Peer Review File

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Reviewer A

In this study, the authors aimed to develop a model based on the genes associated with cellular senescence scores, which could serve as a prognostic tool for individuals diagnosed with HCC. Using TCGA-HCC dataset and the Kyoto Encyclopedia of Genes and Genomes (KEGG), they found that the prognostic outcomes of the HCC patients were predicted using a cellular senescence-related gene model that included VDAC2, CXCL8, MYBL2, RAD9A, LIN52, RHEB, GADD45G, E2F5, MAP2K2, CDC25A, PPP1CB, and HRAS. The Authors concluded that their prognostic model based on cellular senescence-related gene expression may provide insights that can be used to develop novel potential targeted therapies.The study is of interest as it provides novel findings of potential therapeutic impact in the era of "precision medicine". However, some issues should be provided to further improve the manuscript.

1) HCC is characterized by high biological and heterogeneity. This is significantly related to the etiology (viral vs non-viral liver, alcohol, autoimmune, metabolic) of underlying liver disease causing HCC development. Therefore, it would be important to describe (if available) the main etiologies of HCC patients in the TCGA-HCC dataset. This would help to understand whether the results are realistically applicable to all patients regardless of aetiology.

Reply: We sincerely appreciate the reviewer's insightful comment regarding the etiological heterogeneity of HCC and its potential impact on the generalizability of our findings. We fully agree that etiological diversity (viral vs non-viral liver, alcohol, autoimmune, metabolic) represents a critical biological variable in HCC pathogenesis. However, the patients from TCGA database did not have the above information (viral vs non-viral liver, alcohol, autoimmune, metabolic).

Changes in the text: line 70-80.

2) Of potential interest, recent cohort studies have demonstrated that capecitabine, an oral 5-fluorouracil pro-drug, has a promising anti-tumor efficacy in HCC patients failing first-line systemic therapy with a good safety profile, as demonstrated (J Cancer Res Clin Oncol. 2018;144:403-414. doi: 10.1007/s00432-017-2556-6; Dig Liver Dis. 2015;47:518-22. doi: 10.1016/j.dld.2015.03.010). Since capecitabine causes cell senescence (doi: 10.1111/bph.13725.), the authors should expand the discussion about the potential therapeutic impact of their study results establishing a significant correlation between cellular senescence-related genes, and tumor classification/immune response in HCC. The cellular senescence-related signature could serve as promising prognostic tools for identifying HCC patient responding to systemic treatment(s). In particular, this approach can be suggested in future studies to investigate the prognostic significance of cellular senescence-related genes and response to capecitabine that cause cellular senescence and have anti-tumor efficacy in HCC patients, as demonstrated (J Cancer Res Clin Oncol. 2018;144:403-414. doi: 10.1007/s00432-017-2556-6; Dig Liver Dis. 2015;47:518-22. doi: 10.1016/j.dld.2015.03.010.). This point should recalled and discussed to improve the clinical/therapeutic impact of the study.

Reply: We sincerely appreciate the reviewer's insightful suggestion regarding the clinical relevance of cellular senescence-related signatures and their potential interplay with

capecitabine therapy in HCC. We fully agree that bridging our findings to therapeutic strategies targeting senescence holds significant translational value. In response to this constructive feedback, we have substantially expanded the Discussion section as follows:
Notably, our senescence-related gene signature (SRGS) holds particular promise for optimizing patient stratification in systemic therapy. Recent clinical studies highlight capecitabine—an oral fluoropyrimidine prodrug—as an emerging option for advanced HCC patients refractory to first-line therapies (Zhu et al., J Cancer Res Clin Oncol 2018; Dig Liver Dis 2015). Mechanistically, capecitabine induces tumor cell senescence through thymidylate synthase inhibition and DNA damage response activation, mirroring key pathways enriched in our SRGS-high subgroup (e.g., p16INK4a/CDKN2A upregulation and SASP factor overexpression).
Changes in the text: line 354-362.

Reviewer B

Introduction
Strengths

- Clearly establishes the significance of hepatocellular carcinoma (HCC) as a major health concern.

