Identification of metabolism-related long non-coding RNA (lncRNA) signature predicts prognosis and immune infiltrates in hepatocellular carcinoma

Xiaodong Wang[1#], Jing Qian[2#], Ninghua Yao[2#], Christine Pocha[3], Koo Jeong Kang[4], Roberta Angelico[5], Guodong Zhu[6]

[1]Department of Interventional, the First Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine, Guangzhou, China; [2]Department of Cancer Center, Affiliated Hospital of Nantong University, Nantong, China; [3]Avera McKennnan Hospital and University Medical Center, Sanford School of Medicine, University of South Dakota, Sioux Falls, SD, USA; [4]Department of Surgery, Keimyung University School of Medicine, Dongsan Medical Center, Daegu, Republic of Korea; [5]HPB and Transplant Unit, Department of Surgical Sciences, University of Rome Tor Vergata, Rome, Italy; [6]Department of Hepatobiliary Surgery, Affiliated Nantong Hospital 3 of Nantong University, Nantong, China

Contributions: (I) Conception and design: X Wang, G Zhu; (II) Administrative support: G Zhu; (III) Provision of study materials or patients: J Qian, N Yao; (IV) Collection and assembly of data: X Wang, J Qian; (V) Data analysis and interpretation: X Wang, C Pocha, K Kang, R Angelico, G Zhu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Guodong Zhu. Department of Hepatobiliary Surgery, Affiliated Nantong Hospital 3 of Nantong University, 60 Qingnian Middle Road, Nantong 226001, China. Email: 165685178@qq.com.

Background: Cancer-associated metabolic reprogramming promotes cancer cell differentiation, growth, and influences the tumor immune microenvironment (TIME) to promote hepatocellular carcinoma (HCC) progression. However, the clinical significance of metabolism-related lncRNA remains largely unexplored.

Methods: Based on The Cancer Genome Atlas (TCGA) Liver hepatocellular carcinoma (LIHC) dataset, we identified characteristic prognostic long non-coding RNAs (lncRNAs) and construct metabolism-related lncRNA prognostic signature for HCC. Gender, age, grade, stage and TP53 status were used as covariates were used to assess the prognostic capacity of the characteristic lncRNA signature. Subsequently, the molecular and immune characteristics and drug sensitivity in metabolism-related lncRNA signature defined subgroups were analyzed.

Results: We identified 34 metabolism-related lncRNAs significantly associated with the prognosis of HCC (P<0.05). Subsequently, we constructed a multigene signature based on 9 characteristics prognostic lncRNAs and classified HCC patients into high- and low-risk groups based on cutoff values. We found the lncRNA signature [hazard ratio (HR) =3.55 (2.44–5.15), P<0.001] to be significantly associated with survival. The receiver operating characteristic curve (ROC) curves area under the curve (AUC) values for 1-, 3-, and 5-year survival were 0.811, 0.773, and 0.753, respectively. In univariate and multivariate Cox regression analyses, prognostic characteristic lncRNAs were the most crucial prognostic factor besides the stage. The prognostic signature was subsequently validated in the test set. In addition, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and gene set enrichment analysis (GSEA) analyses revealed potential biological features and signaling pathways associated with the prognostic signature. We constructed a nomogram including risk groups and clinical parameters (age, gender, grade, and stage). Calibration plots and decision curve analysis (DCA) showed that our nomogram had a good predictive performance. Finally, we found reduced expression of immune-activated cells in the high-risk group.

Conclusions: The metabolism-related lncRNA signature is a promising biomarker to distinguish the prognosis and an immune characteristic in HCC.

Introduction

Primary liver cancer is one of the 7 most common malignancies worldwide and is also a leading cause of cancer-related death (1). Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, accounting for approximately 80% of all cases (2). To date, multiple risk factors have been associated with the development and progression of HCC, including chronic infection with viruses, alcohol abuse, chronic obesity, or exposure to aflatoxins (2,3). Despite significant advances in clinical cancer treatment, the overall prognosis of patients with HCC remains poor due to high rates of postoperative tumor recurrence and metastasis (4). Previous study has shown that the progression of HCC is a complex process involving multiple factors and genes (5). The underlying molecular mechanisms of HCC development are unknown, and therefore, a better understanding of these is important for the prognosis and targeted therapy of HCC.

Cancer cannot develop without the metabolic reprogramming of cells, which is a direct and indirect consequence of oncogenic mutations (6,7). Cancer metabolic reprogramming can take many forms, such as the classic Warburg effect (8). Regardless of the mode of metabolic reprogramming, a common feature of cancer cells is the ability to obtain nutrients necessary for growth from a nutrient-deficient acidic environment and use these nutrients to maintain their growth transfer viability and build new material (9). The altered intra- and extra-cellular metabolites accompanying cancer-associated metabolic reprogramming are accompanied by corresponding changes in gene expression, which further affects cell differentiation and can even influence the tumor immune microenvironment (TIME) (10,11). The liver is the central metabolic organ that maintains blood glucose levels, ammonia metabolism, biotransformation of xenobiotics and endogenous metabolic by-products of metabolism, and bile synthesis under the action of multiple enzymes and pathways (12). Targeting cancer metabolic reprogramming therapy is expected to improve the risk of recurrence and metastasis in patients with HCC.

RNA-sequencing have revealed distinct long non-coding RNA (lncRNA) expression profiles in HCC tissues and several previously published papers have reported that lncRNAs affect metabolic reprogramming in HCC (13,14). Perturbation of the CCT3-LINC00326 regulatory network led to decreased lipid accumulation and inhibited HCC growth (15). LINC01234 was highly expressed in HCC and associated with poor prognosis. Mechanistically, LINC01234 downregulated the expression of Argininosuccinate synthase 1 (ASS1), upregulated aspartate level and activation of the mammalian target of rapamycin pathway (16). Nevertheless, a single metabolism-related lncRNA cannot reflect the metabolic panorama of tumor tissue. Therefore, we aimed to construct a metabolism-related lncRNA prognosis signature for HCC and clarify the molecular and immune characteristics in defined subgroups. We present the following article in accordance with the TRIPOD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-2194/rc).

