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ORIGINAL RESEARCH

Identification of a Functional ceRNA Network to Explore Potential Biomarkers for Hepatocellular Carcinoma

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Purpose: To establish a novel circRNA–miRNA–mRNA network associated with the poor prognosis of hepatocellular carcinoma (HCC).

Materials and Methods: Quantitative real-time PCR was used to verify the differentially expressed circRNA. Moreover, the competing endogenous RNA networks were established using bioinformatics methods. Meanwhile, the prognostic value and potential mechanism of ceRNA network in hepatocellular carcinoma (HCC) were analyzed.

Results: This work found that *circ_0130911* was highly expressed in HCC tissues and early recurring HCC. Further, we effectively constructed a ceRNA network. The ceRNA network regulated by *circ_0130911* might influence the prognosis of HCC by regulating cell cycle-related pathways.

Conclusion: The ceRNA network proposed here can be used as a novel biomarker for the prognosis of HCC, thereby providing new insights for the targeted therapy of HCC.

Keywords: circRNAs, ceRNAs, biomarker, hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent malignant tumors with its incidence and mortality rates ranking 6th and 4th respectively across the globe.[1,2] Due to the current limitation of medical technology, HCC patients in the Asia-Pacific region are primarily treated via radical resection.[3] Nonetheless, after radical resection of HCC, nearly 70% of patients still develop recurrence of HCC. As a consequence, the overall treatment level of HCC and the improvement of the survival rate of HCC patients remain severely affected.[2] Therefore, it is necessary to identify the risk factors and potential mechanisms related to the early recurrence of HCC after radical resection geared towards providing novel treatment approaches for controlling the early recurrence and metastasis of HCC.

Circular RNAs (circRNAs) are a type of single-stranded endogenous non-coding RNA (ncRNA) with a covalently closed-loop structure. The ncRNA on the other hand is produced by reverse splicing and exhibits a circular structure that cannot be easily degraded by RNA exonuclease. Therefore, it has a more stable structure compared to linear RNA.[4,5] Notably, circRNAs are widely distributed in cells and have tissue-specific expressive characteristics. At the same time, circRNAs might participate in miRNAs sponge, epithelial-mesenchymal transition, and tumorigenesis.[6–9] Thus, the dysregulation of circRNAs expression might influence the development of cancer. For instance,

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circSHKBP1 (*hsa_circ_0000936*) regulates the *HUR-VEGF* pathway to inhibit *HSP90* degradation by sponging *miR-582-3p*, thereby promoting the progression of gastric cancer.[10] Also, recent studies have shown that *circ0003998* regulates the expression of *FOSL2* and *PCBP1-CD44v6* through sponge *miR-143-3p* to participate in the process of epithelial-mesenchymal transition of HCC.[11] Moreover, *circPTK2 (hsa_circ_0005273)* is highly expressed in colorectal cancer and positively correlated with tumor growth and metastasis. Based on various studies, *circPTK2* could promote EMT of colorectal cancer cells by physically binding to the phosphorylation sites *Ser38, Ser55,* and *Ser82* of vimentin thereby improving the metastasis of colorectal cancer. Therefore, *circPTK2* has been predicted to become a potential target for the treatment of colorectal cancer metastasis.[12]

In this study, 3 early recurrence and 3 early recurrence-free cases were selected for circRNA high-throughput sequencing in the preliminary work of our group. As a result, *hsa_circ_0130911* was highly expressed in patients with early recurrence of HCC. Through qRT-PCR and bioinformatics, we found that the expression of *circ_0130911* was upregulated in HCC tissues, and positively correlated with tumor growth and metastasis. Besides, the expression of *circ_0130911* was positively correlated with lower mediocre survival rates and poorer tumor-free survival in HCC patients. Additionally, a complete ceRNA network was constructed to predict the potential mechanism of *circ_0130911*. This work thus provides a strong foundation for the study of circRNAs related to the early recurrence of HCC. However, further experiments should be conducted to explore the specific regulatory mechanisms.

Materials and Methods

circRNA High-Throughput Sequencing

We selected 3 early recurrence cases and 3 early recurrence-free HCC tissues for circRNA high-throughput sequencing to detect the expression of circRNA.

Specimen of HCC Tissues

A total of 106 pairs of primary HCC tissues and 106 matched normal liver tissue samples used in this study were provided by the Affiliated Tumor Hospital of Guangxi Medical University. The diagnosis and cut-off values of clinical indicators for primary hepatocellular carcinoma were based on the Chinese guidelines for the diagnosis and treatment of primary HCC.[13] First, 106 tissues from patients were removed during liver resection, frozen in liquid nitrogen, and stored at −80°C before RNA isolation. After the operation, all patients were followed up via outpatient review, inpatient review, and telephone follow-up. The follow-up date ended in May 2018. Table 1 records the clinical characteristics of 106 patients. The Ethics Committee of the Affiliated Tumor Hospital of Guangxi Medical University approved the study, and all 106 patients signed the relevant informed consent.

Quantitative Real-Time PCR

The total RNA of 106 pairs of HCC tissues was extracted using TRIzol reagent (Invitrogen, USA). PrimeScript RT reagent Kit with gDNA Eraser kit (Takara, Japan) was used to reverse transcribed™ into cDNA, while FastStart Universal SYBR Green Master (ROX) (Roche, Germany) was used for real-time quantitative polymerase chain reaction (qRT-PCR) analysis. The expression level of circRNA was calculated by the $2^{-\Delta\Delta CT}$ method (standardized with *β-Actin*). The primers for *circ_0130911* were 5′-CCTTGGAGGCTGAGTGG −3′ and 5′-CTTCATCACCTGCTTACTTTC −3′. The primers for *β-Actin* were 5′- TGCGTGACATTAAGGAGAAG −3′ and 5′-GTCAGGCAGCTCGTAGCTCT −3′.

