

The Editors  
World Journal of Stem Cells  
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Pleasanton, CA 94566, USA

Dear Editors:

Thank you for giving us the opportunity to revise our manuscript entitled “Small molecules for mesenchymal stem cell fate determination”. We appreciate the careful review and constructive suggestions. It is our belief that the manuscript is substantially improved after making the suggested edits.

Following this letter are the comments with our responses, including how and where the text was modified. Changes made in the manuscript are marked in red or by tracking. Thank you once again for your reconsideration.

Sincerely,  
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We appreciate the editor's comments. The followings are responses to the comments, and the revisions are labeled in red in the manuscript:

#### Reviewer #1:

We appreciate the reviewer's comments. The followings are our point-by-point responses:

- 1) The title is generalized, it must be meaningful Abstract of the review is general covering stem cells differentiation, while the contents are focused on trilineage differentiation.

Response:

The reason why the term "cell fate determination" instead of "differentiation" was used was that, in the most of the studies, the small molecules present at the beginning of induction in the in vitro experiments, and administrated alone to assess the effect in the animal experiments, indicating the effects of the small molecules are related to the initiation of induction, which is cell fate commitment and followed by differentiation into terminal cell types. Therefore, we used the term "cell fate determination" in the title. However, the administration of the small molecules sometimes last until the end of the differentiation, so they affect the differentiation of MSCs at the same time, which was part of the reason why most of the literature cited only used the term "differentiation".

For the cell fate covered in the article, initially, we did the literature review for all different cell fates of MSCs. The fates not included in the article include neurogenic, cardiogenic and endothelial cell fate. The articles we found are listed below.

- Salvia miltiorrhiza extract promote neurogenic commitment of hMSCs<sup>[1]</sup>.
- 5axa-C facilitate cardiogenic differentiation of both mMSC and hMSC<sup>[2]</sup>.
- 1H-pyrrole-2,5-dione small molecule promotes endothelial commitment of MSCs<sup>[3]</sup>.

However, the literature in the non-trilineage cell fate is very limited, some of them are without exploration of the detailed mechanism. Therefore, most of the references included in the article belonged to one of the three major lineages, which are osteogenic, adipogenic and chondrogenic cell fate.

- 2) References need to be formatted in single style.

Response: As suggested by the reviewer, we have formatted the references based on the style of the World Journal of Stem Cells, containing both PMID and DOI with the names of the author and the journals listed in abbreviation. One of the examples is listed below.

“Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JJ, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002; 13: 4279-4295 [PMID: 12475952 DOI: 10.1091/mbc.e02-02-0105]”

### 3) Language need to be improved.

Response: As suggested by the reviewer, we have done a thorough review of the manuscript and corrected all the typos, tenses, determiner and grammar errors. Some of the examples are listed below:

P.1 Investigations in small molecules effect on MSC fate determination will undoubtedly offer insights into bone marrow microenvironment regulation and therapeutic strategy for pathological conditions. -> Investigations in small molecules effect on fate determination of MSCs will undoubtedly offer insights into bone marrow microenvironment regulation and therapeutic strategies for pathological conditions.

P.4 Plastrum testudinis is a herbal medication-> Plastrum testudinis is an herbal medication

P.5 Zhang et al. showed that treatment naringin can promote proliferation and osteogenic differentiation of human BM-MSCs -> Zhang et al. showed that the treatment of naringin promoted proliferation and osteogenic differentiation of human BM-MSCs

P.6 Cui et al. demonstrated the association between SalB and osteogenesis by showing that treatment of SalB at a dose of 40 mg/kg/day to the steroid induced osteoporotic rats reversed the osteoporotic phenotype, presenting elevated bone mineral density, increased cancellous bone mass and thicker trabeculae. -> Cui et al. demonstrated the association between SalB and osteogenesis by showing that treatment of SalB at a dose of 40 mg/kg/day to the steroid induced osteoporotic rats reversed the osteoporotic phenotype. The rats present elevated bone mineral density, increased cancellous bone mass, and thicker trabeculae after the treatment.

