

Bioinformatics analysis and experimental verification of five metabolism-related lncRNAs as prognostic models for hepatocellular carcinoma

Wei Wang, MD^{a,b}, Zhenfeng Deng, MD^{a,b}, Zongrui Jin, MD^{a,b}, Guolin Wu, MD^{a,b}, Jilong Wang, MD^{a,b}, Hai Zhu, MD^{a,b}, Banghao Xu, MD^{a,b}, Zhang Wen, MD^{a,b}, Ya Guo, MD^{a,b,*}

Abstract

Background: The number of patients with hepatocellular carcinoma (HCC) is showing a growing trend all over the world. The metabolic microenvironment has been shown to play a key role in the pathogenesis of HCC in recent studies. The expression of metabolites and metabolic processes in tumor cells can be regulated by gene regulation mediated by long non-coding RNAs (lncRNAs), the abnormal expression of which is closely related to tumor occurrence and metastasis. However, the fundamental mechanism of applying metabolism-related lncRNAs to predicting HCC is still unclear.

Methods: With the complete RNA sequence data and clinical data obtained from The Cancer Genome Atlas database and metabolism-related genes downloaded from the Kyoto Encyclopedia of Genes and Genomes database, with false discovery rate < 0.001, log fold change > 1.5 selected as the screening criteria for lncRNA, the relationship between the expression level of metabolism-related lncRNAs (MRLs) and the overall survival rate was determined by the Univariate Cox regression analyses with the establishment of the metabolic prognosis model by the application of Multivariate Cox regression analyses, revealing the different biological processes and signaling pathways in both high-risk groups and low-risk groups by Gene Ontology, Kyoto Encyclopedia of Genes and Genomes enrichment analysis, and gene set enrichment analysis, leading the expression of lncRNA to be assessed by the reverse transcription-polymerase chain reaction results.

Results: The overall survival rate of HCC patients is significantly correlated with signature of 5-MRLs. The prognosis characteristics of lncRNA reveal the relatively high death rate of patients in the high-risk groups, with the predicted signals by functional and pathway enrichment analysis related to biosynthesis, metabolic process, and metabolic pathway, with the prognostic characteristics of 5-MRLs by the combined analysis showing that it is an independent factor of HCC superior to the traditional clinical indicators in predicting the prognosis. A trend of high-expression was shown in MRLs in tumors by reverse transcription-polymerase chain reaction.

Conclusion: The new 5-MRLs as potential biomarkers provide more powerful prognostic information for HCC patients. In the future clinical treatment of HCC, it will provide doctors with more methods.

Editor: Jianxun Ding.

WW, ZD, YG, and ZW contributed equally to this work.

This work was supported by the National Natural Science Foundation of China (grant no. 81560387, 81902983), the Guangxi Natural Science Foundation of China (grant no. 2018JJB140382), "Medical Excellence Award" funded by the creative research development grant from the first affiliated hospital of Guangxi Medical University (grant no.180327), the Guangxi medical and health technology development and application project (grant no. S2019097, S2018100). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials: The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate: All patients provided written informed consent, and the study was approved by the Ethics Committee of The First Affiliated Hospital of Guangxi Medical University (Nanning, China).

Patient consent for publication: Not applicable.

The authors declare that they have no competing interests.

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

The datasets generated during and/or analyzed during the current study are publicly available.

^a Department of Hepatobiliary Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, China, ^b Guangxi Key Laboratory of Enhanced Recovery after Surgery for Gastrointestinal Cancer, The First Affiliated Hospital of Guangxi Medical University, Nanning, China.

* Correspondence: Ya Guo, Department of Hepatobiliary Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China, Guangxi Key Laboratory of Enhanced Recovery after Surgery for Gastrointestinal Cancer, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China (e-mail: guoygxmu@163.com).

Copyright © 2022 the Author(s). Published by Wolters Kluwer Health, Inc.

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Wang W, Deng Z, Jin Z, Wu G, Wang J, Zhu H, Xu B, Wen Z, Guo Y. Bioinformatics analysis and experimental verification of five metabolism-related lncRNAs as prognostic models for hepatocellular carcinoma. *Medicine* 2022;101:4(e28694).

Received: 14 July 2021 / Received in final form: 17 December 2021 / Accepted: 4 January 2022

<http://dx.doi.org/10.1097/MD.00000000000028694>

Abbreviations: FDR = false discovery rate, GO = Gene Ontology, GSEA = gene set enrichment analysis, HCC = hepatocellular carcinoma, KEGG = Kyoto Encyclopedia of Genes and Genomes, MRG = metabolism-related gene, MRL = metabolism-related lncRNA, OS = overall survival, PCA = principal components analysis, ROC = receiver operating characteristic, TCGA = The Cancer Genome Atlas.

Keywords: hepatocellular carcinoma, metabolism-related long non-coding RNA, prognostic signature, The Cancer Genome Atlas

1. Introduction

Ranking in the sixth of the most common cancers, primary liver cancer comes in the fourth leading cause of cancer-related death, leading to, according to the 2020 statistic, about 906,000 new cases and 830,000 deaths annually,^[1] with the hepatocellular carcinoma (HCC) accounting for 75% to 85% the confirmed liver cancer cases, thus making advancement in the diagnosis, treatment, and prognosis of HCC in light of the limitedness of the current ones.^[2–4] Hepatitis A virus (HBV), hepatitis C virus (HCV) infection, obesity, and type 2 diabetes are the main causes of high risk of HCC.^[5] Cancer cells receive, by generating cumulative changes in metabolism, regular and unconventional nutrition, with these nutrients used to create new biomass to maintain uncontrolled proliferation and metabolites selected to affect the survival of cancer cells themselves and various normal cell types in the tumor microenvironment. As the result of the selective evolution of tumor growth, proliferation and survival, support for the fundamental needs of cancer cells, such as increasing energy production, macromolecular biosynthesis, and maintaining redox balance is provided by metabolic changes,^[6] pathway of which is favorable for tumor proliferation and growth with ensured energy and substrate, thus creating essential conditions for accelerating tumor growth in the tumor microenvironment.^[7] These processes are reprogrammed in cancer metabolism, resulting in the hepatocarcinogenesis and development with the presence of abnormal cell metabolism. Innumerable studies have shown that lipid metabolism, glucose metabolism, purine metabolism, and inositol phosphate metabolism play an active role in the carcinogenesis of HCC which usually manifests a variety of metabolic changes such as increased aerobic glycolysis, enhanced synthesis of new lipids, depletion of glutamine, and imbalance of oxidative metabolism, through which energy and biological macromolecular substances are provided for the rapid growth and proliferation of tumor cells.^[8] Also, tumor metabolism, with its correlation between HCC disclosed by these studies, is regulated by a variety of factors, such as changes in metabolic enzyme activity, aberrant gene expression, disturbance of signal pathways.

With more cancer data published by The Cancer Genome Atlas (TCGA) through which the required shared data sets can be accessed and assessed by the researchers at any time,^[9] a powerful platform was provided by the development of high-throughput sequencing technology and TCGA for the discovery of novel biomarkers and metabolic targets of liver cancer, leading to the results that it is of great significance to obtain the characteristic metabolic genes and their regulatory mechanisms by bioinformatics in predicting the survival rate of HCC patients. This study was designed to identify metabolic-related lncRNAs associated with HCC prognosis on the basis of TCGA and Kyoto Encyclopedia of Genes and Genomes (KEGG) database, with the prognostic risk model established based on univariate and multivariate Cox regression analysis, prognostic characteristics analyzed according to the expression of lncRNA, and revealed the different biological processes and signaling pathways in both

high-risk groups and low-risk groups by Gene Ontology (GO), KEGG enrichment analysis, and gene set enrichment analysis (GSEA). The consistency of these results of the samples verified by utilizing reverse transcription-polymerase chain reaction. Our goal is to use the expression profile of lncRNA to identify metabolism-related lncRNA, which will help us find potential prognostic indicators and promising treatment targets of HCC.

2. Materials and methods

2.1. Dataset source

From the TCGA database (<https://xenabrowser.net/>, accessed October 21, 2019) were the tumor samples and normal samples of HCC patients obtained with gene expression profiles, clinicopathological information, and from KEGG database (<https://www.genome.jp/kegg/>, accessed October 21, 2019) were the metabolism-related genes obtained with the sample collecting conforming to the TCGA guidelines and data access policies, and from TCGA was all the available clinical information downloaded, making it not necessary to gain the approval of the ethics committee.

2.2. Differential expression lncRNA screening

RNA-seq data were obtained from the whole genome expression profile including mRNA and lncRNA expression data, with false discovery rate (FDR) < 0.001, log (fold change) > 1.5 selected as the screening criteria for lncRNA with differential expression using the “limma” package on R (version 3.6.1). With the gene expression data obtained from TCGA intersecting with metabolism-related genes downloaded from KEGG and the selected genes used as metabolism-related genes (MRGs) in expression data, metabolism-related lncRNAs (MRLs) were identified according to the Pearson correlation coefficient between MRGs ($|r| > 0.5$, $P < .001$) and extracted lncRNAs, which were adopted in the following studies.

2.3. Construction of prediction features based on MRL expression

The expression of MRLs and their predictive roles by the “survival” package were determined with the performance of univariate Cox regression analyses by identifying P value < .01 as prognosis MRLs, revealing significant correlation between the expression level and overall survival (OS) of HCC patients. The prognosis of MRL was estimated with the “step” function in survival analyses by selecting the most suitable combination for predicting prognosis. With the incorporation of MRL identified by “step” function into multivariate Cox regression analyses by taking OS as a dependent variable, the multivariate Cox regression coefficient expression and the weight based on multivariate Cox regression coefficient (β) were brought together to construct a risk score model expressing prognosis,^[10] thus forming the risk score formula: Risk Score = expression of MRL 1 \times β 1 MRL 1 + expression of MRL 2 \times β 2 MRL 2 + expression

of $MRL_n \times \beta_n MRL_n$,^[11] leading the accuracy of predicting the prognostic characteristics of HCC to be determined by receiver operating characteristic (ROC) curve of “survival” packages on R.^[12]

2.4. Evaluation of MRL-based prognostic signature

With the prognosis model assessed by prediction features based on MRL expression, the survival status of the high and low response groups compared, and the predictive value of MRL on the prognostic characteristics of HCC patients evaluated, a nomogram was built on the basis of risk score and clinicopathological features to predict the prognosis survival rate, with the prognostic value of risk score and the impact of the risk score on the survival rate assessed according to stratified analysis and combined effect analysis, thus rendering the distribution pattern of high and low-risk cases determined by the principal component analysis (PCA).