Prediction of circRNA–miRNA–mRNA Interaction Network

Notably, Cancer-Specific CircRNAs Database (CSCD)[14] understands the structural model of *circ_0130911*. The circbank[15] was used to predict the sponge miRNA of *circ_0130911*. Unlike the normal liver tissue samples, the differentially down-regulated miRNAs (DEmiRNAs) in HCC were identified by OncomiR and miRCancer. Besides, the intersection of miRNA predicted in circbank and miRNA in OncomiR[16] and miRCancer[17] was considered as the target molecule of *circ_0130911* in HCC. Of note, mRNA was predicted by miRTarBase database.[18] The GEPIA database[19] was used to determine the differentially up-regulated mRNAs in HCC and normal liver tissues. The intersection of mRNA in miRTarBase and GEPIA database was considered as the target gene of *circ_0130911* in HCC. Finally, Cytoscape software 3.7.2 was used to visualize the CircRNA-miRNA-mRNA network.

GO and KEGG Pathway Enrichment Analysis

The clusterProfiler software package in R was used to perform GO annotation and KEGG pathway analysis on the selected mRNA and the most frequently changed neighboring genes.

Table 1 A Comparison Between *circ_0130911* Expression and Clinicopathological Characteristics in HCC Patients

Characteristic	Total	*hsa_circ_0130911*		OR (95% CI)	*p* value
		Low n=53	High n=53		
Gender					0.308
Female	4	3	1	Ref.	
Male	102	50	52	3.120 (0.314–31.002)	
Age (years)					0.093
<65	100	52	48	Ref.	
≥65	6	1	5	5.417(0.611–48.041)	
Tumor diameter					0.338
<5cm	22	9	13	Ref.	
≥5cm	84	44	40	0.629(0.243–1.630)	
Tumor number					0.620
<3	86	44	42	Ref.	
≥3	20	9	11	1.280 (0.482–3.402)	
Child-Pugh					0.119
A	48	28	20	Ref.	
B	58	25	33	1.848(0.852–4.008)	
Portal vein thrombosis					**0.023**
No	87	48	39	Ref.	
Yes	19	5	14	3.446(1.141–10.406)	
Microvascular invasion					0.437
No	52	28	24	Ref.	
Yes	54	25	29	1.353 (0.631–2.905)	
AFP					**<0.001**
<400	53	37	16	Ref.	
≥400	53	16	37	5.348 (2.333–12.256)	
Metastasis					**0.030**
No	90	49	41	Ref.	
Yes	16	4	12	3.585 (1.074–11.966)	
Early recurrence					**0.006**
No	50	32	18	Ref.	
Yes	56	21	35	2.963 (1.343–6.537)	
BCLC stage					0.845
0+A	59	29	30	Ref.	
B+C	47	24	23	0.926 (0.430–1.994)	

Note: Bold, a significant difference.

Abbreviations: HR, hazard ratio; CI, confidence interval; AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma.

Construction and Analysis of Protein-Protein Interaction (PPI) Network and Hub Gene

The PPI networks were established using String database[20] and Cytoscape software 3.7.2 visualization. The Hub genes were identified according to the top ten node degree (the number of genes related to the target gene). Oncomine database[21] and HPA database[22] verified the expression of the selected 10 Hub genes at the transcription and translation levels. The influence of the 10 Hub genes on the prognosis of HCC were verified by the GEPIA database. Using the survivalROC package in R to draw ROC curve and calculate AUC to evaluate the risk value of Hub genes for the prognosis of HCC. Cox P<0.05 was considered to statistically significant.

Gene Set Enrichment Analysis (GSEA)

A total of 10 Hub genes were grouped based on their high and low expressions in the TCGA-LIHC gene set. With the GSEA 4.0.3 software via the Java platform, we derived the "https://software.broadinstitute.org/gsea/downloads.jsp" to evaluate the potential biological functions that might be involved. The enriched signaling pathways with FDR < 0.25 or nominal p < 0.05 were defined as statistically significant.

Statistical Analysis

SPSS 22.0 statistical software was used for all statistical analyses, while GraphPad Prism 8 was used to draw graphs. The chi-square test was used to evaluate the relationship between *circ_0130911* and clinicopathological characteristics. Moreover, the Cox regression model was applied for single factor and multivariate analysis. The Kaplan-Meier method was used to draw OS and DFS curves, and the Log rank test was used to evaluate their statistical significance. With a P value of less than 0.05 (P<0.05), the difference was considered statistically significant. Notably, bioinformatics analysis was mostly performed through the above-mentioned bioinformatics tools and R language. Genes or miRNAs with |log2(fold change)| >1 and P<0.05 were considered statistically significant.

Results

circRNA High-Throughput Sequencing Analysis

In the sequencing results, based on the analysis of the relationship between the expression of the HCC tissues with 3 early recurrence and 3 early recurrence-free, and statistical correlation analysis. We found *circ_0130911* showing a trend of highest expression in HCC tissues with early recurrence (Supplement Table 1).

Structural Characteristics of circ_0130911

The results of the circbase database revealed that *circ_0130911* originated from exons 43 to 50 of the UTRN gene, located at chr6: 144,858,717–144,898,424, and finally formed a mature sequence of 1246 nt in length (Figure 1). The CSCD database showed that *circ_0130911* comprised the structure of microRNA

