Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

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.

Response: Thank you for your comment. Unfortunately, it was a typo error. We have purchased "Bone Marrow-Derived Mesenchymal Stem Cells; Normal, Human" (PCS-500-012™) from American Type Culture Collection (Manassas, VA, USA). We have updated the information in the revised manuscript.

We have performed "EVs" treatment not the "cell treatment/therapy". The previous studies have used to isolate EVs/Exosome from MSCs passage of 4 to 6 (Theranostics, 2018; 8(22): 6163-6177. doi:10.7150/thno.28021) and MSCs passage of 4 to 8 (BioMed Research International, Volume 2019, Article ID 9742765, 12 pages, https://doi.org/10.1155/2019/9742765). In our current study we isolated hMSC-EVs from culture medium of human BM-MSCs (from Passage 3 to 6), We have updated the information in the revised manuscript.

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.

Response: Thanks for your comments and observation. We agree that *In vitro* culture of cells affects the MSCs properties. But to isolate EVs, we must grow cells in cell culture plates. Moreover, in our current study human BM-MSCs from Passage 3 to 6 was used for isolation of hMSC-EVs. In addition to that the changes in extracellular vesicle production and secretion in senescence cells were studied. Many studied results suggested that senescence cells secreted more EVs than non-senescence/young cells, which is beneficial for EV-based therapies. Another study showed that upregulation of RAB27b (a key component of EV secretory pathways) in senescent human fibroblasts than non-senescence/young cells (Mech Ageing Dev. 2020 Jul; 189:111263. doi: 10.1016/j.mad.2020.111263. and Aging Cell. 2018 Apr; 17(2):e12734. doi: 10.1111/acel.12734.). The senescence cells not only secreted more EVs but also increased levels of cargoes (for eg. EphA2), which promoted the cellular proliferation (Nat Commun 8, 15729 (2017). https://doi.org/10.1038/ncomms15728). In both way senescence cells derived EVs are beneficial in EV-based therapies.

- 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).

Response: Thank for your comments. Two different sets of primers ordered for experiments at different time. So, there are two different housekeeping gene used for experiments. We have used a DP cells and ORS cells at passage number 2 or 4. Based on the figure 2B and 3B both DP cells and ORS cells were proliferating by hMSC-EVs treatments. So, we believe that β-actin and GAPDH not make inconsistency in this experiments.

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

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.

Response: Thanks for your comment. We have made all the modification in the revised manuscript.

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

Response: Thanks for your suggestion. We agree with reviewer's comment, and we have changed the title as your and other reviewers' suggestion "Application of BM-MSC-Derived Extracellular Vesicles Promotes Hair Growth through Activation of Human Dermal Cells and Augmentation of Hair Shaft Elongation"

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.

Response: Thank for your comment. We have made the appropriate changes in "core tip" as per your suggestion.

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.

Response: Thank for your comment. We have made the appropriate changes in "Introduction" as per your suggestion.

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].

Response: Thank for your comment. We have made the appropriate changes in "Materials and Methods" as per your suggestion.

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% CO2. DP or ORS cells were seeded (0.5 × 104/well) in 96-well plates and maintained overnight at 37°C and 5% CO2. 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% CO2.

Response: Thank for your comment. We have made the appropriate changes in "Materials and Methods" as per your suggestion.

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 ……

Response: Thank for your comment. We have made the appropriate changes in "Discussion and Conclusion" as per your suggestion.

7. Others: • Biostatistics Certificate should have complete name and address of the Biostatistician.

Response: Thanks. I have attached the certificate.

Reviewer #3:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

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.

Response: Thanks for your comment. We have rewritten the title as per you suggestion as "Application of BM-MSC-Derived Extracellular Vesicles Promotes Hair Growth through Activation of Human Dermal Cells and Augmentation of Hair Shaft Elongation"

2. On page 5 line 99, perhaps the authors meant "hMSC" rather than "hMSC-EVs"

Response: Thanks for pointing out typo error. We have rectified it.

Reviewer #4:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

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.