Reply: Thank you for your comment. Hepatocellular carcinoma (HCC) is indeed a significant global health concern, being one of the most prevalent types of liver cancer and a leading cause of cancer-related mortality. It is often associated with underlying liver diseases such as cirrhosis and hepatitis, which are prevalent worldwide, particularly in regions with high rates of viral infections. The increasing incidence of HCC, coupled with its typically late diagnosis and poor prognosis, underscores the urgent need for improved understanding and management of this disease. Our research focuses on the molecular characterization of senescence-related gene signatures in HCC to enhance prognostic assessments and develop targeted therapeutic strategies, ultimately addressing this pressing health issue.

Changes in the text: line 70-81.

- Highlights the relevance of cellular senescence in cancer progression, which is a novel angle for prognosis modeling.

Reply: Thank you for your feedback. Cellular senescence is increasingly recognized as a crucial factor in cancer progression, including hepatocellular carcinoma (HCC). Our study aims to explore the role of senescence-related genes in HCC to provide deeper insights into the tumor microenvironment and its implications for disease progression. By integrating senescence markers into our prognostic models, we hope to offer a novel perspective that enhances the predictive accuracy for patient outcomes. This approach not only sheds light on the biological mechanisms underlying HCC but also paves the way for potential therapeutic interventions targeting cellular senescence pathways.

Changes in the text: line 99-114.

- Provides a logical rationale for investigating senescence-related gene signatures.

Reply: Thank you for your valuable feedback. The rationale for investigating senescence-related gene signatures in liver cancer stems from the critical role that cellular senescence plays in tumorigenesis and cancer progression. Senescent cells within the tumor microenvironment can influence various aspects of tumor biology, including inflammation, immune response, and tissue remodeling. By identifying and analyzing specific gene signatures associated with senescence, we aim to uncover potential biomarkers that can predict patient outcomes and inform treatment strategies. This approach not only enhances our understanding of liver cancer biology but also paves the way for developing targeted therapies aimed at modulating senescence in tumors.

Changes in the text: line 99-121.

Suggestion for improvement

- Clarify whether "nearly one million new cases" refers to global incidence or a specific region.

Reply: Thank you for your valuable feedback. It refers to global incidence.

Changes in the text: line 70-71.

- The first paragraph ends abruptly—explain why treatment options are limited (e.g., tumor size, metastasis), leading to poor prognosis and high mortality in HCC.

Reply: Thank you for your valuable comment. We acknowledge that the first paragraph may benefit from additional context. The limitations in treatment options for hepatocellular carcinoma (HCC) primarily stem from factors such as tumor size, the presence of metastasis, and underlying liver conditions like cirrhosis. As HCC often presents at an advanced stage, surgical interventions become less viable, and systemic therapies may have limited efficacy due to the tumor's heterogeneity and resistance mechanisms. These challenges contribute to the overall poor prognosis and high mortality associated with HCC. We will revise the paragraph to incorporate this explanation for clarity and completeness.

Changes in the text: line 75-79.

- Break the first sentence of the second paragraph into two for better readability.

Reply: Thank you for your suggestion. I have revised the first sentence of the second paragraph to enhance its readability. The new version breaks the original sentence into two distinct sentences, making the information clearer and easier to understand. Please see the updated paragraph in the revised manuscript.

Changes in the text: line 82-86.

- Specify the gap in knowledge regarding prognostic prediction for HCC—are current models inadequate or is there a lack of studies?

Reply: Thank you for your insightful question. The gap in knowledge regarding prognostic prediction for hepatocellular carcinoma (HCC) lies primarily in both the inadequacy of current

models and the lack of comprehensive studies. While existing prognostic models provide some insights, they often fail to account for the molecular heterogeneity of HCC and do not integrate key factors such as aging-related genetic alterations. Additionally, there is a scarcity of research focusing on the specific impact of these aging-related genes on prognosis, which limits our understanding of their role in HCC progression. Addressing these gaps is crucial for improving prognostic accuracy and patient outcomes.

Changes in the text: line 82-92.

- Make the call for novel biomarkers more impactful by mentioning how they could improve early detection or guide personalized treatment.

Reply: Thank you for your insightful suggestion. To enhance the impact of our call for novel biomarkers, we will emphasize that these biomarkers can significantly improve early detection of liver cancer by enabling more precise identification of at-risk individuals and facilitating timely interventions.

