Dear the Editor-in-Chief of WJG,

We will re-submit our manuscript titled "A single amino acid mutant of SR-BI decreases the infectivity of HCVcc in cell culture model" to WJG.

Par 1

Response to editors:

The following files have been submitted online.

1 34325-Revised manuscript
2 34325-Answering reviewers
3 34325-Copyright assignment
4 34325-Scientific research process
5 34325-Audio core tip
6 34325-Institutional review board statement
7 34325-Institutional animal care and use committee statement
8 34325-Animal care and use statement
9 34325-Biostatistics statement
10 34325-Conflict-of-interest statement
11 34325-Data sharing statement
12 34325-Google Scholar
13 34325-Grant application form(s)
14 34325-Language certificate

1. Please provide the grant application form(s). If you can't provide it, please delete this part.

Response:Certification of two grants have been attached in the "34325-Grant application form(s)".

2.Please provide your email address from your Organization real name mailbox from Second Military Medical University.

Response: The mail box with my real name from Second Military Medical University has been added to the revision text.

3.ADD
Institutional review board statement:
Conflict-of-interest statement:
Data sharing statement:
Institutional animal care and use committee statement:
Animal care and use statement
Biostatistics statement

Response: The above requirements have been added in the revision text.

4. Add Core tip:

Response: The Core tip has been added in the revision text.

5. Please write the COMMENTS section at here.
Response: The COMMENTS section has been added in the revision text.

6. Please add PubMed citation numbers and DOI citation to the reference list and list all authors. Please revise throughout. The author should provide the first page of the paper without PMID and DOI.
Response: The above requirement have been revised in the revision text.

Par 2
Response to reviewers:
The manuscript has been revised carefully in response to the comments of the two reviewers.
In the revised manuscript, changes have been made according to the reviewers' comments. Here we address the reviewers' comments below point to point.

Reviewer #1

In the manuscript entitled "A single amino acid mutant of SR-BI decreases the infectivity of HCVcc in cell culture model" by Gao et al., the authors did site directed mutagenesis in SR-BI and studied the decrease in infectivity of HCVcc in Huh7 cell lines. The paper is well written and well presented. I will suggest you to add couple of lines about the situation of Hepatitis C in China and what efforts government is taking to control hepatitis. The single mutant S112F did big change in SR-BI activity, please explain the location of S112 amino acid in the SR-BI protein e.g. In which domain/region of SR-BI S112 amino acid is present, what is the function of that region. What is expected to happen in protein if an amino acid from Polar uncharged group is changed to aromatic amino acid. Additional comments (not mandatory but to be added if easily manageable). If you add a figure of SR-BI protein by using bioinformatics tools and highlights the amino acid 112 with both wild and mutant amino acid. The paper is accepted for publication after minor revision.

1. The situation of Hepatitis C in China and what efforts government is taking to control hepatitis.

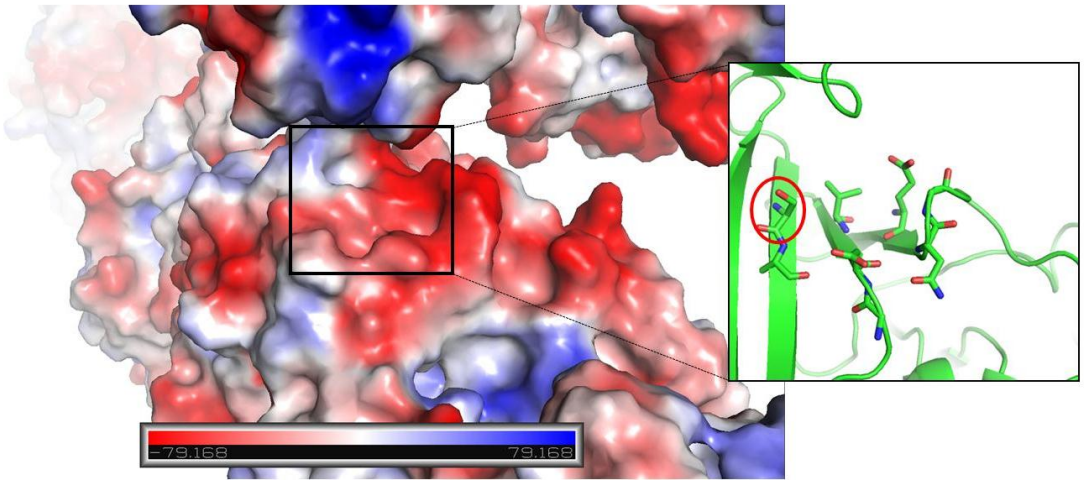
Response: The prevalence of chronic HCV infection in China was 3.2% in 1992 and 0.4% in 2006. Recent reports from the Chinese Ministry of Health have identified 70861 cases in 2006 and 201622 cases in 2012[3]. The most recent investigation showed a prevalence of HCV infection of 3.0% in northeastern China[4]. In recent years, the Chinese government has increased its investment in the prevention and control of viral hepatitis. However,

2.The single mutant S112F did big change in SR-BI activity, please explain the location of S112 amino acid in the SR-BI protein e.g. In which domain/region of SR-BI S112 amino acid is present, what is the function of that region.
Response: Please see the following explain which has been added to the

discussion section.

