Peer Review File

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Reviewer A

The paper titled “Effect of urokinase-type plasminogen activator combined with clinical stage and Barcelona Clinic Liver Cancer stage on the prognosis of patients with hepatocellular carcinoma” is interesting. The decreased expression of PLAU can prolong the DSS, OS, and PFI in LIHC patients, and can be utilized as a novel predictive index. PLAU combined with CS staging and BCLC staging has good clinical value in the early screening and prognosis of LIHC. These results reveal an efficient approach for developing anticancer strategies against LIHC. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) The description of some methods and results in this study is too simplistic, please describe in detail.

Reply: Thank you for your valuable suggestion. Relevant adjustments and modifications have been made in the methods and result description.
Change in the text: Page 4-5, line 132-153. Page 7-8, line 231-250.

2) Is PLAU related to the malignant features of LIHC? If relevant, what is the correlation with tumor size, differentiation grade and absence of tumor encapsulation? It is recommended to add relevant content.

Reply: Thank you for your valuable comment. We first found that PLAU expression was higher in LIHC tumor tissues than in adjacent tissues in TCGA database. Moreover, it was found that LIHC patients with high expression of PLAU had better OS, DSS, and PFI. These results indicate that PLAU plays a role as a "oncogenic gene" in the biological events of LIHC. By analyzing the correlation between PLAU expression and clinicopathological characteristics of LIHC patients, we found that PLAU expression was correlated with T stage and Edmondson's grade of patients, and high PLAU expression was more manifested in T stage III-IV and Edmondson's grade III-IV. These results indicate that high expression of PLAU has more malignant pathological features.
Change in the text: None.

3) In the introduction of the manuscript, it is necessary to clearly indicate the characteristics of infiltrating immune cells in LIHC and the clinical significance of this study.

Reply: Thank you for your valuable comment. We have added features of immune cell infiltration and clinical implications of this study in the introduction.
Change in the text: Page 4, line 113-125.

4) All figures are not clear enough. It is recommended to provide clearer figures again.

Reply: Thank you for your suggestion. We have provided the figures with higher clarity.
Change in the text: All of figures.

5) What are the characteristics of dynamic migration and state transition of immune cells in LIHC? What role does PLAU play in this process? It is recommended to add relevant content.

Reply: Thank you for your suggestion. If we want to clarify the dynamic change characteristics of immune cells in the LIHC microenvironment, we need to carry out single cell RNA sequencing technology (10× Genomics and SMART-seq2), separate immune cells from tumor tissue and other immune related parts for single cell sequencing, and generate a single cell immune map of LIHC. At present, we do not have any research results or basic experiments for this part. We have added relevant content and references in the discussion section.
Change in the text: Page 11-12, line 353-369.

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Urokinase plasminogen activator predicts poor prognosis in hepatocellular carcinoma, J Gastrointest Oncol, PMID: 34532133". It is recommended to quote the articles.

Reply: Thank you for your suggestion. We have added this reference in the introduction section.
Change in the text: Reference 11.

7) It is recommended to add in vivo and in vitro experimental validation of the results of this study.

Reply: Thank you for your valuable comment. Given the current limited resource conditions, in future research, we will further improve the relevant in vitro and in vivo cytology experiments to better support our research results and provide better reference value for clinical work.
Change in the text: None.

Reviewer B

1) First of all, my major concern is the poor prognosis prediction accuracy of the C-index of the nomogram developed, which was 0.643 only and suggests that this is a failed prediction model. The question appropriate to the current data should be the prognostic role of PLAU. Because of this, the authors need to revise the title and elsewhere of this paper accordingly.

Reply: Thank you for your valuable comment. This study found that PLAU is highly expressed in LIHC tumor tissue through analysis of TCGA data. Prognostic analysis also suggests that patients with low PLAU expression have better prognosis, and the correlation with clinical data suggests that low PLAU expression has better pathological characteristics. These results indicate that PLAU may play a role as a "oncogenic gene" in the biological events of LIHC. Although the C-index of the column chart is not high, it also has certain reference value. In the later stage, we will incorporate more independent prognostic factors with higher correlation and construct a more reliable prognostic model.
Change in the text: None.

2) Second, the title needs to indicate the clinical research design of this study, i.e., a retrospective cohort study and a bioinformatics analysis.

