Peer Review File

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Reviewer A

The manuscript TCR-23-463-CL consists of a great deal of analysis of data and is a potentially valuable addition to the literature, but requires improvement of its language, and clarification of a few contradictions to be acceptable for publication.

I do not have the time to indicate all the smaller language corrections needed. However, I have suggested alterations to the Title, the Abstract and the Conclusion that would make these important elements more consistent with the data and analysis given in the text. I have pointed out a few apparently glaring inconsistencies in the Discussion which should be corrected. In addition, I have indicated some smaller corrections in the manuscript. Similar small corrections are needed throughout the text.

I have attached a Word version of the manuscript with my suggested changes in color.

Reply: Thank you for your reminding. Your modification helped me too much. Thank you again. That's very kind of you. All the changes have been marked in red.

Reviewer B

1) Have potential confounding factors, such as patient age, tumor stage, and treatment regimens, been considered in this analysis?

Reply: Sincerest thanks for your response and reviewers comments on our manuscript. Our answer to your question is yes, of course, in our analysis we took into account potential confounding factors that could have affected the result. Our study carefully considered patient age, tumor stage, and treatment options. We performed a stratified analysis to assess how these factors affected the result. Specifically, we performed analyses across different age groups and tumor stages to examine the consistency of our findings across these subgroups. In addition, in the multivariate Cox regression analysis that construct the predictive model, we included these factors as covariates to adjust for their potential impact on the risk assessment. This approach allows us to better understand the independent contribution of COL6A6 mutation status while controlling for the influence of patient age, tumor stage, and treatment regimen. To provide a comprehensive understanding, we also performed a subgroup analysis based on these confounding factors. For example, we evaluated the relationship between risk scores and patient outcomes for different age groups and tumor stages, which allowed us to explore how risk

assessments might vary in specific patient subgroups. Overall, we have taken steps to address potential confounding factors to ensure the robustness and reliability of our analysis. Thank you again for your valuable feedback.

2) How do the identified DEGs contribute to the tumorigenesis and progression of COAD?

Reply: Thanks for your review. The DEGs (differentially expressed genes) identified in our analysis play a crucial role in uncovering the underlying mechanisms of COAD tumor occurrence and progression. These genes show significant differences in expression between different mutant groups, and changes in their expression levels suggest that they may be involved in the disease process. We performed functional enrichment analyses of these DEGs to gain insight into their biological significance. These analyses include pathway enrichment and gene ontology (GO) analysis, which allows us to identify key biological processes, molecular functions, and cellular components associated with DEGs. In addition, many DEGs are known to participate in pathways related to processes such as lipid metabolism, cell cycle regulation, and immune response. Alterations in these pathways are associated with the onset and progression of cancer. Up-regulation or down-regulation of specific genes in these pathways may contribute to various aspects of COAD pathogenesis, such as cell proliferation, invasion, and immune evasion. It is important to note that the exact contribution of individual DEGs to the progress of COAD may vary, and a full understanding requires further experimental validation. However, our analysis provides a basis for identifying potential molecular actors that could be targeted for further COAD research and therapeutic interventions. Thank you again for your valuable feedback.

3) Given the findings related to HLA family gene expression, what might be the implications of higher MHC I and II expression in the high-risk group?

Reply: Thanks for your review. Regarding the higher expression of HLA (Human leukocyte antigen) family genes, particularly MHC Class I and Class II molecules, in high-risk populations, there are several potential effects on colorectal cancer (COAD) : 1, Immune recognition and tumor surveillance: Increased expression of MHC Class I and Class II molecules may mean enhanced antigen presentation to immune cells, such as T cells. This may enhance immune recognition of tumor-specific antigens, thereby facilitating immune surveillance and targeted destruction of cancer cells. 2, Tumor antigen presentation: MHC Class I molecules present intracellular derived antigens to cytotoxic T lymphocytes (CTLS), eliminating cells that present abnormal or tumor-associated antigens. Higher MHC Class I expression in high-risk populations may indicate an active immune response against tumor antigens, which may imply a more aggressive anti-

tumor immune response. 3, Tumor infiltrates immune cells: Elevated MHC expression may attract and stimulate immune cell infiltration into the tumor microenvironment. Although the specific immune cell composition needs further study, an increase in immune cells, such as CD8+ T cells, may be associated with a more powerful anti-tumor response. 4, The potential of immunotherapy: High MHC expression may indicate that tumors in high-risk populations are more immunogenic, potentially making them more sensitive to immunotherapy approaches such as immune checkpoint inhibitors or adoptive T cell therapy. 5, Immune escape mechanism: On the other hand, elevated MHC expression may also reflect an attempt by tumor cells to evade immune surveillance by overexpressing antigens as a form of antigen masking. Tumor cells may "exhaust" immune responses by presenting antigens that lead to immune tolerance or dysfunction. 6, Tumor heterogeneity: Variability in MHC expression may reflect tumor heterogeneity, in which some subclones within the high-risk group may have a more active immune response, while others may have immune escape mechanisms. It is important to note that the effect of higher MHC expression in high-risk populations is complex and may be influenced by a number of factors, including the specific tumor microenvironment, interactions between tumor cells and immune cells, and the overall immune response. Further experimental and clinical studies are needed to validate these effects and determine the functional consequences of MHC expression in the progression of COAD. Thank you again for your valuable feedback.

4) Have you considered potential interactions or crosstalk among the five genes and their potential implications for COAD prognosis?
Please address these questions in your revised manuscript and discuss any limitations of the study and suggest potential avenues for future experimental validation and clinical correlations.
Reply: Thanks for your review. In response to your question, we have considered potential interactions and crosstalk between five genes (MUC16, ASNSP1, PRR18, PEG10, and RPL26P8), which we have identified as independent prognostic factors for COAD. These genes may not function individually, but collectively influence COAD prognosis as components of a complex molecular network. While the exact mechanism of their interaction requires further study, we can suggest some potential effects: 1, Pathways crosstalk: The identified genes may be involved in common pathways or networks associated with cancer progression. They may be involved in shared signaling cascades, transcriptional regulation, or metabolic pathways that have synergistic or antagonistic effects on tumor development and therapeutic response. 2, Photosynthesis: Some of these genes may cooperate to regulate specific cellular processes. For example, they can work together to regulate cell cycle progression, apoptosis,

immune escape, or angiogenesis, which are critical for cancer progression. 3, Immune response regulation: These genes may play a role in shaping the tumor microenvironment and immune response. The interaction between them may affect the recruitment and activity of immune cells within the tumor, thereby affecting overall immune surveillance and response to therapy. 4, Therapeutic potential: Understanding the interactions between these genes could have therapeutic implications. Targeting multiple genes in a network may be more effective at altering disease trajectories than targeting a single gene. 5, Biomarker characteristics: Combinations of these genes may form prognostic or predictive biomarker signatures that provide a more comprehensive view of COAD prognosis than individual genes alone. Thank you again for your valuable feedback.