International Cardiovascular Research Journal
Volume 16 · Issue 3 · e129983
OPEN PEER REVIEW

The Effect of Human Cardiac Myocyte-Derived Conditioned Medium on Differentiation of Mesenchymal Stem Cells

Nadia Rousta, Sirous Naeimi, Shahrokh Zare, Mahintaj Dara, Iman Razeghian-Jahromi

SUBMITTED	REVISED	ACCEPTED
16 Jul 2022	17 Aug 2022	5 Sep 2022

About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

More about open peer review: brieflands.com/knowledgebase/opr

Contents

1 **Original submission** Saied Salehi, Editor-in-Chief 2
2 **Revision 1** Referee 1, Associate Editor, Editor-in-Chief 3
Authors' reply to reviewers 4
Reviewer and editor comments 11

1 Original submission

2 comments · 1 Aug 2022 – 6 Aug 2022

Saied Salehi Reviewer

MINOR REVISION 1 Aug 2022

The authors evaluated the differentiation of rat MSCs toward the cardiogenic lineage using CM of human cardiac myocytes. The study is innovative in terms of using human cardiac myocytes-CM to differentiate MSCs and has the potential to open a novel avenue in the arena of stem cell therapy for myocardial infarction. However, I have a few comments that can greatly improve this work:

1. Please specify all materials, reagents, and kits by catalog number and company name.
2. I suggest including the importance of GATA4 in the introduction.
3. In the material and methods section, under the GATA4 expression heading, more information should be provided, such as the type of master mix, the qRT-PCR instrument, and the thermal cycles.
4. How many MSCs were seeded for the treatments and MTT assay?
5. The specifications of the lyophilizer and plate reader should be provided.
6. I suggest presenting GATA4 expression levels in a column chart instead of presenting the data in a table.
7. I suggest combining figures 3 and 4 (osteogenic and adipogenic differentiation potential).

Editor-in-Chief

MINOR REVISION 6 Aug 2022

Please make the revisions based on the comments.

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Point-by-point response to reviewers

To the EIC of International Journal of Cardiovascular Research

Hi,
Hope this message finds well.

First of all, I would like to express my gratitude for giving us the opportunity to revise the manuscript. In return to your email dated 06-AUG-2022, the authors considered the reviewers' comments thoroughly. I appreciate the reviewers for spending their valuable time in this regard. The criticisms offered by the reviewers are immensely insightful. The authors edited the manuscript after requesting for revision. The responses to the comments (*in italic*) have been brought at the bottom of this letter after each criticism, and the main manuscript was changed as appropriate.

I believe that with incorporating the reviewer's comments, the manuscript improved substantially.

Once again thank you for your team's efforts. We will be grateful for sending us any further comments if necessary.

Iman Razeghian-Jahromi
Corresponding author
Cardiovascular Research Center
Shiraz University of Medical Sciences
Shiraz, Iran

Reviewer 1:

The authors evaluated differentiation of rat MSCs toward cardiogenic lineage by CM of human cardiac myocytes. The study is innovative in terms of using human cardiac myocytes-CM to differentiate MSCs and has a potential to open a novel avenue in the arena of stem cell therapy for myocardial infarction. However, I have a few comments that can greatly improve this work:

1. Please specify all materials, reagents and kits by catalogue number and company name.

- *The required information was added.*

2. I suggest to include the importance of GATA4 in the introduction.

- *Some explanations regarding the importance of GATA4 has been added to the introduction section:*

  “Expression of early cardiac genes such as GATA4 indicates that cells have commenced the process of differentiation. GATA4 plays a fundamental role for regulation of endoderm and mesoderm development in postgastrula embryo (26). In particular, this protein regulates the expression of critical genes during cardiomyogenesis. It was shown that cardiac differentiation is promoted in pluripotent or embryonic stem cells by induction of GATA4 overexpression (27). Intriguingly, GATA4 possesses self-regulation via suppression of its own expression (26).”

3. In the material and methods, GATA4 expression headline, more information should be provided such as type of master mix, the qRT-PCR instrument and thermal cycles.

- *The required information was added.*

4. How many MSCs were seeded for the treatments and MTT assay?

- *About 400,000 cells were cultured in every T25 flasks for treatment experiments. For MTT assay, 96 well culture plate (5,000 cells/200 µl/well) was used. This information was added to the text.*

5. The specification of lyophilizer and plate reader should be provided.

- *The relevant statements were completed by adding the required information:*

  “This medium was air-dried at -56˚C under negative pressure (vacuum degree < 10 pas) by a lyophilizer for 48 hours (BIOBASE BK-FD10PT FREEZE DRYER)”
  “Light absorption was measured at wavelength of 570 nm on a microplate reader (FLUOstar Omega, BMG LABTECH, Germany).”