Methods

Data acquisition

After approval from the database of Genotypes and Phenotypes (dbGaP) data access committee, the raw RNA sequencing (RNA-Seq) datasets and clinical information for LIHC patients were downloaded and extracted from TCGA (https://portal.gdc.cancer.gov/) database. All available raw RNA-seq data in the database were downloaded and normalized by using R language (version 4.1.2; https://www.r-project.org/). Patients with a precise pathological diagnosis, gene expression, and clinical data were included in our study. In the end, we obtained 343 samples after excluding patients with no mutation information. The clinical data collected from 343 LIHC patients included age, gender, grade, pathologic stage, tumor-node-metastasis (TNM), TP53 mutational status, survival status, and survival time. Baseline characteristics of all included LIHC cases are summarized in *Table 1*. Then, TCGA-LIHC patients with complete clinical information were split into

Table 1 Correlation between clinicopathologic characteristics and the metabolism-related lncRNA signature in the TCGA-LIHC cohort

Characteristics	Training (n=207)	Testing (n=136)	Total (n=343)	P value	FDR
Age (years)					
Mean ± SD	60.58±13.31	57.59±12.97	59.40±13.24		
Median (min–max)	64.00 (16.00–90.00)	59.00 (18.00–85.00)	61.00 (16.00–90.00)		
Gender, n (%)				0.98	1
Male	140 (40.82)	93 (27.11)	233 (67.93)		
Female	67 (19.53)	43 (12.54)	110 (32.07)		
Grade, n (%)				0.79	1
Unknown	2 (0.58)	3 (0.87)	5 (1.46)		
G4	7 (2.04)	5 (1.46)	12 (3.50)		
G3	68 (19.83)	44 (12.83)	112 (32.65)		
G2	95 (27.70)	66 (19.24)	161 (46.94)		
G1	35 (10.20)	18 (5.25)	53 (15.45)		
Stage, n (%)				0.39	1
Unknown	13 (3.79)	9 (2.62)	22 (6.41)		
Stage IV	3 (0.87)	0 (0.0e+0)	3 (0.87)		
Stage III	44 (12.83)	36 (10.50)	80 (23.32)		
Stage II	44 (12.83)	33 (9.62)	77 (22.45)		
Stage I	103 (30.03)	58 (16.91)	161 (46.94)		
T, n (%)				0.45	1
Unknown	3 (0.87)	0 (0.0e+0)	3 (0.87)		
T4	8 (2.33)	5 (1.46)	13 (3.79)		
T3	44 (12.83)	31 (9.04)	75 (21.87)		
T2	46 (13.41)	38 (11.08)	84 (24.49)		
T1	106 (30.90)	62 (18.08)	168 (48.98)		
M, n (%)				0.23	1
Unknown	61 (17.78)	34 (9.91)	95 (27.70)		
M1	3 (0.87)	0 (0.0e+0)	3 (0.87)		
M0	143 (41.69)	102 (29.74)	245 (71.43)		
N, n (%)				0.57	1
Unknown	63 (18.37)	38 (11.08)	101 (29.45)		
N1	1 (0.29)	2 (0.58)	3 (0.87)		
N0	143 (41.69)	96 (27.99)	239 (69.68)		

lncRNA, long non-coding RNA; TCGA, The Cancer Genome Atlas; LIHC, liver hepatocellular carcinoma; FDR, false discovery rate.

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a training cohort (n=207) and a testing cohort (internal validation set; n=136) randomly. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Identification of hub metabolism-related lncRNAs

Metabolism-related lncRNAs were searched for through Pearson correlation analysis between messenger RNA (mRNA) and lncRNA expression matrices extracted from the enrolled TCGA-LIHC cohort and metabolism-related genes obtained from gene set enrichment analysis (GSEA). The screening criteria were correlation coefficient $|R^2|>0.4$ and P value <0.001. Tumor and paired normal tissue samples were screened from the samples to which metabolism-related lncRNAs belonged. The "limma" R package was used to select differentially expressed metabolism-related lncRNAs. The screening threshold was false discovery rate (FDR) <0.05 and $|\log_2 FC| \geq 1$. Univariate Cox regression analysis was performed to identify the metabolism-related lncRNAs significantly correlated with the overall survival (OS) in TCGA-LIHC cohort (P<0.05) by using the "survival" R package. To obtain the hub genes, we set the threshold as hazard ratio (HR) >1.35 or HR <0.65 and P<0.01 in Cox regression analysis and finally obtained a total of 34 hub metabolism-related lncRNAs. These identified hub prognosis-related characteristic lncRNAs were used to construct a multigene signature.

Construction and validation of the metabolism-related lncRNAs prognostic signature

The least absolute shrinkage and selection operator (LASSO) Cox regression analysis

and construction of metabolism-related lncRNAs prognostic signature were performed as previously described (17). All HCC cases in the training set were separated into two groups by the cutoff point. Survival curves of two groups were constructed by the Kaplan-Meier method. To evaluate the predictive accuracy of the prognostic metabolism-related lncRNA signature, time-dependent ROC curves were derived with the "survival ROC" R package and the corresponding area under curve (AUC) values calculated. Furthermore, the training set applied univariate and multivariate Cox regression analyses to determine the effect of metabolism-related lncRNAs prognostic signature and clinical parameters (including age,

gender, grade, stage, and TP53 mutational status) on OS. Finally, we used the testing set as internal validation set to examine the reliability of the prognostic metabolism-related lncRNA signature.

Identification of the metabolism-related lncRNAs prognostic signature associated biological characteristics

