Peer Review File
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Reviewer A

The authors reported "the identification and prediction of lung adenocarcinoma prognosis using a novel gene signature associated with DNA replication".
The authors concluded "The contents of this manuscript may provide novel insights into the diagnosis and treatment of LUAD". I could not find novel insights into the diagnosis and treatment in this manuscript. Please describe the novel insights for the diagnosis and treatment of LUAD.
Response: Thanks very much for the reviewer's suggestion. We have revised the problem provided by the reviewer for the manuscript (line 288-303 and line 339-346). Some reasons are as following:

Lung cancer is the most prevalent and lethal type of cancer, and lung adenocarcinoma (LUAD) accounts for approximately 60% of non-small-cell lung cancer cases (1,2). Patients with LUAD usually undergo treatments such as surgery, chemotherapy, radiation therapy, and molecular targeted therapy. However, due to rapid tumor growth and resistance to existing treatments, only about 30% of LUAD patients survive beyond five years (1, 3). Consequently, it is critical that research is conducted to gain an understanding of the mechanisms underlying LUAD. In addition to curative surgeries like lobectomy or segmental lung resection with or without adjuvant chemotherapy, or radiotherapy, more precise treatments like epidermal growth factor receptor (EGFR)-targeted therapy and immunotherapy have enhanced the survival of LUAD patients (4).

Genome instability is a characteristic feature of cancer, and DNA replication is the cellular process most susceptible to it (7). High levels of DNA damage can lead to replication stress, causing genome instability (7). A novel and promising therapeutic strategy focusing on enhancing the integration of damaged deoxynucleoside triphosphates (dNTPs) in cancer cells has been suggested. Targeting NUDT1 (also known as MTH1), a protein that prevents the misincorporation of oxidized dNTPs during replication and is essential in cancer cells but dispensable in normal cells, can lead to the selective killing of cancer cells (7-9). Understanding the molecular mechanisms underlying replication stress is essential in understanding tumorigenesis.

In this study, a total of 2,412 prognostic genes were obtained, and the DNA replication-related pathways closely associated with LUAD were identified by a KEGG enrichment analysis. TCGA-LUAD patients were divided into low- and high-risk

groups based on the 15 DNA replication-related genes (i.e., *FEN1*, *MCM5*, *POLD2*, *MCM4*, *MCM6*, *SSBP1*, *POLE2*, *RFC2*, *MCM2*, *PCNA*, *POLA2*, *MCM7*, *RFC3*, *POLE4*, and *RPA3*). The upregulated genes were mainly related to the hallmarks of cancer (e.g., chromosome segregation, DNA replication, the cell cycle checkpoint, and DNA helicase activity), while the downregulated genes were mainly related to leukocyte activation involved in inflammatory macrophage activation, and passive transmembrane transporter activity. The immune cells, including the B cells, endothelial cells, NK cells, $CD4^+$ T cells, and $CD8^+$ T cells, in the G1 LUAD samples were clearly different from those in the G2 LUAD samples, and five of the 10 ICI-related genes were higher in the G1 LUAD samples than the G2 LUAD samples. The tumor stemness of the two LUAD subtypes differed significantly. Further, a six-gene (*FEN1*, *MCM5*, *POLD2*, *MCM4*, *SSBP1*, and *POLE4*) prognostic model was constructed to predict the prognosis for LUAD patients.

Changes in the text: Thus, this study highlights the significance of a comprehensive bioinformatic analysis in understanding LUAD. By identifying key genes associated with prognosis, the developed six-gene prognostic model can be a valuable tool for clinicians. It has the potential to enhance patient stratification and inform treatment strategies, ultimately aiming to improve outcomes for LUAD patients. Focusing on DNA replication-related genes as prognostic markers indeed represents a novel and insightful approach to LUAD research. This emphasis addresses the critical need for improved patient stratification, which can significantly enhance personalized treatment strategies. By identifying specific genes involved in DNA replication that correlate with patient outcomes, this study not only fills a gap in the existing literature but also paves the way for further exploration into how these genes can influence tumor behavior and response to therapy. This unique perspective could lead to new biomarkers and therapeutic targets, ultimately contributing to better management of LUAD patients. Such predictive models are crucial for personalized medicine, allowing for more tailored and effective interventions based on individual patient profiles.

Reviewer B

The identification and prediction of lung adenocarcinoma prognosis using a 9 novel gene signature associated with DNA replication

Comments on the Manuscript

It is my pleasure to review your manuscript on the prognostic potential of DNA replication-related genes in LUAD. The study presents a comprehensive bioinformatic

analysis and offers a promising perspective on the classification and prognosis of lung adenocarcinoma (LUAD). Below are my observations and suggestions for improvement:

Strengths

1. Innovative Approach: The focus on DNA replication-related genes as prognostic markers is novel and addresses a critical need for better LUAD patient stratification. This unique angle contributes significantly to the existing literature.

Response: Thanks very much for the reviewer's affirmation.

2. Comprehensive Analysis: The study uses diverse methodologies, including KEGG pathway enrichment, immune activity assessment, and prognostic model construction, to derive meaningful insights.

Response: Thanks very much for the reviewer's affirmation.

3. Potential Clinical Utility: The constructed six-gene prognostic model has potential for practical application in predicting LUAD patient outcomes and guiding therapeutic decisions.

Response: Thanks very much for the reviewer's affirmation.

Concerns

Major Issues

1. Limited Model Accuracy: The reported AUC values (1 year: 0.629, 3 years: 0.634, 5 years: 0.582) suggest moderate predictive ability. This raises concerns about the practical utility of the model in clinical settings. Including a comparison with existing prognostic models would clarify its relative performance.

Response: Thank you for your valuable feedback on our research. Regarding the moderate performance of our model in terms of predictive ability at 1 year (AUC=0.629), 3 years (AUC=0.634), and 5 years (AUC=0.582) that you mentioned, we highly value your attention and are willing to explore this further (line 339-346).

Changes in the text: Although these AUC values reflect a moderate level of predictive ability of the model, it should be emphasized that this does not necessarily mean that the model has no value in clinical applications. In fact, in many clinical settings, especially in the prognostic assessment of complex diseases, moderate predictive ability can still provide important references for clinical decision-making. We will further emphasize this point in the discussion section and discuss how the model can be combined with clinical practice to assist doctors in making more comprehensive decisions.

Secondly, we also consider improving the performance of the model in future research, such as by integrating other biomarkers or clinical parameters to enhance predictive ability. Your feedback will help us provide more insights in this area.

Thank you again for your attention and suggestions on our work. We will carefully consider your opinions to enhance the overall quality of our work.

2. Immune Microenvironment Analysis: While the immune activity differences between G1 and G2 groups are highlighted, the underlying mechanisms remain unexplored. A deeper discussion of how DNA replication genes influence immune cell behavior and checkpoint gene expression would add substantial value.

Response: Thanks very much for the reviewer's suggestion. We have revised the problem provided by the reviewer for the manuscript (line 328-338).

Changes in the text: The interplay between DNA replication and immune checkpoint regulation is particularly relevant in the context of cancer. Tumor cells often exploit DNA replication stress to evade immune detection, leading to poor prognoses and treatment outcomes. For example, cancer stem cells (CSCs) can exhibit increased DNA repair capabilities and immune evasion, which contributes to their radioresistance and therapeutic failure [16]. Moreover, the accumulation of cytosolic DNA as a result of replication stress can trigger innate immune responses, highlighting a complex relationship where DNA replication not only influences immune cell activity but also shapes the immune landscape within tumors [17]. Thus, investigating the effects of DNA replication on immune cell function and checkpoint regulation is crucial for advancing our understanding of immune responses in health and disease, particularly in cancer immunotherapy [18, 19].

