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Title: Immunomodulation: the next target of MSC-derived exosomes in the context of ischemic stroke

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We would like to thank all the reviewers for their explicit review and constructive comments. We have made all the changes as suggested, and incorporated them in the revised manuscript. Listed below are our point-by-point responses to their comments. The reviewer comments are laid out below in *blue italicized* font and specific concerns have been numbered. Our response is given in normal font.

Response to Comments of Reviewer #1:

Critique #1: *The authors are only advised to check one of the heading, "EXOSOMES AS A REPLACEMENT THERAPY FOR MESENCHYMAL STEM CELLS ON STROKE", is this in context of stroke or ischemic stroke as the other headings given by authors are in the context of ischemic stroke.*

Response: Thank you for your advice. We have rewritten the Heading as “EXOSOMES AS A REPLACEMENT THERAPY FOR MESENCHYMAL STEM CELLS ON IS”.

Response to Comments of Reviewer #2:

Critique #1: *In the Abstract, Line 8, the authors should notice that exosomes could not represent all important features of MSCs, for example, cell differentiation and paracrine function.*

Response: Thank you for your advice. We have modified the expression in the line 8 of the Abstract to read "Also, a growing number of studies suggest that the therapeutic IS effect after transplantation of MSCs is mainly attributable to MSCs-derived exosomes (MSC-Exos) ".

Critique #2: *In the Abstract, Line 14, please pay attention to the grammar, especially the plural form of nouns and following verbs.*

Response: Thanks for your careful checks. We are sorry for our carelessness. We have carefully checked the English, and corrected the grammar mistakes in the Abstract.

Critique #3: *In the Abstract, Line 16, the word "stimulating" would be replaced by "promoting".*

Response: Thanks for your correction. We have amended "stimulating" to "promoting" in line 16 of the Abstract.

Critique #4: *In the Abstract, Line 18, "immune inflammation" is not a common phrase, "inflammation" alone is enough.*

Response: Thank you for your reminder. We have amended "immune inflammation" to "inflammation" in line 18 of the Abstract.

Critique #5: *In the 1st paragraph of Introduction, Line 5-7, the "4.5-hour time window" is based on studies of thrombolysis. Many studies about thrombectomy have already surpassed this restriction. Ref.: DOI: 10.1056/NEJMoa2207576 .*

Response: We sincerely thank you for your professional comments. We have carefully read the reference you provided and have made the following changes to this section in response to your reminders. In clinical practice, however, the thrombolytic treatment conditions are strictly limited to presentation within 4.5 hours of symptom onset. Although the therapeutic window for thrombectomy has been extended to 24 hours, there may be a risk of cerebral hemorrhage, occlusion after revascularization, and over-perfusion brain injury.

Critique #6: *In the 1st paragraph of Introduction, Line 10, like the previous comment, due to the expansion of treatment window of intraarterial thrombectomy, nowadays most patients who did not receive either thrombolysis or thrombectomy is because very severe or very mild clinical manifestation, or large core infarction based on radiologic results.*

Response: We sincerely thank you for your professional comments. We have carefully read the reference you provided and have made the following changes to this section in response to your

reminders. In clinical practice, however, the thrombolytic treatment conditions are strictly limited to presentation within 4.5 hours of symptom onset. Although the therapeutic window for thrombectomy has been extended to 24 hours, there may be a risk of cerebral hemorrhage, occlusion after revascularization, and over-perfusion brain injury.

Critique #7: *In the 1st paragraph of Introduction, Line 13-14, there is lack of citation(s).*

Response: Thanks for your careful checks. We have added citation to line 13-14 of the 1st paragraph of the Introduction. In conjunction with your suggestion in the previous comment, we have amended this section: lines 13-14 of the 1st paragraph of the Introduction have been removed.

Critique #8: *In the 1st paragraph of Introduction, Line 20-24, do IS patients be commonly prescribed with antibiotics?*

Response: Currently, in clinical practice, antibiotics are not usually used for IS patients. In previous years, studies have suggested that prophylactic antibiotic therapy may be an appropriate way to prevent post-stroke infection. However, in recent years studies have found that prophylactic antibiotic therapy has disadvantages and may not be appropriate for acute stroke care. (Reference: DOI: 10.1016/S0140-6736(15)60076-9). We propose this therapeutic approach in order to review the shortcomings of past exploration of therapeutic strategies for IS to justify the current need to seek new targets for the treatment of IS. Thanks again for your valuable comments.

Critique #9: *In the 1st paragraph of Introduction, Line 24-25, "By 2050, more than 500 million people per year will experience a stroke "is not a precise description.*

Response: We are sorry for not describing this part accurately. We have amended lines 24-25 of the 1st paragraph of the Introduction to read "By 2050, there will be more than 200 million stroke survivors and almost 300 million disability-adjusted life-years, 25 million new strokes, and 13 million deaths from stroke annually". Thanks again for your valuable comments.

