

Development and Validation of a Prognostic Signature Based on Autophagy-related Long Non-coding RNA Analysis in Hepatocellular Carcinoma

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Abstract

Background: The present aimed to establish a prognostic signature based on autophagy-related long non-coding RNAs (lncRNAs) analysis to predict the clinical outcome of hepatocellular carcinoma (HCC) patients using a comprehensive analysis of micro array data from The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC) databases.

Methods: Based on co-expression network analysis, autophagy-related lncRNAs were obtained using edge R package. Univariate and multivariate analyses were used to develop an autophagy-related lncRNAs risk signature and a predictive nomogram by data from the TCGA database. Subgroup analysis and internal validation were performed to confirm the predictive power. Subsequently, the predictive autophagy-related lncRNA prognostic signature and nomogram were externally explored and validated by HCC patients from the ICGC database.

Results: According to univariate and multivariate analyses, four autophagy-related lncRNAs (BACE1-AS, SNHG3, MIR210HG, and ZEB1-AS1) with prognostic value were identified in patients with HCC. Then, an autophagy-related lncRNAs prognostic signature was constructed. Based on the prognostic signature, all patients were subdivided into high- and low-risk groups. The autophagy-related lncRNA prognostic signature showed strong predictive power independent of all the clinicopathological features. A predictive nomogram based on the prognostic signature and multiple clinicopathological features was established through a survival analysis with the HCC data in TCGA. Internal validation demonstrated that the prognostic signature and nomogram have a strong power for predicting overall survival in patients with HCC. Whereas external validation further confirmed the robustly predictive power. Subsequently, functional enrichment analysis indicated the high-risk group mainly enriched in autophagy- and cancer-related pathways.

Conclusions: The four autophagy-related lncRNAs prognostic signature and the predictive nomogram established in the study might support clinician in individual treatment optimization and clinical decision-making for patients with HCC.

Background

Hepatocellular carcinoma (HCC) is one of the most lethal malignancies globally due to its aggressive biologic behavior, dramatically growing incidence, and high mortality[1, 2]. Undoubtedly, early diagnosis and initial surgical intervention are one of the biggest challenges facing most clinicians[3]. Despite diagnosis and multimodal therapies have significantly improved, the survival benefit remains to be limited, mainly due to the high heterogeneity [4–6]. Hence, there is a requirement to discover reliable predictive and prognostic biomarkers to increase risk prediction ability and guide individualized therapy.

Autophagy is a multi-step lysosomal degradation pathway that promotes nutrient cycling and metabolic adaptation. Those functions are biological processes of maintaining cellular function[7–9]. Autophagy has also been extensively proven involved in diverse pathologies including cancer[10]. What is more, the

function of autophagy is bilateral. On the one hand, autophagy could provide the necessary circulating metabolic substrates and enzymes to response to various of poor circumstance; on the other hand, inadequacy autophagy also promotes malignant cells rapidly growing, especially in advanced cancer[11, 12]. Novel potential target therapies involved in autophagy pathways have also been extensively investigated by many studies[13, 14].

Long non-coding RNA (lncRNA), usually more than 200 nucleotides, is a batch of newly-discovered RNA transcripts that are lack of protein-coding ability.[15] An increasing number of lncRNAs were proven to be related to different physiological and physiological progresses such as genetic expression regulation, RNA decay, microRNA regulation and protein folding by regulating transcriptionally or post-transcriptionally biological processes including autophagy[16, 17]. Increasing studies revealed that lncRNA inhibited or activated autophagy by regulating autophagy-related genes or pathways[17–19].

With the rapid development of RNA-sequencing techniques, the potential for utilizing lncRNA as biomarker to facilitate the detection, treatment, or prognosis of malignancy has been gradually unfolded[20, 21]. Hence, the present aimed to establish a prognostic signature based on autophagy-related lncRNAs analysis and a predictive nomogram to predict the clinical outcome of HCC patients using a comprehensive analysis of micro array data from The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC) databases.

Materials And Methods

Patient data acquisition

Microarray datasets including the raw RNA-sequencing and related clinical data of HCC patients were downloaded from TCGA, (<https://portal.gdc.cancer.gov/>) and ICGC (<https://icgc.org/>). 374 HCC patients from the TCGA microarray were used as a training set to develop an autophagy-associated lncRNA prognostic signature, whereas 243 HCC patients from the ICGC database were included as a validation dataset. Patients with a follow-up time of less than 1 month were removed for survival analysis. Finally, 343 HCC patients from the TCGA database and 230 HCC patients from the ICGC database were registered in the present study.

Due to all the data was collected directly from public databases, not any protocol was required from the ethical committee.

Autophagy-related lncRNAs screening

232 autophagy-related genes were obtained from the Human Autophagy Database (HADb, <http://autophagy.lu/clustering/index.html>). Then the profiles of those autophagy-related genes and lncRNAs were freely retrieved from the TCGA and ICGC RNA transcription data, respectively.

The correlations between the expression of the lncRNAs and the corresponding autophagy-associated genes were investigated. lncRNAs with a correlation coefficient $|R| > 0.5$ and $P < 0.050$ were considered to

be autophagy-related lncRNAs.

Construction of an autophagy-related lncRNA signature

The autophagy-related lncRNAs that were related to autophagy-associated genes in both TCGA and ICGC databases were considered as candidate indicators to construct a prognostic signature. According to Kaplan-Meier (KM) method and univariate Cox proportional hazards model, survival analysis of each autophagy-related lncRNA was done to screen prognostic biomarker with both significant *P* values less than 0.050. Then, multivariate Cox regression analysis was used to assess their contribution as prognostic factors among those nominated autophagy-related lncRNAs. Optimal autophagy-related lncRNA signature was acquired based on the lowest Akaike information criterion (AIC) value. Subsequently, a prognostic signature was established by the sum of the value of coefficients multiply expression of autophagy-related lncRNAs. Subsequently, each HCC patients obtained a prognostic risk score.

Evaluation and validation of the prognostic signature

Based on the median value of their risk score, all the patients were classified into high- (high risk score) and low- (low risk score) groups, respectively. K-M survival curve was used to compare the prognosis of the high- and low-risk group patients. Whereas the difference between the two groups was evaluated by a two-sided log-rank test. The receiver-operating characteristic (ROC) curve was performed to evaluate the predictive accuracy of the prognostic signature. Area under the ROC curve (AUC), which is a measure of discrimination, was performed to assess the prognostic accuracy. AUC ranges from 0.5 (no predictive power) to 1 (perfect prediction).

Stratified survival analysis by clinicopathological characteristics was conducted to evaluate the accuracy of the prognostic signature. Moreover, the performance of the prognostic signature constructed by the TCGA training cohort was validated in ICGC testing dataset by a similar approach.

Independence of the prognostic signature from clinicopathological parameters

To evaluate whether the predictive power of the prognostic signature could be independent of clinicopathological parameters, we performed univariate and multivariate Cox proportional hazards regression analyses in the TCGA and ICGC cohorts.

Establishment and validation of a predictive nomogram

A predictive nomogram to accurately predict the overall survival probability by using risk score calculated from the autophagy-related lncRNAs prognostic signature and clinicopathological parameters in the TCGA cohort. Harrell's concordance index (C-index) was performed to evaluate the prognostic accuracy. C-index ranges from 0.5 (no predictive power) to 1 (perfect prediction). Calibration plot was used to assess the performance characteristics of the nomogram. Subsequently, external validation from ICGC

testing cohort further confirmed the robustly predictive power of the nomogram. Two-sided P value < 0.050 was considered to be statistically significant.

