

LETTER TO EDITOR

Development and validation of a mitochondrial metabolism-associated nomogram for prediction of prognosis in clear cell renal cell carcinoma

Dear Editor:

Renal cancer remains a malignant disease worldwide. In 2018, 63 000 new cases and 15 000 deaths due to renal cancer occurred in the United States and about 350 000 new cases occurred yearly.¹ Clear cell renal cell carcinoma (ccRCC) was long defined by its aberrant metabolism alterations. Mitochondrial metabolisms were found to relate to cancer progression, metastasis by providing enough energy supplies and other intermedial metabolites.^{2,3} Besides, mitochondrial functions were essential to oxidative homeostasis and immune repression in cancer cells.^{4,5}

To fully understand its roles in ccRCC, we used TCGA cohort (N = 515) as a discovery cohort. For external validation group, a Fudan University Shanghai Cancer Center (FUSCC; N = 96) group was used. The differences of clinical characteristics between two groups were not significant (Table S1). Thus, the FUSCC cohort was set as external validation group.

The flow chart of the entire research is shown in Figure S1A. The 2981 genes were extracted from the original article of Birsoy et al⁶ and were filtered with patients' transcriptional data to clear out genes with too many missing values. We used univariate COX analysis to rule out genes unrelated to overall survival, we chose 1217 genes significantly correlated to patients' overall survival times. These genes were input in LASSO (least absolute shrinkage and selection operator) Cox regression model to detect proper combinations that are highly associated with prognosis in TCGA cohort. The coefficient profile and a minimal partial likelihood deviance plot are shown

in Figure S1B,C. Taken together, we constructed a ten metabolism-associated genes signature (Table S4). The genes are ABCC10, B3GNTL1, CUBN, GFPT2, IMPDH1, MBOAT7, SLC11A1, SLC16A12, SLC26A6, and SMPD4. The signature separate all patients into high-risk group and low-risk group using the X-tile⁷ for the best cutoff value. Age, TNM stages, gender, ISUP stages, tumor volume, tumor laterality, and metabolism-associated gene signature were put into a univariate COX regression correlation with patients' prognosis (Table S2). Except for gender, all other factors are significantly correlated to patients' prognosis. Multivariate COX regression was used to form a formula (Table S4). The result of the formula was a mitochondrial metabolism-associated signature.

The metabolism-associated signature came up with risk scores that could divide patients with ccRCC into two risk groups, low-risk patients and high-risk patients. Significant differences in survival time and status is found within TCGA cohorts ($P < .0001$, HR: 6.416, 95%CI: 4.624-8.902). Low-risk patients generally had better prognostic outcomes while high-risk patients show shorter living times. We used FUSCC patients with ccRCC as a validation cohort. The results were consistent ($P < .001$, HR: 4.929, 95%CI: 1.841-13.2). In TCGA, FUSCC and entire cohorts, the area under the ROC curve (AUC) values of ROC curves were 0.835, 0.743, and 0.817, respectively. ROC curves suggested that metabolism-associated signature remained efficacy in the validation group and perform better than traditional TNM classification staging system in all three situations (showed in Figure 1A-C). Based on the results of multivariate analysis, we constructed a nomogram to predict prognosis of ccRCC patients. Age, pM stages, tumor laterality, and ten-gene signature were included to evaluate 1-, 3-, and 5-year prognosis (Figure 1D). The risk score of each variable can be measured by the top point ruler, and the probability can be seen by comparing the risk score to the ruler below. Calibration curves for this model are plotted in Figure S2A.

Abbreviations: AUC, the area under the curve; ccRCC, clear cell renal cell carcinoma; CI, confidence interval; ESTIMATE, Estimation of STromal and Immune cells in MAlignant Tumors using Expression data; FUSCC, Fudan University Shanghai Cancer Center; HR, hazard ratio; MCP-counter, the Microenvironment Cell Population-counter; OS, overall survival; ROC, receiver operating characteristic; TNM, tumor-node-metastasis

