



PEER-REVIEW REPORT

Name of journal: *World Journal of Stem Cells*

Manuscript NO: 64840

Title: Transcription regulators differentiate mesenchymal stem cells into chondroprogenitors and their in vivo implantation regenerated the intervertebral disc degeneration

Provenance and peer review: Invited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 03565491

Position: Peer Reviewer

Academic degree: PhD

Professional title: Senior Research Fellow

Reviewer's Country/Territory: Serbia

Author's Country/Territory: Pakistan

Manuscript submission date: 2021-02-28

Reviewer chosen by: Ya-Juan Ma

Reviewer accepted review: 2021-03-10 13:45

Reviewer performed review: 2021-04-01 16:58

Review time: 22 Days and 3 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input checked="" type="checkbox"/> Major revision <input type="checkbox"/> Rejection



Re-review	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Peer-reviewer statements	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

The authors of this manuscript evaluated characteristics of genetically modified human umbilical cord-derived mesenchymal stem cells (hUC-MSCs), as well as capacity of these cells to regenerate the intervertebral disc degeneration (IVDD) following the in vivo xenotransplantation. General remarks: This article deals with the important subject related to the application of MSCs in regenerative medicine, accompanied with approaches to increase the therapeutic capacity of these cells by expressing specific transgenes whose products would promote regeneration of damaged tissues (IVDD in this case). Since this topic belongs to a group of the newest methods in the biomedicine field it could be useful for a scientific audience of "World Journal of Stem Cells". Thorough in vitro analysis of overexpression of Sox9 and Six1 transcription factors is presented. However, there are serious shortcomings in the way important conclusions of the in vivo part of this study are made and written, because of which the value of the article is significantly reduced. Major remarks: The main problem is related to the Histological Examination after transplantation in IVDD Model (Figure 9). What are the real conclusions of this part of the study - are they precisely summarized in the (C) part of Figure (Histological grading showing the score for regeneration) and adequately conveyed to the Title of the manuscript? Even if conclusion is that the synergistic combination of Sox9 and Six1 is not the best option for the regeneration of IVDD - this manuscript still has its value, but conclusions need to be precise, made solely based on obtained and presented data. In addition, Discussion is basically just the repetition of the results without real comparison of obtained data with ones already published by other



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groups. The clear statements, answering questions – What is the original contribution of obtained results, what is the novelty of this work? – are missing. Therefore, in my opinion, this manuscript should be accepted for publication after major revision and introduction of the indicated changes. Minor remarks: MSCs are not “fibroid-like cells”, but fibroblast-like cells. Figure 7. – the accurate part C is missing (instead of results on day 21 post-transfection, the results after 48h are shown - the same as in part A of this Figure). Spelling errors, such as: “chondroproginators” Partial or unclear sentences, such as: “Loss of interconnection with AF and fissuring manifestation in NP area [40], [41].”



RE-REVIEW REPORT OF REVISED MANUSCRIPT

Name of journal: *World Journal of Stem Cells*

Manuscript NO: 64840

Title: Transcription regulators differentiate mesenchymal stem cells into chondroprogenitors and their in vivo implantation regenerated the intervertebral disc degeneration

Provenance and peer review: Invited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 03565491

Position: Peer Reviewer

Academic degree: PhD

Professional title: Senior Research Fellow

Reviewer's Country/Territory: Serbia

Author's Country/Territory: Pakistan

Manuscript submission date: 2021-02-28

Reviewer chosen by: Chen-Chen Gao (Online Science Editor)

Reviewer accepted review: 2021-05-06 11:06

Reviewer performed review: 2021-05-08 09:15

Review time: 1 Day and 22 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input type="checkbox"/> Grade B: Minor language polishing <input checked="" type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input checked="" type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection



Peer-reviewer statements	Peer-Review: [<input checked="" type="checkbox"/>] Anonymous [<input type="checkbox"/>] Onymous Conflicts-of-Interest: [<input type="checkbox"/>] Yes [<input checked="" type="checkbox"/>] No
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SPECIFIC COMMENTS TO AUTHORS

