May 17, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 17333-review.doc).

Title: IGF-1 promotes the growth and metastasis of hepatocellular carcinoma *via* the inhibition of proteasome-mediated Cathepsin B degradation

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 17333

The manuscript has been improved according to the suggestions of reviewers:
1. The manuscript has been strictly formatted according to the style of *World Journal of Gastroenterology*.

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

Reviewer #1
We thank this Reviewer's encouraging comments and valuable suggestions to our manuscript. We have conducted new experiments and modified our manuscript following these opinions to improve the quality of our manuscript.

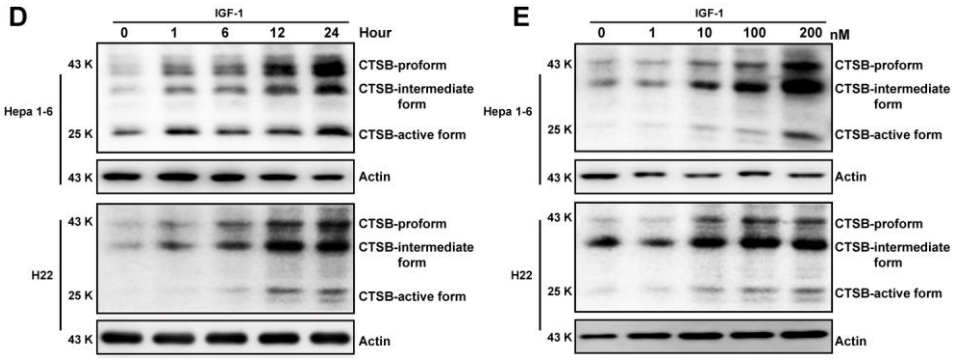
(1) Figure 1C. In the Methods section, the authors indicate that the scratch assays were performed for 48h in 10% serum, but the Figure/legend, indicates IGF or 1% FBS were utilized for the assays and that the assays proceeded for 24h. Obviously, it is unclear how this experiment was performed. If it was done in 10% serum, the authors cannot conclude that there is an effect on migration, as the cells are likely to fill the wound via proliferation during this time period.

Re: Thank you for your reminding about the discrepancy of IGF-1 treatment in Methods section and Figure 1 legend. That was due to our carelessness. Actually, Hepa 1-6 and H22 cell monolayers were wounded with a pipette tip, washed with PBS and cultured in 1% FBS medium or 1% FBS medium with IGF-1 (100 nM) for 24 hours. Images were captured at 0 and 24 hours after wounding with an microscope and the lesion area was measured. We have corrected the error and modified the description of MATERIALS AND METHODS section in the revised MS.

(2) Figure 1D. Cathepsins are present in proforms, intermediate and active forms. It is unclear which form is shown. The authors need to show the entire blot with MW markers indicating each form of the protein. This is a critical point because if the authors are visualizing the active band, they aren't observing an increase in expression, but instead are visualizing inhibition of activation.

Re: Thank you for your suggestion. Because we focus on the proform of CTSB, we only displayed the proform band of CTSB from the entire blot and cut the other forms of CTSB. Following your suggestion, we redone the experiment and showed the entire blot with MW markers indicating each form of CTSB in the revised MS. We found that IGF-1 treatment also increased the expression of the intermediate and

active forms of CTSB. These data are shown below and illustrated in new Figure 1D and 1E in the revised MS.

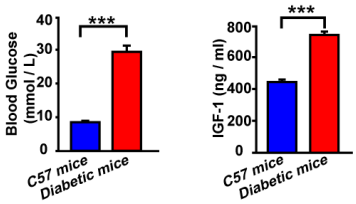


(3) Figure 1E. The Figure Legend indicates the presence of a panel (E); however, there is no panel (E) in the figure. Perhaps they are referring to the right panel of Fig. 1D?
Re: Thanks for your reminding. We have corrected the error in the revised MS.

(4) Figure 2B, C are not referenced in text.
Re: Thanks for your reminding. We have corrected the error in the revised MS.

(5) Figure 3A. Although the data in the diabetic mice are interesting, there is no definitive data showing that the effect is mediated by IGF-1.

Re: Actually, we detected the blood glucose and plasma IGF-1 levels of the diabetic mice. We found that these diabetic animals had higher blood glucose and plasma IGF-1 levels than normal C57 mice, which is consistent with the previous report (Gene P. Ables, 2014). These data are shown below and also described in MATERIALS AND METHODS section in the revised MS.



(6) Figure 4A. This RT-PCR experiment either needs to be quantitative (qRT-PCR) or semi-quantitative (actin primers within the same reaction, taking aliquots at various cycle#s). Without some sort of quantitative measure one can't be certain that the reaction isn't maxed out. In fact, the Method section does not even indicate how many cycles were utilized. Also, the authors indicate that there isn't a change in transcription, when, in fact, this isn't a transcriptional assay. The authors can only say there are no changes in mRNA levels (if they perform an quantitative experiment).

Re: Thanks for your suggestion. We have modified the description of the RT-PCR experiment and added the detailed protocol of RT-PCR in the MATERIALS AND METHODS section. Following your suggestion, we also changed the description from "no change in transcription" to "no changes in mRNA levels" in the revised MS.

(7) Figure 4B,C,F. See comment for Fig. 1D.

