Peer Review File

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Reviewer comment-Reviewer A

1) First of all, my major concern regarding this study is no predictive accuracy data of the pyroptosis-related model but in the title the authors described the focus as prognosis prediction model. In the main text, the authors only examined the prognostic role of the model, not its prognosis predictive accuracy. The authors need to revise the title and elsewhere of this paper accordingly. Please also indicate the research design in the title, i.e., a bioinformatics analysis.

   Reply 1: Thanks for the Reviewers. We changed the title “A novel pyroptosis-related model for prognostic prediction in esophageal squamous cell carcinoma” to “A novel pyroptosis-related model for prognostic prediction in esophageal squamous cell carcinoma: a bioinformatics analysis” of this article. Then, we changed the running title “Risk model of pyroptosis-related genes in ESCC” to “A bioinformatics analysis of pyroptosis-related genes in ESCC”. Thanks again.

   Changes in the text: We changed the text in Page 1, line 3-4 and line 13.

2) Second, the abstract is not adequate. The background did not indicate the clinical needs for the new prognostic factor in ESCC and what the clinical significance and knowledge gap are. The methods need to describe the clinical sample, clinical factors, and prognosis outcomes in the database, as well as statistical method for assessing the independent diagnostic role of the model. The results need to quantify the results by providing detailed effect size measure such as HR and accurate P value. The conclusion needs comments for the clinical implications of the findings.

   Reply 2: Thanks for the Reviewers. We rewrote the abstract according to your reviews. Thanks again.

   Changes in the text: We changed the text in Page 1-2, line 28-53.

3) Third, the introduction of main text needs to briefly review all known biomarkers associated with the prognosis in ESCC and have comments on their limitations and predictive accuracy to indicate the needs for new biomarkers. The authors need to further have comments on the potential strengths and clinical implications of pyroptosis.

   Reply 3: Thanks for the Reviewers. In our introduction, we added the associated papers in our article and gave the comments on the potential strengths and their limitations.

   Then, we added to indicate the needs for new biomarkers clinical implications of pyroptosis. Thanks again.

Changes in the text: We changed the text in Page 4, line 92-96; Page 5, 109-113.

4) Fourth, in the methodology of the main text, the authors need to describe the dataset in detail, including clinical factors and prognosis outcomes. In statistics, please describe the procedures of multiple Cox regression analysis to ascertain the independent prognostic role of the pyroptosis-based model. The results from univariae analysis are biased.
Reply 4: Thanks for the Reviewers. We refined the description of the database. TNM stage is closely related to prognosis and is the most widely used prognostic model in clinical treatment. We evaluated the relationship between the pyroptosis model and TNM stage to evaluate the predictive effect of the model on prognosis. Thanks again.
Changes in the text: We changed the text in Page 5, line 104-105.

5) Finally, please consider to cite the below papers: 1. Deng J, Weng X, Chen W, Zhang J, Ma L, Zhao K. A nomogram and risk classification model predicts prognosis in Chinese esophageal squamous cell carcinoma patients. Transl Cancer Res 2022;11(9):3128-3140. doi: 10.21037/tcr-22-915. 2. Lian L, Teng SB, Xia YY, Shen XM, Zheng Y, Han SG, Wang WJ, Xu XF, Zhou C. Development and verification of a hypoxia- and immune-associated prognosis signature for esophageal squamous cell carcinoma. J Gastrointest Oncol 2022;13(2):462-477. doi: 10.21037/jgo-22-69. 3. Pu Y, Lu X, Yang X, Yang Y, Wang D, Li M, Guan W, Xu M. Estimating the prognosis of esophageal squamous cell carcinoma based on The Cancer Genome Atlas (TCGA) of m6A methylation-associated genes. J Gastrointest Oncol 2022;13(1):1-12. doi: 10.21037/jgo-21-686. 4. Yan K, Wei W, Shen W, Du X, Zhu S, Zhao H, Wang X, Yang J, Zhang X, Deng W. Combining the systemic inflammation response index and prognostic nutritional index to predict the prognosis of locally advanced elderly esophageal squamous cell carcinoma patients undergoing definitive radiotherapy. J Gastrointest Oncol 2022;13(1):13-25. doi: 10.21037/jgo-21-784.
Reply 4: Thanks for the Reviewers. We cited the forward essays in our article. Thanks again.
Changes in the text：We changed the text in Page 4, line 79-91.

Reviewer comment-Reviewer B

The authors of this study constructed a prognostic model with three pyroptosis-related genes. Generally, this work is of clinical relevance. The topic is novel, and the methods used are correct. I have some comments.