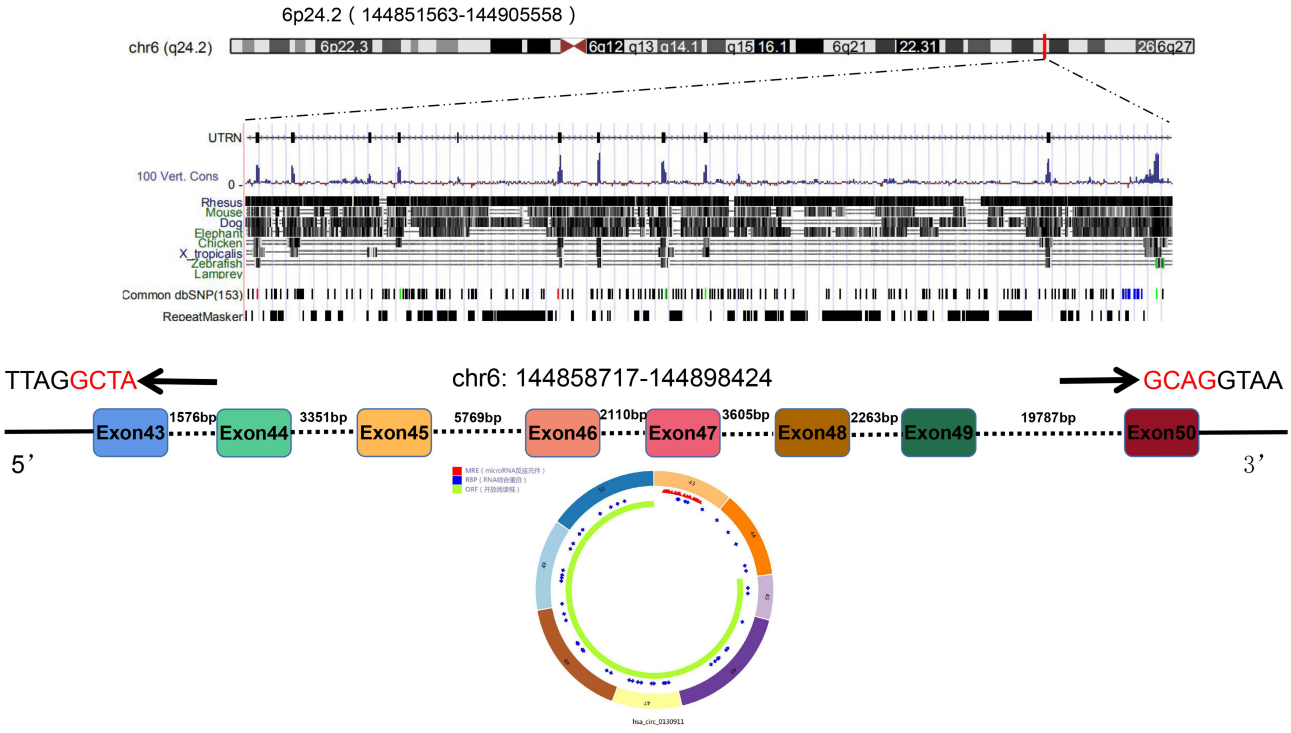

Figure 1 The schematic diagram showing the genomic locus of *circ_0130911* in the *UTRN* gene. Structure patterns of *circ_0130911* based on the CSCD database.

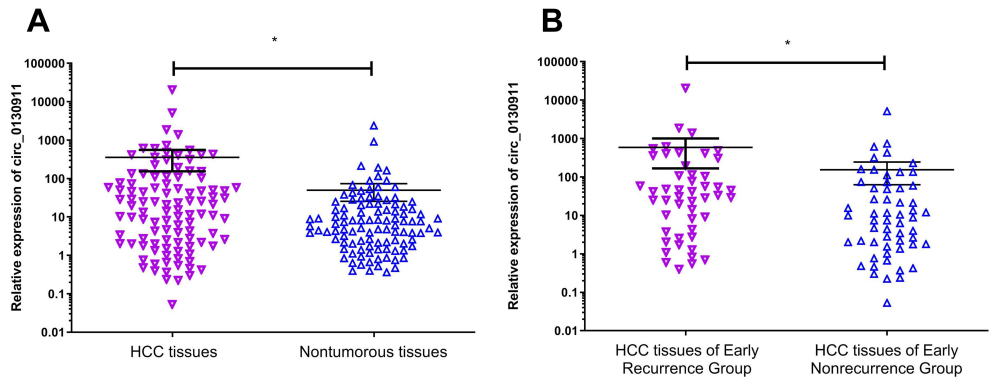

Figure 2 (**A**) The expression level of *circ_0130911* in HCC tissues is higher than that in corresponding adjacent nontumorous tissues. (**B**) The expression of *circ_0130911* in the HCC tissues of the early recurrence group is higher compared to that in HCC tissues of the early nonrecurrence group (*p<0.05).

response elements (MREs), suggesting that *circ_0130911* potentially regulates key circRNA of HCC development through sponge miRNA.

Up-Regulation of circ_0130911 in HCC

qRT-PCR technology was used to detect *circ_0130911* in 106 pairs of HCC tissues and corresponding adjacent tissues. Statistical analysis using Mann–Whitney U rank-sum test showed that the relative expression of *circ_0130911* in HCC tissues was significantly higher compared to that in adjacent tissues (p<0.05, Figure 2A); *circ_0130911* was relatively expressed in cancer tissues of patients with early recurrence of HCC. The expression level of *circ_0130911* was significantly higher compared to that of cancer tissues in patients with early HCC without recurrence (p<0.05, Figure 2B). This suggests that *circ_0130911* might be closely related to the early recurrence of HCC after surgery.