Construction of the lncRNA-mRNA co-expression network

The lncRNA-mRNA co-expression network was constructed to explore the correlations between the autophagy-related lncRNAs and corresponding mRNA. Cytoscape Software (version 3.7.2) was used to visualize co-expression network. Sankey plots were utilized to reveal the detail correlations by R studio software using the package of ggalluvial.

Functional analysis

Based on co-expressed genes of the autophagy-related lncRNAs, the possible functional pathways and categories were enriched by Gene Ontology (GO) and the Kyoto Gene and Genomic Encyclopedia (KEGG). P and q values are less than 0.050 were considered to be significant.

Gene set enrichment analysis (GSEA)

GSEA was utilized to interpret the functional enrichment of gene expression data. This method derives its function by analyzing gene sets, so it can be also used to determine whether the gene set shows a statistically significant difference between the two biological states. Underlying mechanisms were investigated within "Molecular Signatures Database" of c2.cp.kegg. v6.2. Symbols through GSEA with a Java program. The random sample permutation number was set as 1,000, and the significance threshold was $P < 0.050$

Results

Identification of autophagy-related lncRNAs in HCC patient tissue samples

TCGA HCC transcriptome profiling contained 14142 lncRNAs and 19658 mRNAs transcripts. A total of 226 lncRNAs and 22688 mRNAs were identified by analyzing RNA-seq data of HCC patient tissue samples from the ICGC database.

The expression level of 232 autophagy genes were also extracted from the TCGA and ICGC databases, respectively. Pearson correlation analysis was performed to identify autophagy-related lncRNAs using $|R| > 0.5$ and $P < 0.050$ as the selection criteria. Finally, 349 and 67 autophagy-related lncRNAs were selected from the TCGA and ICGC databases, respectively. After autophagy-related lncRNAs were intersected from the two cohorts, 19 autophagy-related lncRNAs were obtained (Fig. 1A).

Identification of prognostic risk expressed autophagy-related lncRNAs in HCC patients from TCGA database

According to KM method and univariate Cox proportional hazards model, survival analysis of each autophagy-related lncRNA was done to screen prognostic biomarker with both significant P values less

than 0.050. Figure 1B showed that a total of 9 autophagy-related lncRNAs might have prognostic value for patients with HCC. Subsequently, Multivariate Cox regression analysis showed that 4 of 9 autophagy-related lncRNAs were well candidates to construct a prognostic risk signature on the basis of the lowest Akaike information criterion (AIC). The four autophagy-related lncRNAs (BACE1-AS, SNHG3, MIR210HG, and ZEB1-AS1) were confirmed to be unfavorable factors for HCC (Fig. 2).

Construction and evaluation of the autophagy-related lncRNAs prognostic signature

The above lncRNAs were performed to construct a prognostic lncRNAs signature as follows: Risk Score = $(0.142 \times \text{expression level of BACE1-AS}) + (0.032 \times \text{expression level of SNHG3}) + (0.067 \times \text{expression level of MIR210HG}) + (0.112 \times \text{expression level of ZEB1-AS1})$.

According to the prognostic signature, each patient obtained a risk score in connection with the patient's prognosis. All patients were divided into high- (n = 171) or low-risk groups (n = 172) by the median risk score, respectively. KM survival analysis demonstrated that the OS of HCC patients with low-risk score had significantly longer than those with high-risk score (shown in Fig. 1C). The 1-, 3-, and 5-year survival rates for the high-risk group patients were 75.60%, 49.90%, and 41.50%, respectively, whereas 1-, 3-, and 5-year survival rates for the low-risk group patients were 93.40%, 76.30% and 57.00%, respectively. Distribution of patients' risk scores in different groups was ranked in Fig. 1D. The scatter dots plot showed that the correlations of dead status with the risk score (Fig. 1E). Those results demonstrated that patients with a higher risk score suffered from a shorter survival time and lower survival rates. Moreover, heat map showed different expression levels of the autophagy-related lncRNAs in the high- and low-risk groups. As shown, patients with high-risk score also expressed higher levels (Fig. 1F). Time-dependent ROC curve analysis further showed that the AUC value for the prognostic signature was 0.728 (Fig. 1G).

The correlation of the autophagy-related lncRNA prognostic signature with clinicopathological features

Subsequently, analysis was done to investigate the clinical value of the prognostic signature in different subgroups stratified by patients' clinicopathological characteristics. As shown in Table 1, patients with high riskscore were prone to find in those with higher level of creatinine or AFP. Interesting, riskscore tends to increase in the advanced T stage and TNM staging, suggesting that the autophagy-related lncRNA prognostic signature might be significantly associated with HCC progression. No significant difference of the other clinicopathological variables with the prognostic signature was found.

Table 1
Clinical value of the autophagy-related lncRNA prognostic signature for HCC

Characteristics	Group	Risk score				
		n	mean	SD	t	Pvalue
Age (years)	< 60	157	1.154	0.891	0.271	0.787
	≥ 60	186	1.183	1.038		
Gender	Male	233	1.114	0.921	1.560	0.120
	Female	110	1.289	1.066		
Alcohol consumption	Present	109	1.106	0.749	0.704	0.482
	Absent	218	1.185	1.045		
Liver cirrhosis	Present	131	1.254	1.032	0.931	0.353
	Absent	65	1.112	0.984		
HBV	Present	98	1.223	1.166	0.800	0.424
	Absent	229	1.131	0.853		
HCV	Present	51	1.079	0.678	0.642	0.521
	Absent	276	1.173	0.999		
Family history	Present	104	1.063	0.789	1.394	0.165
	Absent	194	1.216	0.958		
Histological grade	G1 + G2	211	1.159	0.892	0.387	0.699
	G3 + G4	138	1.202	1.109		
Albumin (g/dl)	< 4.0	128	1.055	0.958	0.478	0.633
	≥ 4.0	153	1.105	0.799		
Creatinine (mg/dl)	< 1.1	189	0.926	0.375	2.842	0.005
	≥ 1.1	92	1.169	1.044		
BMI (kg/cm ²)	< 25	143	1.163	1.035	0.285	0.776
	≥ 25	152	1.196	0.987		
AFP (ng/ml)	≤ 200	187	0.977	0.635	2.806	0.006
	> 200	73	1.408	1.251		
ECOG	= 0	156	1.094	0.954	1.297	0.196
	> 0	117	1.253	1.059		

Characteristics	Group	Risk score				
		n	mean	SD	t	P value
T stage	I + II	252	1.042	0.675	3.111	0.002
	III + IV	88	1.551	1.483		
TNM stage	I + II	238	1.025	0.659	3.329	0.001
	III + IV	83	1.576	1.457		

Note: Bold font represents $P < 0.05$ and the relevant variables are statistically significant

Abbreviations: HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HCV, hepatitis C virus; AFP, alpha-fetoprotein; BMI, body mass index; ECOG: Eastern Cooperative Oncology Group; TNM, tumor node metastasis; SD, standard deviation.

Prognostic value of the autophagy-related lncRNA prognostic signature in different subgroups

Subgroup analysis was done to investigate the prognostic value of the autophagy-related lncRNA prognosis signature among different subgroups stratified by clinicopathological variables. As shown in Table 2, the risk scores prognostic signature showed better performance in male patients without liver cirrhosis, the history of HBV or HCV infection as well as family history, whereas obese patients with poor physical condition could benefit more in prognosis based on the autophagy-related lncRNA prognostic signature. Considering laboratory index, the prognostic signature seemed more applicable to patients with relatively lower expression levels of serum AFP, albumin, and creatinine. Besides, the prognostic signature showed strong predictive power independent of clinicopathological features including gender, age, the history of alcohol consumption, tumor stage and histological grade.

