PEER-REVIEW REPORT

Name of journal: *World Journal of Stem Cells*

Manuscript NO: 74752

Title: Application of Extracellular Vesicles from Mesenchymal Stem Cells Promotes Hair Growth by Regulating Human Dermal Cells and Follicles

Provenance and peer review: Invited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05684808

Position: Peer Reviewer

Academic degree: MD

Professional title: Associate Chief Physician

Reviewer's Country/Territory: China

Author's Country/Territory: South Korea

Manuscript submission date: 2022-03-04

Reviewer chosen by: AI Technique

Reviewer accepted review: 2022-03-07 09:59

Reviewer performed review: 2022-03-07 10:16

Review time: 1 Hour

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [Y] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [ ] Grade B: Minor language polishing [Y] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [ ] Major revision [Y] Rejection
Re-review	[Y] Yes [ ] No

| Peer-reviewer statements | Peer-Review: [Y] Anonymous   [   ] Onymous |
| | Conflicts-of-Interest: [   ] Yes   [Y] No |

SPECIFIC COMMENTS TO AUTHORS

1. The possible mechanism of enrichment of wnt3a in Evs should be studied. 2. translocated β-catenin should be studied by western blots. 3. How about combined wnt inhibitors on Ex vivo experiments to support the conclusion. 4. The comparing effect of wnt3a-enriched Evs and normal Evs should be performed. 5. In vivo study is highly recommended for supporting the conclusion.

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No

| Peer-reviewer statements | Peer-Review: [Y] Anonymous   [   ] Onymous |
| | Conflicts-of-Interest: [   ] Yes   [Y] No |

SPECIFIC COMMENTS TO AUTHORS

I would like to congratulate the authors for this manuscript. The study is interesting. I have some comments about the manuscript: Regarding the title, please make it more specific, as it will reflect your study. Instead of "a class" of MSC, if human BM-MSCs are used, they should be mentioned for better clarity.   Introduction: Page 4 line 6, please expand the sentence appropriately to accommodate all the included references (4, 12-21).

Materials and methods: Cell culture: please specify which specification of ATCC cells were used in your study for clarity (e.g., if you used human BM-MSC PCS-500-012TM please state so).   Discussion: Please point out the limitations of your study within the methodology. Regarding the determination of EV concentrations used, please give brief explanation (1-5 mcg/mL for proliferation assay and 0-1 mcg/mL for hair follicles). If they are based on your previous study, please clarify.   Figure 1A: please insert the measurement on the scale bar for clarity.   Figure 1E: please use the same color, refer to figure 1D.

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Provenance and peer review: Invited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 03738702

Position: Editorial Board

Academic degree: MSc, PhD

Professional title: Senior Researcher, Senior Scientist

Reviewer's Country/Territory: Italy

Author's Country/Territory: South Korea

Manuscript submission date: 2022-03-04

Reviewer chosen by: AI Technique

Reviewer accepted review: 2022-03-04 13:38

Reviewer performed review: 2022-03-09 09:39

Review time: 4 Days and 20 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No

Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

I read with great interest this manuscript by Dr. Rajendran and colleagues investigating the effects of human mesenchymal stem cell-derived extracellular vesicles (hMSC‑EVs) on hair growth. In particular, in this work it has been shown that a class of hMSC‑EVs that expressed Wnt3a was able to promote in vitro the proliferation of both dermal papillae and outer root sheath cells, and to promote in ex vivo the hair follicle growth. Moreover, the analysis highlighted the value of β‑catenin pathways in mediating the observed effects. This work adds new knowledge that can help in pharmacological strategies to reduce hair loss. The topic is interesting, the methods well reported and conclusions are consistent with results.   Minor concerns before publication in "World journal of stem cells": 1.   The title should be rewritten referring to the promising results of this work obtained in vitro, on dermal cells, and ex vivo on a hair follicle model. The effects of exosomes on hair growth should only be assumed potentially.   2.   On page 5 line 99, perhaps the authors meant "hMSC" rather than "hMSC-EVs"

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Provenance and peer review: Invited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 02560127

Position: Editorial Board

Academic degree: PhD

Professional title: Professor

Reviewer's Country/Territory: Pakistan

Author's Country/Territory: South Korea

Manuscript submission date: 2022-03-04

Reviewer chosen by: AI Technique

Reviewer accepted review: 2022-03-04 09:31

Reviewer performed review: 2022-03-13 17:23

Review time: 9 Days and 7 Hours

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Re-review	[ ] Yes [Y] No

Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The manuscript by Rajendran et al describes the functions of hMSC-derived EVs on inducing hair growth and the possible molecular mechanisms. They also described hMSC-EV-treated human DP and ORS cells, and human HFs for the activation of DP and ORS cells and their effects on hair shaft elongation in human HFs. The manuscript is well written with only a few typographical and formatting errors which are listed below:

 1. Throughout the manuscript the format adopted by the authors is different than WJSC permitted format. Font style is different, as well as references in text and in the reference section. Statistical significance is mentioned as asterisks rather than a, b, and c denoting p< 0.05, p< 0.01 and p< 0.001. In vitro, ex vivo etc. should be in italics. 2. Title should be corrected to: A Class of Human Mesenchymal Stem Cells derived Extracellular Vesicles Promotes Hair Growth by Regulating Dermal Cells In Vitro and Enhances Human Hair Follicle Growth Ex Vivo 3. Core Tip: The changes recommended are underlined: This study reveals that human mesenchymal stem cell-derived extracellular vesicles (hMSC‑EVs) were enriched with wnt3a and some proteins associated with their membrane. This study provides a new insight into how EVs modulate the recipient cells in promotion of hair growth. hMSC‑EVs could be clinically used as a promising inducer against alopecia. 4. Introduction: The change recommended is underlined: Another treatment is hair transplant surgery; however, this is not possible for all because of low HF viability, limited number of donors, and immune rejection.   5. Materials and Methods: The changes recommended are underlined: hMSCs were cultured and culture media was collected, and EVs were isolated from the media as previously described[11].