2.5. Functional assessment and enrichment analysis

The “cluster Profiler” R package was used to analyze the co-expressed MRLs for GO terminology and Kyoto gene and genome Encyclopedia (KEGG) database path analysis, with the GO analysis revealing the MRL function in the biology process, cell component, and molecular function, and the KEGG analysis showing the pathway enrichment of MRLs. As P -value $< .05$ is of statistical significance in GO and KEGG enrichment analysis, the different functional phenotypes between the high and low-risk groups were explored for GSEA (version 4.0.1, <http://www.broadinstitute.org/GSEA/index.jsp>), and the Molecular Signatures Database (MSigDB) of c2 (c2.cp.kegg.v7.0.symbols.gmt) and c5 (c5.all.v7.0.symbols.gmt) was used to GSEA, in which $P < .05$ and $FDR < 0.05$ were considered to be of statistical significance.

2.6. RNA extraction and real time polymerase chain reaction assay

With the ethical approval, approval number: 2020 (KY-E-172), granted by the First Affiliated Hospital of Guangxi Medical University of the Ethics Committee, and written informed consent obtained from all patients. Inclusion criteria: age from 18 to 75 years old, no gender limit; cases undergoing hepatectomy, and postoperative histopathological diagnosis of HCC. Six tumor tissues and the adjacent tissues from hepatectomy patients from 2020 to 2021 were collected from the First Affiliated Hospital of Guangxi Medical University of the Hepatobiliary Surgery Department, with subjects identity information accessible during or after data collection. The specimens were collected during the operations and frozen in liquid nitrogen immediately afterward, with total RNA extracted by using RNAiso Plus (TaKaRa, Japan) complying with the manufacturer’s protocol and reversely transcribed into complementary DNA (cDNA) by using a PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa, Japan) in the condition that real-time PCR in the ABI7500 real-time PCR system was carried out by using the TB Green *Premix Ex Taq II* (Tli RNaseH Plus) (2 × conc) (TaKaRa, Japan).

The primers pairs were:

MIR210HG Forward CCCTTTCTCTGGAGCACAC
MIR210HG Reverse TTCCCTGTTCCCTGCCT
AL365203.2 Forward ACACCTTCATGGGCTTACTGCT

AL365203.2 Reverse GCTGCCTTTTCACCTCTTACAC
AL031985.3 Forward CACCTATTCAACTTCCCCATT
AL031985.3 Reverse CCAAGGATTCCCCTAAACATC
AC099850.3 Forward GAAAATATGGAAACAGGAACAG-
GAC
AC099850.3 Reverse GGAAATCTCAAAAACCCAAAGG
LUCAT1 Forward GGTGCCAAGGTCCCATA
LUCAT1 Reverse AAGCTCGGATTGCCTTAGA

2.7. Statistical analysis

The measurement data conforming to the normal distribution are expressed by the mean \pm standard deviation, the comparison between groups is by t test; the count data is expressed by percentage, and the comparison between groups is by Chi-squared test; survival status comparisons between different subgroups were carried out via Kaplan–Meier survival analysis by log-rank test, with the clinical characteristics and survival time analyzed by the Cox hazard model and all statistical analyses implemented by SPSS version 22.0 (IBM Corporation, Armonk, NY) and R 3.6.1.

3. Results

3.1. Identification of based MRLs

lncRNA ($FDR < 0.001$, $|\log \text{fold change}| > 1.5$) in 374 HCC samples and 50 normal samples were identified by using the “limma” package on R, with 370 HCC samples with complete survival information further analyzed, under the condition that a total number of 937 MRGs were obtained from KEGG database and the expression data of these genes in the liver cancer cohort of the TCGA database were extracted (Table S1, Supplemental Digital Content, <http://links.lww.com/MD/G599>). Meanwhile, a total number of 2052 different lncRNAs of cancer tissues and the adjacent tissues were screened by 14,141 lncRNAs, with the different analysis results shown in Fig. 1A and B, indicating the result of 166 MRLs being identified between 937 MRGs and 14,141 lncRNAs by Pearson correlation analysis ($|r| > 0.5$ and $P < .001$).

3.2. Construction of the MRLs prognostic signature predicts survival

Univariate Cox analysis was used to analyze the expression level and survival time of 166 MRLs with “survival” package, with 20 of them significantly correlated with the survival time ($P < .01$, Table S2, Supplemental Digital Content, <http://links.lww.com/MD/G600>). The best combination (5-MRLs: AC099850.3, AL031985.3, AL365203.2, LUCAT1, MIR210HG) was selected by the “step” function (Table 1). for the purpose of constructing a multivariate COX analysis model of MRLs related to prognosis, with prognostic analysis of 5-MRLs in the MRLs prognostic model falling into high and low-risk groups (Fig. 2), thus leading the contribution of prognostic MRLs to prognosis prediction to be determined by the multivariate Cox analysis. The risk score grades, survival outcomes, and prognosis of the 5-MRLs expression in different groups are shown in Fig. 3A–D, with the prognostic analysis of clinicopathological features and risk score showing that tumor stage and radical resection score were of statistical significance and the 2 factors were significantly related to the prognosis and survival of HCC (Table 2). Based on

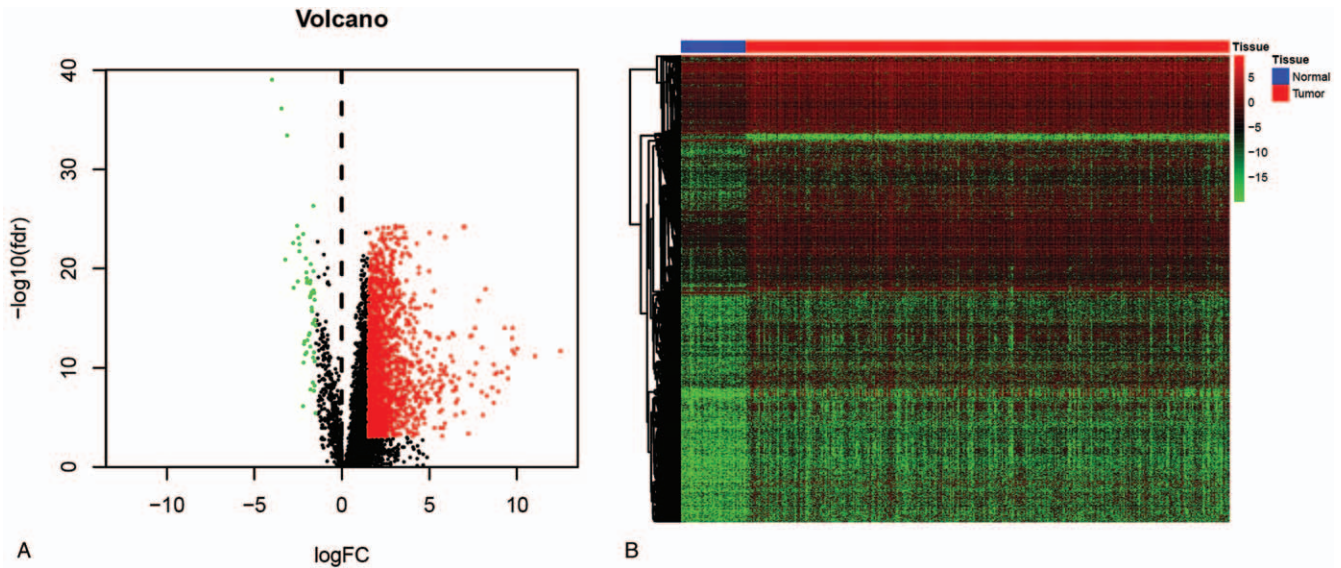


Figure 1. Differential expression LncRNA screening (A). Volcano plot of MRLs (B). Heatmap of MRLs in 374 HCC samples and 50 normal samples. HCC=hepatocellular carcinoma, MRL=metabolism-related LncRNA.

the median risk score of MRL expression, HCC patients were subdivided into high-risk groups and low-risk groups. Results show that, despite significant improvement made in the risk of death with high-risk score ($P < .001$; hazard ratio=2.206; 95% confidence intervals =1.539–3.163; Table 2, Fig. 3A–D), the median survival time (MST) of high-risk groups was still significantly shorter than that of low-risk groups (high risk vs low risk: 1149 days vs 2131 days). As displayed in ROC analysis using the survival package, the 1, 2, 3, and 5-year survival rates were respectively 0.769, 0.729, 0.703, and 0.648 (Fig. 3E), result in the conclusion that this prognostic feature based on the expression of LncRNA also is well-performed in predicting the survival rate of HCC, with the expression levels distribution of 5 MRLs in different subgroups shown in Fig. 4A and B.

3.3. Combined analysis of the prognostic characteristics of 5-MRLs

According to the combined effect analysis, the prognostic characteristics of 5-MRLs combined with the clinicopathological characteristics have a good performance in predicting the survival rate of HCC patients (Table 3, Fig. 5A–I), making it reasonable for the risk scores and clinical information to be integrated to construct a prognostic nomogram for predicting 1 to 0.3 to 0.5-year survival rates (Fig. 6). In this research, PCA analysis was

carried out to describe the distribution pattern of prognosis in high-risk and low-risk populations, leading to the conclusion that, compared with traditional prognostic indicators, the risk score of nomogram has a more significant impact on the prognosis of HCC. Significantly different prognosis distributions of the high-risk and low-risk groups were obtained base on the 5 MRL prognostic models (Fig. 7A), while there was no significant difference in prognostic distributions of all genes, 166 MRLs, and 20 prognostic-related MRLs (Fig. 7B–D).

3.4. Function and pathway enrichment analysis

Previous researches have established that proteins could not be encoded by the biological function of lncRNA, with many of them acting as cis regulators and their functions linked to co-expressed mRNAs with the protein-coding ability.^[13,14] Pearson correlation analysis showed that MRLs were highly correlated with metabolism and biosynthesis in function and pathway enrichment analysis, by the result of which these lncRNAs were mainly concentrated in 30 GO items and 18 KEGG pathways ($P < .001$), with GO enrichment analysis showing the obvious enrichment effects of the co-expressed MRLs on nucleoside phosphate, nucleotide, purine-containing compounds, nucleoside phosphate biosynthesis, and glycoside compound metabolism (Fig. 8A), and KEGG analysis disclosing the close connection between most co-

Table 1
The 5 MRLs of prognostic signature identified from Cox regression analysis.

LncRNA	Ensemble ID	Hazard ratio [*]	Coefficient [†]	P value [*]
AC099850.3	ENSG00000265303	1.523	0.188	.001
AL031985.3	ENSG00000260920	3.205	0.597	.001
AL365203.2	ENSG00000273038	1.654	0.225	.001
LUCAT1	ENSG00000248323	1.692	0.367	.001
MIR210HG	ENSG00000247095	1.519	0.196	.001

HCC=hepatocellular carcinoma, MRLs=metabolism-related long noncoding RNAs.

^{*}Data from the univariate Cox regression analysis in HCC cohort.

