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Original Article

High expression of C10orf91 and LINC01224 in hepatocellular carcinoma and poor prognosis

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Abstract: Background: The dysregulation of long non-coding RNAs (lncRNAs) has been implicated roles in the pathogenesis of many human diseases, including hepatic diseases. Several lncRNAs have been associated with the progression of hepatocellular carcinoma (HCC), but their function as diagnostic markers for liver cancer remain to be determined. Objective: This study aimed to identify the potential diagnostic markers for liver cancer. Methods: The Cancer Genome Atlas (TCGA) database was used to obtain the gene transcriptome data of liver cancer. In addition, this study enrolled 70 liver cancer patients admitted to the Yiwu Central Hospital and 50 healthy people who concurrently underwent physical examinations from February 2017 to January 2020. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to detect the expression of C10orf91 and LINC01224 in the patients' tissues and serum. A 5-year follow-up was conducted for survival observation. The potential and targeted miRs of C10orf91 and LINC01224 were predicted by online database for miRNA target prediction. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted and competing endogenous RNA (ceRNA) network was plotted. Results: A total of 175 differentially expressed lncRNAs were screened out, of which 173 were upregulated and 2 were downregulated. C10orf91, and LINC01224 were independent prognostic factors for liver cancer ($P<0.05$). C10orf91 and LINC01224 had diagnostic value for differentiating liver cancer, tumor node metastasis (TNM) staging, and lymphatic metastasis. GO and KEGG enrichment analysis showed that C10orf91 and LINC01224 were involved in 23 significant biological functions and 35 significant signal transduction pathways respectively. Conclusion: C10orf91 and LINC01224 are highly expressed in liver cancer patients withpoor prognosis.

Keywords: C10orf91, LINC01224, TCGA, ceRNA network, prognosis, liver cancer

Introduction

Liver cancer, a clinically common gastrointestinal tumor, is the third leading cause of cancer-related death around the world [1]. Although its incidence has been decreasing in recent years due to improvement of treatment, the long-term prognosis of patients with liver cancer remains unsatisfactory [2]. According to Allemani et al., the 5-year survival rate of the patients from 2010 to 2014 was 14.1%, which was higher than that between 2005 and 2009 [3]. This is basically attributed to the rather delayed diagnosis as most patients are already in the middle or advanced stage, resulting in the missing of the optimal timing for surgical treatment. Additionally, the lack of diagnostic markers with high specificity is also an important reason [4, 5]. A recent study has shown that as a clinical biomarker for liver cancer, alpha fetoprotein (AFP) is differentially expressed in patients with benign liver lesions, which is bound to reduce its specificity in liver cancer [6]. Therefore, it is essential to find new diagnostic markers.

Long non-coding RNAs (lncRNAs) are over 200 nt in length [7], which have been shown to be unable to code proteins [8]. Nonetheless, with the advancement in equipment and instruments, various mechanisms and functions of lncRNAs have been gradually unveiled [9]. According to Wang et al., lncRNA miR503HG, a prognostic indicator for liver cancer. It can inhibit tumor metastasis of hepatocellular carcinoma (HCC) by regulating the hnRNPA2B1/NF-κB pathway [10]. Xin et al. revealed that lncRNA HULC accelerated liver cancer through miR15a

autophagy and PTEN inhibition [11]. Zhang et al. proposed that the overexpression of lncRNA SNHG20 indicated the somber prognosis of liver cancer patients [12]. All these studies suggest the involvement of lncRNAs in the occurrence and progression of liver cancer, which indicates their potential to be indicators for the prognosis of the disease.

The Cancer Genome Atlas (TCGA) database is one of the most well-known databases that plots the genome maps of human tumors through large-scale high-throughput DNA sequencing and microarray technology [13-15]. In this study, differentially expressed lncRNAs in patients with liver cancer were screened out and analyzed through the TCGA database, and clinical experiments were conducted, to find potential diagnostic markers for the disease.

Materials and methods

Download and analysis of TCGA data

The website https://portal.gdc.cancer.gov/ was logged in. After Repository → Cases → Liver and intrahepatic bile ducts → TCGA-LIHC → File → Transcriptome Profiling → Gene Expression Quantification → HTSeq-Counts were chosen, Add all Files to cart was added to download Manifest, Cart, and Metadata data. The data were collected by the TCGA repository (http://can-cergenome.nih.gov/cancergenomics/tissuesamples), and then sequenced and analyzed by standardized treatment schemes. Altogether 374 cancer sample data and 50 matched paracancerous sample data were retrieved through IlluminaHiSeq2000. Perl scripts were used to combine the files into mRNA.symbol matrix files, which included protein-coding genes, lncRNAs, and pseudogenes. LncRNAs were extracted and then analyzed by edgeR package. The website http://gdac.broadinstitute.org/runs/stddata__2016_01_28/data/LIHC/20160128/ was logged in to download the clinical data of liver cancer patients. The patients' lncRNA expression and the clinical data were combined into matrix files. Screening criteria were FoldChange =4 and P=0.0001. Patients, whose survival time was shorter than 30 days as well as whose survival time and condition were lost, were deleted. After the log (X+1, 2) conversion was performed on the lncRNA expression, univariate and multivariate Cox regression was carried out. Finally, Kaplan-Meier (K-M) survival curves were plotted according to the indicators with differences in the multivariate Cox regression analysis.

Collection of clinical data

Seventy patients with liver cancer admitted to the Yiwu Central Hospital from February 2017 to January 2020 (the patient group) and 50 healthy controls who concurrently underwent physical examinations (the normal group) were enrolled in this study. In the patient group, there were 49 males and 21 females, with an average age of 55.3±5.3 years old. The normal group had 32 males and 18 females, with an average age of 54.8±4.9 years old. The two groups did not differ in their general data ($P>0.05$).

