

Dear editors,

We deeply appreciate the precious comments from reviewers on our manuscript (NO 53991, Invited NO.05084565). We have revised the original manuscript according to the reviewers' suggestion with highlight part in red in the revised manuscript, and here is the point-to-point response to the comments. We hope that this revised manuscript could be accepted by the reviewers and editors. And we're looking forward to receiving your positive response as soon as possible.

Thanks.

Meijiang Feng

Dep. of Geriatrics, Key Lab for Aging & Disease,

Nanjing Medical University

03/20/2020

#### **Reviewer 1**

1. Nice and fluently written review of literature.

**Response:** We appreciate the reviewer's positive evaluation of our work.

2. It would be prudent if the authors provide some information on protein therapy with NTFs as well.

**Response:** We deeply appreciate the reviewer's suggestion. There are elaborate reviews of the protein therapy with NTFs and the stem cell therapy respectively for NDDs, and the emphasis of our review is their combination therapy. Although we have provided some information on NTFs protein therapy combined with stem cells in our original manuscript [Line1-2 of the last paragraph on Page 5, Line3-5(NSC transplantation...NSCs alone) of the first paragraph on Page 6], the supplementary information was also added in this revised manuscript [Line1-5(A study...function improvement) on Page 8, the last 4 lines(In addition...ALS mice) of the 2nd paragraph on Page 10], and some corresponding revisions were made in Table 2(the last Row)and Table 4(Raw 6).

3.Page# 5 second paragraph from bottom, the clinical trial mentioned reads out of context as the author is talking about gene therapy and out of nowhere provides an example of cell therapy which may be confusing for the reader. This needs a bit of more clarity

**Response:** We agree with the comment. We think it more appropriate to delete this little part to avoid confusing, so we have deleted it in this revised manuscript.

#### **Reviewer 2**

In this Review Article, the Authors have been focusing on the analysis of the advances provided by the combinatorial use of stem cells with different Neurotrophic Factors (NT) in the treatment of neurodegenerative diseases. This is a kind of systematic review which is highly needed, due to the lack of consistent improvement afforded by the currently available

conventional therapies. The Review has been essentially focused on the dissection of the effects provided by stem cell transplantation alone or by the transplantation of genetically modified stem cells, capable of secreting targeted individual NTs or a mixture of them. The description and analysis of the underlying experimental data is well conducted, and balanced across the different forms of neurodegenerative disorders considered. The presence of detailed Tables is highly appreciated, and it is further supporting the discussion of experimental and clinical findings. The conclusions are in keeping with the overall narrative under consideration.

**Response:** We appreciate the reviewer's positive evaluation of our work.

### **Reviewer 3**

The topic of neurodegenerative diseases and of the possible advances in the therapy is very intriguing, thus several papers published recently evidence the possible therapeutic strategies. The novelty of this manuscript is the consideration of the use of stem cells in association with neurotrophic factors for neurodegenerative disorders.

1. It is necessary a revision of the text giving more attention to the importance of MSCs (including exosomes, vesicles, miRNA that are not cited in this version of the manuscript) and neurotrophic factors for the treatment of neurodegenerative disorders.

**Response:** We deeply appreciate the reviewer's kind suggestion. Considering that excellent comprehensive reviews of MSCs and NTFs for neurodegenerative diseases have been published, and the focus of our review is the combination of stem cells and NTFs, we added information of MSCs-derived exosomes (MSC-exosomes) in the treatment of neurodegenerative diseases in the revised manuscript (the 2nd paragraph on Page 6, the 2nd paragraph on Page 8, the 2nd paragraph on Page 9, the last paragraph on Page 10). We also made corresponding revisions in the Abstract (line 6-8, 12-13) and Introduction (line 7,9,13 of paragraph 2 on Page 3, the 2nd and 3rd paragraph on Page 4).

2. In addition, I would to suggest the authors to insert a scheme illustrating the possible effects of MSCs and neurotrophic factors for each disorder described in the paper. This could help readers for a more appropriate lecture of the manuscript.

**Response:** We deeply appreciate the reviewer's suggestion. We have inserted a schematic illustrating of the possible effects of MSCs and NTFs for each disorder described in the paper (Fig 1 on Page 27). We also made corresponding revision in line 5-8 of paragraph 1 on Page 3.

3. In addition the authors should choose more appropriate reference for some parts of the manuscripts. In several cases, in fact the references are far from the context of the sentence (in particular references n. 1 and n. 2).

**Response:** We deeply appreciate the reviewer's suggestion. We have chosen more appropriate references for some parts of this revised manuscript to make it more rigorous.

4. In addition, the tables 1-4, should be reorganized giving more emphasis on cell types and neurotrophic factor (first and second column). Reference should be the last column. In addition, the study design sign and the outcome should be described in synthetic manner.

**Response:** We agree with the comment and reorganized the tables (cell types and neurotrophic factor in the first and second column, reference in the last column). In addition, we synthetically described the study design sign and the outcome.

#### **Review 4**

1. In the present review, the authors reviewed the roles (neuroprotection and neuroregeneration) of neurotrophic factors (NTFs) such as growth factors (GFs) and neurotrophic factors (NFs) in major CNS diseases. Therefore, they emphasized that MSCs over-expressing GF/NF could be good tools for continuous delivery of the NTF. The mini-review is well organized, and may provide readers and investigators with good information.

**Response:** We appreciate the reviewer's positive evaluation of our work.

2. However, in the review, only NTFs were included as functional molecules adopted in genetically-engineered stem cells. There are important functional molecules such as choline acetyltransferase (ChAT) for acetylcholine synthesis (for AD) and tyrosine hydroxylase (TH) for dopamine synthesis (for PD). It is recommended that in possible, the authors review papers on NSC-ChAT, NSC-TH-GTPCH1, etc.

**Response:** It's true that ChAT and TH are important functional molecules for AD and PD, respectively. Considering that the focus of this review is the combination of stem cells with NTFs in the treatment of neurodegenerative diseases, and ChAT and TH are beyond the category of neurotrophic factors. So we didn't include these molecules in this review.

3. The authors pointed out the limited penetration of stem cells and functional proteins (NTFs) into the injured regions of brain due to BBB. Nowadays, extracellular vesicles (EVs, so called exosomes) containing NTFs released from stem cells are an emerging tool to deliver the functional molecules. The nano-sized (50–100 nm) exosomes were found to readily penetrate BBB, nasal mucosa, and even the skin, and to regenerate damaged neurons. Review on these points are also recommended.

**Response:** We deeply appreciate the reviewer's suggestion. We added information of MSCs-derived exosomes(MSC-exosomes) in the treatment of neurodegenerative diseases in the revised manuscript (the 2nd paragraph on Page 6, the 2nd paragraph on Page 8, the 2nd paragraph on Page 9, the last paragraph on Page 10). We also made corresponding revisions in the Abstract (line 6-8, 12-13) and Introduction (line 7,9,13 of paragraph 2 on Page 3, the 2nd and 3rd paragraph on Page 4).