Table 2
Prognostic value of the autophagy-related lncRNA prognostic signature in different subgroups stratified by clinicopathological variables

Characteristics	Group	Low /High	HR (95% CI)	P value
Overall		172/171	2.249 (1.559–3.256)	< 0.001
Age	< 60	76/81	2.832 (1.586–5.056)	< 0.001
	≥ 60	95/91	1.754 (1.079–2.853)	0.023
Gender	Male	124/109	3.125 (1.925–5.072)	< 0.001
	Female	47/63	1.290(0.7285–2.285)	0.383
Alcohol consumption	Present	60/49	3.718 (1.921–7.194)	< 0.001
	Absent	106/112	1.794 (1.136–2.833)	0.012
Liver cirrhosis	Present	26/39	2.108 (1.010–4.396)	0.047
	Absent	70/67	1.922 (0.962–3.836)	0.064
HBV	Present	48/20	1.650 (0.720–3.780)	0.237
	Absent	118/111	2.513 (1.653–3.820)	< 0.001
HCV	Present	28/23	3.242 (0.805–13.057)	0.098
	Absent	138/138	2.206 (1.496–3.251)	< 0.001
Family history	Present	63/41	1.828 (1.019–3.277)	0.043
	Absent	83/111	2.944 (1.688–5.133)	< 0.001
Histological grade	G1 + G2	102/109	2.154 (1.338–3.466)	0.002
	G3 + G4	66/61	2.549 (1.411–4.607)	0.002
Albumin(g/dl)	< 4.0	76/52	1.908 (1.040–3.501)	0.037
	≥ 4.0	75/78	1.644 (1.893–3.025)	0.110
Creatinine(mg/dl)	< 1.1	98/91	1.721 (1.034–2.862)	0.037
	≥ 1.1	53/39	1.736 (0.825–3.656)	0.147
BMI (kg/cm ²)	< 25	70/73	1.670 (0.917–3.040)	0.093
	≥ 25	77/75	3.805 (2.105–6.787)	< 0.001
AFP (ng/ml)	≤ 200	116/171	1.985 (1.156–3.408)	0.013
	> 200	21/52	2.041 (0.757–5.506)	0.159
ECOG	= 0	91/65	1.878 (0.924–3.817)	0.081

Characteristics	Group	Low /High	HR (95% CI)	P value
	> 0	53/64	3.016 (1.679–5.416)	< 0.001
T stage	I + II	140/112	2.064 (1.280–3.329)	0.003
	III + IV	29/59	1.926 (1.054–3.520)	0.033
TNM stage	I + II	135/103	1.915 (1.155–3.174)	0.012
	III + IV	26/57	2.025 (1.046–3.921)	0.036

Note: Bold font represents $P < 0.050$ and the relevant variables are statistically significant

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; AFP, alpha-fetoprotein; BMI, body mass index; ECOG: Eastern Cooperative Oncology Group; TNM, tumor node metastasis; HR, hazard ratio.

Validation of the autophagy-related lncRNA prognostic signature in the ICGC database

Next, we further assessed the predictive power of the autophagy-related lncRNA prognosis signature in the ICGC database. A total of 230 HCC patients were enrolled. All the patients were separated into low and high-risk groups based on the median value of the risk score. Consistent with the results derived from the TCGA database, KM survival curve analysis demonstrated that the OS of HCC patients in the low-risk group was significantly higher than that of the patients in the high-risk group (Fig. 3A). The expression of each autophagy-related lncRNA showed that those lncRNAs were down-regulated in the low-risk group and up-regulated in the high-risk group (Fig. 3B). Time-dependent ROC curve analysis further demonstrated that the AUC value for the autophagy-related lncRNA prognostic signature was 0.685 in ICGC database, which further confirmed the robust prediction of the signature among HCC patients (Fig. 3C). The distribution of risk score and survival status was shown in Fig. 3D-E. Those results further confirmed that the signature could accurately predicted the prognosis of HCC patients.

Determination of the autophagy-related lncRNA prognostic signature as an independent prognostic factor

Subsequently, univariate, and multivariate Cox regression analyses were performed to assess whether the prognostic significance was a prognostic factor independent of clinicopathological features in the TCGA and ICGC databases. As shown in Fig. 4A, Univariate analysis indicated that ECOG [HR: 2.390; 95% CI: 1.894–3.016; $P < 0.001$], TNM stage [HR: 1.784; 95% CI: 1.446–2.202; $P < 0.001$], HBV [HR: 1.587; 95% CI: 1.006–2.504; $P = 0.047$], HCV [HR: 2.446; 95% CI: 1.239–4.828; $P = 0.010$], liver cirrhosis [HR: 2.426; 95% CI: 1.516–3881.; $P < 0.001$], and autophagy-related lncRNAs prognostic riskscore [HR: 1.539; 95% CI: 1.339–1.769; $P < 0.001$] were significantly prognostic factors in HCC patients cohort from the TCGA database. To avoid the occurrence of collinearity of TNM stage with T stage, T stage was not enrolled into multivariate Cox regression modeling because TNM stage was calculated based on T stage, N stage, and M stage. Multivariate analysis further screened out ECOG [HR: 1.589; 95% CI: 1.101–2.294; $P = 0.013$] and autophagy-related lncRNAs prognostic riskscore [HR: 1.376; 95% CI: 1.114–1.699; $P = 0.003$] as independent prognostic parameters (Fig. 4B). Besides, based on univariate and multivariate analyses,

autophagy-related lncRNAs prognostic riskscore was also proven to be an independent prognostic factor in the ICGC validation cohorts (Fig. 4C-D).

Constructing and Evaluating a nomogram to predict OS in patients with HCC

We constructed a predictive nomogram to accurately predict the 1-, 3-, and 5-year OS rate by using riskscore calculated from the autophagy-related lncRNA prognostic signature and clinicopathological parameters including age, gender and AJCC stage in the TCGA cohort (Fig. 5A). Calibration plots further identified that the nomogram performed well in predicted 1-, 3-, and 5-year survival probabilities with an ideal model, which indicated that the nomogram was perfectly calibrated to predict OS at assessing the performance characteristics (Fig. 5B-D). The C-index value for the nomogram was 0.734. Therefore, internal validation demonstrated that the nomogram was accurate and reliable.

Subsequently, external validation was investigated in the ICGC external validation cohort. The C-index of the nomogram was 0.701 in validation cohort. The calibration plots showed excellent agreement between the actual observation and the nomogram prediction in terms of the 1-, 3-, and 5-year survival probabilities (Fig. 5E–6G). Hence, external validation from ICGC testing cohort further confirmed the robustly predictive power of the nomogram.

Construction of the prognostic autophagy-associated lncRNA–mRNA co-expression network and functional enrichment analysis

To figure out the potential functions of the four-prognostic autophagy-associated-lncRNAs in HCC, we analyzed the lncRNAs mediated mRNA network by using cytoscape 3.6.1. As showed in Fig. 6A, 4 lncRNAs, 91 mRNAs and 141 lncRNA-mRNA pairs were shown in lncRNA-mediated genes network. Sankey plot also showed the detail relationships of prognostic autophagy-associated lncRNAs with autophagy-related-genes and risk types (Fig. 6B). Then, GO and KEGG pathway analyses further demonstrated that these genes were main correlated with autophagy and enriched in pathways in cancer (Fig. 6C-D).

Gene set enrichment analysis

Further functional annotation was performed by GSEA. GSEA results showed the altered gene sets in the high-risk group were mainly found to be directly involved in the tumorigenesis and progression of malignant tumor (Fig. 7A). Besides, the differentially expressed genes between the two groups were main enriched in the autophagy-associated and tumor-related pathways including ERBB signaling pathway, MAPK signaling pathway, mTOR signaling pathway, VEGF signaling pathway, WNT signaling pathway, and P53 signaling pathway (Fig. 7B). In addition, the altered gene sets in the high-risk group were also found to be involved in the physical actions of autophagy (Fig. 7C). Whereas, the protective pathways involved in metabolism were significantly enriched in the low-risk group (Fig. 7D).

