Format for ANSWERING REVIEWERS

September 27, 2015

Dear Editor,

Please find the revised manuscript in Word format (file name: 21958_Review.docx).

Title: Adult Stem Cells as a Tool for Kidney Regeneration

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Name of Journal: *World Journal of Nephrology*

ESPS Manuscript NO: 21958

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 reviewer
Please see below.

3 References and typesetting were corrected.

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

Sincerely yours,

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Responses to Reviewers
Responses to Reviewer #02398400
We appreciate your review of our manuscript.

1. The authors state that clinical use of ESCs is limited due to ethical concerns and the "small number of stem cells". While the later may be true a more prominent concern is their propensity to form teratomas in vivo.

We wanted to mention that stem cells in the embryonic kidney are a promising source for kidney regeneration, although their clinical use is limited. "Embryonic stem cells" in the sentence refers to stem cells in the embryonic kidney rather than to ES cells (pluripotent stem cells isolated from the inner cell mass of blastocysts). We have modified the sentence to clarify the meaning.

--- Although stem cells in the embryonic kidney are a promising source for kidney regeneration,---(page 6, line 13).

2. It would be interesting to indicate whether anyone attempted to isolate LRCs from the kidney and examine their phenotype.

Oliver et al. (J Am Soc Nephrol 20; 2315-2327: 2009) examined the expression of stem cell-specific markers in LRCs. They reported that LRCs isolated from the kidney expressed a higher amount of CD133 and Pax2 than non-LRCs. However, they used real-time PCR analysis to examine their expression by LRCs; they did not examine their expression at protein levels. We therefore did not cite this paper in the manuscript.

3. When discussing transplantation studies of SP cells, CD133+ and other putative kidney stem cell populations, the authors may want to provide more details of how engraftment was analyzed as the term is widely misused to imply functional incorporation into a tissue. In many cases labeled cells injected into a tissue survive short term, and/or get trapped in the tissue via the circulation but never adopt a phenotype of tissue resident cells and/or contribute to tissue function. These distinctions are important in evaluating such studies.

In the papers we cited, some proportions of injected stem/progenitor cells were incorporated into kidney structures (mostly into renal tubules). Stem/progenitor cells were labeled with a fluorescent dye or modified to express β-galactosidase prior to injection into the kidney, and their localization in the kidney was examined under a microscope. Otherwise, human stem/progenitor cells were injected into the kidneys of other species (usually mice) so that injected cells could easily be detected using antibodies that specifically react with human proteins. The co-localization of a marker for stem/progenitor

cells (e.g., a fluorescent dye) and renal tubule markers was examined in some studies. Our understanding is that in most studies, the term “engraftment” is used to describe the incorporation of stem cells into injected tissue and the expression of the phenotype of resident cells by stem cells. However, as you have pointed out, in some studies, “engraftment” does not always mean functional incorporation. We therefore changed the term “engraftment” to “incorporation” or “integration” in the revised manuscript. We also changed the word “Engraftment” to “Incorporation” in Table 1. However, for conciseness, we did not explain in detail how the incorporation of stem/progenitor cells was confirmed in those studies. The following parts have been changed in the revised manuscript:

-- some cells were incorporated into renal tubules.—(page 7, line 13)

-- and integrated into—(page 8, line 6)

-- these cells were barely incorporated into the renal tissues—(page 8, line 9)

--- they were incorporated predominantly into the proximal and distal tubules. (page 8, bottom)

-- These cells were integrated predominantly into renal tubules (page 9, line 5)

--, some of these cells were integrated into renal tubules. (page 9, line 10)

--- These cells were incorporated into renal tubules—(page 9, line 7 from the bottom)

Incorporation into kidney tubules (In Table 1)

4. In describing the Phase I clinical trial using BMMSCs for the prevention of AKI after open heart surgery, the authors suggest the trail was a success because no patients required hemodialysis. The authors should indicate how the study was powered and if outcomes were significant as compared to controls or placebo.

We introduced this trial because this is the only clinical trial published so far in which adult stem cells were used to treat kidney disease. As this is a phase I study, the primary objective was to confirm the safety of BMMSC administration to patients. However, the authors also analyzed the efficacy of BMMSC administration in the prevention of AKI. Although this trial was not a prospective study and controls in this study were historical controls, the renal function of patients who were administered BMMSCs was stable after open-heart surgery, compared with a 20% occurrence of AKI in controls. Therefore, this treatment appears to be effective. It seems that the final report of this study has not been published. We have modified sentences in the revised manuscript:

