

Peer Review File

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Reviewer A

Comment 1:

First of all, the title needs to accurately indicate the two focuses of this study, the prognostic role of TPGS2 and its relationship with immune infiltrates. The title also needs to indicate the research methodology of this study, i.e., a bioinformatics analysis. Because the current study did not assess the predictive accuracy of TPGS2 for predicting the prognosis, so terms such as “a powerful biomarker to predict the clinical outcomes” are misleading and should not be used in the whole paper.

Reply 1:

At first, thanks for your sincere comments. According to the suggestions, we have indicated our research methodology of this study in the title (Page 1, line 1). In addition, according to the reviewer's requirements, we have carefully modified the manuscript. Then considering that our study did not assess the predictive accuracy of TPGS2 for predicting the prognosis, terms such as “a powerful biomarker to predict the clinical outcomes” have been removed from the manuscript (Page 1, line 35).

Changes in the text: Page 1, line 1; Page 1, line 35

Comment 2:

Second, the abstract needs some revisions. The background did not describe the potential clinical significance of this research focus. The methods part is not informative. The authors need to describe the data and prognosis outcomes in the datasets used and main statistical methods for analyzing the prognostic role of TPGS2 and its associations with immune indicators. The results should provide details data and statistics such as expression levels, HR values, and accurate p values to support the findings. The conclusion needs to be more detailed for the possible implications.

Reply 2:

Thanks for your rigor. We've stated the potential clinical significance of this research focus in the background (Page 1, line 15). Besides, after careful consideration of the reviewer's suggestions, we included the description of the data and prognosis outcomes in the datasets and main statistical methods for analyzing the prognostic role of TPGS2

and its associations with immune indicators in the methods (Page 1, line 17). Given that the entire pan-cancer analysis includes multiple dimensions for multiple tumors, it is difficult to present specific statistical values in short conclusions, given the completeness and comprehensiveness of the summary results. However, we have also made minor changes in response to the reviewer's suggestions (Page 1, line 25). Finally, we have detailed our conclusions (Page 1, line 34).

Changes in the text: Page 1, line 15; Page 1, line 17; Page 1, line 25; Page 1, line 34

Comment 3:

Third, the introduction of the main text needs to review what has been known on the prognostic biomarkers and their associations with immune-cell infiltration, analyze the limitations and knowledge gaps of prior studies, and why TPGS2 deserved to be studied.

Reply 3:

We accepted the reviewer's suggestion, and added the relationship between prognostic indicators and immune infiltrating cells in the introduction, and then introduced TPGS2. Since TPGS2 and tumor immunity are the main topics of this paper, we have shortened the introduction of prognostic indicators (Page 2, line 67).

Comment 4:

Fourth, in the methodology of the main text, it is necessary to have an overview of the research procedures of this study and describe the clinical factors and prognosis outcomes in the databases used. In statistics, please ensure $P < 0.05$ is two-sided.

Reply 4:

Thank the reviewers for their sincere comments. We have added a description of the research process and related database information to the methodology of the main text. Other parts of the manuscript also contain presentations and descriptions of prognostic outcomes and clinical information. And in general, $p < 0.05$ is two-sided in our manuscript.

Reviewer B

Comment 1:

The result description and figure legends in Figure 8 both describe 8G-K, but it is not indicated in the figure. Please carefully check and make corrections.

Reply 1:

We are so sorry for our carelessness. We have attached the corresponding letters (G-K) in figure 8, and carefully checked the figure legends and the description of the result carefully.

Comment 2:

The result describes " As for MSI, a positive correlation with the expressions of TPGS2 was found in BRCA, COAD, and STAD; a negative correlation was found in DLBC and PCPG (Figure 11B). Should this part of the result be figure 12B? Please double check and correct this.

Reply 2:

First of all, I'd like to apologize for our carelessness. We have corrected the error and checked it carefully to ensure that the article is correct and accurate. We apologize again for our mistake, and thank the reviewer for his sincere correction.

Comment 3:

What is the relationship between TPGS2 and tumor-infiltrating immune cells? What role does TPGS2 play in prognosis in tumor? It is recommended to add relevant content.

Reply 3:

Because there are few studies on TPGS2 at present, we have not found the correlation between TPGS2 and tumor-infiltrating immune cells. Therefore, the relationship between TPGS2 and tumor-infiltrating immune cells is not clear at present. Our study established the link between TPGS2 and tumor immune infiltration for the first time, which will not only increase people's cognition of TPGS2, but also improve the research level of human tumor immunity. Similarly, although we can guess from a small number of relevant studies that TPGS2 is likely to be related to tumor prognosis, so far, few studies have clearly indicated that TPGS2 is related to tumor prognosis. In other words, the relationship between TPGS2 and tumor-infiltrating immune cells and tumor prognosis is not clear. Therefore, we have not attached the relevant content.

Comment 4:

Some fonts need to be enlarged, as shown in Figure 4.

Reply 4:

It is a very good reminder. In the process of writing the manuscript, we were faced with

this problem. As suggested by reviewers, we have reduced the number of figures in Figure 4 and adjusted its font size to improve viewing efficiency. We move the other results into the supplementary results.

Comment 5:

The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “A pan-cancer analysis of the expression of gasdermin genes in tumors and their relationship with the immune microenvironment, *Transl Cancer Res*, PMID: 35116710”. It is recommended to quote the article.

Reply 5:

Thank you for your valuable suggestions. This is a very meaningful article of the similar types. This paper illustrates the important value of pan-cancer analysis in the preliminary exploration of tumor immunity research. So we have cited it in our manuscript (Page 2, line 69). In the meantime, thank you again for your recommendation.

Comment 6:

It is recommended to increase the functional experimental study of the TPGS2 gene.

Reply 6:

The experimental study on the function of TPGS2 is an important part of our research. We very much hope that the tumor immune effect of TPGS2 can be further verified in cells and animals. This is a very pertinent suggestion and one that we are considering. However, due to the short revision time and the special situation of our team, we have not yet achieved further experimental validation. Thank you again for your sincere advice.

Comment 7:

It is suggested to increase the in-depth study on the function of TPGS2 in the occurrence and metastasis of different cancers, which may make this study more complete.

Reply 7:

The profound ideas have greatly broadened our thinking, which is of great significance to the improvement of our research. We once tried to find the correlation between TPGS2 and the occurrence and metastasis of different cancers, but combined with the results of our previous research and considering the exploration of TPGS2 in tumor

prognosis and treatment, it is easier to achieve clinical transformation, and finally we focused on the study of tumor immunity. Thank you for your valuable comment.