To explore the associated biological characteristics and potential signaling pathways, all the hub prognosis-related characteristic lncRNAs were subjected to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. The Cell-type Identification by Estimating Relative Subsets of RNA Transcripts (CIBERSORT) algorithm and single sample gene set enrichment analysis (ssGSEA) were performed as previously described (18). The "GSVA" R package was used to calculate the enrichment score of each sample. The "pRRophetic" R package was used to predict differences in drug sensitivity between high- and low-risk groups. Somatic mutation analysis between the 2 groups was performed using the "maftools" R package.
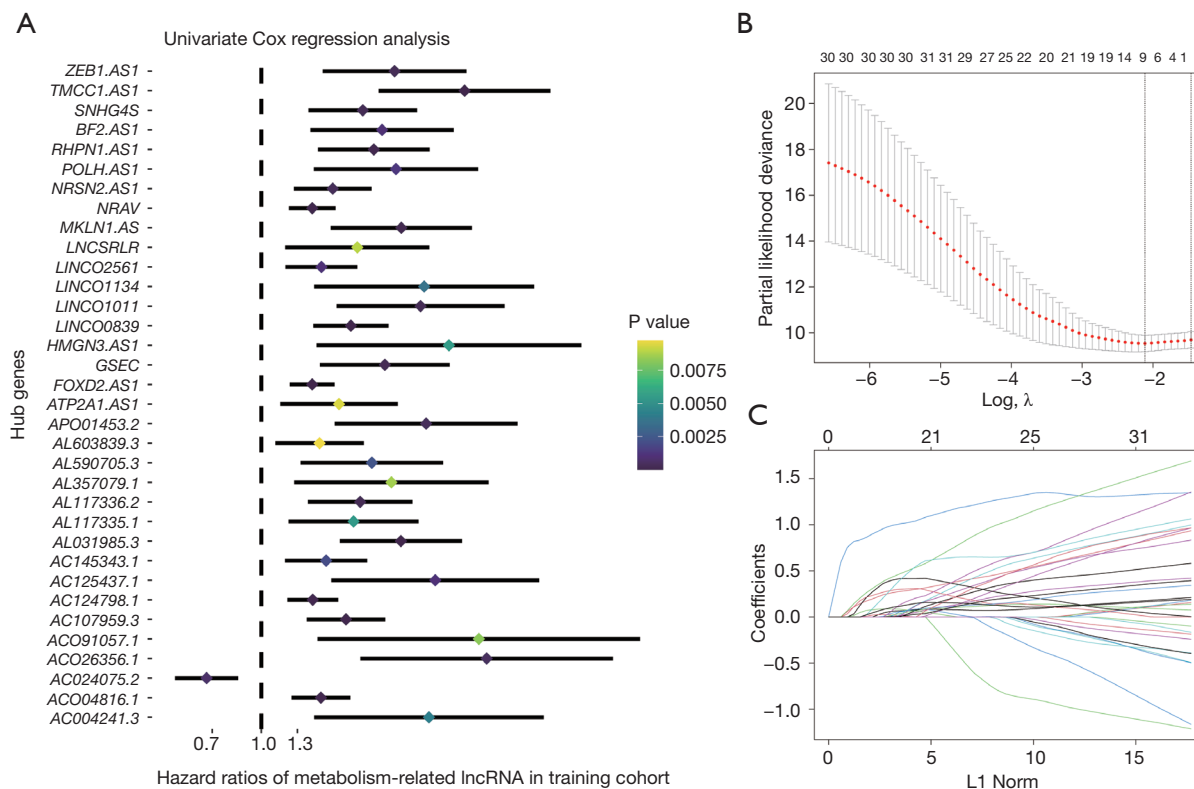
Evaluation of the clinical validity of the prognostic signature

We used age, gender, grade, stage, and risk score to construct a nomogram for the training set via the "rms" R package. Calibration curves were plotted to evaluate actual and predicted survival consistency. The "ggDCA" R package was used to plot decision curve analysis (DCA) curves to verify the accuracy of prognostic signature.

Statistical analysis

Wilcoxon and Kruskal-Wallis tests were used to compare continuous variables. Categorical variables were analyzed using the chi-square test. Mann-Whitney test was used to compare the ssGSEA scores of immune characteristics between the high- and low-risk groups. We adjusted P values with the Benjamini & Hochberg method. The OS between different groups was compared by Kaplan-Meier analysis with the log-rank test. Significant variables on univariate Cox regression analysis (P<0.05) were subjected to multivariate Cox regression analysis to identify independent predictors of OS. All P values were based on a 2-sided statistical test, and P<0.05 was considered statistically significant. All statistical analyses were

Figure 1 Acquisition of hub metabolism-related lncRNAs and biological functions based on TCGA database in LIHC patients. (A) Flowchart for bioinformatics analysis of publicly available data from TCGA databases. (B,C) The heatmap showed differential expression in metabolism-related genes (B) and metabolism-related lncRNAs (C). Red means up-regulated, green means down-regulated, and grey means no significance. (D,E) GO, and KEGG analysis demonstrated the biological functions of hub metabolism-related lncRNAs. lncRNA, long non-coding RNA; TCGA, The Cancer Genome Atlas; LIHC, liver hepatocellular carcinoma; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

conducted using the R software.

Results

Identification of differential metabolism-related lncRNAs and visualization of their biological functions

The acquisition of metabolism-related lncRNAs in the current study is displayed in the flowchart in *Figure 1A*. All differentially expressed genes and metabolism-related lncRNAs are presented in the form of volcano maps (*Figure 1B,1C*). In GO and KEGG analysis, characteristic lncRNAs were significantly associated with metabolic pathways, such as glycolytic pathways, certain amino acid metabolism, and so on (*Figure 1D,1E*).

Figure 2 Construction of the risk score model in the training cohort. (A) Univariate Cox analysis of 34 hub metabolism-related lncRNAs. (B) LASSO coefficient profiles. The two dotted vertical lines indicate the optimal values using the minimum and 1-SE criteria. (C) Candidate metabolism-related lncRNAs from the univariate Cox regression analysis were filtered by the LASSO algorithm. Each colored line represents a lncRNA, and the axis above indicates the number of nonzero coefficients at the current lambda(λ). lncRNA, long non-coding RNA; LASSO, least absolute shrinkage and selection operator.

Construction of the metabolism-related lncRNAs prognostic signature model

Univariate Cox regression analysis was performed on enrolled HCC patients to explore the prognostic value of metabolism-related lncRNAs. A total of 34 lncRNAs were screened for prognosis-related lncRNAs based on the criterion of P<0.05. The forest map showing the risk ratios and corresponding confidence intervals for each prognosis-related lncRNAs showed that most of these genes were risk genes (*Figure 2A*). Next, these prognostic lncRNAs were included in further LASSO Cox regression analysis. The prognostic lncRNAs with the highest contribution to the OS of HCC were generated by performing LASSO Cox regression analysis in the training cohort. The corresponding regression coefficients were calculated. *Figure 2B* illustrates that the model performed best with 9 lncRNAs involved. *Figure 2C* indicates the spectrum of

LASSO regression coefficients for the 9 lncRNAs.