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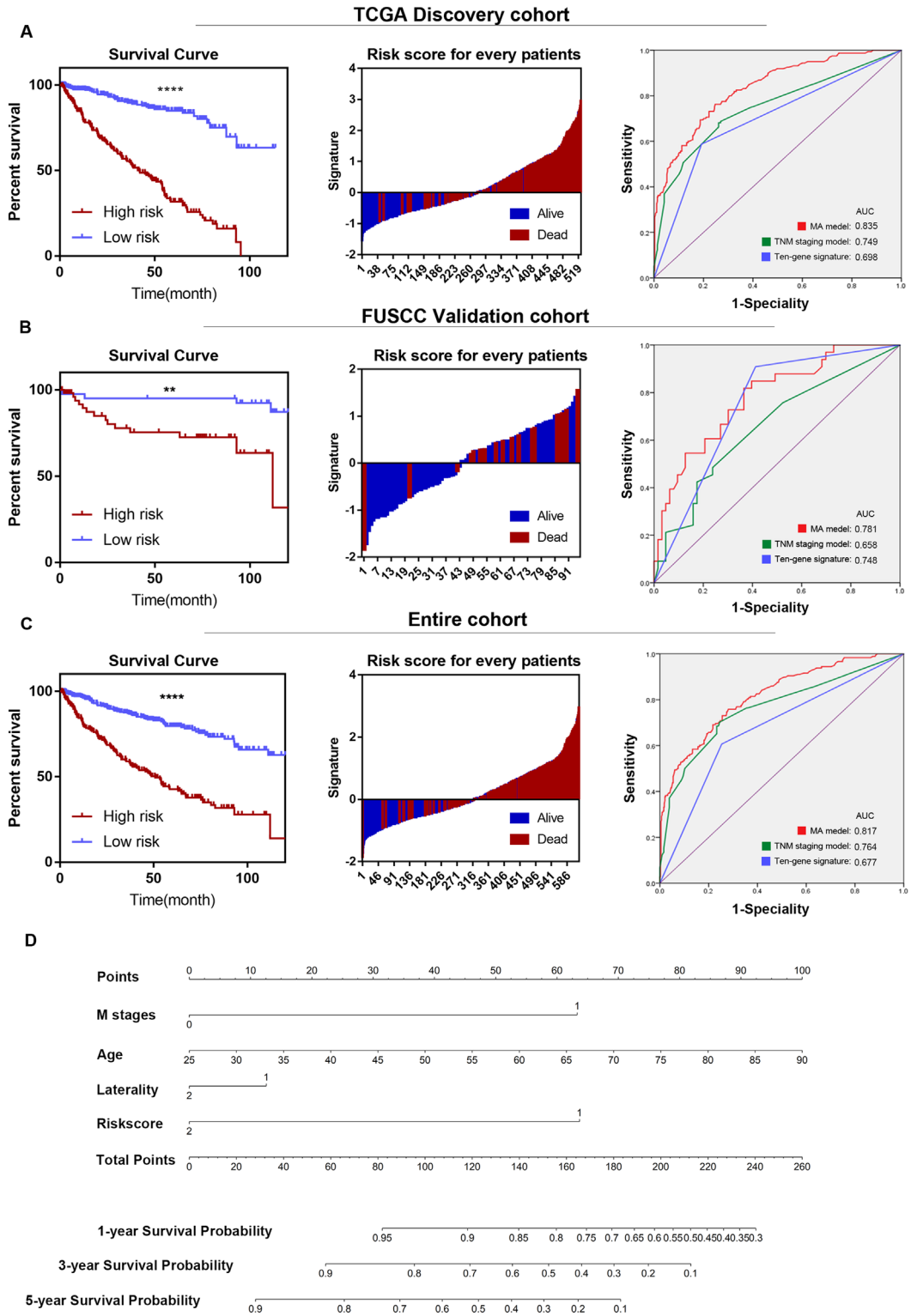


FIGURE 1 The survival curves plotted by Kaplan-Meier method (Low-risk group versus High-risk group); the boxplot of risk score for every patients with vital status; the cumulative ROC curve and AUC indexes for MA model, TNM staging model, and ten-gene signature, respectively. A, TCGA discovery cohort. B, FUSCC validation cohort. C, The entire cohort. D, The nomogram. (** P -value $< .01$, **** P -value $< .0001$)

