

Lian-Sheng Ma, Science Editor, Company Editor-in-Chief, Editorial Office
World Journal of Gastroenterology

June 22, 2021

Re: **Manuscript NO: 67569**

Dear Editor-in-Chief Ma,

We have attached a copy of our revised manuscript entitled "**Hepatitis B core antigen modulates exosomal miR-135a to target VAMP2 promoting proliferation and Doxorubicin chemoresistance in hepatocellular carcinoma**". We want to thank you and the reviewers for carefully reading the manuscript and the helpful comments.

We have carefully addressed the reviewer's questions and the editorial office's comments point-by-point (please refer to the following pages for the details of change).

As suggested, we have revised and supplemented the text of the article according to the comments of reviewers, and polished the language of the article again. We have adjusted the content of the supplementary material.

We believe that the revised manuscript has been substantially improved and hope it meets the standard for publication in the World Journal of Gastroenterology. We look forward to hearing from you.

Sincerely yours,

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RESPONSES TO THE REFEREE'S COMMENTS (blue texts are the original comments)

Many thanks for the valuable comments on the manuscript. We have made a substantial revision to the manuscript. Please see below for details of our responses to your comments.

Reviewer #1:

Scientific Quality: Grade A (Excellent)

Language Quality: Grade A (Priority publishing)

Conclusion: Accept (High priority)

Specific Comments to Authors: I would like to commend the authors for the excellent work. This in vitro study has shown that miR-135a-5p decreases apoptosis and increases the proliferative capacity of HCC cell lines through the VAPM signalling. It has very good insights to the HBV driven hepatocarcinogenesis.

Response: We are very grateful for your comments on the manuscript.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Major revision

Specific Comments to Authors: Review report General comments: This study was carried out with the goal at trying to elucidate the role of exosomal miR-135a in the chemoresistance of hepatocellular carcinoma. The assays covered the expression levels of miR-135a-5p and target gene VAMP2 in HCC tissue and cell culture samples, various functional tests with mimic and inhibitor of miR-135a-5p, and specific assays for detection of apoptosis, cell proliferation and drug dox effects. The manuscript provided ample experimental findings and computational prediction of miR-135a-5p target gene. All these works were quite straight forward towards the elucidation of potential role of miR-135a that could play in the chemoresistance of HBV-associated

hepatocellular carcinoma. While the study is interesting, the writing needs a great of efforts to improve the manuscript to a professional grade, both wording/grammar and clear/accurate description of the findings.

(1) Authors carried out a lot of wet-lab experiments and bioinformatic analysis, with a main goal at trying to elucidate the role of miR-135a in hepatocellular carcinoma (HCC). Among the wet-lab assays, the level of miR-135 and target gene VAMP2 were measured in both HCC cells and derived exosomes. However, authors chose to make conclusions/claims for findings only in exosomes and did not mention the role of miR-135 in whole cells. This includes the title of the manuscript and the first 2 subtitles in Results section. Exosomal miRs would be good to be utilized as diagnostic tools when they reflect the changes in cellular activities. They may also exert their biological role by affecting adjacent and distal cells. Since miR-135a was elevated in both HCC cells and exosomes, the claim “HBV up-regulates the expression levels of miR-135a-5p in exosomes” would be better worded as “HBV may up-regulate the expression levels of miR-135a-5p in HCC cells and exosomes”. The in vitro experiments revealed that miR-135a played a role in cell survival and proliferation by targeting VAMP2 and even induced chemoresistance via exosome route. It is suspected that miR-135a would play the same role in whole cell level. Thus, why had authors to make conclusion “Hepatitis B core antigen modulates exosomal miR-135a to target VAMP2...” in the title of the manuscript? Did authors exclude the possibility that those effects of miR-135a might play out at the whole cell level?

