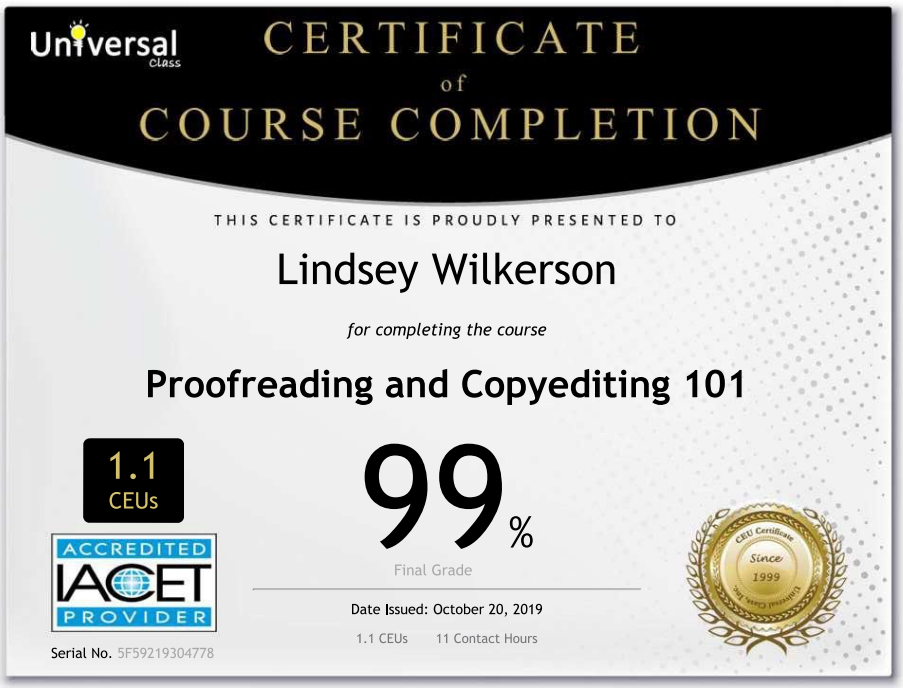
Cover letter

Dear Editor：

On behalf of my co-authors, we thank you very much for allowing us to revise our manuscript, we appreciate editor and reviewers very much for their positive and constructive comments and suggestions on our manuscript entitled “Construction of a risk score prognosis model based on hepatocellular carcinoma microenvironment”. (Manuscript NO.: 51680).

We have made revision which you mentioned in the edited manuscript file that needs to be modified, some of which are important to explain as follows:

First, considering the Editor and Reviewer’s suggestion, we have tried our best to improve the manuscript and made some changes in the manuscript. Also, we have polished the overall language by Lindsey Wilkerson (linnylikescoffee@hotmail.com), the language editing certificate was uploaded.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Construction and verification of a risk score prognosis model based on hepatocellular carcinoma microenvironment

prepared by

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and have found it thorough and acceptable with respect to grammar and composition.

signature over printed name

Professor, Guangdong Province Laboratory of Epigenetics and Cancer Regulation, +8613926401219

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26 September 2019

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Second, we have prepared and arranged the figures using PowerPoint to ensure that all graphs or text portions can be reprocessed. We modified the color and axis of some of the images (figure 2A-D, figure 6B-D, figure 7A-D, and supplementary figure 4A, B), but we didn't change the data. The figure files were uploaded on the system.

Third, we have checked the references and removed the duplicates.

Fourth, we have uploaded the "Audio core tip" on the system.

Fifth, we have submitted "Approved Grant Application Forms" and "Institutional review board statement" on the system.

Sixth, we did not submit the ARRIVE guidelines because the study does not involve animal experiments.

Besides, we have studied the reviewer's comments carefully and have made revision which marked in red in the paper. We have tried our best to revise our manuscript according to the comments.

Attached please find the revised version, which we would like to submit for your kind consideration.

We would like to express our great appreciation to you and reviewers for comments on our paper. Looking forward to hearing from you.

Thank you and best regards.

Sincerely yours,

Lei-Bo Xu, M.D., Ph.D.

Department of Hepato-Pancreato-Biliary Surgery, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou 510120, China

Phone: +86-20-34071172;

Fax: +86-20-83755229;

Email: xuleibo3@mail.sysu.edu.cn

Answering Reviewers

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "Construction of a risk score prognosis model based on hepatocellular carcinoma microenvironment" (Manuscript NO: 51680).Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made corrections which we hope meet with approval. The revised portion is marked in red in the paper. The main corrections in the paper and the response to the reviewer's comments are as flowing:

Responds to the reviewer's comments:

Reviewer #1 (code: 03259512):

1. Comments:

The abstract is too generalized and does not indicate with genes/what gene signature was identified as the most important. NOTUM, PAGE4, PEG10…etc-genes and signaling pathways were identified among the relevant to the prognosis.

