**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:** 22 Jan 2016

**Reviewer:** Mark Dockrell

**Reviewer’s report:**

The manuscript describes a potentially exciting topic in the treatment of renal fibrosis, the use of Thymosin β4 a therapeutic. The investigators use an established model of tubulointerstitial fibrosis and focus specifically on the effects of treatment on TGFβ and its direct effects.

There are some points that need to be addressed before the manuscript is suitable for publication.

I am very surprised to see such levels of proteinuria in a UUO model as the injured kidney is completely obstructed and has no urine output. The authors need to discuss this point and explain their findings.

For the in situ work, sections without probes are not an appropriate negative control as they don't compensate for any non-specific binding; it requires a labelled sense strand. Can the authors defend their choice of negative control?

In the Western Blot for TGFβ, did the authors see a band at 12.5 KDa? One might expect that, under the conditions described, a protein of this size would be detectable.

When the authors refer to "The area of the renal interstitial tissue was measured", are they really excluding all cells and if so how was this done?

Regarding the statistical analysis, it seems to me that not all of the data being analysed in normally distributed; did the authors check this?

In the e-cadherin data presented in figures 6 & 7, Thymosin β4 appears to increase the e-cadherin levels above "sham"; did the authors test this statistically and were they powered to assess such a difference? The authors should comment on this as it consistent across both techniques. Loss of E-cadherin can be required prior to reparative proliferation.

TGFβ is Transforming Growth Factor, not tumour growth factor; also in some parts of the manuscript it is written TFG-beta.

Although supported by citations some of the statements in the introduction do not seem to be reasonable and the phrasing should be adjusted.
For example: 1) "Overall lifetime incidence of CKD is 59.1% for an estimated glomerular filtration rate (eGFR) <60 ml/minute/1.73 m², 33.6% for an eGFR <45 ml/minute/1.73 m², 11.5% for an eGFR <30 ml/minute/1.73 m², and 3.6% for end-stage renal disease (ESRD)." These figures represent "risk" of attaining the given levels of eGFR in a US population and are based on estimates of prevalence; they should not be quoted as incidence.

2) "The main characteristics of CKD are the accumulation of extracellular matrix (ECM) and fibrosis," this is possibly the main renal characteristic, in reality the main characteristic is renal related cardiovascular disease.

3) "TGF-β can induce fibroblast cells to express α-SMA and to transform into fibroblasts.". Fibroblasts do not transform into fibroblasts.

The authors state "In this study, different doses of Tβ4 decreased alleviated kidney fibrosis, suggesting that Tβ4 could antagonize the expression of TGF-β and α-SMA, inhibit cell apoptosis, and reduce EMT." This may be true but I'm not sure the authors have included any data to support the anti-apoptosis claim.

**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.

No

**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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I am able to assess the statistics

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