References:

[16] Meyer F, Engel AM, Krause AK, et al. Efficient DNA Repair Mitigates Replication Stress Resulting in Less Immunogenic Cytosolic DNA in Radioresistant Breast Cancer Stem Cells. Front Immunol. 13:765284.

[17] Sugimura N, Kubota E, Mori Y, et al. Reovirus combined with a STING agonist enhances anti-tumor immunity in a mouse model of colorectal cancer. Cancer Immunol Immunother. 2023;72(11):3593-3608.

[18] Sayson SL, Fan JN, Ku CL, Lo JF, Chou SH. DNAJA3 regulates B cell development and immune function. Biomed J. 2024;47(2):100628.

[19] Murayama T, Nakayama J, Jiang X, et al. Targeting DHX9 Triggers Tumor-Intrinsic Interferon Response and Replication Stress in Small Cell Lung Cancer. Cancer Discov. 2024;14(3):468-491.

Recommendations

To enhance the impact and robustness of the manuscript, I recommend the following:

• Include a comparative analysis of your model with existing prognostic models to demonstrate its strengths and limitations.

Response: Thanks very much for the reviewer's suggestion. We have revised the problem provided by the reviewer for the manuscript (line 347-353).

Changes in the text: Compared to the previous study, they identified 2- transcription factor signature (1-year AUC = 0.73, 2-year AUC = 0.60 and 3-year AUC = 0.61) based on the TCGA database [20]. In Ai et al. research, fatty acid synthesis and metabolism were used to construct the prognosis model for hepatocellular carcinoma, and the ROC curves indicated that the AUC for the high-risk group exceeded 0.7 at the 1, 3, and 5-year marks [21]. We have added the limitations of our study in the discussion.

References:

[20] Zhengdong A, Xiaoying X, Shuhui F, Rui L, Zehui T, Guanbin S, Li Y, Xi T, Wanqian L. Identification of fatty acids synthesis and metabolism-related gene signature and prediction of prognostic model in hepatocellular carcinoma. Cancer Cell Int. 2024 Apr 7;24(1):130.

[21] Yang Y, et al. A novel transcription factor-based signature to predict prognosis and therapeutic response of hepatocellular carcinoma. Front Genet. 2022; 13: 1068837.

• Provide a more detailed discussion of the immune microenvironment findings, including potential mechanisms and therapeutic implications.

Response: Thanks very much for the reviewer's suggestion. We have revised the problem provided by the reviewer for the manuscript (line 328-338).

Changes in the text: The interplay between DNA replication and immune checkpoint regulation is particularly relevant in the context of cancer. Tumor cells often exploit DNA replication stress to evade immune detection, leading to poor prognoses and treatment outcomes. For example, cancer stem cells (CSCs) can exhibit increased DNA repair capabilities and immune evasion, which contributes to their radioresistance and therapeutic failure [16]. Moreover, the accumulation of cytosolic DNA as a result of replication stress can trigger innate immune responses, highlighting a complex relationship where DNA replication not only influences immune cell activity but also shapes the immune landscape within tumors [17]. Thus, investigating the effects of DNA replication on immune cell function and checkpoint regulation is crucial for advancing our understanding of immune responses in health and disease, particularly in cancer immunotherapy [18, 19].

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[17] Sugimura N, Kubota E, Mori Y, et al. Reovirus combined with a STING agonist enhances anti-tumor immunity in a mouse model of colorectal cancer. Cancer Immunol Immunother. 2023;72(11):3593-3608.
[18] Sayson SL, Fan JN, Ku CL, Lo JF, Chou SH. DNAJA3 regulates B cell development and immune function. Biomed J. 2024;47(2):100628.
[19] Murayama T, Nakayama J, Jiang X, et al. Targeting DHX9 Triggers Tumor-Intrinsic Interferon Response and Replication Stress in Small Cell Lung Cancer. Cancer Discov. 2024;14(3):468-491.

I appreciate the effort and thoughtfulness that went into this work and believe it holds promise with these improvements. Thank you for the opportunity to review this manuscript.

Response: Thanks very much for the reviewer's affirmation.