" We performed a literature search to determine why the S112F mutant significantly decreased the expression of SR-BI and found that Ser 112 is located in the extracellular domain of SR-BI. SR-BI and LIMP II belong to same family and share 34% sequence identity and 56% sequence homology. The X-ray crystal structure of the extracellular domain of human LIMP II has been solved. Therefore, we used the LIMP II structure as a guide to generate a homology model of human SR-BI. Ser 112 in SR-BI is located in a hydrophilic pocket, which is conserved in SR-BI. If the serine (hydrophilic amino acid) is mutated to phenylalanine (hydrophobic amino acid), then this hydrophilic pocket will be destroyed. Thus, the protein will not fold correctly, which might be responsible for the down regulation of SR-BI expression in cells expressing the SR-BI S112F mutant."

The following picture showed the SR-BI Ser112 formed a hydrophilic pocket, which is important to the protein folding if it is mutated.



Reviewer #2:

In this paper by Gao and cols., the authors describe the effect on infectivity exerted by one single amino acid mutation in the SR-BI gene. The work aims to identify host factors contributing to the HCV infectivity.

Major comments:

-The authors should describe in much more detail their method section. Particularly, the generation of the SR-BI mutant cell line.

Response: We described more method details in the text. And, the SR-BI mutant cell line is transient.

Briefly, the SR-BI shRNA was designed, cloned into the pGP-Lenti3 vector (Biovector, Science Lab, Beijing), and the positive recombinant Lv-SR-BI-shRNA vector was verified. This vector and helper plasmids were co-transfected into HEK 293T cells. The recombinant lenti-SR-BI-shRNA virus was used to infect Huh7 hepatocellular carcinoma cells. Puromycin was added for screening, and real-time PCR and Western blottings were conducted to detect the levels of the SR-BI mRNA and protein, respectively; finally, the Huh7-siSR-BI cell line was obtained[29].

- Is this a transient or stable transfection?

Response: The transfection of SR-BI/S112F into SR-BI knock down Huh7-siSR-BI cell line is transient transfection. The reason is the expression of SR-BI is very high in Huh7 cell line, it took us quite long time to screen the Huh7-siSR-BI cell line, even though, the expression of SR-BI could not be completely deleted. So, we tried to do SR-BI mutant transient transfection based on SR-BI knock down Huh7-siSR-BI cell line which already published before.

- Is the plasmid integrating into the genomic DNA? How many copies of the mutated gene were identified in the cell line? How the expression of the native gene (unmutated), is suppresed after transfection with the mutated construction?

Response: We did not check the plasmid integration into the genomic DNA and the mutant copies in the cell line. The reason is that it is very difficult to knock out the SR-BI. SR-BI/S112F showed same molecular with the wild type, and it is hard to tell the how much the native gene suppressed and how much the mutant gene expressed, and we used the vector control to help telling how mutant gene affected the expression of SR-BI and the infectivity of HCVcc.

- Why the authors did not include the T175A mutant in their study?

Response: We actually included the T175A and P297S mutants in our study. However, although S112F and the other two mutants has same effects of HDL metabolism, they showed different sensitivity of HCVcc infection, which confused us. We tried to find out the reason, we repeated at least four times and got the similar results, finally we decided to present our S112F data first.

- Why the authors did not include other mutants not expected to have an effect on infectivity as control/reference?

Response: The reason is partially because that S112F and other mutant showed different results, and the main reason is that there is no reports about the SR-BI mutants on infectivity of HCVcc the time we carried out the experiment, so we decided to find what the effects of one amino acid change of S112F compared to the wild type.

- The authors should be encouraged to explain in more detail the behavior of their vector control. Both, HCV infectivity and SR-BI expression seemed to be significantly affected by the vector without the insert. How the authors explain this behavior?

Response: For the HCV infectivity assay, we used the cell line previous established of SR-BI knock-down Huh7-siSR-BI cells[29]. The vector control is pCDNA 3.1 empty vector, without the SR-BI insert, so the expression of SR-BI (similar level of SR-BI in knock-down Huh7-siSR-BI cells) is also low, and therefore the infectivity of HCVcc is low too. And for SR-BI/S112F, since the mutant decreased the expression of SR-BI, and hence the infectivity of HCVcc is low, but a little bit higher than of vector control.

Thank you and the reviewers again for the comments and suggestions, and we hope that our corrections will meet with your approval.

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