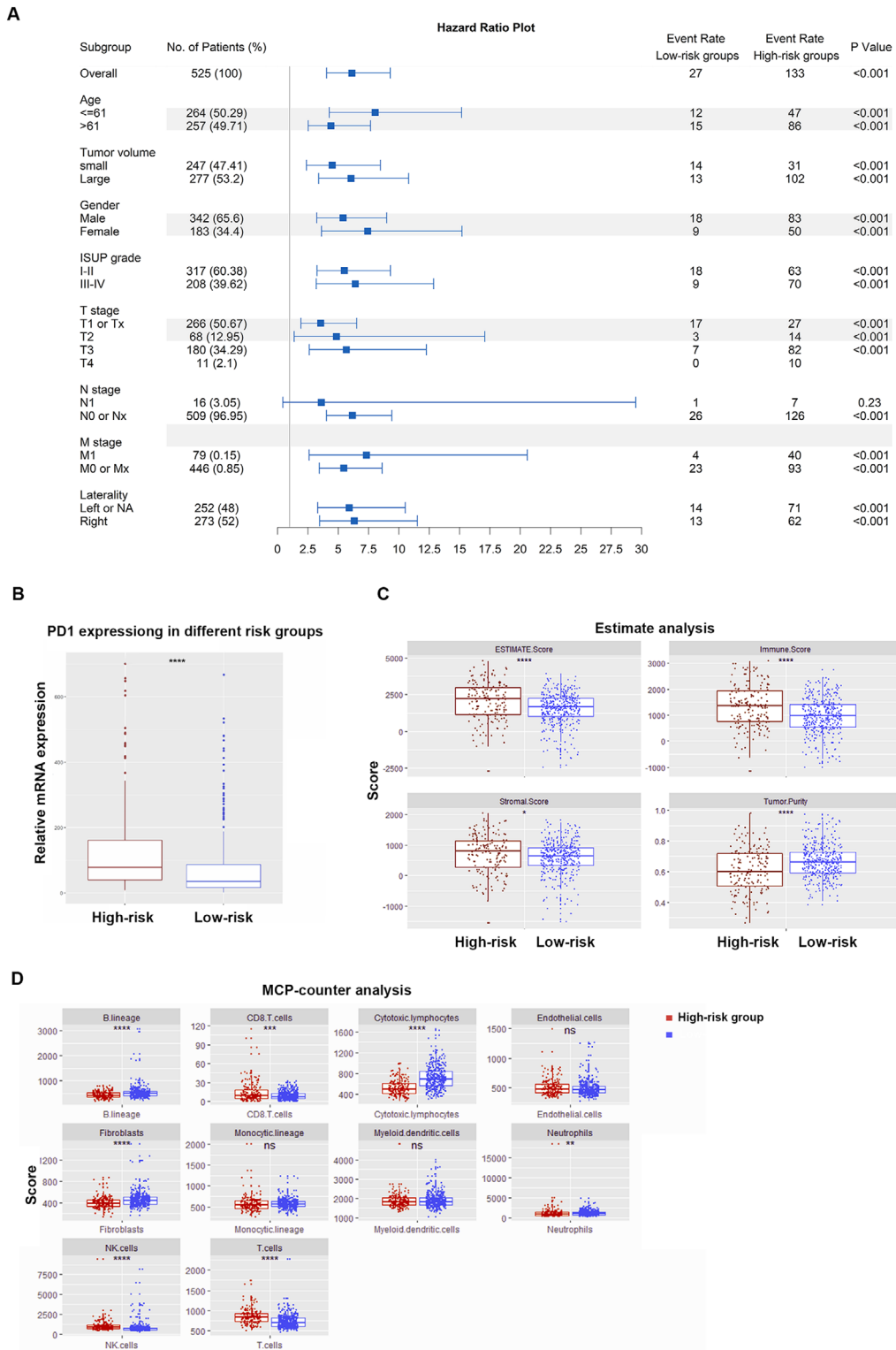


FIGURE 2 A, The hazard ratio of MA model (High-risk group versus Low risk group) in different subgroups including ages, genders, ISUP grades, T stages, M stages, N stages, tumor volume, and tumor laterality. B, The relative mRNA expression amount in different risk groups ($P < .0001$). C, Estimate analysis of overall transcriptional profiles of patients in different groups. D, MCP-counter analysis of transcriptional profiles of patients in different groups to measure specific immune cells

The signature was examined by measuring death events between risk groups in different subgroups. Except for pN1 group, high-risk groups showed higher hazard ratio significantly in all subgroups ($P < .001$; Figure 2A). Survival curves of each subgroups are shown in Figure S2B.

Tumors were found to attenuate mitochondrial functions in immune cells to escape anti-PD-1 Therapy.⁵ Mitochondrial metabolisms might be used to predict effectiveness of PD-1 blockage therapy. We tested several immune related indexes. We found that PD-1 mRNA expression significantly higher in high-risk group (Figure 2B). In ESTIMATE analyses,⁸ the high-risk group had more immune score and ESTIMATE score, but lower tumor purity compared to the low-risk group (Figure 2C). Meanwhile, in MCP-counter analyses,⁹ the high-risk group has more T cells, CD8⁺ T cells and NK cells while having lower density of cytotoxic lymphocytes (Figure 2D). It indicated that patients in high-risk group might have an immunocompromised tumor microenvironment, marked by hinder of differentiation to cytotoxic T cells. Thus, PD-1 blockage therapy might suitable for patients in high-risk group.

In conclusion, our research constructed a mitochondrial metabolism-associated signature to better predict prognosis in ccRCC patients. External validation showed great efficiency and robustness of this model. Furthermore, we found that high-risk patients might be suitable for anti-PD-1 therapy due to its unique immune components.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was in accordance with the recommendations of the Research Ethics Committee of Shanghai Cancer Center, Fudan University, China according to the provisions of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013). The protocol was approved by the Research Ethics Committee of Shanghai Cancer Center, Fudan University, China.

AUTHOR CONTRIBUTIONS

BZ and SJ interpreted the data and drafted the manuscript. YH and KC help validate signature. KC, BD and DY designed the study. BZ prepared all figures. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

TCGA database can be from UCSC Xena (<https://xenabrowser.net/datapages/>) or through “RTCGA”

(<https://rtcg.github.io/RTCGA.>) in R. Fudan University Shanghai Cancer Center (FUSCC) cohort can be requested from corresponding authors.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.