Author's response to reviews

Title: Thymosin β4 alleviates renal fibrosis and tubular cell apoptosis through TGF-β pathway inhibition in UUO rat models

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Author’s response to reviews:

Dear Editor-in-chief, Associate Editor and Anonymous Reviewers,

We would like to thank you for your efforts in reviewing our manuscript and providing many helpful comments and suggestions. Those comments are all valuable and very helpful for revising and improving our paper. We have studied comments very carefully. Based on your comments and suggestions, we have revised the manuscript accordingly. The details are explained below, where the number of the response is in correspondence with the number of the reviewers’ comments and suggestions.

Reviewer #1

1. The authors did not answer the question whether Thymosin treatment increased the e-cadherin levels above "sham".

Reply: This is a very good question, and we are so sorry that rats in the sham group suffered damage in the previous experimental process, but the results had not been verified before
submission. We redid the sham operation procedure, and found that e-cadherin levels between sham group and high-dose Tβ4 group had no significance difference. Thymosin treatment didn’t increase the e-cadherin levels above "sham".

2. Tautological sentences like that in the Background, end of paragraph 1 should be removed.

Reply: The tautological sentences have been removed in the manuscript.

3. The authors use reference 14 to justify the statement that UUO is regarded as the best animal model of progressive tubulointerstitial fibrosis; reference 14 does not make this claim or even imply it.

Reply: This reference has been replaced by an appropriate literature.

4. I can’t see any indication of the species of the cells used in the in vitro experiments or whether they are transformed or primary.

Reply: Information of tubular epithelial cells has been added.

5. Have the authors demonstrated that beta actin is not altered by the treatments? If not then it is not an appropriate control.

Reply: The expression of beta actin had no difference.

6. The authors do not present any data showing cells treated with Thymosin beta4 alone; they should do this as it appears from their data that it changes the cell phenotype.

Reply: This is an excellent question. Cells were treated with Tβ4 alone in our supplementary experiment section, and we found the protein levels of E-cadherin, α-SMA, cleaved caspase3, Bax and Bcl2 between control group and Tβ4 treated group had no significance difference (Figure S1).

7. The authors must address the clear discrepancy between alpha smooth muscle actin mRNA and protein.

Reply: The results of alpha smooth muscle actin mRNA and protein in the figure 4 have been mended and verified.
Reviewer #2

1. There is still important information missing from the methods:

   a. No methods are detailed for any of the apoptosis methods in vitro or in vivo (TUNEL methods, antibodies used for Cleaved Caspase 3, Bax, Bcl-2). What criteria did the authors use to assess apoptosis by morphological analysis of cell nuclei?

   b. The n number for the in vitro studies needs to be specified.

   c. Could the authors provide more details for the tubular epithelial cells they are using? Is this a cell line, or primary cells? Are they human, rat, mouse-derived?

Reply:

   a. The method of TUNEL and anti-Cleaved Caspase 3, Bax and Bcl-2 antibodies had been represented in manuscript.

   b. The n number for the in vitro studies had been added in the illustration of figure 4 and figure 6.

   c. Information of tubular epithelial cells has been added.

2. Could the authors justify the dose of Tβ4 used? 1 or 5ug/ml seems quite how compared to 100ng/ml that has been previously reported in literature.

   Reply: We couldn’t repeat experimental results according to the concentration of 100ng/ml. The drug doses in this study had been justified by our preliminary experiment.

3. Figure 5A: The pictures are very small and it is difficult to see the TUNEL positive nuclei.

   Reply: The pictures of figure 5A have been enlarged.

4. Figure 6A: A better quality picture is needed that can allow the reader to assess the morphological changes indicating cell apoptosis.

   Reply: The pictures in the figure 6A have been changed.

5. The references to Figure 5 in the text do not match the numbering of the panels in the Figure. There are also many typos that need to be addressed.

   Reply: The illustration of figure 5A has been mended.