The Relationship Between circ_0130911 Level in HCC and Clinical Characteristics of Patients

Based on the median relative expression of *circ_0130911* in HCC tissues, HCC patients were subdivided into *circ_0130911* high expression group (53 cases) and low expression group (53 cases). Chi-square test was used to analyze the relationship between *circ_0130911* high expression group and low expression group and clinicopathological characteristics of HCC patients. Consequently, the high expression level of *circ_0130911* was significantly related to

Table 2 Univariate and Multivariate Cox Regression Analyses of Overall Survival

Clinicopathological Parameters	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*p*	HR	95% CI	*p*
Gender	2.705	0.373–19.602	0.325			
Age	0.248	0.034–1.794	0.167			
Tumor diameter	1.577	0.767–3.243	0.216			
Tumor number	2.701	1.473–4.954	**0.001**	2.632	1.404–4.932	**0.003**
Child-Pugh	1.408	0.787–2.521	0.249			
Portal vein thrombosis	2.757	1.482–5.131	**0.001**	1.436	0.715–2.887	0.309
Microvascular invasion	2.426	1.380–4.264	**0.002**	2.091	1.151–3.798	**0.015**
AFP level	1.722	0.995–2.979	0.052			
BCLC stage	1.492	0.869–2.560	0.147			
hsa_circ_0130911 expression	2.295	1.314–4.011	**0.004**	2.254	1.240–4.096	**0.008**

Note: Bold, a significant difference.
Abbreviations: HR, hazard ratio; CI, confidence interval; AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma.

Table 3 Univariate and Multivariate Cox Regression Analyses of Disease-Free Survival

Clinicopathological Parameters	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*p*	HR	95% CI	*p*
Gender	21.88	0.118–4064.671	0.247			
Age	0.928	0.290–2.970	0.899			
Tumor diameter	0.741	0.405–1.358	0.332			
Tumor number	2.183	1.206–3.951	**0.01**	2.325	1.240–4.359	**0.008**
Child-Pugh	1.938	1.113–3.374	**0.019**	1.662	0.929–2.974	0.087
Portal vein thrombosis	1.924	1.050–3.527	**0.034**	0.837	0.407–1.721	0.629
Microvascular invasion	2.138	1.242–3.680	**0.006**	1.922	1.070–3.452	**0.029**
AFP level	1.661	0.978–2.823	0.061			
BCLC stage	1.662	0.982–2.813	0.059			
hsa_circ_0130911 expression	2.260	1.313–3.889	**0.003**	2.286	1.280–4.080	**0.005**

Note: Bold, A significant difference.
Abbreviations: HR, hazard ratio; CI, confidence interval; AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma.

portal vein tumor thrombus, alpha-fetoprotein, metastasis, and early tumor recurrence (Table 1).

The Relationship Between circ_0130911 Level and Poor Prognosis of HCC Patients

Single-factor and multi-factor COX regression analysis was applied for all the above indicators. The results showed that the high expression of *circ_0130911* was an independent risk factor for death and recurrence of HCC patients. In terms of overall survival rate, the HR of the *circ_0130911* high expression group was 2.295 (95% CI 1.314–4.011, p=0.008, Table 2) whereas, in terms of the tumor-free survival rate, the HR of the *circ_0130911* high expression group was 2.260 (95% CI 1.313) −3.889, p=0.005, Table 3).

Kaplan-Meier statistics showed that the overall survival rate of patients in the *circ_0130911* high expression group (p=0.002, Figure 3A) and the tumor-free survival rate of early (within one year) recurrence (p=0.002, Figure 3B) were both significantly lower compared to the low expression group. This indicates that the higher the content of *circ_0130911* in HCC tissue, the worse the prognosis of HCC patients, and the higher the possibility of early recurrence.

Prediction and Construction of ceRNA Network

Based on the ceRNA theory, circRNA regulates the up-regulation of related target genes by competitively binding miRNA response elements with miRNA. Therefore, this study established an HCC-related

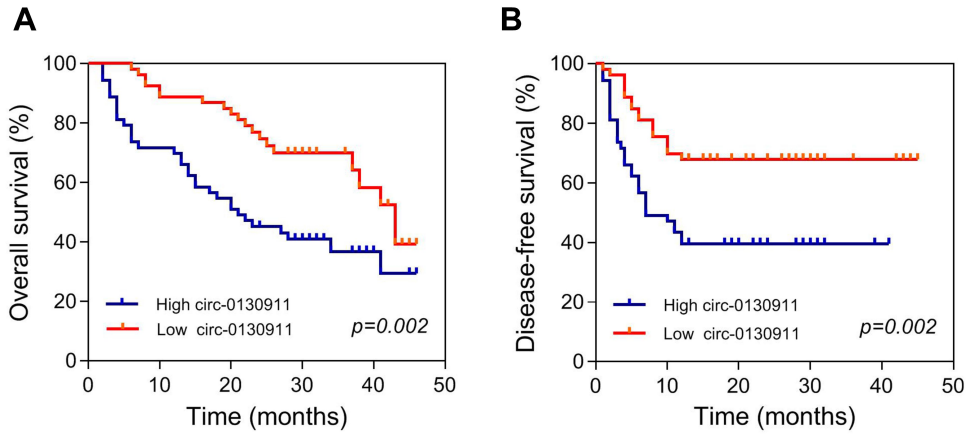

Figure 3 (**A**) The high level of *circ_0130911* expression in HCC tissue indicates that the overall survival rate of the patients is worse. (**B**) The high level of *circ_0130911* expression in HCC tissue indicates that the disease-free survival rate of the patient is worse.