 They were then incubated with non-labeled hMSC-EVs (5 μg/mL) and DiD-labeled

hMSC-EVs (2.5 and 5 µg/mL; hMSC-EVs/DiD) for 2 h at 37°C in 5% CO_2. DP or ORS cells were seeded (0.5×10^4/well) in 96-well plates and maintained overnight at 37°C and 5% CO_2. Cells treated with hMSC-EVs (DP cells: 2, 4, 6, 8, and 10 µg/mL and ORS cells: 1–5 µg/mL) were maintained for 24 h at 37°C and 5% CO_2. 6. Discussion and Conclusion: The changes recommended are underlined: Our results revealed that hMSC-EVs increased hair-inducing transcription factors (Axin2, EP2, and LEF1), which agrees with other studies[15, 17, 18, 32]. Our findings agree with previous studies that treated human HFs with EVs[15, 17, 18]. (This sentence is not clear, something is missing. In conclusion, the present study suggests ……… 7. Others: •Biostatistics Certificate should have complete name and address of the Biostatistician.

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Provenance and peer review: Invited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05115902

Position: Editorial Board

Academic degree: BSc, PhD

Professional title: Associate Professor

Reviewer's Country/Territory: Italy

Author's Country/Territory: South Korea

Manuscript submission date: 2022-03-04

Reviewer chosen by: AI Technique

Reviewer accepted review: 2022-03-07 10:11

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Review time: 11 Days and 3 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

- The authors have to provide a clear definition of MSCs. Mesenchymal stromal cells, present in the stromal component of several tissues are a heterogeneous population, including multipotent stem cells, progenitors, and differentiated cells (doi.10.2106/JBJS.19.00189; doi.org/10.1007/s12015-021-10231-w). Therefore, only a fraction of the population (multipotent stem cells and progenitors) exhibit the ability of self- renewal and multidirectional differentiation into osteocyte, chondrocyte and adipocytes. - In Materials and Methods, is not clear if the authors use hMSC-EVs, and so Extracellular Vesicles purchased from American Type Culture Collection (Cell culture section) (Manassas, VA, USA)" or if hMSC were cultured (as reported in "Isolation of hMSC-EVs" section). In the latter case, however, the authors did not take in account of remarkable aspect. During cell therapy, in vitro expansion, by several passages, is a necessary procedure to guarantee the elevated number of MSCs employed in each administration. Nevertheless, ex vivo expansion greatly affects MSC properties, and it has been demonstrated that in vitro growth of MSCs can give rise to replicative senescence (doi.10.18632/aging.100971). Many researchers have focused their analysis of senescent secretomes on specific proteins: The SASP, senescence-associated secretory phenotype, constitutes a hallmark of senescent cells and mediates many of their patho-physiological effects. Most differences between the molecular signatures of presenescent and senescent cells entail cell-cycle- and metabolism-related genes, as well as genes encoding the secretory proteins that constitute the SASP. - The interpretation of qPCR data strongly depends on the employ of a normalization factor which is frequently calculated based on the expression of a reference gene, whose

levels remain unchanged among the different conditions analyze. In the interpretation of qPCR data, the authors use different housekeeping gene: figure 2D b-actin and figure 3E GAPDH. Please, to describe a rational explanation to explain this discrepancy. For example, GAPDH, a common housekeeping gene used for qPCR normalization, is unstable in many conditions and cell types (doi.org/10.1007/ s13353-013-0173-x). Always related to the senescence, recent experiments of single‐cell qPCR—a variation of the qPCR that does not rely on the use of reference genes for normalization—reported changes in GAPDH expression in senescent vs. proliferating cells (doi.org/10.1111/acel.12632).

RE-REVIEW REPORT OF REVISED MANUSCRIPT

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Provenance and peer review: Invited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05684808

Position: Peer Reviewer

Academic degree: MD

Professional title: Associate Chief Physician

Reviewer's Country/Territory: China

Author's Country/Territory: South Korea

Manuscript submission date: 2022-03-04

Reviewer chosen by: Jia-Ru Fan

Reviewer accepted review: 2022-05-27 06:25

Reviewer performed review: 2022-05-29 14:56

Review time: 2 Days and 8 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [Y] Accept (General priority) [ ] Minor revision [ ] Major revision [ ] Rejection
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

| statements | Conflicts-of-Interest: [   ] Yes   [Y] No |

SPECIFIC COMMENTS TO AUTHORS

The answer were find.

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [Y] Accept (General priority) [ ] Minor revision [ ] Major revision [ ] Rejection
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

no comments