Professor Ya-Juan Ma
Science Editor,
World Journal of Gastroenterology

Re: Manuscript NO 27286
Title: Pretreatment AKR1B10 expression predicts the risk of HCC development after HCV eradication

Dear Editors:
We are most grateful to you and the reviewers for the valuable comments on the original version of our manuscript. We have considered all the comments and revise the paper accordingly; the revised manuscript is submitted herewith.

We have addressed all the comments by reviewers, as indicated in our responses presented with this letter, and we hope that our explanations and revisions are satisfactory. We hope that the revised version of our paper is now suitable for publication in *World Journal of Gastroenterology*. We look forward to hearing from you at your earliest convenience.

Thanking you in advance for your attention to this matter.

Yours sincerely,

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Reply to reviewer #58381:

Comment 1:

Page 12, first paragraph: “153 patients (50.5%) presented scarce AKR1B10 expression (0%).” - What is meant by “0%”?

Reply to comment 1:

As pointed out by the reviewer, this sentence was confusing. We have revised the sentence as follows: “153 patients (50.5%) presented scarce AKR1B10 expression, whose AKR1B10 positive staining area was estimated at 0%.”

Comment 2:

Page 11, third paragraph: “Figure 2 shows representative of immunohistochemical staining for AKR1B10 in liver tissues.” - This sentence should be improved.

Reply to comment 2:

We have revised the sentence as follows:

“Figure 2 shows representative immunohistochemical staining of AKR1B10 in liver tissues.”

Reply to reviewer #8874:

Major point 1:

In this study, the cut off value of AKR1B10 expression was 8%. However, similar study from this group (J Gastroenterol Hepatol. 2016 Jan 13. doi: 10.1111/jgh.13295. [Epub ahead of print]) took 6% as the cut off value of AKR1B10 expression. Authors should discuss how decided the cut off value of AKR1B10 expression.

Reply to major point 1:

Thank you for your valuable comment. As pointed out by the reviewer, the ROC analysis-determined AKR1B10 cut-off value in the present study (8%) was higher than that in the previous report (6%). A fundamental difference between these studies was non-SVR patients included in the study cohort: 42% non-SVR patient in the previous study, whereas 0% in the present study. The difference of AKR1B10 cut-off value might indicate the impact of SVR achievement on subsequent change of AKR1B10 expression. We have added the discussion about this point in page 15 paragraph 2.

Major point 2:

Page 5, Core Tips. Authors concluded that “Thus, AKR1B10 is not only a novel biomarker for assessing the risk of HCC after SVR: it might also be involved in the very early stages of hepatocarcinogenesis.” The results of this study leaded the conclusion “AKR1B10 is not only a novel biomarker for assessing the risk of HCC after SVR.” It is true. However, the sentence “it might also be involved in the very early stages of hepatocarcinogenesis” is speculation. This

sentence should be removed.

Reply to major point 2:

We agree the reviewer's suggestion, and have revised "Core Tips" as follows:

A cancer-related oxidoreductase, aldo-keto reductase family 1 member B10 (AKR1B10) expression in the liver was upregulated in patients with chronic hepatitis C (CHC). High AKR1B10 expression was associated in a statistically significant manner with the risk of hepatocellular carcinoma (HCC) development even after sustained virological response (SVR) was achieved through interferon-based antiviral therapy. Pretreatment AKR1B10 expression of 8% was associated with a >15-fold-increased risk of HCC development. Thus, AKR1B10 is not only a cancer biomarker but also a novel predictive marker for assessing the risk of HCC development in CHC patients who achieved SVR.

Major point 3:

This study analyzed the association between the pretreatment AKR1B10 expression and HCC development after SVR in patients with chronic hepatitis C. How about the association between the post-treatment AKR1B10 expression after SVR and HCC development? I think it is difficult to take liver biopsy samples after treatment. If authors have data, please discuss the AKR1B10 expression before and after IFN treatment.

Reply to major point 3:

This comment is well taken. It is a major issue whether or not HCV eradication per se affect AKR1B10 expression in the liver. However, as you mentioned, it is difficult to take liver biopsy samples after SVR, and unfortunately, we have no data regarding AKR1B10 expression after SVR. In the revised discussion, we have commented about this point (Page 15, paragraph 2).