6. I suggest to present GATA4 expression levels by a column chart instead of presenting data in a table.

- *As requested, table was substituted by a column chart.*

7. I suggest to combine figures 3 and 4 (osteogenic and adipogenic differentiation potential)

- *Figures 3 and 4 were combined.*

Reviewer 2:

Dear Editor-in –Chief

The manuscript entitled "The effect of human cardiac myocyte-derived conditioned medium on differentiation of mesenchymal stem cells" described the beneficial impact of conditioned medium of human cardiac myocyte-derived conditioned medium on differentiation of rat mesenchymal stem cells. According to findings of this study, the conditioned medium of human cardiac myocyte owing to presenting verity of growth factors acts as an inductive cocktail that promote differentiation of MSCs. I believe this manuscript is well designed and conducted. Also it was skillfully written and its finding can benefit future preclinical studied. Hence, I find it suitable to be published in the International Cardiovascular Research Journal. However, there are several points that needs to be revised as follow:

Line 97: To be more accurate, mention that cells were fixed before staining.

- *The required change was applied.*

Line 102: Since you followed the kit instruction for RNA isolation, you need to add the cat number of used reagents and kits.

- *The required information was added.*

Line 103: You need to re-write this line- PCR (Ampliqon).

- *The mentioned lines were revised.*

Line 119: Replace the star symbol with ×.

- *The required change was applied.*

Line 120: Add a ref. for Vendor protocol.

- *The vendor reference was added.*

Line 126: Replace term of “Numeration” with count. If you mean cell counting.

- *“Numeration” was replaced by “cell counting”.*

Line 128: it is better to briefly explain that following treatment of MSCs with conditioned medium, in the beginning of this sentence.

- *An extra explanation was added to the manuscript:*

  “Following treatment of cells with conditioned medium, cells in both control and treatment groups were detached from the flasks at day 1, 3, and 7 as previously described.”

Line 135: Instead of P0, it is better to address it as the first days of in vitro culture.

- *The required change was applied.*

Line 146: It is better to combine Figure 1 and 2 (osteogenesis and adipogenesis). Also, the legends of both figures are not complete.

- *The figures were combined and their legends were completed.*

Line 148-152: The section related to confirming the identity of MSCs at P3, needs to be re-write and also explain the finding in this part.

- *The mentioned section was rewrote and the relevant findings were also added.*

  “Following treatment of cultured cells at P3 with trypsin/EDTA, centrifugation of the cell suspension produced two phases, pellet and

supernatant. They contained mesenchymal and hematopoietic cells, respectively. Both phases were subjected to RNA extraction, cDNA synthesis, and PCR using primers of mesenchymal specific markers, CD73 and CD90. As shown (Figure 2), the cells in the pellet expressed CD73 (208bp) and CD90 (197bp) while the expression of these markers was absent in the supernatant."

I find it important to mention that all figures need complete legend and a brief explanation. Also, figures need to be rearrange as they are not in line with the text.

- *All the figures were reviewed and legends were completed by adding a brief explanation. Also, they were rearranged in line with the text.*

Line 153: Regarding the HCM morphology and culture condition, it would be better to explain this part more accurately. Also I cannot find the related figure.

- *This section was enriched by adding some more information about HCM morphology and culture condition. Sorry about the figure. It was corrected.*

  "At the first days of culture, HCM was mostly appeared in the form of round shapes, some with short projections to different directions. However, it became more fibroblast-like at P1, which is the usual appearance of these cells (Figure 3). They need a specific culture medium that contains a variety of ingredients including Ham's F12+DMEM, FBS, insulin, and basic fibroblast growth factor. It is recommended to subculture of the cells using poly-L-lysin coated flasks."

Line 161: demonstarated → demonstrated

- *Sorry. It was corrected.*

Line 181: Add more information about the selected marker of GATA4. However, there are more about this factor in discussion.

- Given that some information were added to the introduction section about GATA4 and considering existence of more explanations in the discussion, only two sentences were added to this part in order to fulfill this comment and to avoid redundancy as well.

  “Expression of GATA4 is assumed as a sign that reveals the commencing of cardiac differentiation. In fact, one of the earliest molecular markers that is associated with initiation of cardiac gene expression is GATA4 (27).”

2 Revision 1

3 comments · 5 Sep 2022 – 5 Sep 2022

Referee 1 Reviewer — ACCEPT 5 Sep 2022

accept

Associate Editor — ACCEPT 5 Sep 2022

accept

Editor-in-Chief — ACCEPT 5 Sep 2022

accept