Additionally, we will highlight that these biomarkers can guide personalized treatment approaches, allowing for tailored therapies based on the specific molecular characteristics of a patient's tumor. This individualized strategy has the potential to improve treatment efficacy and patient outcomes.

We appreciate your feedback and will incorporate these points to strengthen our manuscript.

Changes in the text: line 82-121.

- Explain how senescence-related genes contribute to tumor suppression or promotion in HCC.

Reply: Thank you for your valuable question. Senescence-related genes play a dual role in hepatocellular carcinoma (HCC), contributing both to tumor suppression and promotion depending on the context. On one hand, these genes can induce cellular senescence, a state that halts the proliferation of damaged or stressed cells, thereby preventing the progression of potential tumors. This tumor-suppressive effect is crucial in maintaining tissue homeostasis and preventing the accumulation of mutations that could lead to cancer. On the other hand, in the tumor microenvironment, senescent cells can secrete pro-inflammatory cytokines, growth factors, and proteases, collectively known as the senescence-associated secretory phenotype (SASP). This phenomenon can promote tumor progression by creating a supportive environment for neighboring malignant cells, enhancing their growth and metastasis. In summary, senescence-related genes contribute to the complex interplay of tumor suppression and promotion in HCC, highlighting the need for a deeper understanding of their context-dependent roles in cancer biology. We will ensure to elaborate on this in our revised manuscript. Thank you for the opportunity to clarify this important aspect.

Changes in the text: line 99-121.

- Provide more detail on the role of therapy-induced senescence in HCC progression.

Reply: Thank you for your valuable question. We have provided more detail for the role of therapy-induced senescence.

Changes in the text: line 99-121.

- Clarify whether senescence is beneficial or detrimental in HCC progression and why it is important for prognosis.

Reply: Thank you for your valuable question. Senescence-related genes play a dual role in hepatocellular carcinoma (HCC), contributing both to tumor suppression and promotion depending on the context. On one hand, these genes can induce cellular senescence, a state that halts the proliferation of damaged or stressed cells, thereby preventing the progression of potential tumors. This tumor-suppressive effect is crucial in maintaining tissue homeostasis and preventing the accumulation of mutations that could lead to cancer. On the other hand, in the tumor microenvironment, senescent cells can secrete pro-inflammatory cytokines, growth factors, and proteases, collectively known as the senescence-associated secretory phenotype (SASP). This phenomenon can promote tumor progression by creating a supportive environment for neighboring malignant cells, enhancing their growth and metastasis. In summary, senescence-related genes contribute to the complex interplay of tumor suppression and promotion in HCC, highlighting the need for a deeper understanding of their context-dependent roles in cancer biology.

Changes in the text: line 99-121.

- Compare current prognostic models with the cellular senescence-based model to highlight its advantages.

Reply: Thank you for your question. The aging model has not yet been established in HCC and has not been discussed in the introduction. Thank you for your valuable feedback. Our hypothesis is that altered expression of senescence-related genes in hepatocellular carcinoma (HCC) influences tumor behavior and patient prognosis. Specifically, we propose that the upregulation or downregulation of these genes may affect the cellular senescence pathways, leading to changes in tumor progression, metastasis, and response to therapy. By examining the expression profiles of these genes, we aim to establish a correlation between their levels and clinical outcomes, providing insights into their potential as biomarkers for prognosis in HCC.

Changes in the text: line 99-114.

- Include a clear hypothesis about how senescence-related gene expression correlates with prognosis in HCC.

Reply: Thank you for your valuable feedback. Our hypothesis is that altered expression of senescence-related genes in hepatocellular carcinoma (HCC) influences tumor behavior and patient prognosis. Specifically, we propose that the upregulation or downregulation of these genes may affect the cellular senescence pathways, leading to changes in tumor progression, metastasis, and response to therapy. By examining the expression profiles of these genes, we aim to establish a correlation between their levels and clinical outcomes, providing insights into their potential as biomarkers for prognosis in HCC.

Changes in the text: line 99-121.

- Update claims with more recent citations, particularly post-2022 studies, to support the link between senescence and tumor aggressiveness.

