Reviewer's report

**Title:** Gremlin induces cell proliferation and accumulation of ECM in mouse mesangial cells under high glucose via ERK1/2 pathway

**Version:** 1  **Date:** 20 August 2012

**Reviewer:** Phillip Kantharidis

**Reviewer's report:**

The manuscript investigates the role of gremlin in mesangial cells and how it may regulate the ERK signalling pathway. Once elevated, gremlin causes increased expression of TGFb and CTGF and these presumably drive the pro-fibrotic response under HG conditions. This area is interesting as the investigators propose that inhibiting gremlin may be a therapeutic approach to preventing or attenuating renal fibrosis.

**Major Compulsory Revisions**

1. Many of the experiments look at the role of gremlin in the context of HG. Because the treatments with gremlin or si-Gremlin are concurrent with the addition of the HG stimulus, it is difficult to assess whether gremlin has an effect on its own, or whether it transiently enhances the effect of HG. These experiments should be conducted in normal glucose conditions and also in MCs adapted to HG for the specific effects of gremlin to be identified.

2. Because of the HG stimulus, the control experiments for HG should be either mannitol or L-Glucose as an osmotic control. It is well established that osmotic pressure can activate signalling through the MAP kinase pathways. In the absence of an osmotic control, the data is difficult to interpret.

3. The transfection efficiency claimed for figure 2 appears to be exaggerated. At best it may be 20-30%, resulting in a 1.6 fold increase in gremlin expression. However the effect of the transfection of gremlin or the siRNA into MCs appears to be in every cell in Figures 3 and 4, as evidenced by the PCNA staining. Why the difference? Maybe a gremlin immuno could be performed on transfected cells to confirm the presence of gremlin in most cells.

**Minor revisions**

At the end of the first paragraph of the results section the claim is made that gremlin is involved with the HG-induced effects in MCs. This claim is premature so early in the manuscript when the only thing the investigators have shown till now is that gremlin gets upregulated by HG.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable
**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests’