Rebuttal letter

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: - The authors explore the expression of P-Rex1 in HCC, and further evaluate its potential application in the diagnostic and predictive prognosis for HCC, especially in the diagnosis in HBV-related patients -The idea is very good. The study is well-designed. The manuscript was well, concisely and coherently organized but I want to clarify some points.

Reply: We thank the reviewer's positive comments.

1- what is the temperature of the refrigerator? Do you put the resected specimens as a whole in the refrigator or you cut the specimens and then preserve the slices in the refrigator).

Reply: Thanks for your question. The temperature of the refrigerator is -80°C. And the whole resected tissues including tumor and surrounding normal tissues were stored at the refrigerator respectively. And the detailed information was also supplied in the revised manuscript.

2- Did you do resection of the tumour for patients have lymph node invasion, distant metastasis????)

Reply:

Reply: We thank the reviewer's constructive question, and the metastatic tumor tissues including lymph node invasion and distant metastasis were not included in this study, only primary tumor tissues were included in this study. This question is very good, but in this study, we highlighted the significance of P-Rex1 in the primary HCC. And in the next study, we would further study the clinical significance of P-Rex1 in the metastatic HCC.

3-(How you do score of the immunohistochemistry staining and please can you add a figure of these staining in the tumour and the adjacent tissue)

Reply: We thank the reviewer's question. As we showed in Figure 4B, the P-Rex1 staining of liver tumor and adjacent normal tissues were provided, and the score of the immunohistochemistry staining were achieved by Quantity One software.

4-You do not explain how you divide the cases into low or high expression.

Reply: We thank the reviewer's question, and in the "Survival analysis with Kaplan-Meier methods" and the Table 1 of the primary manuscript, we have showed that the median level of P-Rex1 was used as the cut-off of high or low expression group.

5-You do not even mention if the staining is nuclear or cytoplasmic.

Reply: We thank the reviewer's question, and in the revised manuscript, we have supplied the description of the staining.

6- Please you do not mention figures in the discussion)

Reply: We thank the reviewer's question, and the related figures were included in the discussion section. In the section paragraph of discussion section, the detailed figures were discussed and the potential association was also discussed in the discussion section.

7-(please , how you can explain the prolong survival time in higher P-Rex1 expression and in the same time, the P-Rex1 high expression was associated with lymph node invasion and distant metastasis which are bad prognostic factors)

Reply: Thank the reviewer's question. This question is constructive, and as the reviewer pointed out lymph node invasion and distant metastasis are poor prognostic factors, and P-Rex1 expression has a positive association with lymph node invasion and distant metastasis. However, P-Rex1 as an important immune function regulator, which could regulate the tumor immune response, the high expression of P-Rex1 might be a compensatory phenomenon.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: The authors clearly demonstrated that P-Rex1 expression was increased in HCC and P-Rex1 is a diagnostic and favorable prognosis biomarker; however, there are several issues for acceptance in this study.

Reply: We thank the reviewer's constructive comments, and we carefully revised the manuscript according to the reviewer's suggestion.

Major points

1. Ninety HCC patients were included in this study; however, the detailed clinical backgrounds are not described. The authors should describe the patient's background, such as tumor size and numbers, BCLC stage, vascular invasion, and Child Pugh grade, which are associated with HCC prognosis. Especially, vascular invasion plays an important role in HCC prognosis, thus authors should evaluate P-REX1 expression in patients with positive or negative vascular invasion.

Reply: We thank the reviewer's question. And the detailed information about patients were supplied in the section of materials and methods of the revised manuscript. And due to the deficiency of the patient samples, the vascular invasion was not included in this study, the low samples about vascular invasion could not support the significance of P-Rex1 in the positive or negative vascular invasion, but the lymph node invasion was included in this study, and the results showed P-Rex1 expression has a certain association with lymph node invasion.

2. Why are the patients with HBV infection higher P-Rex1 expression?

Reply: We thank the reviewer's question. P-Rex1 as an important immune function regulator, which could regulate the tumor immune response, and HBV infection has a significant effect on tumor immune response, and P-Rex1 might be a compensatory factor.

