

Manuscript number: 52207

Manuscript title: Exosomes Secreted from CCR2-Overexpressing Mesenchymal Stem Cells Promote Cognitive Function by Enhancing Microglia/Macrophage M2 Polarization and Remyelination via the Suppression of Macrophage Infiltration Following Transient Middle Cerebral Artery Occlusion

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Journal full title: World Journal of Stem Cells

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Dear editor and reviewers,

Thank you for reviewing our above-numbered manuscript and providing us with very constructive comments. We believe these comments will significantly strengthen the manuscript.

We have accordingly revised the manuscript by providing a point-by-point response as shown below.

We hope the current version now satisfies the requirements for publishing in WJSC.

We look forward to your final decision on this manuscript.

Sincerely yours,

Xi-Quan Hu

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A point-by-point reply

Comments/suggestions from Reviewer #1

1. Figure 2A is mentioned in first place in the document. Please fix so that Figures appear ordered from the beginning of the document.

Answer: the sentence “The experimental design is indicated in Figure 2A” in methods section has been deleted, so that the figure numbering becomes more logic.

2. The methods section “Transfection of HUC-MSCs...” needs to be improved. Authors refer cells “infected” “transfection” transfected” The protocol described is not clear in its current form, neither the osteogenesis and lipogenesis inductions referred at the end of the section.

Answer: We have re-written the methods section “Transfection of HUC-MSCs...” and the protocol for “osteogenesis and lipogenesis”. The revised manuscript is now considerably improved.

3. Please review text in sections of methods “Exosome...and “Western blotting” The connector “First” is used in both parts without being followed by “Secondly or other. Please review grammar in this sense.

Answer: The sections “Exosome...” and “Western blotting” have been revised using appropriate transitional words. The connector “First” has been replaced by “Briefly”.

4. Readers would appreciate a short explanation to justify marker selection in some parts, i.e CD13, CD29...NG2+ cells, iba-1; CD68 etc (some arguments provided in the discussion section for iba-1 could be replaced in the text to help readers understand the selection of markers in the results section)

Answer: In the section “CCR2-overexpressing HUC-MSCs load the CCR2 receptor into their exosomes”, relevant references have been provided to justify the selection of HUC-MSC surface markers (CD13, CD29, CD44, CD34, CD45, CD73, CD90, CD105, HLA-DR) and the exosome specific markers (CD9, CD63, and CD81). In the section “Exo^{CCR2} showed more beneficial effects with regards to oligodendrogenesis and remyelination than Exo^{Ctrl}”, references have been added to justify the use of antibodies such as anti-MBP, anti-BrdU, and anti-NG2. In the section of “Exo^{CCR2} promoted microglia/macrophage M2 polarization and inhibited microglia/macrophage M1 polarization *in vivo* compared to that by Exo^{Ctrl}”, the cited references should justify the selection of the M1 and M2 markers, as well as the selection of iba-1 antibody and CD68 antibody.

5. Catalogue numbers in addition to providers for all materials are needed. The authors miss to provide these details for several important components such as the antibodies used, cell line RAW 264.7. Please review all materials in this sense. The concentration or dilutions of antibodies used should also be indicated in each case.

Answer: The catalogue numbers for all antibodies we used, together with their dilutions have been provided in the revised manuscript. The detailed information for the RAW 264.7 cell line has been provided.

6. Journal name for reference 17 is missing; Replace the term “thrice” for: three times. On page 6 Morris appears in lower case letters (morris). On page 8, mNSS appears once with the “m” in capital letters (MNSS), which is confusing. “et al” should appear with a final dot and in italics “ et al.,”

Answer: Journal name for reference 17 has been added. The term “thrice” has been replaced by “three times”. In the results section, the “Morris” has been used. The acronym for modified neurological severity score (mNSS) has been made consistent throughout the manuscript. “et al” has been replaced with italic “*et al.*,” throughout the revised manuscript.

Reviewer #2: The presented work was performed at a high methodological level. In the course of the study, the authors obtained new data on the mechanisms of the therapeutic activity of MSCs and their effect on a specific population of central nervous system macrophages - microglia. To study the mechanisms of therapeutic activity of MSCs, a model of cerebral ischemia was used. The authors used Cd206 as a marker of M2 macrophages, which corresponds to the classical concept of the M1 / M2 paradigm. Currently, the markers of the corresponding phenotypes are Arg1, iNOs, transcription factors STAT1, STAT3, STAT6. Murray P.J., Allen J.E., Biswas S.K., Fisher E.A., Gilroy D.W., Goerdt S., Gordon S., Hamilton J.A., Ivashkiv L.B., Lawrence T., et al. Macrophage Activation and Polarization: Nomenclature and Experimental Guidelines. *Immunity*. 2014;41:14–20. doi: 10.1016/j.immuni.2014.06.008.

Answer #2: Thank you very much for your positive feedback. CD206 has been reported as a marker of M2 macrophages in many experimental stroke studies such as Suenaga’s work (PMID: 25836044), Pan’s work (PMID: 25889216) and Han’s work (PMID: 26243225). Based on previous studies, we choose CD206 as a marker of M2 macrophages.

Comments/suggestions from editors

- 1. Comments/Suggestions:** The title should be no more than 12 words. The running title, author contributions, funding, institutional review board statement, institutional animal care and use committee statement, conflict-of-interest statement, data sharing statement, ARRIVE guidelines statement, the details title and address of corresponding author, structured abstract, core tip, details of materials, article highlights should be added in the manuscript.

Answer: We thank the editor for such meticulous comments. All components raised by the editor have now been made available in the revised manuscript.

- 2.** Please provide the decomposable figure of Figures, whose parts are movable and editable. So you can put the original pictures in PPT and submit it in the system.

Answer: The decomposable/editable figures have been provided in PPT format.