Within the submitted document “64840-Answering-Reviewers-revision”, authors responded: All the modifications and suggestions highlighted by the reviewer is now incorporated in the revised version. Minor remarks: MSCs are not “fibroid-like cells”, but fibroblast-like cells. Corrections has been made. Spelling errors, such as: “chondroproginators” Spelling and typographical errors are corrected. Unfortunately, many of the suggested corrections have not been incorporated in the revised version of the manuscript, moreover, new mistakes were created during this process, which is very disappointing! Discussion and conclusions are improved, but that approach has not been applied throughout the whole manuscript. Therefore, please find detailed list of my remarks related to the submitted “64840-Manuscript-File-revision” that need to be introduced into the final version of the manuscript: Abstract: Methodology MSCs were transfected with sox-9 and six-1 transfection factors Instead of - transfection factors, it should be - transcription factors Result Isolated cells showed fibroid morphology Instead of - fibroid, it should be - fibroblast-like Core tip In this study, we highlighted that overexpression of Chondrogenic transcription factors in hUC-MSCs accelerated their differentiation potential into chodroprogenitor cells. Instead of - Chondrogenic, it should be - chondrogenic Instead of - chodroprogenitor, it should be - chondroprogenitor Instead of - The synergistic effect of sox9, and six1 transcription factors leads the MSCs to differentiate into chondrogenic cells in the basal medium produced the same effect as the chondro-induction medium. It should be - The synergistic effect of sox9 and six1 transcription factors lead the MSCs to differentiate into chondrogenic cells in the basal medium, which produced the same effect as the



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chondro-induction medium. *in vitro* - should be in Italic INTRODUCTION Instead of -
In the present study we hypothesized that if MSCs are preconditioned for
overexpressing chondrogenic transcription factors *six1*, *sox9* and their synergistic
combination, and pre-differentiate MSCs into chondro-progenitor cell (CPCs),
transplanted in the form of induced chondro-progenitor cell (iCPCs) into damage IVD,
the disc can be regenerated and normal physiological function can be restored. It should
be - In the present study we hypothesized that damaged IVD can be regenerated and
its normal physiological function can be restored if MSCs are preconditioned by
overexpressing chondrogenic transcription factors *six1*, *sox9* or by their synergistic
combination, as well as by MSCs pre-differentiated into chondro-progenitor cells (CPCs),
transplanted into the damaged disc in the form of induced chondro-progenitor cells
(iCPCs). MATERIALS AND METHODS Transfection of Human Umbilical
Cord-derived Mesenchymal Stem Cells (hUC-MSCs) by Electroporation Instead of -
Each subset of transfected MSCs was cultured in a basic growth medium for 48 hours
followed by incubation in a chondrogenic induction, and normal growth medium for
day 21. It should be - Each subset of transfected MSCs was cultured in a basic growth
medium for 48 hours, followed by incubation in a chondrogenic induction and normal
growth medium for 21 days. Protein Expression Analysis Instead of - Normal MSCs
and transfected MSCs cultured in the basal and chondro-induction medium for day 21...
It should be - Normal MSCs and transfected MSCs cultured in the basal and
chondro-induction medium for 21 days... The same correction has to be introduced in
the text that is part of the graphs within Figure 7. Instead of - at 21 day It should be - at
day 21 RESULTS Isolation, Proliferation, and Characterization of MSCs from a Primary
Culture of Human Umbilical Cord Tissue Instead of - Fibroid-like cells It should be -
Fibroblast-like cells Characterization of Differentiated Transfected MSCs Instead of -
The transfected cells completely lost their fibroid shape. It should be - The transfected



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cells completely lost their fibroblast-like shape. Instead of - called induce It should be - called induced Instead of - The fluorescent intensity was quantified and plotted and showed that MSCs transfected with sox-9, six-1, and the synergistic group expressed chondrogenic markers in 21 days of culturing in basal medium. The 21 days culturing of transfected MSCs in chondro-induction medium expressed chondrogenic markers sox-9 and six-1 as shown in figure 5. It should be - The fluorescent intensity was quantified and results showed that MSCs transfected with sox-9, six-1 and the synergistic group expressed chondrogenic markers following 21 days of culturing in the basal medium. Also, the 21 days culturing of transfected MSCs in chondro-induction medium lead to expression of chondrogenic markers sox-9 and six-1 as shown in figure 5. The second part of Figure 5 is missing in the submitted PowerPoint presentation!! Gene Expression Dynamics of Sox9 and Six1 Transfected MSCs Instead of - Expression of Sox9 has significantly up-regulated at 21 days post-transfection transfected in the basal medium... It should be - Expression of Sox9 has significantly up-regulated at 21 days post-transfection in the basal medium... DISCUSSION Instead of - To analyzed It should be - To analyze Instead of - denerated disc It should be - degenerated disc