ANSWERING REVIEWERS

February 04, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: **15189**-review.doc).

Title: Fetal kidney stem cells ameliorate cisplatin induced acute renal failure and promote renal angiogenesis

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Name of Journal: *World Journal of Stem Cells*

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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. The point-wise response to the comments of the reviewers is given below.

Reviewer-1

- **Reviewer's Comment:** Characterization of Endothelial cells is poor. The Authors should provide some data on migration (J Clin Invest, 2014; 124: 4102-4114).

  Response: The aim of this study was to demonstrate the angiogenic potential of fetal kidney stem cells (fKSC) which we have successfully demonstrated by matrigel induced tubularization of these stem cells and expression of endothelial markers CD31 and vWF on the tubules. Similar to our study, Ramkisoensing *et al*., 2011, PMID: 21931658 have also demonstrated angiogenic potential of embryonic stem cells derived mesenchymal stem cells by matrigel induced tubularization. Hence, we have not done a detailed characterization of endothelial cells present in the tubular structure formed by fKSC. As far as their migration potential is

concerned, the engraftment of intravenously infused fKSC into injured kidney demonstrates *in vivo* migration of these stem cells.

- **Reviewer's Comment:** Blood pressure of the animals should be provided. Indeed, endothelial dysfunction, hypertension and kidney damage are strictly related (J Am Heart Assoc. 2012 Aug;1(4):e001081). The role of hypertension in determining the risk of coronary artery disease (Coronary heart disease risk factors and mortality. JAMA. 2012 Mar 21; 307(11):1137 - PMID: 22436947) should be mentioned.

  Response: The parameters like blood pressure, endothelial dysfunction, and hypertension are particularly important in the coronary artery diseases and may have a relation with chronic kidney injury but these parameters are not of much relevance in the induction of acute renal failure (ARF). Since our study was on ARF, the above mentioned parameters have not been used in this study. Regarding hypertension, the Reviewer himself has mentioned that it is a major risk factor for coronary artery disease.

- **Reviewer's Comment:** Fig. 8A: the molecular markers on the gel should be shown. At least two lines per sample should be provided.

  Response: We have revised Fig 8A as suggested by the Reviewer.

- **Reviewer's Comment:** The study limitations should be extensively addressed. The conclusions should be toned down. Language needs some polishing for better flow.

  Response: The study limitations have been incorporated, the conclusions of the study have been toned down (highlighted in yellow) and the language flow has also been improved in the revised manuscript as pointed out by the Reviewer.

Reviewer-2

The Reviewer has appreciated the study and mentioned that it is acceptable for publication. We appreciate the Reviewers' opinion about our manuscript.

Reviewer-3

The Reviewer has stated that the manuscript is well written and its data are convincing. However, he has given certain comments that are addressed below.

❖ **Major comments:**

- **Reviewer's Comment:** As Pax2 over expression has been shown to reduce WT1, why the fKSC cells express both markers highly? This should be discussed.

  Response: The high expression of both Pax2 and Wt1 by fKSC has now been discussed in the revised manuscript as suggested by the Reviewer (highlighted in yellow).

- **Reviewer's Comment:** This should be discussed. HIF-1α does not only activate VEGF, but also WT1. In Western Blots, is there a difference in Wt1 expression?

  Response: It has been reported that HIF-1α not only activates VEGF but also Wt1, which in turn activates VEGF resulting in increased angiogenesis (Wagner et al., 2003, PMID: 12738801; McCarty et al., 2011, PMID: 22030397; Katuri et al., 2014, PMID: 24810959). Since fKSC that were infused in ARF rats were already expressing Wt1, we did not study its expression in the kidney tissues. In western blots we studied the expression of HIF-1α, VEGF and eNOS and observed their increased expression in the kidney tissues of fKSC treated compared to saline treated rats. Since fKSC therapy up-regulated HIF-1α, it is likely that kidney tissues also have up-regulation of Wt1. However, since the angiogenic role of Wt1 has mostly been reported in tumors, it is not known whether Wt1 also has a similar role in normal tissues like kidney. This has now been incorporated in the revised manuscript (highlighted in yellow).

