Peer Review File

Article information: https://dx.doi.org/10.21037/tcr-23-2270

Reviewer A

The title of the article fully corresponds to the content. In the article, the authors report on a new signature associated with PD-1/PD-L1, which can be used to differentiate breast cancer and prognosis of the disease.

"Abstract" fully covers the main aspect of the work. In the "Background information" section, the authors pointed out the task to which the study was devoted: the functional role of the protein programmed cell death 1/ligand programmed cell death 1 (PD-1/PD-L1) in patients with breast cancer was analyzed. The main research methods are indicated in the "Methods" section. The Results section presents the results of a study using data from The Cancer Genome Atlas (TCGA). In the Conclusions section, the authors concluded that BC subtypes associated with the PD-1/PD-L1 pathways were identified, which were closely related to the immune microenvironment, ferroptosis status, and m6A in patients with BC. The identified gene signature involved in the PD-1/PD-L1 pathway may help to distinguish and predict the prognosis in patients with breast cancer.

The "Keywords" presented in the article correspond to the content of the article and are necessary.

In the Introduction section, the authors pointed out that breast cancer is a global health problem. A significant proportion of patients with breast cancer do not receive effective anti-cancer therapy. This is due to the existence of an unmet need for new biomarkers for the early detection, prediction and survival of patients with breast cancer.

In this study, the authors set themselves the task of conducting a systematic study to identify prognostic genes in patients with breast cancer using Cancer Genome Atlas (TCGA) sequencing data. At the same time, the authors focused on assessing the functional role of the genome in PD-1/PD-L1 status in patients with breast cancer.

In the Methods section, the authors indicated that transcriptome data and clinical information on patients with breast invasive cancer (BRCA) were obtained from the TCGA dataset. The following studies were conducted: "Identification of prognostic genes in PD-1/PD-L1 pathway", "Consensus clustering analysis of prognostic genes of the PD-1/PD-L1 pathway", "Identification of differently expressed genes (DEGs) and functional enrichment analysis", "Comparative analysis of ferroptosis and m6A between BC subtypes", "Development of a prognostic gene model in the TCGA-BC cohort", "Immunohistochemical analysis". The section presents the research that allowed us to solve the problem. The design of the study is clear.

In the Discussion section, the authors analyzed their results. Published data from other research groups were used for this purpose. It is important that the authors point out a number of

limitations inherent in computational analysis. In this regard, the authors rightly point out that experimental studies are needed in the future to uncover the potential mechanisms of these important genes.

The Key findings section presents the main findings of the study. The conclusion is fully consistent with the results of the study.

This article is interesting, timely and important for the clinic. The manuscript did not cause any ethical problems. The patient's anonymity is protected.

The figures and tables are clear and legible.

The text of the article is clearly written. The manuscript did not cause any ethical problems. All links to publications in the "References" section are necessary and correct, made in the correct style. I have no concerns about the similarity of this article to other articles published by the same authors.

Competing interests of the authors do not create bias in the presentation of results and conclusions.

Response: Thank you very much for your affirmation of our work.

Reviewer B

1) First, the title needs to indicate the development and validation of the prognosis prediction model. Since the authors did not use the seven-gene signature for differentiation, the authors should rewrite the title accordingly.

Response: Thank you very much for your helpful comments. We have followed the reviewer's suggestion by rewriting the title accordingly (line 3-4).

2) Second, abstract is inadequate. The background did not describe the current knowledge gap and the potential clinical significance of this research focus. The methods need to describe the prognosis outcome in the TCGA dataset and method for assessing the predictive accuracy of the seven-gene signature model. The results need data to support these findings including the AUC values of the seven-gene signature model. The conclusion needs to be tone down due to the unsatisfactory AUC values and no data on the external validity of the predictive model.

Response: Thank you very much for your helpful comments. We have followed the reviewer's suggestion by revising the background in the abstract (line 39-42). We have added the prognosis outcome and the predictive accuracy of the seven-gene signature model in the method (line 51-52). We have added the AUC values of the seven-gene signature model in the results (line 66-68). We have followed the reviewer's suggestion by revising the conclusion (line 71-72).

3) Third, in the introduction, the authors need to briefly review what has been known in the prognostic biomarkers and the prognosis prediction models based on these biomarkers and analyze the limitations and knowledge gaps of prior studies. Please also explain why the PD-1/PD-L1 pathway-related biomarkers could accurately predict the prognosis in BC.

Response: Thank you very much for your helpful comments. We have followed the reviewer's suggestion by revising the background in the abstract (line 39-42). We have added what has been known in the prognostic biomarkers and the limitations in the abstract (line 88-96). We have added the reasons of the PD-1/PD-L1 pathway-related biomarkers for BC in the abstract (line 101-118 and line 120-125).