Reply: Thank you for your suggestion. We appreciate the importance of incorporating recent studies to strengthen our claims regarding the link between senescence and tumor aggressiveness in hepatocellular carcinoma (HCC). We will review the literature for relevant post-2022 publications and update our manuscript to include these citations, ensuring that our arguments are supported by the most current research findings. This will enhance the rigor of our discussion on the role of senescence-related genes in HCC progression and prognosis.

Changes in the text: line 99-121.

Methods

Strengths

The methods section is well-structured, using established bioinformatics tools like ConsensusClusterPlus, limma, pheatmap, and clusterProfiler for robust analysis. The integration of clustering, differential gene expression, immune profiling, and prognostic modeling adds depth, while examining clinical characteristics such as TNM classification and tumor grade enhances translational value.

Reply: Thank you for your positive feedback on the methods section of our manuscript. We appreciate your recognition of the structured approach and the use of established bioinformatics tools such as ConsensusClusterPlus, limma, pheatmap, and clusterProfiler. The integration of clustering, differential gene expression analysis, immune profiling, and prognostic modeling indeed provides a comprehensive framework for understanding the molecular characterization of aging-related genes in liver cancer. Additionally, by examining clinical characteristics like TNM classification and tumor grade, we aim to enhance the translational value of our findings, making them more applicable to clinical settings.

Changes in the text: line 137-203.

Suggestions for improvement

- The choice of k-values (2 to 6) for hierarchical clustering should be justified further. Did the study use methods such as the elbow method or silhouette score to determine the optimal cluster number?

Reply: Thank you for your insightful question regarding the choice of k-values for hierarchical clustering. In our study, we initially explored a range of k-values from 2 to 6 based on biological relevance and previous literature. To further justify our selection, we employed the silhouette score as one of the methods to assess the optimal number of clusters within this range. We found that the silhouette scores provided meaningful insights into the clustering structure, highlighting the robustness of our chosen k-values.

Changes in the text: line 137-203.

- The study uses $P < 0.05$ and $|log2\ fold\ change| > 1$ as cutoffs for DEGs. Were other thresholds considered? Was multiple testing correction applied (e.g., FDR adjustment)?

Reply: We appreciate your suggestion and will ensure to elaborate on this justification in the revised manuscript. Thank you for your valuable question regarding the thresholds used for identifying differentially expressed genes (DEGs). In our study, we utilized $P < 0.05$ and $|log2\ fold\ change| > 1$ as our primary cutoffs based on their biological relevance and to ensure a balance between sensitivity and specificity.

We did consider other thresholds during our analysis but found that the chosen criteria provided the most meaningful results in the context of our research objectives. Additionally, we applied multiple testing correction using the false discovery rate (FDR) adjustment to account for multiple comparisons, ensuring the robustness of our findings.

We appreciate your feedback and will clarify these points in the revised manuscript.

Changes in the text: line 137-207.

- Were the gene expression values normalized (e.g., TPM, FPKM, log-transformation)?

Reply: Thank you for your insightful question. In our study, the gene expression values were normalized using the TPM (Transcripts Per Million) method. This normalization approach was chosen to account for both sequencing depth and gene length, allowing for a more accurate comparison of gene expression levels across samples.

Changes in the text: line 166-170.

- Were batch effects corrected (e.g., using ComBat or other methods)?

Reply: Thank you for your important question. Yes, we corrected for batch effects in our study using the ComBat method. This approach allowed us to adjust for potential confounding variables related to batch variations, ensuring that our results reflect true biological differences rather than technical artifacts.

Changes in the text: line 137-207

- The term "immunogenomics" is used but is not a standard immunological analysis term. A brief explanation or reference to a relevant study would help.

Reply: Thank you for your insightful comment. The term "immunogenomics" in our study refers to the integration of genomic data with immunological profiles to analyze the immune response in the context of cancer, particularly HCC. While it may not be a standard term in all immunological analyses, it encompasses the exploration of how genetic variations influence immune responses and tumor microenvironments.

Changes in the text: line 137-207.

- How were immune cell infiltration levels assessed? Were specific algorithms (e.g., CIBERSORT, xCell, MCP-counter) used?

