Peer Review File

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Reviewer comments：

Comment 1:

SOD, MDA and GSH Assay

Did you determine the extracellular (as it's described in this part) or the intracellular content of enzyme activities (as it's written in the figure legend of figure 3)? This is very confusing. What are your controls? Which kits did you use (manufacturer)? If anything is described before, please cite the primary source. Please improve this part.

Reply 1:

Thanks for your careful revision and constructive suggestions. The SOD, MDA and GSH were detected after the cellular protein extraction of SCs, which were actually intracellular. So the unclear description in the original method in manuscript has been revised. In addition, the primary SCs subjected to no treatments were set as the negative control. Meanwhile, the kits used and the primary source have been complemented.

Changes in the text:

Line 10-11, page 11; Line 16-17, page 28.

Comment 2:

LDH release assay

Which kits did you use? If anything is described before, please cite the primary source.

Reply 2:

Thank you for the constructive comments! The literature for original source has been cited. The kit used has been added.

Changes in the text:

Line 2, page 12.

Comment 3:

Western Blot Analysis

How much μg protein per lane is loaded?

Reply 3:

Thanks for your careful revision! The 30μg protein per lane was loaded, and explanation in the manuscript was modified.

Changes in the text:

Line 6, page 12.

Comment 4:

RNA-seq Analysis

Can you give a short explanation, why you have 60 RNA samples? How many time points and replicates were analyzed? Otherwise the reader could not get into it.

Reply 4:

Thanks a lot! The comprehensive and accurate information is very important for the publication of our article. There were 5 time points, 4 groups and 3 replicates, so 60 samples were collected. The detailed description have been supplemented in the manuscript.

Changes in the text:

Line 17-21, page 12.

Comment 5:

In figure 1 is shown 56 % cells are viable after 4-hour serum deprivation. But in figure 2 you determined 10 % death cells and approximately 35 % (please describe the real numbers in the text) apoptotic cells. How did you explain this mismatch?

Reply 5:

Thanks for thequestions! As a dynamic process, apoptosis has different phases in early and late stages. As two methods used to detect apoptosis, Flow cytometry

can simultaneously detect the proportion of early (lower right quadrant) and late apoptosis (upper right quadrant), that is, 36.3% after serum deprivation in Figure 2 is the proportion of early apoptosis, 14.0% is the proportion of late apoptosis; while the TUNEL is to detect the extensive DNA degradation during the late stage of apoptosis. So, the result of about 10% is consistent with the early result 14% of flow cytometry. In addition, the total proportion of apoptosis in the flow cytometry results should be 14+36.3, which is 50.3%, which is also consistent with 56% of the cell viability.

Changes in the text:

No changes.

Comment 6:

The immunoblot of cleaved caspase in figure 3 is not an appropriate representative result. Please replace this blot.

Reply 6:

Thanks for the suggestions! A representative and clearer image has replaced the original result part B involving the statistical graph. Meanwhile, the error of "Bcl/2" in part A has also been corrected to "Bcl-2". The related description in manuscript was also be changed.

Changes in the text:

The figure 3- revised has been re-uploaded.

Line 15, page 16.

Comment 7:

Figure 7 is not viewable. Please replace this figure through a high-resolution picture.

Reply 7:

Thanks for your comments, and the quality of the pictures is very important. The picture has been redrawn and re-uploaded. On the basis of ensuring the structure and aesthetics of the figure 7, the font is enlarged as much as possible, and the resolution is also improved.

Changes in the text:

The figure 7- revised has been re-uploaded.

Comment 8:

Discussion

In my opinion, the meaning of the results is discussed very superficially. Some citations are missing, for example in line 404/404 "..that was further researched.." or line 421 "..the results of previous studies…". The authors discussed the results not in the content of the existing literature.

Reply 8:

Thanks for the important suggestions! The part of "...that was further researched…" refered to the further molecular level research in our experiments, so no literature was cited; and some modification was done. The section of "...the results of previous studies…" was the comparison with the previous related literature, the new citations has been added. Since our research on ABPPk in the protection of nerve injury is not very much, the previous reports were mainly on its role in central nerve injury, so the results of this experiment were mainly compared with those results, and confirmed that it may indeed have Advantages of vascular regulations.

Changes in the text:

Line 5-8, page 22. Line 4, page 23; Line 13-17, page 29.

Comment 9:

What means the qPCR validation results?

Reply 9:

Thanks for your comments! The validation is firstly to verify the accuracy of sequencing data, which is the basis of all bioinformatics analysis results and the biological role of molecules. The second aim is to verify the contents and phases of different factors regulating biological effects through the comparison of a few of slected important and key gene expression in different groups. Therefore, in

routine experimental design, the qPCR verification of key genes is necessary.

Changes in the text:

No changes.

Comment 10:

Why you analyzed these genes over the 24 hours?

Reply 10:

Thanks for your question! The correct choice of observation time points is critical to the results and conclusions of the entire experiment. The choice of time points in our experiment is mainly based on the following aspects: 1. Damaged cells can be restored to about 80% of their vitality by adding factors for 24 hours, suggesting that the protective effect of nutritional factors may be mainly within 24 hours (of course, this is also related to the choice of experimental models, and in vitro cell models are more suitable for earlier studies.). 2. To study the effects of Achyranthes bidentata active ingredients on the protection of peripheral nerve injury, we are more interested in its ability to protect or repair in the early stage of injury (especially for the resarch of early key transcription factors, which is of great significance for the development of new targets for clinical treatment). The active factor play a positive and repairing role at the early stage, which is very important, is also expected in the treatment of clinical peripheral nerve injury repair.

Changes in the text:

No changes.

Comment 11:

What are the supposed or expected consequences of different gene expression (in comparison to NGF)?

Reply 11:

The purpose of this experiment is to study the role of active ingredients of traditional Chinese medicine Achyranthes bidentata in clinical peripheral nerve

protection and injury treatment. While NGF is one of the earliest researched factors that have the effect of promoting nerve regeneration (our group has also done many studies), used as a positive control to better evaluate the role of Achyranthes bidentata active molecules.

Through the analysis and exploration of the different expressions of the molecules behind the phenomenon, we hope that in addition to affirming the effect of Achyranthes bidentata active molecules on protecting and repairing peripheral nerve damage, the bioinformatics research of molecular mechanisms will reveal its characteristics and advantages that are different from NGF, so as to provide experimental basis and theoretical guidance for the application of traditional chinese medicine Achyranthes bidentata and its active substances in the treatment of clinical nerve injury repair. Through our research, it do shows the advantages of Achyranthes bidentata active extract in vasculartur and immune regulation.

Changes in the text:

No changes.

Comment 12:

Please also include detailed future perspectives (related to the results).

Reply 12:

Thank you for the constructive comments! Based on the results, the detailed future perspectives of ABPPk for clinical treatments have been added. It will be more recommended for the treatment of patients with peripheral nerve injury during the acute phase, and/or patients with severe vascular injury and inflammation due to trauma; thus, Schwann cell survival can be maintained earlier, blood vessels and immunity can be better regulated, and the clinical nerve peripheral regeneration and quality of life can be improved.

Changes in the text:

Line 5-12, page 24.

Comment 13:

I am not a native speaker, but I think the paper would benefit from careful proofreading.

Reply 13:

Thanks for your suggentions! The language of the entire document has been carefully revised to reach the publishing requirements.

Changes in the text:

The errors or deficiencies especially the discussion in text have been revised in the corresponding place.