Peer Review File

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Review comments

Comment 1: Please indicate the clinical research design of this study in the title

Reply 1: This is a retrospective study. We have indicated the clinical research design of this study in title of the revised manuscript.

Changes in the text: We have modified our text as advised in the title of manuscript. (see Page 1, line 2)

Comment 2: In the background of abstract, please indicate the clinical significance of this research topic.

Reply 2: The clinical significance of our research topic was to find alternative biomarkers for early HCC detection. This has been added in the background of abstract.

Changes in the text: We have modified our text as advised in the background of abstract. (see Page 4, line 54)

Comment 3: In the part of method, please specify the inclusion criteria for the patient samples and normal controls and explain whether the two groups were matched.

Reply 3: The inclusion criteria have been specified in the method part of abstract.

Since paired adjacent noncancerous tissues were used for controls, there were no inclusion criteria for control group.

Changes in the text: We have modified our text as advised in the Method part of abstract.(see Page 4, line 56)

Comment 4: In the part of results, please also report sensitivity and specificity.

Reply 4: The mean methylation level of the miR-657 promoter region could distinguish cancerous tissues from paired normal tissues of HCC patients with a sensitivity of 95.50% and a specificity of 70.01%, by using 59.50% as the optimal cut-off.

Changes in the text: We added some data in the result part of abstract. (see Page 4, line 67)

Comment 5: English language of this paper needs further editing.

Reply 5: The paper was revised by medical writing service.

Changes in the text: Editorial certificate was uploaded in attachment.

Comment 6: In the introduction part, the authors emphasize “early diagnosis” but in the research design, they did not compare the biomarker level between patients with early HCC and late HCC.

Reply 6: Thank you for the advice. We calculated the methylation level of each CpG site in miR-657 promoter region for different stage of HCC patients. The result

showed that there was no difference for methylation level of miR-657 promoter region in early and late stage of HCC patients. This indicated that the methylation level of miR-657 promoter region decreased in early stage of HCC.

Changes in the text: We have modified our text as advised in the Method part and Result part. (see Page 9, line 160 and Page 11 line 197) The detailed data was listed in the revised Table 3.

Comment 7: In this part, the authors should have a brief overview of known biomarkers on the diagnosis of HCC, have comments on their limitations, and knowledge gaps. Please explain why methylation status of miR-657 deserved to be studied and its relative potential strengths in comparison to other known biomarkers. Please also indicate the clinical significance of the current research topic.

Reply 7: Thanks for the suggestion. The commonly used biomarker for clinical diagnosis of HCC is AFP. But for early diagnosis of HCC, AFP shows low level of sensitivity. As we described previously in the Introduction part, epigenetic modification analysis has several advantages over somatic mutation analysis for cancer detection. So we conducted this research to see whether methylation level of miR-657 could be used for early diagnosis of HCC.

Changes in the text: We have modified our text as advised in the Introduction part (see Page 5, line 81).

Comment 8: In the part of methodology, please describe the inclusion and exclusion

of patients and controls. Please provide the considerations for the current sample size.

Reply 8: Thanks for the advice. The inclusion and exclusion criteria of patients were added in the revised manuscript. As the paired adjacent noncancerous tissues were used for controls, there were no inclusion and exclusion criteria for control group. The current sample size was calculated by using the PASS 15.1.

Changes in the text: We have modified our text as advised in the Methodology part (see Page 7, line 130, Page 9, line 155).

Comment 9: In general, for diagnostic test, controls should be patients with benign tumors in consideration of differential diagnosis.

Reply 9: In this study we mainly checked that hypomethylation status of miR-657 in tissues could distinguish HCC tissues from normal tissues significantly. This can be used as auxiliary molecular diagnosis of puncture biopsy for HCC. We are collecting serum samples of HCC patients and normal patients currently. In our future work we will check whether hypomethylation status of miR-657 in ctDNA could distinguish HCC patients from normal patients. We look forward that hypomethylation status of miR-657 could be used as alternative biomarker for early detection of HCC.

Changes in the text: None.

Comment 10: In the statistics, I do not agree that AUC>0.5 is appropriate, at least 0.8. Please also report the calculation of sensitivity and specificity. Please indicate $P<0.05$ is two-sided or not.

Reply 10: Thanks for the suggestion. We have changed the cutoff value of AUC to 0.8. The diagnostic sensitivity, specificity and Youden's index [sensitivity (%) + specificity (%) - 100] were determined from optimal AUC of methylation levels. $P<0.05$ is two-sided.

Changes in the text: We have modified our text as advised in the Statistical analysis part, result part and discussion part. (see Page 9, line 168, Page 13, line 236 and Page 14, line 264).