Reviewer’s report

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

Version: 0 Date: 05 Feb 2016

Reviewer: Elisavet Vasilopoulou

Reviewer’s report:

In this study, renal injury was induced in rats by unilateral ureteral obstruction (UUO) and the effects of thymosin-β4 administration were tested. The authors found that UUO led to fibrosis accompanied by increased mRNA and protein levels of TGF-β and α-SMA and decreased E-cadherin. Thymosin-β4 treatment attenuated these changes in a dose-dependent manner.

This is a well conducted study confirming previous findings showing the anti-fibrotic properties of thymosin-β4 following kidney injury (Ref 13 in the manuscript). However, there is limited original data presented in this manuscript.

This manuscript would benefit from some in vitro experiments to test if thymosin-β4 can block EMT of tubular epithelial cells. This will provide some insight as to whether thymosin-β4 acts directly on tubular epithelial cells or whether the observed effects are mediated by paracrine interactions with other cells.

The authors postulate in the discussion that the effects they observed may be accompanied by changes in cell proliferation or apoptosis. It would be useful to add quantification of proliferation and apoptosis to this study.

Methods:

Important information is missing:

How much thymosin-β4 was administered per gram of body weight, when was the treatment initiated and how often was it administered?

What is the n number for the in situ quantification per group? How many fields were quantified for each sample?

What is the n number for the immunoblotting quantification per group? Which antibody was used for the TGF-β immunoblotting and at what dilution?

Results:
The figures are not well matched. The magnification appears different between different groups, especially in Figure 1. Figure 1C is not well focused. In Figures 2, 4 and 6 some of the pictures show glomeruli and others show tubules only making comparisons difficult. All the microscopy figures need scale bars.

Page 9, line 58: Please correct the title as this paragraph does not describe E-cadherin levels.

Discussion:

The authors demonstrate that thymosin-β4 treatment attenuated the UUO-induced increase of TGF-β and α-SMA mRNA and protein level and restored the level of E-Cadherin. The authors' interpretation is that thymosin-β4 regulates the expression of these genes as stated in the title of the paper and throughout the manuscript. However, these effects may be secondary to other changes induced by thymosin-β4. As the authors acknowledge in the discussion, the study did not assess the mechanisms underlying the anti-fibrotic effects of thymosin-β4. Therefore, I think the wording should be adjusted to better reflect the presented results.

The discussion should elaborate more on how these findings compare to other studies assessing TGF-β following thymosin-β4 treatment.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

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If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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