Peer Review File

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Reviewer A

My major concerns are listed here:
1. The authors firstly investigated PCK2 expression in TCGA pan-cancer samples and then find that expression of PCK2 was reduced in most tumors. However, it is still unknown which cells have reduced expression of the PCK2. Improving resolution of expression profile further would be more convincing. The authors should consider using the samples in the GTEx database for comparison, at least the manuscript should refer new computational framework e.g. (PMID: 36765073).

Reply: Thank you so much for your valuable comment. The TCGA database is comprehensive and authoritative, but its drawback is that the sequencing data of normal tissue samples is relatively small; And GTEx can make up for this disadvantage very well. The biggest advantage of GTEx is that there is a large amount of sequencing data in normal tissue samples. However, compared to TCGA, the GTEx database has a narrower focus. Our aim in this study is to quickly screen a target gene related to the prognosis of lung adenocarcinoma patients at the histological level, and then further validate and analyze the role of this target gene in biological events of lung adenocarcinoma in different databases, providing better reference for our future cytological experiments.
Change in the text: None.

2.Protein-gene interaction networks were built using GeneMania and STRING databases. However, there is little of interpretation about those two networks in the manuscript. It would be better to interpret them in detail.
Reply: Thank you so much for your valuable comment. We have added an introduction to the two databases in Method section.
Change in the text: Page 5/Line 140-147.

3.In the Figure 6, what functionality of genes positively and negatively correlated with PCK2 in lung cancer patients in cancer progress? Are they related to lung cancer patient survival?

Reply: Thank you so much for your valuable comment. The results in this section mainly focus on exploring genes that are positively and negatively correlated with PCK2 and does not specifically refer to functional upregulation or downregulation. No analysis was conducted on the role of these target genes in the prognosis of lung adenocarcinoma. However, these genes may participate in biological events of lung adenocarcinoma through their interaction with PCK2. These research results have good reference value for further conducting relevant cytological experiments to explore the specific mechanisms in the future.
Change in the text: None.

4.In the Figure 11, the x-axis coordinates overlap. It would be better to redraw it.

Reply: Thank you so much for your kind reminder. We have replaced Figure 11 with a new image to enable readers to better view it.
Change in the text: Figure 11.

Minor Comments:
Some sentences in the article have issues such as unclear sentences and incorrect grammar. Please check the entire manuscript.

Reply: Thank you so much for your valuable suggestion. We have reviewed the whole manuscript and corrected relevant syntax error, so that readers can read it better.

Reviewer B

The author utilized bioinformatics and TCGA data to determine a potential cancer suppressor molecule, PCK2. However, the author still needs to make significant modifications to meet the publishing requirements. Some suggestions are as follows:

1.The research on this molecule in LUAD is limited in the introduction is inappropriate. (PMID: 34520823; PMID: 32777161) The author should cite them, and explain the necessity of your research.

Reply: Thank you so much for your valuable suggestion. Firstly, based on the current epidemiology and treatment prognosis of lung adenocarcinoma, there is an urgent need to seek reliable target molecules for early diagnosis and treatment. There are currently many different perspectives on this research. We checked the relevant literature and found that tumor metabolic abnormalities are the hot topic of research, and most of these studies are around the three major basal metabolism. Our molecular phosphoenolpyruvate carboxyl kinase (PEPCK) is the key enzyme of gluconeogenesis. There are many studies on glucose metabolism in different tumors, including lung adenocarcinoma. Therefore, we chose to first use bioinformatics analysis to analyze the potential role of this molecule in lung adenocarcinoma, providing a better reference for our future cytological experiments, and also serving as a "pre experiment". We have added citations to the relevant references ((PMID: 34520823; PMID: 32777161).
Change in the text: Reference 10 and 13.

2. The image is not aesthetic, and it needs to be remade according to the requirements of the submission to unify the size of the image. In addition, the colors between different groups can be appropriately unified. There are too many figures, some smaller images can be merged.

Reply: Thank you so much for your valuable comment. The small images in each large image also hope to further illustrate the value of our molecular research from different perspectives,

providing a better reference perspective for our future basic experiments. We have made some modifications regarding the images, hoping to provide better reading for readers.

3. The AUC value in Figure 1D is too low, at least around 0.72. As mentioned in comment 1, the author can consider further grouping patients for recalculation to speculate on the role of this molecule (such as being very useful in a specific patient group).

