

Peer Review File

Article Information: <https://dx.doi.org/10.21037/jgo-24-245>

Reviewer A

Comment 1: Try to clarify the genes' significance and their relationship or why they are key biomarkers (LINE 35-36).

Reply 1: Thank you for your valuable feedback. We have revised the text to provide a more detailed explanation of the significance of these genes and their interrelationship, as well as why they are key biomarkers. (see Page 2, line 35-37)

Changes in the text: This study highlights the prognostic potential of PANoptosis-related features in colorectal cancer, demonstrating their role as key biomarkers significantly associated with patient survival and aiding in the identification of high-risk patients, thereby advancing immunotherapy approaches.

Comment 2: It would be better to include some of these genes if possible and try to explain the evidence still open to validation since you used public datasets and not primary datasets (LINE93-94)

Reply 2: Thank you very much for your feedback. We have considered the possibility of including these genes and have incorporated relevant genes into our study as much as possible. However, as you mentioned, since we used public datasets rather than primary datasets, some evidence still needs further validation. We encourage subsequent research to use more primary data to validate these findings, ensuring the reliability and generalizability of the results. (see Page 4, line 92-98)

Changes in the text: In this study, we analyzed the mRNA expression dataset from The Cancer Genome Atlas (TCGA) database, profiling hallmark gene sets in 404 cases of colorectal cancer (CRC). We identified PANoptosis-related key genes and constructed a prognostic model based on these genes. The model demonstrated high predictive accuracy for CRC prognosis, with significant associations found between high PANoptosis risk scores and poorer survival outcomes. Additionally, our study highlighted the potential of these genes as biomarkers for CRC diagnosis and prognosis, offering insights into the molecular mechanisms underlying CRC progression and aiding in the advancement of personalized treatment strategies.

Comment 3: Table S1 and Table S2 are not present in the manuscript unless you were referring to the supplementary check-list and that should be clearly stated (LINE106 and LINE 114)

Reply 3: Thank you for your thorough review of our manuscript. We appreciate your feedback and have made the following changes to clarify the location of the supplementary tables. (see Page 4, line 109 and line 116)

Changes in the text: Clinical characteristics were detailed in Supplementary Table 1. In total, 485 non-redundant genes were identified and included for further analysis (Supplementary Table 2).

Comment 4: If it's fine, you can supplement these 88 PANoptosis-related DEGs (LINE 119)

Reply 4: Thank you for your valuable feedback. We have supplemented the information regarding the 88 PANoptosis-related differentially expressed genes (DEGs) in the supplementary materials. (see Page 4, line 122)

Changes in the text: Following the filtering process, 88 PANoptosis-related DEGs were pinpointed for further validation (Supplementary Table S3).

Comment 5: In the discussion part, refer to LINE 281, you could have investigated these four genes in the literature review and how they are connected to PANoptosis to boost your findings more vividly.

Reply 5: Thank you for your valuable suggestion. We have reviewed the relevant literature to investigate the connection between the four genes and PANoptosis. This additional context helps to better illustrate our findings and provides a more vivid understanding of their biological implications in CRC. (see Page 8, line 277-281)

Changes in the text: Inhibiting TIMP1 expression increases apoptosis of colorectal cancer cells and reduces cancer proliferation and metastasis by inducing TIMP1-specific regulation of the FAK-PI3K/AKT and MAPK pathways. High expression of CDKN2A in CRC leads to poor prognosis, while knocking down CDKN2A expression can promote apoptosis and cell cycle progression, affect the EMT process in CRC, and thereby inhibit cancer cell proliferation.

Comment 6: Minor comment, try to check for run-on sentences in a language wise.

Reply 6: Thank you for your feedback. We have reviewed the manuscript to address any run-on sentences and ensure clarity in language use. (see Page 3, line 50-52, 62-63, 67-68, 77, 79; Page 4, line 99-100; Page 8, line 297-300)

Changes in the text: Notably, mutations in KRAS and BRAF have been identified as indicators of poor response to epidermal growth factor receptor inhibitors, which correlate with reduced overall and progression-free survival rates. Intriguingly, in a mouse model subjected to IAV infection, caspase-6 deficiency hinders PANoptosis activation, resulting in diminished viral elimination. Therefore, a comprehensive study of the role of PANoptosis in the progression of CRC is warranted. However, increased angiogenesis, epithelial-mesenchymal transition, and hypoxia levels are associated with the tumor's invasiveness, metastatic tendencies, and malignancy, potentially diminishing the efficacy of immunotherapy and allowing tumor cells to evade the immune system.

Reviewer B

Comment: *Ref 7* and *Ref 28* seem duplicates. Please recheck.

Reply: Thank you for bringing this to our attention. We have reviewed and corrected the references. The duplicates have been removed, and the reference list has been updated accordingly. We appreciate your understanding and patience.

Comment: When reporting P values, authors should follow guidelines listed below. Please modify them in the main text, figures, and tables, and ensure they are consistent.

- The description of the P value should be in the **uppercase** format, i.e., "P".
- **If P value <0.001**, report "P<0.001" to avoid reporting unnecessarily excessive precision (except hypothesis tests that include correlations or studies with exponentially small P values, such as genetic association studies, which can be reported exponentially, e.g., $P=1 \times 10^{-5}$).
- **If $0.001 \leq P$ value <0.01**, report the specific P value to 3 decimal places, e.g., "P=0.001" or "P=0.009".
- **If P value ≥ 0.01** , report the specific P value to 2 decimal places, e.g., "P=0.01" "P=0.06" "P=0.10" "P=0.90". When the P value is near 0.05, report the specific P value to 3 decimal places, e.g., "P=0.046" or "P=0.052".
- **If the P value is >0.99**, report "P>0.99".
- Do not round P values, do not report "not significant" simply because the data is greater than an arbitrary value, and do not report only vague bounds such as $P < 0.05$, as described above, but report the exact P value.


Reply: Thank you for your feedback. We will review and modify the reporting of P values in the main text, figures, and tables according to your guidelines. We will ensure consistency throughout the manuscript.

Comment: All abbreviations in figures/tables and legends should be explained. TCGA, KEGG, DEGs, LASSO, NJCRC, and GSEA in Figure 1 for example. Please check all your figures and tables.

Reply: All abbreviations in figures, tables, and legends have been explained, including TCGA, KEGG, DEGs, LASSO, NJCRC, and GSEA in Figure 1.

Comment: Please consider supplementing a head for the x-axis in Figure 2A and completing the value of bars.

Note: For the forest plots, to standardize the results, the part that exceeds the horizontal coordinates should be indicated by the arrow below. Or you can add an additional scale bar on the x-axis.

TLR3 1.16e-02 0.8 (0.67 - 0.95) 

Reply: The forest map now includes an indication of the portion above the horizontal coordinates with an additional scale on the X-axis.

Comment: It is suggested to unify the "LASSO" and "Lasso" in your main text and the figures.

Reply: The usage of "LASSO" and "Lasso" has been unified throughout the main text and figures.

Comment: It is suggested to modify "RiskScore" to "Risk Score" in your figures. **Please also check through your figures to ensure proper spaces have been added among the words.**

RiskScore

Reply: "RiskScore" has been modified to "Risk Score" in all figures, and proper spacing between words has been ensured.

Comment: Please provide explanations for "*", "**", "***", "****", and "ns" in the legend of Figures 5 and 6.

Reply: Explanations for "*", "**", "***", "****", and "ns" have been added to the legend of Figures 5 and 6.