Inclusion criteria: Patients met the diagnostic standard of 8th edition of tumor node metastasis (TNM) staging criteria issued by the American joint Committee on cancer (AJCC) in 2017 [16]; patients were diagnosed with HCC by pathology, with complete clinical data; patients had not received targeted cancer treatment before enrollment; patients and their families signed the informed consent form after being fully informed of the purpose and process of the study.

Exclusion criteria: Patients complicated with other tumors; with renal diseases; with infections before admission; with severe cardiac and cerebral dysfunction; reluctant to cooperate with the follow-up; with immune deficiencies.

This study was authorized and approved by the ethics committee of our hospital, with the approval no. of 2016-125-61.

Sample collection

Peripheral blood (5 mL) was collected from all the research subjects, placed still for 30 minutes, and then centrifuged at 3000 rpm and 25°C for 10 minutes, to collect the serum for subsequent detection. Cancer tissues and paracancerous tissues were collected and stored in liquid nitrogen for later detection.

LncRNA detection

The TRIzol reagent (Invitrogen, Carlsbad, California, USA, 15596018) was used for RNA extraction. Ultraviolet (UV) spectrophotometer

Table 1. Top 10 lncRNAs with the most significant differences

Genes	logFC	logCPM	*P* value	FDR
SFTA1P	5.45278672	6.53627089	1.45E-47	4.72E-44
HAGLROS	4.83276827	6.31898833	3.53E-32	1.04E-29
LVCAT1	6.34929961	7.51483444	6.55E-27	9.70E-25
RP11-685F15.1	-4.36784799	5.48771736	1.37E-25	1.65E-23
LINC00176	5.01375438	10.79090480	3.96E-25	4.30E-23
RP11-138J23.1	7.21110208	7.37125460	8.23E-25	8.38E-23
RP1-170O19.14	6.48326074	6.50132750	1.49E-23	1.31E-21
RP11-25H12.1	4.95942990	6.59673685	5.19E-22	3.60E-20
AC011294.3	4.34439089	8.23570279	1.56E-21	1.03E-19
LINC01419	10.99847732	11.73249780	7.06E-21	4.18E-19

and agarose gel electrophoresis were used to detect its purity, concentration, and integrity. 5× TransScript®All-in-One SuperMix for qPCR and gDNA Remover in the TransScript Green Two-Step qRT-PCR SuperMix kit (TransGen Biotech, Beijing, China, AQ201-01) were used for reverse transcription following the manufacturer's instruction. The amplification systems of lncRNA C10orf91 and LINC01224 were as follows: 1 μL of cDNA, 0.4 μL each of upstream and downstream primers, 10 μL of 2× TransScript® Tip Green qPCR SuperMix, 0.4 μL of Passive Reference Dye (50×), and Nuclease-free Water in a final volume of 20 μL. Reaction condition: pre-denaturation at 94°C for 30 s, denaturation at 94°C for 5 s, annealing and extension at 60°C for 30 s, for a total of 40 cycles. The experiment was conducted in triplicate, with GAPDH as an internal standard, and $2^{-\Delta\Delta ct}$ [17] was used to analyze the data. During the experiment, an ABI 7500 quantitative PCR instrument was used. The premier sequences of C10orf91 and LINC01224 are presented in **Table 4**.

LncRNA bioinformatics analysis

The targeted microRNAs (miRs) of lncRNA C10orf91 and LINC01224 were predicted using miRcode and Starbase 3.0. Prediction of microRNA (miRNA) target mRNAs was carried out through miRDB, miRTarBase, and TargetScan. Competing endogenous RNA (ceRNA) network was plotted by Cytoscape. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analyses were performed on target genes through DAVID and KOBAS database.

Follow-up

Follow-up was conducted through telephone and outpatient service for 5 years. In the first year, the patients were followed up at the 1st, 4th, 8th, and 12th months, while at the following 4 years, the patients were followed up once every 3 months.

Statistical analysis

In this study, SPSS20.0 and R3.0.1 were used for data analysis. GraphPad 7 was used to plot figures. Kolmogorov-Smirnov (K-S) test was used for the distribution of measurement data, expressed by mean ± standard deviation (Mean ± SD). Comparison between groups was performed by independent samples t-test, while comparison within groups was analyzed by paired t-test and represented by t. Ranked data were analyzed by rank-sum test and represented by Z. Count data were analyzed by chi-square test. Receiver operating characteristic (ROC) curves were plotted to show the diagnostic value of C10orf91 for HCC. The 5-year survival of patients was visualized by K-M survival curves and compared by Log-rank test. Multivariate Cox regression was used to analyze independent factors affecting the prognosis of patients. Pearson test was used to analyze the correlation between C10orf91 expression in the tissues and the serum. A *P*-value less than 0.05 was considered statistically significant.

Results

Differentially expressed lncRNAs

After the analysis of the TCGA database, 175 differentially expressed lncRNAs were obtained, among which 173 were upregulated and 2 were downregulated. LncRNAs with the most significant differences were SFTA1P, HAGLROS, LVCAT1, RP11-685F15.1, LINC00176, RP11-138J23.1, RP1-170O19.14, RP11-25H12.1, AC011294.3, and LINC01419 (**Table 1**; **Figures 1** and **2**).

