ANSWERING REVIEWERS

January 10, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7568-edited.doc).

Title: *MT1M* and *MT1G* promoter methylation as a biomarker for hepatocellular carcinoma

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Name of Journal: *World Journal of Gastroenterology*

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The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers

(1) Reviewer No. 02444769

Major concern comes from the study design:

1) When you mix HBV(+) and HBV(-) HCCs to compare with the CHB patients, your result will be misleading.

Reply: Thank you for your constructive comments. According to your comments, we have performed the analysis for the diagnostic value of *MT1M* and *MT1G* promoter methylation in discriminating HBV(+) HCC from CHB

patients in the revised manuscript. This issue has been modified in the RESULTS part.

2) There should be exclusion criteria: I cannot find information about any therapy the HCCs received before methylation study. For example, TACE might be a confounding factor. why there is no follow up data?

Reply: Exclusion criteria for the enrolled HCC patients have been added in the revised manuscript according to your comments. Considering the research objective of our present study, we did not include the HCC patients who have a history of receiving any curative treatment such as TACE, surgical resection and radiofrequency ablation. Furthermore, we totally agree with you that the predicative value of *MT1M* and *MT1G* promoter methylation in the prognosis of HCC should be performed in our further work. We have also discussed this issue in the revised manuscript.

(2) Reviewer No. 02444790

General This was a diagnostic trial. The result was interesting and had a clinical relevancy. The experimental design was acceptable. Language should be polished. Specific Conclusion, “MT1M and MT1G promoters methylation may be serum biomarkers for detection of HCC” is OK.

1) "…an valuable tumor” should be “a…”.

Reply: This issue has been modified in the revised manuscript.

2) How to diagnose HCC? How many cases were confirmed by pathological data, and how many cases diagnosed with clinical findings?

Reply: In this present study, HCC patients were diagnosed on the clinical parameters according to the guideline of the American Association for the Study of Liver Disease (AASLD). Furthermore, all the included HCC patients have been confirmed by pathological data before or during the treatment. We have added this issue in the revised section of MATERIALS AND METHODS.

3) How to calibrate the tumor size? Ultrasound or CT? Correlation between the methylation and tumor size should be interpreted. Linear or rank correlation? Table 2 only demonstrated a difference between ≥5 and <5 cm tumors.

Reply: Thank you for your constructive comments. In our study, tumor size was evaluated by CT and reflected with the longest diameter. We have added this point in the MATERIALS AND METHODS part. Since the methylation status of *MT1M* and *MT1G* promoters was measured by a qualitative method (MSP) in our study, the correlation between it and tumor size was reflected by spearman rank correlation. We have further calculated their correlation instead of simply distinguishing the tumor size up or below 5cm in the RESULTS part according to your suggestion.

4) This was a diagnostic trial. I suggest that the 95% confidence interval be listed.

Reply: According to your comments, we have listed the 95% confidence intervals in the revised manuscript

5) There were only 31 cases of normal control. This was a limitation which may lead to a bias. This should be discussed briefly.

Reply: Yes, we totally agree with you that this point is just one of our limitations. This issue has been described in the DISCUSSION part.

6) AFP was used to detect HCC in the present regime. I suggest that the sensitive and specificity of MT1M and MT1G was compared with those of AFP.

Reply: Thank you for your comments. According to your suggestions, we have analyzed the sensitivity and specificity of *MT1M* and *MT1G* promoter methylation in identifying HCC patient from CHB patients, compared with AFP alone in table 5. Moreover, the area of receiver operating characteristic curves (AUC) was also performed to compare the diagnostic value of *MT1M/MT1G* and AFP in Figure 4. Detailed information has been presented in the revised section of Results.

(3) Reviewer No. 02444752

In the present study, MT1M and MT1G promoter methylation are reported as serum biomarkers for HCC, which might be interesting for clinical practice. Overall, the manuscript was well organized.

1) However, the treatment information of patients and the time of blood

sampling should be added in the section of "MATERIALS AND METHODS"

Reply: Thanks for your comments. Considering the research objective of our present study, we did not include the HCC patients who have a history of receiving any curative treatment such as TACE, surgical resection and radiofrequency ablation. And this is the reason why we did not present the treatment information in the manuscript. However, we totally agree with you that the predicative value of *MT1M* and *MT1G* promoter methylation in the prognosis of HCC should be performed in our further work and this issue has been included in the revised manuscript. According to your comments, the time of blood sampling should be added in the revised section of MATERIALS AND METHODS.

2) Furthermore, there are still some grammatical (e.g., "either or neither of MT1M and MT1G methylated" in page 18) and spelling (e.g., "fucused" in page 7) mistakes in the text. Again, "n(%)" in Table 2 and "N(%)" in Table 3 should be instead of "N", because there are no percentage data in these two tables.

Reply: This issue has been modified in the revised manuscript according to your comments. Furthermore, the English writing of this revised manuscript has been given proof-reading by Jing-Yun Ma Office for SCI Biomedical Editing and Publishing (Certificate verification code: 2014-01-0026) according to your suggestion. In the certificate verification, they declared that the revised manuscript has reached grade A as defined by WJG language evaluation.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*

Sincerely yours,

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