Reviewer's report

Title: Bone Morphogenetic Protein (BMP)-7 expression is decreased in human hypertensive nephrosclerosis MS: 1125618792355098

Version: 1 Date: 16 August 2010

Reviewer: Leif Oxburgh

Reviewer's report:

The authors have fundamentally revised the manuscript, focusing on BMP7 in nephrosclerosis. This is a great improvement and they have a number of interesting findings that are very worthy of publication. However, the incomplete nature of the manuscript again makes it difficult to assess as a whole, and I recommend another revision addressing the following questions.

MAJOR COMPULSORY REVISIONS

Samples
Are the tubulointerstitial and glomerular samples from the same individuals?
Table 1: male/female distribution indicate a sample size of 42 rather than 32. Also, could some normal reference values be added to this table for readers who do not have clinical experience?

QPCR
The assay detecting BMP7 does not span an intron, but the authors do not list a DNase treatment step in the cDNA protocol. The authors need to provide some proof that they are not detecting genomic material. This becomes particularly important in assays where the authors use up to 18ug/ml cDNA.

Fluorescent colocalization of BMP7 with molecular markers
Both the anti-BMP7 antibody and the Aquaporin antibodies used in costaining are rabbit polyclonals. Fluorophore conjugated anti-rabbit secondary antibodies were used to label these. Using the approach described, it is not possible to colocalize two different antigens in tissue.

Immunohistochemistry
Although the finding that BMP7 is reduced in nephrosclerotic tissue is based on analysis of multiple sections, only one is shown and there is no control (ie other protein confirming the quality of the section). This analysis would be greatly improved if the authors could quantify the difference in BMP7 staining between control kidney tissue versus nephrosclerotic tissue, while showing that overall protein levels are not depressed in nephrosclerotic tissue by comparing expression of a housekeeping protein.

Cell-based experiments
Throughout these experiments, the horizontal lines above the graphs on which the p values are written are confusing – the authors need to specify which actual comparison have been made.

Smad expression

Although an immunoblot experiment measuring the expression of smads is reported in the results, no data is presented.

TNFa induced apoptosis data

The authors have quantified the effect of BMP7 treatment on TNFa-induced apoptosis in HK-2s by double immunofluorescence, but there is no description of what they have stained in the results section or in the figure legend.

TGFbR1 immunostaining

The anti-TGFbR1 immunofluorescence seems to show a change in localization of the receptor to the nucleus after BMP7 treatment. If that is really the case, it seems worthy of discussion.

MINOR ESSENTIAL REVISIONS

Figure legends for the new figures need to be more informative – for example, it is very difficult to understand what antibodies have been used, how the quantitation has been performed.

The conclusion of the abstract is cryptic and should be revised.

In the statistical analysis section, the sentence on significant changes being regarded as descriptive needs to be explained.

DISCRETIONARY REVISION

It would help the flow of the manuscript if the authors included a couple of sentences on the rationale for doing experiments at the beginning of each results section.

Level of interest: An article whose findings are important to those with closely related research interests

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

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