Evaluation of the prognostic model

Based on the HCC prognostic signature, we divided the cases into high- and low-risk groups. Kaplan-Meier survival curves suggested that HCC patients with higher risk scores had less favorable OS in the training, test, and entire cohorts, respectively (*Figure 3A-3C*, upper). The time-dependent ROC curve indicated that the prognostic signature model could accurately predict prognosis in 3 separate cohorts (training cohort 1-year AUC =0.811, 3-year AUC =0.773, 5-year AUC =0.753; test cohort 1-year AUC =0.757, 3-year AUC =0.72, 5-year AUC =0.671; entire cohort 1-year AUC =0.789, 3-year AUC =0.752, 5-year AUC =0.729; *Figure 3A-3C*, middle). AUC values higher than 0.5 indicate a better prognosis predictive ability.
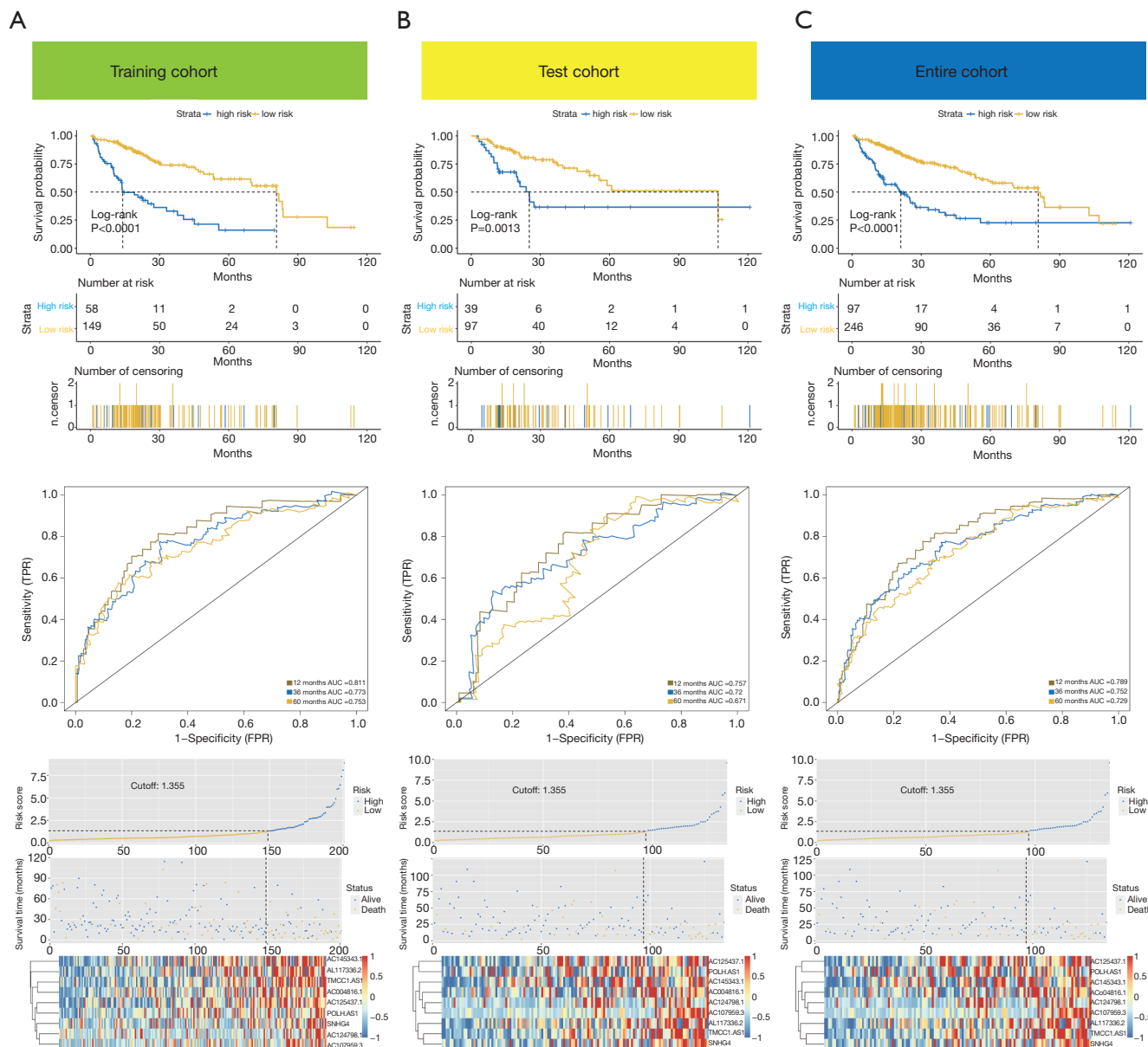
Figure 3 Evaluation of the prognostic signature model in different cohorts. (A) Kaplan-Meier survival curve analysis showed the relationship of survival time of LIHC patients between high- and low-risk scores (upper). The time-dependent ROC curves for 12-, 36-, and 60-month OS predictions by the prognostic signature model in the training cohort (middle). Cases were ranked according to the risk score, and the correlation between survival time and risk scores was demonstrated using scatter plots. Heatmap showed the correlation between characteristic lncRNAs and the prognostic signature model (bottom). The same approach was applied to the test cohort (B) and the entire cohort (C). lncRNA, long non-coding RNA; LIHC, liver hepatocellular carcinoma; ROC, Receiver Operating Characteristic; OS, overall survival; TPR, true positive rate; FPR, false positive rate.