Discussion

HCC is one of the most lethal and common types of primary liver malignant neoplasms worldwide. Despite the diagnosis and multimodal therapies have significantly improved, the survival benefit remains to be limited, mainly because of high heterogeneity of HCC[22]. Clinically, histological grade, tumor stage, molecular subtype, and serum indicator were used to evaluate the prognostic effect among HCC patients[23]. However, those clinicopathological characteristics could not accurately provide prognostic value, which resulted in inaccurate judgment on prognosis. According to the situation, some high-risk patients may experience tumor cell growing uncontrollably due to inadequate treatment, whereas low-risk patients may receive over treatment. Therefore, reliable genetic signatures or biomarkers as prognostic predictors or therapeutic targets are of significance for HCC.

Malignant cell progress and extinction involve in reprogramming of energy metabolism including over-utilization of amino acids as tryptophan, aerobic glycolysis, tricarboxylic acid (TCA) cycle, glutamine, and arginine, dysfunctional mitochondrial bioenergetics as well as oxidative phosphorylation and so on[24, 25]. Moreover, these energy metabolisms are dramatically associated with autophagy. Hence, understanding the detail and direct relationships between autophagy and tumor progress might provide reliable basis for the development of specific agents targeting these mechanisms, and then treating tumors. After decades of researches on genetic prognostic biomarker of tumor-related events like microRNAs, and genes, lncRNAs have aroused much attention in the recent. So far, the roles of lncRNA in tumorigenesis and progression of malignant tumor have been gradually uncovered. The prognostic value of lncRNAs also has been extensively studied. However, so far, there has been no systematic process to identify autophagy-related lncRNAs signatures for predicting the prognosis of HCC patients. Hence, it is critical to develop an autophagy-related lncRNAs signature to predict the clinical outcome among HCC patients.

In the present study, HCC patients from TCGA and ICGC databases were enrolled to explore the prognostic value of autophagy-related lncRNAs. Based on lncRNAs and autophagy genes co-expression network, we firstly identified 19 autophagy-related lncRNAs through analyzing high throughput RNA transcriptome data from the TCGA and ICGC databases. After univariate and multivariate Cox regression analyses, four prognostic autophagy-related-lncRNAs including BACE1-AS, SNHG3, MIR210HG, and ZEB1-AS1 were selected out for establishing a prognostic signature. Each patient obtained a risk score based on the expression of the four autophagy-related-lncRNAs. All the patients were divided into high- and low- risk based on the median value of risk scores. We further found that patients with low scores had significantly better OS than those with high scores in the TCGA patient cohort. In addition, the distribution of risk score, survival status and ROC curve analysis further confirmed the prognostic accuracy of the signature. Similar results were also obtained when the autophagy-related-lncRNAs signature was validated in the ICGC patient cohort, which further confirmed the robust prediction of the prognostic signature for HCC patients. On the basis of univariate and multivariate regression analyses, the autophagy-related lncRNAs prognostic signature showed strongly predictive power independent of all the clinicopathological features including gender, age, history of alcohol consumption, tumor stage and histological grade in both cohorts. Hence, the autophagy-related lncRNAs prognostic signature showed powerful potential for clinical applications.

The associations of the prognostic signature and clinicopathological characteristics were investigated and results showed the signature was significantly related to advanced tumor and a higher level of serum AFP. The correlations were supported by the explanation that autophagy mainly promotes malignant cell rapid growth, invasion and migration, and its alteration might lead to poor prognosis of patients with advanced cancers[14, 26]. Subgroups analyses stratified by clinicopathological variables further verified the steadied prognostic value of the prognostic signature.

Nomogram is an effective and reliable clinical tool used to provide probabilistic prediction for an individual patient with cancer. Therefore, in order to achieve better predictive ability of prognosis for HCC patients, several clinicopathological variables (age, gender, and AJCC stage) and the autophagy-related lncRNAs prognostic signature were incorporated to establish a predictive nomogram in the present study. Calibration plots showed that the predicted 1-, 3-, and 5-year survival rates were comparable with the actual observation. High C-index confirmed that the nomogram showed powerful discrimination, indicating that it might function as a potentially prognostic tool. In addition, external validation from the ICGC testing cohort further confirmed the predictive power of the nomogram.

The role of autophagy in cancer is controversial because it could inhibit and promote tumor. However, with the development of understanding of autophagy, the role of autophagy in cancer has also been gradually uncovered. On the one hand, autophagy could provide the necessary circulating metabolic substrates and enzymes to response to various of poor circumstances such as tumor microenvironment; on the other hand, inadequacy autophagy also promotes malignant cell rapid growth especially in advanced tumor. Recently, lncRNAs' roles in mediating tumorigenesis, progression, metastasis, and drug resistance by regulating autophagy-related genes or microRNAs have gradually been found. In the present study, we firstly identified four autophagy-related lncRNAs for establishing a prognostic signature. BACE1-AS has been widely reported in previous studies to be significantly associated with prognosis of patients with cancer[27, 28]. BACE1-AS could also promote autophagy-related neuronal damage through the miR-214-3p/ATG5 signaling axis in Alzheimer's disease[29]. Additionally, the role of SNHG3 in cancer has been gradually uncovered[30]. Increasing studies indicated that SNHG3 affects the tumor development and progression by regulating autophagy-related microRNAs, genes, or corresponding pathways[31–34]. MIR210HG[35–38], and ZEB1-AS1[39, 40] have also been found to affect tumorigenesis, progression, and tumor metastasis, resulting in a poor prognosis for patients with cancer. Hence, the prognostic signature established by the four robust autophagy-related lncRNAs undoubtedly showed better prognostic value. Subsequently, we also identified autophagy-related genes governed by the four lncRNAs and established the lncRNA-mRNA co-expression network. GO and KEGG functional enrichment analysis showed those genes mainly enrich in autophagy and tumor-related signaling pathways.

GESA functional enrichment analysis showed the significant difference between the low- and high-risk groups. The high-risk group main enriches in autophagy- and cancer-related pathways. The altered gene sets in the high-risk group were also found to be involved in the autophagy-associated and tumor-related pathways as well as the physical actions of autophagy. Whereas the predictive pathways involved in various of metabolisms were significantly enriched in the low-risk group. Therefore, these results further

confirmed that autophagy is a critical modulator of oncogenesis and progression. We also concluded that the four autophagy-related lncRNAs might be potential therapeutic targets.

However, our study has several limitations. For example, functional experiments such as immunohistochemistry, quantitative real-time PCR, and flow cytometry should be conducted to uncover the potential molecular mechanisms for predicting the effect of autophagy-related lncRNAs and confirm our findings.

Conclusions

In conclusion, the four autophagy-related lncRNAs signature was a reliable tool for predicting OS in HCC patients. The prognostic signature might help us better understanding the role of autophagy in carcinogenesis and development of HCC. Hence, the four autophagy-related lncRNAs and their signature might be molecular biomarkers and therapeutic targets for HCC patients. The nomogram incorporating both the autophagy-related lncRNAs signature and clinicopathological parameters might support clinician in treatment optimization and clinical decision-making.

