Title: Murine Hepatocellular carcinoma derived stem cells reveal epithelial-to-mesenchymal plasticity

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Name of Journal: World Journal of Stem Cells

ESPS Manuscript NO: 34591

Column: Basic Study

Dear Editor,

We would like to thank you for reconsidering our manuscript no **34591** entitled "Murine Hepatocellular carcinoma derived stem cells reveal epithelial-to-mesenchymal plasticity" by Jayachandran *et al*, for publication in World Journal of Stem Cells as a basic study. Thank you for your instructions and the comments from reviewers.

We have revised the manuscript and added a point-by-point response to all the reviewer's comments below. For easily viewing the changes, we have highlighted them in red in the manuscript. We have listed reviewer's comments and our response in details below.

We trust that these findings will be of interest to readers of World Journal of Stem Cells and look forward to your response.

Yours sincerely,
Dr Jason C Steel
(On behalf of the authors)

Reviewer 1 comments:

This manuscript is interesting, presenting a feasible method for concentrating a stem-like population from hepatic cancer cells by extending their previously reported technique for enriching a cancer-initiating population from lung cancer cell lines (Morrison BJ et al.,

PLoS One 2012; 7: e49752). They also showed that the concentrated population bears mesenchymal characteristics, suggesting that epithelial-to-mesenchymal transition (EMT) has taken place in that population. I hope that their technique will contribute to an advanced understanding of cancer stem cells. Although the content of the manuscript is clear and solid, there is one concern regarding the use of the term "sphere". As is well known, E-cadherin is expressed in immature stem cells (e.g. morula cells and pluripotent stem cells) and is suggested to play an indispensable role for an implementation of compaction. As authors showed in Figure 3A, E-cadherin expression levels were considerably low in Hepa 1-6 CSCs compared to AML12 CSCs. This finding is consistent with the morphological observation (Figure 1), where Hepa 1-6 CSCs created irregularly shaped aggregates without compaction while AML12 CSCs created typical and compact sphere-like structures. Therefore, it would be appropriate for authors to use the term "aggregates" instead of "spheres" throughout the manuscript.

Response:

1. When grown in a stem cell conditioned serum-free medium, CSCs grow initially as small spheres which eventually form 3-dimensional structures by day 5 (Figure 1A&B). As these 3-dimensional spheres are very distinct from non 3-dimensional aggregates of cells sticking together, we have changed the term spheres to 3-dimensional spheres throughout the manuscript for clarity.

2. The reviewer suggests that differences in the level of E-cadherin expression (Figure 3A&C) between spheres derived from Hepa 1-6 and AML12 may correlate with differences in the morphology of the spheres. This is not the case, as we noted higher Vimentin expression in AML12 derived spheres compared with Hepa1-6 derived spheres (Figure 4).

3. We agree with the reviewer that E-cadherin is crucial for maintaining the pluripotent state of immature embryonic stem cells and a loss in E-cadherin drives EMT that results in differentiation of embryonic stem cells. However, in the context of cancer, E-cadherin is considered a repressor of tumor progression and metastasis. During EMT, E-cadherin downregulation is often accompanied by other synchronized changes in EMT markers, including ZO-1, Vimentin, Fibronectin, Snai1, Snai2, Zeb1, Zeb2, Twist1 and Twist2 (Figures 3-6). Analysing a panel of EMT markers is more informative than assessing individual markers, while investigating the acquisition of CSC-EMT phenotype.

Reviewer 2 comments:

The manuscript entitled "Murine Hepatocellular carcinoma derived stem cells reveal epithelial-to mesenchymal plasticity" addresses very intriguing issue of tumor initiating cells on particular model of hepatocellular cancer. They applied well known sphere formation assay to access whether it is applicable for investigation of hepatic cells stemness and epithelial to mesenchymal transition. Using qRT-PCR they proved that their model maybe a valuable tool to investigate tumor initiating cells that have much common with stem cells.

Response:

We thank Reviewer2 for favourable comments.

Editor's suggestions in the manuscript file:

1. When you send back, please provide the format of doc, not the pdf. Thank you!

Response: We have uploaded the word document file.

2. Please offer the postcode! Thank you!

Response: We have provided the postcodes.

3. Please offer signed pdf file. Thank you!

Response: We have submitted the copyright assignment form signed by all authors.

4. Please offer the grant approval. Thank you!

Response: We have provided the grant approval number.

5. Please offer other statements. Thank you.

Response: We have included all the statements

6. Please offer the audio core tip, the requriment are as follows:

In order to attract readers to read your full-text article, we request that the first author make an audio file describing your final core tip. This audio file will be published online, along with your article. Please submit audio files according to the following specifications: Acceptable file formats: .mp3, .wav, or .aiff Maximum file size: 10 MB

To achieve the best quality, when saving audio files as an mp3, use a setting of 256 kbps or higher for stereo or 128 kbps or higher for mono. Sampling rate should be either 44.1 kHz or 48 kHz. Bit rate should be either 16 or 24 bit. To avoid audible clipping noise, please make sure that audio levels do not exceed 0 dBFS.

Response: We have included the audio core tip

7. Comments. The comments section aims to help readers avoid misunderstanding or over-interpretation of your study by summarizing the content of your article, including technical details, in a precise and simple manner. The comments section is broken down into the following subsections: background, research frontiers, innovations and breakthroughs, significance of the applications, terminology, and comments from peer-reviewers. The specific requirements for each subsection are provided below.

(1) Background To summarize concisely and accurately the relevant background information so that readers may gain some basic knowledge about your study's relevance and understand its significance for the field as a whole.

(2) Research frontiers To introduce briefly the current hotspots or important areas in the research field as related to your study.

(3) Innovations and breakthroughs To summarize and emphasize the differences, particularly the advances, achievements, innovations and breakthroughs, as compared to other related or similar studies in the literature, which will allow the readers to assimilate the major points of your article.

(4) Applications To summarize the practical applications of your research findings, so that readers may understand the perspectives by which this study will affect the field and future research.

(5) Terminology To describe concisely and accurately any terms that may not be familiar to the majority of the readers, but which are essential for understanding your article.

Response: We have included the comments section in the manuscript.

8. Please add PubMed citation numbers and DOI citation to the reference list and list all authors. Pleased provide PubMed citation numbers for the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in the E-version of this journal. Thanks very much for your co-operation. Such as: 1 Nayak S, Rath S, Kar BR. Mucous membrane graft for cicatricial ectropion in lamellar ichthyosis: an approach revisited. Ophthal Plast Reconstr Surg 2011: e155-e156 [PMID: 21346670 DOI: 10.1097/IOP.0b013e3182082f4e]

Response: We have included missing PMID numbers and highlighted this in red.