April 28, 2022

Lian-Sheng Ma, President and Editor-in-Chief
World Journal of Gastroenterology
Baishideng Publishing Group
Email: l.s.ma@wjgnet.com

Re: Revision of Manuscript (ID: 75896)

Dear President Ma:

We would like to thank you very much for your letter dated April 16, 2022, and the insightful review of our manuscript, entitled "**Effects of microwave ablation on serum Golgi protein 73 in patients with primary liver cancer**." Your valuable comments and suggestions, as well as those from the reviewers, have helped us to improve the manuscript.

We have carefully read the letter and considered the comments. In light of the good suggestions, we have revised the manuscript. All questions and comments have been answered. We are resubmitting a thoroughly revised manuscript, one clean copy and one with track changes in red, as well as the point-by-point responses to the reviewers.

This revised manuscript has been edited and proofread by *Medjaden* Bioscience Limited.

We believe that the revised manuscript has been further improved. We hope that you find the revision and replies to be satisfactory and that the revised manuscript is now suitable for publication in the *World Journal of Gastroenterology.*

As always, we sincerely appreciate your interest in our manuscript. Should there be any remaining concerns, please do not hesitate to let us know.

Very truly yours,

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Point-by-Point Responses

Reviewer 1

Specific Comments to Authors:

Nice and useful study. Do the authors have any experience with gp73 during RFA and other ablational modalities?

Response: We sincerely appreciate your recognition of our work and your thoughtful comments. We also examined the levels of GP73 during RFA of primary liver cancer and observed changes similar to those with microwave ablation. As this study focused on the microwave ablation of primary liver cancer, the data with RFA were not presented in this manuscript.

Reviewer 3

Specific Comments to Authors: *The authors studied the dynamic changes of serum GP 73 in 150 primary liver cancer patients treated with MWA. Serum GP73 is markedly increased in response to MWA of liver cancer. Thus, serum GP73 holds potential as a marker to monitor MWA-induced inflammatory liver injury in need of amelioration.*

1. The authors used "primary liver cancer". This terminology includes mainly both HCC and iCCA. Were these 150 cases all HCC or some were HCC? Please clarify and if they were all HCC, please use HCC instead of primary liver cancer. If some were iCCA, I wonder how the authors put together the AFP levels for comparison.

Response: We are grateful for your insightful comments and valuable suggestions, which have been very helpful for us to improve the manuscript.

We fully agree that the terminology “primary liver cancer” includes mainly both HCC and intrahepatic cholangiocarcinoma (iCCA). In this study, the 150 cases included HCC and some iCCA. Among the 150 patients with primary liver cancer, 102 patients had abnormally high levels of AFP. In these 102 patients with primary liver cancer with elevated AFP, we compared the changes of serum AFP before and after MWA treatment. (Line 26, Page 9, and Table 1).

2. The authors mentioned that all of the study subjects were diagnosed as having primary liver cancer, in accordance with the Guidelines for the Diagnosis and Treatment of Primary Liver Cancer in China. Any case had biopsy confirmed diagnosis? Since this is a prospective study, a biopsy diagnosis would be needed for a well-designed study. Also important is to see the status of background liver.

Response: Thank you for your comment. This is a retrospective study in which the study subjects were retrospectively enrolled between January 2016 and October 2018. All of the subjects were diagnosed as having primary liver cancer in accordance with the Guidelines for the Diagnosis and Treatment of Primary Liver Cancer in China. According to the guidelines, space-occupying lesions with typical imaging features of liver cancer usually do not require puncture biopsy of liver lesions for the purpose of diagnosis, mainly due to the possible risks of bleeding and spreading of cancer along the needle track through the liver biopsy. In addition, the study subjects had a single small lesion (≤3 cm in diameter) that could be completely ablated, and a liver biopsy is generally not required.

In regards to your comment on “Also important is to see the status of background liver,” we reviewed the retrieved data. Among the 150 patients with primary liver cancer, 115 patients had background liver cirrhosis, and 35 patients showed no clinical signs of liver cirrhosis. In this study, we also compared the serum GP73 levels between the liver cancer patients with versus without background liver cirrhosis, and the results are presented in Figure 3. These findings suggest that background liver cirrhosis was significantly associated with higher serum GP73 levels. In light of the good suggestion, we have revised the Materials and Methods as well as Results sections.

3. It is unclear so far which cell type produces GP73. In the introduction, the authors mentioned the bile duct epithelial cell is the main cell type to produce GP73, and hepatocyte is very low. The authors' have published before that higher serum GP73 levels are positively correlated with HBV-associated chronic liver diseases. I wonder if the authors have any direct evidence which cell type produces GP73 in HCC patients/HBV patients, bile duct? Hepatocyte? If GP73 can reflect the liver inflammatory status, why there is an increase in GP73 after MWA? Are these GP73 were produced by background liver bile duct epithelial cells or other cells? Any inflammatory cell can produce GP73. That is also very important to have a prior biopsy of the tumor and background liver for IHC stain to identify the resources of GP73, especially after MWA. It might be inflammatory cells are the resources of GP73, since MWA can induce local inflammation. The authors need to clarify this. Otherwise the mechanism of increasing GP73 post MWA is still unclear and it is hard to use it as a marker as the authors suggested.