Reply: Thank you for your question regarding the assessment of immune cell infiltration levels in our study. We utilized a combination of computational algorithms, specifically CIBERSORT and xCell, to estimate the relative abundance of various immune cell types within the tumor microenvironment. These algorithms analyze gene expression data to provide estimates of immune cell infiltration levels accurately.

Changes in the text: line 137-207.

- While LASSO regression is a powerful feature selection method, was cross-validation used to avoid overfitting?

Reply: Thank you for your insightful question regarding the use of LASSO regression in our study. We employed cross-validation as part of our analysis to prevent overfitting and ensure

the robustness of the selected features. Specifically, we utilized k-fold cross-validation to assess the model's performance and to fine-tune the regularization parameter.
Changes in the text: line 137-207.

- Was the model validated using an independent dataset, or was it only trained on TCGA data?

Reply: Thank you for your valuable question regarding the validation of our model. We have conducted additional validation using an independent dataset to ensure the generalizability and robustness of our findings.
Changes in the text: line 137-207.

Results

Strengths

The study identifies two HCC subtypes (k = 2), with C1 patients showing worse prognosis per Kaplan-Meier analysis. C1 correlates with TNM stage, tumor grade, and pathological stage, highlighting cellular senescence-related genes as potential risk biomarkers. Molecular analysis reveals 947 upregulated and 90 downregulated genes, with GO/KEGG linking them to cell cycle, DNA replication, and ECM interactions, while metabolic and immune pathways are downregulated. Immune profiling shows C1 has higher immune cell infiltration (B/NK/macrophages, CD4+/CD8+ T cells) and immune checkpoint expression (PD-1, CTLA4, LAG3, TIGIT), suggesting immune evasion. A prognostic model using Cox and LASSO regression identifies 12 key genes (e.g., VDAC2, CXCL8, MYBL2, HRAS), with AUC values (1-year: 0.764, 3-year: 0.701, 5-year: 0.706) confirming strong predictive potential. Limitation is mentioned.

Suggestions for improvement

- The prognostic model is derived exclusively from TCGA-HCC data without external validation in an independent dataset (e.g., GEO, ICGC, or clinical samples). Without independent cohort validation, the model's generalizability and robustness remain uncertain.

Reply: Thank you for your insightful comments regarding the prognostic model. While we acknowledge the importance of external validation in enhancing the generalizability and robustness of our findings, we currently do not have access to an independent dataset for validation. Our analysis is primarily based on TCGA-HCC data, which provides a comprehensive foundation for the model. We appreciate your understanding of our current limitations and will emphasize this aspect in the discussion section of the revised manuscript to provide clarity on the model's applicability.

Changes in the text:

- Report statistical metrics used to assess cluster validity.

Reply: This study does not involve this method.

- Consider a sensitivity analysis to determine whether k > 2 would yield meaningful subgroups.

Reply: Thank you for your suggestion regarding the sensitivity analysis. We have conducted an additional analysis to evaluate whether increasing the number of clusters ($k > 2$) yields meaningful subgroups in our dataset. This involved assessing the stability and biological relevance of the resulting clusters, as well as their prognostic value. Your input is valuable in ensuring the robustness of our clustering approach.
Changes in the text: line 214-223.

- The study relies solely on bioinformatics analysis without experimental confirmation (e.g., qPCR, Western blot, IHC) of the identified genes in HCC samples. This limits confidence in the functional relevance of the 12 prognostic genes. At least perform experiment with a couple of genes if not 12

Reply: Thank you for your valuable feedback. While we acknowledge the importance of experimental validation, our study primarily focuses on bioinformatics analysis to identify potential biomarkers associated with aging-related genes in HCC. Due to resource constraints and the scope of our current project, we are unable to perform experimental validation at this time. However, we believe that our findings provide a strong basis for future studies aimed at confirming these results through experimental approaches. We appreciate your understanding and hope that our bioinformatics insights will contribute to the broader understanding of liver cancer biology.
Changes in the text: line 282-298.

- The AUC values indicate moderate prognostic accuracy (0.764 for 1-year survival), but there is no comparison to existing HCC prognostic models (e.g., AFP, GPC3, or established transcriptomic risk scores.