[†]Data from the multivariate Cox regression analysis in HCC cohort.

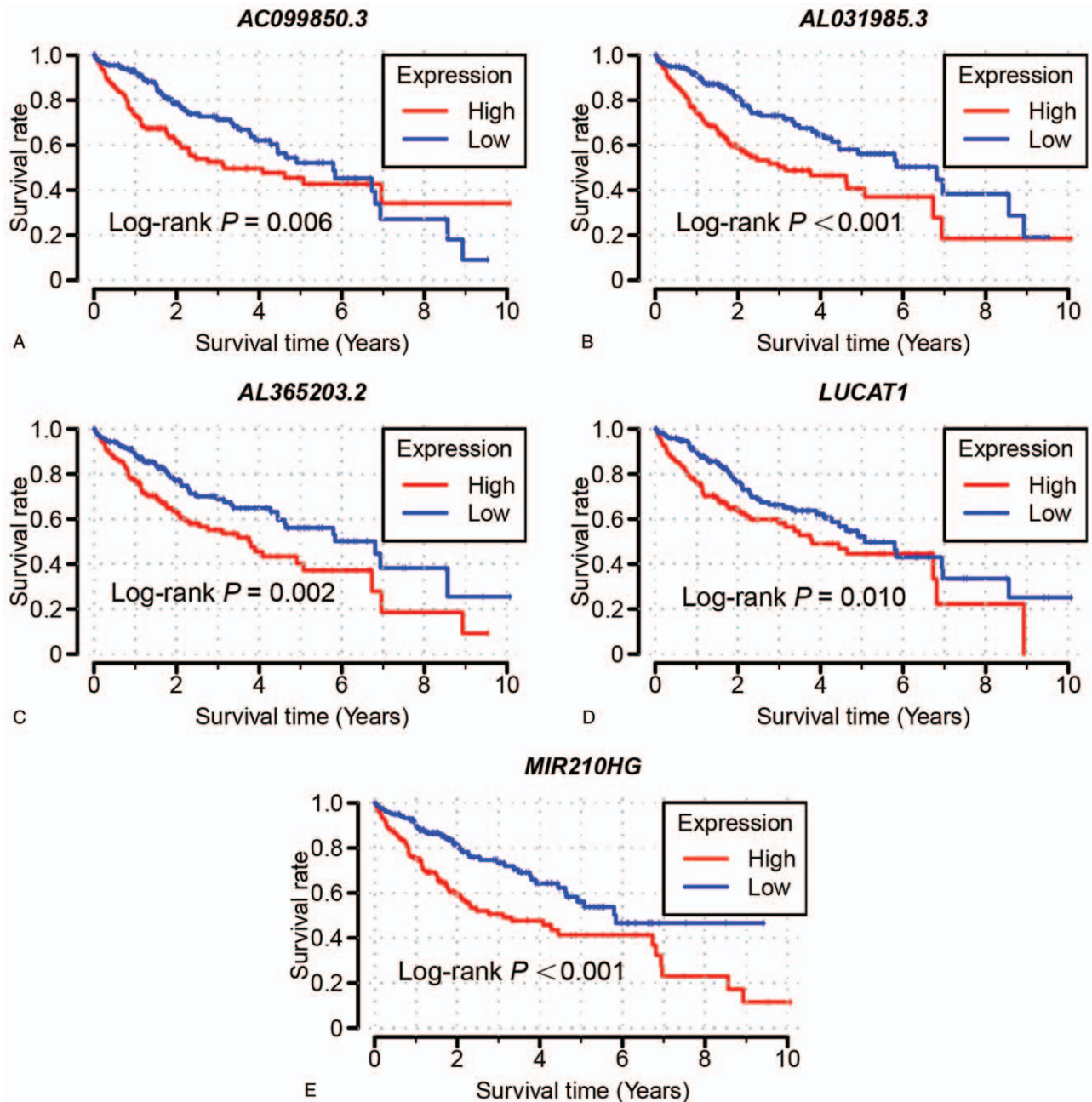


Figure 2. The Kaplan–Meier curve of 5-MRLs divided into high and low expression group. 5-MRLs selected through univariate cox regression analysis and multivariate cox risk model: A AC099850.3; B AL031985.3; C AL365203.2; D LUCAT1; E MIR210HG. MRL=metabolism-related lncRNA.

expressed MRLs and purine metabolism, inositol phosphate metabolism, and DNA replication (Fig. 8B). The results of GSEA analysis on C2, which is enriched with polysaccharide biosynthesis, purine metabolism, pyrimidine metabolism, RNA degradation, and ubiquitin-mediated proteolysis (Fig. 9A–F), and C5, which is rich ATPase activity, DNA catalytic activity, and cell cycle regulation, in genomes of diverse risk populations were consistent with those of GO and KEGG of MRGs (Fig. 9G–I).

3.5. Clinical validation of lncRNA levels of 5-MRLs

Six pairs of tumor and para-cancerous controls were analyzed to validate the lncRNA levels of 5-MRLs, revealing that the

expressions of AC099850.3, AL031985.3, AL365203.2, LUCAT1, and MIR210HG were relatively highly expressed in tumors, indicating that the trend of our experimental results was almost consistent with data analysis (Fig. 10). The flow-process diagram of this experiment is shown in Fig. 11.

4. Discussion

Most cases of hepatocellular carcinoma, a highly lethal tumor, are already at an advanced stage when diagnosed, with its morbidity close to its mortality, along with the continuous change of the metabolic environment resulting in the rapid development of HCC and the incessant changes of metabolic

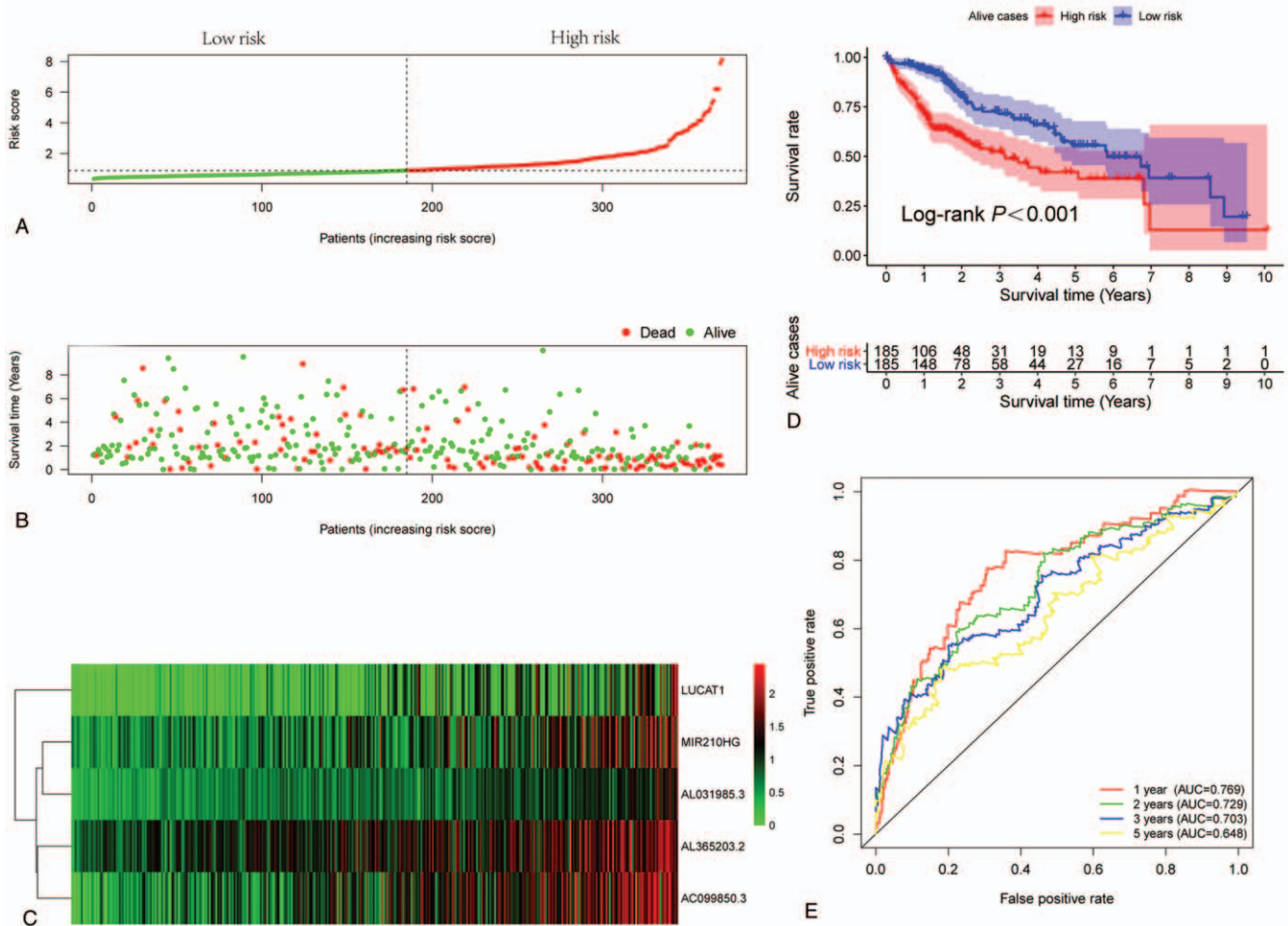


Figure 3. Analysis of risk prognostic models for 5-MRLs. (A) The risk scores of the 5-MRLs of the low- and high-risk groups, (B) the distribution of the patient's survival status, and (C) the expression heat map. (D) Kaplan–Meier curves for low-risk and high-risk groups. (E) ROC curve analysis predicts survival of patients with HCC. HCC=hepatocellular carcinoma, MRL=metabolism-related lncRNA, ROC=receiver operating characteristic.

processes and metabolites leading to the rapid development of liver cancer, thus posing a major challenge to global public health and demonstrating the importance of comprehensively studying the relationship between metabolism-related genes and the prognosis of liver cancer. In this study, with the advancement in science and technology, the cancer prediction researches were carried out by utilizing TCGA in the search for metabolism-related genes that can predict the survival rate of HCC, with 5-MRLs proved by the bioinformatics methods to play an important role in predicting the prognosis of HCC and verified by the real-time PCR to be highly expressed in HCC tumors.