Responses:

Thank you for your advice. Based on your valuable suggestion, we have re-written this part:

Page 4 line 17-23:

Abstrac

BACKGROUND: Hepatocellular carcinoma (HCC) is a common cancer with a poor prognosis. Previous studies revealed that the tumor microenvironment

(TME) plays an important role in HCC progression, recurrence, and metastasis, leading to poor prognosis. However, the influence of genes involved in TME on the prognosis of HCC patients remains unclear. Here, we investigated the HCC microenvironment to identify prognostic genes for HCC.

AIM: To identify a robust gene signature associated with the HCC microenvironment to improve prognosis prediction of HCC.

METHODS: We computed the immune/stromal scores of HCC patients obtained from The Cancer Genome Atlas (TCGA) based on the ESTIMATE algorithm. The results showed that they were related to the prognosis of HCC patients. Additionally, a risk score model based on Differentially Expressed Genes (DEGs) between high- and low-immune/stromal score patients was established.

RESULTS: The risk score model consisting of eight genes was constructed and validated based on HCC patients to divide patients into high- or low-risk groups. And the genes (Disabled Homolog 2 (DAB2), Musculin (MSC), C-X-C Motif Chemokine Ligand 8 (CXCL8), Galectin 3 (LGALS3), B-Cell-Activating Transcription Factor (BATF), Killer Cell Lectin Like Receptor B1 (KLRB1), Endoglin (ENG) and Adenomatosis Polyposis Coli Tumor Suppressor (APCS)) that compose our risk score model could be considered to be potential immunotherapy targets, and they may provide better performance in combination. Functional enrichment analysis showed that the immune response and T cell receptor signaling pathway represented the major function and pathway, respectively, related to the immune-related genes in the DEGs between high- and low-risk groups. The Receiver Operating Characteristic (ROC) curve analysis affirmed the good potency of the risk score prognostic model. Moreover, we validated the risk score model in the International Cancer Genome Consortium (ICGC) and the Gene Expression Omnibus (GEO) database. A nomogram was established to predict the overall survival of HCC patients.

CONCLUSION: The risk score model and the nomogram will benefit HCC

patients through personalized immunotherapy for HCC patients.

The above revisions were marked with red color in the main text, see page 4, lines 17–23.

2. Comments:

Simplified schematic presentation of the analyzed associations could be also presented.

Responses:

Thanks for your constructive suggestion. We have investigated the potential functions of the differentially expressed genes (DEGs) by GO analysis and KEGG pathway (Supplementary Figure 2A-C and Figure 3B). Besides, to help readers to understand our study, we have added the flow chart of this study (Figure 1).

