Peer Review File

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Reviewer A

Feng et al. describe the upregulation of PTER in HCC tumor tissues and correlate its expression to a number of clinicopathological features and survival. Subsequently, the authors suggest that PTER could potentially serve as a prognostic factor for HCC. The current study is scientifically sound and the data presented is sufficiently novel to warrant publication in Chinese Clinical Oncology; however, there are a number of issues which need to be addressed before I would recommend the manuscript be accepted for publication.

Comment 1: There is no explanation as to the function of PTER and its relevance to cancer. In the Introduction, it would be good to include more justification for studying PTER in the liver and, more specifically, HCC.
Reply 1: Thanks for your constructive comments. There are very few existing published studies on the function of PTER, and even fewer related to cancer. Most of these studies have been cited in this study. Meanwhile, we have added a PTER-related study that we have missed before, which reported that downregulation of PTER in the lake trout liver may be associated with persistent expression of inflammatory factors due to parasitism by the sea lamprey. This article also mentions the paucity of studies on PTER. Our study was conducted based on our concern from RNA-seq sequencing of paired HCC and non-tumor tissues that PTER is highly expressed in HCC tissues, and it has been little studied in liver as well as cancer.
Changes in the text: we have added a reference in the manuscript (see Page 4, line 85-93).

Comment 2: In the Materials and Methods section, the details of the IHC and WB protocols are required, it is not sufficient enough to simply reference a previous publication.
Reply 2: Thanks for your kind reminding. We have added the details of the IHC and WB protocols to the material methods.
Changes in the text: we have modified our text as advised (see Page 5, line 132-149; Page 7, line 173-188).

Comment 3: A section on the acquisition and analysis of TCGA data needs to be included in the Materials and Methods section (e.g. how why were the 'high' and 'low' expression groups defined for the Kaplan-Meier curves in Figures 2D & 2E?).
Reply 3: Thanks for your kind advice. In the revised version, the process of acquisition and analysis of TCGA data were supplemented in the section of Materials and Methods 2.6. The results of Figure 2D & 2E are obtained from the Kaplan-Meier plotter online website, which we illustrate in the figure legends to Figure 2D& 2E.
Changes in the text: we have modified our text as advised (see Page 7, line 190-197; see Page 18, line 508-512).

Comment 4: Why do the bars in the quantification of the western blotting in Figure 1B have error

bars and how have the statistics been undertaken between individual 'normal' and 'tumor' pairings? The data would better be presented in a paired plot (as is used in Figure 1E).
Reply 4: Thanks for your pertinent and constructive comments. This was our carelessness. For Figure 1B, we present it in a more appropriate representation, we used ImageJ software to quantify the immunoblot bands, and the PTER bands in non-tumor and tumor tissues were quantified relative to their own actin, respectively, and the ratios of PTER/actin was statistically analyzed by Graphpad prism 8.0 software, which are described in Materials and Methods 2.5.
Changes in the text: we have modified our text as advised (see Page 7, line 185-188).

Comment 5: There are more than 50 non-tumor samples in the TGCA databases, why were only 50 samples selected for Figure 1D and what were the selection criteria?
Reply 5: Thanks for your careful review. Truly as you suggested there might be more none-tumor samples in the TCGA database. However, there were only 50 pairs of normal liver-HCC tissues in the database when we downloaded these data. Maybe, the TCGA database updates along with time.
Changes in the text: we have added the explanation in Materials and Methods 2.6 (see Page 7, line 192-193).

Comment 6: A description of the staining pattern of PTER with tumor and peri-tumor tissues should be included (e.g. is PTER exclusively found in tumor cells or is it found in other cell types?).
Reply 6: Thanks for your pertinent comments, I'm sorry we missed this description. We added it in Result 3.2. Immunohistochemical experiments showed that PTER was expressed in both the cytoplasm and the nucleus of hepatocellular carcinoma cells, and is not found in peri-tumor tissues.
Changes in the text: we have modified our text as advised (see Page 9, line 239-241).

Comment 7: The "Strengths and limitations" section in the discussion does not actually describe the strengths and weaknesses of the study. For example, a strength of the study is the cohort size; nevertheless, ~93% of the patients within the cohort are HBV+ and so a weakness would be that the findings ideally need to be validated in HCC patients with more diverse aetiologies of background disease.
Reply 7: Thanks for your pertinent and constructive comments, and we couldn't agree with you more. The "Strengths and limitations" section is improved and we add your views to the "Strengths and limitations" section.
Changes in the text: we have modified our text as advised (see Page 12, line 309-317).

Comment 8: Throughout the manuscript, the non-tumorous tissues are described as 'normal', given that the samples utilized in the study have tumors on the background of chronically diseased liver tissues, the terminology 'non-tumor' should be used instead.
Reply 8: Thanks for your pertinent comments, I'm sorry we are lack of rigor in our description. We have used non-tumor instead of normal in the manuscript.
Changes in the text: we have modified our text throughout the manuscript as advised.

Comment 9: There are a number of grammatical and spelling errors throughout the manuscript; therefore, I would urge the authors to thoroughly proofread their work and amend the errors as necessary.

Reply 9: Thanks for your pertinent and constructive comments. We apologize for our poor English and many errors. We have carefully revised the errors and improved the language expression, but maybe the English still needs to be improved, please kindly comment and correct us.
Changes in the text: we have modified our text throughout the manuscript as advised.

Reviewer B

The present study provides interesting results highlighting the potential use of PTER as a predictive biomarker in HCC to improve the clinical landscape of this liver tumor. Although the findings here observed are preliminary, they provide a useful insight into the likely role played by this protein PTER as well as the possible tumor-associated pathways that could be modulated by PTER (as observed in the GSEA analysis).

