OPEN PEER REVIEW REPORT 1

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-20-00601
Title: One-year follow up results of human umbilical cord mesenchymal stem cells transplantation in treating animal models of traumatic brain injury
Reviewer's Name: Elena Abati
Reviewer's country: Italy

COMMENTS TO AUTHORS

The manuscript addresses an important topic in the field of stem cell research, and the results obtained are relevant. In this study, authors focus on the safety and efficacy - from an immunomodulatory point of view - of huMSC transplantation in mice.
However, I do have some concerns related to the design of the study, the conclusions drawn by the authors and to language, style and organization of the manuscript.
Please see my suggestions below:

Please check that referencing is appropriate throughout the manuscript.

Major concerns:
1) "CD29 was detected with immunofluorescent slice, indicating there was no polyneuropathy occurred and the immune response is at a very low level". To my knowledge, analysis of CD29 is not a validated method to exclude the presence of polyneuropathy. Furthermore, I recommend you to specify which kind of tissue was the immunofluorescent analysis conducted on. As far as I have understood from the figures, CD29 was analysed on brain biopsies, so I don't understand how authors could draw conclusions on presence or absence of polyneuropathy. If the authors want to support this conclusion, they should consider supporting it with neurophysiological studies or with a more complete study of a nerve biopsy. Otherwise, they should reword the conclusion. Moreover, I recommend to explain better CD29 study in the Results section, as it was not mentioned there, only in the Conclusions.
My second question relates to the significance of polyneuropathy to the study: was was polyneuropathy looked for? Which is the relevance to immunogenicity and tumorigenicity of huMSCs?
2) Which criteria have been used to define and purify huMSC (criteria from scientific panels, existing literature..)? Which markers did you exclude? Please specify with appropriate reference
3) "Immunomodulatory effects of huMSCs in TBI model rats". The authors assessed the effect of huMSC transplantation on cytokine profile. However, in order to make these results relevant, some other points need to be analysed as well:
- Additionally, in the In Situ group (huMSCs delivered in situ of lesion site) and Tail Vein group (huMSCs delivered through tail vein), we found that the levels of pro-inflammatory cytokines decreased and the levels of anti-inflammatory cytokines increased, compared with TBI group" how did this cytokine pattern changed over the study time? Was the effect transitory or long-lasting? Were there differences between the in situ and tail vein groups?
- Effects on phenotype and survival compared to untreated group should be assessed in order to state that therapy had efficacy. If you unable to provide these data, please modify the Discussion and explain the reason
4) "Immunogenic effects of huMSCs in TBI model rats": the title is misleading as immunogenicity was not assessed in mice but only on transplanted cells. Please add data on immunologic effects in mice (immunitary response against transplanted cells, development of autoantibodies...) or reorganize the paper and explain why these tests were chosen in the Discussion

5) The Discussion section looks disorganized; I suggest dividing it in paragraphs and expand better some crucial topics, putting your results in the context of previous works. In addition, I believe that you should focus on the advances brought by this study. Below you may find some suggestions:

- you may use a paragraph to focus on the reasons why you chose to use huMSC (safety, identification and purification of huMSC, their characteristics, advantages and disadvantages over other types of MSC). "Purity and identity of the cells play a crucial role in safety and efficacy of the treatment. Our results showed that huMSCs was correctly acquired through strict managing process and identified with high purity by flow cytometry detection of characteristic surface markers, which paved the way of successful cell therapy." This sentence is very vague. Please explain better: how did you select the cells? How can that determine the success of therapy? Then, "...in huMSCs therapy, which showed advantages of umbilical cord derived MSCs over bone marrow derived MSCs, the latter contains multiple kinds of stem cells, such as hematopoietic stem cells, which can induce immunological damage. Hence, huMSCs transplantation therapy is very safe from the point of immune rejection." This concept can be better developed. Then the section regarding HLA and your related finding can make up the last part of the paragraph.

