April 9, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 9687-edited-WJSC.doc).

Title: Adipose-derived stem cells: implications for tissue regeneraiton

Author: Wakako Tsuji, J. Peter Rubin, Kacey G. Marra

Name of Journal: *World Journal of Stem Cells*

ESPS Manuscript NO: 9687

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers

**Reviewer 1**

(1) The introduction is overly simplistic. For example, definitions used in the introduction to describe MSCs and ASCs are rudimentary, and fail to convey the unique tissue-specific characteristics of these populations. Moreover, the statement that MSCs reside in tumors is ambiguous, as it is unclear whether epithelial cells acquire MSC-like characteristics via an epithelial-to-mesenchymal transition (see Battula et al. Stem Cells 2010; 28:1435). Therefore, the authors should provide a more comprehensive overview of the nature and complex biology of ASCs and MSCs that more accurately reflects the current state of the field. Ironically, in section 2 of the manuscript the authors state that "just as ASCs from different anatomical areas have different characteristics" but fail to elaborate on this statement.

**Answer:**

Further descriptions have been added to the introduction. The description that MSCs in cancer was deleted, and we've discussed the possible relationship between ASCs and cancer cells in another section.

(2) The authors indicate that ASCs become more “homogeneous” as a result of passage in vitro. They should specify whether the term “homogeneous” refers solely to surface phenotype and/or if passage enriches for other functional characteristics. Clarification is needed as the authors go on to discuss how ASCs contain various subpopulations of cells with distinct phenotypes. Are they homogeneous or not?

**Answer:**

The term ‘homogenous’ was used for cell surface phenotype, as we mentioned that the expression of CD34 dissipates and CD29, CD73, and CD90 increases. The description has been changed.

(3) The argument that ASCs possess unique paracrine characteristics that make them suitable for tissue repair is not well substantiated. For example, various cell types secrete VEGF and up regulate expression of this protein in response to hypoxia. How do ASC-based therapies compare to VEGF therapy or use of other cell-based therapies? While it is well established that ASCs secrete paracrine acting factors, the authors do a poor job of describing translational studies that demonstrate the value of ASC-based therapies. Citing one or two papers that indicate ASCs enhance tissue regeneration without providing any context, e.g. controls used, comparative treatment regimens, etc. does not make a convincing argument.

**Answer:**

We have now added further description regarding the efficacy of ASC-based therapy.

(4) The authors state that tri-lineage differentiation of ASCs to connective tissue fates is “less controversial” and describe methods by which cells are induced to undergo lineage commitment. Since these methods are all well-established, these descriptions are viewed as redundant and not very informative. In contrast, a more rigorous comparison of the tri-lineage potential of ASCs compared to other cell types, e.g. marrow, cord blood, placental-derived MSCs, synovial fibroblasts, etc. would be more informative and also further establish that not all MSC-like cells are equivalent. For example, whether ASCs actually generate articular cartilage remains controversial but is an important question regarding their clinical use, e g. does differentiation to hypertrophic chondrocytes preclude formation of articular cartilage?

**Answer:**

Mesenchymal stem cells from different tissues have different levels of differentiation ability. For example, adipose-derived stem cells have superior differentiation ability towards adipose tissue, and bone marrow-derived stem cells have osteogenic

differentiation ability. The comparison of tri-lineage potential of ASCs compared to other cell types are not within the scope of this review article.

(5) The authors point out ASCs can survive in ischemic tissues, and hypoxia induces VEGF secretion, but then state that ASCs exhibit poor survival in vivo due to ischemia. They then provide a discussion about scaffolds but never address how cell survival following transplantation may be augmented.

**Answer:**

The description 'poor cell survival after *in vivo* injection or implantation is common.' is not meant only for ASCs but for all types of injected cells. It is important how quickly angiogenesis occurs after ASCs are implanted with the scaffold. We have now added this description.

(6) Information regarding use of ASCs for bone and cartilage repair is also overly simplistic and descriptions of clinical studies fail to point out that most trials are not randomized and/or are often case reports, and therefore need to be interpreted cautiously.

**Answer:**

Clinical studies using BM-MSCs are relatively common, but clinical trials utilizing ASCs are less common. ASCs are typically less osteogenic compared to BM-MSCs. Therefore, we have added additional descriptions of clinical studies.

**Reviewer 2**

The article "Adipose-derived stem cells: Implications in tissue regeneration" by Tsuji et al gave a comprehensive review of the differences between ADSC and MSC derived from other sources. Minor comments: The authors mentioned that brown adipose tissues expressed uncoupling protein 1 without explanation. The readers are left wondering what is the significance of uncoupling protein 1 expression in MSC? Mesenchymal stem cells can also be derived from beige adipose tissues. Perhaps the authors can comment on that in the text. Please correct all typo and grammatical mistakes.

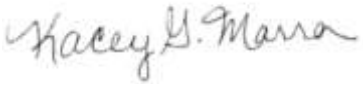
**Answer:**

Thank you for your comments. According to your suggestion, descriptions about beige adipose stem cell have been added.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Stem Cells.*

Sincerely yours,



Kacey G Marra, PhD
Adipose Stem Cell Center
Department of Plastic Surgery Department
University of Pittsburgh
200 Lothrop Street
Pittsburgh PA 15213
Fax: +1-412-383-8924
E-mail: marrak@upmc.edu