- **Reviewer's Comment:** Do the fKSC express nephrin or nestin, which are two important Wt1-dependent molecules in the kidney?

  Response: Nephrin and nestin are activated by Wt1 in the developing kidney and represent important markers of glomerular epithelial cells (podocytes). Thus kidney stem cells that are destined to give rise podocytes and express Wt1 will also have expression of these two podocytic markers (Wagner et al., 2006, PMID: 16614054; Wagner et al., 2004, PMID: 15579507). Since our objective was not to characterize in detail the markers of fKSC but to study their

efficacy in ARF, we have not evaluated expression of nephrin and nestin. However, since fKSC express Wt1, a fraction of these stem cells could also express nephrin and nestin markers.

- **Reviewer's Comment:** As Wt1 has been described recently as important regulator of angiogenesis (Wagner et al., Nat Commun. 2014 Dec 16;5:5852), do the Wt1 expressing fKSC contribute directly to the neo- angiogenesis? All the above mentioned papers should be cited.

  Response: A number of studies published recently on tumor angiogenesis have shown Wt1 to be an important regulator of angiogenesis (Katuri et al., 2014, PMID: 24810959; Wagner et al., 2006, PMID: 16614054; Wagner et al., 2004, PMID: 15579507; Wagner et al., 2014, PMID: 25510679). As already explained above, HIF-1α can induce Wt1 which can directly up-regulate VEGF resulting in increased angiogenesis. The fKSC express Wt1 and their infusion in ARF rats results in increased expression of HIF-1α which in turn can up-regulate Wt1 expression in the kidney tissues. Although we have not evaluated in this study the angiogenic role of Wt1 but the above mentioned studies reveal that it may contribute directly to neoangiogenesis. However, the angiogenic role of Wt1 has mostly been reported in tumors and whether its angiogenic properties are also maintained in normal tissues remains to be seen. This part including its references has been discussed in the revised manuscript as suggested by the Reviewer (highlighted in yellow).

❖ **Minor comments:**

The short title is not really short. Page 7: Retinoic acid Page 7: the animals were given Page 8: "All animals were euthanized by barbiturate overdose (intravenous injection, 150 mg/kg pentobarbital sodium) before fKSC administration on day 0 and after fKSC administration on days 3 and 7" needs clarification. Page 9: room temperature Page 10: horseradish Page 12: TUNEL Page 13 and 14: renal function Page 15: by VEGF instead of these VEGF Figure 4 legend: Scale bars indicate 20µm. Figure 4: Please point to the signs of kidney damage with different types of arrows and explain in the legend.

Response:

- The short (running) title has been further shortened in the revised manuscript as "*Fetal kidney stem cells ameliorate acute renal failure*".
- The corrections pointed out by the Reviewer on page 7 have been made and incorporated in the revised manuscript (highlighted in yellow).

- The sentence referred by the Reviewer on page 8 of the manuscript has been clarified in the revised manuscript as "*Animals were euthanized by barbiturate overdose (intravenous injection, 150 mg/kg pentobarbital sodium) before fKSC therapy (day 0) and after three days (day 3) and seven days (day 7) of fKSC therapy*" (highlighted in yellow).
- The corrections pointed out by the Reviewer on pages 9, 10, 12, 13, 14 and 15 have been made in the revised manuscript (highlighted in yellow).
- In legend of Fig 4 it has now been mentioned that "*Scale bars indicate 20μm*" (highlighted in yellow) and in Fig 4 different signs of kidney damage have been indicated by different types of arrows and the same has been explained in the legend of the figure of the revised manuscript as suggested by the Reviewer.

3. References and typesetting were corrected

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

Sincerely yours,

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