Reply: Thank you for your valuable comment. The content of this study is sourced from the TCGA database and clinical data of LIHC patients in our hospital, and is itself a retrospective study. Secondly, in CS staging and BCLC staging, clinical data is mainly used, and our keyword section already includes bioinformatics. Therefore, we believe that our research topic is more focused on the impact of urokinase type plasmin (PLAU) combined with CS staging and BCLC staging on the prognosis of patients with hepatocellular carcinoma.
Change in the text: None.

3) Third, the abstract needs to be further revised. The background did not indicate the knowledge gap on and clinical significance of this research focus. The methods need to briefly describe the variables and prognosis outcomes in the dataset. Please also describe the inclusion of the clinical sample, follow up, and prognosis outcome assessment. The results need to quantify the findings by using detailed statistics such as expression levels, HR values, and accurate P values. The conclusion need to focus on the prognostic role only. The screening and predictive values of PLAU are problematic; the current study did not provide evidence on the diagnostic accuracy.

Reply: Thank you for your valuable comment. The abstract section has been modified accordingly, as highlighted in bold and red. The main purpose of this study is to quickly obtain some genes that can support the prognosis of LIHC through bioinformatics analysis, and to

predict the relevant mechanisms. The immunohistochemical component of this gene has been added to the combined clinical data, and it is hoped that through the combination of bioinformatics and clinical data, urokinase plasmin activator (PLAU) combined with CS stage and BCLC stage can predict the prognosis of patients with hepatocellular carcinoma. This study also has some limitations, and further multicenter clinical trials are needed in the future to verify the accuracy and utility of our findings.
Change in the text: Page 2, line 41-46.

4) Fourth, the introduction needs to briefly review known prognostic biomarkers in LIHC, have comments on their limitations and knowledge gaps, and clearly explain why PLAU is important and deserved to be studied. Comments on the knowledge gaps and clinical significance on the prognostic role of PLAU are needed.

Reply: Thank you for your valuable comment. We have added known prognostic biomarkers in LIHC in the introduction and supplemented relevant references. PLAU, as one of the main proteolytic enzymes involved in extracellular matrix degradation, has been shown to play a crucial role in biological events such as tissue remodeling and migration during development and tumorigenesis, with recurrence and metastasis of LIHC being one of the main clinical events with poor prognosis. Considering the research reports of PLAU in other tumors, we selected PLAU for relevant research analysis in LIHC.
Change in the text: Page 3, line 85-93.

5) Fifth, the methodology of the main text needs to briefly describe the research procedures in bioinformatics analysis and the analysis within the clinical samples, as well as questions to be answered by these procedures. The authors need to describe the clinical factors and prognosis outcomes in the TCGA dataset and the baseline clinical factors collected in the clinical sample. In statistics, please describe the details for assessing the independent prognostic role of PLAU, not to identify prognostic factors. Please ensure $P<0.05$ is two-sided and revise the wrong term “statistically important”.

Reply: Thank you for your valuable comment. We have made relevant changes to the wording.
Change in the text: Page 4-5, line 132-153.

Reviewer C

1. Abstract
Please define all the abbreviations in the abstract.
*Please note that 350 words is maximum.

Methods: We verified PLAU expression and its correlation with LIHC patients' prognosis in the TCGA database. The interaction network for protein-gene was established in the GeneMania database and the STRING database, and the association between PLAU and immune cells was assessed in TIMER and TCGA databases. The potential physiological mechanism was elucidated by the GSEA enrichment assessment. Finally, the individual clinical data of 100 LIHC patients were retrospectively evaluated

Reply: Thank you for your comment. We have defined all the abbreviations in the abstract. The abstract in 347 words now.

2. Figure 1

a) As there is no symbol "**" in the figure, please delete the explanation in the legend.

b) Please explain ns, TCGA, TPR, and FPR in the legend.

Reply: Thank you for your comment. a) We have deleted the explanation in the legend.

b) We have explained ns, TCGA, TPR, and FPR in the legend.

3. Figure 2

Please explain TCGA in the legend.

Reply: Thank you for your comment. We have explained TCGA in the legend.

4. Figure 3

Please explain AFP and TPM in the legend.

Reply: Thank you for your comment. We have explained AFP and TPM in the legend.