Figure 1

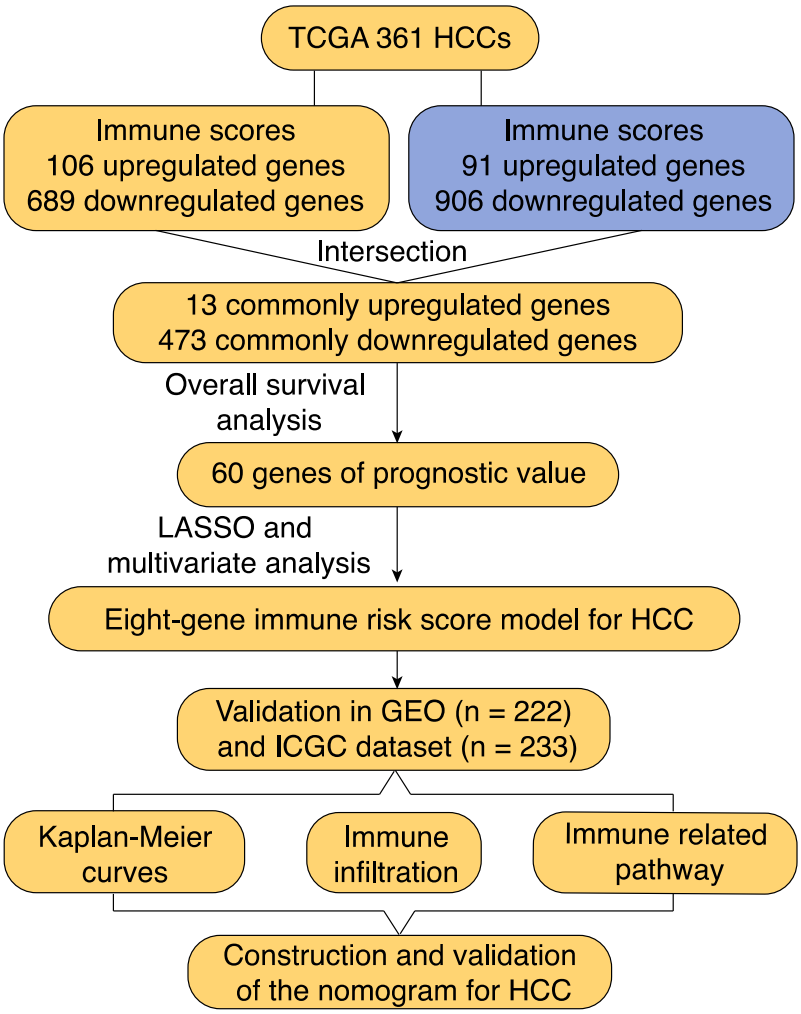


Figure 1 Overall design of the present study. TCGA: The Cancer Genome Atlas database; HCC: Hepatocellular carcinoma; LASSO: Least Absolute Shrinkage and Selection Operator; GEO: Gene Expression Omnibus databases; ICGC: International Cancer Genome Consortium database.

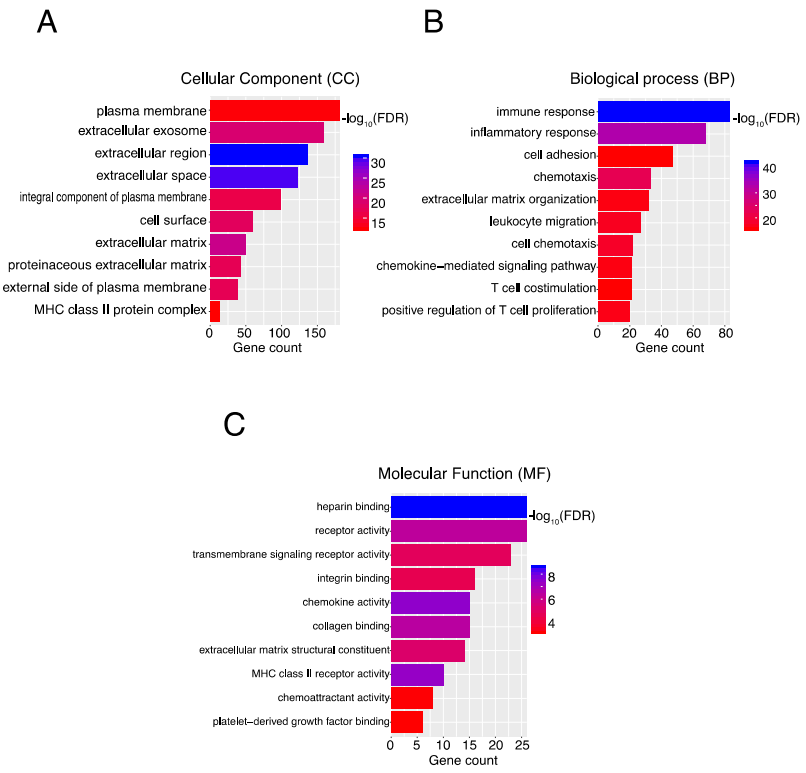
The revisions were marked with red color, see page 35.

3. Comments:

The paper contains many figures, however several of them can be attached as Supplementary material.

Responses:

As suggested, we moved the Figure 3C-E to Supplementary Figure 2 A-C.



Supplementary Figure 2 GO analysis of DEGs. Top 10 GO terms in cellular component (A), biological process (B) and molecular function (C) branches were displayed. False Discovery Rate (FDR) of GO analysis was acquired from the DAVID functional annotation tool. DEGs: Differentially Expressed Genes; GO: Gene Ontology; DAVID: Database for Annotation, Visualization and Integrated Discovery.

The revisions were marked with red color, see page 37 and page 44.

4. Comments:

The simplified scheme of association between the top-related genes/pathway and the observed effect of inflammation/microenvironment are preferable.

Responses:

Thanks for your advance, we have drawn the schematic diagram of the main altered pathway between the high- and low-risk patients (Figure 8).