1) The language quality of this manuscript is poor, and it should be carefully edited by a native speaker. Lines 77 to 79: Searching for the biomarkers or the risk model in ESCC, more and more studies focused on the genes' mutation related to ESCC patients' overall

survival (OS) and progression-free survival (PFS) in recent years. This sentence is difficult to understand. Please rephrase it.

Reply 1: Thanks for the Reviewers and Editors. We changed the title “Searching for the biomarkers or the risk model in ESCC, more and more studies focused on the genes’ mutation related to ESCC patients’ overall survival (OS) and progression-free survival (PFS) in recent years.” to “In recent years, there has been an increasing focus on studying the mutations of genes related to ESCC patients' overall survival (OS) and progression-free survival (PFS), as researchers search for biomarkers or risk models for the disease.” of this article. Thanks again.

Changes in the text: We changed the text in Page 4, line 88-90.

2) Lines 79 to 89: Several examples of the prognostic score were listed here. However, the implications of these scores, as well as the relationship between these scores and the author’s study, are unclear. Please rephrase these sentences. Two examples are adequate if the author only intended to provide some examples.

Reply 2: Thanks for the Reviewers and Editors. We rephrased these sentences and deleted the second and fourth example in our text. Thanks again.

Changes in the text: We changed the text in Page 4, line 91-95.

3) Line 86: What does PNI mean?

Reply 3: Thanks for the Reviewers and Editors. We deleted this example and PNI gene in our text. Thanks again.

Changes in the text: We changed the text in Page 4, line 90.

4) Lines 171 to 173: This paragraph should be removed.

Reply 4: Thanks for the Reviewers and Editors. We removed the paragraph of “#Results ##Pyroptosis-related score according to GSEA”. Thanks again.

Changes in the text: We changed the text in Page 7, line 183.

5) Line 243: what does resent mean?

Reply 5: Thanks for the Reviewers and Editors. We deleted the word and changed the sentence. Thanks again.

Changes in the text: We changed the text in Page 9, line 260.

6) Lines 248 to 249, what does 1-way logistic regression mean?

Reply 6: Thanks for the Reviewers and Editors. We removed the expression in the text. Thanks again.

Changes in the text: We changed the text in Page 9, line 265.

7) The limitations of this study should be discussed in the discussion section.

   Reply 7: Thanks for the Reviewers and Editors. We added the limitations "However, there were several limitations in our study. First, we collected 75 ESCC patients which might not be adequate for a comprehensive analysis. Second, the vertical extent of our study which deeply explored the mechanisms of these 3 genes and the interaction with pyroptosis and immunity was not sufficient. Thrid, this research was short of other cohort and a follow-up for further validation. Thus, the pyroptosis and immunity associated signature explored in this study requires further validation by more prospective studies." in our text. Thanks again.

   Changes in the text: We changed the text in Page 10, line 318-324.

8) Lines 288 to 294, this paragraph should be rephrased. The logic of this paragraph is unclear.

   Reply 8: Thanks for the Reviewers and Editors. We rephrased the paragraph. Thanks again.

   Changes in the text: We changed the text in Page 10, line 311-317.

9) Lines 90 to 91, the authors believe that the previous studies are retrospective and the sample size is small. However, this study is also a retrospective study with a small sample size. Please discuss the strength of this study in the discussion and introduction sections.

   Reply 9: Thanks for the Reviewers and Editors. We added this part into introduction. Thanks again.

   Changes in the text: We changed the text in Page 5, line 116-119.

10) Line 95 to 98, a reference should be cited there.

   Reply 10: Thanks for the Reviewers and Editors. We found the reference and cited the text. Thanks again.

   Changes in the text: We changed the text in Page 5, line 106.

11) Lines 103 to 109, these three sentences are hard to understand. What is the rationale among them? Please rephrase these sentences.

   Reply 11: Thanks for the Reviewers and Editors. We rephrased the sentences of "The dual impact of pyroptosis is believed to have opposing effects on tumor growth. Prolonged and persistent injury may accelerate tumor growth as the exacerbation caused by pyroptosis is amplified by age and an inflammatory microenvironment surrounding the diseased cells. However, on the other hand, the strong impact of pyroptosis triggers the activation of various immune cells to suppress cancer growth". Thanks again.

Changes in the text: We changed the text in Page 5, line 111-116.

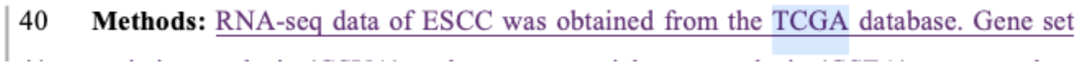
12) The introduction should be reorganized. The current version lacks readability, and the rationale is unclear.