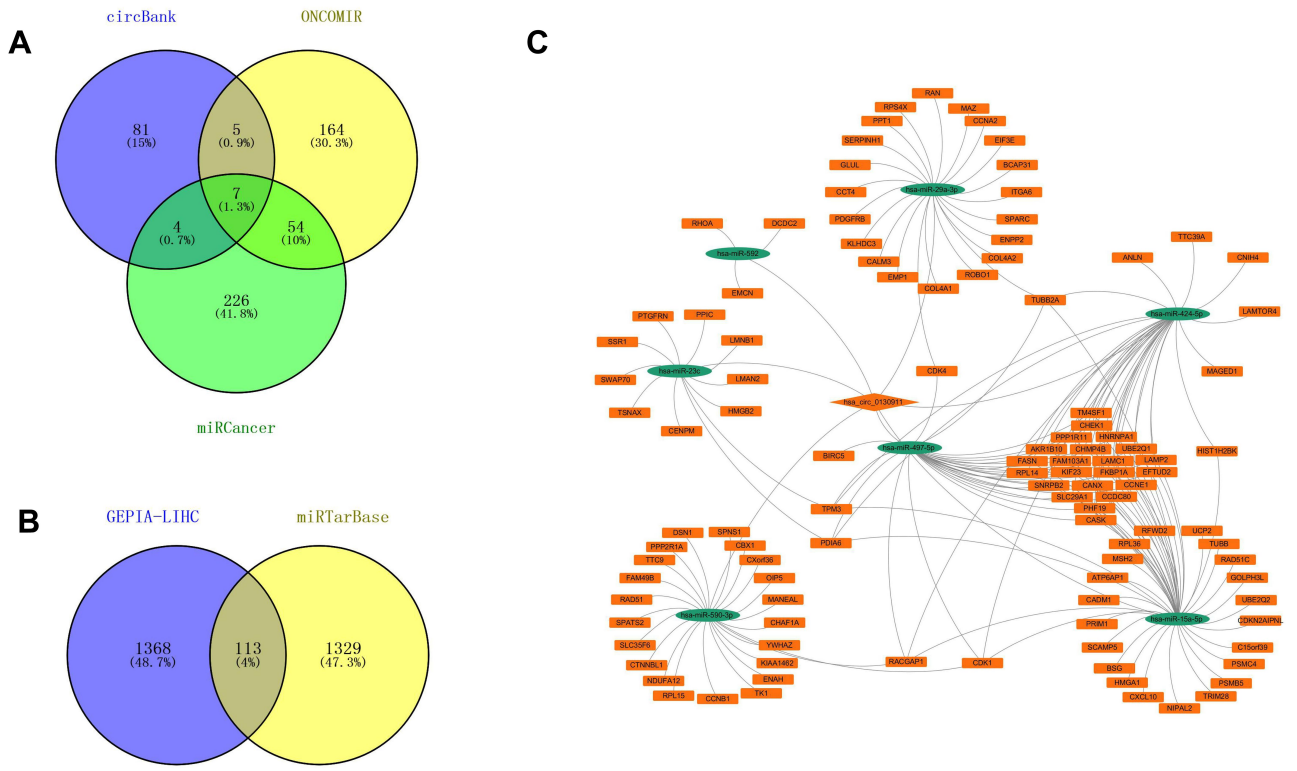

Figure 4 (**A**) Venn diagram of downregulated DEmiRNAs of *circ-0130911*. (**B**) Venn diagram of upregulated target genes of *circ-0130911*. (**C**) A circRNA-miRNA-mRNA regulatory network. The network comprises one circRNAs, 7 miRNAs, and 113 genes. CircRNAs, miRNAs, and mRNAs are respectively represented by diamond ovals and rectangles.

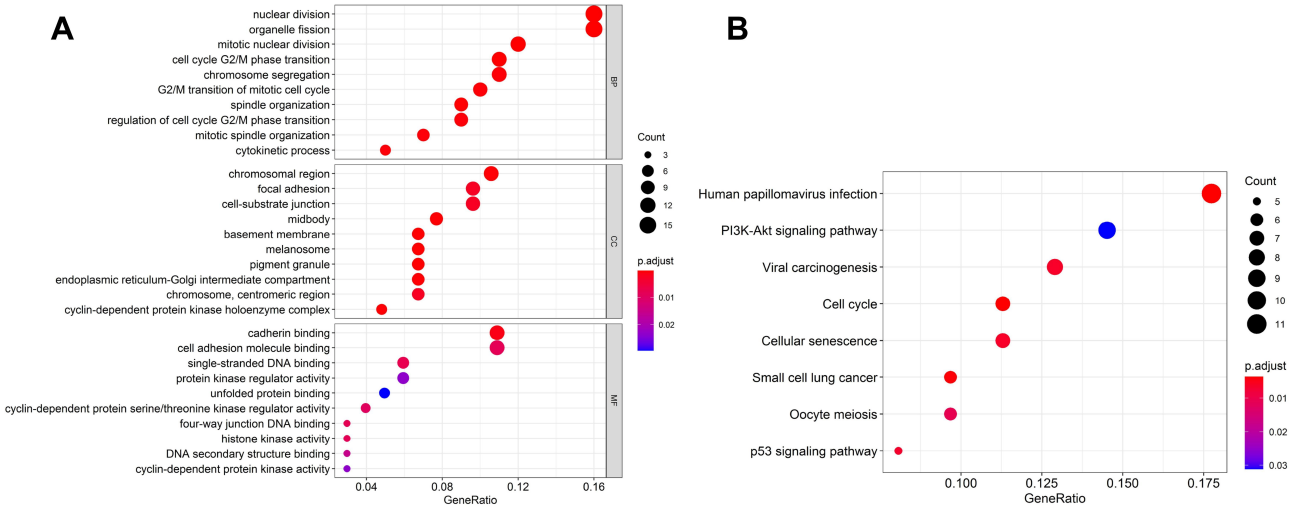

Figure 5 Functional enrichment analysis of the ceRNA network. (**A**) Results of GO analysis, including BP, CC, and MF; (**B**) Results of KEGG biological process analysis. The horizontal axis represents the gene ratio. The vertical axis represents lots of the GO terms or KEGG pathways. The size and color of a point represent the number and P-value of the pathway, respectively.

ceRNA network through the following steps. First, circRNA-miRNA interaction pairs were predicted in the circbank database, and DEmiRNAs that were differentially down-regulated in HCC were screened based on OncomiR and miRCancer databases. After crossing the 3 data sets, 7 down-regulated DEmiRNAs were selected including, *hsa-miR-590-3p, hsa-miR-15a-5p, hsa-miR-23c, hsa-miR-29a-3p, hsa-miR-424 −5p, hsa-miR-497-5p,* and *hsa-miR-592* (Figure 4A). Then, 1442 target genes of 7

DEmiRNAs were predicted through the miRTarBase database. Based on the GEPIA database, this study selected 1481 genes that were differentially and highly expressed in HCC. After the intersection, 113 highly expressed DEmRNAs were screened out (Figure 4B). Finally, based on the DEcircRNA-DEmiRNA pair and DEmiRNA-DEmRNA pair, a circRNA-miRNA-mRNA network was constructed. Cytoscape v3.7.2 was used to visualize the ceRNA network (Figure 4C).

