Author’s response to reviews

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

Authors:

Jingyi Zhou (zhoujingyi@zju.edu.cn)
Shuaihui Liu (liushuaihui@zju.edu.cn)
Luying Guo (guoluying@zju.edu.cn)
Rending Wang (rd_wangjia@zju.edu.cn)
Jianghua Chen (chenjianghua@zju.edu.cn)
Jia Shen (jiashen@zju.edu.cn)

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Author’s response to reviews:

Dear Editor,

Thank you and the reviewers again for the constructive comments to help improve the quality of the manuscript. We believe that the manuscript has been further improved based on the comments. As before, all changes made are highlighted in the manuscript. Following is our point-to-point response:

Abstract

The abstract described about the methods in very detail, however, the results were not presented adequately, which is not supportive to the conclusion. Similarly, the results were not described clearly in the main text.

Thank you for pointing this out. We felt very regret that we didn’t presented the results clearly. It’s extremely helpful for us to get this feedback. We have added more detailed results in Abstract and re-wrote the Results part in the main text.

Materials and methods

Please state what anesthesia was used to euthanize the animals, and the dose used.

Point taken! The detailed information of anesthesia has been added. (Page 7 Line 10)

The company from where the reagents were purchased was not included, especially antibodies for Western blotting, and DXM.

Thank the reviewer for pointing this out. The antibodies for Western blotting were the same as immunohistochemistry, which was added (Page 9 Line 18). The company of DXM was also included. (Page 8 Line 18)

For the in vitro study, E-cadherin, a-SMA etc should be detected after TGF-β treatment to prove the pro-fibrotic response was induced successfully.
Thank the reviewer for this advice, and we added α-SMA fluorescent staining result as Supplementary Figure 2.

Results
The quality of the figures is currently not acceptable. Please can you improve the quality of the figures [Editor]. The resolution of the figures was too low. It's hard to tell the histologic change of the kidney [Reviewer 1]. The resolution ratio of figures should be increased [Reviewer 2].

Yes, we have improved the quality of all the figures. However, the figures in the PDF document of manuscript might be compressed during being uploaded, please download the original ones or contact zhoujingyi@zju.edu.cn or jia.shen@zju.edu.cn for it. Sorry for the inconvenience. We are willing to make it a perfect presentation in the final publication. (Figure 1 to Figure 6, Supplemental Figure 1)

The quantification data of Western blotting should be presented as mean ± SEM normalized to internal reference.

Thank you very much for this advice, we can’t agree more about the data process. Therefore, in this manuscript, the blots were normalized to internal reference in the same sample first, and then compared with WT groups respectively which presented as %WT help readers to understand the quantitative changes compared with the healthy mice in the injure groups. The data was changed to be presented as mean ± SEM.

In figure 1, the pictures of Masson staining are better choosing the cortex region of the kidney.

We can’t agree more with it, therefore, we presented images with views of glomerulus to show the histopathological changes in cortex region indeed. Moreover, the medulla area in our UUO model shrank.

In figure 2, the IHC of α-SMA, the tubular dilation in UUO group looks like slighter than UUO+NR-sh group.

Thank you for your professional comments. The former image of IHC we chose represented the high expression level of α-SMA in UUO model. It’s truly not a very typical histological image of UUO model. Hence, we carefully reviewed the figures of α-SMA in UUO, and chose a more general and typical image to replace it.

In figure 4, it would be better to low power image to show the general expression of all markers.

Yes, it will be perfect if we could provide low power image to show general view, which shall be taken into consideration at the very beginning of this study. And at this time point, we could not rescue this limitation. The expression levels of p-CaMKII and NR1 is homogeneous, thus the images in figure 4 is representative. Thank you very much. We will keep it in mind to guide our future work and presentations.

Thank you again for the professional and helpful advices on our manuscript. We appreciate it very much. We made point-to-point changes and responses. Please feel free to contact us, if any adjustment needed. We look forward to your decision.

Sincerely,
Jia Shen Ph.D.