OPEN PEER REVIEW REPORT 1

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-22-00273
Title: PARP14 silencing accelerates acute spinal cord injury-induced neuronal apoptosis and neuroinflammation through regulating microglia M1/M2 polarization via the STAT1/6 pathway
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COMMENTS TO AUTHORS

The authors did a good job of tying together the in vivo and in vitro work. The data presented are interesting and important to the SCI field. However, a major concern is that some of the conclusions drawn are not demonstrated by statistical analyses. Additional analyses will greatly strengthen their arguments and improve this manuscript. Specific comments for each section of the paper are listed below:

Introduction:
-Add references for the statement - "Among them, the activation of microglia and the subsequent release of inflammatory factors often lead to the direct death of neurons." (lines 34-35)
-Lines 36-37 state that "Inhibition of SCI-induced microglia activation and the subsequent neuroinflammatory response has been shown to improve SCI." What consequences of SCI have been shown to be improved by inhibiting microglial activation?
- Lines 50-51 state that "GEO database (GSE5296 and GSE52763) analysis showed that PARP14 was highly expressed in SCI model." Where was PARP14 highly expressed?
-There is no mention of the rationale of measuring bone loss in the introduction. This should be added to tie together the relationship between SCI, bone loss, PARP14, and microglia.
-There is no description of what STAT1/6 pathway is important for until Lines 233-235 "The STAT1 pathway is identified to mediate M1 microglia/macrophage polarization and the STAT6 pathway is a key signaling pathway for M2-like polarization of microglia/macrophages (Gan et al., 2017; Li et al., 2021)." A statement similar to this should be added to the introduction.

Methods:
-Is there a way to score the "footprint analysis" to quantify gate and coordination?
-Can you include a statement about why only female mice were used?
-The primer sequences used for qRT-PCR should be listed.
-Is there a reason that Nissl and TUNEL and NeuN staining were only done at 7d post-SCI?
-The methods of immunohistochemistry does not list what day after injury the tissue was taken.

Results/Figures:
-All of the western blots need to be quantified and statistical analyses should be run on them, otherwise it cannot be stated that one condition is 'significantly different' than another group. For example, lines 197-199, lines 210-213, 223-226, 245-246, 270-272.

-Can the immunofluorescence or immunohistochemistry be quantified? If so, statistical analyses should be run on that data. If not, then it cannot be stated that one condition is 'significantly different' than another because no statistical analyses have been ran. This language should be dampened.

- Lines 199-201 state that "The behavioral assessment demonstrated that mice receiving LV-shPARP14 exhibited exacerbated motor dysfunction as early as 3 days post-injury, compared with mice receiving the LV-shNC, and the acceleration persisted until the last observation at 28 days post-injury (Fig.2g)." However, there appears to be no statistical analyses run on these data and day 3 looks to be the same as the other groups.

-Lines 207-208 state that "Nissl staining for gray ventral motor neurons proved that SCI mice (7 days post-SCI) showed more neuron loss and PARP14 knockdown further enhanced SCI-induced neuron loss (Fig.3a)" However, nothing was quantified in these images so it cannot be stated that this was "proved". To use the word "proved" these images need to be quantified and statistical analyses ran on them, or another experiment with quantifiable data needs to be ran.

-Lines 250-251 state that "Immunofluorescence staining and western blot analysis showed M1 phenotype microglial markers (iNOS and CD16) were significantly increased in LPS/IFN-γ-treated microglia, and PARP14
inhibition enhanced this trend (Fig.6c-d)." However, no immunofluorescence is shown for CD16.

-Lines 261-262 state that "M2 phenotype microglial markers (Arg-1 and CD206) expression was detected by immunofluorescence staining and western blot". However, only Arg-1 is shown in the staining.

-Lines 276-278 state that "Results of micro CT showed that PARP14 knockdown enhanced the downregulations of BV/TV, Tb. Th, and Tb. N and the upregulation of Tb. Sp (Fig.9a)." Were statistical analyses run on these data? No significance is shown on the graphs, which suggests there are no differences between the groups.

-Lines 281-283 state that "Meanwhile, PARP14 downregulation increased the mRNA expression of RANKL (osteoclast differentiation marker) but decreased OPG (osteogenic differentiation marker) mRNA level (Fig.9d)." Were statistical analyses run on these data? No significance is shown on the graphs, which suggests there are no differences between the groups.

- It would be helpful in each figure legend to describe a takeaway message from each of the figures rather than simply stating "representative images" or "protein expression measured by western blot". Especially for the immunofluorescence images where it is not always obvious what is different between the different groups. For example, something like "PARP14 mRNA expression is significantly increased 1, 7, 14, 28 day after SCI". Also, indicating what the arrows are pointing to in the immunofluorescence images should be added to the figure captions.

-Some of the figure legends (Figure 3, 5, and 8) states that "values were shown as mean +/- standard deviation" but there are no values shown and no quantification. What are these statements referring to?

Figure 2:
-what magnification were the immunohistochemistry and immunofluorescence images taken at?
-what area of the spinal cord are the immunohistochemistry and immunofluorescence staining being done on?
-Figure 2 Legend: says "b-c" when it should say "b-d" to include the immunohistochemistry

Figure 3:
-the figure legend should state somewhere what type of tissue these images were taken from and what tissue the western blot was run on

Figure 4:
-the figure legend should state somewhere what type of tissue these images were taken from, what tissue the western blot was run on, and what tissue the ELISAs were run on

Figure 5:
-the figure legend should state somewhere what type of tissue these images were taken from and what tissue the western blot was run on

Figure 9:
-should list what H&E and TRAP staining is labeling in these images

Discussion and Conclusions:
-more discussion and elaboration could be added about how PARP14 could be related to the statement in lines 368-369 that "significant bone loss occurred after PARP14 silencing"
-it is difficult to determine if the conclusions drawn are appropriate without having additional statistical analyses ran on the data presented.