-" Generally, a molecular war begins between anti-inflammatory cytokines and pro-inflammatory cytokines after TBI." Please expand this topic, explaining the relevance of your findings in the context of other studies

- There are many sentences stating that use of huMSC is definitely safe, e.g. "All these facts comprehensively showed huMSCs transplantation therapy was safe from the view of tumorigenicity", or, in the Abstract "huMSCs are low immunogenicity, will not cause immunologic rejection, tumorigenesis or uncontrolled proliferation." I believe that data are not strong enough to totally exclude the possibility of immunologic rejection or tumor formation. Please consider the use of modal verbs.

- Include a paragraphs discussing the limitations of your study and future steps needed to address them

OPEN PEER REVIEW REPORT 2

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-20-00601
Title: One-year follow up results of human umbilical cord mesenchymal stem cells transplantation in treating animal models of traumatic brain injury
Reviewer's Name: Fei Gao
Reviewer's country: USA

COMMENTS TO AUTHORS

It's much appreciated that the authors contributed lots of time and strength to develop a potential stem-cell therapy in a rodent model of TBI which may become a great benefit for TBI patients. The experimental design and data from this present study are sound. My big concerns are as follows:

1. Both introduction and discussion are not strong enough. It would be helpful for the readers if the authors could introduce the tested proteins, factors or cell markers in introduction but not in discussion. The discussion is expected to include 1) a brief summary of the results; 2) implications of the findings; 3) innovation or improvement compared with previous studies 4) potential directions in future study. Also, a statement or citations are welcomed to explain how the findings in rodent models could be applied to clinical treatment for TBI patients.
2. It would be much appreciated if the author could add more details in the legends, so that the readers could have an idea about the results without going through the whole text.

Other than these, I have some minor comments to the authors:

1. P2-line36: please consider "Sprague-Dawley rat" as a key word.
2. P3-line9: please clarify where TBI "remains a major cause of death and disability", in USA or the world? I am confused about the subject of the "which" clause.
3. P3- line13: how about to write the sentence like this "thus there no therapies are available to effectively treat traumatic brain injury" ? Line15-17, how "stem cells" became a "strategy"? I think the authors wanted to say "stem-cell therapy". It would be helpful to reorganize these lines.
4. P3-line 22, a "have" was missing before "reported". I don't think I understand the sentence of "however these results available …." Please reword it.
5. P3-line 25, please consider using another word like "issues or problems" to replace "worries".
6. P3-line31, reword "should be carried out" to "is necessary to be completed"
7. P3-line33, please remove "preclinical".
8. P3-line44, did the authors want to say "the huMSCs was identified and qualified for the succeeding experiments" ? Also, it would be appreciated if a reference about the huMSCs isolation and identification could be cited here.
9. P3-line51, citations are needed here.

10. P3-line 59, there are always changes or differences between the groups even if p values > 0.05. So I would add “significantly” in the statement if p values were less than 0.05. In addition, it would be much helpful for the readers if “significant(ly)” could be added in the abstract.
11. P7-line25,26: citations are needed here if the authors were following a published cell biology method to isolate and identify huMSCs.
12. P8-line 48: Please add a few words to indicate the aim of detecting serum IL-6, IL-10… …
13. P9-line10 and 11, please reword the two sentences.
14. P9-line 19, Is the “0.5% blocking solution” 0.5% BSA in PBS or a 1:20 diluted solution? Also, it’s no need to state “dripped onto the sections” for it’s obvious an on-slide staining.
15. P9-line25, how long were the slices rinsed in PBS? Or the authors could briefly state that “the sections were rinsed in PBS before incubated in DAPI at 37 C for 30 min” .
16. P9-line14 and 34, after antigen retrieval and before rinsing the slices in PBS, were the sections cool down to room temperature?
17. P9- please adds some more details for HE staining.
18. P11-figure2: The description in legend is not clear and enough. For example, how many animals were included in each image in panel A? It’s obvious that panels B and C illustrated comparisons between control and treatment groups. What does “*” and “**” indicated in panels B and C, as well as what statistical method was applied for those results? In addition, what’s the scale for the images in D and E?
19. Please add more details to the other figures. For example, what the colored arrows mean in the bottom images of panel C in figure 4?
20. P5-line42-44, citations are needed.