---A phase I clinical study evaluated the safety and efficacy of allogenic BMMSC administration for the prevention of AKI after open-heart surgery[39]. This study enrolled 16 patients who required on-pump cardiac surgery and who were at a high risk of postoperative AKI due to underlying chronic kidney disease, advanced age, diabetes mellitus, and congestive heart failure. Allogenic BMMSCs were injected into the suprarenal aorta after surgery. The primary objective was the safety of BMMSC administration. The secondary objective was the efficacy of this treatment compared with well-matched historical controls. This treatment appeared to be both safe and effective as no adverse events related to the procedure were reported, and renal function was well preserved post-operatively, with no patients requiring hemodialysis after surgery, whereas 20% of the controls developed AKI. This is the only clinical trial published so far in which ASCs were used to treat kidney disease. (page 12, line 10)

5. The authors should revise the statement that liposuction is less invasive than harvesting bone marrow aspirates. This statement has been propagated throughout the literature without any clinical justification. Both procedures are invasive and may be painful. Also, while the yield of adherent cells from adipose is greater than bone marrow, the actual number of stem cells is much more difficult to quantify.

We have deleted several sentences from the revised manuscript so that the statement is not implied. The following parts in the original manuscript have been deleted from the revised manuscript.

--, a procedure that is less invasive than bone marrow aspiration,--(page 12, line 7 from the bottom in the original manuscript)

-- Furthermore, the adipose tissue contains a significantly greater proportion of stem cells than bone marrow (5% vs. 0.01%), and it is, therefore, a convenient source of stem cells[40].—(page 12, line 6 from the bottom in the original manuscript)

6. The section on iPS reprogramming was well written, but a critical barrier that remains is the poor efficiency of the process. Therefore, while the technique is relatively straightforward, efficiency remains a limiting factor.

We have modified sentences in the revised manuscript so that this is implied.

-- First, the efficiency of iPS cell preparation from adult somatic cells is still low.—(page 15, line 3 from the bottom)

7. Both ASCs and BMMSCs release extracellular vesicles. Therefore the title of this section should be changed to reflect the fact that vesicle release is not a unique property of ASCs.

In this manuscript, “ASCs” is the abbreviation for adult stem cells that include bone marrow-derived stem cells, adipose tissue-derived stem cells (abbreviated as ADSCs in the manuscript), and other mesenchymal stem cells. To avoid misunderstanding, we changed “ASCs” to “MSCs” (the abbreviation for mesenchymal stem cells) in the revised manuscript.

8. The review would be improved if the authors could include a few comments on which stem cells evaluated to date in translational models appear to yield the best outcomes. This would help inform the reader as to which area of stem cell research is more mature with respect to therapeutic interventions for kidney disease.

Stem cell therapy for kidney disease is an immature field compared with that for cardiovascular disease (CVD). Many clinical trials using BMMSCs and ADSCs have been performed to treat CVD and have proved to be safe and effective. As we have mentioned, the results of only one clinical trial in which BMMSCs were used to prevent AKI after open-heart surgery have been reported so far with regard to the treatment of kidney disease. We believe that it is premature to recommend a specific area of stem cell research to readers.

Responses to Reviewer #02446219
We appreciate your review of our manuscript.

1- *Titles should be uniform, some titles are full name and acronym together, but the others are only acronym.*

We mentioned the abbreviation in parentheses at their first appearance and used the acronym thereafter. For example, we mentioned “EPCs” in the title (page 13) because it is the second appearance of “EPCs”. The first appearance is on page 8 [we described “vascular endothelial progenitor cells (EPCs)”]. We mentioned “Umbilical cord blood (UCB)” in the title (page 13) because this is the first appearance of this term.

2- *In title “Umbilical cord blood ” and “Amniotic fluid” phrase of stem cells should be added to the end of these two titles.*

We have modified the titles:

Umbilical cord blood (UCB)-derived MSCs (page 13, bottom)

Amniotic fluid stem cells (page 14, line 5 from the bottom)

3-Mesenchymal stem cells release EVs, but in the manuscript BMSCs are the only cells which have this property, whereas the other mesenchymal stem cells also have such trait.

Please also refer to our responses to Reviewer #02398400, question #7. We did not state that BMMSCs are the only source of EVs. Although other mesenchymal stem cells do release EVs, EVs released from BMMSCs have been mostly used to treat kidney disease in animal models. That was why we cited papers in which EVs derived from BMMSCs were used. We added sentences to introduce a paper in which EVs harvested from umbilical cord blood-derived mesenchymal stem cells were used to treat kidney disease.

-- Zhou et al. administered EVs harvested from human UCB-derived MSCs to a rat model of cisplatin-induced AKI. EV administration significantly restored renal function and morphology[75]. (page 17, line 3 from the bottom)

Minor:

1) As we have deleted some sentences in the manuscript, reference numbers mentioned in Table 3 have changed.
2) We found a mistake in the use of an acronym and changed “UBC” to “UCB”. (page 14, line 9 from the bottom)
3) To correct a mistake in a title, we changed “Direct/indirect reprogramming of ASCs” to “Direct/indirect reprogramming of adult somatic cells”. (page 15, line 6)