December 19, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 15468-review.doc).

Title: MicroRNA-185-5p mediates the regulation of SREBP2 expression by HCV core protein

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Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "MicroRNA-185-5p mediates the regulation of SREBP2 expression by HCV core protein"(ID: 15468). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper. The main corrections in the paper and the responds to the reviewer's comments are as flowing:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer. Responds to the reviewer's comments:

(1) Reviewer **02861340**:

1) Although the augmentations of mRNA for SREBP2 and HMGCR were slight (Fig. 1c), the difference in protein expressions of these was more remarkable (Fig. 1d). What is the explanation?

Response: Thank you very much for your comments and suggestions. Those comments are all valuable and very helpful for revising and improving our paper. We tried our best to improve the manuscript and made some changes in the manuscript. The responds are as flowing: Regulation of gene expression refers to the regulation of process from DNA to protein. Regulation of gene expression mainly include

the following aspects: ① the level of transcription regulation; ② mRNA processing, regulation on the level of maturity; ③ regulation of translation level. In this study, on one hand, it is found that SREBP2 promoter activities are enhanced by HCV core. On the other hand, microRNAs primarily regulate gene expression by inhibiting the translation process. And we confirm that HCV core could attenuate the inhibition of miR-185-5p on SREBP2. So the increase of SREBP2 resulted from two parts: ① the enhance of transcription level and ② the increase of translation level. That is why the difference in protein expressions of these was more remarkable (Fig. 1d) than the augmentations of mRNA (Fig. 1c).

2) In Fig. 3E, it seems to the reviewer that there is no difference of SREBP2 expression between the two lanes.

Response: We do note that in HepG2 cell line, the effect of miR-185-5p inhibitor seemed not strong as the mimic, because of that the endogenous miR-185-5p expression level is low in this cell line. However, Band intensities of SREBP2 were quantified using the Image J program, and the values from control samples were set to 1. The quantity result shown that the SREBP2 level of cells treated with miR-185-5p inhibitor was higher than that of control group (data not shown).

3) The authors cannot conclude that the increase of SREBP2 expression by HCV core is mediated by miR-185-5p from Fig. 4. It is still possible that the increase of SREBP2 by HCV core and the decrease of SREBP2 by miR-185-5p occur independently that is not related with each other.

Response: It is really true as you suggested that the increase of SREBP2 by HCV core and the decrease of SREBP2 by miR-185-5p occur independently if there was no relationship between HCV core and miR-185-5p. However, Preliminary results has revealed that miR-185-5p is tightly regulated by HCV core. So the conclusion is credible. At least, the results suggest the miR-185-5p involves the regulation of SREBP2 by HCV core, directly or indirectly.

4) Is depletion or inactivation of miR-185-5p able to increase cholesterol level?

Response: Special thanks to you for your good comments. As mentioned above, the endogenous miR-185-5p expression level is low in this cell line, so most of the research data are based on the mimics. It has been confirmed that miR-185 as a regulator of de novo cholesterol biosynthesis and low density lipoprotein uptake by Yang Yang M and Nickels JT Jr.'s group and the results were published in J Lipid Res 2014 Feb;55(2):226-38. It is really true as Reviewer suggested that we should establish the regulation-ship between miR-185-5p and cholesterol in our own system. Considering the Reviewer's suggestion, we have performed experiments to make complements.

(2) Reviewer **02995238**:

1) The author mainly confirmed the expression of SREBP2 was regulated by miR-185-5p, so the title is very necessary to be revised.

Response: Thank you very much for your comments and suggestions. Those comments are all valuable and very helpful for revising and improving our paper. We tried our best to improve the manuscript and made some changes in the manuscript. The responds are as flowing: It is really true as you suggested that we spend a lot of energy to confirm that the expression of SREBP2 was regulated by miR-185-5p. This part is the foundation for next work. In this group we focus on the pathogenesis of HCV infection including steatosis, apoptosis and autophagy. This paper focus on the intracellular cholesterol regulation by HCV core protein via SREBP2. So we consider that this title is rational.

2) HepG2 cell line was commonly used to study the pathogenesis of liver neoplasm. The author use it to study the liver cholesterol metabolism whether a credible report support or not.

Response: Special thanks to you for your good comments. It is really true as you suggested that HepG2 cell line is one kind of hepatoma cell line. So it is commonly used to study the pathogenesis of liver neoplasm. However, it is also used by many researchers to study the lipid metabolism, because that the 3-hydroxy-3-methyl glutarate coenzyme A reductase and triglyceride lipase are expressed by this cell line. For instance, it was reported that HCV core protein induces hepatic lipid accumulation by activating SREBP1 and PPAR-γ in HepG2 cells (Kim KH et al Biochem Biophys Res Commun. 2007. 355(4): 883-8). Clement S et al reported that down-regulation of phosphatase and tensin homolog by Hepatitis C virus core 3a in hepatocytes triggers the formation of large lipid droplets in HepG2 cells (Hepatology. 2011. 54(1): 38-49).

3) P value should be an exact figure.

Response: P value has been shown as an exact figure which is marked in red in revised paper.

4) Editing of language by a native English speaker is recommended.

Response: The manuscript has been provided for language certificate by professional English language editing company named Jing-Yun Ma Editorial Office.

3 References and type setting were corrected.

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

Sincerely yours,

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