Response: Many thanks for your reminder. First of all, exosomes are small vesicular structures that are released by cells. Exosomes contain various molecular constituents of their cell of origin. The constituents of exosomes are also in origin cells. If the miR-135a is not in cells, it can not exit in exosome. This is one of the reasons that we only indicate exosomal miR-135a in our title and our conclusion. By the way, as an invited article, and the title of the manuscript can not be changed. So we do not change the title.

(2) In addition, VAMP2 was identified as the target gene of miR-135a. However, it is suspected that miR-135a would target other genes as well. Thus, the claim “VAMP2 is the target gene of miR-135a-5p in HCC cells” should be modified as “VAMP2 is a potential target gene of miR-135a-5p in HCC cells.”

Response: Many thanks for reminding us of that. As suggested, we have revised it. (page 11, paragraph 3)

(3) Inappropriate figure legends: Figure legends should mainly be used to explain things shown in the figures, such as the purpose of figure (i.e., what is this figure for?), labels, group definition, etc. Authors did not write up the necessary information. Instead, they put in a lot of interpretations for the findings, which should be placed in the Results section.

Response: Thank you for reminding us of that. We have modified the figure legend as suggested.

(4) Poor English: Ambiguous, inaccurate, and incorrect English wording and grammar were found throughout the whole manuscript, from abstract to introduction, methods, results and to discussion. Although authors provided a Certificate Service Confirmation on manuscript editing and proofreading service, these language errors indicate that no serious English editing and proofreading was performed responsibly. Specific comments: The title of the manuscript would be better worded as “Hepatitis B core antigen modulates cellular and exosomal miR-135a to target VAMP2 and induce doxorubicin chemoresistance in hepatocellular carcinoma.” The “clinical” word was used in multiple places in the manuscript. By reading through the whole contents of the study, the reviewer found that it was wrongfully worded. Anything “clinical” would be referred to as patient characteristics such as age, gender, symptoms, etc. or at most could cover some clinical lab testing results but not the tissue samples taken from patients for basic research. Thus, authors should modify it accordingly. The reviewer has taken a liberty to correct some of those as indicated in the tracking changes file.

The reviewer has taken a liberty to correct some of those as indicated in the tracking changes file.

Response: Many thanks for your reminder. As suggested, after we finished the content modification, we re-polished the manuscript.

(5) The “Eighteen paired HCC tissues” description was not entirely clear. Since “paired” was used, authors should make sure what was the pair, normally it refers to as normal and cancer. See the “tracking changes” file for details.

Response: We agree with Reviewer #2’s comments. As a result, we have revised manuscript content to “Eighteen paired HCC and adjacent tissues”. (page 6, paragraph 3).

(6) The “Hepatitis B virus ... and human VAMP2 ... were amplified separately according to sequences of these genes” needs a clarification. The “amplified” means a template should have been used. Otherwise, the “synthesized” may be the correct word here. See details in the “tracking changes”

Response: Thank you for reminding us of that. As your suggestion, we have added a description of the template used (page 7, paragraph 2). The method used here is PCR amplification. In biological research, synthesis usually refers to artificial synthesis, and amplification is better in PCR experiments.

(7) The “Total RNA was extracted using TRIzol reagent” was not clear for readers to understand what samples of origin was used in this process. Please show what samples were used in this process. See details in the “tracking changes” file.

Response: We agree with Reviewer #2’s comments. As a result, we have added sample types to the manuscript (page 8, paragraph 4).

(8) The “R software for bioinformatics analysis.” was not clear for readers to know/replicate how analysis was performed in R environment. Please list which R packages were used. See details in the “tracking changes” file.

Response: Many thanks for your reminder. As suggested, we have listed the R packages used in our study (page 10, paragraph 3).

(9) Authors have put some results in supplementary figures. However, those results had been used by authors to make major claims. Thus, these supporting findings should be presented in main figures. Particularly the supplementary figures 1-3 should be merged into Figure 1-2. See details in the “tracking changes” file.

Response: Thanks. As you suggested, we have revised the figure. As suggestion, we have added supplementary figures 1-2 to Figure 1, and supplementary figures 3 to Figure 2. The detailed changes are shown in revised Figure 1 and Figure 2.