Cox regression analysis

Univariate and multivariate Cox regression analyses were performed on the combined

Table 2. Cox regression analysis

Genes	Univariate Cox			Multivariate Cox		
	P value	HR	95% CI	*P* value	HR	95% CI
C10orf91	<0.001	0.641	(1.278-1.908)	<0.001	0.696	(1.159-1.781)
RP11-440G9.1	<0.001	0.721	(1.148-1.678)			
LINC01224	0.001	0.721	(1.139-1.6900)	0.015	0.740	(1.061-1.723)
CDKN2A-AS1	0.002	0.680	(1.150-1.881)			
LINC01234	0.002	0.760	(1.103-1.569)			
RP11-57A1.1	0.003	0.736	(1.108-1.666)			
RP11-776H12.1	0.006	0.782	(1.074-1.521)			
RP11-495P10.5	0.007	0.781	(1.071-1.529)			
LINC00942	0.015	0.795	(1.045-1.513)			
LINC00648	0.017	0.827	(1.0347-1.413)			
MGC39584	0.021	0.837	(1.027-1.389)			
AC009014.3	0.024	1.214	(0.696-0.975)	<0.001	1.425	(0.579-0.850)
AC079466.1	0.039	0.949	(1.003-1.108)			

Table 3. Correlations of C10orf91 and LINC01224 with pathological data

Factors	lncRNA C10orf91		*P* value	lncRNA LINC01224		*P* value
	High expression group (n=35)	Low expression group (n=35)		High expression group (n=35)	Low expression group (n=35)	
Gender			0.192			0.434
Male (n=49)	22 (62.86)	27 (77.14)		26 (74.29)	23 (65.71)	
Female (n=21)	13 (37.14)	8 (22.86)		9 (25.71)	12 (34.29)	
Age (Years)			0.467			0.225
<55 (n=29)	16 (45.71)	13 (37.14)		12 (34.29)	17 (48.57)	
≥55 (n=41)	19 (54.29)	22 (62.86)		23 (65.71)	18 (51.43)	
Tumor size (cm)			0.337			0.150
≥5 (n=32)	14 (40.00)	18 (51.43)		19 (54.29)	13 (37.14)	
<5 (n=38)	21 (60.00)	17 (48.57)		16 (45.71)	22 (62.86)	
HBV			0.450			0.382
Yes (n=55)	25 (71.43)	20 (80.00)		29 (82.86)	26 (74.29)	
No (n=15)	10 (28.57)	5 (20.00)		6 (17.14)	9 (25.71)	
TNM staging			0.008			0.03
Stages I+II (n=37)	13 (37.14)	24 (68.57)		14 (40.00)	23 (65.71)	
Stages III+IV (n=33)	22 (62.86)	11 (31.43)		21 (60.00)	12 (34.29)	
Lymph node metastasis			0.003			0.015
Yes (n=28)	20 (57.14)	8 (28.57)		19 (54.29)	9 (32.14)	
No (n=42)	15 (42.86)	27 (77.14)		16 (45.71)	26 (61.90)	
Differentiation			0.454			0.803
Lowly differentiated (n=25)	14 (40.00)	11 (31.43)		12 (34.29)	13 (37.14)	
Moderately + well differentiated (n=45)	21 (60.00)	24 (68.57)		23 (65.71)	22 (62.86)	
Vascular invasion			0.314			0.615
Yes (n=24)	10 (28.57)	14 (40.00)		13 (37.14)	11 (31.43)	
No (n=46)	25 (71.43)	21 (60.00)		22 (62.86)	24 (68.57)	

Note: HBV: Hepatitis Bvirus.

Table 4. Primer sequences of C10orf91 and LINC01224

Primer		
C10orf91	F	5'-CTAACCCTAACCCTAACCCTAACCCTAA-3'
	R	5'-CCCTAACCCTAACCCTAACCCTAACC-3'
LINC01224	F	5'-CATTCAGCGGGGCTGCGGCTCCACGGCC-3'
	R	5'-ATGCCCAGTCCCCTGCAGGCCGCACC-3'

matrix files using R scripts (survival, qvalue). The univariate Cox regression analysis showed 13 factors of prognostic relevance. Further analysis showed that AC009014.3, C10orf91, and LINC01224 were independent prognostic factors for liver cancer patients

