Reviewer's report

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

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Reviewer: Motoko Yanagita

Reviewer's report:

In this manuscript, Huang et al. demonstrated that high glucose (HG) induced the expression of Gremlin, TGF-b1 and CTGF, and the accumulation of collagen in mouse mesangial cells. They showed that the overexpression of gremlin in mouse mesangial cells further induced TGFb1 and CTGF expression, and accumulation of collagen, whereas knockdown of gremlin by siRNA inhibited these effects. They further demonstrated that HG induced the proliferation of mesangial cells and phosphorylation of ERK, and overexpression of gremlin further enhanced the induction, whereas the inhibition of gremlin abrogated these effects.

Taken together, authors tried to conclude that Gremlin induces the expression of TGF-b1 and CTGF in HG condition, accumulates collagen, stimulates ERK phosphorylation and cellular proliferation.

Although the topic is clinically relevant, there are many concerns about the manuscript.

Overall comment:

1) To study certain functions of gremlin, authors should test whether the functions are BMP independent.

2) Transfection efficiency was low in primary cultured mesangial cells, and authors are dealing with the mixed population: cells with plasmid and cells without plasmid. To analyze the function of gremlin, one should try the administration of recombinant Gremlin, because the protein is commercially available, or at least more efficient transfection method such as letrovirus.

Specific comments:

1) Figure 1: The induction of gremlin in HG condition is already reported by McMahon et al. in JBC (2000).

2) Figure 2: The efficacy of Gremlin plasmid transfection was not high (A), and the induction of Gremlin expression was not prominent in western blotting (E). To determine the function of Gremlin, the administration of recombinant Gremlin protein is more reliable than the overexpression of plasmid.

3) Figure 3, 4: Intensity of PCNA staining is not a reliable marker for cell proliferation (A). Authors should try more quantitative methods to analyze cell
proliferation (e.g. BrdU elisa assay).
To my knowledge, the induction of TGF-b1 and CTGF by Gremlin is novel (B, C), whereas the induction of collagen accumulation by Gremlin is already reported (D).

4) Figure 5: Phosphorylation of ERK by Gremlin is already reported in endothelial cells by Stabile H et al. in Blood (2007). Enhancement of ERK phosphorylation by Gremlin overexpression was not significant in the gel in Figure 5B.

**Level of interest:** An article of insufficient interest to warrant publication in a scientific/medical journal

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests.