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Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "Mesenchymal stem cells rescue acute hepatic failure by polarizing M2 macrophages" (ID: 36006). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper. The main corrections in the paper and the responds to the reviewer's comments are as flowing:

Responds to the reviewer's comments:

Reviewer #1:

Response to comment: The definition of M1 markers to select TNF alpha and INF gamma may be described more in detail. It seems like polarization in the manuscript means differentiation.

Response: There is no specific marker for M1, and we identify the M1 by the expression levels of CD68, tumor necrosis factor alpha (TNF- α), interferon- γ (IFN- γ), inducible nitric oxide synthase (iNOS). IFN- γ is produced by activated CD4⁺ T helper (Th) 1 cells, and converts resting macrophages into potent cells with enhanced antigen-presenting capacity, increased synthesis of pro-inflammatory cytokines and toxic mediators, and augmented complement-mediated phagocytosis. This initial description of macrophage activation became known as M1. M1 are induced by IFN- γ , and liberate

pro-inflammatory cytokines such as TNF- α . So TNF- α and IFN- γ are selected to defined M1.

The polarization of macrophages in the manuscript means differentiation.

Reviewer #2:

2. Response to comment: This is a very well carried out bench study that provides greater insight to the role of MSCs in acute liver failure. M2 polarization contributes to the therapeutic effects of MSCs in AHF by altering levels of anti-inflammatory and pro-inflammatory factors. This is a major finding and does offer a potential therapeutic application. It will need to be followed up by more bench studies but this is potentially an opportunity for a new clinical approach.

Reviewer #3:

3. Response to comment: Please tell me the detail etiology which MSCs transfused into rats inhibit apoptotic hepatocytes and promote hepatocyte regeneration.

Response: The implanted MSCs promote hepatocytes regeneration and improve local cell function by paracrine effects after treatment, which been involved in the releasing of growth factors and signaling molecules. In addition, implanted MSCs reduce apoptotic hepatocytes to inhibit inflammatory necrosis.

Reviewer #4:

4. Response to comment:

Major

1. The authors compare the immunohistochemistry and real-time PCR of liver between dead and survival group. I wonder how to get liver tissue and in the dead group. Does this mean that liver tissue was obtained after dead? Please provide how and when you get the liver tissue in dead group. You should also provide the number of each group in dead and survival group in Figure 3,4,5 and 6.
2. In AHF animal models, rats were divided into group A (D-GaIN ip, N=16) and Group B (PBS ip, N=10). In MSC transplantation, rat were also divided into two groups (MSC group, N=16, and DPBS group, N=10). I recognized this as two-by-two method, which resulted into 4 groups. Please revise about this points as understandable.
3. CD68 was a pan-macrophage marker, not a M1 macrophage marker. CD68 positive macrophage did not mean M1 macrophage. The other marker for M1 macrophage was necessary to assess macrophage polarization.

Minor

1. Please spell out D-GaIN and DPBS.
2. In results 1 and figure 1, please provide the histology of the PBS group after 5 days.
3. Please provide more precise explanation of HE histology.
4. In the second paragraph of Discussion.

Response:

Major

1. The liver tissue was obtained in death group before they died, when rats were in very poor physical condition or in the state of death after treatment. All rats in the death group were taken within 48 hours after treatment. The number of each group in dead and survival group in Figure 3,4,5 and 6 has 4-6 samples, and the specific values are marked in the paper.
2. A total of 52 rats were randomly divided into four groups: Group A (n=16), the experimental group; Group B (n=10), the control group; Group C (n=16), MSC-treated group; Group D (n=10), DPBS-treated group. Rats in Group A were injected intraperitoneally (i.p.) with D-galactosamine (DGalN) (1.2 g/kg; Sigma-Aldrich, St. Louis, MO, USA). Rats in Group B were injected i.p. with 2 ml of 0.9% phosphate buffered saline (PBS). At 12h after DGalN-treated, rats in Group C underwent intravenous tail vein transplantation of 5.5×10^5 MSCs dissolved in 1.0 ml Dulbecco phosphate-buffered saline (DPBS) and 1.0 ml DPBS in Group D.
3. So far, there is no specific marker for M1, and we identify the subtypes of macrophages by the expression levels of cell surface markers and cytokine. CD68 is associated with lysosomal membranes, particularly the phagosomes of macrophages, so increased expression of CD68 is consistent with increased lysosomal activity which may in turn imply enhanced phagocytosis. Therefore, to some extent, the elevated expression of CD68 may reflect an increase in the distribution of M1. And it has been reported in related literatures^[1-3].

REFERENCES:

1. Kavindra Kumara Wijesundera, Takeshi Izawa, Jyoji Yamate.

M1-/M2-macrophage polarization in pseudolobules consisting of adipophilin-rich hepatocytes in thioacetamide (TAA)-induced rat hepatic cirrhosis. *Experimental and Molecular Pathology* 2016;101:133–142.

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3. Barros MHM, Hauck F, Dreyer JH, Kempkes B, Niedobitek G. Polarisation: an Immunohistochemical Approach for Identifying M1 and M2 Macrophages. *PLoS ONE* 2013;8(11): e80908.

Minor :

1. Dulbecco phosphate-buffered saline (DPBS)

D-galactosamine (D-GalN)

2. At 48h after treatment, the mortality of rats was 90% in DPBS-treated group, and the liver inflammation in the survival rats was very mild, and there was no model significance, so there was no liver histology of 5D after DPBS treatment.

3. HE staining of liver sections in each group. Compared with the blank control group (H), we observed necrosis of centrilobular hepatocytes, characterized by cell shrinkage and lost nuclei, interstitial hemorrhage and inflammatory cell infiltration in the DPBS-treated group (I). Liver histomorphology at 48 h after MSC treatment (J) did not change significantly compared with the DPBS-treated group, but the number of hepatocytes with edema, shrinkage and lost nuclei decreased significantly, lots of inflammatory cells infiltration and increased number of cells were observed. The liver histomorphology was gradually repaired after 5 days (K).



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4. In contrast, CD68+macrophages and levels of TNF- α and iNOS were significantly up-regulated in the death group.

Special thanks to you for your good comments. We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in yellow in revised paper. We appreciate for Editors' warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.