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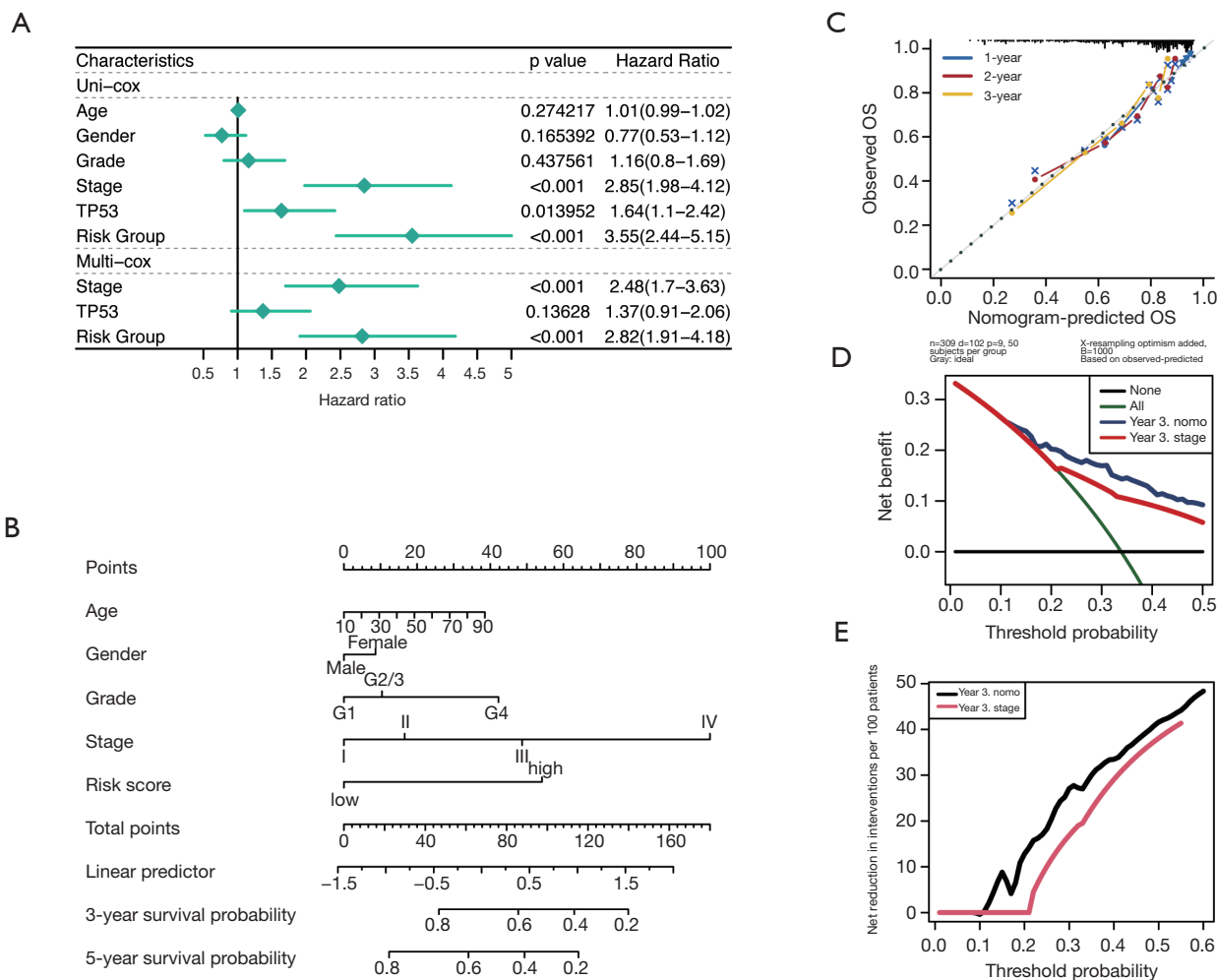
Figure 4 Assess the accuracy and clinical applicability of the prognostic signature model in LIHC patients. (A) Univariate and multivariate Cox regression analysis showed the correlation between OS and various clinicopathological parameters such as age, gender, grade, stage, TP53, and characteristic lncRNAs prognostic signature risk group. The stage and prognostic signature risk group significantly correlated with prognosis (P<0.001). (B) The prognostic nomogram with characteristic lncRNAs prognostic signature risk group and clinicopathological features were constructed to predict the prognosis of LIHC patients. (C) Calibration curve for nomogram-predicting 1-, 3-, and 5-year OS. The X-axis is nomogram-predicted survival probability, and the Y-axis is observed survival probability. (D) Decision curve analysis for nomogram and stage. (E) A net reduction in intervention per 100 patients results from the two risk prediction models. lncRNA, long non-coding RNA; LIHC, liver hepatocellular carcinoma; OS, overall survival; DCA, decision curve analysis.

Survival status distributions and risk scores were plotted. In addition, the characteristic lncRNAs associated with the prognostic model in each cohort are presented in the form of heat maps (*Figure 3A-3C*, bottom).

Description and evaluation of the metabolism-related lncRNAs as a crucial prognostic factor

Univariable and multivariable Cox Regression analysis revealed that metabolism-related lncRNAs signatures and stage are independent predictors of HCC survival (P<0.001; *Figure 4A*). Next, a nomogram containing the prognostic signature and traditional clinicopathological features was used to assess further the accuracy and clinical applicability of the prognostic (*Figure 4B*). The calibration plots indicated that the observed versus predicted rates of 1-, 2-, and 3-year OS had an excellent concordance in the HCC patients (*Figure 4C*). Similarly, the DCA curves showed the

accuracy of the prognostic model (*Figure 4D,4E*). The above results indicated that the metabolism-related lncRNAs signature could be identified as a crucial prognostic factor for HCC patients.

Stratified analysis of clinical parameters

Based on the optimal cut-off value of the training cohort calculated by "survminer" package, entire cohorts were classified into high- and low-risk groups. We aimed to ascertain whether the prognostic signature could be applied to different clinicopathologic subgroups (*Figure 5*). However, subgroup survival analyses found that whether age ≥60 or <60 years, gender male or female, tumour stage I/II or III/IV, and TP53 mutant or not, the patients in the high-risk group were significantly associated with a poor prognosis (all P<0.001).

Correlation between metabolism-related lncRNAs and status of TIICs

The TIICs influence tumorigenesis by affecting the immune microenvironment. The TIICs influence tumorigenesis by affecting the immune microenvironment. We inferred the absolute level of immune infiltrate within each sample in the high- and low-risk groups using the CIBERSORT algorithm (*Figure 6A*). The ssGSEA showed the differences between the 28 TIICs in the high- and low-risk groups more clearly (*Figure 6B*). It could be seen that immune-activated cell infiltration was significantly reduced in the high-risk group, such as activated B cells, activated CD8+T cells, Th1 cells, and NK cells.

Molecular characteristics of patients in the high- and low-risk groups

We performed GSEA to determine the gene sets enriched between the high- and low-risk group. In the low-risk group, the enriched gene sets included BILE ACID METABOLISM and CHOLESTEROL HOMEOSTASIS (*Figure 7A*), where KRAS SIGNALING DN was enriched in the high-risk group (*Figure 7B*). We predict the drug sensitivity of cisplatin and sorafenib using the pRRophetic algorithm in the high- and low-risk groups, and the results suggested that the high-risk group was less sensitive to both drugs (*Figure 7C*).