4) Fourth, in the methodology, the authors need to describe the research design, the clinical variables and prognosis outcomes in the TCGA dataset, and explain why the authors did not set an independent sample to validate the prediction model. Please provide the threshold AUC values for a good prediction model. The AUCs valued presented in this paper poor, so I think the prediction model has little clinical implications.

Response: Thank you very much for your helpful comments. We have followed the reviewer's suggestion by adding the research design, the clinical variables and prognosis outcomes in the TCGA dataset in the methodology (line 152-156). We have added threshold AUC values in the methodology (line 201-203). Considering the AUCs valued in this study, we have followed the reviewer's suggestion by revising the conclusion (line 71-72).

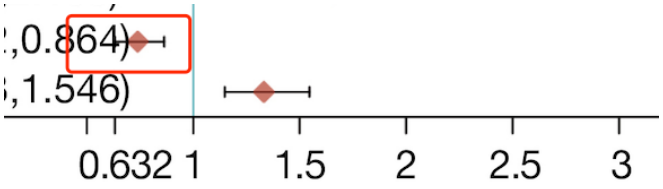
5) Finally, please cite several related papers: 1. Zhong S, Jia Z, Zhang H, Gong Z, Feng J, Xu H. Identification and validation of tumor microenvironment-related prognostic biomarkers in breast cancer. Transl Cancer Res 2021;10(10):4355-4364. doi: 10.21037/tcr-21-1248. 2. Zheng M, Wu L, Xiao R, Zhou Y, Cai J, Chen W, Chen C, Sun K, Shen S. Integrated analysis of coexpression and a tumor-specific ceRNA network revealed a potential prognostic biomarker in breast cancer. Transl Cancer Res 2023;12(4):949-964. doi: 10.21037/tcr-23-313. 3. Wang X, Li X, Jiang W. High expression of RTN4IP1 predicts adverse prognosis for patients with breast cancer. Transl Cancer Res 2023;12(4):859-872. doi: 10.21037/tcr-22-2350. 4. Xu M, Chen Z, Lin B, Zhang S, Qu J. A seven-lncRNA signature for predicting prognosis in breast carcinoma. Transl Cancer Res 2021;10(9):4033-4046. doi: 10.21037/tcr-21-747.

Response: Thank you very much for your helpful comments. We have cited several related papers in the Discussion (line 348-353).

Reviewer C

1. Figure 1

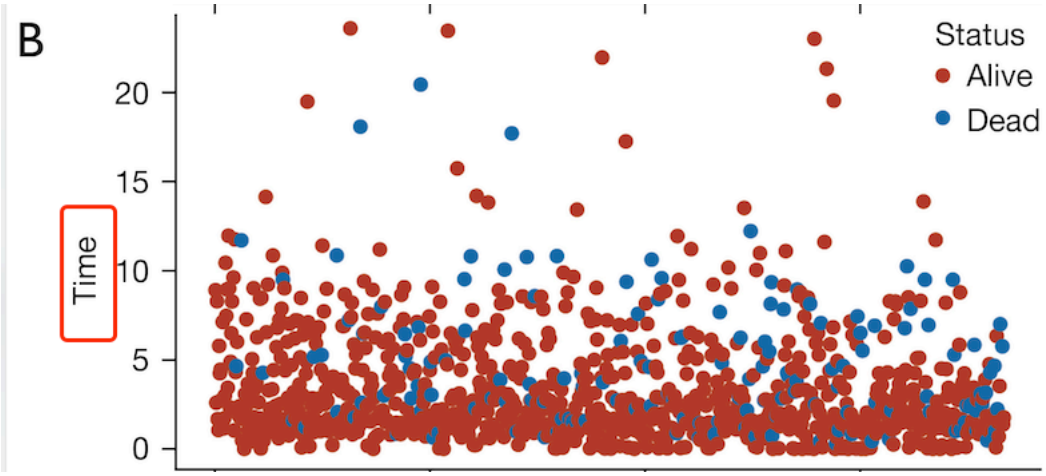
The bar was covered, please revise.



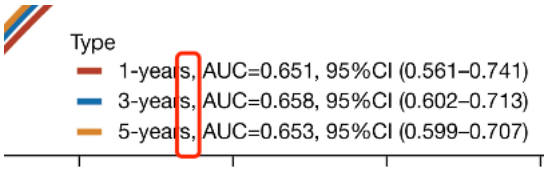
Response: Thank you very much. We have revised the Figure 1.

2. Figure 5

a) Please provide the unit of y-axis.



b) Please remove the “s”.



Response: Thank you very much. We have followed your suggestion by revising the Figure 5.

3. Supplementary Tables

Please define all the abbreviations appeared in the table title and header.

Response: Thank you very much. We have followed your suggestion by revising this problem.