Response to Reviewers Comments Manuscript # 84723

Reviewer #1:

Specific Comments to Authors: I would like to thank you for your suggestion to review the paper entitled " IWP-4, a potent Inhibitor of the Wnt signaling pathway promotes MSCs differentiation into cardiac progenitor cells in vitro and improves cardiomyopathy in vivo" for World Journal of Stem Cells: According the check list I have added the following comments; The title reflects the main subject/hypothesis of the manuscript Generally, it is an interesting study, however there are some comments and questions the authors should address all were detailed below: Major concerns

Thank you very much for the in-depth review of the manuscript and for highlighting the shortcoming, which helped us to improve the manuscript. We have incorporated all the changes as mentioned and suggested by the reviewers. Concerns raised by the reviewers have also been justified. The reviewers will now find the manuscript substantially revised concerning the suggestions.

1. The components of culture media used for induction of cardiomyocyte differentiate weren't provided.

The components of culture media used for induction of cardiomyocyte is already mentioned in the materials and methods section heading 'Treatment of compound'. Briefly, 5 µM concentration was optimized for the treatment. 20µM stock compound was dissolved in 50mL of complete DMEM. This conditioned media was used for the differentiation of MSCs into cardiomyocytes.

2. Based on cardiomyocyte phenotype and electrophysiology, cardiogenic differentiation must be confirmed.

Cardiogenic differentiation was confirmed by morphology, immunocytochemistry, and gene expression analysis. The morphology of differentiated cells was flattened and appeared larger with myotube like structures shown in Figure 5A. Immunocytochemistry and cardiac gene expression further confirmed the cardiogenic differentiation as shown in Figure 5B and Figure 3 respectively. In the current study, our aim was to differentiate MSCs into cardiac like cells that were transplanted *in vivo*. These transplanted cardiac like cells converted into mature cardiomyocytes and repaired the fibrotic tissue. These differentiated cells were not matured cardiomyocytes *in vitro* that's why electrophysiology was not performed.

3. What was the rational for induction of trilineage differentiation of stem cells?

To characterize MSCs from umbilical cord tissue, we analyze their morphological appearance, immunostaining and immunophenotyping for the presence of MSCs specific markers. Similarly, MSCs possess trilineage differentiation potential i.e. they can differentiate into mesodermal lineage i.e adipocytes, chondrocytes, and osteocytes.

In order to validate that isolated cells were MSCs, we performed trilineage differentiation using adipogenic, osteogenic and chondrogenic induction media.

4. IWP-4 is a well known inhibitor for Wnt, its effect on the expression of cardiac markers and stimulation of stem cell differentiation into cardiomyocytes was previously established in stem cell research by Chen et al. 2015" Development of a scalable suspension culture for cardiac differentiation from human pluripotent stem cells", however, the newly added part in this study is the in vivo work, so the authors are encouraged to focus more on the in vivo results.

We agree with the reviewer that no in vivo studies were reported for cardiac regeneration. To the best of our knowledge, no work has been performed on Mesenchymal Stem cells using IWP-4. The published work was carried out on induced pluripotent stem cells. For this reason, we tried to focus on both *in vitro* as well as *in vivo*.

5. additional work is needed to highlight the effectiveness of preconditioning on optimizing stem cell therapy.

Pre-differentiated MSCs have various advantages over undifferentiated MSCs. Pre-differentiated MSCs have better survival and homing as compared to undifferentiated MSCs. As previous studies showed that undifferentiated cells escape from the site of injury while differentiated cells homed and reside there. It was further confirmed by the tracking of cells labeled with DiI dye as discussed in the discussion part.

6. Please, provide more clear figure for differentiated stem cells (figure 5).

The aim of this study is to pre-differentiate MSCs into cardiac progenitor cells so that cells home in the infarcted myocardium, where they survive, proliferate, and differentiate into mature cardiomyocytes. The fully differentiated cells lost the ability to divide. Additionally, these cells are unable to survive the harsh microenvironment of the infarcted heart.

However, we provided pieces of evidence in figure 3, 4, and 5 that cells differentiated into cardiac like cells.

7. The abstract must be summarized.

It is summarized.

8. Introduction is too long.

It has been reduced.

Reviewer #2:

Specific Comments to Authors: This MS mainly investigated the potential of "treatment of

hUC-MSCs with IWP-4 for differentiation of MSCs into cardiomyogenic lineage via inhibiting Wnt pathway and their succeeding role in the cardiac function restoration in the rat MI model." and proposed that preconditioning of MSCs with IWP-4 may be a promising approach for the treatment of this debilitating heart disease, which provided a means for the production of cardiomyocytes from discarded human umbilical cord tissue for cardiac cell therapy. However, below some revisions are still needed before being published in World Journal of Stem Cells.

Thanks to the reviewer for understanding the importance of the topic. We acknowledge the reviewer for critically analyzing our work and suggesting improvements.

1. Please refer to recent papers published in World Journal of Stem Cells and correct the format, such as: In page 1, "Running title", "Author contributions" and "Supported by" section should be added. Before REFERENCES section, ARTICLE HIGHLIGHTS section including Research background, Research motivation, Research objectives, Research methods, Research results, Research conclusions, and Research perspectives section should be added according to the journal's requirements. In page 1 and 2, "Background", "Aim", "Methodology", "Results", and "Conclusion" should be changed to "BACKGROUND", "AIM", "METHODS", "RESULTS", and "CONCLUSION". In page 3, 12, and 16, "Introduction", "Results", and "Discussion" should be changed to "INTRODUCTION", "RESULTS" and "DISCUSSION". In page 4, "[7][8]" should be changed to "[7, 8]".

All the suggested corrections have been incorporated in the revised manuscript.

2. In page 29, "Figure1:" should be changed to "Figure 1". In figure legends, all abbreviations should be given in full text according to the journal's requirements. Additionally, In Figure3 legend, it is suggested that "Cardiac markers gene expression analysis: Cardiac markers gene expression analysis by qPCR" should be changed to "Cardiac markers gene expression analysis by qPCR". Not *P < 0.05, **P < 0.01, and ***P < 0.001 while aP < 0.05, bP < 0.01, and cP < 0.001 < 0.001 in all bar charts? As shown in Figure 4B, GSK expression was increased in the fourteen days treatment while significant decrease was described in the text. Please check it. In Figure5, it is suggested that Figure 5C should be placed before Figure 5A, and Day 0, Day 7, Day 14 should be put above the picture. Additionally, α-actinin, connexin-43, cTnI, Desmin, GATA-4, Nkx2.5 should be put beside the vertical axis while not under the horizontal axis. There are too many images in Figure 7 and Figure 8. It is recommended to remove some images, and a local enlarged image could be given in the right side of each Masson's trichrome or HE stained image. In Figure9, in order to facilitate a comparison between the transplanted untreated MSCs and fourteen days IWP-4 treated MSCs group, it is suggested that all alpha actinin images could be listed as Figure 9A, and the corresponding quantification of bar graph could be used as Figure 9B. Similarly, all cTnI images could be listed as Figure 9C, and the corresponding quantification of bar graph as Figure 9D. All GATA-4 images could be listed as Figure 9E, and the corresponding quantification of bar graph as Figure 9F.

All corrections have been made. All suggested changes in figures have been performed.

3. In references, all authors, PMID and DOI should be provided, and the first author and volume number should be bold. Additionally, all journal names should be abbreviated and italicized. Please check all references including content and format carefully according to the journal's requirements. Other suggestiones have been listed in the uploaded revised version.

All references have been corrected.

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