Peer Review File

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Reviewer A

The study provides translational insights into the mechanisms underlying stenosis and thereby help elucidate potential pathways to preventing or treating this disorder.

I had a few recommendations that might improve clarity of the submission:

1. Please state the source of the comparison tracheal tissue used to obtain normal fibroblasts in the same section of the manuscript where the source information for the LTS fibroblasts is discussed. If possible, please clarify if there was any way to match age, gender, or other sociodemographic or biological attributes.

Reply: Thank you for the recommendations. we have modified our text as advised (see Page 2, line 37-38; Page 4, line 129)

2. The title of the paper could be improved slightly to clarify that fibroblasts "from laryngotracheal stenosis" were "from patients with acquired laryngotracheal stenosis" so that it is clear that the source was human subjects rather than an animal model or otherwise.

Reply: Thank you for your recommendations. We have modified our title as advised (see Page 1, line 1-2)

3. The text in the figures, particularly the first figure Part a [heatmap] and Part b [classification] are too small to read/ interpret. The size of the font needs to be increased in relation to the rest of the figure.

Reply: Thank you for your recommendations. The images in the first figure are all vector graphics, each of which is 300dpi and can be seen clearly after magnification.

4. In the abstract/introduction the authors note that surgical or procedural approaches to LTS (laryngotracheal reconstruction, or dilation) have risk of restenosis. It should be noted that all available treatments have risk of restenosis. Although finding a treatment with higher success rate would be ideal, perhaps the most compelling argument for research is to spare patients the morbidity and risks associated with these procedures.

Reply: Thank you for your kind recommendations. We have modified our text as advised (see Page 1, line 31-32; Page 3, line 84-85).

5. There appears to be some discrepancy between what is stated in the abstract regarding LTS

migration and proliferation versus what is states in the key points. The key points convey that, "Cell proliferation and migration of LTS derived fibroblasts were shown to be significantly faster." Although this finding was shown in the authors' prior work, was it replicated in the present study? If not, then the statement should be revised to emphasize only those findings from the present study.

Reply: We feel great thanks for your professional review work on our article. In our previous work, we revealed the heterogeneity of fibroblasts between LTS and skin hypertrophic scar in pediatric patients. We found that cell proliferation and migration of LTS derived fibroblasts were significantly faster. However, in the present study, by comparing fibroblasts of LTS tissues with those of normal trachea tissues, we found that cell migration of LTS derived fibroblasts was significantly faster. Although different normal control cells were chosen in these two researches, similar conclusions were reached.

6. Although these data are experimental, it might be of interest to share with readers the potential implications of the study findings for targeted therapies that would inhibit CXCR7 and potentially impede migration? For example, although inhibiting this pathway would be predicted to cause less LTS, would there also be a potential risk for delayed wound healing, if defect closure is delayed?

Reply: Thank you for your valuable advice. Your questions and suggestions are of great significance to our follow-up research plans and strategies (such as in mice model of LTS). We can reduce the generation of LTS by extending the administration time, such as adding the pathway inhibitors one week after wound healing.

7. A **figure** would be helpful to elucidate the model summarized below:
Reply: Thank you for your recommendations. We added a figure (see Page 20).

8. There are some minor language corrections that would be helpful. Likely most can be addressed by the copyeditor, but a couple of examples are below:

Please change "CXCR7 was knockdown" to either "CXCR7 knockdown was performed using..." or "CXCR7 was knocked down..." in the excerpt that follows:

36 CXCR7 was knockdown using specific siRNAs and activated by CXCR7
37 agonist VUF11207.

Please change "inhibit" to "inhibitory" in the sentence below:

299 The inhibit effect of siRNAs was shown
300 by qPCR and western blot assay (figure 4a and b)

Reply: We are very sorry for our careless mistakes. We have modified our text as advised (see Page 2, line 38-39; Page 10, line 303).

9. Please add a limitations discussion to the section. The study has limitations relating to experimental model and translatability. For example, cell culture can result in loss of context cues and alter gene expression and other attributes of in situ tissues. In addition, there may have been confounders if the experimental tissues in the LTS and normal group were not matched optimally.

Reply: We appreciate the detailed and constructive comments provided by professors. we added discussion of limitations (see Page12-13, line 366-372)

Reviewer B

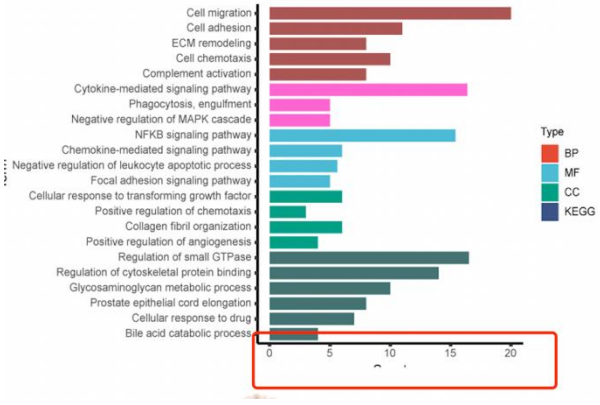
1. Abstract, please introduce the abbreviation PCR in its first use.

knockdown was performed using specific siRNAs and activated by CXCR7 agonist VUF11207. Cell proliferation and migration were analyzed by EdU proliferation, wound healing and transwell assays. The expressions of CXCR7, E-cadherin and NF-κB signaling pathway were analyzed by quantitative PCR, western blotting, immunohistochemistry, and immunofluorescence.

Reply: Thank you for your advice. We have defined the abbreviation PCR in its first use (see Page 2, line 48).

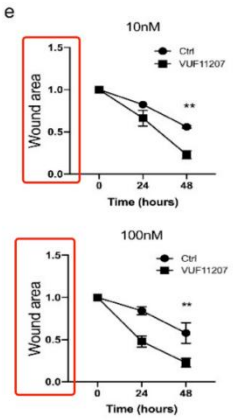
2. Figures

1) Figure 1a, please check if any description/unit should be added to the X-axis.



2) Figure 1b, scale bar should be added either in the figure or in the figure legend.

3) Figure 2e, please check if any unit should be added to the Y-axis.



3) Reply: Thank you for your advice. In Figure 2e, we define wound area in 0 hour as 1.0, so wound areas at other times in the figure are the ratio between these two.

4) The figure 2g is not mentioned in the legend. Please check the legend of figure 2f and 2g, should it be '(f) Culture medium with different concentrations of VUF11207 (10nM and 100 nM) were applied to measure LTS derived fibroblasts. (g) Quantification of the percentages of cell migration in (f)'?

515 areas. (e) Quantification of the wound areas in (d). (e) Culture medium with different
516 concentrations of VUF11207 (10nM and 100 nM) were applied to measure LTS
517 derived fibroblasts. (f) Quantification of the percentages of cell migration in (e). Data

5) Figure 2f and figure 4g, scale bar should be added either in your figure or in the figure legend. Staining method should also be mentioned either in legend or in main text. Please add it.

6) Figure 4f, please indicate the meaning of the black solid lines.

7) Any abbreviations used in figures and tables or their description should be defined in a footnote beneath each corresponding table/figure. Even if they were explained in the main text, full terms must be presented again in the corresponding figures and tables, so that figures and tables can be read on their own.

Reply: Thank you for your advice. We have revised our figures and footnotes as required.