Reply: Thank you for your insightful comment. We recognize the importance of comparing our prognostic model to established HCC prognostic models such as AFP, GPC3, and existing transcriptomic risk scores. Unfortunately, due to the scope of our study, we did not include these comparisons. However, we agree that such analysis would strengthen our findings and provide more context for the prognostic accuracy of our model. We appreciate your suggestion and will consider it for future research to enhance the validation of our results against existing benchmarks in HCC prognosis.

Changes in the text: line 282-298.

- While clinical characteristics (age, sex, TNM stage) are considered, other confounders (e.g., underlying liver disease, viral status, treatment history) are not accounted for in the analysis.

Reply: Thank you for your valuable feedback. We understand the importance of considering additional confounders such as underlying liver disease, viral status, and treatment history in our analysis. However, we would like to clarify that the TCGA database primarily focuses on tumor genomic and transcriptomic data and does not consistently provide detailed information on all clinical characteristics, including the ones mentioned. As a result, our analysis was limited to the available clinical data. We appreciate your suggestion and acknowledge that incorporating these factors could provide a more comprehensive understanding of the prognostic model in future studies.
Changes in the text: line 282-298.

Discussion

Suggestions for improvement

While the prognostic model is promising, there is little discussion on its real-world application.

- Explain how this 12-gene model could be integrated into clinical practice, e.g., could it enhance existing HCC staging systems, guide treatment decisions, or predict response to therapies (e.g., immunotherapy or targeted therapy)?

Reply: Thank you for your insightful comment regarding the real-world application of our 12-gene prognostic model for liver cancer. We believe that this model has significant potential to enhance existing HCC staging systems by providing a more nuanced assessment of prognosis based on molecular characteristics. By integrating these gene expression profiles into clinical workflows, clinicians could stratify patients more effectively, potentially leading to tailored management strategies.

For treatment decision-making, our model could guide the selection of therapies, particularly in identifying patients who are likely to respond to immunotherapy or targeted therapies. For instance, patients with specific gene expression patterns may benefit more from certain treatments, allowing for a more personalized approach.

Additionally, this model can serve as a complementary tool to traditional staging systems, helping to refine prognosis and enabling better risk stratification. We envision that with further validation in larger cohort studies, our 12-gene model could be incorporated into clinical guidelines, assisting healthcare providers in making informed decisions that improve patient outcomes.

Overall, the integration of this model into clinical practice holds promise for advancing precision medicine in HCC management.

Changes in the text: line 323-331.

- Emphasize the necessity of validating the model in independent datasets to improve generalizability.

Reply: Thank you for your insightful comment regarding the validation of our model. We acknowledge that validating our prognostic model in independent datasets is essential for enhancing its generalizability and confirming its applicability across diverse patient populations.

In our discussion, we have addressed this limitation and emphasize the importance of independent validation to establish the model's robustness and predictive accuracy. This step is crucial for ensuring that our findings can be reliably applied in clinical practice. We appreciate your suggestion and will incorporate it into our manuscript to highlight this important aspect of our research.

Changes in the text: line 335-340.

- Discuss potential future validation steps, such as retrospective patient cohort analysis or prospective clinical trials.

Reply: Thank you for your insightful question regarding potential future validation steps. To further validate our findings on the molecular subtyping and prognosis of aging-related genes in liver cancer, we propose the following steps:

Retrospective Patient Cohort Analysis: We plan to conduct a comprehensive analysis of existing patient databases to evaluate the correlation between identified molecular subtypes and clinical outcomes. By stratifying patients based on these subtypes, we can assess overall survival, disease-free survival, and response to treatment, which will help to reinforce the prognostic value of our molecular classifications.

Prospective Clinical Trials: Following the retrospective analysis, we aim to initiate prospective clinical trials designed to test the predictive accuracy of our molecular subtyping in real-time. These trials will involve recruiting patients with liver cancer and applying our classification system to guide treatment decisions. We will monitor treatment outcomes and progression rates, providing a robust dataset to validate our findings prospectively.

Through these validation steps, we hope to establish a solid foundation for the clinical application of our research, ultimately improving patient management and outcomes in liver cancer. Thank you for your consideration.

Changes in the text: line 335-340.

