Author’s response to reviews

Title: NMDA Receptor-Mediated CaMKII/ERK Activation Contributes to Renal Fibrosis

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Version: 1 Date: 09 Jan 2020

Author’s response to reviews:

Dear Editor,

Thank you and the reviewer so much for the sincere and inspiring comments. According these advices, we revised the manuscript carefully, and all changes made are highlighted in the manuscript. Following is our point-to-point response:

Introduction (Changed to Background)
<It would be helpful to better introduce the NMDA receptor for a broader scientific community, notably its molecular organization and functional domains.>

Thank for the suggestion, more detailed introduction of NMDAR including molecular organization and functional domains has been added. (Page 5 Line 6 to Page 5 Line 11)

Materials and methods (Changed to Methods)
<S100A4 must be introduced as fibroblast specific protein.>

Thank you very much for this advice, and S100A4 is introduced as fibroblast specific protein in Introduction along with other markers. (Page 4 Line 12 to Page 4 Line 16)

Results
Figure 1
<Figure 1: there is an overstatement of data presented in this figure. WB analyses should be presented as mean ± SEM in each group (n=6) rather %WT, which help the readers to appreciate the quantitative changes. The Masson’s trichrome staining is not convincing. The typical lesions
of AKI are scarce or lacking. It would be needed to quantitate renal fibrosis by digital morphometry of fibrogenic proteins (collagen, S100A4, etc.). Without these data, it is difficult to claim that NR1 is involved in UUO-induced renal fibrosis. Please indicate the scale bar in each figure. Please use the expression “original magnification instead "the images were photographed at 20°—objective".>

Thank you for these suggestions in this part, and followed are our response.

The statement has been changed in this part based on the result. While WB analyses presented as %WT help readers to understand the quantitative changes compared with the healthy mice in the injure groups, which is a quantify method widely used in literatures, such as articles published in BMC Nephrology before (DOI: 10.1186/s12882-019-1550-4, DOI: 10.1186/s12882-019-1540-6, etc.) and others (DOI: 10.1681/ASN.2018030270, DOI: 10.1681/ASN.2014050457, DOI: 10.1681/ASN.2016111248, etc.).

The Masson’s trichrome staining in Figure 1 was intended to demonstrate fibrosis produced by UUO operation, and is widely accepted as a basic staining method to discriminate collagen fibers from tissues on histological slides. The quantifications of fibrogenic proteins including S100A4 have been presented in Figure 2.

The statement of Figure 1 has been changed into “NR1 was overexpressed in UUO-injured kidneys in C57BL/6 mice”. (Figure 1 legend)

The scale bars and the original magnification of the pictures have been added in all figures, thank you so much.

Figure 2

<Figure 2: not easy to understand. Usually, the figures must be selfexplanatory. No legend and no scale bar were included within the histochemistry panels. Panels should be spaced and numbered closely. Here again, it would be advisable to quantitate renal fibrosis by digital morphometry of fibrogenic proteins. The authors should explain why the tubulin labeling is not homogenous between different WB>

Thank you very much for the suggestions, and these were really the deficiency in the manuscript. As improvement, the legends in this manuscript have been re-written, the pictures and graphs have been rearranged, and the quantification graphs have been added.

Thank you for the careful review, and it is our fault to make this mistake. In this research, the housekeeping protein standard of WB is α-tubulin, and has been labeled in all WB pictures in this new edition manuscript.

Figure 4

<Figure 4: Scale bars must be included. In the panels in the TGFb panels (pCamKII and pErk), I am not sure that the lower panels correspond to magnified images from the respective merges.>

Thank you for the suggestion, and the bars are added.

The lower panels magnified images are all from the merges above respectively, and the frames have been labeled on the merges to figure out the original locations of these images.

Figure 5

<I have some trouble about the B1 panel. There is a sharp contrast between the tubulin (housekeeping protein) and the Erk expression. Blots seem to originate from different WB.>

Thank you for the careful review on the figures. The blots of α-tubulin and ERK were marked by the different antibodies, and the contrast between them might because of the different tilters of antibodies.

<Based on findings in Fig 4 and Fig 5, it was suggested that MK801 and KN 93 act downstream of TGFb, while NR1-sh mimicked their effects on the phosphorylation of CamKII and Erk in
UUO model. So, it would be interesting to show the expression level of TGFβ in renal tissues of mice treated by NR1-sh.

Thank you for the useful suggestion, and it really inspired us. And we have performed the TGF-β1 staining in mice kidney sections, and the results have been presented in the supplemental materials.

Figure 6

<Please correct the figure number in the text (Fig 7?) Please include Scale bars and legend in figure (IR vs Con). It is needed to quantitate renal fibrosis by digital morphometry of fibrogenic proteins on higher magnification images. It is also advisable to include blood and urine tests to evaluate the renal function and pathological changes at the time of sacrifice.>

Thank you very much for figuring out this mistake, it should be Fig 6, and the error has been corrected. The figure numbers in the text are corrected. (Page 12 Line 19 and Page 13 Line 10)

The scale bars, legends, and quantification graphs have been added.

Kidney function was described in the text without figures because there was no difference with a normal kidney left. Figures are added. It helps to show the safety of DXM.

Figure legends
<Overall, the legend text should be rewritten in appropriate language and need to be completed in many instances.>

Thank for your suggestion. All the legend texts have been rewritten with more detailed information.

Discussion
<Although well documented, the authors did not integrate a synthetic view of their findings, notably how NMDAR-mediated CaMKII/ERK activation induces renal fibrogenesis and do not demonstrate whether NMDAR blocking is enough to inhibit the fibrogenic process, regardless of TGFβ expression.>

Thank you for the useful advice in this part. Combined the result of TGF-β1 expression staining in vivo, the mechanism of NMDAR blocking became much more specific, and the discuss has been added.

Others
Shuaihui Liu spent much time and effect on the revised manuscript, thus become the co-first author.

Reference list has been updated.
The funding numbers have been added in Fund.

Thank you again for the reviewer’s professional and useful advice on our manuscript which helped us to improve the quality a lot, and we indeed appreciate them, and made point to point change and response. Please let us know, if any adjustment needed.

Sincerely,
Jia Shen Ph.D.