P.7 Risedronate is a bisphosphate that is used to treat osteoporosis clinically by inhibiting osteoclastic differentiation. -> Risedronate is a bisphosphonate medication that is used to treat osteoporosis clinically by inhibiting osteoclastic differentiation.

### 4) References of the table need to be included in complete list of references.

The reference table is merged into the content, sharing the complete lists of references.

References:

- 1 Ma L, Feng XY, Cui BL, Law F, Jiang XW, Yang LY, Xie QD, Huang TH. Human umbilical cord wharton's jelly-derived mesenchymal stem cells differentiation into nerve-like cells. *Chin Med J (Engl)* 2005; 118: 1987-1993 [PMID: 16336835]
- 2 Qian Q, Qian H, Zhang X, Zhu W, Yan Y, Ye S, Peng X, Li W, Xu Z, Sun L, Xu W. 5-azacytidine induces cardiac differentiation of human umbilical cord-derived mesenchymal stem cells by activating extracellular regulated kinase. *Stem Cells Dev* 2012; 21: 67-75 [PMID: 21476855 DOI: 10.1089/scd.2010.0519]
- 3 Song BW, Kim IK, Lee S, Choi E, Ham O, Lee SY, Lee CY, Park JH, Lee J, Seo HH, Chang W, Yoon C, Hwang KC. 1h-pyrrole-2,5-dione-based small molecule-induced generation of mesenchymal stem cell-derived functional endothelial cells that facilitate rapid endothelialization after vascular injury. *Stem Cell Res Ther* 2015; 6: 174 [PMID: 26373837 DOI: 10.1186/s13287-015-0170-6]

## Reviewer #2:

We appreciate the reviewer's comments. The followings are our point-by-point responses:

- 1) The key words reflect the focus of the manuscript. But I recommend adding “natural compounds” or something like this.

Response: As suggested by the reviewer, we have added the “natural compounds” to the list of keywords to help the reader identify the complete subjects that the article covers.

- 2) The table has its own list of references – it's very comfortable for the reader. But it would be better to add in the table one more column with the origin/natural source of the small molecule.

Response: We have merged the table to the article to prevent two separate reference tables, and added an additional column listed the origin or the natural source of the small molecules.

### Reviewer #3:

We appreciate the reviewer's comments. The followings are our point-by-point responses:

- 1) At page 2, the paper refers to a Dai et al study (ref 45). The authors could summarize, in few words, about this study and the explanation about the possible mechanisms mentioned.

Response:

We have briefly summarized the experimental design and findings of the involvement of the BMP signaling pathway in the article. The content is labeled in blue on Pg.2.

"A concentration ranged from 0.1 to 10  $\mu$ M Genistein were tested, and the osteogenic stimulations were statistically significant at 0.1 and 1  $\mu$ M with the highest ALP activity at 1  $\mu$ M. The gene expression profile showed that osteogenic genes, such as Runx2 and osteocalcin were highly expressed in genistein-treated cells compared with untreated cells. In the meantime, the BMP signaling pathway related mediators were upregulated, while BMP signaling pathway inhibitors such as SMAD6 and 7 were downregulated."

- 2) Attention to typing errors. For example, at end of page 4.

Response: As suggested by the reviewer, we have done a thorough review of the manuscript and corrected all the typos, tenses, determiner and grammar errors. Some of the examples are listed below:

P.1 Investigations in small molecules effect on MSC fate determination will undoubtedly offer insights into bone marrow microenvironment regulation and therapeutic strategy for pathological conditions. -> Investigations in small molecules effect on fate determination of MSCs will undoubtedly offer insights into bone marrow microenvironment regulation and therapeutic strategies for pathological conditions.

P.4 Plastrum testudinis is a herbal medication-> Plastrum testudinis is an herbal medication

P.5 Zhang et al. showed that treatment naringin can promote proliferation and osteogenic differentiation of human BM-MSCs -> Zhang et al. showed that the treatment of naringin promoted proliferation and osteogenic differentiation of human BM-MSCs

P.6 Cui et al. demonstrated the association between SalB and osteogenesis by showing that treatment of SalB at a dose of 40 mg/kg/day to the steroid induced osteoporotic rats reversed the osteoporotic phenotype, presenting elevated bone mineral density, increased cancellous bone mass and thicker trabeculae. -> Cui et al. demonstrated the association between SalB and osteogenesis by showing that treatment of SalB at a dose of 40 mg/kg/day to the steroid induced osteoporotic rats reversed the osteoporotic

phenotype. The rats present elevated bone mineral density, increased cancellous bone mass, and thicker trabeculae after the treatment.

