

PEER-REVIEW REPORT

Name of journal: World Journal of Stem Cells

Manuscript NO: 67900

Title: ARPE-19 conditioned medium promotes neural differentiation of adipose-derived mesenchymal stem cells

Reviewer's code: 03726486

Position: Editorial Board

Academic degree: PhD

Professional title: Professor

Reviewer's Country/Territory: China

Author's Country/Territory: Italy

Manuscript submission date: 2021-05-05

Reviewer chosen by: AI Technique

Reviewer accepted review: 2021-05-28 10:04

Reviewer performed review: 2021-05-31 01:27

Review time: 2 Days and 15 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input checked="" 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 Grade E: Do not publish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

In this paper, Mannino, et al. reported that the conditioned medium from ARPE-19 cells promoted ASCs differentiate to a neural cell-like phenotype, the authors isolated human ASCs from healthy donors and compared their differentiation under four culture conditions, they found that neural cell markers and ASC proliferation/viability were upregulated when the cells were cultured in ARPE-19 conditioned medium compared to other serum-free mediums. This paper is interesting and the results are of potential medical relevance, however, several problems including scientific questions throughout the manuscript need to be solved before final acceptance: 1. There are some mistakes in English grammar throughout the manuscript, please check with a native speaker or a language editor before submission. 2. Type setting of the manuscript needs to be carefully checked and normalized, for example, the reference part appears two times in the manuscript, one consists 37 papers (page 15), while one consists 36 papers (page 24), the figures also appear two times, one consists 5 pictures (page 19) and one consists 6 pictures (page 29), this should not happen in a submitted manuscript. 3. As the ASCs were primary cells isolated from human tissues, specific markers of ASCs shall be checked to confirm the purity of the cells. 4. In the method part of immunofluorescence and western blot analysis, product code of the indicated antibodies shall be provided to ensure the reproducibility of the research, only dilution information is not enough, as a company always has several different antibodies for one kind of protein. 5. The authors should compare cell differentiation when ASCs are cultured in complete conditioned medium (including FBS) from ARPE-19 cells, as Figure 1 showed that control ASCs cultured in basal DMEM have better cell viability compared to ASCs cultured in serum-free conditioned medium from ARPE-19, while the differentiation markers (Figure 2-6) were more obvious in serum-free conditioned medium from ARPE-19, it is

important to exclude the influence of FBS. 6. In Figure 2-5, the pictures are only representative images, how is the status of other cells? In order to make the results more convincing, statistical results of different pictures from biologically independent experiments shall be performed and added in the manuscript.

PEER-REVIEW REPORT

Name of journal: World Journal of Stem Cells

Manuscript NO: 67900

Title: ARPE-19 conditioned medium promotes neural differentiation of adipose-derived mesenchymal stem cells

Reviewer's code: 06081653

Position: Peer Reviewer

Academic degree: MD

Professional title: Doctor

Reviewer's Country/Territory: Australia

Author's Country/Territory: Italy

Manuscript submission date: 2021-05-05

Reviewer chosen by: AI Technique

Reviewer accepted review: 2021-05-11 03:02

Reviewer performed review: 2021-06-04 03:16

Review time: 24 Days

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 aim of the present study is to demonstrate that ARPE-19 conditioned medium is able to upregulate neural specific genes in adipose MSCs. The data presented goes part way towards supporting this hypothesis by showing differences in expression using immunofluorescence (Figs 2-5) and Western blots. The authors point out that PGP9.5 expression is similar across all conditions at D1. However, immunofluorescent data (Fig 3) clearly show higher intensity levels between ASC vs CM/ASC at D1, but not by Western analysis. Although these fluorescence intensities remain similar within conditions (ie. D1 vs D8, it is not clear if the difference seen between is necessarily due only to upregulation of expression per cell, or if there is some degree of selection occurring, whereby the growth of PGP9.5high cells is favoured by conditioned medium.

This possibility has not been addressed in the context of differential cell growth and viability between ASC controls and CM/ASCs across time points (Fig 1). The effect at D1 seen across conditions is not addressed in the manuscript, however, this observation that 24 hours is sufficient to increase levels of neural markers, (with little opportunity for the effects of selective growth) supports their interpretation that ARPE-19 CM does indeed cause upregulation of neural markers, rather than favour their growth. I suggest direct analysis using flow cytometry to determine changes in cell MSC population markers, as well as to measure the proportion and intensity of neural markers tested here by immunostaining. Related literature missed: 1. The increased cell viability (inhibition of apoptosis) in CM/ASCs vs serum free cultures should be mentioned from previous studies. It is understood that the cells are not exactly the same, however, the mechanism may be. Pigment epithelium-derived factor from ARPE19 promotes proliferation and inhibits apoptosis of human umbilical mesenchymal stem cells in serum-free medium. Ding DC, Wen YT, Tsai RK. Exp Mol Med. 2017 Dec



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15;49(12):e411. doi: 10.1038/emm.2017.219. Final note, authors should be careful not to describe upregulation of neural markers as neural differentiation itself. For example, hypoxia can upregulate OCT4 (pluripotent marker) in MSCs, but this does not make the cells human induced pluripotent stem cells. Suggest adjustment of language.

PEER-REVIEW REPORT

Name of journal: World Journal of Stem Cells

Manuscript NO: 67900

Title: ARPE-19 conditioned medium promotes neural differentiation of adipose-derived mesenchymal stem cells

Reviewer's code: 03448879

Position: Editorial Board

Academic degree: MD

Professional title: Chief Doctor, Deputy Director, Professor, Surgeon

Reviewer's Country/Territory: China

Author's Country/Territory: Italy

Manuscript submission date: 2021-05-05

Reviewer chosen by: AI Technique

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Review time: 9 Days and 13 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input 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
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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

The study reported that the conditioned medium of ARPE-19 could promote neural differentiation of ASCs. The study design is logical. Experimental methods and statistical methods are appropriate. Questions raised: 1. The company and product number of the culture medium should be added. 2. The neural differentiation of ASCs should be proven from multiple aspects. Several markers were used in this study, including western blotting and immunofluorescence imaging. However, it would be better if functional indicators are added. 3. Language editing is required.