Functional Analysis of mRNAs

To better understand the potential biological functions of *circ_0130911* in the development of HCC, GO (Figure 5A) and KEGG (Figure 5B) pathway enrichment analyses were performed on 113 target genes in the established ceRNA network. GO biological process (BP) analysis showed that up-regulated target genes were implicated in nuclear division, organelle fission, and mitotic nuclear division. For cellular component (CC) analysis, target genes were significantly enriched in the chromosomal region, focal adhesion, and cell-substrate junction. Additionally, the molecular function (MF) analysis for these DEmRNAs included cadherin binding, cell adhesion molecule binding, and single-stranded DNA binding.

Moreover, the enrichment results of KEGG showed that DEmRNAs exhibit a clear relationship with various cancer-related pathways, such as the *PI3K-Akt*signaling pathway, Cell cycle, Cellular senescence, and *p53* signaling pathway which were closely related to the ceRNA network. The significant GO enrichment terms and KEGG pathways were shown in bubble

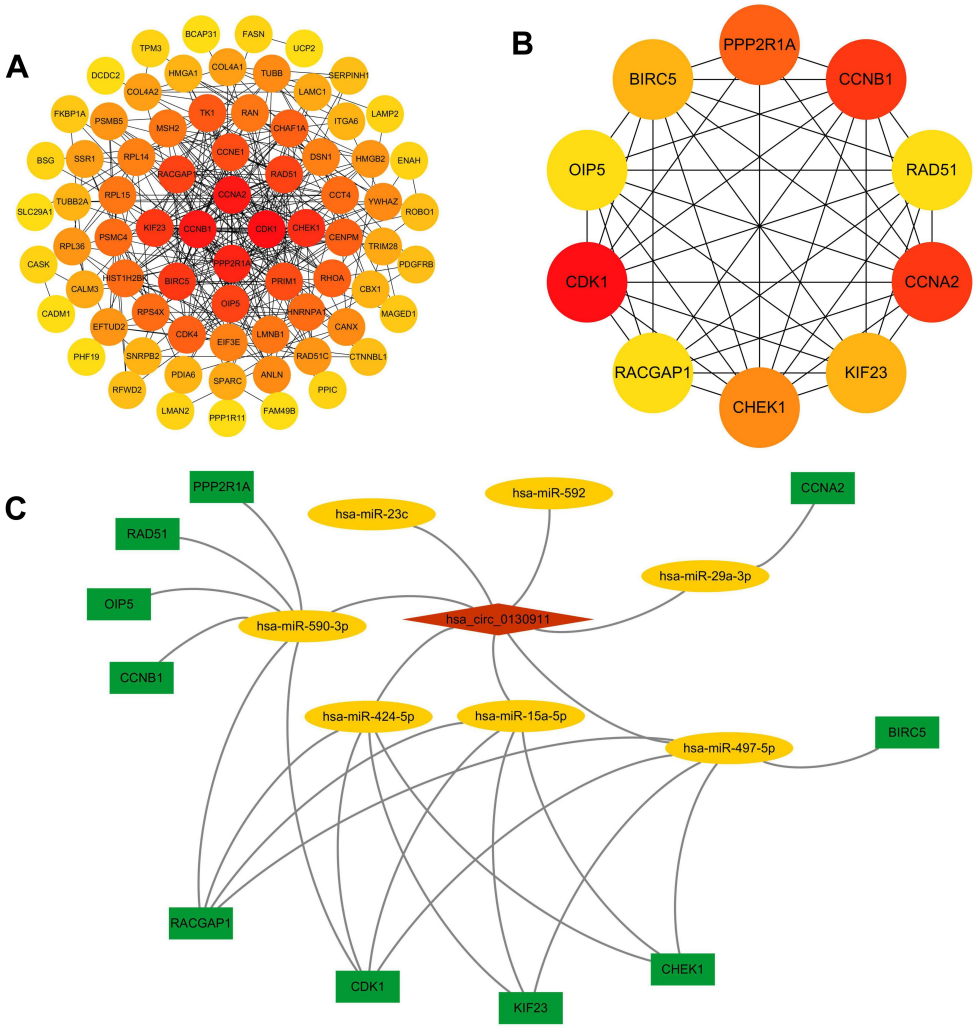

Figure 6 (**A**) PPI network of 113 target genes of *circ-0130911* in HCC. (**B**) 10 Hub-genes (*CDK1, CCNA2, CCNB1, PPP2R1A, CHEK1, KIF23, BIRC5, OIP5, RAD51, RACGAP1*) in the PPI network. (**C**) The circRNA-miRNA-Hub-genes axes.

maps using the ggplot2. R package with P<0.05. The results revealed that the constructed ceRNA network was related to multiple pathways regulating the growth of HCC.

Construction of PPI Network and Hub Gene

The STRING database was used to analyze the relationship between the 113 DEmRNAs in the ceRNA network and constructed a PPI network with 78 and 318 edges on the node. Cytoscape 3.7.2 was used to visualize the PPI network (Figure 6A). The top 10 Hub genes were identified using the cytoHubba plugin, including *CDK1, CCNA2, CCNB1, PPP2R1A, CHEK1, KIF23, BIRC5, OIP5, RAD51, RACGAP1* (Figure 6B). Interestingly, an interactive network was also formed between these Hub genes. Then, Cytoscape was used to built the circRNA-miRNAs-Hubs gene networks (Figure 6C).