Critique #10: *In the 3rd paragraph of Introduction, Line 2-3, it is not wrong to emphasize*

immunosuppression in the prognosis of IS, but whether it is the primary cause is not sure yet. If the author found any article hold the opinion then it should be cited.

Response: We sincerely appreciate the valuable comments. We are sorry for not describing this part accurately. We have amended lines 2-3 of the 3rd paragraph of the Introduction to read "Immunosuppression is the important cause of IS patients' poor prognosis and increased susceptibility".

Critique #11: *In the Section "IMMUNE RESPONSE AFTER IS", it would be better if the author would re-organize this section. Since the section talks the immune responses mainly by cell types, a sequence like "myeloid cells" "lymphoid cells" "granulocytes" might make it easier for read and understand.*

Response: We have re-organize this section according to your suggestion.

Critique #12: *In the 2nd paragraph of the Section "IMMUNE RESPONSE AFTER IS", Line 29-31, the author should give the specific biomarkers that microglia recognize on ischemic neurons and cite relevant article.*

Response: Thanks for your suggestion. As suggested by you, we have added the specific biomarkers CD47-SIRPα and CD200-CD200R that microglia recognize on ischemic neurons and cited relevant article.

Critique #13: *In the 3rd paragraph of the Section "IMMUNE RESPONSE AFTER IS", Line 1-2, there is lack of citation about the sentence "Neutrophils are the initial blood-derived immune cells to cross the BBB and invade ischemic tissues, and they can be detected as soon as 1h after the event."*

Response: We sincerely thank you for careful reading. We have added citations in the in 3rd paragraph of the Section "IMMUNE RESPONSE AFTER IS", lines 1-2. Thanks again for your valuable comments.

Critique #14: *In the 1st paragraph about Microglia of the Section "MSC-Exos regulate the immune response through cells", for contents in this section, I would recommend to talk more about details of some important studies.*

Response: Thank you for your advice. We have adjusted the first paragraph of the Section "MSC-Exos regulates immune responses through cells" and added some details of the important studies. Thanks again for your valuable comments.

Critique #15: *In the 1st paragraph about Astrocytes of the Section "MSC-Exos regulate the immune response through cells", about the sentence "It has been demonstrated that MSC-Exos reduces A1-Ast expression and improves inflammation. They act primarily through the Nrf2-NF-κB signaling pathway in the regulation of Ast activation", whether these results came from studies of ischemic stroke or other brain diseases? Detailed methods of these articles should be added.*

Response: These results come from other neuroinflammatory diseases. We have added these article detail methods after the results.

Critique #16: *In the 1st paragraph about Astrocytes of the Section "MSC-Exos regulate the immune response through cells", the sentence "Moreover, a recent study identified high LCN2 expression in a mouse transient middle cerebral artery occlusion (tMCAO) model and detected that IS patients with higher plasma LCN2 levels were more likely to develop a post-stroke infection" is confusing, did they do both human and animal studies?*

Response: They have conducted both human and animal studies. They examined LCN2 expression in the ischemic brain in an animal model and measured plasma levels of LCN2 in ischemic stroke patients. Immunohistochemistry revealed expression of LCN2 in the mouse brain already at one day following tMCAO, and the amount of LCN2 subsequently increased with a maximum at 2 weeks after tMCAO. In ischemic stroke patients, higher plasma levels of LCN2 were associated with a worse clinical outcome at 90 days and with the occurrence of post-stroke infections. Thanks again for your valuable comments.

Critique #17: *In the 2nd paragraph about Astrocytes of the Section "MSC-Exos regulate the*

immune response through cells", what does the word "mast" mean in the sentence "Recently, the in vitro studies reported that MSCs improved brain function after transplantation mainly by reducing the number of mast Ast and GFAP overexpression through inhibition of p38 MAPK, JNK, and its downstream targets p53 and STAT1 activation by paracrine factors"?

Response: We are very sorry that the word "mast" was a translation error. We intended to express the reduction in the number of hypertrophic astrocytes and GFAP overexpression. We have corrected "mast" into "hypertrophic". Thank you again for your careful examination.

Critique #18: *About the sub-title "FROM BENCH TO BEDSIDE: LIMITATIONS OF EXOSOMES THERAPY IS AND THE COUNTERMEASURES", please re-check the grammar of the sentence.*

Response: Thank you for your advice. We have rewritten the Sub-title as "FROM BENCH TO BEDSIDE: RESPONSES TO THE LIMITATIONS OF MSC-EXOS THERAPY FOR IS".

Critique #19: *In Figure 2, the word "Muccle" should be "Muscle".*

Response: We were really sorry for our careless mistakes. Thank you for your reminder. We have modified "Muccle" to "Muscle" in Figure 2.