(10) Throughout the whole manuscript, there are words, especially verbs that were used inaccurately, such as illustrate (better to use elucidate), manifest (better to use show), certify (better to use verify), signified (better to use suggested), disclose (better to use demonstrate), etc. See details in the “tracking changes” file.

Response: Many thanks for your reminder. As suggested, we went through the manuscript carefully, and we have revised the manuscript with the more appropriate verb (page 3, paragraph 2; page 3, paragraph 3; page 6, paragraph 2; page 11, paragraph 1; page 13, paragraph 1; page 14, paragraph 3).

(11) A lot of more English polishes were made by the reviewer. It is too many to list here. See details in the “tracking changes” file.

Response: Thank you for reminding us of that. For the language problem, we have revised the manuscript according to the “tracking changes” file and re-polished.

EDITORIAL OFFICE'S COMMENTS

Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

1 Scientific quality: The manuscript describes a Basic Study of the Chemoresistance role of Hepatitis B core antigen in hepatocellular carcinoma. The topic is within the scope of the WJG. (1) Classification: Grade A and Grade C; (2) Summary of the Peer-Review Report: The manuscript has high priority for publication. The writing needs a great of efforts to improve the manuscript to a professional grade, both wording/grammar and clear/accurate description of the findings. The questions raised by the reviewers should be answered;

Response: Many thanks for your reminder. We have revised the manuscript according to the reviewers and given a point-by-point answer to all questions. We hope that the revised paper meets the standard for publication.

(3) Format: There are 3 tables and 8 figures; (4) References: A total of 67 references are cited, including 20 references published in the last 3 years; (5) Self-cited references: There are 2 self-cited references.

Response: Thank you very much for your help.

2 Language evaluation: Classification: Grade A and Grade C. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the Institutional Review Board Approval Form. No academic misconduct was found in the Bing search. 4 Supplementary comments: This is an invited manuscript. The study was supported by National Natural Science Foundation of China. The topic has not previously been published in the WJG. 5 Issues raised: (1) The language classification is Grade C. Please visit the following website for the professional English language editing companies we recommend: <https://www.wjgnet.com/bpg/gerinfo/240>;

Response: Thank you for your suggestion. We have re-polished the manuscript.

(2) The authors did not provide the approved grant application form(s). Please upload

the approved grant application form(s) or funding agency copy of any approval document(s);

Response: We have uploaded the approved grant application forms attached to the revised manuscript.

(3) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor;

Response: We have provided original pictures using PowerPoint to ensure all graphs or arrows or text portions can be reprocessed by the editor.

(4) The “Article Highlights” section is missing. Please add the “Article Highlights” section at the end of the main text;

Response: We have added the “Article Highlights” section at the end of the main text.

(5) Please upload the primary version (PDF) of the Institutional Review Board’s official approval, prepared in the official language of the authors’ country to the system; for example, authors from China should upload the Chinese version of the document, authors from Italy should upload the Italian version of the document, authors from Germany should upload the Deutsch version of the document, and authors from the United States and the United Kingdom should upload the English version of the document, etc. Example: Download our sample of institutional review board approval, at <https://www.wjgnet.com/bpg/GerInfo/287>. 6 Recommendation: Conditional acceptance.

Response: We have uploaded the primary version (PDF) of the Institutional Review Board’s official approval attached to the revised manuscript.

Company editor-in-chief: I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript

is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Before its final acceptance, please upload the primary version (PDF) of the Institutional Review Board's official approval in official language of the authors' country to the system; for example, authors from China should upload the Chinese version of the document, authors from Italy should upload the Italian version of the document, authors from Germany should upload the Deutsch version of the document, and authors from the United States and the United Kingdom should upload the English version of the document, etc.

Response: Thank you very much for your help. We have uploaded the primary version (PDF) of the Institutional Review Board's official approval attached to the revised manuscript.