Abbreviations

lncRNAs: long non-coding RNAs; TCGA: The Cancer Genome Atlas; ICGC: International Cancer Genome Consortium; HCC: Hepatocellular carcinoma; HADb: Human Autophagy Database; KM: Kaplan-Meier; AIC: Akaike information criterion; ROC: receiver-operating characteristic; AUC: Area under the ROC curve; C-index: Harrell's concordance index; GO: Gene Ontology; KEGG: Kyoto Gene and Genomic Encyclopedia; GSEA: Gene set enrichment analysis; OS: Overall survival.

Declarations

Acknowledgments:

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Authors' contributions:

YD and FZ designed and draft the current study. All authors contributed to data analysis and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. SW and ZG S are co- corresponding authors.

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Availability of data and materials:

Publicly available datasets from the Cancer Genome Atlas (<https://portal.gdc.cancer.gov/>), International Cancer Genome Consortium (<https://icgc.org/>), and Human Autophagy Database (HADb, <http://autophagy.lu/clustering/index.html>) were analyzed in this study. All data supporting the findings of the study are available from the corresponding author on reasonable request. All data in our study are available upon request.

Ethics approval and consent to participate:

Due to all the data was collected directly from public databases, not any protocol was required from the ethical committee.

Consent for publication:

Consent for publication was obtained from all authors

Competing interests:

The authors declare that there is no conflict of interests.

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Figures

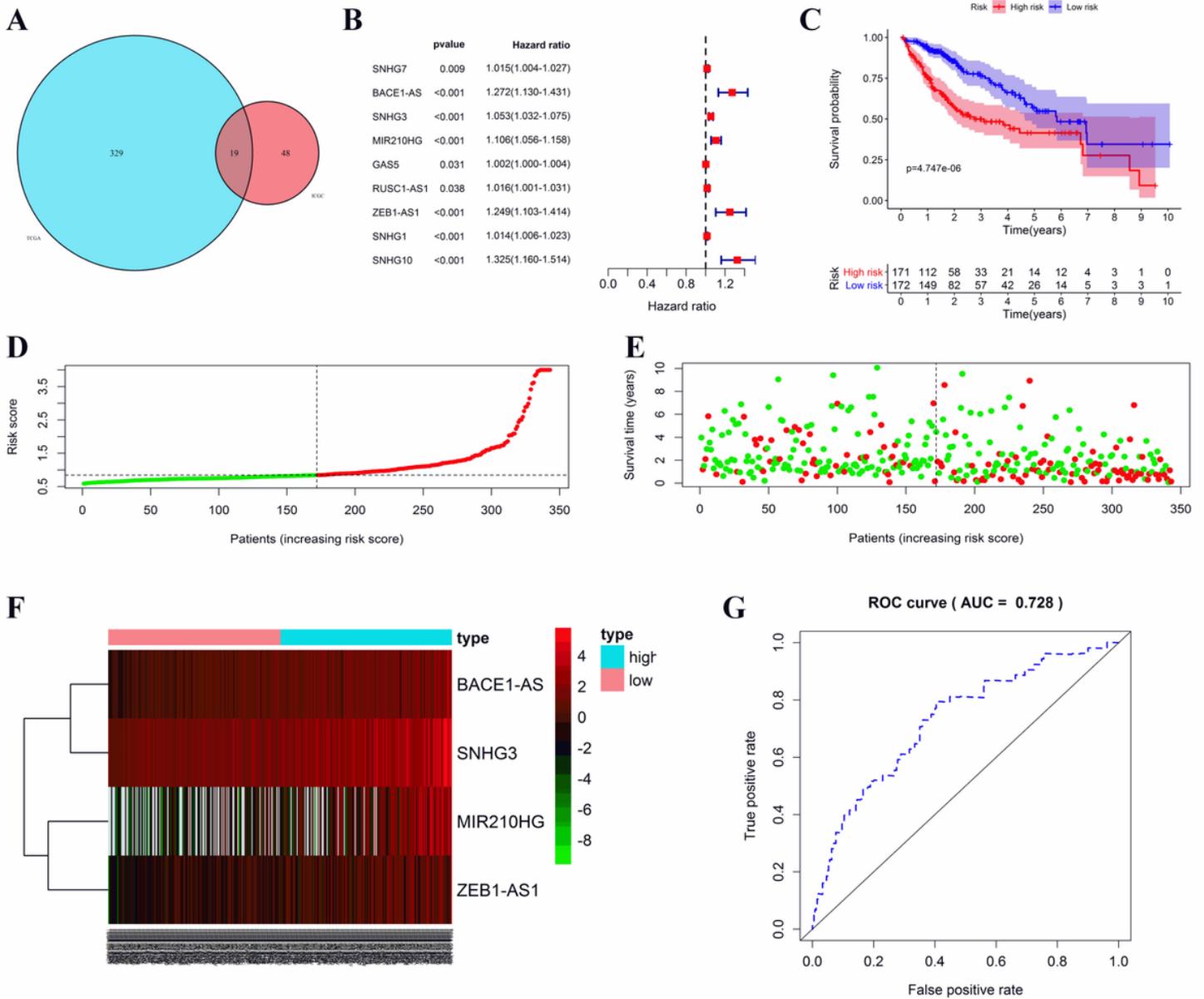


Figure 1

Construction and validation of the autophagy-related lncRNAs prognostic signature in HCC patients. (A) Venn diagram showing the 19 autophagy-related lncRNAs (the intersection of autophagy-related lncRNAs from the TCGA and ICGC databases). (B) KM method and univariate Cox regression analysis showed that 9 autophagy-related lncRNAs might correlated with overall survival (OS) in the TCGA dataset. (C) Kaplan-Meier survival curve analysis of OS in the high-risk (red line) and low-risk (blue line) in the TCGA dataset. Patients with a high-risk score exhibited poorer OS. (D) Distribution of risk scores of low- and high-risk HCC patients based on the autophagy-related lncRNA prognostic signature. (E) Scatter plot shows the relationship between risk score and survival time. (F) Heatmap showed that high-risk patients expressed higher levels of autophagy-related lncRNAs in the TCGA dataset. (G) Time-dependent

ROC curve analysis further showed the accuracy of the autophagy-related lncRNA prognostic signature in predicting prognosis of HCC patients from the TCGA database.