Reply 12: Thanks for the Reviewers and Editors. We rephrased all the part of Introduction. Thanks again.

Changes in the text: We changed the text in Page 4-5, line 71-155.

Reviewer comment-Reviewer C

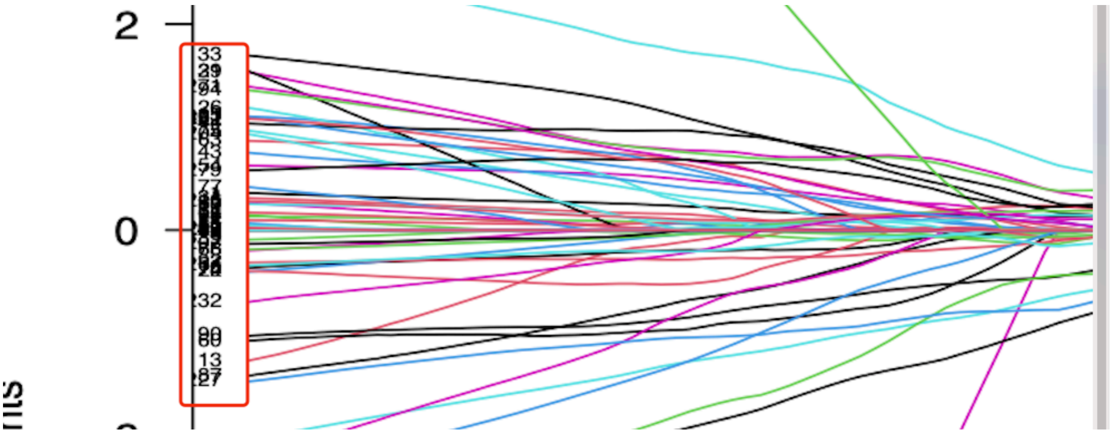
1. Please define TCGA in Abstract.



Methods: RNA-seq data of ESCC was obtained from the TCGA database. Gene set

Reply: We added the definition of TCGA. Thanks again.

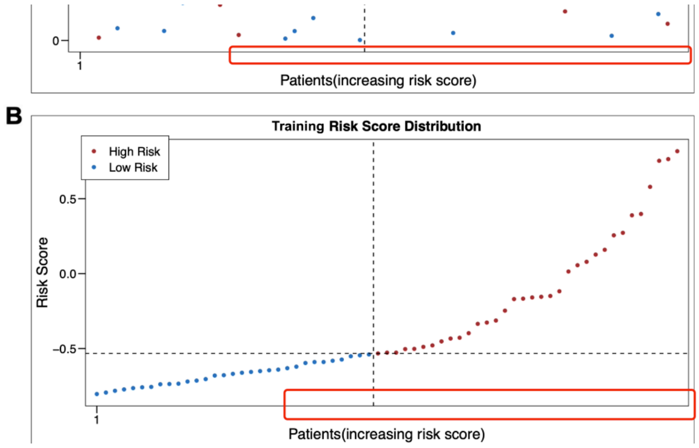
2. Figure 2: The numbers are overlapped, please check and revise.



Reply: We revised the Picture. Thanks again.

3. Figure 3

a. Are there any missing numbers on the X-axis? Please check.



Reply: We revised the Picture. Thanks again.

b. Please define ESCC in figure legends.

Reply: We defined ESCC in figure legends. Thanks again.

4. Figure 4: Please define below abbreviations in figure legends.

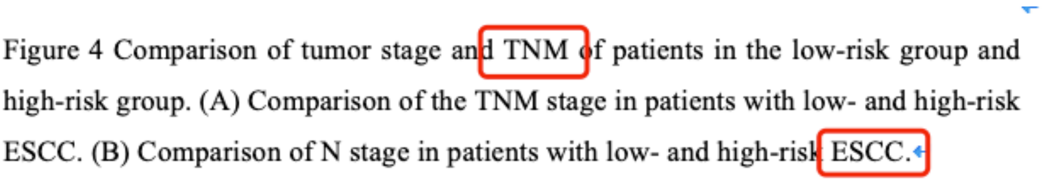


Figure 4 Comparison of tumor stage and TNM of patients in the low-risk group and high-risk group. (A) Comparison of the TNM stage in patients with low- and high-risk ESCC. (B) Comparison of N stage in patients with low- and high-risk ESCC.

Reply: Thanks for Editors. We defined TNM and ESCC in figure legends. Thanks again.

5. Please define all abbreviations in Figure 5 legends.

Reply: We defined all abbreviations in figure legends. Thanks again.

6. Please define OR and CI in Table 1 footnote.

Reply: We defined all abbreviations in Table legends. Thanks again.