

Name of Journal: World Journal of Stem Cells

Manuscript NO: 41840

Dear members of the Editorial Board

Thank you for your positive feedback and valuable comments by the editor and the reviewers. Enclosed please find the original copy of the revised manuscript.

We consider the editor's and referees' comments fair and very useful. I have revised the manuscript according to the provided proposals and I believe that we were able to answer the questions to satisfy the reviewers. In addition, the manuscript is grammatically polished.

Yours sincerely,

Ramin Radpour, MSc, PhD
Tumor Immunology,
Department for BioMedical Research (DBMR),
University of Bern, Murtenstrasse 35,
3008 Bern, Switzerland.
Email: ramin.radpour@dbmr.unibe.ch
Telephone: +41-31-6320956
Fax: +41-31-6323297

Reviewer's comments:

Reviewer 1

This review described the recent methodologies advances for single-cell analysis and discuss the challenges and prospects for molecular characterization and profiling of CSCs as a hallmark of biomarker discovery in oncology. It has important significance to understand the methodologies for single-cell analysis. But the depth and logicality about single-cell analysis for CSCs is not enough. In addition, the following issues should be addressed. The title in the part "MOLECULAR BIOMARKERS OF CANCER CELLS AND CSCs" should be consistant to the following content "Proteomics..." Totally, the writing of the manuscript is good, but there are some grammer errors. The authors can make a list to describe different Single-cell based analytic approches for different CSC markers in different tissue derived tumors.

Authors: to be more precise by the title, it is changed to "Single-cell analysis of tumors: creating new value for molecular biomarker discovery of cancer stem cells and tumor-infiltrating immune cells".

In order to make a list of different Single-cell based analytic approaches for different human tumors, we added Table 1 as "Single-cell sequencing studies on variety of human tumors". In addition, Figure 2 is added to illustrate the main applications of single-cell based profiling in cancer research.

The manuscript is grammatically polished!

Reviewer 2

The mini-review proposed by Forouharkhou and Radpour discusses about the use of the new “single cell” technology to discover new molecular biomarkers in cancer diagnostic/prognostic/prediction. Overall, the review includes all the interesting and new methods to try to find biomarkers for cancer diagnostic, prognostic, and predictive in cancer cells and cancer stem cells. It however lacks, deeper discussion on all aspects: technologies, analyses, and perspectives. The review starts with a general introduction followed by a paragraph on cancer stem cells (CSC) and high-throughput technologies to isolate cancer cells and CSC. This is followed by a paragraph on technologies used to detect biomarkers in cancer cells and CSC. In this paragraph where technologies (proteomics, genomics, epigenomics, transcriptomics) are rapidly described, it would be nice to develop and describe a bit more these technologies that are not always well-known by readers and more particularly to discuss the advantages and drawbacks for each technology and conclude by telling which is more appropriate for each application. In the paragraph transcriptomics, it would be interesting to develop a bit the recent works performed to get molecular signatures of cancer that highlight the great heterogeneity within a tumor but also within single cells. In the single cell based approaches paragraph it would be interesting to develop the part about microfluidic systems as a lot of bibliography exists now. What has been done and what are the trends for the years to come? It would also be interesting to develop a bit more the paragraph on scRNA-Seq and its value for cancer treatment orientation. The authors should also discuss the problem of sequencing errors associated with NGS. What is done at the informatics level (algorithms) to avoid/decrease this problem? Again authors briefly describe single cell methods adapted to biomarker search but then what? A discussion about the best method(s) for diagnostic, prognostic, predictive biomarkers should be included based on the literature.

Authors: Thanks for your valuable comments. We tried to improve the second part of manuscript accordingly by adding the following parts:

- 1- Improving the “figure 1”.
- 2- The following paragraph is added to the “SINGLE-CELL BASED APPROACHES” section as:
“Applied methods for single-cell isolation have rapidly enhanced in the past few years from manual micromanipulation, cell-search antibody-based isolation or flow-sorting of cells to high-throughput isolation methods using DEP-arrays, microfluidics, emulsion-based platforms or 10X genomics Chromium™ single cell controller system. This technical advances could provide massive advantages in terms of significantly increase the throughput sensitivity and accuracy of employed approaches (Figure 1B).”
- 3- Adding “figure 2” to illustrate the main applications of single-cell based profiling in cancer research.
- 4- Adding “table 1” to list single-cell sequencing studies on variety of human tumors. Also the following paragraph is added to the manuscript: “Important findings using single-cell sequencing studies on variety of human primary tumors including bladder, blood, brain, breast, colorectal, kidney, lung and ovarian cancer, are summarized in Table 1”.
- 5- Adding extra section for “single-cell T cell receptor sequencing of tumor infiltrating lymphocytes”.

Reviewer 3

The manuscript highlight the recent methodologies advances for molecular characterization and profiling of CSC. Very interesting the section related to single-cell based approaches for the detection of molecular biomarkers. However, it would be suitable for this part to be more detailed and with more examples, this being the main purpose of the manuscript (according to the title). It would be appropriate to change the manuscript with the reduction of the first part referred to whole cell population analysis, and the extension of the second part focusing on single-cell RNA-Seq, multiplexed error robust fluorescence (MERFISH), quantitative hybridization chain reaction (qHCR), lineage tracing by nuclease-activated editing of ubiquitous sequences (LINNAEUS), single-cell whole exome sequencing (scWES), with a broader description of the method and some examples of use in diagnosis. Overall, the manuscript is well written and definitely helpful.

Authors: We tried to improve the second part of manuscript accordingly by adding the following parts:

- 6- Improving the “figure 1”.
- 7- Adding “figure 2” to illustrate the main applications of single-cell based profiling in cancer research.
- 8- Adding “table 1” to list single-cell sequencing studies on variety of human tumors.
- 9- Adding extra section for “single-cell T cell receptor sequencing of tumor infiltrating lymphocytes”.

Reviewer 4

The present manuscript focuses on recent advancements towards the discovery of cancer related biomarkers of diagnostic, prognostic and predictive significance. Molecular heterogeneity at cell level and the presence of cancer stem cells advocate single cell based analysis/ profiling as a better approach towards improved investigation of more specific biomarkers to identify and target cancer stem cells. Authors should discuss in detail genomics, epigenomics, transcriptomics and proteomics approaches for diagnosis and prognosis in tumor cells as well as CSCs.

Authors: Focusing on genomics, epigenomics, transcriptomics and proteomics approaches for diagnosis and prognosis in tumor cells is not a direct aim of our review manuscript and only was used to highlight the weaknesses of those methods and necessity of introducing novel single-cell based approaches in cancer biomarker discovery.