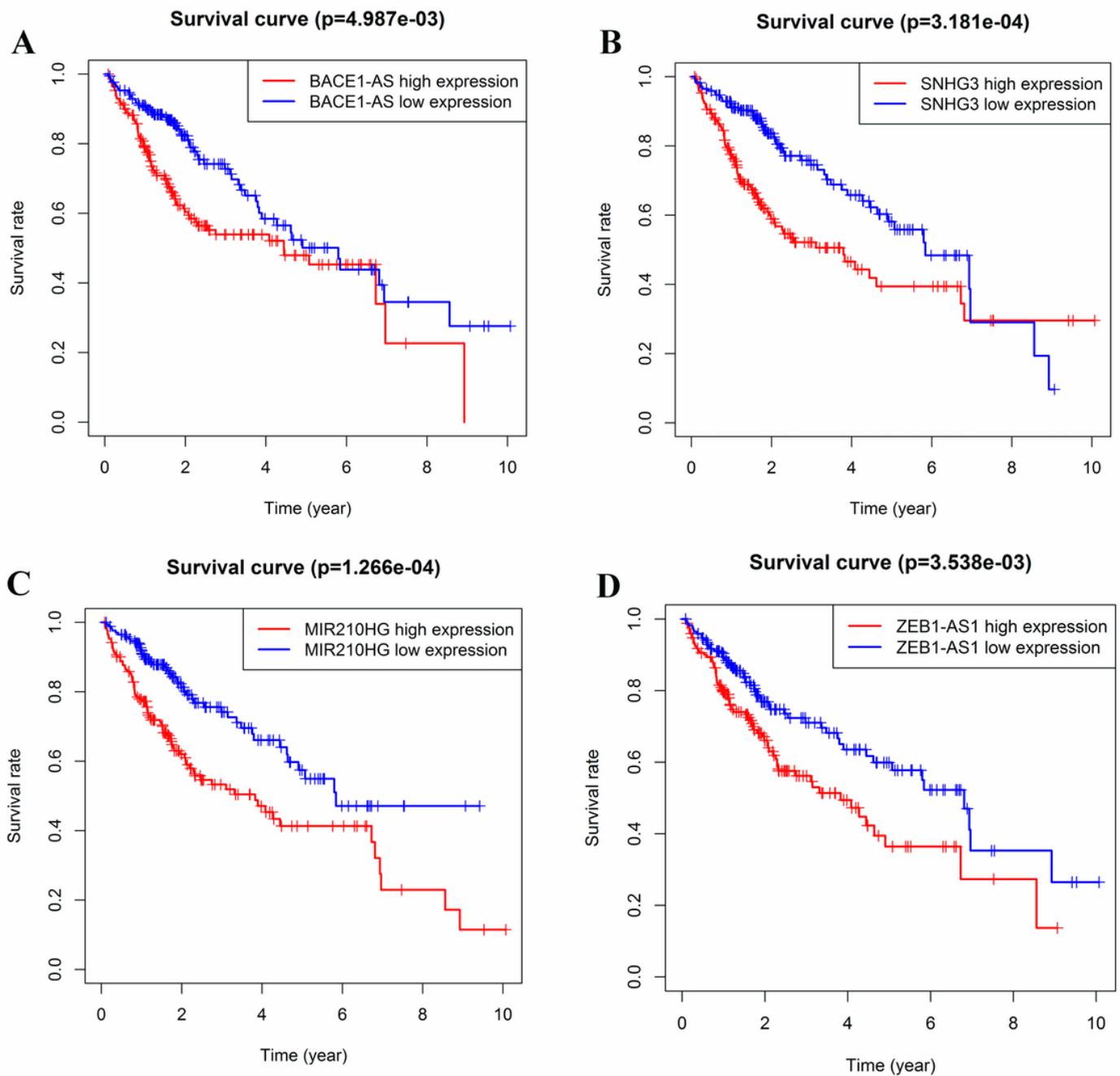


Figure 2

Kaplan–Meier survival curves for the four-prognostic autophagy-related-lncRNAs in HCC patients from the TCGA database. The four autophagy-related lncRNAs were confirmed to be risk factors.

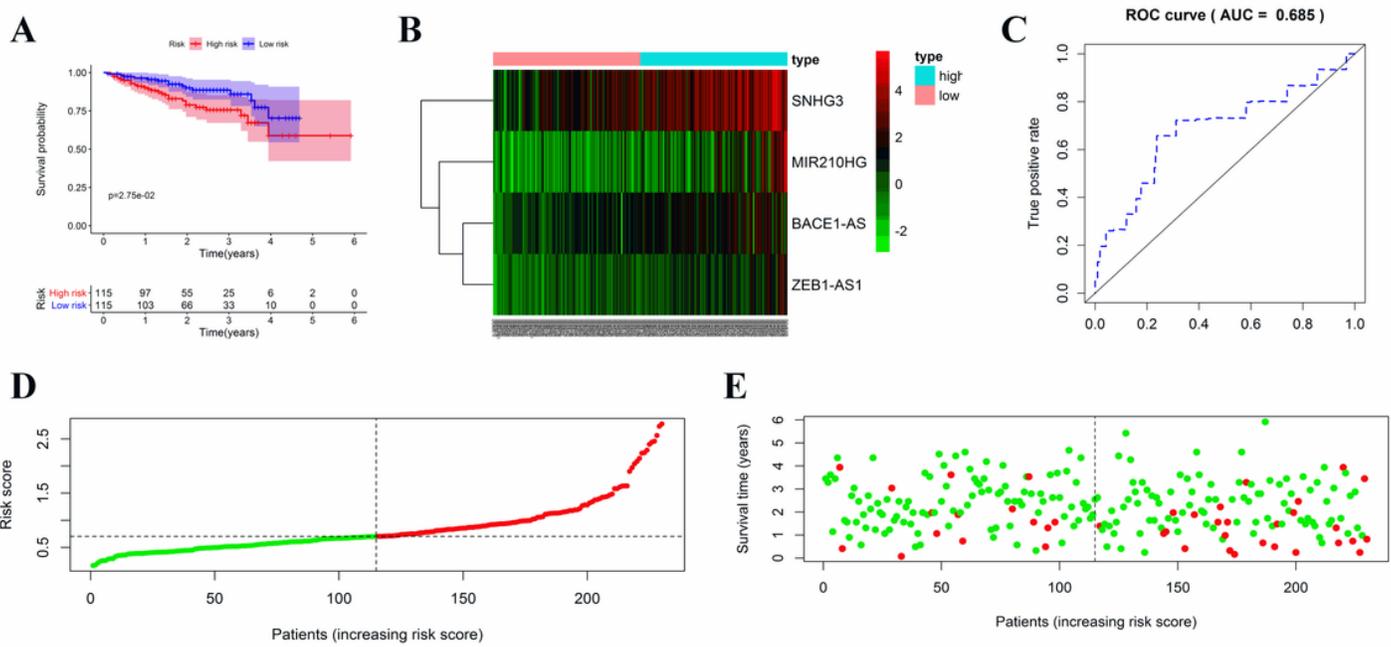


Figure 3

Validation of the autophagy-related lncRNA prognostic signature in the ICGC database. (A) K-M survival curve analysis of OS in the high-risk (red line) and low-risk (blue line) in the ICGC testing cohort. The high-risk groups had significantly poorer OS rates than the low-risk groups. (B) Heatmap showed that high-risk patients expressed higher levels of autophagy-related lncRNAs in the ICGC dataset. (C) The prognostic performance of the autophagy-related-lncRNAs signature demonstrated by the time-dependent ROC curve in the ICGC testing cohort. (D) Distribution of risk score curve of low- and high-risk HCC patients. (E) patient survival status and time distributed by risk score.

