

PEER-REVIEW REPORT

Name of journal: World Journal of Stem Cells

Manuscript NO: 48747

Title: Applications of single cell RNA sequencing to research of stem cells

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SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	<input type="checkbox"/> Accept	Peer-Review:
<input type="checkbox"/> Grade B: Very good	<input checked="" type="checkbox"/> Grade B: Minor language	(High priority)	<input checked="" type="checkbox"/> Anonymous
<input type="checkbox"/> Grade C: Good	polishing	<input type="checkbox"/> Accept	<input type="checkbox"/> Onymous
<input checked="" type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade C: A great deal of	(General priority)	Peer-reviewer's expertise on the
<input type="checkbox"/> Grade E: Do not	language polishing	<input type="checkbox"/> Minor revision	topic of the manuscript:
publish	<input type="checkbox"/> Grade D: Rejection	<input checked="" type="checkbox"/> Major revision	<input type="checkbox"/> Advanced
		<input type="checkbox"/> Rejection	<input checked="" type="checkbox"/> General
			<input type="checkbox"/> No expertise
			Conflicts-of-Interest:
			<input type="checkbox"/> Yes
			<input checked="" type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

Specific comments to the authors are attached below

The manuscript by Zhang and Liu provides an overview of the applications of scRNA-seq with a focus on stem cells research, a topic worth deep studying considering its wide clinical application. Indeed, single-cell genome sequencing is rapidly evolving, expanding our understanding of the cell as a functional unit.

I suggest the authors to increase the impact of the manuscript by addressing the following recommendations:

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).
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.
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).

I recommend this paper for publication after suggested revisions.

INITIAL REVIEW OF THE MANUSCRIPT



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