Jing Yu
Scientific editor
World Journal of Gastroenterology

Manuscript No.: 24559
Title: miR106b promotes cancer progression in hepatitis B virus-associated hepatocellular carcinoma

Dear Dr. Yu,

We appreciate very much your effort and the reviewer's insightful suggestions and comments. We revised the manuscript to address the issues raised by the reviewers. We here submitted a modified manuscript for a further review. The amendments are highlighted in yellow in the text of revised manuscript. Hope our manuscript is now suitable for publication in *World Journal of Gastroenterology*.

Sincerely yours,

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To the Reviewers

Thank you very much for taking time to review our manuscript. We have revised the manuscript according to your comments. Please find attached the revised manuscript with all changes. Our point-by-point answers to the comments are as follows.

Reviewer 02438890

Comments: In this manuscript the authors investigated the effect of miR-106b on tumor progression in hepatitis B virus-associated hepatocellular carcinoma (HCC) in a clinical model. The authors concluded that miR-106b was up-regulated and co-transcribed with its host gene MCM7 in HBV-associated HCC. They also suggested that HBx enhance transcription of miR-106b to promote tumor progression in HBV-associated HCC. This is a well-designed study in HCC material and the author's method is acceptable to draw the conclusion. I believe that this study can be published in the WJG. To further improve the study, the correlation of miR-106b with tumor parameters such as tumor grade, size, vascular invasion, neuroinvasion etc. can be included in the analysis. This is not absulately necessary but may be advantagous.

Reply: Thank you very much for giving a positive evaluation about our manuscript. According to your suggestion, we have added a correlation analysis of miR-106b level with tumor size in Table 2 of revised manuscript (Page 38-39).

Reviewer 02530754

Comments:

1- In methods, the authors said that: "A total of 120 patients who underwent surgery for HCC… were enrolled". Was HBV infection present in the whole cohort? What is meant by surgery (resection, LT, both)? Inclusion and exclusion criteria should be clearly stated here.

Reply: We divided total 120 patients into two cohorts. 12 HCC patients with HBV infection (5 patients), HCV infection (6 patients) and non-B non-C (1 patient)

were included in the first cohort to screen the miRNA expression profile using miRNA array, the other 108 HCC patients all with HBV infection were enrolled in the second cohort for further analysis of the role of miR-106b in HBV-associated HCC. The item “surgery” represented in the text for description of patients and HCC tissue means “receive liver resection”. We have amended it and highlighted in yellow in the text of revised manuscript (Page 4 and 10) for clear description. Thank you for your questions and guidance.

2- In the statistical analysis, both parametric and not-parametric methods are included. Did the authors check for normal distribution of variables before choosing one test or another? If so, please include this information together with the methodology used in this section.

Reply: We did check for normal distribution of variables before choosing the test of statistical analysis. *W* value of Shapiro-Wilk method and *D* value of Kolomogorove method were used in the tests for normality. The information has been included in the *Statistical analysis* section of Materials and Methods of revised manuscript (Page 12).

3- In results, it can be read “12 patients with distinct types of HCC…”. Did these patients belong to the whole cohort of 120 patients? Please provide additional information in “methods” about how these patients were selected, and further data about their clinical features in “results” as compared with the remaining cohort.

Reply: As the reply to comment 1, 12 patients with distinct types of HCC were included in the first cohort and the other 108 HBV-associated HCC patients were enrolled in the second cohort. Total 120 patients that were divided into 2 cohorts were enrolled in this study. We have added clear information about the description of patients in Materials and Methods (Page 10) and provided further data about the clinical features of patients in Table 1 (Page 37) of revised manuscript.

4- It seems to me that the initial 12 patients formed the "training cohort" and the remaining 108 patients formed the "validation cohort" but it is not clear in the text. Please provide a detailed description of both cohorts to ensure that they are comparable.

Reply: Thank you for your comment. We have amended the description in the text regarding patients in both cohorts (Page 10).

5- The expression of miR-106b was evaluated as a predictor of poor outcomes. For that purpose the patients were divided into two groups (high expression and low expression of miR-106b). The definition of "high expression" should be provided. In addition the methodology to define the threshold to consider high or low expression should be included in the paper.

Reply: The mean of miR-106b level in adjacent non-tumor tissue was defined as the threshold (0.4 arbitrary unit from q-RT-PCR analysis). The miR-106b expression level higher than threshold was classified into "high-expression of miR-106b" group. On the other hand, the level lower than threshold was classified into "low-expression of miR-106b" group. The non-tumor tissue is beside to the tumor tissue and the miR-106b level in non-tumor tissue is suggested to be between in tumor tissue and normal liver tissue. We believe that it is an objective point for the definition of the threshold. We have added the description in the text of Material and Methods in revised manuscript (Page 10-11).

6- It is not clear to me whether both resected and transplanted patients were included or not. Overall survival and recurrence-free survival are different in these two groups of patients, and therefore they cannot be analyzed altogether. I would recommend reproducing survival analyses in patients who underwent liver resection, and those who received a liver transplantation separately.

Reply: All 120 HCC patients were operated for liver resection. No liver transplanted patients were included in this study. We have described more detail

about patients in the text of revised manuscript (Page 10).

7- Vascular invasion means microvascular invasion? Correct if needed.

Reply: The item “vascular invasion” in this original manuscript means “microvascular invasion”. We have replaced ““vascular invasion” to “microvascular invasion” both in the text and Table (Page 17 and 38). Thank you for the correction.

8 - Table 1: provide % values, and not only number of patients. Where do the p values come from? There are no groups of comparison in this table. Diameter of the largest nodule should be included.

Reply: We have added the % values in Table 2 (Table 1 in original submitted manuscript) of revised manuscript. Some description regarding statistical analysis has been corrected in Material and Methods (Page 12-13) and Table 2 (Page 38-39) of revised manuscript. In Table 2 of revised manuscript, all *P* value are from Mann-Whitney *U* test of the comparison of miR-106b expression levels between two different groups. Diameter of the largest nodule has been included in Table 2 of revised manuscript (Page 39).

9- Follow-up protocol after surgery should be included, together with the median follow-up length in the whole cohort.

Reply: We have added this information to Material and Methods of revised manuscript (Page 10).

10- Minor English polishing is required.

Reply: Our revised manuscript has been edited for English language usage, grammar, spelling and punctuation by native English-speaking editors at Nature Publishing Group Language Editing (NPG Language Editing). The certificate letter is as file attachment. Thank you for your comment.