- While the roles of VDAC2, CXCL8, MYBL2, etc. are mentioned, the mechanistic link between cellular senescence and HCC progression remains underdeveloped.

Reply: Thank you for your valuable feedback regarding the mechanistic link between cellular senescence and hepatocellular carcinoma (HCC) progression, particularly concerning the roles of VDAC2, CXCL8, MYBL2, and other genes.

We acknowledge that while we have highlighted the involvement of these genes in our study, further elucidation of their specific mechanisms is critical. To address this, we plan to undertake in-depth functional studies that will investigate how these genes influence cellular senescence pathways and contribute to HCC progression.

Changes in the text: line 370-375.

- Discuss whether these senescence-related genes promote tumor progression via SASP (senescence-associated secretory phenotype).

Reply: Thank you for your question regarding the role of senescence-related genes in tumor progression, specifically in relation to the senescence-associated secretory phenotype (SASP). Our research indicates that senescence-related genes may indeed contribute to tumor progression through the SASP. The SASP is characterized by the secretion of various pro-inflammatory cytokines, growth factors, and proteases that can modify the tumor microenvironment. This secretory phenotype has the potential to promote tumor growth, invasion, and metastasis by enhancing cellular proliferation and survival of adjacent tumor cells. Furthermore, components of the SASP can recruit immune cells to the tumor site, which might initially have a tumor-suppressive effect but can also lead to immune evasion strategies employed by the cancer cells over time. In our study, we aim to explore the specific mechanisms by which these senescence-related genes influence SASP activation and subsequent tumor

behavior. Understanding this relationship could provide insights into new therapeutic strategies aimed at targeting senescent cells or their secretory factors to inhibit liver cancer progression. Thank you for allowing us to clarify this important aspect of our research.

Changes in the text: line 358-367.

- Explain how senescence contributes to immune modulation in HCC—does it create a pro-inflammatory or immune-suppressive environment?

Reply: Thank you for your inquiry regarding the role of senescence in immune modulation within HCC. Senescence can have a dual impact on the immune environment in HCC, exhibiting both pro-inflammatory and immune-suppressive characteristics depending on the context. On one hand, senescent cells often secrete a variety of pro-inflammatory cytokines and chemokines as part of the senescence-associated secretory phenotype (SASP). This secretion can recruit immune cells to the tumor microenvironment, promoting inflammation and potentially enhancing anti-tumor immune responses. On the other hand, prolonged senescence can lead to an immune-suppressive environment over time. The continuous presence of SASP factors can create a chronic inflammatory state that may ultimately contribute to immune evasion. The factors released by senescent cells can induce the recruitment of immunosuppressive cell types, such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), which can inhibit effective anti-tumor immunity and support tumor progression. In summary, while senescence initially may create a pro-inflammatory environment that could stimulate immune responses, the long-term implications can shift towards immune suppression, facilitating HCC progression. Ongoing research in our study aims to clarify these mechanisms and their implications for therapeutic interventions. Thank you for your thoughtful question.

Changes in the text: line 324-331.

- Discuss the potential for targeting senescence-related pathways (e.g., using senolytics, immune checkpoint inhibitors, or metabolic modulators) in high-risk HCC patients.

Reply: Thank you for your question regarding the potential for targeting senescence-related pathways in high-risk HCC patients. Targeting senescence-related pathways presents a promising therapeutic strategy for high-risk HCC patients. Senolytics, which selectively eliminate senescent cells, could potentially reduce the burden of the senescence-associated secretory phenotype (SASP) that contributes to tumor progression and immune evasion. By clearing these cells, we may enhance the effectiveness of existing therapies and improve immune responses against the tumor. Additionally, combining senolytics with immune checkpoint inhibitors could further potentiate anti-tumor immunity. The removal of senescent cells may improve the tumor microenvironment, making it more susceptible to immune-mediated attack by inhibiting pathways that tumors use to evade the immune system. Metabolic modulators are another avenue worth exploring. Since senescent cells often exhibit altered metabolic profiles, targeting these changes could disrupt the support that senescent cells provide to tumor growth and survival. Overall, integrating therapies that target senescence-

related pathways in high-risk HCC patients might improve clinical outcomes by not only reducing tumor burden but also by reinvigorating anti-tumor immunity. Ongoing research is essential to better understand the optimal combinations and timing of these interventions. Thank you for your insightful question.