Next, gene variations were detected to more clearly elucidate these characteristic lncRNAs' biology. The top 10 mutated genes shared in the 2 groups were ranked by mutation rate (*Figure 7D*). We found that only TP53 mutation frequency exceeded 20% in both groups. The gene variations in both groups were predominantly deletion mutations.

Discussion

Abnormal cancer metabolism, such as increased glycolytic and glutamine metabolic pathways, has an important role in tumor development, metastasis, drug resistance, and cancer stem cells (19-21). Tumor neovascularization causes cancer cells to grow in an environment of hypoxia and nutrient deficiency (22). To adapt to these harsh environments, cancer cells constantly alter their metabolic pathways to obtain external metabolites and maximize the maintenance of high metabolic enzyme activity (6). Classical cancer-related signaling pathways, such as the PI3K/AKT and myc pathways, can mediate aberrant metabolic gene expression and increase key metabolic enzyme activities (23,24). Cancer-associated metabolism is based on a differential metabolic stress response between cancer and normal cells; targeting cancer-associated metabolism is expected to selectively inhibit cancer progression with less cytotoxicity to normal cells.

In this study, we explored the role of metabolism-related prognostic signature lncRNAs in HCC. A prognostic model containing 9 metabolism-related lncRNAs was first constructed and validated in the HCC training set. In addition, immunoassays using various bioinformatics tools showed significant differences in TIME and TIICs between high- and low-risk groups. Functional analysis revealed that the signature lncRNAs were associated with many cancer-related metabolic pathways. These findings strongly suggested a huge potential for cancer-related metabolic reprogramming in HCC.