Long non-coding RNAs (lncRNAs), a common type of non-coding RNAs, are >200 nucleotides in length, with a great volume of published literature describing their biological processes, including gene activation, autophagy, metabolism, inflammation, and immune response,^[15,16] and out-of-control lncRNA usually inducing cancer cells related to the occurrence, development, and metastasis of cancer as well,^[17,18] proved by such examples as lncRNA-HEIH promoting the progression of liver cancer through the enhancer of Zete homolog 2,^[19] lncRNA-UFC1 promoting tumor growth by targeting micro-

RNA-34a,^[20] lncRNA-PRAL being the key stimulator to the development of liver cancer,^[21] lncRNA FAL1 being positive in HCC tissues and playing a role as an oncogene,^[22] LINC00460 being a prognostic marker for lung cancer and has also been reported in recent literature.^[23] And, in prostate cancer, the up-regulated lncRNA-HULC being associated with low overall survival of patients with prostate cancer.^[24] In the latest study, Elhendawy et al^[25] detected expression profiles of miRNAs in serum samples of 20 HCC patients and 10 healthy controls were detected and they have detected a panel of serum miRNAs that can be used as a reliable noninvasive screening biomarker of HCC. Despite the fact that, in previous studies, many abnormal lncRNAs have been found in various cancers, it is rare to see the reports of metabolic biomarkers on risk assessment and prognosis prediction in the early diagnosis and treatment of HCC.

To better understand the biological status and clinical status of cancer patients and help them to choose better treatment options, a diagnostic screening platform using metabolomics was established by the researchers in previous researches,^[26] with the focus of identifying metabolism-related biomarkers of HCC,^[27] as identifying the changes of HCC cells in every step

Table 2
Clinical and pathologic characteristics of HCC patients and prognostic analysis.

Variables	Count of events/total (n = 142)	MST, d	HR (95% CI)	P value
Age, y				.143
≤60	52/177	2532	1	
>60	74/193	1622	1.303 (0.913–1.860)	
Gender				.362
Female	48/121	1560	1	
Male	78/249	2486	0.845 (0.588–1.214)	
Serum AFP, ng/mL*				.852
≤400	60/213	2456	1	
>400	21/64	2486	1.049 (0.633–1.738)	
Child-Pugh grade†				.077
A	57/216	2542	1	
B/C	9/22	1005	1.872 (0.924–3.795)	
Ishak fibrosis score‡				.847
0	29/74	2456	1	
1/2	7/31	1791	0.757 (0.325–1.762)	
3/4	6/28	NA	0.686 (0.281–1.675)	
5	2/9	1386	0.720 (0.170–3.056)	
6	17/69	NA	0.750 (0.408–1.380)	
Tumor stage§				<.001
I	41/171	2532	1	
II	25/85	1852	1.436 (0.871–2.369)	
III/IV	47/90	770	2.751 (1.803–4.198)	
Histologic grade				.786
G1	18/55	2116	1	
G2	58/177	1685	1.148 (0.676–1.950)	
G3	41/121	1622	1.180 (0.676–2.060)	
G4	5/12	NA	1.825 (0.648–5.140)	
MVI¶				.185
No	59/206	2131	1	
Yes	34/108	2486	1.331 (0.870–2.034)	
Radical resection#				.003
R0	106/323	2116	1	
R1/R2/RX	17/40	837	2.137 (1.276–3.581)	
Risk index				<.001
Low	51/185	2131	1	
High	75/185	1149	2.206 (1.539–3.163)	

HCC=hepatocellular carcinoma, HR=hazard ratio, MST=media survival time.

*Ninety three patients' data were unavailable.

†One hundred thirty two patient's data were unavailable.

‡One hundred fifty nine patient's data were unavailable.

§Twenty four patients' data were unavailable.

|| Five patients' data were unavailable.

¶ Fifty six patients' data were unavailable.

Seven patients' data were unavailable.

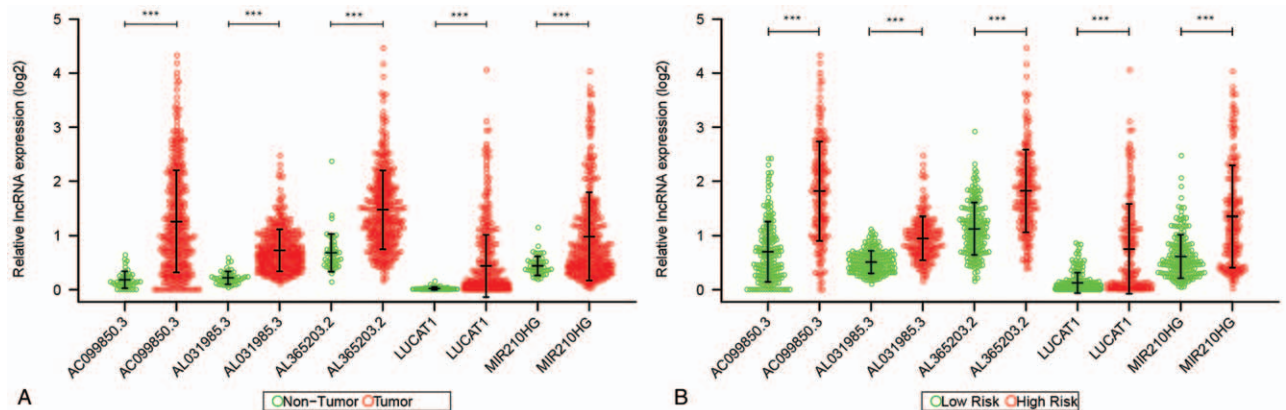


Figure 4. Comparison of the expression levels of 5-MRLs. (A) Expression of 5-MRLs between cancer and para-cancerous, (B) expression of 5-MRLs between high and low-risk group. ****P* < .001. MRL=metabolism-related LncRNA.

Table 3
Combined effects analysis of clinicopathologic characteristics and the MRLs risk score in HCC patients.

Groups	Risk	Variables	Count of events/total (n = 370)	MST, d	HR (95% CI)	P value
Age, y						
A	Low risk	≤60	20/87	2532	1	
B	Low risk	>60	31/98	1791	1.224 (0.692–2.166)	.487
C	High risk	≤60	32/90	2542	2.211 (1.260–3.880)	.006
D	High risk	>60	43/95	1005	3.046 (1.765–5.258)	<.001
Gender						
a	Low risk	Female	24/59	2116	1	
b	Low risk	Male	27/126	NA	0.628 (0.356–1.108)	.108
c	High risk	Female	24/62	1135	1.595 (0.875–2.908)	.128
d	High risk	Male	51/123	1149	1.598 (0.971–2.630)	.065
Serum AFP, ng/mL*						
1	Low risk	≤400	30/118	2456	1	
2	Low risk	>400	7/27	NA	0.791 (0.341–1.834)	.584
3	High risk	≤400	30/95	2542	1.828 (1.090–3.065)	.022
4	High risk	>400	14/37	2486	1.911 (1.004–3.638)	.049
Child-Pugh grade [†]						
(A)	Low risk	A	29/121	3125	1	
(B)	Low risk	B/C	4/12	1624	1.668 (0.582–4.777)	.340
(C)	High risk	A	28/95	2542	1.765 (1.034–3.012)	.037
(D)	High risk	B/C	5/10	601	5.323 (1.623–11.511)	.003
Ishak fibrosis score [‡]						
(a)	Low risk	0	16/45	2456	1	
(b)	Low risk	1/2/3/4/5/6	17/75	NA	0.820 (0.401–1.676)	.587
(c)	High risk	0	13/29	1005	2.069 (0.964–4.443)	.062
(d)	High risk	1/2/3/4/5/6	15/62	NA	1.245 (0.584–2.657)	.570
Tumor stage [§]						
(1)	Low risk	I/II	28/136	2532	1	
(2)	Low risk	III/IV	15/35	931	2.852 (1.507–5.397)	.001
(3)	High risk	I/II	38/120	1852	2.448 (1.469–4.079)	<.001
(4)	High risk	III/IV	32/55	412	4.410 (2.637–7.374)	<.001
Histologic grade						
I	Low risk	G1/G2	39/139	2131	1	
II	Low risk	G3/ G4	11/44	NA	0.855 (0.437–1.671)	.646
III	High risk	G1/G2	37/93	1271	2.128 (1.350–3.354)	.001
IV	High risk	G3/G4	35/89	1149	2.262 (1.408–3.633)	<.001
MVI [¶]						
AA	Low risk	No	29/123	2456	1	
AB	Low risk	Yes	12/41	3258	1.614 (0.818–3.184)	.168
AC	High risk	No	30/83	1372	2.591 (1.522–4.412)	<.001
AD	High risk	Yes	22/67	2486	2.253 (1.279–3.970)	.005
Radical resection [#]						
i	Low risk	R0	45/167	2456	1	
ii	Low risk	R1 + R2 + RX	5/14	837	4.402 (1.656–11.704)	.003
iii	High risk	R0	61/156	1271	2.232 (1.510–3.300)	<.001
iv	High risk	R1 + R2 + RX	12/26	1135	3.387 (1.775–6.465)	<.001

Abbreviations: HCC = hepatocellular carcinoma, HR = hazard ratio, MST = media survival time, MVI = microvascular invasion.

* Ninety three patients' data were unavailable.

† One hundred thirty two patient's data were unavailable.

‡ One hundred fifty nine patient's data were unavailable.

§ Twenty four patients' data were unavailable.

|| Five patients' data were unavailable.

¶ Fifty six patients' data were unavailable.

Seven patients' data were unavailable.

of metabolism is essential for the tumor staging and treatment progress,^[28] which was proved by the examples like significant reduction of, in the metabolomic analysis of liver cancer, both the metabolism related to the glycolysis process and the metabolism of malic acid, alanine, and linoleic acid.^[29] Blood lipid measurement in patients with hepatitis C virus (HCV) and HCV-HCC showed that cholesterol, bile acid synthesis, and fatty acid oxidation were down-regulated compared with normal

subjects,^[13] with the increase of glycolysis, glycine, serine, threonine, aromatic amino acid metabolism, nucleotide metabolism, and succinate 5 secretion during HCC recurrence, leading a variety of metabolites and abnormal subtle changes in metabolic processes to be found before the occurrence and development of HCC owing to the above mentioned advantages of metabolomics.^[14] It is a known fact that there are 3 obvious metabolic disorders found in tumor and hepatoma cell lines in the