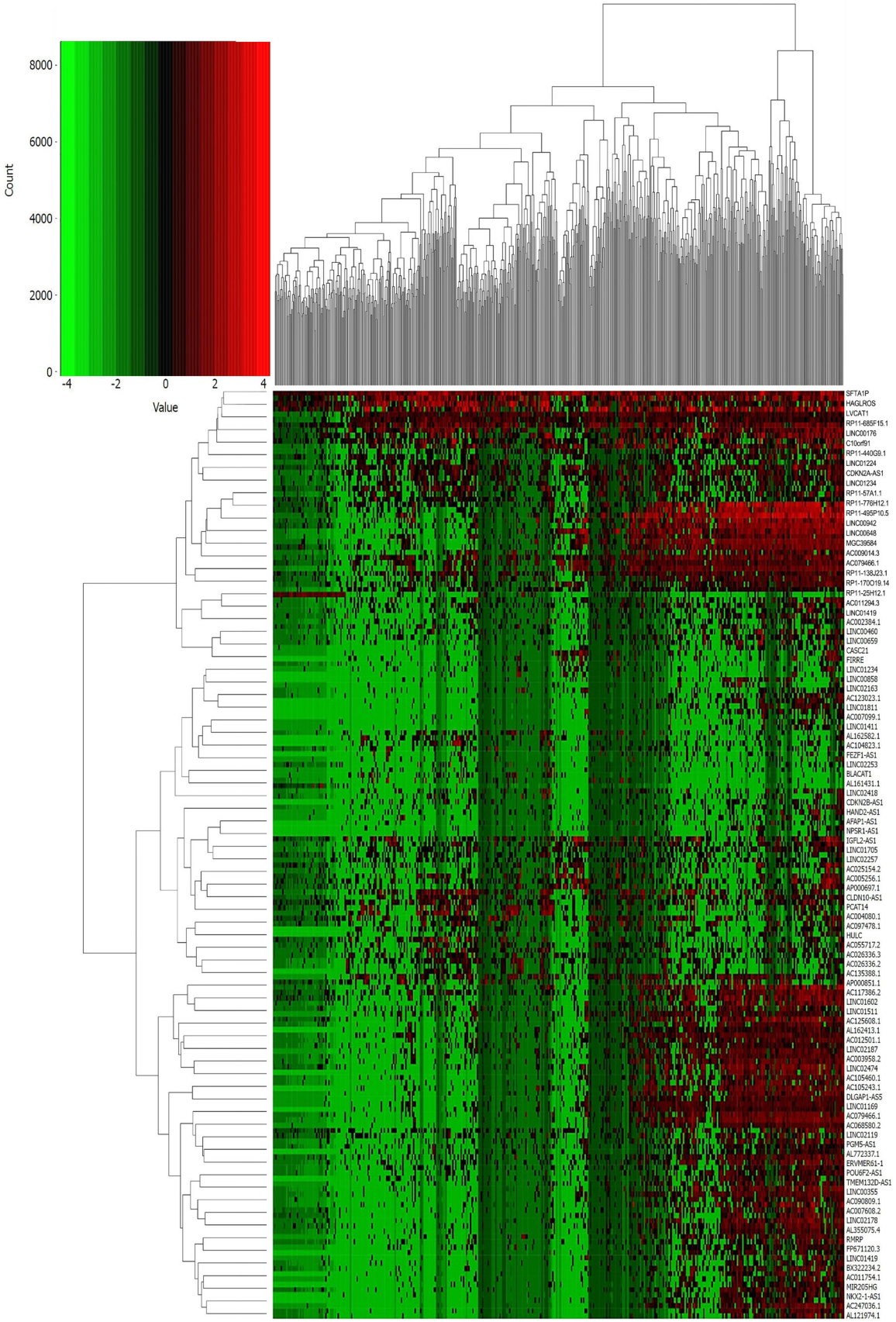

Figure 1. Heatmap of differentially expressed lncRNAs based on TCGA database. Red represents high expression and green represents low expression.

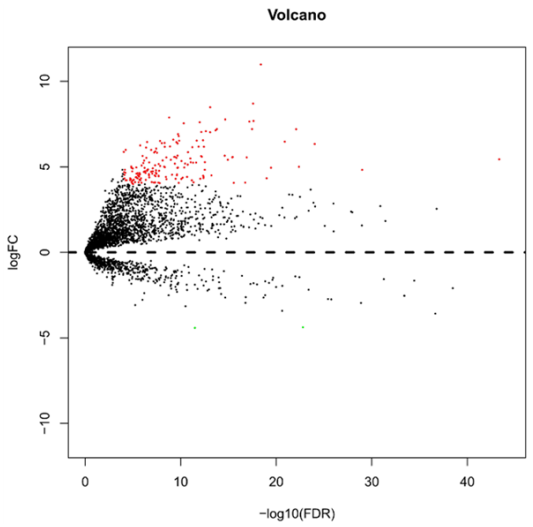

Figure 2. A volcano map. Red represents high expression and green represents low expression.

(**Table 2**). No significant difference was found in the overall survival rate between the high and low AC009014.3 expression groups (P=0.166), while higher survival rates were observed in C10orf91 and LINC01224 low expression groups compared with their corresponding high expression groups ($P_{C10orf91}$< 0.001, $P_{LINC01224}$=0.047; **Figure 3**).

Expression and clinical significance of C10orf91 and LINC01224

QRT-PCR analysis showed that the expressions of C10orf91 and LINC01224 in the patients' tissues and serum was higher than that in patients' paracancerous tissues and the serum of healthy controls (**Figure 4A**, **4B**; all P<0.001). The areas under the curves (AUCs) of C10orf91 and LINC01224 were 0.905 and 0.809 respectively (**Table 5**; **Figure 4F**). The expression levels of the two genes in the serum were positively correlated with those in the cancer tissues of patients (**Figure 4C**; P<0.001). Further, we analyzed the relationship between the expression of C10orf91 and LINC01224 and the pathological data of patients. It was found that, compared with the low expression groups, patients in the high expression groups showed a higher risk of stages III+IV of liver cancer and lymphatic metastasis (P<0.05). Moreover, C10orf91 and LINC01224 were of diagnostic value for distinguishing stages I+II from stages III+IV and the existence of lymphatic metastasis (**Tables 3**, **5**; **Figure 4D**, **4E**).

Correlations of C10orf91 and LINC01224 with prognosis

All patients were followed up for 5 years, and 57 cases died, with a survival rate of 18.57%. According to the median expression of C10orf91 and LINC01224, the patients were divided into the high and low expression groups to observe the correlation of these two genes with the 5-year survival. A higher 5-year survival rate was recorded in the C10orf91 and LINC01224 low expression groups ($P_{C10orf91}$<0.001, $P_{LINC01224}$<0.001). Therefore, C10orf91 and LINC01224 could be used as prognostic factors of patients with liver cancer. The multivariate Cox regression analysis revealed that LINC01224 (HR: 2.013, 95% CI: 1.148-3.529) and C10orf91 (HR: 3.736, 95% CI: 1.157-12.065) were independent factors affecting the prognosis of the patients (**Figure 5** and **Table 6**).

ceRNA network diagram as well as KEGG and GO enrichment analyses

A total of 16 potential targeted miRs of C10orf91 and LINC01224 were predicted by miRcode and Starbase 3.0. Altogether 77 downstream mRNAs of the miRNAs were predicted by miRDB, miRTarBase, and TargetScan. After that, Cytoscape was used to construct the interaction map between lncRNA-miRNA-mRNA. DAVID and KOBAS were used to carry out GO enrichment and KEGG pathway enrichment analyses on the 77 mRNAs in the ceRNA network. The results showed 23 GO biological functions and 35 signal transduction pathways (all P<0.05; **Figure 6**; **Tables 7** and **8**).