Reply: Thank you so much for your valuable comment. The current research is based on the TCGA database, and the reliability of positive results for this molecule is very high in certain angles. Although the positive results for certain angles are not high, they also have certain reference value. Although the AUC in Figure 1D is only 0.670, it also reflects a certain reference value. This is the result of our analysis of relevant data based on the TCGA database, and we are currently unable to further improve the processing. The research value of this molecule still depends on its role in the biological events of lung adenocarcinoma, which requires further cytological experimental verification and clinical data analysis and exploration in the future.
Change in the text: None.

4. The P-value in Figure 2A3 is not significant and the description in the results is not appropriate.

Reply: Thank you so much for your valuable comment. Although there is no statistical difference in the DSS in Figure 2A, we can see that the prognosis of patients with high expression of PCK2 is better than those with low expression of PCK2. This graph reflects this trend, although there is no statistical difference, it also has reference value.
Change in the text: None.

5. Figure 2B is very confusing. Why did you suddenly analyze in specific T2 and N0 groups? If it doesn't significant in other groups, please explain. Or provide other reasons to explain why you are doing so.

Reply: Thank you so much for your valuable comment. Based on the comment, we have deleted the group KM survival curve analysis of T2 and N0 groups and update the figure.
Change in the text: Figure 2.

6. Figure 3 seems to have two pathological stages. Is it a mistake that one of them is TNM. In addition, the M group is missing; And it seems that the T and N groups have not been included in all categories, are there no such patients or are they missing?

Reply: Thank you so much for your valuable comment. This is our mistake, we have replaced Figure 3. The patients in the M stage group were not included in the analysis results due to the lack of patient data between groups and the lack of effective statistical analysis.
Change in the text: Figure 3.

7. Before doing NOMOGRAM in Figure 4, it is recommended to supplement the COX regression results.

Reply: Thank you so much for your valuable suggestion. Because our research is mainly based on TCGA database analysis, our goal is also to explore and analyze the research value of PCK2 as much as possible using biological analysis methods. Therefore, it is necessary to further accurately explore the value of this molecule, and further analyze it through our clinical data in the future, as well as further validate it from the perspective of cytological experiments.
Change in the text: None.

8. The descriptions in Figures 5 and 6 are too simple, and this result should be reflected in the results to speculate on the function of the protein.

Reply: Thank you so much for your valuable comment. The results in this section mainly explore the genes that may interact with PCK2, which may participate in the biological events of lung adenocarcinoma through a certain mechanism of action with PCK2. These research results have good reference value for further conducting relevant cytological experiments to explore the specific mechanisms in the future. However, the main direction of our research is centered around PCK2, and therefore, no analysis has been conducted on the functions of these interacting protein molecules.
Change in the text: None.

9.The result title in Figure 6 is incorrect (CK2).

Reply: Thank you so much for your valuable comment. We have revised the result title in Figure 6 to “PCK2 co-expression gene screening”.
Change in the text: Page 7/line 223.

10. In the section of immune cell infiltration, Bulk data typically represents the expression level of genes within dominant cells. Therefore, these correlation may not necessarily be true, and words such as “may” and “would” should be used. Otherwise, it is recommended to supplement single cell data for further clarification.

Reply: Thank you so much for your valuable suggestion. We have made changes in language expression.
Change in the text: Figure 7 legend.

11. Figure 9 lacks P-value. What is ZC3H13 in the results? Please explain.

Reply: Thank you so much for your valuable comment. Firstly, this result mainly comes from the UALCAN database. Our aim is to compare the promoter methylation levels of different lung adenocarcinoma patients in the UALCAN database. What we need to obtain is a trend that can indicate that different levels of promoter methylation may be related to different

expressions of PCK2, so there is no statistical analysis of P-values. At the same time, this is also a reference perspective for us to explore the specific mechanisms through basic cytology experiments in the future. Regarding 'ZC3H13' as our writing error, we have made corresponding changes, as highlighted in red.
Change in the text: Page 8/line 261-262.

12. Use italics for P values and gene names.
Reply: Thank you so much for your comment. We have made corresponding changes in the article.

13. Appropriate references should be cited for materials and methods.

Reply: Thank you so much for your valuable comment. Because the databases we analyzed are relatively common; Secondly, in our introduction and discussion sections, some of the questions we have cited are also articles on bioinformatics, and the methodology section is also for our reference and reference.
Change in the text: Reference 16.

14. The discussion is not in-depth enough, please do not introduce background content anymore. The discussion should be a summary of the results, and the findings in Figure 5.6.7 should be further discussed. The author should think carefully about the significance of conducting these studies and the similarities and differences between them and previous findings.

Reply: Thank you very much for your valuable suggestion. We have revised Discussion section of the text.
Change in the text: Page 10-11/line 325-333.