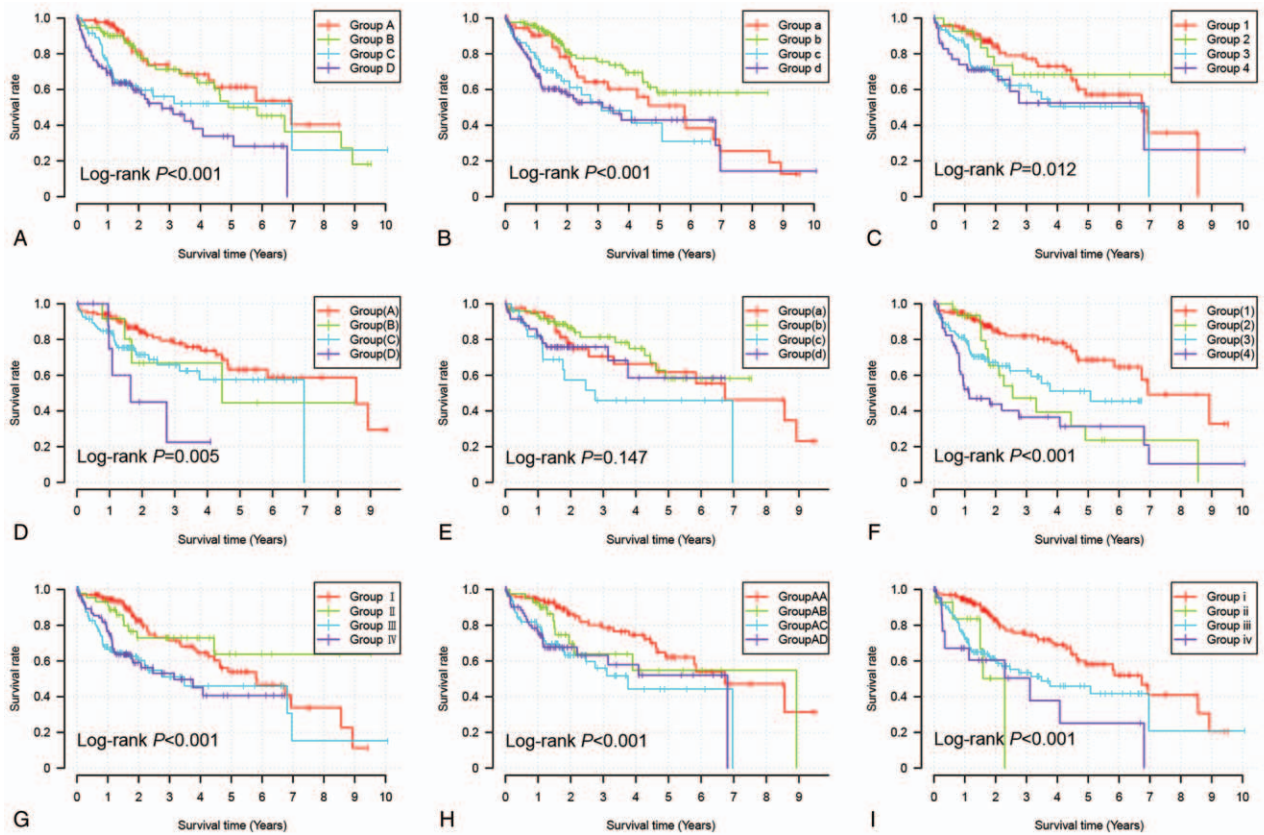


Figure 5. Combined analysis of survival time by risk score and clinicopathological characteristics. (A) Age, (B) gender, (C) serum AFP, (D) Child-Pugh grade, (E) Ishak fibrosis score, (F) tumor stage, (G) histologic grade, (H) microvascular invasion, (I) radical resection.

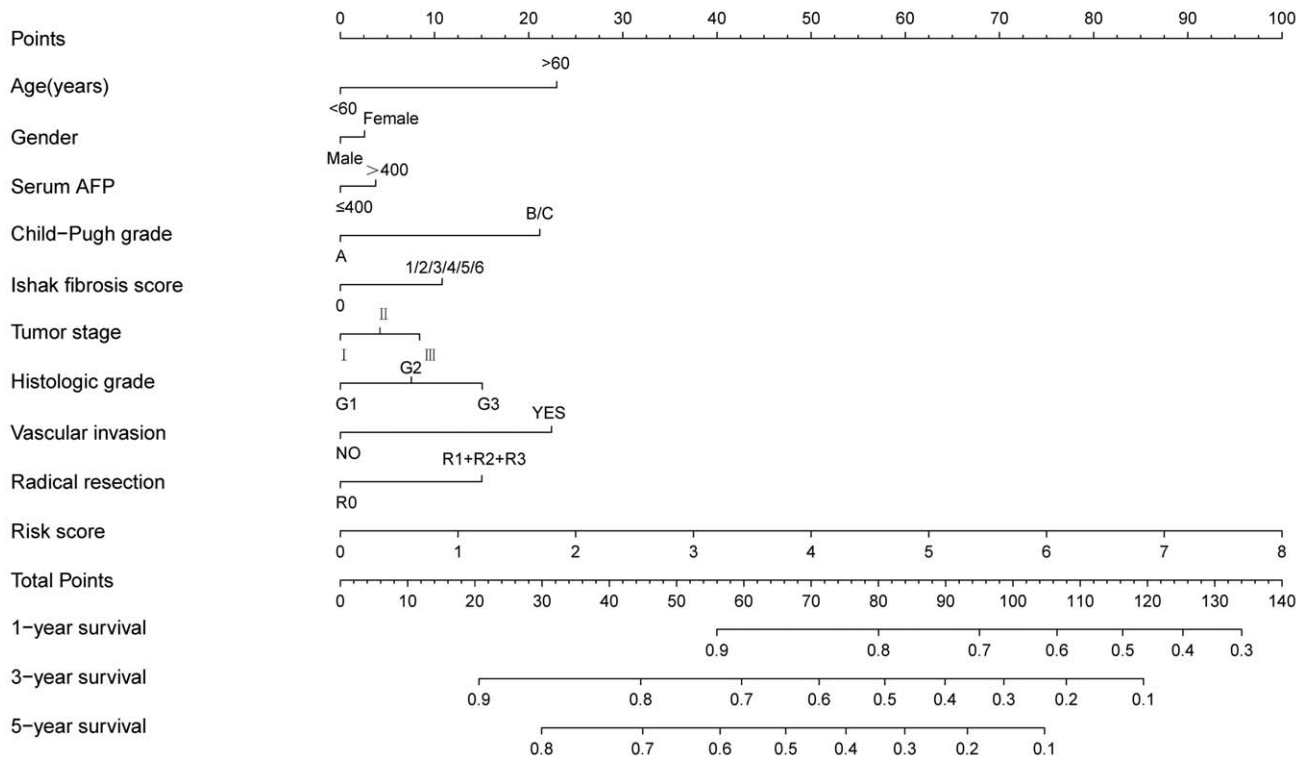


Figure 6. A nomogram between risk score and clinical data that predict 1-, 3-, and 5-year survival rates.

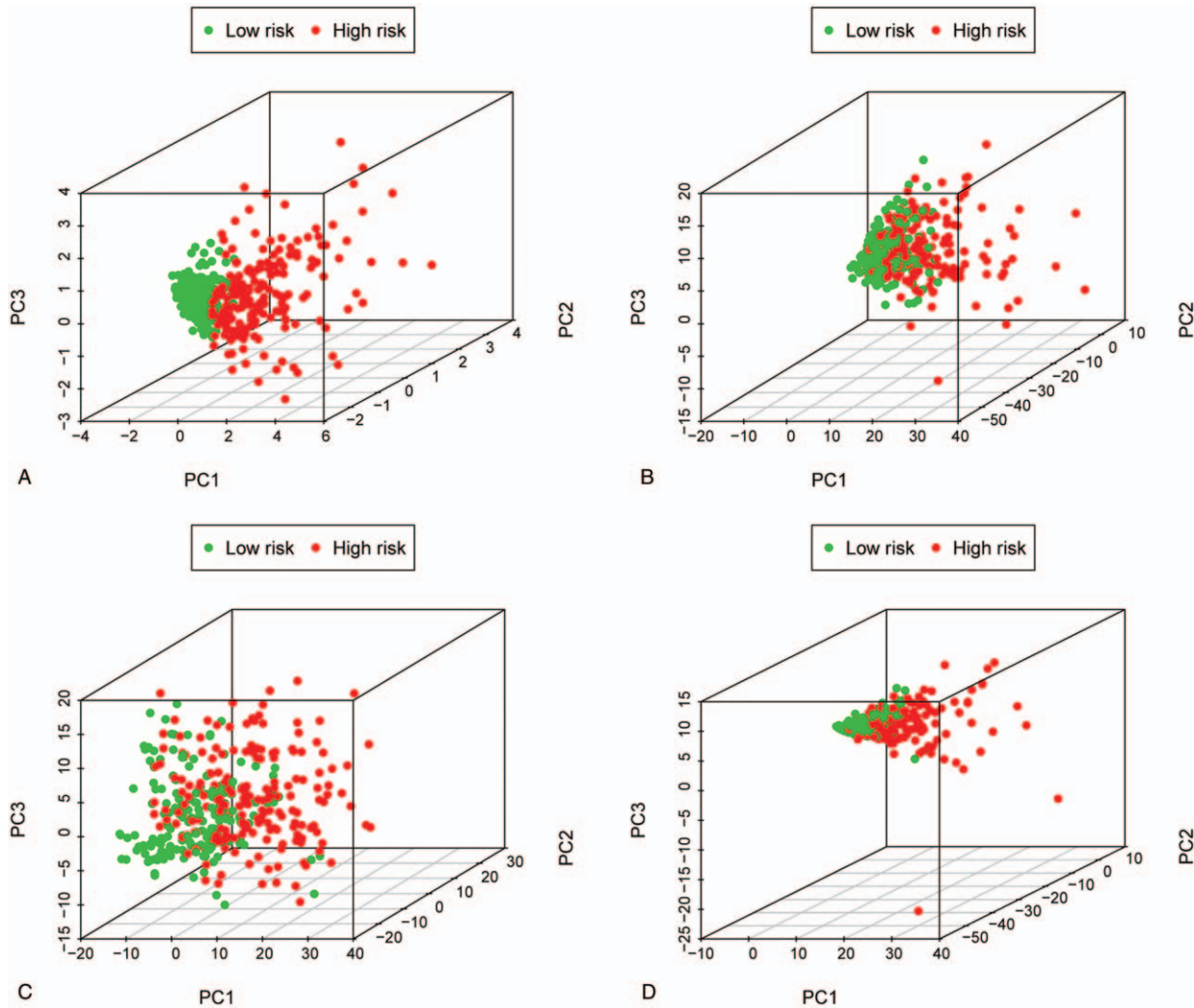


Figure 7. PCA between the high and low-risk group of the genome expression set. (A) Prognostic model set; (B) all genes set; (C) 166 MRLs set; (D) 20 prognostic-related MRLs set. MRL = metabolism-related LncRNA.

occurrence of liver cancer^[30]: molecular processes related to hepatocyte function involving lipid/fatty acid/bile synthesis, inflammatory processes related to cytokine, sphingomyelin, and chondroitin sulfate metabolism, and nucleotide rich metabolic processes involving purine/pyrimidine and glucose-mediated, and glucose-mediated catabolism. With these results in agreement with the results of this paper. Lipid metabolism has been investigated as an energy source, microenvironment adaptation, and cell signal transduction of tumor cells, revealing the fact of the occurrence and development of liver cancer accelerated Fatty acid synthesis, β -oxidation, and changes in cell lipid composition.^[31]