P.7 Risedronate is a bisphosphate that is used to treat osteoporosis clinically by inhibiting osteoclastic differentiation. -> Risedronate is a bisphosphonate medication that is used to treat osteoporosis clinically by inhibiting osteoclastic differentiation.

- 3) The article is an interesting and well-done review work, but the information brought could be more elaborate. For example, about the scientific / clinical applications. The title leads us to understand that the use of the mentioned molecules approached, so the authors could discuss more about clinical applications. What obstacles encounter for its use?

Response: As suggested by the reviewer, we have included the clinical trial studies that were published and related to the small molecule listed in the article. However, we didn't review the articles published in languages other than English. It is possible more clinical studies have done on the listed small molecules. The added contents are labeled in red on Pg. 2, 5 and 6. We also summarized the current progress of clinical translation and foreseeing obstacles, which are labeled in the last paragraph (Pg.9)

Second revision about the format:

1. Please provide language certificate letter by professional English language editing companies (Classification of manuscript language quality evaluation is B). For manuscripts submitted by non-native speakers of English, please provided language certificate by professional English language editing companies mentioned in 'The Revision Policies of BPG for Article'.

The revised manuscript was reviewed and edited by the native speaker. The Editing-Certificate for the revision has uploaded.

2. Audio core tip: In order to attract readers to read your full-text article, we request that the author make an audio file describing your final core tip, it is necessary for final acceptance. Please refer to Instruction to authors on our website or attached Format for detailed information. The accepted formats are mp3 or wma.

Completed.

3. Please provide the decomposable figure of all the figures, whose parts are all movable and editable, organize them into a PowerPoint file, and submit as "Manuscript No. - image files.ppt" on the system. Make sure that the layers in the PPT file are fully editable. For figures, use distinct colors with comparable visibility and consider colorblind individuals by avoiding the use of red and green for contrast.

Thank you for your comments. We have prepared the decomposable figures and uploaded with the according name "47741 – image files.ppt" to the designation "manuscript".

4. Your manuscript should be prepared with Word-processing Software, using 12 pt Book Antiqua font and 1.5 line spacing with ample margins.

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5. Please rearrange all the authors' affiliations with Department, University or Institute, City, Postcode, Country, etc. (without any symbol or figure like \* or 1, postcode must be there)  
Such as: full name, address, ORCID number, Author contributions

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6. You need to provide the grant application form(s) or certificate of funding agency for every grant, or we will delete the part of "Supported by...".



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7. Abbreviations and acronyms are often defined the first time they are used within the main text and then used throughout the remainder of the manuscript. Please consider adhering to this convention.

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8. Please distinguish between the title of the article series. Three levels of subtitles are allowed: (1) First subtitle: All in bold and capital; (2) Second subtitle: All in bold and italic; and (3) Third subtitle: All in bold.

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9. REFERENCE: Please check and confirm that there are no repeated references! Please correct all cited references number like [number], then keep them superscript. Please add PubMed citation numbers (PMID NOT PMCID) and DOI citation to the reference list and list all authors. Please revise throughout. The author should provide the first page of the paper without PMID and DOI. PMID (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed>) (Please begin with PMID: ) DOI (<http://www.crossref.org/SimpleTextQuery/>) (Please begin with DOI: 10.\*\*)

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10. Figure/table For figures, use distinct colors with comparable visibility and consider colorblind individuals by avoiding the use of red and green for contrast. Please don't include abbreviations in the title of the figure/table. Please explain all the abbreviations in the figure/table legends as full name (abbreviation). Please explain all the abbreviations of each figure/table under each piece of figure/table legends. Please don't include any \*, #, ...in your manuscript; Please use superscript numbers for illustration; and for statistical significance, please use superscript letters. Please create another file for all the supplementary figures and tables and name the file as "xxxxx-Supplementary material.pdf"

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