Identification of Hub Genes

To further understand the biological functions of these 10 Hub genes in HCC, the Oncomine database was used to detect the expression of the Hub genes in HCC. Results suggested that the level of Hub genes in HCC tissues was significantly higher compared to that in normal tissues. Moreover, the Human Protein Atlas database showed the difference between the actual expression of *CDK1, CCNA2, CCNB1, PPP2R1A, CHEK1, KIF23, BIRC5, OIP5, RAD51,* and *RACGAP1* in normal liver tissue and HCC through immunohistochemistry (Figure 7A–J). The patient data of the IHC are listed in Table 4. Subsequently, the GEPIA database was used to evaluate the impact of these Hub genes on the prognosis of HCC. As a result, elevated expressions of *CDK1, CCNA2, CCNB1, PPP2R1A, CHEK1, KIF23, BIRC5, OIP5, RAD51,* and *RACGAP1* suggested a shorter survival time (Figure 8A–J) and tumor-free survival (Figure 9A–J).

Using the TCGA-LIHC database to incorporate these Hub genes into a single-factor, multi-factor COX risk regression model, *CCNB1, OIP5*, and *RACGAP1* showed significant prognostic value for HCC patients. The risk values of these 3 Hub genes were combined to draw the ROC curve, and the AUC value was calculated as 0.771 (Figure 10A). This indicates that *CCNB1, OIP5,* and *RACGAP1* in the established ceRNA network might be potential biomarkers for predicting the prognosis of HCC.

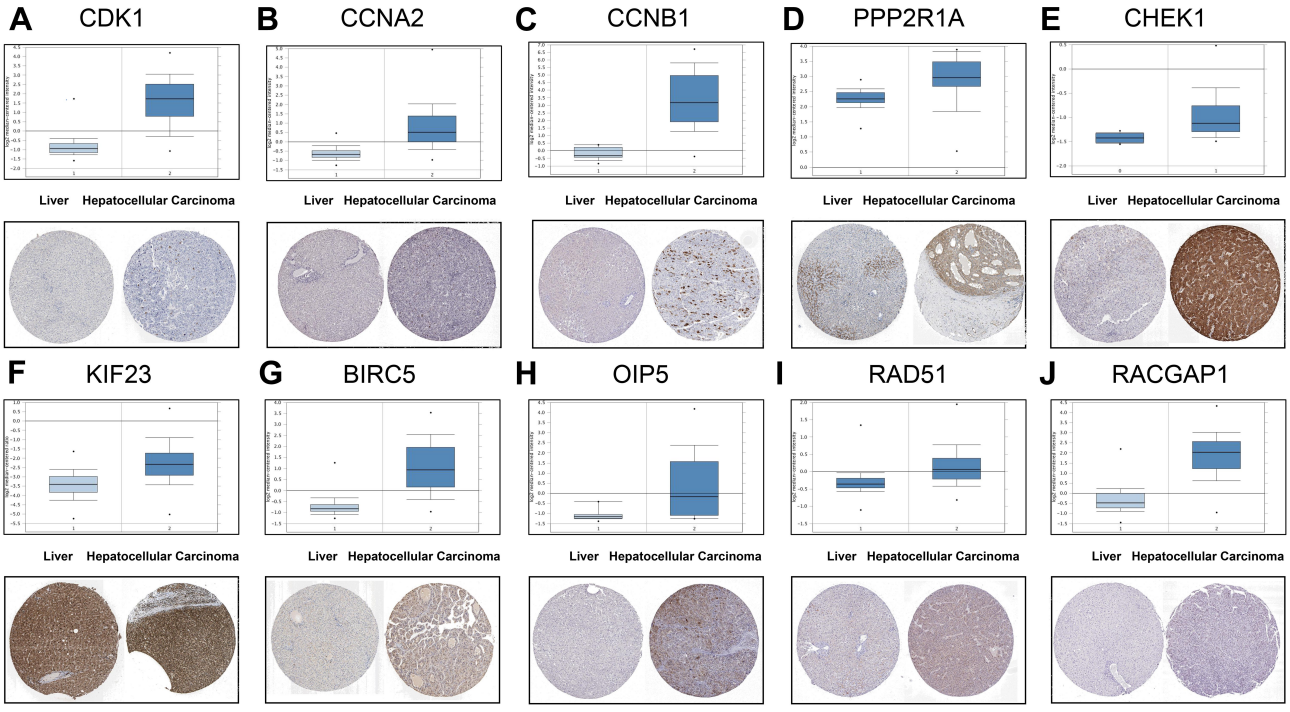

Figure 7 Validating the expression of 10 Hub genes on transcriptional and translational level by the Oncomine database and HPA database (immunohistochemistry). (**A**) *CDK1*. (**B**) *CCNA2*. (**C**) *CCNB1*. (**D**) *PPP2R1A*. (**E**) *CHEK1*. (**F**) *KIF23*. (**G**) *BIRC5*. (**H**) *OIP5*. (**I**) *RAD51*. (**J**) *RACGAP1*.

Table 4 Clinical Information of IHC in the Human Protein Atlas Database

Gene Symbol	Tissue Type	ID	Age	Gender	Staining
CDK1	Liver	1899	29	Female	Not detected
CDK1	Carcinoma hepatocellular	2325	76	Male	Medium
CCNA2	Liver	2251	50	Female	Not detected
CCNA2	Carcinoma hepatocellular	5177	60	Female	Medium
CCNB1	Liver	1720	67	Male	Not detected
CCNB1	Carcinoma hepatocellular	929	49	Male	Medium
KIF23	Liver	3378	73	Male	High
KIF23	Carcinoma hepatocellular	2177	58	Female	High
BIRC5	Liver	1846	32	Female	Low
BIRC5	Carcinoma hepatocellular	2325	76	Male	Medium
OIP5	Liver	3402	54	Female	Not detected
OIP5	Carcinoma hepatocellular	5034	76	Male	Medium
RAD51	Liver	3402	54	Female	Low
RAD51	Carcinoma hepatocellular	983	53	Female	High
RACGAP1	Liver	3402	54	Female	Not detected
RACGAP1	Carcinoma hepatocellular	3196	65	Male	Medium
PPP2R1A	Liver	3402	54	Female	Low
PPP2R1A	Carcinoma hepatocellular	2766	73	Female	Medium
CHEK1	Liver	1720	67	Male	Not detected
CHEK1	Carcinoma hepatocellular	2556	72	Male	High

