Manuscript NO: 36612

RE: Hepatitis C virus core protein-induced miR-93-5p inhibits IFN signaling pathway through targeting IFNAR1

COMMENTS TO AUTHORS (Reviewer 1)

Dear Authors Thank you for submitting the manuscript entitled, "Hepatitis C virus core protein-induced miR-93-5p inhibits IFN signaling pathway through targeting IFNAR1 " for WJG. The manuscrpt is well written and the topic is interesting and timely, however, I wonder if the experiments were properly performed and the data were properly analyzed. Several ciricisms should be addressed as major commnets.

Authors: We thank the reviewer for giving us constructive suggestions. We have studied the comments carefully and revised them one by one.

Reviewer: 1) I think the Western blot method is very difficult, and I have experienced it. The authors descrbed that the exam have performed two or three times on the exams of Figure 3. ABCDE and Figure 4. CD. what are the participants on the experiments? all participants, several, some or only one case? Please represent all the actuall quantification data or films of westerm blot exams to me and editors for confirmation. After our confirmation, the authors should add tha short commnets, "(data not shown)" in the descriptions.

Authors: We are sorry that we cannot understand the meaning of the sentence exactly: “what are the participants on the experiments?”. For western blotting assays, we used GAPDH as the reference. For qRT-PCR experiments, β-actin and U6 were used as the references. We used image-pro plus software to analyze the quantification of the western blotting data. The films of the western blotting were presented in a PPT (submit as supporting data). We hope can meet with the reviewer’s approval.

Reviewer: 2) Figure legends: No description of methodology, qRT-PCT, Western blot, or other procedures. Please cralify it on all the figures. No description of these in the section of "Result".

Authors: We have added the description of methodology of qRT-PCR and Western

blotting, lines 12-15, page 5, and lines 15-28, page 6. We have checked the description of all the figures and confirmed that they were described.

Reviewer: 3) Please add the schamtic fugure on the relations among HCV-!b core protein, miR-93-5p, IFNAR1, STAT1, pcDNA31, adn so on, refering the manuscript No.8-13.

Authors: We have added the schematic figure as Figure 6. We hope can meet with the reviewer's approval.

Reviewer: 4) What are the participants and samples in Figure 1,2,3,4,5? All participants, several, some or only one, healthy or illness ? Please cralify it and do proper descriptions.

Authors: In Figure 1 and 2. 168 serum samples from 84 patients and 84 healthy subjects were examined. In Figure 3, 4 and 5, Hu7 cell line was used to perform the experiments rather than human serum samples.

COMMENTS TO AUTHORS (Reviewer 2)

Authors: We thank the reviewer for giving us constructive suggestion, and we have revised the manuscript according to the suggestion one by one.

Reviewer: 1. Introduction, lines 16-18; "Several studies indicated that the expression of multiple miRNAs was regulated by IFN in inhibiting HCV replication[9].", please add more references. Also, explaine which "miRNAs"?

Authors: We have added references and exemplified associated miRNAs, lines 21-22, page 3.

Reviewer: 2. In Cell culture, "DMEM/HIGH GLUCOSE (Hyclone)" should be written as "DMEM high glucose (Hyclone, USA)". "10% FBS (Gibco)" should be "10%".Fetal Bovine Serum (FBS)".

Authors: We have revised them, lines 22-23, page 4.

3. In RNA extraction; “TRIzol reagent (Invitrogen)” should be “TRI reagent (Invitrogen, Carlsbad, CA, USA)”.

Authors: We have revised it, lines 25-26, page 4.

4. In RNA extraction; “C.elegans miR-39 (GenePharma)” should be “GenePharma (Shanghai, China)”. Other instruments and substances should be followed by the company name and also the country in paranthesis. I gave some examples, in 2 and 3, please carefully revişe your manuscript not to miss any reagent, instruments etc.

Authors: We have revised them.

Reviewer: 5. In Quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR); “Two microliter” should be “Two microliters”.

Authors: We have corrected it, line 10, page 5.

Reviewer: 6. In Statistical analysis “Graphpad Software Inc, Caligornia” should be revised as “Graphpad Software Inc, California”.

Authors: We have corrected it, line 7, page 7.