Discussion

Liver cancer is the most common gastrointestinal malignancy worldwide, with increasing incidence and mortality in recent years [18]. The reasons for the increase may be attributed to the following: First, liver cancer screening is limited. Second, the clinical symptoms of early liver cancer are rather hidden, which may result in delayed diagnosis. Third, the optimal timing for surgical treatment is missed, as the disease is usually in the middle or advanced stage once diagnosed. Fundamentally, these are due to the lack of markers with high specificity for liver cancer screening, which underlines the significance of new potential markers.

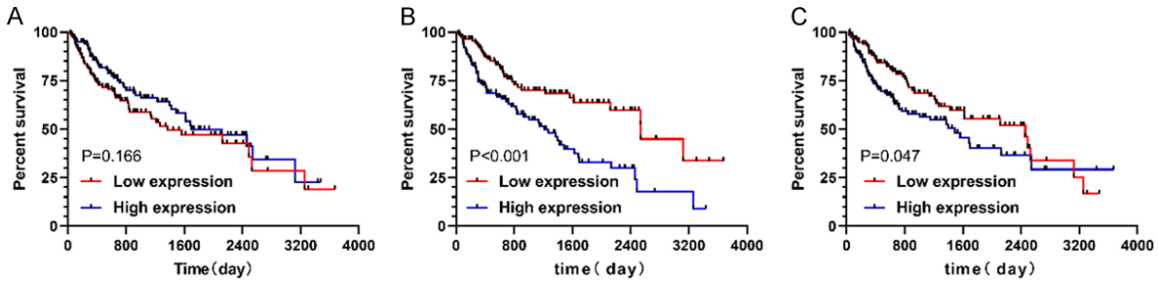

Figure 3. Correlations of AC009014.3, C10orf91, and LINC01224 with patient survival. A: There was no significant difference in the overall survival rate between the AC009014.3 high and low expression groups; B: Patients with high expression of C10orf91 had a poor prognosis; C: Patients with high expression of LINC01224 had a poor prognosis.

AFP, which is a clinically common tumor marker for liver cancer screening, has a positive value for the screening and diagnosis of the disease [19]. A recent study has shown that AFP is differentially expressed in patients with benign liver lesions, which reduces its specificity [20]. Previous research has shown that lncRNAs, with a length of over 200 nt, cannot code proteins [21]. However, recent studies have revealed that lncRNAs play a role in epigenetic changes, transcriptional regulation, and post-transcriptional modification [22], which are involved in the development and progression of various diseases through the targeted regulation of miRNAs and target proteins [23]. According to Li et al., lncRNA HOTAIR down-regulated the expression of SETD2 and promoted the growth of liver cancer stem cells [24]. Huang et al. reported that low expression of lncRNA DGCR5 indicated a somber prognosis of liver cancer patients [25]. These studies suggest the pertinence of lncRNAs to the progression of liver cancer and their potential to be its diagnostic markers. Therefore, in this study, differentially expressed lncRNAs in patients with liver cancer were analyzed based on the TCGA database to provide new and potential markers for the clinical diagnosis and prognosis of the disease.

In this study, differentially expressed lncRNAs in the TCGA database were first analyzed, with the thresholds set as FoldChange =4 and P=0.0001. A total of 175 differentially expressed lncRNAs were retrieved. The results of univariate and multivariate Cox regression analysis demonstrated that AC009014.3, C10orf91, and LINC01224 could be employed as independent prognostic factors for liver cancer. The survival curves were plotted to further verify the relationship between the three genes and patients' overall survival. The results presented no significant difference in the overall survival rate between the groups with high and low expression of AC009014.3, while a higher overall survival rate was observed in the C10orf91 and LINC01224 low expression groups compared with their corresponding high expression groups, indicating the potential of C10orf91 and LINC01224 as prognostic indicators for patients with liver cancer. Currently, there are few studies on the two genes that have been found in multiple expression profiling analyses [26, 27]. Through the analysis of the TCGA database, we found that C10orf91 and LINC01224 were expected to be potential prognostic markers for liver cancer, but whether their clinical expression had similar effects remains unclear. Therefore, further clinical research was carried out.

The expression profiles of C10orf91 and LINC01224 in the tissues and serum of patients with liver cancer were first analyzed. The expression of the two in the tissues and serum of the patients reduced significantly, which was consistent with the screening results of the database. The correlation between C10orf91 and LINC01224 expression in the cancer tissue and the serum of the patients was analyzed, which yielded a result that the expression of the two genes in the serum were positively correlated with that in the tissues, suggesting that the detection of their serum expression can reflect their expression in cancer tissues. Further, the correlation of C10orf91 and LINC01224 with patients' pathological data was analyzed. The results showed that patients with high expression of C10orf91 and LINC01224 was more likely to experience stag-

