ANSWERING REVIEWERS

January 9, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7269-review.doc).

Title: IGFBPrP1 induces liver fibrosis by Smad2/3 signaling via hepatic stellate cell activation and hepatocyte apoptosis

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 7269

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) Reviewer 00006459

Many experiments are n=3 and this is a concern. In the 4th para of results, "similar results for RNA"is not appropriate and needs to be closed. The next page has a "data not shown" statement and need to be closed.

We agree and have clarified the experiments more than 3 times.
We agree and have cancelled the sentences of "similar results for RNA".
We agree and have cancelled the statement of data not shown.

In the abstract please define AdshSmad3.Western blotting needs to be written with capital W every time. Do not use the term" mediating", intend to use "stimulated" or "treated".

We agree and have defined AdshSmad3 and correct the Western blotting.
We agree and have changed "mediating" to "stimulated"

The graphs all need to be changed to scatter plots and mean ±SE need to be changed to mean ±SD.

We agree and have changed mean ±SE to mean ±SD. But about the graphs, we prefer to use the column graphs.

(2) Reviewer 00069371

Check and correct all abbreviation such as Western, AdIGFBPrP1, cAd.

We have checked and corrected all abbreviation including Western, mediated, AdIGFBPrP1, cAd, AdshNC, AdshSmad3.

Cell culture model – should show hepatocyte culture.

In this experiment, we only cultured HSC-T6 cells not hepatocytes. We have shown the cell culture in methods. We found the hepatocyte apoptosis only in animal model.

Quantitative real-time PCR should be read by copy number- relative results in fig 1, did not show what related to what.

We agree and have clarified in the method that all of the mRNA results were expressed as number of folds relative to the control group.

Sirius Red stains collagen, TUNEL assay and immunohistochemistry results should be mentioned in the method % positive staining cells based on intensity or per area/ field or total number?

We agree and have clarified in the method that the results of Sirius Red staining, TUNEL assay and immunohistochemistry were expressed as the percentage of the area of occupied by positive cells.

Detail described in texts that included numbers/digits are confusing. Please find the better way to made clearer interpretation these numbers. Fig3 Western blotting of phosphor-Smad2/3, band density should be grouping in the same figure. The decreased approximately 3 folds in the figure, at different time (48, 72 h) were not got along well with 0.6 and 1.5 folds in the text. What do you mean by the transfection efficiency?

We agree and have changed another way to describe the Western blot results. Figure 3, we have described the results in the same figure. In figure 3, Western blot showed the Smad3 protein, which decreased 3 folds. But in figure 2, Western blot showed the phosphorated-Smad2/3 protein, which increased 0.6 and 1.5 folds. So it was got along well.

We want to give the evidence that AdshSmad3 has a best knock down effect on Smad3 gene at MOI 100 and explain why we used this concentration in the following experiments.

Fig 3 did not show positive cells after varied MOI transfection. Although the figures showed the representative results, measurement methods must be declared. The knock down result showed abrogated of SMA, but the figures in parenthesis were reversed (0.196-0.723). Fig 4 since you are localizing GFP positive cells, Smad3 and p-Smad3 cells, please use arrows/arrowheads to point whether they are HSC or hepatocyte. The observed time 14d or 28 d was lacking in the text. Fig 5 missed legend and labeling of figures A and B.

We have done three different concentrations and have the pictures. In Figure 3, we have showed the transfection efficiency at MOI 100. We agree and declared the transfection efficiency was expressed as a percentage of the numbers of EGFP or RFP positive cells to the total cells in the methods. We have corrected the parenthesis about the α -SMA and have used arrows/arrowheads to point about the hepatocyte and HSCs in the figure at good magnification. We also referred to the observed time in the method and supplemented the labeling of figure.

(3) Reviewer 02444774

Is it pathway etiology dependent? Is it only applicable in NASH but not viral hepatitis? The authors should elaborate more in details about the clinical implications of the

results.

It is a good question. Our results showed that IGFBPrP1 induced hepatocytes steatosis and fibrosis in rats. We will continue to do further experiment to evidence whether it is only applicable in NASH or not.

3 References and typesetting were corrected.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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