15. If possible, please supplement the experimental verification appropriately.

Reply: Thank you very much for your valuable suggestion. In the future, we will increase in vivo and in vitro cytology experiments to verify possible related mechanisms. Currently, due to limitations in experimental conditions, our research results cannot be further validated from different perspectives.
Change in the text: None.

16. English editing is necessary.
Reply: Thank you very much for your valuable comment. We have conducted language polishing and some grammatical changes to make our research results more accessible to readers.

Reviewer C

The manuscript aimed to identify the role of PCK2 in the development of lung cancer. Through analyzing the TCGA database, the authors found that PCK2 was lowly expressed in lung cancer tissues. Decreased PCK2 gene expression levels were associated with overall survival, disease-specific survival, and progression-free interval. Additionally, PCK2 was found to be involved in EMT, hypoxia, senescence, and immune evasion of tumor cells. The work provides potential targets for the treatment of lung cancer. However, there are still a few issues that need to be addressed:

1. At the end of each result, the authors should add a conclusion to summarize the section.

Reply: Thank you very much for your valuable comment. We have made corresponding changes to the wording of the article.

2. While the manuscript has identified PCK2's involvement in oxidative stress-induced senescence, gene silencing, cell cycle, and more, all conclusions are based solely on database analysis. Therefore, the authors should perform experiments or discuss related references to validate their findings.

Reply: Thank you very much for your valuable comment. Our research objective is mainly to use bioinformatics analysis methods to quickly identify a gene involved in the prognosis and biological events of lung adenocarcinoma, providing better reference for our future research. In the future, we will increase in vivo and in vitro cytology experiments, while improving the validation analysis of clinical data, in order to better validate the value of our molecular research from different perspectives of bioinformatics, clinical, and cytology.
Change in the text: Page 10-11/line 325-333.

3. The manuscript's English should be rechecked for errors, as several mistakes are present throughout the text.

Reply: Thank you very much for your valuable comment. We have changed some syntax error in the article so that readers can read it better.

Reviewer D

1. Please check all abbreviations in the abstract and main text, such as below. All abbreviated terms should be full when they first appear.

34 the efficiency of lung adenocarcinomas, and a T-SNE map was constructed to show the
35 expression profile of PCK2 in single cells in TCGA lung adenocarcinoma samples. The
36 potential mechanism of action was finally investigated using GSEA enrichment

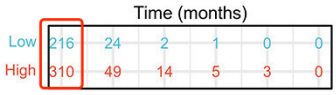
43 expression, and its mutation rate in lung adenocarcinoma was 0.53%. CancerSEA
44 research revealed that in lung adenocarcinoma, PCK2 was negatively correlated with
45 EMT and hypoxia. Gene ontology and KEGG enrichment analysis revealed PCK2-

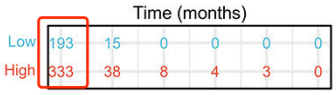
211 **##PCK2 and lung cancer prognosis in TCGA database**
212 In the TCGA database, we discovered that patients with high expressions of PCK2 in
213 their lung adenocarcinoma had superior OS, DSS, and PFI (Figure 2A).

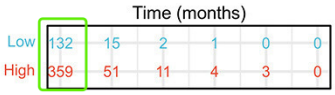
Reply: Thank you for your comment. We have checked and defined all abbreviations in the abstract and main text.

2. The number of Keywords should be 3-5, but you have 7. Please revise.
Reply: Thank you for your comment. We have reduced the keywords to 5.

3. Figure 2:
Please check whether the data in below green box is correct. The data in below green box should be equal to below two red boxes.





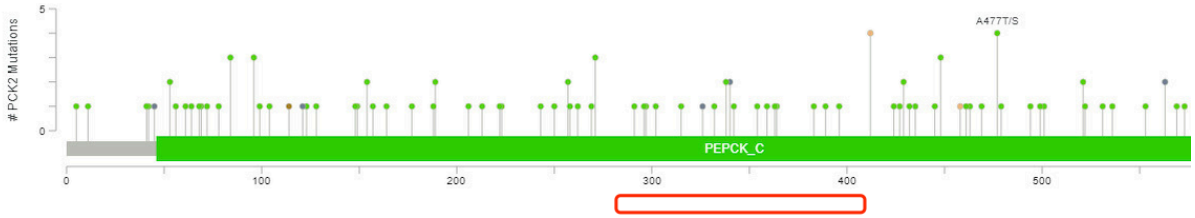


Reply: Thank you for your comment. The data in green box is right. The pictures were obtained in the database at that time, and it is possible that a small number of patients' information was missing, and the prognostic evaluation criteria of the analysis were inconsistent, so the total number of patients' information was inconsistent.