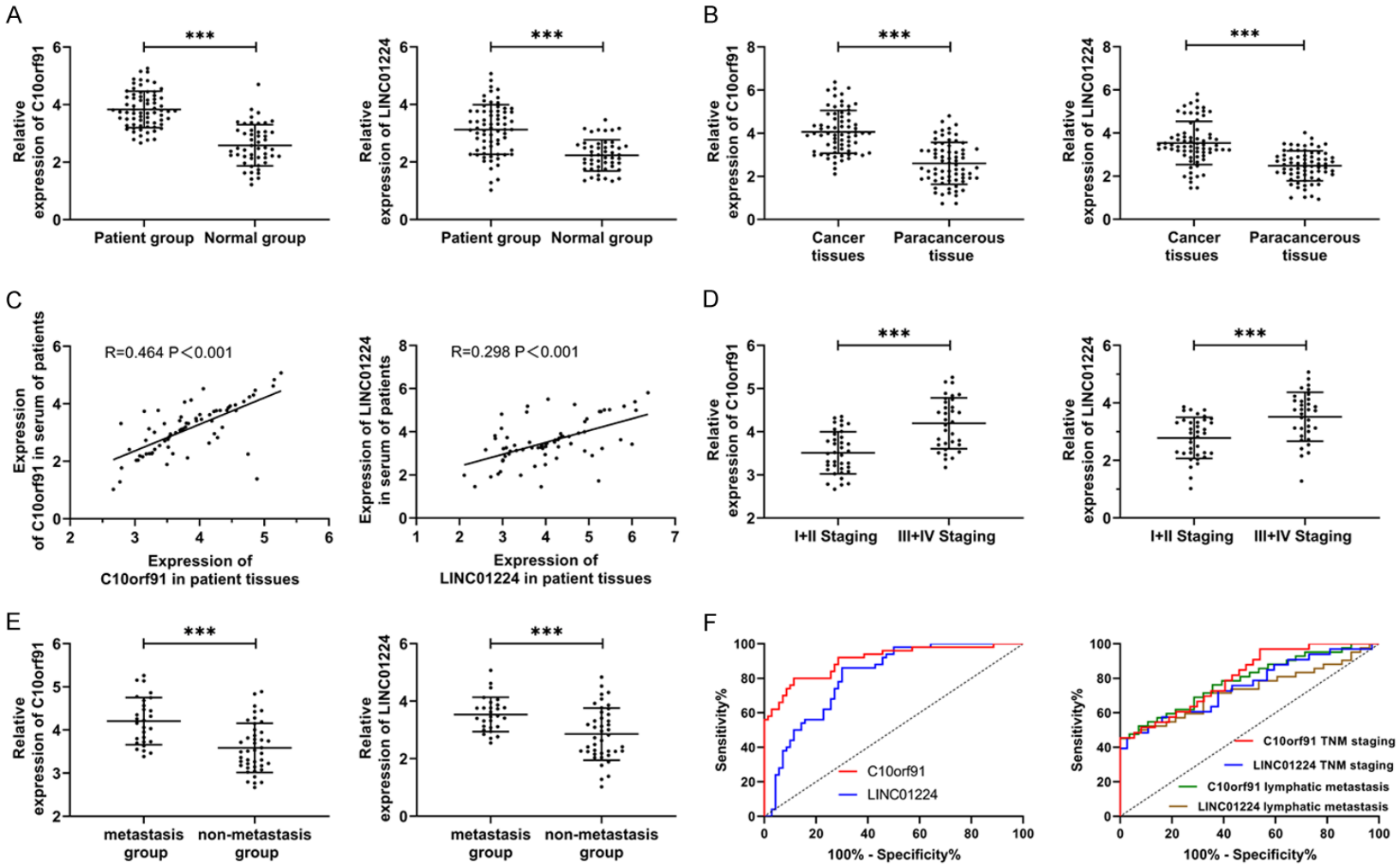

Figure 4. Expression and diagnostic value of C10orf91 and LINC01224. A: C10orf91 and LINC01224 were highly expressed in the serum of patients with liver cancer; B: C10orf91 and LINC01224 were highly expressed in the tissues of patients with liver cancer; C: The correlations between C10orf91 and LINC01224 expression in the serum and in the tissues; D: The expression of C10orf91 and LINC01224 in patients with stages I+II and III+IV of liver cancer; lymphatic metastasis; E: The expression of C10orf91 and LINC01224 in patients with and without lymph node metastasis; F: The ROC curves of serum C10orf91 and LINC01224 expression for diagnosing liver cancer as well as distinguishing stages I+II from stages III+IV and the presence or absence of lymph node metastasis. *** indicates P<0.001.

Table 5. ROC parameters

Factors	AUC	95% CI	Specificity	Sensitivity	Youden index	Cut-off
C10orf91 diagnosing liver cancer	0.905	0.849-0.960	88.57%	80.00%	68.57%	<3.134
LINC01224 diagnosing liver cancer	0.809	0.733-0.886	70.00%	86.00%	56.00%	<2.697
C10orf91 diagnosing staging	0.802	0.706-0.905	100.00%	45.45%	45.45%	>4.388
LINC01224 diagnosing staging	0.756	0.641-0.871	94.59%	48.48%	43.08%	>3.760
C10orf91 diagnosing lymph node metastasis	0.787	0.683-0.890	92.86%	52.38%	45.24%	<3.515
LINC01224 diagnosing lymph node metastasis	0.730	0.615-0.846	100.00%	45.24%	45.24%	<2.520

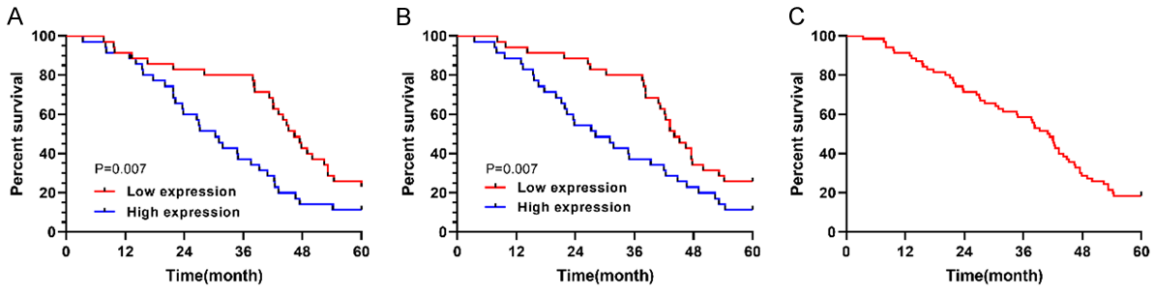

Figure 5. Correlations of C10orf91 and LINC01224 with 5-year survival. A: Differences in the 5-year survival rate between the C10orf91 high and low expression groups; B: Differences in the 5-year survival rate between the LINC01224 high and low expression groups; C: The 5-year overall survival.