Response: Thanks for your suggestion. We agree with reviewer's comment, and we have changed the title as "Application of BM-MSC-Derived Extracellular Vesicles Promotes Hair Growth through Activation of Human Dermal Cells and Augmentation of Hair Shaft Elongation"

Introduction: Page 4 line 6, please expand the sentence appropriately to accommodate all the included references (4, 12-21).

Response: Thanks for your comment. We have rephrased the sentence and referenced according to cells.

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).

Response: Yes. Bone Marrow-Derived Mesenchymal Stem Cells; Normal, Human (PCS-500-012™) was purchased from ATCC, and information is included in the revised manuscript.

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 ug/mL for proliferation assay and 0-1 ug/mL for hair follicles). If they are based on your previous study, please clarify.

Response: Thank you for your comments. We have used a non-toxic concentration for all experiments. For, hair follicle elongation study, in our previous studies with more than 1 ug/ml (others EVs) have showed a toxicity to HFs, where hair shaft did not grow significantly. So, we haven't tested more than 1ug/ml of hMSC-EVs.

Figure 1A: please insert the measurement on the scale bar for clarity.

Response: Thank you so much. I have inserted the measurement on the scale bar for clarity.

Figure 1E: please use the same color, refer to figure 1D.

Response: Thank you so much. I have changed same colour in Figure 1E and 1D.

Reviewer #5:

Scientific Quality: Grade D (Fair)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Rejection

Specific Comments to Authors:

1. The possible mechanism of enrichment of wnt3a in Evs should be studied.

Response: Thanks for your suggestion. The enrichment of Wnt proteins into/onto EVs is well reported in several studies including our own previous studies (see below). At present, the sorting mechanism of proteins into/onto EVs are not well known. We have discussed it in discussion.

References:

1. Nat Cell Biol. 2012 Oct;14(10):1036-45. doi: 10.1038/ncb2574.

2. BMB Rep. 2021 Nov 8:5464.

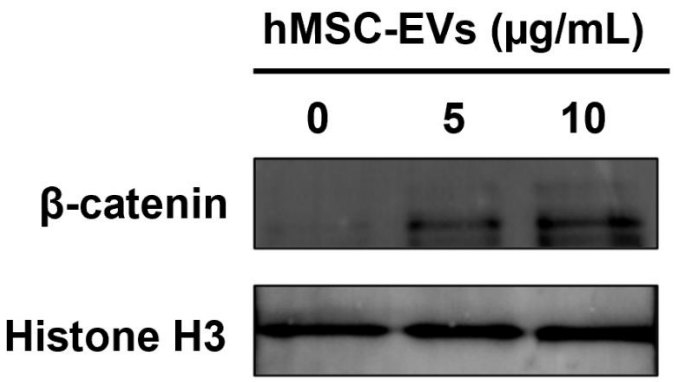
3. Int J Mol Sci. 2019 Mar 21;20(6):1436. doi: 10.3390/ijms20061436.

