injected with an RAB17-overexpressing rAAV vector had a higher wound perfusion and a significant acceleration of wound closure^[11]. Theocharidis *et al*^[9] profiled 174962 single cells from the foot, forearm and peripheral blood mononuclear cells using scRNAseq; based on the differential expression of genes, the fibroblast population was divided into 14 subclusters, in which an unique population of fibroblasts overexpressing matrix metalloproteinase 1 (MMP1), MMP3, MMP11, hypoxia inducible factor 1-alpha (HIF1A), chitinase 3-like protein 1 and tumor necrosis factor (TNF)-alpha-stimulated gene-6 was defined as a new type of fibroblast, namely HE-Fibro. They were also found to preferentially locate at the wound bed compared to the wound edge or unwounded skin and increase M1 macrophage polarisation in the DFU patients with healing wounds, suggesting that particular subtypes of fibroblasts play pivotal roles in the healing process of DFUs, implying that targeting these specific fibroblast subtypes may

represent a viable therapeutic strategy^[9]. The differential gene expression revealed by scRNAseq also suggested the activation or inhibition of the corresponding signalling pathway in the process of diabetic wound healing. The diabetic healing-related differentially expressed gene analysis and gene ontology functional enrichment analysis identified significant differential genes, including CD19, Integrin Subunit Alpha M, HLA-DR, CXC chemokine ligand 11, MMP1, heparan sulfate 3-O-sulfotransferase 2, CALML, interleukin (IL)7R, IL6, TCF7, CCR7, IL1B, S100A8, HIF1 α , TNF, CD44, transforming growth factor β 1, C-C chemokine ligand 5, SOX4, RAB17, CD200 and vascular endothelial growth factor A. These genes were predominantly associated with the immune and inflammatory signalling pathways, oxidative phosphorylation and cytokine receptor interactions, suggesting that the immune and inflammatory environment is critical for diabetic wound healing. Alterations in the metabolic processes of cells within diabetic wounds have also been implied^[7,10]. Additionally, genes such as ANPEP, BID, CYBA, CYBB, FCER1G, ITGA1 and PLAUR, which are overexpressed in the diabetic wound microenvironment, might serve as potential drug targets^[7]. Currently, scRNAseq is extensively applied in the analysis of pathological tissues, the identification of cell populations and the discovery of novel cell subpopulations in diabetic wound healing. However, advanced applications, such as spatial transcription and research into cell development and differentiation, remain underexplored and represent promising frontiers for investigation.

CONCLUSION

The advent and integration of scRNAseq into the study of diabetic wound healing have afforded unprecedented insights into cellular functions, pathophysiological processes and the intricate microenvironment of wounds. This technology enables the precise delineation of cellular subpopulations, the elucidation of pivotal molecular mechanisms and the identification of novel therapeutic targets. Future longitudinal studies that build a map of the diabetic wound healing timeline, and combine scRNAseq and spatial transcription may provide a better way to explore tissue regeneration and repair

mechanisms. In essence, the application of scRNAseq in diabetic wounds has provided new insights into the mechanism of diabetic wound healing and possible directions for further treatment.

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