5. Figure 4

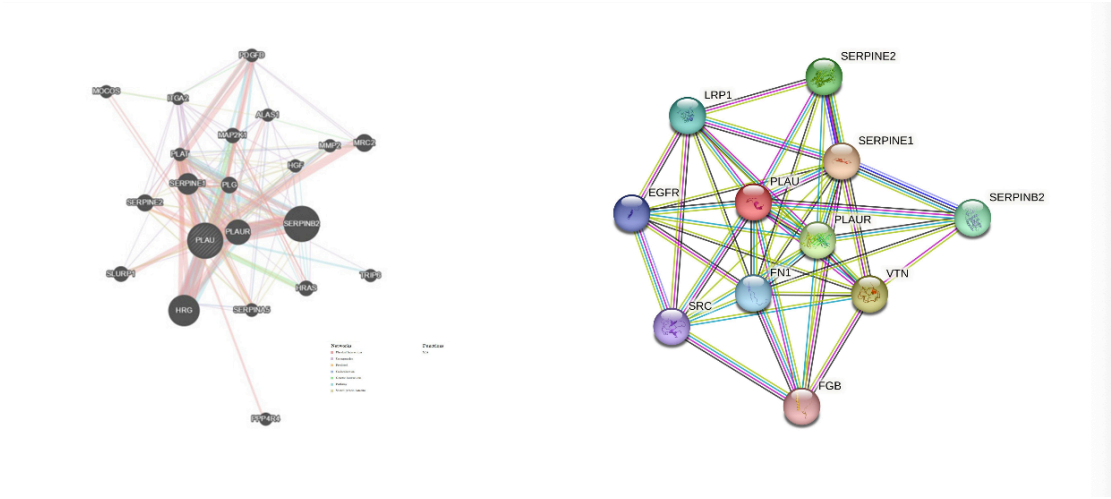
Please explain AFP in the legend.

Reply: Thank you for your comment. We have explained AFP in the legend.

6. Figure 5

a) Please explain STRING in the legend.

b) Please add A and B in the figure.



##Determining the PLUA-interacting genes and proteins

A PLUA gene-gene interaction network was generated, and the neighboring genes were altered via GeneMania (Figure 5A). The PPI network of PLUA was established with the help of the STRING database (Figure 5B).

Reply: Thank you for your comment. a) STRING is an international database name, the full name is STRING.

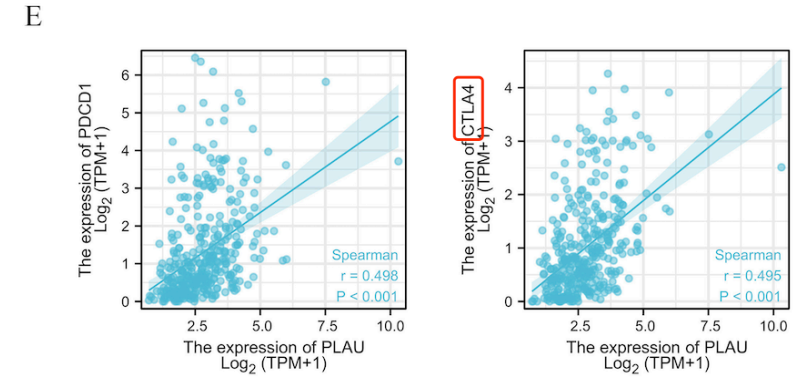
b) We have added A and B in the figure.

7. Figure 6

a) Please explain TCGA, TPM, and TIMER in the legend.

b) Please unify the name.

cell infiltration levels of LIHC in TCGA database; (E) PLUA expression in LIHC scatterplot of correlations with CTLA-4, PDCD1, and CD274. P<0.05, the variability



Reply: Thank you for your comment. a) We have explained TCGA, TPM, and TIMER in the legend.

b) CTLA4 was written uniformly.

8. Figure 7

Please explain TCGA and TPM in the legend.

Reply: Thank you for your comment. We have explained TCGA and TPM in the legend.

9. Figure 9

Please explain GSEA in the legend.

Reply: Thank you for your comment. We have explained GSEA in the legend.

10. Table 1

Please explain CTD in the table footnote.

Reply: Thank you for your comment. We have explained CTD in the table footnote.

11. Table 3-4

Please explain CI in the table footnote.

Reply: Thank you for your comment. We have explained CI in the table footnote.