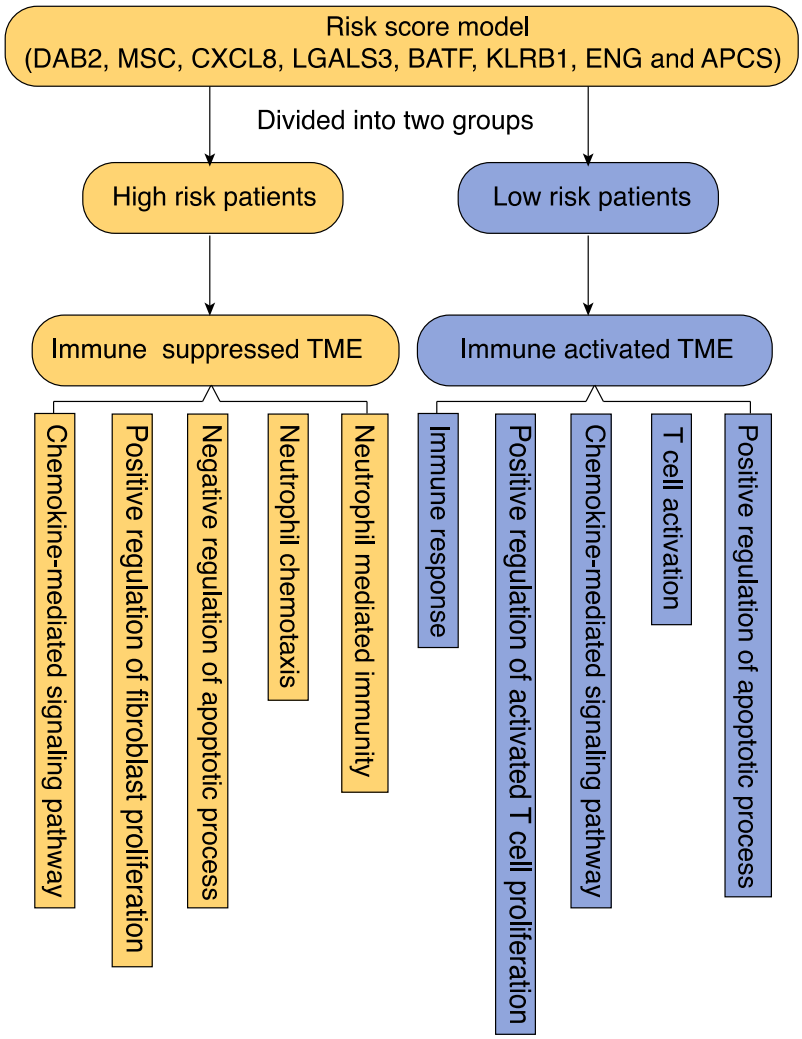


Figure 8 Schematic diagram of the main altered pathway between the high- and low-risk patients. TME: tumor microenvironment.

The revisions were marked with red color, see page 43.

Special thanks to you for your good comments.

Reviewer #2 (code: 04737441):

1. Comments:

I would like to see how these scores would perform prospectively in guiding treatment algorithms but this would need a new prospective study. For now, may be out of spectrum but it would be interesting to see how these scores would perform in comparison to other established and novel scoring systems as BCLC, ALBI etc. This could be even added to the supplemental materials.

Responses:

We thank the reviewer for this perspective. We are also interested in how the risk score model would perform in comparison to other established and novel scoring systems (eg BCLC, ALBI, etc.). As suggested, we have retrieved the database, however, neither TCGA nor GEO or ICGC database has corresponding data. And we agree with the reviewer's opinion that the risk score model needs to be further validated in multicenter clinical trials and prospective studies.

2. Comments:

Besides, some typos need to be corrected and an overall language polishing is recommended.

Responses:

Considering the Reviewer's suggestion, we have tried our best to improve the manuscript and made some changes in the manuscript. Also, we have polished the overall language by Lindsey Wilkerson (linnylikescoffee@hotmail.com), and the language editing certificate was uploaded.

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Special thanks to you for your good comments.

Other changes:

1. Page 4 Line 15-16, the statements of "Functional analysis demonstrated that the genes which were differentially expressed between high- and low-immune/stromal score patients were mainly associated with immune

response." were deleted.

2. Page 4 line 15-16, "In addition, a risk score model consisting of eight genes were constructed and validated based on HCC patients to divide patients into high- or low-risk group." were corrected as "The risk score model consisting of eight genes was constructed and validated based on HCC patients to divide patients into high- or low-risk group."

3. Page 9 line 5, "as" was deleted.

4. Page 11 line 11, "from" was deleted.

We tried our best to improve the manuscript and made some changes to the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in red in the revised paper.

We appreciate for Editors/Reviewers' warm work earnestly and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions.

Sincerely yours，

Lei-Bo Xu, M.D., Ph.D.

Department of Hepato-Pancreato-Biliary Surgery, Sun Yat-Sen Memorial Hospital, Sun Yat-Sen University, Guangzhou 510120, China

Phone: +86-20-34071172;

Fax: +86-20-83755229;

Email: xuleibo3@mail.sysu.edu.cn