Re: Because we focus on the proforms of CTSB, we only displayed the proform band from the entire

blot and cut the other forms of CTSB. Following your suggestion, we showed the MW markers indicating the proforms of CTSB in new Figure 4B, C and F in the revised MS.

(8) Figure 4C. The second upper panel is labeled CTSB rather than CTSB. MG132 and baflomycin doses needed to be indicated either in the figure or in the figure legend. The authors also need to include a control to show that the baflomycin is working.

Re: Thank you for your reminding. We have corrected the error in the revised MS. Following your suggestion, the doses of MG132 and baflomycin have been indicated both in the figure and the figure legend. LC3 protein has been reported to be the substrate of auophagy and the degradation of LC3 could be interrupted by baflomycin. Hence, we used LC3 protein as a control to show the effects of baflomycin. We found that the degradation rate of LC3 was slow down by baflomycin but not by MG132. These new data have been illustrated in Figure 4C in the revised MS.

(9) Figure 5. A Figure 5 is shown, but there is no figure legend and it isn't referenced in the text. It appears to be a repeat of Figures 4D-F. This is very sloppy and indicates that the authors didn't reread the manuscript prior to submission.

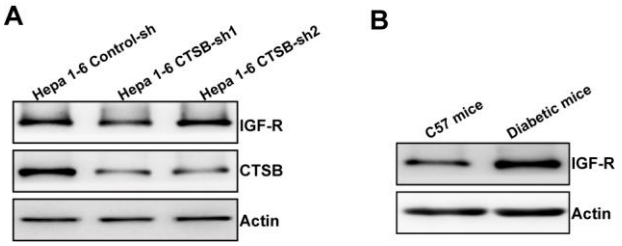
Re: Thank you for your reminding. We have already corrected this error in the revised MS.

Reviewer #2

We thank this Reviewer's encouraging comments and valuable suggestions to our manuscript. We have conducted new experiments and modified our manuscript following these opinions to improve the quality of our manuscript.

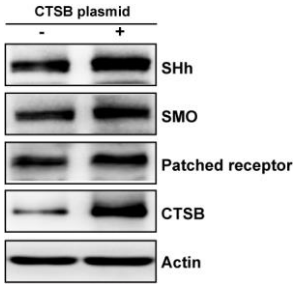
(1) expression level of IGF-1 receptor with CTSB knockdown, and also the level of IGFR on WT and Diabetic mice.

Re: Following your suggestion, we detected the expression level of IGF-1 receptor in CTSB knockdown cells and WT or diabetic mice. We found that CTSB knockdown did not affect the level of IGF-R expression in HCC cells. Whereas the liver tissue of diabetic mice showed higher IGF-R expression than that of C57 mice. The data are shown below and provide an important clue for our consequent work about IGF-1/IGF-R pathway involved in the progression of HCC.



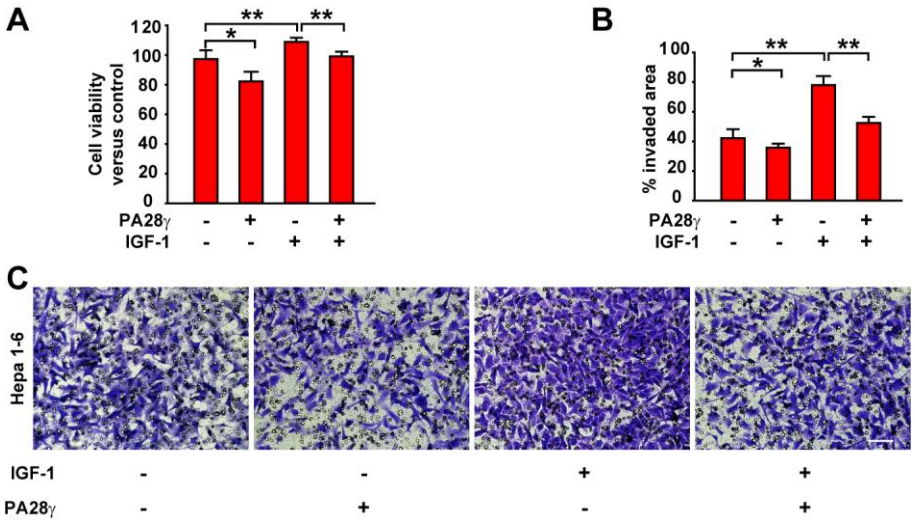
(2) CTSB has been reported to be a Hedgehog target in pancreatic cancer. Does CTSB accumulation cause an upregulation in Hedgehog pathway (SHh, SMO, Patched receptor) etc, hence causing the increase migration and metastatic ability.

Re: Following your suggestion, we detected the expression levels of SHh, SMO, and Patched receptor in Hepa 1-6 cells which transfected with CTSB expressing plasmid. We found that CTSB accumulation indeed moderately increased SHh, SMO, and Patched receptor expression. The data are shown below and we will explore this in our consequent study in which we want to know whether Hedgehog pathway is involved in tumor-promoting effect of CTSB.



(3) Xenograft experiment showing decrease in tumour size after overexpression of PA28

Re: Thank you for your suggestion. Because of the time limit of revision, we detected whether PA28γ overexpression impeded the tumor-promoting effect of IGF-1 *in vitro*. We found that PA28γ overexpression indeed inhibited the proliferation and metastasis of Hepa 1-6 cells with or without IGF-1 treatment. These data are shown below and illustrated in new Figure 5 in the revised manuscript.



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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