

Dear editor and reviewers,

I am glad to receive your letter. Thank you very much for your hard work and kind suggestions. I have read the comments carefully and revised the manuscript according to your suggestions. Thanks again for your excellent work.

If you have any question, please don't hesitate to contact me.

With best regards,

Sincerely yours

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Response to Reviewer:

Thanks for your valuable comments and suggestions which have led to significant improvement on the presentation and quality of this paper. The following are point-by-point responses to your concerns. And we shall detail the changes we have made on the paper.

- 1) *A careful editing is needed to correct minor spelling mistakes, for example on page 8 "residinge" instead of "residing"; repetitions should be also amended (such as "step"/"steps" on page 6). Abbreviations should be utilized for frequent used term (such as stem cells).*

Reply: Many thanks for your comments. Corrections on spelling and grammatical errors could be seen in the revised manuscript.

- 2) *Chapter "Introduction" should be rephrased and shortened. The paragraph "One of the most striking phenomena is that even most stem cells show obvious homogeneity within a single tissue, there are diverse subpopulations of cells, all of which have unique distinct functions, morphologies, developmental statuses, or gene expression profiles of that tissue type compared with the other cell subpopulations[1,2]. Such diversity represents the heterogeneity of stem cells[3,4]. Previous studies have indicated that the heterogeneity of cellular states is not only caused by the cell's own physiology and differentiation state[5], but also their inherent plasticity[6-8]. Because of the presence of heterogeneity, further studies of biological characteristics and applications of stem cells are hindered[9]. Although bulk-based approaches using microarrays of high throughput RNA sequencing (RNAseq) techniques provide certain important insights into stem cells, these approaches are limited because results about structures and functions reflect average measurements from large populations of cells or the results are predominantly obtained from cells with superior numbers[10,11]. In addition, they overlook unique biological behaviors of individual cells, conceal cell-to-cell variations and prevent us from learning more about the heterogeneity at the molecular level as a basis to understand the biological complexity of stem cells. As a consequence, heterogeneity is still a major issue to be resolved in the research and applications of stem cells. Studies conducted at the single cell level are the only means to understand the heterogeneity of stem cells." contains redundant terms and phrases.*

Reply: Many thanks for your comments. Following your suggestions, we have made some modifications in the **Introduction** section.

- 3) *Chapter "Single cell RNA sequencing" (page 6) should be renamed "Single cell RNA challenges" and typed in capital letters (as " Applications of scRNA-seq to research of stem cells" on page 7). Also, other technical challenges should be discussed (except isolation of target single cells and transcriptome amplification).*

Reply: Thanks for your suggestion. Following your suggestion, relative information was added to the **Single cell RNA challenges** section.