OPEN PEER REVIEW REPORT 1

Name of journal: Neural Regeneration Research
Manuscript NO: NRR-D-21-00191
Title: Promoting axon regeneration in the central nervous system by increasing PI3-kinase signaling
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COMMENTS TO AUTHORS

The manuscript NRR-D-21-00191 entitled "Promoting axon regeneration in the CNS by increasing PI 3-kinase signalling" is a well-organized and carefully written manuscript that covers in depth recent knowledge on the mechanisms by which the PI3K/PTEN pathway promotes axon regeneration in the CNS after injury.

The authors have addressed all the recent themes/developments considering PI3K isoforms, PTEN and the abundance of the 2nd messenger PIP3 in neurons, as well as the downstream PI3K players and the biological mechanisms that take place during the axonal regeneration process.

I have only some minor comments and suggestions to make:

1. Page 5, line 27-32: Correct to "It is important….."

2. Page 5, line 39-44: "PIP3 signalling regulates developmental neuronal events such as axon specification, axon elongation, and the formation of growth cone filopodia and lamellipodia (Menager et al., 2004; Zhou et al., 2004; Cosker and Eickholt, 2007; Kakumoto and Nakata, 2013)."
It is not entirely clear that PIP3 controls axon specification per se. Although initial papers did indeed suggest that (e.g. Menager et al., 2004), more recent studies have failed to provide strong evidence for a causal relationship of high abundance of PIP3 on specific neurites and axon specification (e.g. Kakumoto and Nakata, 2013). Perhaps the authors would like to rephrase here ?

3. Page 9: the second and third sentences begin both with "In addition"; perhaps the authors should consider replacing with another word, e.g. "furthermore".

4. Page 16, lines 17-24: "Whilst it is not known whether mTOR functions within the axon to control regenerative mechanisms, it has been identified at the growth cones of axons extending through the brain during development (Poulopoulos et al., 2019). It is possible then that mTOR may function here to regulate local translation"
The authors should include here the study by Terenzio et al., 2018 (Science, 359(6382): 1416-1421) which provides evidence for activation and upregulation of local translation of mTOR itself in injured axons, together with increase of mTOR-controlled local translation. This has been observed in peripheral nervous system injury (sciatic nerve); it is worth discussing to what extent this occurs in CNS axons.

5. Page 17, lines 24-34: "This suggests that axon growth capacity might be more tightly regulated by controlling PTEN activity rather than its expression levels, and there is evidence for this. During development, axonal PTEN activity is controlled by binding to PRG2, a protein which targets PTEN to nascent axon branch points and inhibits its activity leading to localised increases in PIP3 generation (Brosig et al., 2019)."
Perhaps the authors could include here additional supporting references that discuss the axonal PTEN/PIP3 relationship focusing on PTEN and its regulation along the axonal membrane during development (e.g., Fuchs et al., 2020 Neurosci Insights Sep 13;15:2633105520959056).

6. Table 1 summarizes in a comprehensive way the existing literature on the results of PTEN downregulation/deletion in in vivo axonal regeneration animal models. Perhaps the authors could comment in the text on other, non-genomic, pharmacological approaches on activating downstream PI3K/PTEN/PIP3 signaling to achieve axonal regeneration (for example see the review by Ohtake et al., 2015, Neural Regen Res. 10(9):1363-8). I do not suggest an extensive discussion of this issue; a couple of sentences with specific recent references in order to alert the reader to these additional approaches would suffice.

7. Figures 1 and 2 summarize all the concepts and ideas of the authors. I would only suggest some minor stylistic modifications in Figure 1. If the authors agree they could modify the figure accordingly.
(a) the cartoons for receptors are a bit disproportionally large
(b) the red phosphate (P) on PIP3 apparently represents the D3-position of the inositol headgroup, but this is not specified in the legend
(c) the cartoons downstream of Arf6 are somewhat ambiguous; I suspect they represent motor proteins operating along microtubules for transport but perhaps they could be drawn more clearly or specified in the legend.