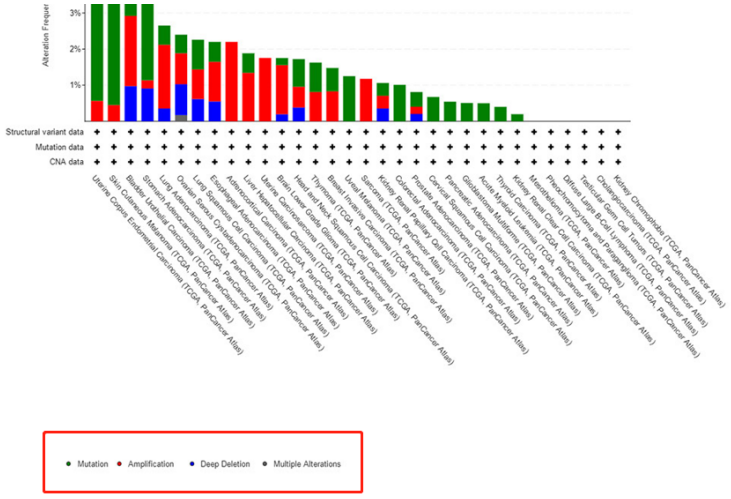
4. Figure 6:
1) Figure 6 is not clear enough. Please resubmit it in higher resolution.
2) Please indicate the meaning of *, **, *** in the legend.
3) Please indicate the full name of "TPM" in the legend.
Reply: 1) We have resubmitted figure in higher resolution.
2) We have indicated the meaning of *, **, *** in the legend.
3) We have indicated the full name of "TPM" in the legend.

5. Figure 7:
1) Please indicate the meaning of **, *** in the legend.
2) Please indicate the full name of "TPM" in the legend.
Reply: 1) We have indicated the meaning of **, *** in the legend.
2) We have indicated the full name of "TPM" in the legend.

6. Figure 8:
1) Figure 8 is not clear enough. Please resubmit it in higher resolution.
2) Figure 8A, B legends don't match with Figure 8A, B. You mixed up them.
3) Please check whether it's needed to add the description of x-axis in Figure 8B.



Reply: 1) We have resubmitted figure in higher resolution.
2) We have changed the arrangement of figure A and B.



B

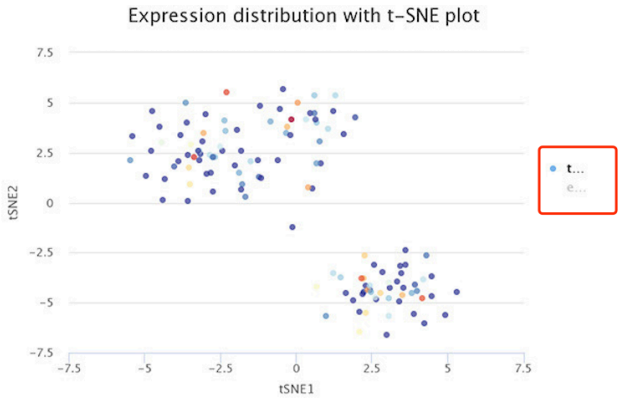
3) Because the X axis can be retrieved from the public website, it is not necessary to add relevant information.

7. Figure 9:
Figure 9 is not clear enough. Please resubmit it in higher resolution.
Reply: We have resubmitted figure in higher resolution.

8. Figure 10:
1) There are no *, **, *** in Figure 10, but you indicated them in the legend.

adenocarcinoma; and (C) expression profile of PCK2 at the single-cell level in lung adenocarcinoma as shown by the T-SNE map. *P<0.05; **P<0.01; ***P<0.001. PCK2,

2) The figure labels in below red box are not clear. Please modify. And what is the meaning of "t.." and "e…"?

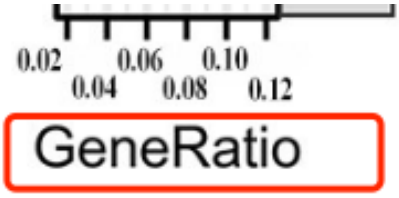


Reply: 1) We have deleted *, **, *** in Figure 10 legend.

2) This is caused by the author's mistake screenshot, "t.." and "e..." are not belong to this picture, we have made changes.

9. Figure 11:

Please revise all “GeneRatio” to “Gene Ratio”.



Reply: we have revised all “GeneRatio” to “Gene Ratio”.