Reply to reviewer #13203:

In the present study the authors tried to investigate the association between aldo-keto reductase family 1 member B10 (AKR1B10) expression and hepatocarcinogenesis after hepatitis C virus eradication. Although the results of this study look interesting. The sample size and the incomplete evaluation are the major problems. Also there is a previous publication of this group of researches with similar information. Sato S, Genda T, Ichida T, Murata A, Tsuzura H, Narita Y, Kanemitsu 1, Ishikawa S, Kikuchi T1, Mori M, Hirano K, Iijima K1, Wada 4, Nagahara A, Watanabe S. Impact of aldo-keto reductase family 1 member B10 on the risk of hepatitis C virus-related hepatocellular carcinoma. J Gastroenterol Hepatol. 2016 Jan 13. doi: 10.1111/jgh.13295.

Comment 1:

It is important to clarify what information is new in this study compared to the previous one (J Gastroenterol Hepatol. 2016 Jan 13. doi: 10.1111/jgh.13295)

Reply to comment 1:

We appreciate this proposition. In the present study, we aimed to clarify the association between pretreatment AKR1B10 expression and HCC development after SVR. Therefore, the present study included only SVR patients, and exclude a patients with HCC development within 1 year after EOT, because HCC developed within 1year might exist before treatment. We have emphasized exclusion of pre SVR-existing HCC in the MATERIAL AND METHODS section (page 7, paragraph 1), and discussed the difference of the results (Page 15, paragraph 2).

Comment 2:

The sample size of 8 patients is too small and the results with this methodology is difficult to evaluate.

Reply to comment 2:

As the reviewer mentioned, the number of patients developed HCC was small in the present study, because the incidence of HCC development is generally very low under 5% at 5 years. Although the significance of high AKR1B10 expression for the risk of HCC development could be demonstrated in both multivariate Cox analysis and log-rank test, this point was one of the limitations of the present study. In accord with the comment, we have added the discussion about the limitation of the present study as follows (Page 17, paragraph 2):

The main limitations of this study were its monocentric aspect and retrospective nature. Cases of HCC development was very small because the incidence of HCC development after SVR was generally low. A future multicenter prospective analysis will be required to validate the association between AKR1B10 expression and risk of HCC development in patients with chronic hepatitis C who achieve HCV eradication.

Comment 3:

Did you have a chance to use RT-PCR to evaluate de expression of AKR1B10?

Reply to comment 3:

This concern is valid. It had been verified in the previous reports if the quantification of AKR1B10 immunoreactivity was well correlated with its mRNA expression levels (ref 14 and 15 in the revised manuscript). We have added this point in the MATERIALS AND METHOD section (Page 10, paragraph 1).

Comment 4:

Those patients who developed HCC had advanced fibrosis that is in part of the reason why they had more altered their liver function tests. What do you think about that?

Reply to comment 4:

In general, liver function tests alter in accordance with hepatic fibrosis progression. AKR1B10 expression is also associated with stage of hepatic fibrosis, however, factors other than fibrosis are also associated with AKR1B10 expression in the liver (Sato et al. J Gastroenterol Hepatol 2016 PMID2678591). In the present study, the multivariate analysis did not identify histological stage of hepatic fibrosis as an independent risk factor for HCC development. In addition, it was also demonstrated that AKR1B10 expression could stratify the risk of HCC development both in patients with and without advanced hepatic fibrosis (Sato et al. J Gastroenterol Hepatol 2016 PMID2678591). Taken together, hepatic fibrosis might not be an only risk for HCC development.

Comment 5:

The advanced liver fibrosis, genotype 1 in my opinion are the most important factors in the development of HCC. Could you please discuss it in the discussion section?

Reply to comment 5:

According to the reviewer's comment. We have added the discussion about the risk factors of HCC development as follows (Page 14, paragraph 2):

To date, several factors have been reported to predict the risk of HCC development in patients with chronic hepatitis C, such as older age, male gender, alcohol intake, and hepatic fibrosis. Before DAA became available, Genotype 1 infection was refractory to treatment, and might be correlated with risk of HCC. Pretreatment existence of advanced hepatic fibrosis are recognized as a significant risk for HCC development after achieving SVR, however, not all patients with advanced hepatic fibrosis developed HCC.

Comment 6:

I suggest to create one table with the main characteristics of the 8 patients with HCC.

Reply to comment 6:

According to the reviewer's suggestion, we have added new Table which showed the characteristics of the 8 patients with HCC (Table 3).

Comment 7:

The discussion is very poor. I suggest to rewrite it. Focus on the main results

Reply to comment 7:

According to the reviewer's suggestion, we had revised the Discussion section in our manuscript to emphasize a main result with comparing to the previous report (Page 15, paragraph 1, 2).