Response: We thank the reviewer for the insightful comments and excellent questions. Under normal conditions in the liver, GP73 is mainly expressed in the epithelial cells of the bile duct, while its expression in hepatocytes is considerably lower. However, in a variety of acute and chronic liver diseases, hepatic GP73 expression has been reported to be upregulated. Our previous study demonstrated that GP73 was expressed in the cytoplasm of hepatocytes, but not in the infiltrating inflammatory cells in patients with chronic HBV infection, and that changes in the hepatic and serum levels of GP73 were positively correlated with hepatic necroinflammatory activity in CHB patients (Xu *et al.*, 2015). Few hepatocytes expressed GP73, and the serum GP73 levels were low in patients with chronic HBV infection but without indications of liver injury. However, once hepatic necrosis was triggered, the affected hepatocytes started to release more GP73 into the blood, resulting in elevated hepatic and serum levels of GP73. Our previous study also found that elevated serum GP73 levels were positively associated with a higher hepatic necroinflammatory activity grade (Xu *et al.*, 2018). The current study demonstrated that the serum GP73 levels were markedly elevated in response to MWA treatment for primary liver cancer. Although further in-depth studies are needed to gain insight into the exact mechanisms of increasing GP73 post MWA, we proposed that the MWA-induced liver aseptic inflammatory injury may contribute to the elevated hepatic and serum GP73 levels. The findings of this study provide evidence supporting the serum GP73 level as a potential marker to monitor MWA-mediated inflammatory injury in patients with primary liver cancer.

References

Xu *et al.*, Serum Golgi protein 73 (GP73) is a diagnostic and prognostic marker of chronic HBV liver disease. Medicine 2015; 94:e659

Xu *et al.*, Predictive value of serum Golgi protein 73 for prominent hepatic necroinflammation in chronic HBV infection. J Med Virol 2018; **90**:1053-1062

4. Inflammatory response may not always be a bad thing. In the situation of MWA, the inflammation might be good to clean the dead tumor cells. Have the authors correlated the initial level of GP73 post MWA with AFP levels, recurrence and survival?

Response: Thank you very much for the very thoughtful comments and interesting questions. We reviewed the retrieved patient data and did not find any available data to answer any of these questions. Due to the retrospective nature of this study, we were unable to examine the correlation between the initial level of GP73 after MWA and the AFP level, recurrence rate, or survival rate. We have added this information as one of the limitations of this study in the Discussion section. We will keep this in mind for a future prospective study.

Again, we sincerely appreciate your valuable suggestions and help. If you have any remaining concerns, we shall make changes under your guidance.

EDITORIAL OFFICE'S COMMENTS

Science editor

1. What type of primary liver cancer does the author refer to in the manuscript?

Response: Thank you for your question. In this study, the 150 cases included HCC and some iCCA. We

reviewed the retrieved data. Among the 150 patients with primary liver cancer, 143 patients had space -occupying lesions with typical imaging features of liver cancer and did not receive a liver biopsy, and the remaining 7 patients underwent a liver biopsy and had a pathological diagnosis of hepatocellular carcinoma. In response to your question, we have included the above information in the Materials and Methods section.

2. The research background of liver cancer should be summarized in the preface.

Response: We have followed your suggestion and included a brief research background of liver cancer in the Introduction section.

3. Specify which cells produce GP73

Response: Your good suggestion has been well received, and we have specified the cells that produce GP73 in the revised manuscript.

Under normal conditions in the liver, GP73 is mainly expressed in the epithelial cells of the bile duct, while its expression in hepatocytes is considerably lower. However, in a variety of acute and chronic liver diseases, hepatic GP73 expression has been reported to be upregulated. Our previous study demonstrated that GP73 was expressed in the cytoplasm of hepatocytes, but not in the infiltrating inflammatory cells in patients with chronic HBV infection, and that changes in the hepatic and serum levels of GP73 were positively correlated with hepatic necroinflammatory activity in CHB patients (Xu *et al.*, 2015). Few hepatocytes expressed GP73, and the serum GP73 levels were low in patients with chronic HBV infection but without indications of liver injury. However, once hepatic necrosis was triggered, the affected hepatocytes started to release more GP73 into the blood, resulting in elevated hepatic and serum levels of GP73. Our previous study also found that elevated serum GP73 levels were positively associated with a higher hepatic necroinflammatory activity grade (Xu *et al.*, 2018).

References

Xu *et al.*, Serum Golgi protein 73 (GP73) is a diagnostic and prognostic marker of chronic HBV liver

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As always, we are grateful for your valuable suggestions and comments. If you have any remaining concerns, we shall make amendments under your guidance.