A total of 9 hub metabolism-related lncRNAs (AC124798.1, TMCC1.AS1, AC145343.1, AC125437.1, SNHG4, AC004816.1, AL117336.2, POLH.AS1, and AC107959.3) were identified in our prognostic model. The lncRNA TMCC1-AS1 promotes epithelial mesenchymal transition for tumor metastasis in HCC (25). The lncRNA SNHG4 can promote cancer development as a competing endogenous RNA (ceRNA) (26,27). In addition, AC124798.1 (28), AC145343.1 (29), AC004816.1 (30), and AC107959.3 (31) were found to be strongly associated
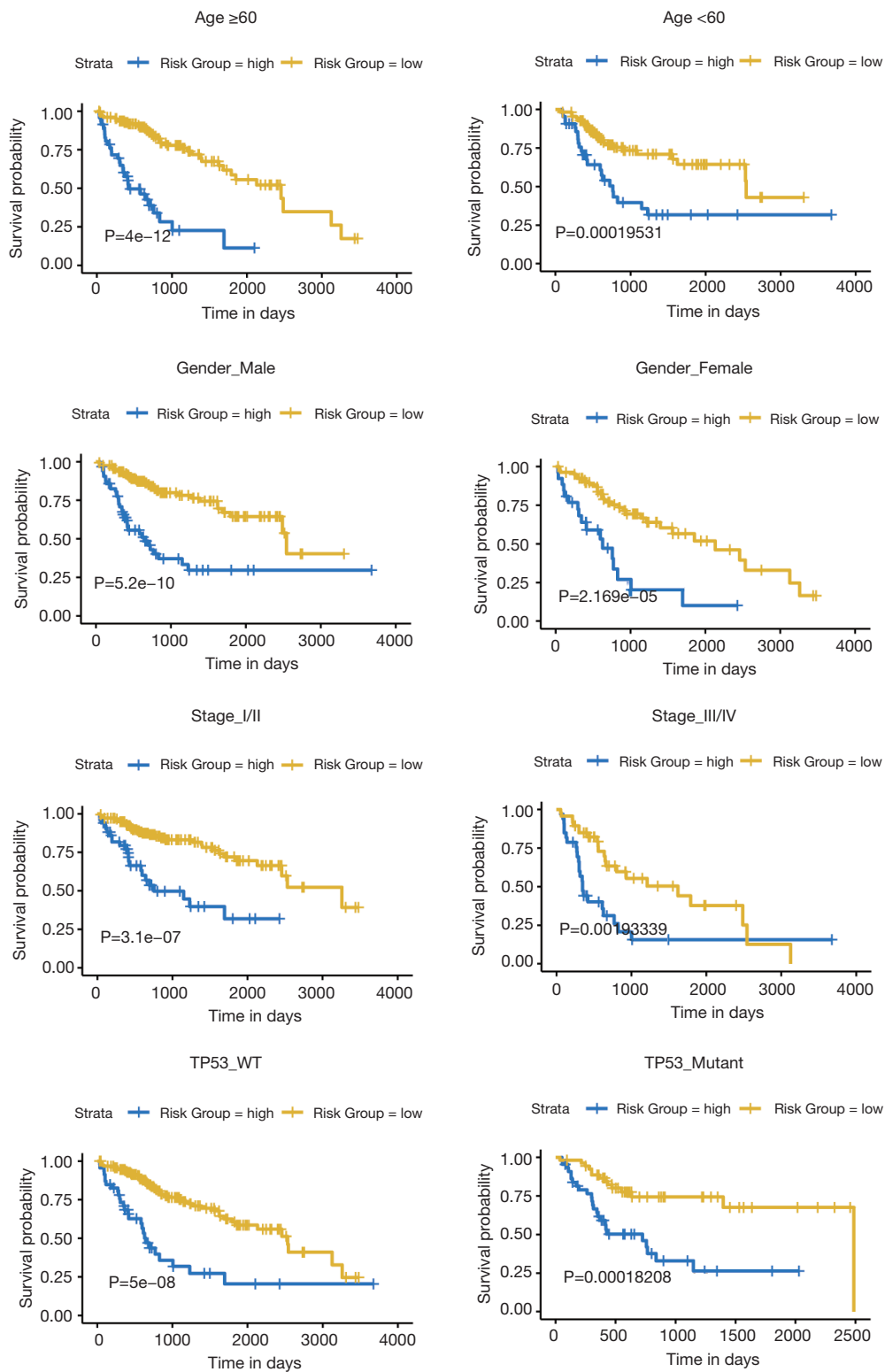
Figure 5 Stratification analysis based on clinical parameters. Kaplan-Meier analysis demonstrated the survival of LIHC patients according to different ages, gender, stage, and TP53 status based on different prognostic signature risk groups. The respective P values are displayed under the survival curves. LIHC, liver hepatocellular carcinoma.
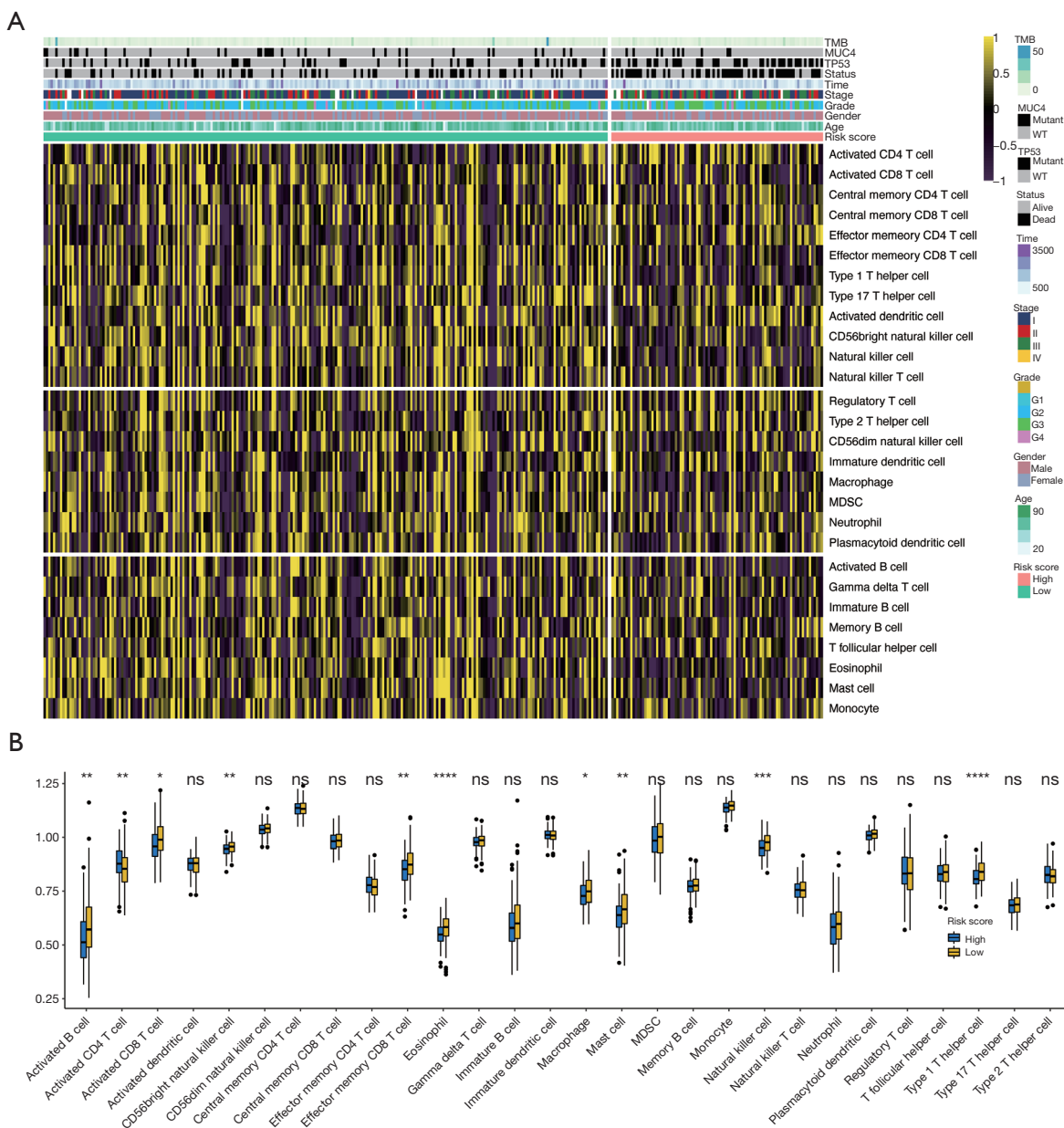
Figure 6 Association between prognostic signature risk score and TIICs. (A) Heatmap for metabolism-related lncRNAs prognostic signature risk score, MUC4, TMB, and clinicopathological features based on CIBERSORT. TP53, status, time, stage, grade, gender, and age are patient annotations. (B) ssGSEA for the association between TIICs and related functions in different risk groups. Adjusted P values were showed as: ns, not significant; *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001. TIICs, tumor-infiltrating immune cells; TMB, tumor mutational burden; ssGSEA, single-sample gene set enrichment analysis; CIBERSORT, Cell-type Identification by Estimating Relative Subsets of RNA Transcripts.

with poor prognosis of cancer by bioinformatics analysis. Although only a few lncRNAs have been experimentally reported as associated with cancer development, it is still possible to hypothesize that our prognostic model was

negatively associated with prognosis of HCC based on the results of previous bioinformatic analysis and our results.

There is growing evidence that cancer-associated metabolic reprogramming not only plays a crucial role