Table 6. Cox regression analysis

Factors	Univariate Cox			Multivariate Cox		
	P value	HR	95% CI	*P* value	HR	95% CI
Gender (male VS female)	0.471	0.816	(0.470-1.418)			
Age (<55 years VS ≥55 years)	0.148	1.481	(0.870-2.520)			
Tumor size (≥5 cm VS <5 cm)	0.340	1.289	(0.765-2.172)			
Complicated with hepatitis (yes VS no)	0.965	1.012	(0.591-1.734)			
TNM staging (stages I+II VS stages III+IV)	0.250	0.737	(0.438-1.240)			
Lymph node metastasis (yes or no)	0.514	1.193	(0.702-2.029)			
Differentiation (low VS moderate + well)	<0.001	2.775	(1.610-4.783)	0.853	1.150	(0.263-5.036)
Vascular invasion (yes VS no)	0.585	1.162	(0.678-1.994)			
C10orf91 (lowly expressed VS highly expressed)	<0.001	4.519	(2.705-7.550)	0.011	3.736	(1.157-12.065)
LINC01224 (lowly expressed VS highly expressed)	<0.001	6.121	(3.419-10.961)	0.015	2.013	(1.148-3.529)

es III+IV of liver cancer and lymph node metastasis. According to the ROC curves, C10orf91 and LINC01224 were both of high diagnostic value for liver cancer, TNM staging, and lymph node metastasis. It has been reported that liver cancer patients have a low survival rate in consequence of the late diagnosis and the deficiency of prognostic indicators for the disease [28]. Subsequently, higher 5-year survival rates were determined in C10orf91 and LINC01224 low expression groups compared with their corresponding high expression groups. C10orf91 and LINC01224 were considered independent factors affecting the prognosis of the patients. This study demonstrated that the two genes can be used as potential prognostic markers for liver cancer. Then, bioinformatics analysis was performed to explore its relevant mechanism. ceRNA network was plotted through online prediction websites and Cytoscape, and KEGG and GO enrichment analyses were carried out according to mRNAs with potential binding targets. GO enrichment analysis showed 23 significant biological functions while KEGG enrichment analysis identified 35 significant signal transduction pathways. Among them, PI3K-Akt and MAPK signaling pathways are the major transduction pathways of tumors [29, 30], which suggest potential research directions in the future.

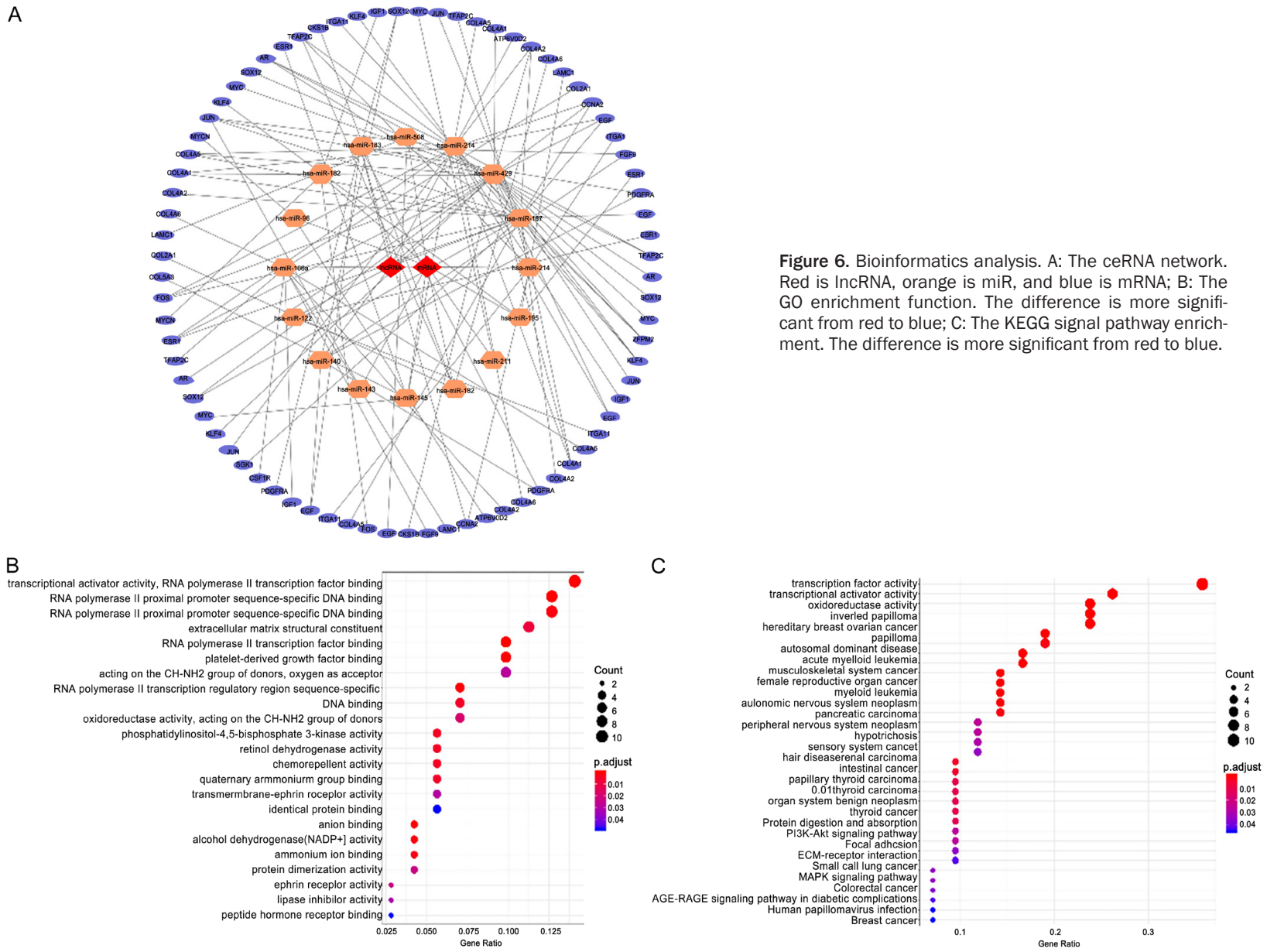

Figure 6. Bioinformatics analysis. A: The ceRNA network. Red is lncRNA, orange is miR, and blue is mRNA; B: The GO enrichment function. The difference is more significant from red to blue; C: The KEGG signal pathway enrichment. The difference is more significant from red to blue.

