Title: Stromal cell-derived factor-1α promotes recruitment and differentiation of nucleus pulposus-derived stem cells

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Dear Editor,

We would like to thank you for reconsidering our manuscript NO 43395 entitled "Stromal cell-derived factor-1α promotes recruitment and differentiation of nucleus pulposus-derived stem cells" for publication in World Journal of Stem Cells as a basic study. Thank you for the comments from reviewers. These comments are all valuable and very helpful for improving our paper.

We have studied comments carefully and have made correction which we hope to meet with your approval. The revised portions have been highlighted in red in the manuscript. The point-by-point answers to the reviewers' comments have been listed below.

We earnestly appreciate for editors and reviewers' warm work and look forward to your response.

Yours sincerely,

Di-Ke Ruan
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(On behalf of the authors)

Reviewer 1 (NO.03370303) comments:

This study was very well performed, showing proinflammatory signal-induced SDF-1α production by NP cells in vivo and SDF-1a-depedent migration and chondrogenic differentiation of NPSCs in vitro. I believe that this manuscript will contribute to an advanced understanding of the pathophysiology of IVD.

However, there is one concern regarding the description about the expression of CD105 in NPSCs (Fig. 1d). Before publication in World Journal of Stem Cells, this point should be addressed.

Minor concerns:

In the 1st paragraph in Result section, there is no description about the finding of CD105 in the main text although its flow cytometry result was shown in Fig. 1d.

It is known that CD105 positivity considerably differs among MSCs of different tissues (Maleki M et al. International Journal of Stem Cells 7:118-126, 2014). Moreover, bone marrow-derived MSCs consist of mixed populations with $CD105^{high}$ and $CD105^{low}$ (Anderson P et al., Plos One 8: e76979, 2013). Therefore, it is a very interesting finding that NPSCs consist of a single $CD105^{high}$ populations. CD105 is known as an auxiliary receptor for the TGF-β receptor complex and, as authors suggested (in page 15, lines 6-

7), SDF-1α synergistically promotes TGF-β1-induced chondrogenic differentiation of NPSCs.

I suggest that authors would add descriptions about CD105 expression in NPSCs in the 1st paragraph in Result. For example, the sentence "The cells were positive for the MSC markers CD29 and CD90, and negative for the hematopoietic markers CD45 and CD34 (Figure 1d)" should be corrected as "The cells were positive for widely used MSC markers CD29 and CD90, positive for CD105, which is expressed in certain populations of MSCs to serve as an auxiliary receptor for the TGF-β receptor complex, and negative for the hematopoietic markers CD45 and CD34 (Figure 1d)".

Response:

We would like to thank the reviewer for this valuable comment. We absolutely agree that CD105 is highly expressed in NPSCs and the descriptions about CD105 expression in NPSCs should be added in the 1st paragraph in Result section.

According to the reviewer's suggestion, we have corrected the sentence "The cells were positive for the MSC markers CD29 and CD90, and negative for the hematopoietic markers CD45 and CD34 (Figure 1d)" into "The cells were positive for widely used MSC markers CD29 and CD90, positive for CD105, which is expressed in certain populations of MSCs to serve as an auxiliary receptor for the TGF-β receptor complex, and negative for the hematopoietic markers CD45 and CD34 (Figure 1d)" in Result section (Page 14, Line 8 – 12).

Reviewer 2 (NO. 03478635) comments:

This is a very important study about SDF1alpha on chondrogenic differentiation of stem cells. The effect of CXCR4 on MSC differentiation may be discussed more in detail.

Response:

We thank the reviewer for this comment. We agree with the reviewer that the effect of CXCR4 on MSC differentiation may be discussed more in detail.

CXCR4 is the primary transmembrane receptor of SDF-1α and expressed in MSCs (Kim et al., Int Endod J 6: 534-41, 2014). SDF-1α and its receptor CXCR4 play an important role in the regulation of MSCs migration. In addition, the collected evidence showed that MSCs with CXCR4 expression had the powerful chemotaxis to SDF-1α, and SDF-1α could increase the expression of CXCR4 itself and promote the migration of the MSCs (Li et al., Orthopedics 34:450, 2011). It has been reported that SDF-1/CXCR4 signal axis plays an important role in mediating BMP-9-induced osteogenic differentiation of MSCs (Liu et al., Int J Med Sci 10:1181-92, 2013), BMP-2-induced osteogenic and TGF-β1-induced chondrogenic differentiation of MSCs (Chim et al., Cell Tissue Res 350:89-94, 2012). However, its effects on NPSCs differentiation into chondrocytes for repairing IVD are seldom reported. In the present study, we firstly reported that CXCR4 is expressed in cytomembrane and cytoplasm of NPSCs and SDF-1α upregulates the expression of CXCR4 in NPSCs, which influences chondrogenic differentiation in response to TGF-1β for promoting IVD regeneration. Similar to the results of the previous study (Guang et al., Int J Biochem Cell Biol 44:1825-33, 2012), we propose that the effect of SDF-1 on TGF-β1-chondrogenic induction is regulated via binding to CXCR4 and phosphorylating intracellular R-Smads and Erk, and then the activated R-Smads and Erk1/2 translocate to nucleus and regulate the transcription of chondrogenesis-related genes. Our future studies will investigate whether SDF-1 and TGF-β1 signaling pathways interact at receptor activation and/or upstream regulators for R-Smads and Erk1/2 activation in chondrogenic differentiation.

Now, we provide the detailed description of the effect of CXCR4 on NPSCs differentiation in Discussion section (Page 18, Line 7 – 22).

Reviewer 3 (NO. 02728252) comments:

It is a well-designed comprehensive study aimed to evaluate the effects of stromal cell-derived factor-1α on recruitment and chondrogenic differentiation of nucleus pulposus-derived stem cells. The authors concluded that stromal cell-derived factor-1α has the potential to enhance recruitment and chondrogenic differentiation of nucleus pulposus-derived stem cells via SDF-1/CXCR4 chemotaxis signals that contribute to intervertebral disc regeneration. The study is interesting, well written and has a rational with no further comments.

Response:

We thank Reviewer 3 for favorable comments. We hope it could be finally published and we can continue working on this field of knowledge.