A poor prognosis was caused by high expression of adipose triglyceride lipase (ATGL) in HCC, the expression of which was regulated by LncRNA-NEAT1 which destroys the lipolysis of hepatoma cells and promotes the proliferation of hepatoma cells.^[32] With the deregulation of lipid metabolism in hepatocellular carcinoma was regulated by LncRNA-HULC activating of the acyl CoA synthase subunit ACSL1, HULC mRNA level was shown, by real-time PCR detection, to be positively correlated

with ACSL1 level in 60 HCC patients. About 77% (180/233) of HCC tissues were revealed, by immunohistochemical analysis of tissue microarray, to be positive for ACSL1, and the proliferation of HCC cells was promoted by the overexpression of cholesterol producing ACSL1, thus leaving the growth of HCC accelerated by the abnormal lipid metabolism enhanced by HULC.^[33] Considered to be a marker of cancer is reprogrammed glucose metabolism (or Warburg effect) of aerobic glycolysis,^[34] in which LINC01554 promotes the degradation of PKM2 proteasome, inhibits Akt/mTOR signaling pathway, and reduces the level of aerobic glycolysis in HCC cells, indicating the association between the down-regulation of LINC01554 and poor prognosis of HCC patients.^[35] A rate-limiting enzyme implicated in purine metabolism is xanthine dehydrogenase, a low mRNA level of which is associated with a higher tumor stage and poor prognosis of HCC, with the migration and invasion of HCC cells promoted and the expression of xanthine dehydrogenase down-regulated by means of blocking or inhibiting xanthine dehydrogenase, which is conducive to the occurrence and development of HCC.^[36]

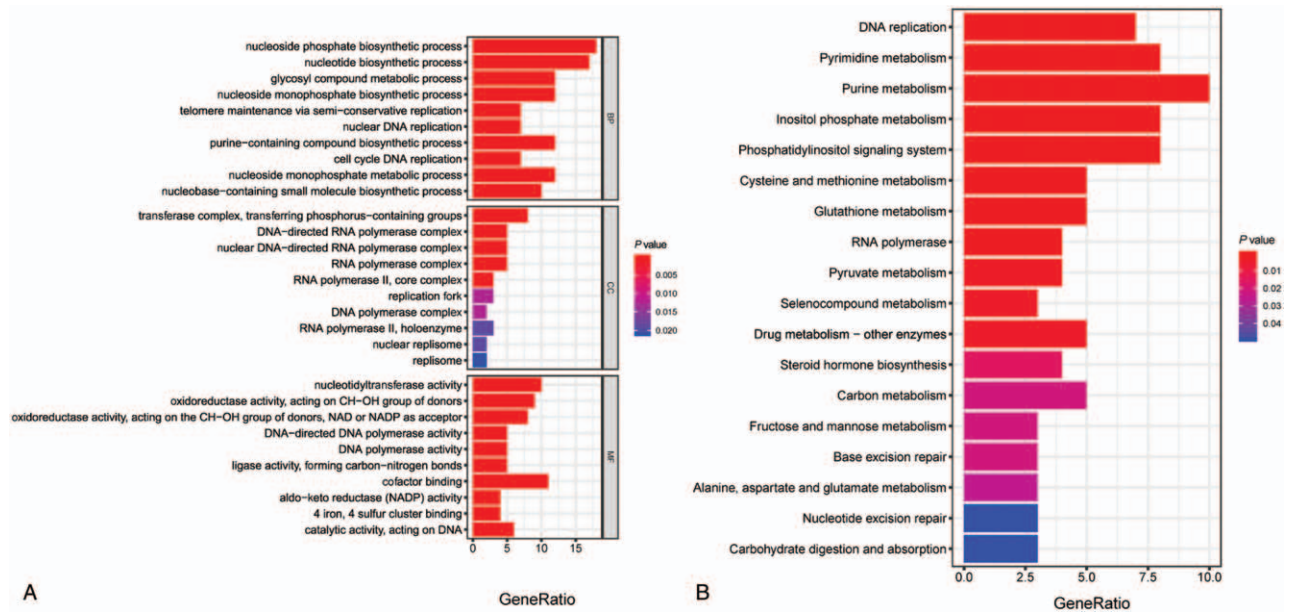


Figure 8. Enrichment analysis of functions and pathways in 5-MRLs. (A) GO results reveal the MRL function in the biology process, cell component, and molecular function. (B) KEGG result shows the enrichment pathway of MRLs. KEGG=Kyoto Encyclopedia of Genes and Genomes, MRL=metabolism-related lncRNA.

In this study, it was investigated when 5-MRLs were used as an important prognostic signal to predict the survival rate of HCC patients, and 937 MRLs by “limma” package were identified to clarify the effect of 5-MRLs on HCC OS, with the construction of a multivariate prognostic analysis model based on MRLs. Meanwhile, the best combination of “step” function were further evaluated and screened, leaving 5-MRLs (AC099850.3, AL031985.3, AL365203.2, LUCAT1, MIR210HG) identified suitable for constructing prognosis characteristics, leading to the ROC analysis determination that the area under the curve values for 1-, 2-, 3-, and 5-year survival was 0.769, 0.729, 0.703, and 0.648, respectively, leaving the prognostic characteristics of 5-MRLs shown to be significantly correlated with HCC OS and the prognostic characteristics shown to be having a high accuracy in predicting the survival of HCC, by the survival analysis.

Stronger than the traditional clinical prediction index, prognostic characteristics obtained through the final analysis were confirmed by the stratified analysis to be independent factors of HCC, causing the combination of risk score and clinicopathological characteristics to make the prediction effect more obvious with combined effect analysis. The significant impact of the risk score on the survival rate of HCC was supported by the prognostic indicators of nomogram, with 5-MRLs shown to be divided into 2 different subgroups by PCA, indicating the significant difference between prognosis of HCC patients in high-risk group and that in low-risk group.

The 5-MRLs in biological process analysis by GO analysis were shown mainly to be enrichment in nucleoside phosphate, nucleotide biosynthesis, and glycosyl compounds metabolism, with the genes analyzed by cellular component which is rich in transferase complex, phosphorous group transfer, DNA, and nuclear DNA oriented RNA polymerase complex and the genes analyzed by molecular function which is abundant in nucleotide transferase activity and oxidoreductase activity. Most of the co-expressed MRGs were shown by KEGG analysis to be closely

linked to DNA replication, purine metabolism, and inositol phosphate metabolism. Besides, according to GSEA analysis, C2 genes were significantly rich in polysaccharide biosynthesis, purine metabolism, pyrimidine metabolism, RNA degradation, and ubiquitin-mediated proteolysis, while C5 genes were abundant in ATPase activity, DNA catalytic activity, and cell cycle regulation. From a variety of studies, these 5-MRLs, which can serve as potential independent risk factors and prognostic indicators for HCC, have profound potential value in the diagnosis and prognosis of HCC patients.

The expression of 5-MRLs in HCC was finally confirmed by PCR, with the expressions of AC099850.3, AL031985.3, AL365203.2, LUCAT1, and MIR210HG relatively highly expressed in tumors, revealing the experimental results were generally consistent with the results from the TCGA, thus confirming the reliability of the model designed in this research.

AC099850.3, AL031985.3, AL365203.2, LUCAT1, and MIR210HG were included in the 5-MRLs, among which MIR210HG overexpression was found to be one of the independent factors of hepatocarcinogenesis by Wang et al,^[37] with MIR210HG serving as a carcinogenic lncRNA in liver cancer. MIR210HG has proved to be the strongest candidate lncRNA for predicting the prognosis of colon cancer, with the high expression of MIR210HG confirmed, by Ruan et al,^[38] to be related to the shortening of overall survival time in colon cancer in an investigation into MIR210HG which was assumed to be an important biomarker for glioma diagnosis by Min et al,^[39] LUCAT1 expression proved to be an independent predictor of poor prognosis and assumed to be a useful biomarker for patients with liver cancer by Jiao et al,^[40] and the lncRNA-miRNA-mRNA ceRNA network constructed to investigate lncRNA by Zhou et al,^[41] revealing a new lncRNA (AC099850.3) related to ceRNA, which is conductive to the diagnosis and treatment of squamous cell carcinoma of the tongue. On the basis of RNA sequencing, including AL031985.3, a 9-lncRNAs prognosis

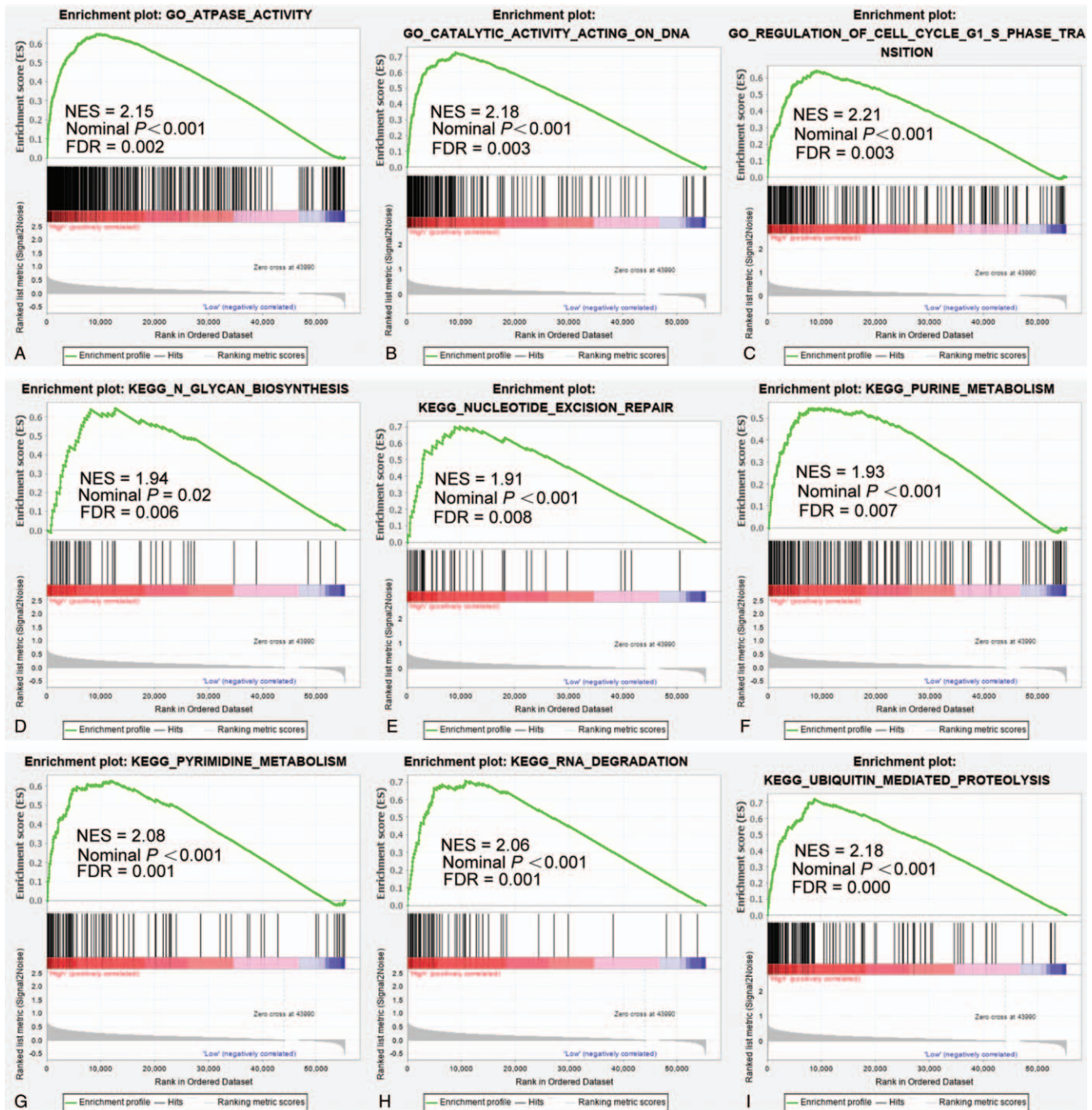


Figure 9. Gene set enrichment analysis results of 5-MRLs between high and low-risk groups. C2 (A–C); and C5 (D–I) gene sets. MRL = metabolism-related lncRNA.