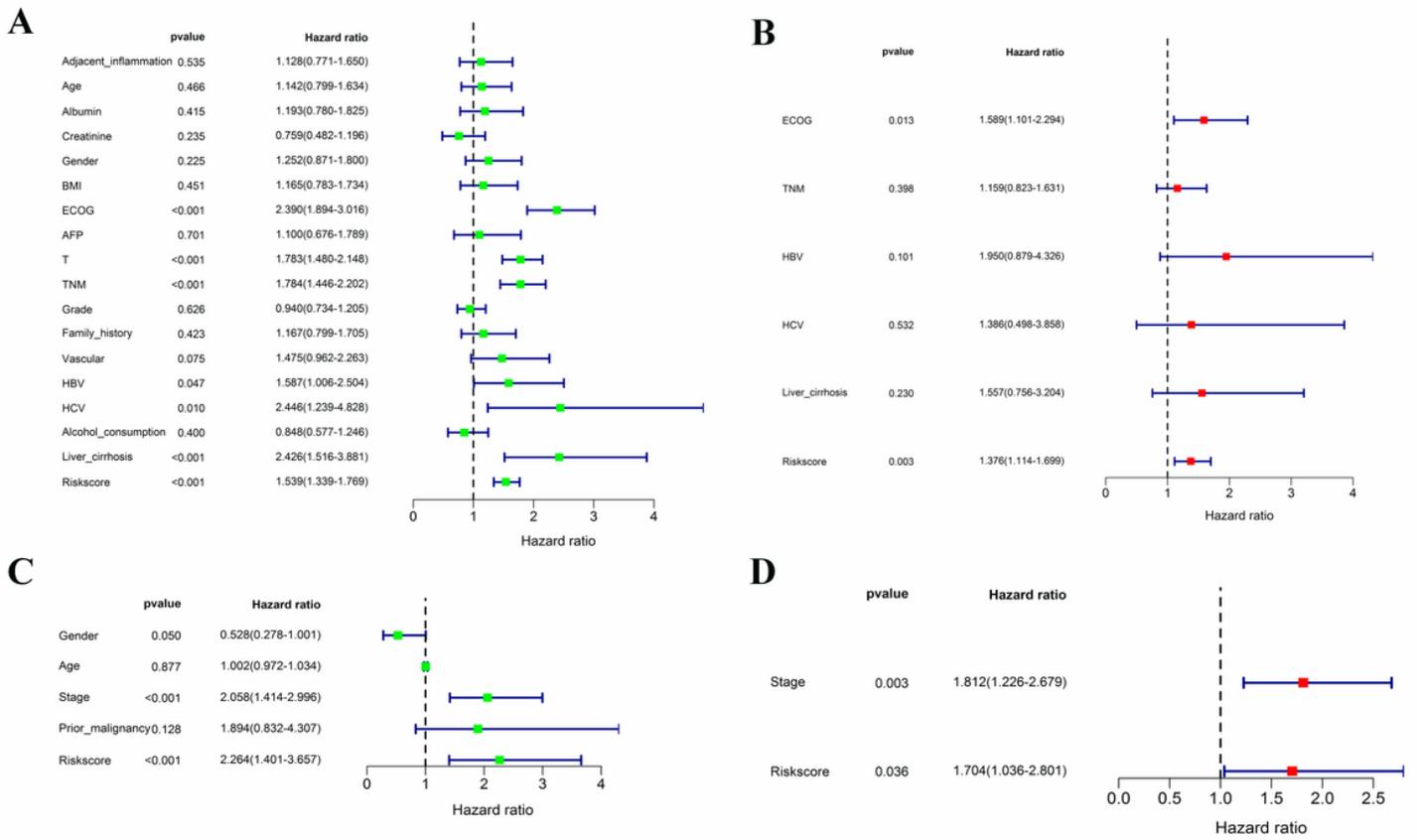


Figure 4

Evaluation of the prognostic accuracy of the autophagy-related lncRNAs prognostic signature and other clinicopathological variables in the HCC patients from the TCGA (AB) and ICGC (CD) databases. Univariate Cox regression analysis showed the correlations between various clinicopathological variables and OS (AC). The remaining parameters significantly associated with OS were enrolled into multivariate analysis. Multivariate Cox regression analysis showed that the established risk score was independent prognostic indicators for overall survival rate of HCC patients in both databases (BD).

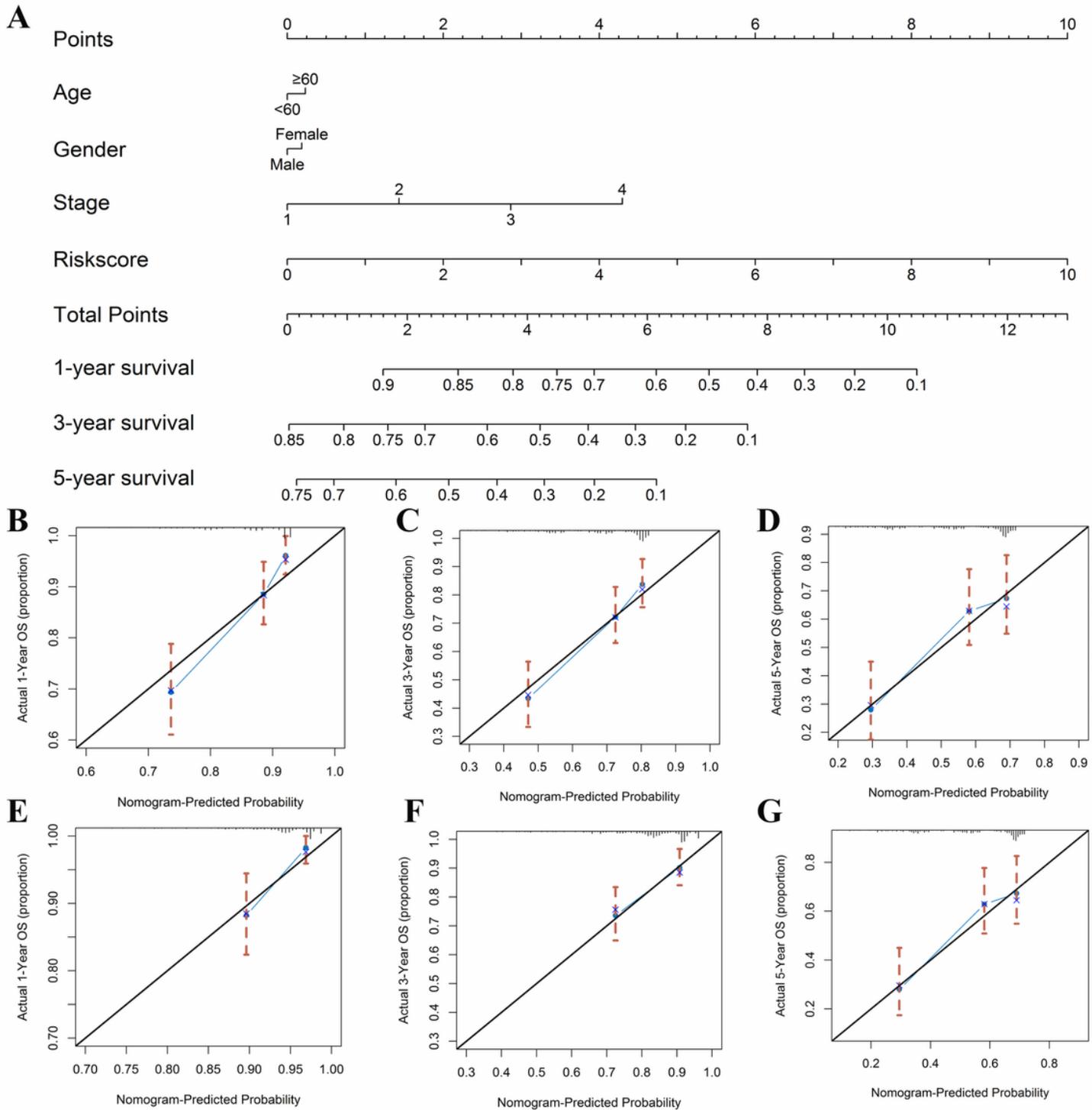


Figure 5

Constructing and Evaluating a nomogram to predict OS in patients with HCC. Nomogram for predicting 1-, 3-, and 5-year OS based on the prognostic signature and clinicopathological features (A). Calibration plot of the nomogram for 1- (B), 3- (C), and 5-year survival in the TCGA patients' cohort (D). Calibration maps used to predict the 1- (E), 3- (F), and 5-year (G) survival in the ICGC external validation cohort. The black line represents the "ideal" line of a perfect match between predicted and observed survival. The blue

line indicates the performance of the proposed nomogram. The X-axis is nomogram predicted probability of survival and Y-axis is actual survival. Blue dots are sub-cohorts of the data set; Red vertical bars represent 95% confidence interval.