4. Front Cell Dev Biol. 2021 Oct 13;9:735888. doi: 10.3389/fcell.2021.735888.

5. Cells. 2020 Apr 1;9(4):856. doi: 10.3390/cells9040856.

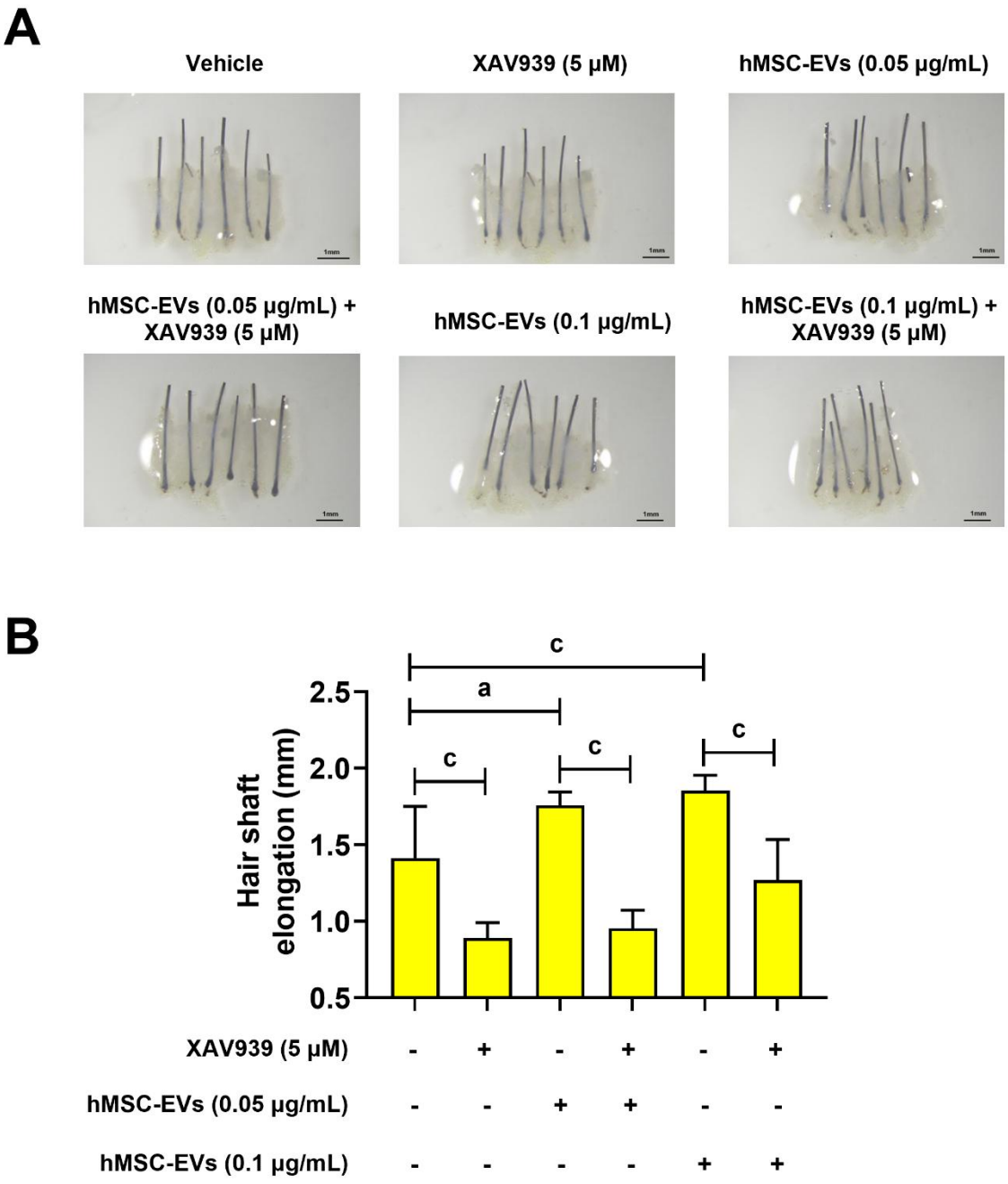
2. translocated β-catenin should be studied by western blots.

Response: Thanks for your suggestion. As per your suggestion we have performed the western blotting analysis of β-catenin translocation in nuclear fraction of DP cells treated with hMSC-EVs (Revised Figure 2D)



3. How about combined wnt inhibitors on Ex vivo experiments to support the conclusion.

Response: Thanks for your suggestion. As per your suggestion we have performed the Ex vivo experiments with Wnt inhibitors and presented the results in Figure 4.



4. The comparing effect of wnt3a-enriched Evs and normal Evs should be performed.

Response: Thanks for your suggestion. Doing the experiments with EVs (wnt3a-enriched) and normal EVs (No Wnt3a) would add some impact to the study, but it would require an additional study, time, and funds. Moreover, we also not ruled out that other proteins and/or miRNAs may play roles in hair regrowth and discussed in "Discussion". We have showed that Wnt inhibitor (XAV939) have inhibited the therapeutical functions of hMSC-EVs in ex vivo experiments (Figure 4), indirectly proved that therapeutical function of hMSC-EVs may be due to Wnt in them.

5. In vivo study is highly recommended for supporting the conclusion.

Response: Thanks for your suggestion. Our main goal of the study is to find the therapeutical functions of human MSC-EVs on human Cells (DP and ORS cells) and human HFs and find out the possible mechanism in promotion of hair growth. To support the result, we have showed the effect in a possible human HF model.