model was established by Deng et al.^[42] In those study, showing the predictability of the prognosis of HCC, achieving the results similar to results for MIR210HG, AL031985.3, AC099850.3, LUCAT1 in this study. However, there are no other relevant literature about the effects of lncRNA-AL365203.2 on cancer occurrence, development, and prognosis, leading the functions of these lncRNAs to be supported by future experiments and in-depth studies.

Previous works with different focuses on lncRNA analysis of HCC prognosis by TCGA have been found in literature, while in this study, a prognostic risk score model for 5-MRLs, with better performance than traditional prognostic indicators, was established to foresee HCC through comprehensive survival analysis of prognostic characteristics. However, in spite of a good application prospect in predicting the prognosis of liver cancer, there are still some limitations in the current research: first, a

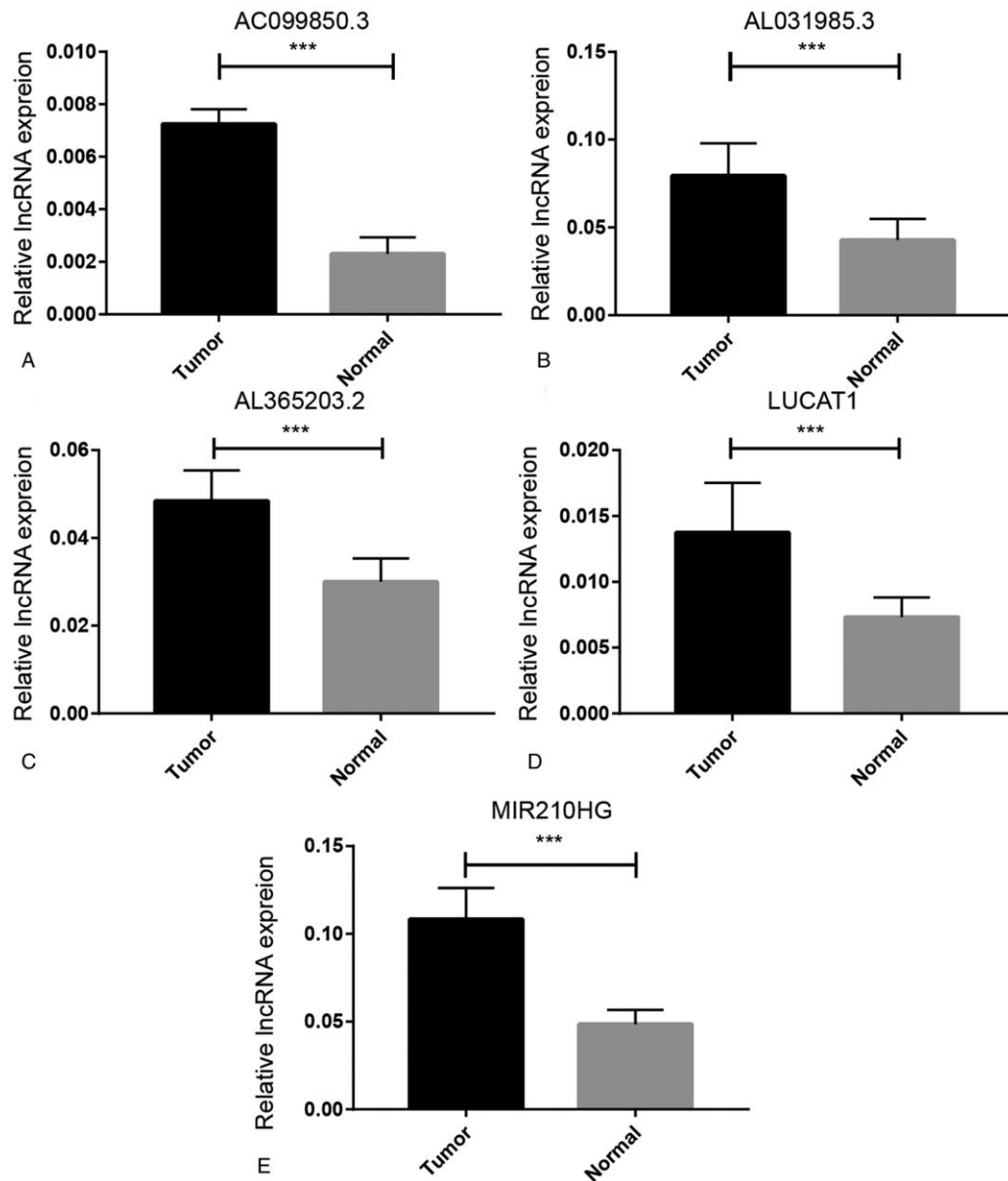


Figure 10. RT-PCR tests on 5-MRLs in HCC tissues and para-cancerous tissues. (A) AC099850.3; (B) AL031985.3; (C) AL365203.2; (D) LUCAT1; (E) MIR210HG. *** $P < .001$. HCC=hepatocellular carcinoma, MRL=metabolism-related lncRNA, RT-PCR=reverse transcription-polymerase chain reaction.

comprehensive survival analysis based on lncRNA expression is unable to be carried out due to such incomplete clinical parameters in TCGA as the unavailable details of postoperative treatment; second, results in this study may be biased owing to the relative small number of patient samples; third, a lncRNA was rarely reported in the literature, thus leaving the research work to be further experimented and verified for the purpose of remedying the limitations listed.

5. Conclusion

Identified by genome-wide screening analysis of the HCC cohort from TCGA database the 5 MRLs were consistent with the

characteristics of MRLs and had independent prognostic significance in predicting the clinical prognosis of HCC patients, with the potential significance, as the result of constructing MRL based prognostic markers, in the clinical application of potential prognostic biomarkers and therapeutic targets for HCC and other malignant tumors.

Acknowledgments

The author thanks Junxin He, Jian Li, Jue Wang, Qilin Yi, Zhujing Lan, and Keyu Huang for their help in surgery and follow-up data collection. The authors would like to thank Editage for English language editing.

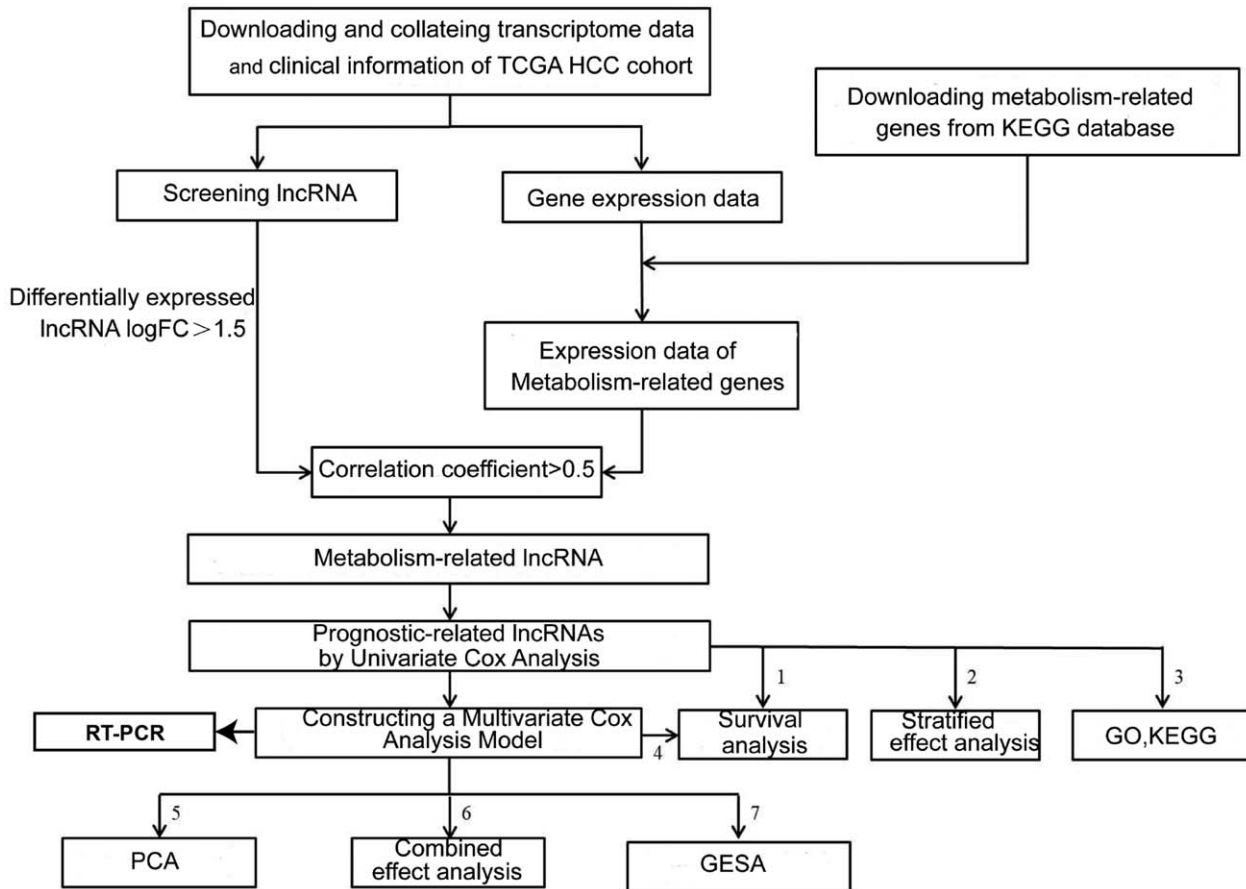


Figure 11. Research flow-process diagram.