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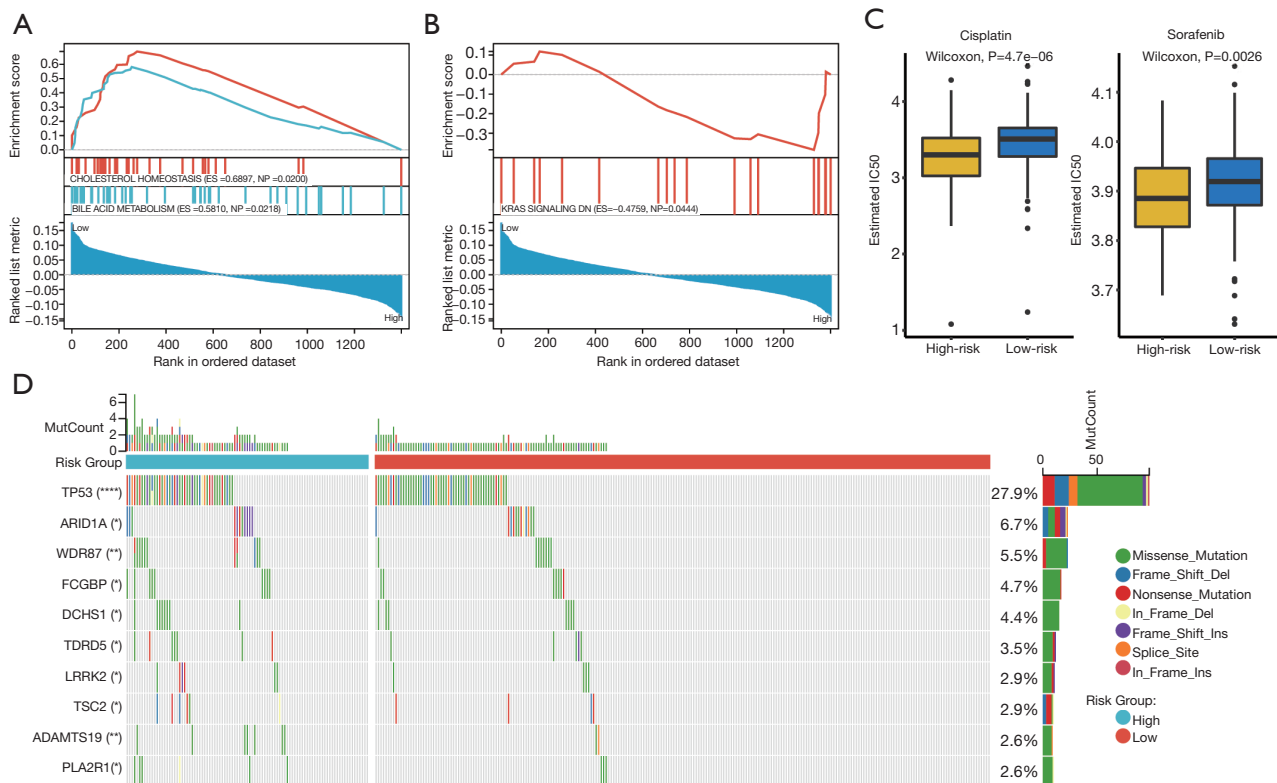
Figure 7 Molecular characteristics of patients in the high- and low-risk group. (A,B) GSEA analysis showed that the high-risk group was negatively associated with metabolic-related pathways and positively associated with tumor proliferation-related pathways. (C) IC50 values of cytotoxic chemotherapeutic agents between different risk groups based on the pRRophetic algorithm. The high-risk group had significantly lower IC50 values for cisplatin and sorafenib. (D) Significantly mutated genes in LIHC patients in the high- and low-risk group. The top 10 mutated genes in 2 groups ranked by mutation rate are shown. The mutation rate is shown on the right, and the mutation counts are shown on the top. Adjusted P values are shown as: *, P<0.05; **, P<0.01; ****, P<0.0001. GSEA, gene set enrichment analysis; LIHC, liver hepatocellular carcinoma.

in cancer signaling, but also has broad implications in regulating antitumor immune responses through metabolite release and influencing the expression of immune molecules, such as lactate (32). Metabolic reprogramming is not unique to cancer cells; it is present in some immune cells, such as activated T cells, regulatory T cells (Tregs), and neutrophils (33) Like cancer cells, TIICs require certain nutrients from TME to maintain their own proliferation and differentiation. Previous study has shown that cancer cells limit glucose consumption by T cells through glycolysis, thereby inducing T cell failure and immune escape (34). In our study, immune infiltration analysis and enrichment analysis revealed a significant decrease in the percentage of immune activated cells in the high-risk group compared to the low-risk group. In *Figure 1D* and *Figure 1E*,

we found that characteristic lncRNAs were significantly associated with glycolysis, oxidative phosphorylation, and amino acid metabolism. Glycolysis has been reported to be a major metabolic pathway for M1 macrophages, dendritic cells, naive T cells, and effector T cells (35). Tregs, M2 macrophages, and memory T cells rely mainly on OXPHOS, a source of fatty acid oxidation, for energy supply (36). Certain amino acids, such as glutamine, are essential for the maintenance and regulation of immune cell function (37). In addition, GSEA found that the high-risk group was significantly associated with the KRAS pathway. KRAS mutation aberrantly activates its downstream signaling pathways, including PI3K/AKT and MEK/ERK, thus contributing to the promotion and maintenance of cancer malignancy. Recently, KRAS has been reported to

mediate immune escape in TME, which is consistent with our findings (38).

In addition, we explored the differences in drug sensitivity between high and low risk groups. Cisplatin and sorafenib are the most classical HCC drugs, and our results suggest that the high-risk group is less sensitive to both drugs. Both cisplatin and sorafenib were reported to promote immune activation to maintain the anticancer effect (39,40). This suggests that we can improve drug sensitivity to cisplatin and sorafenib by improving the immunosuppressive status of HCC patients. We are looking forward to see if it is the same with the new antitumoral drugs for HCC, such as lenvatinib, cabozantinib, or regorafenib, as well as the antiangiogenic antibody ramucirumab.

With the rapid development of bioinformatics technology, an array of models has been constructed to predict the prognosis of HCC based on lncRNA. However, in contrast to our study, these studies neglected the value of traditional clinical parameters. We combined clinical parameters with metabolism-related signature lncRNAs to predict the survival of HCC, which would provide great value to the clinic. In addition, we hypothesized a behind-the-scenes reason why signature lncRNAs affect prognosis of HCC-immunosuppression, which will provide new insights for future experimental design. However, our study still had some limitations. First, it was conducted retrospectively and therefore needs further validation in prospective studies. Second, HCC is highly heterogeneous and has different biological behaviors, the information from TCGA database was limited and incomplete. Third, to define the prognostic clinical outcome of patients with HCC there are different variables that have not been considered such has the histopathology of the tumor, level of alfa-fetoprotein and type of treatment (systemic or surgery as resection or transplantation). Fourth, more experiments are needed to further reveal the potential mechanisms by which the characteristic lncRNAs affect the prognosis of HCC.

In conclusion, our study identified 9 characteristic lncRNAs' prognostic features and validated the accurate type and reliability of the model by ROC, DCA, and nomogram. Our prognostic model can help clinicians to provide personalized treatment strategies, and the 9 signature lncRNAs also provide some insights into new prognostic markers and potential therapeutic targets for HCC.

Acknowledgments

Footnote

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