Peer Review File

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Reviewer A

The paper titled "Healing mechanism of diabetic foot ulcers using single-cell RNA-sequencing" is interesting. The CCL2-ACKR1 axis is closely associated with DFU healing. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1: Some cell subsets were mentioned in this study, and it is suggested to analyze the heterogeneity and functional changes of different cell subsets in diabetes. Which cells can be used as immune regulators in wound healing of refractory diabetes? It is suggested to add relevant contents.

Reply 1: Thanks for your comments, various cell subtypes all play important roles in the wound healing process of diabetic foot, and we have increased the specific role of related cells in wound healing. (see page 8, line 228 to 235)

Changes in the text: Among them, the increased infiltration of neutrophils, monocytes / macrophages and T lymphocytes into the wound site occurs in the inflammatory stage to remove bacteria and other microorganisms and prevent infection; fibroblasts are the key cells responsible for initiating angiogenesis, epithelialization and collagen production; keratinocytes, fibroblasts, endothelium, endothelial cells, epithelial cells and tissue stem cells are involved in the formation, epithelialization and tissue remodeling of granulation tissue.

Comment 2: The identifications in the figure are inconsistent with those in the figure legends, for example, a and b are used in Figure, but A and B are used in the figure legends. Uniform identification is recommended.

Reply 2: Thanks for your comments, we have replaced it with uniform lower case letters.(see page 9 to 11)

Comment 3: The description of some methods in this study is too simplistic, please describe in detail.

Reply 3: Thanks for your comments, we have made detailed descriptions of some experimental methods of this study.(see page 5, line 145 to 150; page 6,line 158 to 163)

Changes in the text:

1) Quality control has two main parameters: 1. The number of the unique characteristics measured in each cell (the unique feature represents the number of genes detected in a cell, can be adjusted according to the quality of the data) 2. The proportion of mitochondrial genes detected in each cell, compared with the nuclear genome, theoretically mitochondrial genome only a small part. So cells with excessive expression of mitochondrial genes are filtered out.

2) Parameter selection: Feature_RNA represents the number of genes measured per cell, selection is greater than 200 and less than 7500, nCount represents the sum of expression of all genes measured per cell, selects the proportion of mitochondrial genes measured by 100000,percent.mt represents the proportion of the mitochondrial genes measured, less than the 20%,percent.HB represents the proportion of the red blood cell genes, selected for less than 5%.

Comment 4: What factors play a role in determining the healing or deterioration of diabetic foot lesions? It is recommended to add relevant content.
Reply 4: Thanks for your comments, we have added relevant factors that promote the healing of diabetic foot lesions.(see page 11, line 337 to 344)
Changes in the text: The healing process of diabetic foot ulcer belongs to pathological healing, which is easy to form chronic refractory wound, and it is difficult to appear the step reaction of normal physiological healing. In addition to paying attention to the treatment of systemic factors, such as the control of blood sugar, blood pressure, blood lipid, anti-infection and the improvement of microcirculation, we must also pay attention to the standardized treatment of chronic wound itself. Only the comprehensive coordinated treatment of whole body and local can promote the healing of chronic difficult wound.

Comment 5: This study is based on bioinformatics analysis. It is recommended to increase in vivo and in vitro experimental studies, which may be more meaningful.
Reply 5: Thanks for your comments, the lack of experimental validation is one of the limitations of this paper. I am deeply sorry for that.

Comment 6: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Randomized research on the mechanism of local oxygen therapy promoting wound healing of diabetic foot based on RNA-seq technology, PMID: 33440964”, “IL-1B can serve as a healing process and is a critical regulator of diabetic foot ulcer, PMID: 35280410”. It is recommended to quote the articles.
Reply 6: Thanks for your comments, we have cited relevant literature to enrich the article.

Comment 7: It may be more meaningful to add functional research on key genes and signaling pathway.
Reply 7: Thanks for your comments, relevant experiments proved that CCL 2 can induce the expression of matrix metalloproteinase MMP-2 and MMP-9, thus promoting the wound healing of diabetic foot; ACKR 1 has been confirmed to be expressed in erythroid lineage to regulate hematopoietic function, and can also act as a plasma chemokine to trigger the adhesion and migration of leukocytes through endothelial cells. However, the CCL 2 / ACKR 1 axis has not

proved its specific role in the wound healing of diabetic foot, so the functional study of this signaling pathway cannot be analyzed for the time being.

Comment 8: What is the guiding significance of this study for the treatment of diabetic foot ulcers? How to further study on the basis of this research? What experimental methods can be used? It is recommended to add relevant content.

Reply 8: Thanks for your comments. We have added the relevant content.(see page 14, line 430 to 435)

Changes in the text: But, this study is based on bioinformatics analysis, lack of experimental validation is one of the limitations of this paper. Next, we will conduct animal modeling test for functional studies of key genes and signaling pathways, including immunofluorescence(IF) analysis, flow cytometry(FCM), western blot(WB) and qPCR, in order to provide a theoretical basis for clinical diagnosis and treatment through animal verification.

Reviewer B

1. Please check if any more references need to be added in the below 3 sentences since you mentioned "Studies", but only one reference was cited. If not, "studies" should be changed to "a study/a previous study".

81 extremities (5,6) and varying degrees of vasculopathy (6). Pathological studies have
82 indicated that the causes of difficult healing of DF wounds include microbial invasion,
83 epithelial cell rupture, and impaired immune function (7). Among all possible

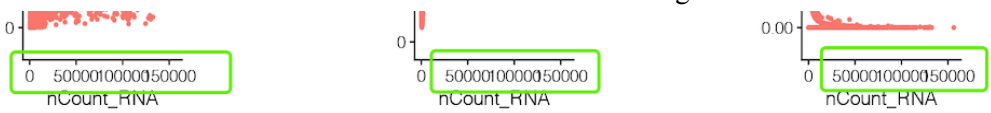
102 Related studies have reported that autologous PRP has become an important treatment
103 for most chronic wounds due to its richness of various growth factors [Epidermal
104 Growth Factor (EGF), Platelet-Derived Growth Factor (PDGF), Transforming Growth
105 Factor-β 1 (TGF-β1)], and ability to promote cell proliferation differentiation and
106 inhibit inflammation (15).

442 (42). Recent studies have shown that erythrocyte infusion upregulates ACKR1
443 expression, affects the apoptosis of macrophages, and reduces inflammatory cytokine
444 production (43) in a sepsis model.

Reply: We've revised it.

2. Figure 1:

The numbers are too close and not clear in the x-axis of Figure 1C. Please revise.



Reply: We've revised it.

3. Figure 3:

There are two words cells.

Reply: We've revised it.

4. Figure 4:

The below words should be "DEGs". Please revise.

Reply: We've revised it.

5. Figure 5:

Please indicate the full name of "BP", "CC", "MF" in the legend.

Reply: We've revised it.

6. Figure 6:

Your Figure 6C legend and Figure 6D legend is the same one. Please combine them.

634 the wound healing process of each cell; (C) comparative analysis of cell subsets in the
635 wounds of healing DFU and DFU; (D) comparative analysis of cell subsets in the
636 wounds of healing DFU and DFU; (E) overall signal pattern of DFU signal pathway;

Reply: We've revised it.