Author contributions

Conceptualization: Wei Wang, Zhenfeng Deng, Zongrui Jin, Guolin Wu, Jilong Wang, Hai Zhu, Banghao Xu, Zhang Wen, Ya Guo.

>Data curation: Wei Wang, Zhenfeng Deng, Guolin Wu.

Formal analysis: Wei Wang, Zhenfeng Deng, Zongrui Jin, Guolin Wu.

Funding acquisition: Wei Wang, Zhenfeng Deng, Zhang Wen, Ya Guo.

Investigation: Wei Wang, Zhenfeng Deng, Zongrui Jin, Jilong Wang, Hai Zhu, Banghao Xu, Zhang Wen, Ya Guo.

Methodology: Wei Wang, Zhenfeng Deng, Zongrui Jin, Guolin Wu, Jilong Wang, Hai Zhu, Banghao Xu, Zhang Wen, Ya Guo.

Project administration: Wei Wang, Zhenfeng Deng, Jilong Wang, Hai Zhu, Banghao Xu, Zhang Wen, Ya Guo.

Resources: Wei Wang, Zhenfeng Deng.

Software: Wei Wang, Zhenfeng Deng.

Supervision: Jilong Wang, Hai Zhu, Zhang Wen, Ya Guo.

Validation: Wei Wang, Zhenfeng Deng, Guolin Wu.

Visualization: Wei Wang, Zhenfeng Deng, Guolin Wu.

Writing – original draft: Wei Wang, Zhenfeng Deng.

Writing – review & editing: Wei Wang, Zhenfeng Deng, Zongrui Jin, Guolin Wu, Jilong Wang, Hai Zhu, Banghao Xu, Zhang Wen, Ya Guo.

References

- [1] Sung H, Ferlay J, Siegel R, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2021;71:209–49.
- [2] Liver EAftSot. EASL Clinical Practice Guidelines: management of hepatocellular carcinoma. *J Hepatol* 2018;69:182–236.
- [3] Yang J, Hainaut P, Gores G, Amadou A, Plymoth A, Roberts L. A global view of hepatocellular carcinoma: trends, risk, prevention and management. *Nat Rev Gastroenterol Hepatol* 2019;16:589–604.
- [4] Forner A, Reig M, Bruix J. Hepatocellular carcinoma. *Lancet* 2018;391:1301–14.
- [5] Miller K, Ortiz A, Pinheiro P, et al. Cancer statistics for the US Hispanic/Latino population, 2021. *CA Cancer J Clin* 2021;71:466–87.
- [6] De Matteis S, Ragusa A, Marisi G, et al. Aberrant metabolism in hepatocellular carcinoma provides diagnostic and therapeutic opportunities. *Oxid Med Cell Longev* 2018;2018:13.
- [7] Mossenta M, Busato D, Dal Bo M, Toffoli G. Glucose metabolism and oxidative stress in hepatocellular carcinoma: role and possible implications in novel therapeutic strategies. *Cancers (Basel)* 2020;12.
- [8] Ning Z, Tan G. Cancer metabolism: a novel perspective on precision diagnosis and treatment for liver cancer. *Zhonghua Wai Ke Za Zhi* 2020;58:31–6.
- [9] Wang M, Wen T, He L. A six-microRNA set as prognostic indicators for bile duct cancer. *Int J Clin Exp Med* 2015;8:17261–70.
- [10] Lossos IS, Czerwinski DK, Alizadeh AA, et al. Prediction of survival in diffuse large-B-cell lymphoma based on the expression of six genes. *N Engl J Med* 2004;350:1828–37.
- [11] Deng Z, Wang J, Xu B, et al. Mining TCGA Database for tumor microenvironment-related genes of prognostic value in hepatocellular carcinoma. *Biomed Res Int* 2019;2019:12.

- [12] Combesure C, Perneger T, Weber D. Prognostic ROC curves: a method for representing the overall discriminative capacity of binary markers with right-censored time-to-event endpoints. *Epidemiology* 2014;25:103–9.
- [13] Wu J, Skill N, Maluccio M. Evidence of aberrant lipid metabolism in hepatitis C and hepatocellular carcinoma. *HPB* 2010;12:625–36.
- [14] Ye G, Zhu B, Yao Z, et al. Analysis of urinary metabolic signatures of early hepatocellular carcinoma recurrence after surgical removal using gas chromatography-mass spectrometry. *J Proteome Res* 2012;11:4361–72.
- [15] Susan C, Fitzgerald KA. Cytokines and long noncoding RNAs. *Cold Spring Harb Perspect Biol* 2017;10:a028589.
- [16] Mathy NW, Chen X. LncRNAs and their transcriptional control of inflammatory responses. *J Biol Chem* 2017;292:
- [17] Yuan J, Yang F, Wang F, et al. A long noncoding RNA activated by TGF- β promotes the invasion-metastasis cascade in hepatocellular carcinoma. *Cancer Cell* 2014;25:666–81.
- [18] Tang J, Jiang R, Deng L, Zhang X, Wang K, Sun B. Circulation long non-coding RNAs act as biomarkers for predicting tumorigenesis and metastasis in hepatocellular carcinoma. *Oncotarget* 2015;6:4505–15.
- [19] Yang F, Zhang L, Huo X, et al. Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. *Hepatology* 2011;54:1679–89.
- [20] Cao C, Sun J, Zhang D, et al. The long intergenic noncoding RNA UFC1, a target of MicroRNA 34a, interacts with the mRNA stabilizing protein HuR to increase levels of β -Catenin in HCC cells. *Gastroenterology* 2015;148:415.e18–26.e18.
- [21] Zhou C, Yang F, Yuan S, et al. Systemic genome screening identifies the outcome associated focal loss of long noncoding RNA PRAL in hepatocellular carcinoma. *Hepatology* 2016;63:850–63.
- [22] Li B, Mao R, Liu C, Zhang W, Tang Y, Guo Z. LncRNA FAL1 promotes cell proliferation and migration by acting as a CeRNA of miR-1236 in hepatocellular carcinoma cells. *Life Sci* 2018;197:122–9.
- [23] Ye J, Cheng Y, Deng J, Tao W, Wu L. LncRNA LINC00460 promotes tumor growth of human lung adenocarcinoma by targeting miR-302c-5p/FOXA1 axis. *Gene* 2019;685:76–84.
- [24] Zheng P, Li H, Xu P. High lncRNA HULC expression is associated with poor prognosis and promotes tumor progression by regulating epithelial-mesenchymal transition in prostate cancer. *Arch Med Sci* 2018;14:679–86.
- [25] Elhendawy M, Abdul-Baki E, Abd-Elsalam S, et al. MicroRNA signature in hepatocellular carcinoma patients: identification of potential markers. *Mol Biol Rep* 2020;47:4945–53.
- [26] Wei J, Xie G, Zhou Z, et al. Salivary metabolite signatures of oral cancer and leukoplakia. *Int J Cancer* 2011;129:2207–17.
- [27] Xue R, Lin Z, Deng C, et al. A serum metabolomic investigation on hepatocellular carcinoma patients by chemical derivatization followed by gas chromatography/mass spectrometry. *Rapid Commun Mass Spectrom* 2008;22:3061–8.
- [28] Wang X, Zhang A, Sun H. Power of metabolomics in diagnosis and biomarker discovery of hepatocellular carcinoma. *Hepatology* 2013;57:2072–7.
- [29] Beyoğlu D, Imbeaud S, Maurhofer O, et al. Tissue metabolomics of hepatocellular carcinoma: tumor energy metabolism and the role of transcriptomic classification. *Hepatology* 2013;58:229–38.
- [30] Ramesh V, Ganesan K. Integrative functional genomic analysis unveils the differing dysregulated metabolic processes across hepatocellular carcinoma stages. *Gene* 2016;588:19–29.
- [31] Sangineto M, Villani R, Cavallone F, Romano A, Loizzi D, Serviddio G. Lipid metabolism in development and progression of hepatocellular carcinoma. *Cancers (Basel)* 2020;126:
- [32] Liu X, Liang Y, Song R, et al. Long non-coding RNA NEAT1-modulated abnormal lipolysis via ATGL drives hepatocellular carcinoma proliferation. *Mol Cancer* 2018;17:90.
- [33] Cui M, Xiao Z, Wang Y, et al. Long noncoding RNA HULC modulates abnormal lipid metabolism in hepatoma cells through an miR-9-mediated RXRA signaling pathway. *Cancer Res* 2015;75:846–57.
- [34] Lunt S, Vander Heiden M. Aerobic glycolysis: meeting the metabolic requirements of cell proliferation. *Annu Rev Cell Dev Biol* 2011;27:441–64.
- [35] Zheng Y, Li L, Jia Y, et al. LINC01554-mediated glucose metabolism reprogramming suppresses tumorigenicity in hepatocellular carcinoma via downregulating PKM2 expression and inhibiting Akt/mTOR Signaling pathway. *Theranostics* 2019;9:796–810.
- [36] Chen G, Ye T, Chen H, et al. Xanthine dehydrogenase downregulation promotes TGF β signaling and cancer stem cell-related gene expression in hepatocellular carcinoma. *Oncogenesis* 2017;6:e382.
- [37] Wang Y, Li W, Chen X, Li Y, Wen P, Xu F. MIR210HG predicts poor prognosis and functions as an oncogenic lncRNA in hepatocellular carcinoma. *Biomed Pharmacother* 2019;111:1297–301.
- [38] Ruan Z, Xu Z, Li Z, Lv Y. Integral analyses of survival-related long non-coding RNA MIR210HG and its prognostic role in colon cancer. *Oncol Lett* 2019;18:1107–16.
- [39] Min W, Dai D, Wang J, et al. Long Noncoding RNA miR210HG as a potential biomarker for the diagnosis of glioma. *PLoS One* 2016;11:e0160451.
- [40] Jiao Y, Li Y, Ji B, Cai H, Liu Y. Clinical value of lncRNA LUCAT1 expression in liver cancer and its potential pathways. *J Gastrointest Liver Dis* 2019;28:439–47.
- [41] Zhou R, Zhang E, Sun Q, et al. Integrated analysis of lncRNA-miRNA-mRNA ceRNA network in squamous cell carcinoma of tongue. *BMC Cancer* 2019;19:779.
- [42] Deng B, Yang M, Wang M, Liu Z. Development and validation of 9-long Non-coding RNA signature to predicting survival in hepatocellular carcinoma. *Medicine (Baltimore)* 2020;99:e20422.