Changes in the text: line 358-367.

- Mention whether the identified genes are potential druggable targets.

Reply: Thank you for the inquiry regarding the potential druggability of the identified genes related to senescence in hepatocellular carcinoma (HCC). The genes we identified in our study play significant roles in the pathways associated with cellular senescence and HCC progression. Several of these genes are indeed promising candidates for being druggable targets. For instance, if they encode proteins that are involved in critical signaling cascades or metabolic processes, they could be modulated by small molecules or biologics. Additionally, we observed that some of these genes are implicated in pathways that are currently being targeted in other malignancies, such as immune checkpoint inhibitors or specific kinase inhibitors, suggesting a potential for repurposing existing drugs or developing new therapeutic agents. Further functional validation and screening will be necessary to determine the efficacy and specificity of targeting these genes. Our findings lay a foundation for future studies aimed at exploring these therapeutic avenues in the context of HCC treatment. Thank you for your valuable question.

Changes in the text: line 323-340.

- The conclusion repeats key findings but lacks a strong take-home message regarding the study's potential impact on clinical management. Clearly state why this model is important for prognosis, how it advances current knowledge, and what the next steps should be.

Reply: Thank you for your valuable feedback regarding the conclusion of our study. We appreciate your insights and have taken them into consideration. In this study, we developed a molecular classification model for hepatocellular carcinoma (HCC) based on senescence-related genes, which provides a novel approach to prognostication in this heterogeneous disease. The importance of this model lies in its potential to enhance clinical management by enabling more personalized treatment strategies. By identifying distinct molecular subtypes, clinicians could better predict patient outcomes and tailor therapies accordingly, ultimately improving patient survival rates. Our findings advance current knowledge by elucidating the role of cellular senescence in HCC progression, highlighting how senescence-associated pathways can influence tumor behavior and patient prognosis. This understanding not only deepens our comprehension of HCC biology but also paves the way for the development of targeted therapies aimed at these pathways. The next steps should include validation of our model in larger, independent cohorts and exploration of the functional roles of the identified genes in HCC. Additionally, we recommend investigating the therapeutic implications of targeting senescence pathways, which could lead to innovative treatment options for HCC patients. Thank you for encouraging us to clarify the significance of our work.

Changes in the text: line 324-376.

Reviewer C

1. Fig 2

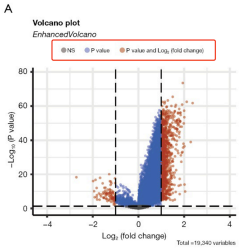
Please explain the meaning of “obs” in the legend.

n_{obs} =370

Reply: Thank you very much. It means 370 patients in this experiment.

2. Fig3A

The below explanation (red box) looks unusual. Please check whether the explanation is correct.

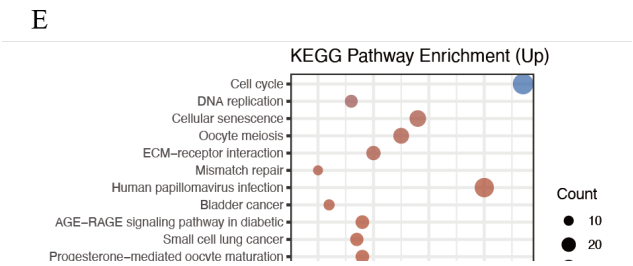


Reply: Thank you very much. We have revised.

3. Figure 3

There seems to be no “ECM” in Figure 3, while it was explained in the legend. Please check and revise.

Reply: Thank you very much. “ECM” is in Figure 3E.



4. Figure 4

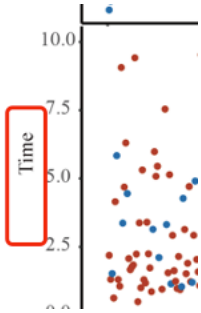
1) No “**” in Figure 4, while it was explained in the legend. Please check and revise.

1) “ns” in Figure 4: please explain its meaning in the legend.

Reply: Thank you very much. We have deleted.

5. Figure 5B

1) Please provide the unit for “Time”



Reply: Thank you very much. We have added.