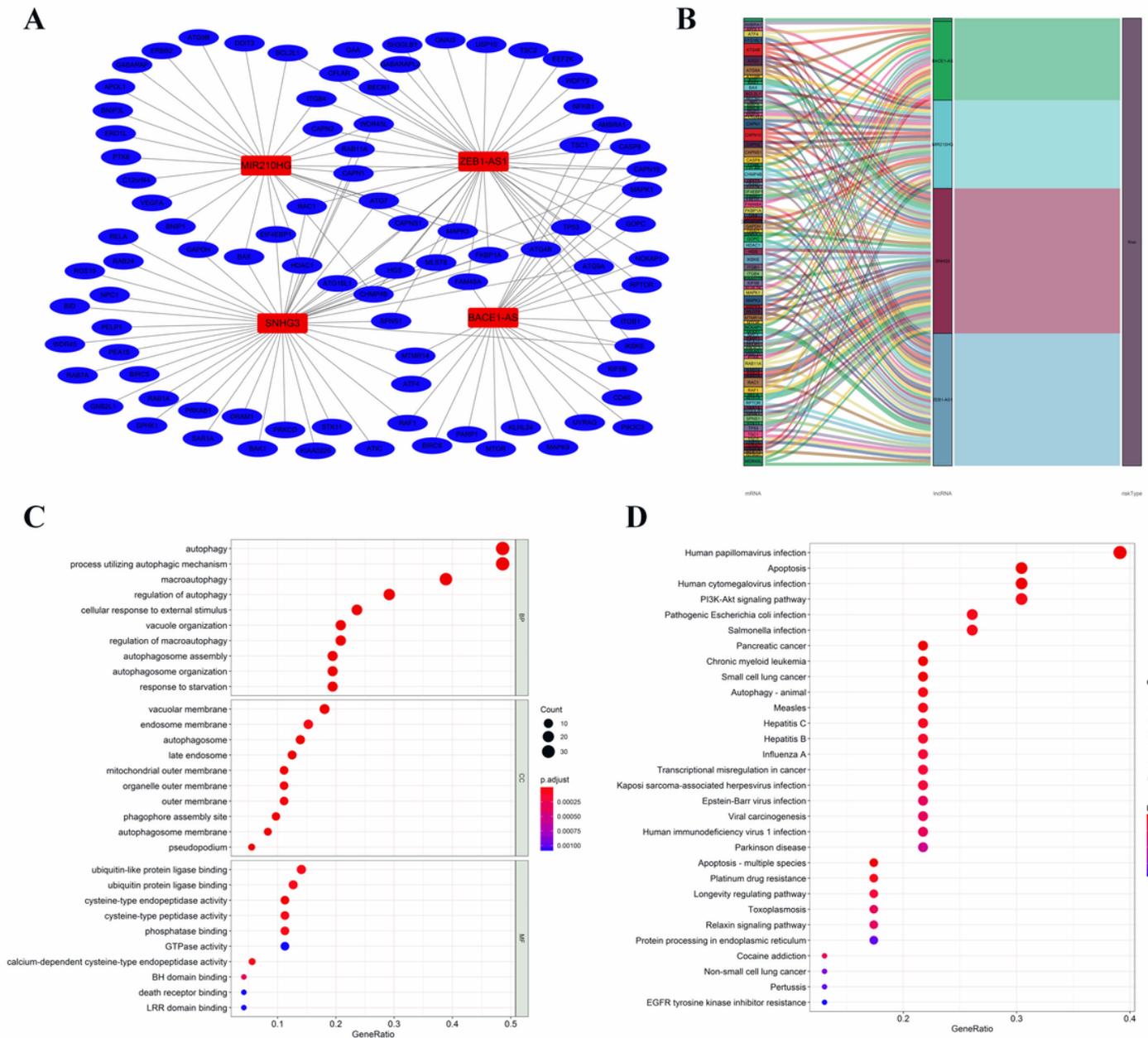


Figure 6

Construction of the prognostic autophagy-associated lncRNA-mRNA co-expression network and functional enrichment site analysis. Network of four-prognostic autophagy-related-lncRNAs with co-expressed autophagy genes in HCC. The red nodes correspond to autophagy-related lncRNAs, the blue nodes represented autophagy genes, and each edge represented a co-expression relationship (A). Sankey plot also showed the detail relationships of four autophagy-related lncRNAs with autophagy genes and risk types (B). Gene Ontology (GO) (C) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (D) pathway

analyses showed the enriched signaling pathways related to the mRNAs that co-express with the 4 autophagy-related lncRNAs.

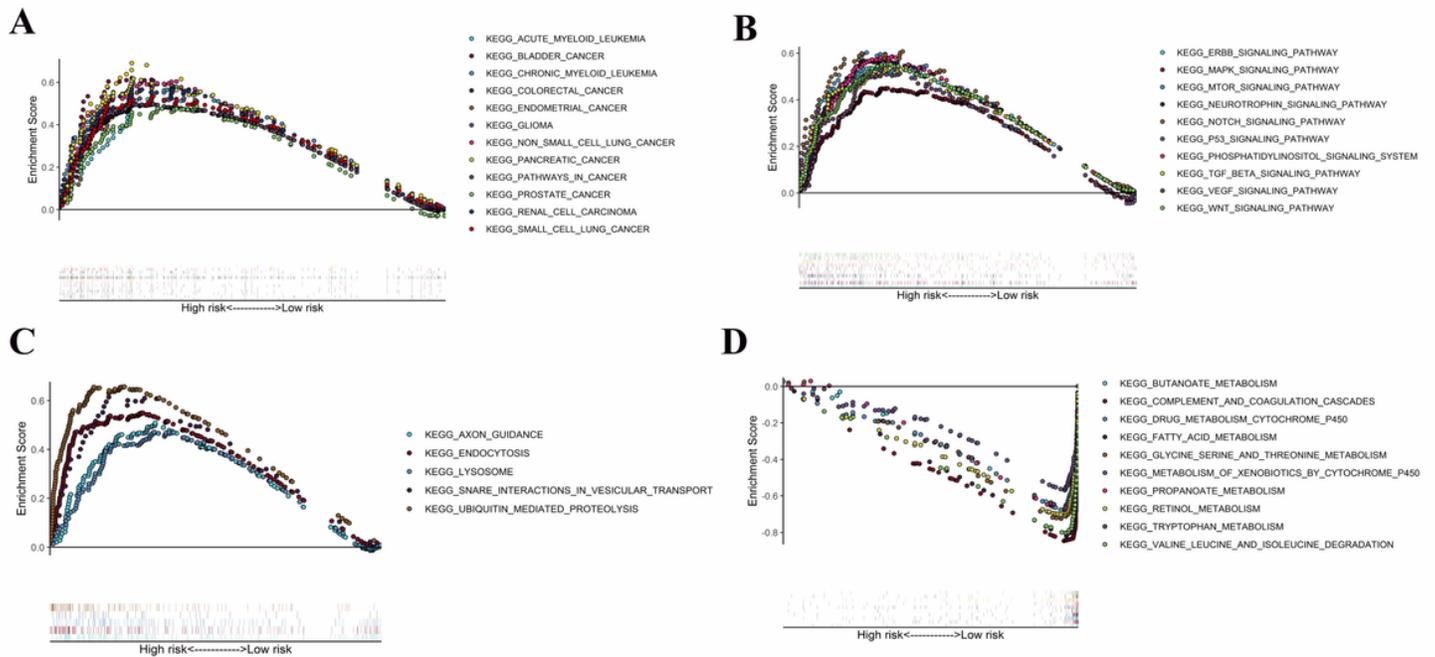


Figure 7

Gene set enrichment analysis of high- and low-risk HCC on the basis of the autophagy-related lncRNA prognostic signature. GSEA results showed the altered gene sets in the high-risk group were mainly significantly enriched in tumorigenesis (A), cancer- and autophagy-related signaling pathways (B), and the physical actions of autophagy (C). The protective pathways involved in metabolism were significantly enriched in the low-risk HCC patients (D).