Table 7. Top 10 GO terms of mRNA in liver cancer

ID	Terms	Count	Genes	*P* value
GO:0005201	extracellular matrix structural constituent	7	COL4A5/COL4A1/COL4A2/COL4A6/LAMC1/COL2A1/COL5A3	2.52E-08
GO:0001085	RNA polymerase II transcription factor binding	7	FOS/ESR1/AR/HDAC4/ZFPM2/KLF4/JUN	1.61E-06
GO:0001077	transcriptional activator activity, RNA polymerase II proximal promoter sequence-specific DNA binding	9	FOS/MYCN/ESR1/TFAP2C/AR/SOX12/MYC/KLF4/JUN	1.80E-06
GO:0000982	transcription factor activity, RNA polymerase II proximal promoter sequence-specific DNA binding	10	FOS/MYCN/ESR1/TFAP2C/AR/SOX12/MYC/ZFPM2/KLF4/JUN	6.26E-06
GO:0001190	transcriptional activator activity, RNA polymerase II transcription factor binding	5	FOS/SOX12/ZFPM2/KLF4/JUN	6.43E-06
GO:0048407	platelet-derived growth factor binding	3	COL4A1/COL2A1/PDGFRA	1.02E-05
GO:0016641	oxidoreductase activity, acting on the CH-NH2 group of donors, oxygen as acceptor	3	LOX/LOXL2/LOXL4	4.14E-05
GO:0001228	transcriptional activator activity, RNA polymerase II transcription regulatory region sequence-specific DNA binding	9	FOS/MYCN/ESR1/TFAP2C/AR/SOX12/MYC/KLF4/JUN	4.48E-05
GO:0016638	oxidoreductase activity, acting on the CH-NH2 group of donors	3	LOX/LOXL2/LOXL4	9.25E-05
GO:0046934	phosphatidylinositol-4,5-bisphosphate 3-kinase activity	4	FGF9/ESR1/PDGFRA/EGF	1.65E-04

Table 8. Top 10 KEGG terms of mRNA in liver cancer

ID	Description	Count	Genes	*P* value
hsa04974	Protein digestion and absorption	10	COL4A5/COL4A1/COL4A2/COL4A6/COL15A1/COL2A1/SLC36A2/COL7A1/COL21A1/COL5A3	3.83E-11
hsa04151	PI3K-Akt signaling pathway	15	MCL1/FGF9/COL4A5/COL4A1/COL4A2/COL4A6/LAMC1/COL2A1/MYC/SGK1/CSF1R/PDGFRA/IGF1/EGF/ITGA11	1.48E-10
hsa04510	Focal adhesion	11	COL4A5/COL4A1/COL4A2/COL4A6/LAMC1/COL2A1/PDGFRA/IGF1/EGF/JUN/ITGA11	4.34E-09
hsa04512	ECM-receptor interaction	8	COL4A5/COL4A1/COL4A2/COL4A6/LAMC1/COL2A1/FREM2/ITGA11	1.51E-08
hsa05222	Small cell lung cancer	7	COL4A5/COL4A1/COL4A2/COL4A6/CKS1B/LAMC1/MYC	4.88E-07
hsa04010	MAPK signaling pathway	10	FGF9/FOS/TGFB2/IRAK1/MYC/CSF1R/PDGFRA/IGF1/EGF/JUN	2.35E-06
hsa05210	Colorectal cancer	6	FOS/TGFB2/DCC/MYC/EGF/JUN	5.41E-06
hsa05165	Human papillomavirus infection	10	COL4A5/COL4A1/ATP6V0D2/COL4A2/COL4A6/LAMC1/COL2A1/CCNA2/EGF/ITGA11	6.41E-06
hsa05224	Breast cancer	7	FGF9/FOS/ESR1/MYC/IGF1/EGF/JUN	1.06E-05
hsa04933	AGE-RAGE signaling pathway in diabetic complications	6	COL4A5/TGFB2/COL4A1/COL4A2/COL4A6/JUN	1.30E-05

This study has confirmed the clinical value of C10orf91 and LINC01224 in liver cancer, but it still has limitations. First, basic research was not carried out, and the relevant mechanisms of C10orf91 and LINC01224 in liver cancer remain elusive. Second, the sample size was small, so the representativeness of the two genes needs further verification. Third, it is still unclear whether C10orf91 and LINC01224 are polymorphic. Therefore, we will further analyze their roles in liver cancer through more basic research and increasing sample diversity (such as race and region). Moreover, no drug target study was conducted on C10orf91 and LINC-01224. And given that the high expression of C10orf91 and LINC01224 in liver cancer tissues suggests a somber prognosis of patients with liver cancer, we will down-regulate their expression in future studies for further research, so as to obtain new clinical data and provide new ideas for future treatment.

Conclusion

In summary, C10orf91 and LINC01224 are highly expressed in liver cancer patients, which indicates a somber prognosis of the patients.

Disclosure of conflict of interest

None.

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