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:

1. Please also ensure that your revised manuscript conforms to the journal style, which can be found in the Instructions for Authors on the journal homepage.

Reply: Yes, thanks!

2. In particular, Figures 5 and 6 require attention because we don't think as presented, it is possible to see what is described in the text and this calls into question the interpretation of the data and hence the conclusions.

Reply: The text and conclusion had been revised.

3. Looking at some of the graphs, we find it very surprising that the apparently very small differences are statistically significant - e.g. Figure 3C compare low and high dose TB4 groups. Figure 4D and E likewise. Can you please provide further explanation to support your statements.
Reply: Compare low with high dose TB4 groups, there are very small statistical differences. This shows that the effects of Tβ4 on UUO rat model might be concentration-independent. We will further investigate to about this.

4. We have also identified several grammatical errors that will need to be addressed. We have included some examples where the language/presentation require revisions (please note that this is not the full list, so you will need to have your manuscript checked by a copy-editor):

- In the letter to the Editor they refer to "tumor growth factor beta" which is in fact transforming growth factor beta

Page 11: "In vitro experiments of tubular epithelial cells revealed that alpha-SMA mRNA and protein levels decreased, E-cadherin mRNA and protein levels increased by TB4 treatment, and similarly these changes were more significant in the UUO+high-dose TB4 group." This appears to be muddling in vitro and in vivo experiments?

Reply: it had been revised.

5. Page 4: "regeneration as healing process is adequate", "ECM extension"

Reply: "regeneration as healing process is adequate", had change to "especially when healing process was applied by regeneration inadequate " ;"ECM, extension" had change to "ECM and extension "

- Page 9: "The 15 samples per group were detected", "The data were analysed in normal distributed with Kolmogorov-Smirnov", "The kidney tissues were regularly arranged"

Reply: "The 15 samples per group were detected" had been revised to "The 15 samples per group was detected" "The data were analysed in normal distributed with Kolmogorov-Smirnov" had been revised to "The data was analysed in normal distributed by Kolmogorov-Smirnov"

Reply: "The kidney tissues were regularly arranged" had been revised to" organization of kidney tissue was in good order".

- Repeated reference to "tubulointerstitial tissue" when they mean cortical tissue since they include both glomeruli and tubulointerstitium.

Reply:" tubulointerstitial tissue" had been revised to" renal tissue"

- Page 11: The wrong portions of figures are referred to. In the 2nd paragraph lines 6 and 9 Figure 4 D should be Figure 4 E.

Likewise on Page 12 Figure 5 C should be Figure 5F
5. There are other issues, for example, Reviewer 1 pointed out that a reference cited to support
the statement that "UOO is regarded as the best animal model of progressive tubulointersitial
fibrosis" (which, incidentally, it is not) was incorrect in the original manuscript the authors have
replaced the reference with an equally inappropriate reference.

Reply: It had been revised.

6. Methods: Details are currently lacking, and we would suggest that you refer to the ARRIVE
Guidelines. This is so your methodology can be fully evaluated and utilised. For example, for the
in vitro experiment, there is no indication of the concentration of TB4 used or length of
treatment, were cells made quiescent before treatment, how confluent were the cells at the time
of treatment.

Reply: It has been revised (page 4)

7. They state they used an RNAse Mini kit to isolate RNA which seems unlikely; I think they
may mean RNAEasy minikit?

Reply: We have replaced "RNAse Mini kit" to "RNAEasy minikit"

8. There is no method provide for Hoescht staining of epithelial cells.

Reply: It’s my error about this, Hoescht staining wasn’t applied, we replaced it using tunnel
staining.

9. There is no indication of how morphological alteration was assessed or what that actually
meant.

Reply: Normal morphology of kidney tissue is the base of kidney function.