Reviewer: 7. In miR-93-5p concentration in serum of patients with HCV-1b infection is involved in pegylated IFNα resistance; “The results showed an AUC value of 0.8846 for serum miR-93-5p in distinguishing HCV-1b-infected patients from healthy subjects, with sensitivity of 76.19% and specificity of 100%, cut-off value of 0.009774 amol/μl (Figure 2A), an AUC value of 0.8562 in distinguishing HCV-1b-infected patients with pegylated IFNα sensitivity from healthy subjects, with sensitivity of 70% and specificity of 100%, cut-off value of 0.009774 amol/μl (Figure 2B), an AUC value of 0.9265 in distinguishing HCV-1b-infected patients with pegylated IFNα resistance from healthy subjects, with sensitivity of 85.29% and specificity of 100%, cut-off value of 0.01087 amol/μl (Figure 2C), an AUC value of 0.8359 in distinguishing HCV-1b-infected patients with pegylated IFNα resistance from those with pegylated IFNα sensitivity, with sensitivity of 76.47% and specificity of 100%, cut-off value of 0.03030 amol/μl (Figure 2D).”. Please exclude these results

from the text and draw a plain table indicating these values, you should also keep the Fig 2,. This is an optional suggestion, if you desire, you can keep the same format but it is very difficuklt to understand.

Authors: According to the reviewer's suggestion, we have added a now table, Table 3, lines 1-3, page 20. We have also redescribed the associated results, lines 3-12, page 8. We hope can meet with the reviewer's approval.

Reviewer: 8. In Discussion "miR-93-5p has been shown to target several mRNAs in HCC cells, such as PTEN, CDKN1A, and also to regulate the c-Met/PI3K/Akt pathway[14],", When I evaluated reference 14, I noticed they used miR-93 not miR-93-5p, please explaine or revise it as "miR-93". Please add the following sentence from the reference 14 as "They indicated the mechanisms through which miR-93 inhibits PTEN and CDKN1A, thereby activating proliferation through the c-Met/PI3K/Akt pathway and inhibiting apoptosis in HCC.", this information is needed.

Authors: miR-93 is also termed miR-93-5p, and miR-93* is termed miR-93-3p (http://www.mirbase.org/cgi-bin/mirna_entry.pl?acc=MI0000095). According to the reviewer's suggestion, we have revised the description, lines 26-28, page 10.

Reviewer: 9. In Discussion, use miR-93-5p/IFNAR1 axis promotes gastric cancer metastasis through activating the STAT3 signaling pathway as "Using an mRNA microarray, Ma et al., (reference) found that miR-93-5p significantly downregulated IFNAR1 expression in GC cells by promoting gastric cancer metastasis , which was further identified as a direct target of miR-93-5p.", reference DOI: 10.1016/j.canlet.2017.08.017 Cancer Lett. 2017 Nov 1;408:23-32. doi: 10.1016/j.canlet.2017.08.017. Epub 2017 Aug 24. miR-93-5p/IFNAR1 axis promotes gastric cancer metastasis through activating the STAT3 signaling pathway. The reference indicates other cancer but with the same mechanisms due to the downregulation of IFNAR1 expression.

Authors: When we submitted this manuscript, we had not found this literature. Its results support our finding. Now, we have cited this literature as supporting data, lines

1-2, page 11.

Reviewer: 10. In Discussion, page 2, line 2-3;" by synthesizing five databases five databases" What do you mean, by investigating or evaluating etc.. but "by synthesizing" does not seem an adequate word.
Authors: We have replaced this word with "integrated", line 28, page 10.

Reviewer: 11. In Discussion, line 3, "we found IFNAR1, a receptor of IFNα,". But as I know, the protein encoded by IFNAR1 is a type I membrane protein that forms one of the two chains of a receptor for interferons alpha and beta. Please revise it.
Authors: We have added "and IFNβ" in this sentence. We hope can meet with the reviewer's approval, line 10, page 29.

Reviewer: 12. In Discussion, line 5, " but we believed" is not scientific, please use " but we suggested".
Authors: We have deleted this sentence, and we hope can meet with the reviewer's approval.

Reviewer: 13. In Discussion, lines 19-23, "In addition, hepatitis delta virus (HDV) can also impair the phosphorylation level of STAT1 and STAT2 through blocking the IFN-α-stimulated tyrosine phosphorylation of IFN receptor-associated JAK kinase Tyk2, without affecting the expression of type 1 IFN receptor subunits[23]. Although HDV infection also regulates IFN signaling pathway, the molecular mechanism may be different from HCV infection.". Why did you write down this part. There is no relation with your study. Dou you have any case with HDV. This is another possible pathogenesis mechanism related to HDV. I suggest to excludr this part from the text.
Authors: According to the reviewer's suggestion, we have excluded this part.

Reviewer: 14. In Figure 1, miR-93-5p should be written inside the figures, miR-93-5p in the legend is not informative, please write down miR-93-5p inside the Figures.

Authors: According to the reviewer's suggestion. We have written miR-93-5p inside the Figure 1. We hope can meet with the reviewer's approval.