Open peer review report 1

Reviewer: Yiren Hu, University of California San Diego, Medicine, CMM-WEST Room 345, School of Medicine, La Jolla, California 92037-0648, UNITED STATES.

Comments to the authors:

In this manuscript, the authors described the function of SB216763, a GSK3β inhibitor on Schwann cells and muscle cells based on cell line study. Specifically, they found that GSK3β inhibitor would promote Schwann cells migration and muscle cell contraction. Also, they discovered that the inhibitor would induce proliferation of both Schwann cells and muscle cells. They further checked the nuclear localization of β-catenin and suggested that GSK3β inhibitor effects were mediated through Wnt/β-catenin signaling pathway. Overall, the findings are of potential interest to readers of Neural Regeneration Research. However, there are several issues that the authors need to address to meet the standard of Neural Regeneration Research.

First, for figure 1, proper measurement of the wound size and changes after 24 hours is desired such that quantification and statistical analysis are applicable to draw solid conclusion.

Second, for all the experiments mentioned in the paper, how many biological replicates have the authors conducted, for example, figure 2? Also, the authors need to specify the appropriate statistical analysis for all of the figures in the corresponding method part.

Third, for figure 4, the y-axis labeling is not clear. Presumably, the authors meant the relative gene expression level compared to GAPDH mRNA. Is it the case?

Last but not the least, the authors tried to link the Wnt/β-catenin signaling pathway to the effects of GSK3β inhibitor. However, without blocking the Wnt/ β-catenin signaling and check if the GSK3β inhibitor still works, the authors might not make a convincing argument on that.

Open peer review report 2

Reviewer: Dirk Montag, Head Neurogenetics Special Laboratory, Leibniz Institut for Neurobiology, Neurogenetics Special Laboratory, Brenneckestr. 6, Magdeburg, D-39118, GERMANY.

Comments to the authors:

Most experiments were conducted only once without quantification, repetition of experiments are generally missing, statistics are at best rudimentary. Therefore, the manuscript is scientifically not sound (low beginner level in a students practical course).

The experiments are simple cell culture assays without relevance for neural regeneration or muscle atrophy related to peripheral nerve injury.

This manuscript describes 5 in vitro assays on RSC96 and C2C12 cells after exposure to the GSK3ß inhibitor SB216763. The results are presented in 5 figures.

1) For the test of RSC96 migration after SB216763 treatment using a scratch assay, only 1 single photograph (Fig 1) is provided without quantification and statistics. Repetition and number of experiments are lacking.

2) Myotube formation after carbachol treatment of C2C12 cells and after SB216763 treatment is determined by measuring integrated "option" density - shall this read optical density?- and a contraction index is calculated as average of differences in density with time. However, evidently only 1 experiment was performed, therefore, standard deviation, statistics, repetition of experiments are lacking.

3) Cell proliferation of RSC96 and C2C12 cells after SB216763 treatment was analyzed using OD450 measurement. Here, at least values with supposedly standard error are provided. However, repetition

and number of experiments are lacking. Furthermore, it is stated that SB216763 treatment significantly stimulates cell growth, but statistics are not provided. Fig 3 legend is poor.

4) For SB216763 treated RSC96 cells, expression of MAG and CCND1 genes was determined by quantitative PCR. For SB216763 treated C2C12 cells, expression of MyoG and nAChRs genes was determined by quantitative PCR. Control values are only shown in Fig 4, number of repetitions/experiments is not stated.

5) IHC for ß-catenin expression after SB216763 treatment of RSC96 and C2C12 cells is shown in Fig 5. However, quantification and statistical analysis are missing.

In conclusion, the manuscript has fundamental experimental flaws and deficits that do not allow any conclusions on the reproducibility and significance of the effects. Even if the experiments could be properly conducted and would be statistically sound, conclusions from these limited in vitro assays on myelination or for denervated muscle atrophy are not possible.