Nonetheless, there are some minor issues that should be amended:
Comment 1: English should be further improved. Authors should use an English editing service. Some major and minor mistakes have been observed.
Reply 1: Thanks for your pertinent and constructive comments. We apologize for our poor English and many errors. We have carefully revised the errors and improved the language expression, but maybe the English still needs to be improved, please kindly comment and correct us.

Changes in the text: we have modified our text throughout the manuscript as advised.

Comment 2: Background section of the introduction could be improved. It is true that hepatectomy remains the first-choice for the treatment of HCC patients. However, this is not always possible, and it remains as the first-choice only in early stages of HCC (where only a minority of patients are diagnosed). In intermediate stages, TACE is established as the preferred therapeutic option, while chemotherapy administration is the eligible treatment in advanced HCC. This should be clarified in this part of the introduction.
Reply 2: Thanks for your pertinent and constructive comments. We referred to the relevant studies, background section of the introduction has been improved.
Changes in the text: We have modified our text as advised (see Page 3, line 65-76).

Comment 3: In the introduction, authors have focused not only in the background related to PTER in liver pathologies but also in renal. Due to the main objective of this study is to assess the potential role of PTER in the HCC prognosis and other clinicopathological features, the introduction should be more focused on the background related to liver, while the studies performed in renal pathologies and models could be used in the discussion section.
Reply 3: Thanks for your pertinent and constructive comments. We have modified the introduction section as advised.
Changes in the text: we have modified our text as advised (see Page 4, line 85-93).

Comment 4: Please, check all the abbreviations. Some abbreviations have been found defined twice or even three times (e.g., TMA). In other cases, abbreviations are not defined in the first use (e.g., OS and DFS).
Reply 4: Thanks for your kind reminding. We have checked all the abbreviations and corrected these mistakes.
Changes in the text: we have modified our text throughout the manuscript as advised.

Comment 5: For the performance of the qRT-PCR, how did the authors select the samples to perform this technique? Was a random selection or was it based in some specific criteria?
Reply 5: Thanks for your constructive comments. We randomly selected 26 pairs of non-tumor and HCC samples for PCR experiments to detect mRNA levels of PTER, the explanation was added in the Materials and Methods 2.4.
Changes in the text: we have added the description in the manuscript (see Page 6, line 160).

Comment 6: Specify the sense of the primers (5' and 3') to avoid confusion.
Reply 6: Thanks for your kind reminding. This was our carelessness. We have specified the 5' and 3' ends of the primer sequences.
Changes in the text: we have modified our text as advised (see Page 7, line 165-166).

Comment 7: When using a public database for analyzing data from HCC patients, TCGA in this study, authors must cite the source as it is specified in the webpage. Please, check this and cite properly the TCGA source.
Reply 7: Thanks for your kind reminding. This was our carelessness. We have added this section to the Material and Method 2.6.
Changes in the text: we have added the section in the txt (see Page 7, line 190-197 and 201).

Comment 8: For facilitating reproducibility and replicability, a suitable and detailed description of methodology and analysis should be included. In this regard, how immunoblots of PTER were quantified must be described in the figure legend or in the methodology. For example, figure 1A and 1B: it is not specified if the quantification of PTER levels were relative to actin levels. Moreover, there is deviation, however, the number of replicates use for this is also not described.
Reply 8: Thanks for your pertinent comments. This was our carelessness. We used ImageJ software to quantify the immunoblot bands, and the PTER bands in non-tumor and tumor tissues were quantified relative to their own actin, respectively, and the ratios of PTER/actin was statistically analyzed by Graphpad prism 8.0 software. Meanwhile, for figure 1B we present it in a more appropriate representation.
Changes in the text: we have modified our text as advised (see Page 7, line 185-188).

Comment 9: Add in the second column of table 1 that the percentage is given in brackets: "Value (%)" instead of "Value".
Reply 9: Thanks for your kind reminding. This was our carelessness. We have used Value (%)" instead of "Value".
Changes in the text: we have modified our text as advised (see table 1).

Comment 10: In the lines 199-204, the significant correlations of PTER high expression with different clinicopathological characteristics analyzed are described. However, the correlation with the levels of AFP is missing and, as observed in Table 2, there is a significant association.
Reply 10: Thanks for your kind reminding. This was our carelessness. We have added AFP.
Changes in the text: we have added it in the manuscript (see Page 10, line 254).

Comment 11: There is a mistake in the figure legend 2: PTEN is written instead of PTER. Please, amend this. This has been also observed in line 233.
Reply 11: Thanks for your kind reminding. This was our carelessness. We have corrected those mistakes.
Changes in the text: we have modified our text as advised (see Page 11, line 284; Page18, line 507) throughout the manuscript.

Comment 12: Discussion must be highly improved, mainly the 4.3 section. Although authors have compared results here described with previous obtained by other studies conducted in the same research group, a broader discussion with investigations conducted in different groups and/or tumor types would be of great interest. Some studies that performed similar analyses and that could be used to discuss these results are:
o PMID: 36046831 (HM13 upregulated in HCC and also in kidney renal papillary cell carcinoma)
o PMID: 33046796 (SIX4 is upregulated and promoted invasion and metastasis in HCC)
o PMID: 35611367 (SPINDOC is upregulated in HCC and also in kidney renal papillary cell carcinoma)
o PMID: 35884516 (NRP1 in liver cancer related to prognosis and invasion)
o PMID: 35196258 (THRSP identified as a potential biomarker in HCC)
o PMID: 34771514 (FOXO3 upregulated in HCC)
Reply 12: Thanks for your kind suggestion. We have read these articles you recommended which helped us a lot. We have added the content in section 4.3.
Changes in the text: we have modified our text as advised (see page 12, line 320-333 and line 340-345).