3. P-Rex1 expression was significantly higher in patients with positive of lymph node invasion and distance metastasis, and high AFP level (Figure 2 B, C, and D). These findings seemed to indicate that P-Rex1 is a poor prognostic factor for HCC patients; however, the results are contrary to the expectation as Figure 5 shown. The authors should discuss these discrepancy.

Reply: We thank the reviewer's question, and in the discussion section, the related discussion was supplied. P-Rex1 was reported as an important factor which regulate the immune signaling pathways, and the complex regulation mechanism of tumor immune was also considered as the most important factor in the development of liver cancer. Thus, the high P-Rex1 correlated with development of liver cancer, but the high expression of P-Rex1 showed the favor survival time, suggesting that the high expression of P-Rex1 might be a compensatory

phenomenon.

4. The authors should discuss the reason why P-Rex1 is associated with a favorable outcome, such as overall survival, progression free survival, and relapse free survival in HCC patients.
Reply: We thank the reviewer's question. And in the revised manuscript, the discussion was included in the discussion section.

Minor point Figure 3B and C are not mentioned in RESULTS.
Reply: We thank the reviewer's question, and in the revised manuscript, we have supplied in the results section, we thank the reviewer's careful review again.

Editorial Office's comments
The author must revise the manuscript according to the Editorial Office's comments and suggestions, which listed below:
(1) Science Editor: 1 Scientific quality: The manuscript describes a retrospective study of P-Rex1 in the diagnostic for hepatocellular carcinoma. The topic is within the scope of the WJCC. (1) Classification: Grade B and Grade C; (2) Summary of the Peer-Review Report: The authors explore the expression of P-Rex1 in HCC, and further evaluate its potential application in the diagnostic and predictive prognosis for HCC, especially in the diagnosis in HBV-related patients. The idea is very good. The study is well-designed. The manuscript was well, concisely and coherently organized. The questions raised by the reviewers should be answered; and (3) Format: There are 2 tables and 6 figures. A total of 35 references are cited, including 19 references published in the last 3 years. There are no self-citations. 2 Language evaluation: Classification: Two Grades B. A language editing certificate issued by YHYSCI was provided. 3 Academic norms and rules: The authors provided the Biostatistics Review Certificate, the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement, the Institutional Review Board Approval Form, and the written informed consent. No academic misconduct was found in the CrossCheck detection and Bing search. 4 Supplementary comments: This is an unsolicited manuscript. No financial support was obtained for the study. The topic has not previously been published in the WJCC. 5 Issues raised: (1) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor. 6 Re-Review: Required. 7 Recommendation: Conditional acceptance.
(2) Editorial Office Director: I have checked the comments written by the science editor.
(3) Company Editor-in-Chief: I have reviewed the Peer-Review Report, the full text of the manuscript, the relevant ethics documents, and the English Language Certificate, all of which have met the basic publishing requirements of the World Journal of Clinical Cases, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.
Reply: We thank the editor's constructive comments, and we carefully studied the editor and reviewer's suggestion and revised according to these comments. The point-to-point response were showed as previous. And the revised content was highlighted in red. Thank you for your cooperation.

RE-REVIEW REPORT OF REVISED MANUSCRIPT

Response letter

Reviewer #1

I highlighted my comments in yellow in your response to reviewers

Reply: Thank you for your review, we found that the uploaded review document is another manuscript, which submitted to "Pathology & Oncology Research" journal. Thus, it is not applied to our manuscript. And we carefully revised our manuscript according to the first and second review, thank you for your time.

Reviewer #2

This manuscript was well revised according to the comments. Minor point: The detailed information of the patients including its table should be described in "RESUTLS".

Reply: Thank you for your review, as the former revised manuscript showed, the second section of results, "P-Rex1 expression was closely associated with clinical features of HCC", the results of Table 1 have been described, and Table 2 has been described in fifth and sixth section of results. Thank you again for your time on our manuscript