Dear Editors and Reviewers:

Thank you for your letter and for the editorial comments concerning our manuscript entitled “Single cell RNA sequencing reveals mesenchymal heterogeneity and critical functions of *Cd271* in tooth development” (Manuscript NO.: 83772, Basic Study). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have made correction which we hope meet with approval. The main corrections in the paper and the responds to the reviewer’s comments are as following:

Response to Reviewers’ Comments

Reviewer #1:

There are no comments. The research can be interesting as it contains new fundamental knowledge.

Response: Thanks for your time and effort. We are very glad to receive your comments on our manuscript.

Reviewer #2:

The authors used the latest molecular biology methods to study the role of CD271 in the development of the facial skeleton bones and, as the title of the article states, tooth development. The authors used animals with cd271 gene knockout in their work. However, with such a powerful set of methods, the results are more than modest. The authors themselves point out in the introduction that the role of cd271 in neural crest cell function is key and is being actively studied. And as a consequence of this, it was obvious to expect some abnormalities when this gene was knocked out, which is what the authors got: a decrease in proliferative ability, and the ability to mineralize.

1. Heterogeneity of MSCs has long been discussed in the literature, the authors again confirmed this conclusion. Among the MSCs the authors distinguished subpopulations of progenitor cells, osteoblasts and fibroblasts. What is new and surprising in this is not clear.

   Response: Thanks for pointing out this important issue. Heterogeneity of MSCs has

long been discussed, however, most of the previous studies have been at the multicellular level. In addition, previous studies based on the cultured MSCs in vitro and the culture environment in vitro may lead to their differentiation. The multicellular level and phenotypic instability hinder the reliability. We distinguished MSCs as progenitor cells, osteoblasts and fibroblasts by unsupervised clustering. The high-throughput and high-resolution transcriptome at the single-cell level provides us with reliable evidence to the composition of MSC found from previous studies.

2. because it is already known that from the MSCs that settle out of the neural crest, the connective tissues of the facial skeleton develop and it is quite expected that among morphologically similar cells there are already those that have entered different differentiation pathways.

   Response: Thank you for your comment. It is already known that MSCs enters different differentiation pathways. We make it possible to observe the cell differentiation dynamics, temporal and spatial expression of key genes over time by pseudo time trajectory.

3. In this connection, the authors should analyze the obtained data more deeply and clearly formulate what is the novelty of their study.

   Response: Thanks for the suggestion. This is well taken. We have carefully revised the first paragraph of conclusion section in the revised manuscript. (Page 18)

4. By the way, what is the role of CD 271 in tooth development remains unclear to me.

   Response: Thanks for pointing out this important issue. *Cd271* is implicated in various biological functions, such as migration, proliferation, differentiation, survival and apoptosis. We found that *Cd271* involved in regulating biomineralization in tooth development by analyzing the differential genes between *Cd271* knockout and wildtypes. Also, we demonstrated that *Cd271* deletion suppressed mesenchymal cells migration, proliferation and odonto/osteogenic differentiation on our functional experiments. *Cd271* of MSCs during tooth

development could impact the communications between different cell types, finally influence the mesenchylial cell fate of differentiation. We further found *Cd271* regulates *Mdk* to influence osteogenesis in the CellChat. In summary, we obtained molecular evidences that *Cd271* involved in the initiation of dental development and the regulation of mineralization and preliminarily explored the possible signaling mechanism of regulation.

Response to Company editor-in-chief:

Thank you for the comments. I prepare the figures in PowerPoint file and I submitted the revised manuscript again to English language editing and obtained an Editing Certificate.

In our reference, there are 40 literatures also available in RCA. And I searched the literature using RCA database according to the editor's suggestion. I added these articles as references. There are totally 44 literatures which available in RCA listed as references.

Previous:

Previous studies focused on cellular physiological functions in processes, such as migration, proliferation, differentiation, survival and apoptosis [5-7].

Cd271 is involved in the regulation of morphogenesis and the development of various tissues, including nerves, fat, liver and teeth [9-12].

Nevertheless, accumulating evidence suggests that MSCs are functionally and morphologically heterogeneous in essence [18].

scRNA-Seq also provides insights into specific changes in cell lineages, trajectory inference, and the identification of biomarkers [21].

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13. Smith RJP, Faroni A, Barrow JR, Soul J, Reid AJ. The angiogenic potential of cd271+ human adipose tissue-derived mesenchymal stem cells. Stem Cell Res Ther 2021; 12: 160 [PMID: PMC7927269 DOI: 10.1186/s13287-021-02177-0

19. Chen Y, Zhang Z, Yang X, Liu A, Liu S, Feng J, Xuan K. Odontogenic msc heterogeneity: Challenges and opportunities for regenerative medicine. Front Physiol 2022; 13: 827470 [PMID: PMC9061943 DOI: 10.3389/fphys.2022.827470

22. Li Y, Ju S, Li X, Li W, Zhou S, Wang G, Cai Y, Dong Z. Characterization of the microenvironment of diabetic foot ulcers and potential drug identification based on scrna-seq. Front Endocrinol (Lausanne) 2022; 13: 997880 [PMID: PMC9845942 DOI: 10.3389/fendo.2022.997880

Thank you very much for your attention and time. Look forward to hearing from you.

Sincerely yours,

Xin Nie

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