GSEA

The TCGA database was used to further determine the possible pathways and mechanisms of these 3 Hub genes in regulating the progress of HCC. Based on their median, the Hub genes were divided into high expression and low expression groups (Figure 10B–D). GSEA was conducted to search for the GO and KEGG pathways enriched in the highly-expressed samples. The results revealed that the high expression of these 3 Hub genes were enriched in the pathways such as "kegg_cell_cycle", "go_cell_cycle_g1_s_phase_transition" (FDR q<0.05). Therefore, the ceRNA network might promote the development of tumors by regulating the cell cycle and other pathways.

Discussion

Based on the latest data released by the National Cancer Center, hepatocellular carcinoma (HCC) is the 4th leading cancer in China with its 2014 incidence being about 26.67 per 100,000. It causes the cancer-related deaths of approximately 319, 000 people annually, with its death rate only second to lung cancer.[23] The existing guidelines recommend that hepatectomy is a vital method to ensure the long-term survival of HCC patients, and significantly improves the prognostic quality of life among patients.[24] Nonetheless, the high recurrence rate and short survival time of HCC patients negatively impact the improvement of the overall treatment level of HCC. Also, this is attributed to its biological characteristics including strong invasiveness, high malignancy, and poor prognosis.

Accumulating evidence has shown that circRNA plays an important role in the progression of HCC. As hypothesized by the ceRNA network, circRNA regulates the expression of mRNA through the sponge miRNA of MREs structure.[9] As such, circRNA exerts its biological functions through an extensive gene expression regulatory network, and can as well be used as a diagnostic and prognostic biomarker. For instance, Exosomal *circPACRGL* acts as a sponge for *miR-142-3p/miR-506-3p* and promotes progression of colorectal cancer via the *miR-142-3p/miR-506-3p- TGF-β1* axis. Shang et al established a ceRNA network that might play a role in the early recurrence of HCC, thereby providing new ideas for monitoring the early recurrence of HCC.[25]

Furthermore, studies conducted by Xu et al have shown that the upregulated expression of *circ-CCAC1* in cholangiocarcinoma increases cell progression by sponging *miR-514a-5p* and up-regulating YY1. Besides, it promotes the occurrence and metastasis of cholangiocarcinoma, and might be an important organism markers for the treatment of cholangiocarcinoma.[26]

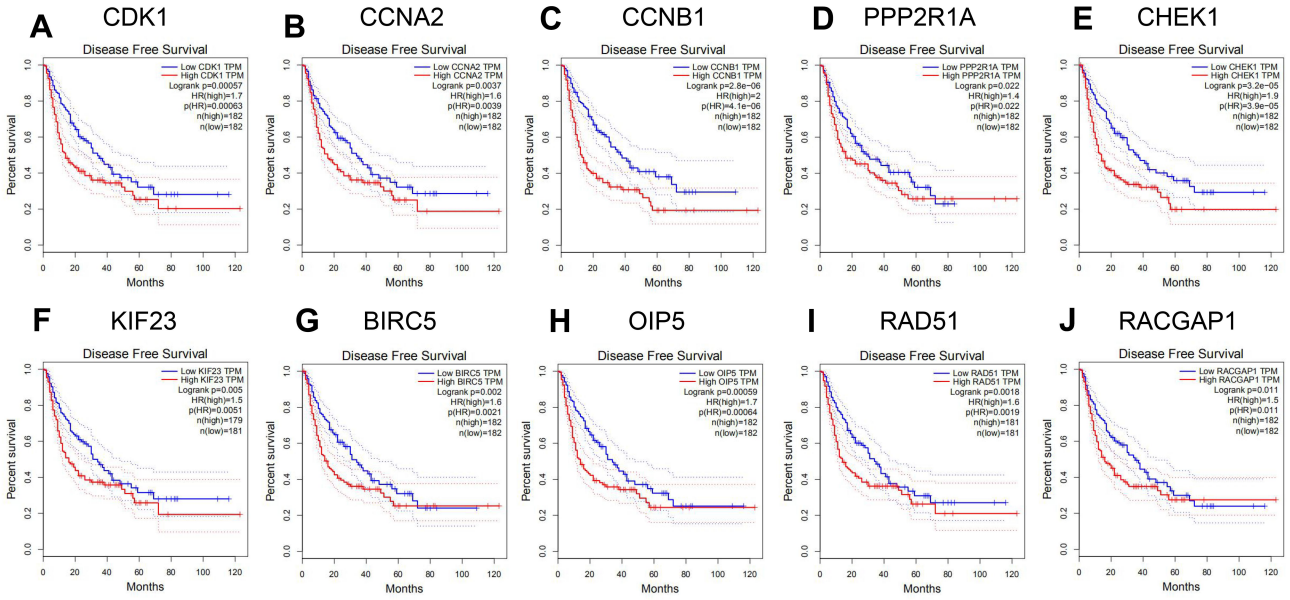

Figure 8 Association of expressions among 10 upregulated Hub genes and the overall survival of patients with HCC. (**A**) *CDK1*. (**B**) *CCNA2*. (**C**) *CCNB1*. (**D**) *PPP2R1A*. (**E**) *CHEK1*. (**F**) *KIF23*. (**G**) *BIRC5*. (**H**) *OIP5*. (**I**) *RAD51*. (**J**) *RACGAP1*.

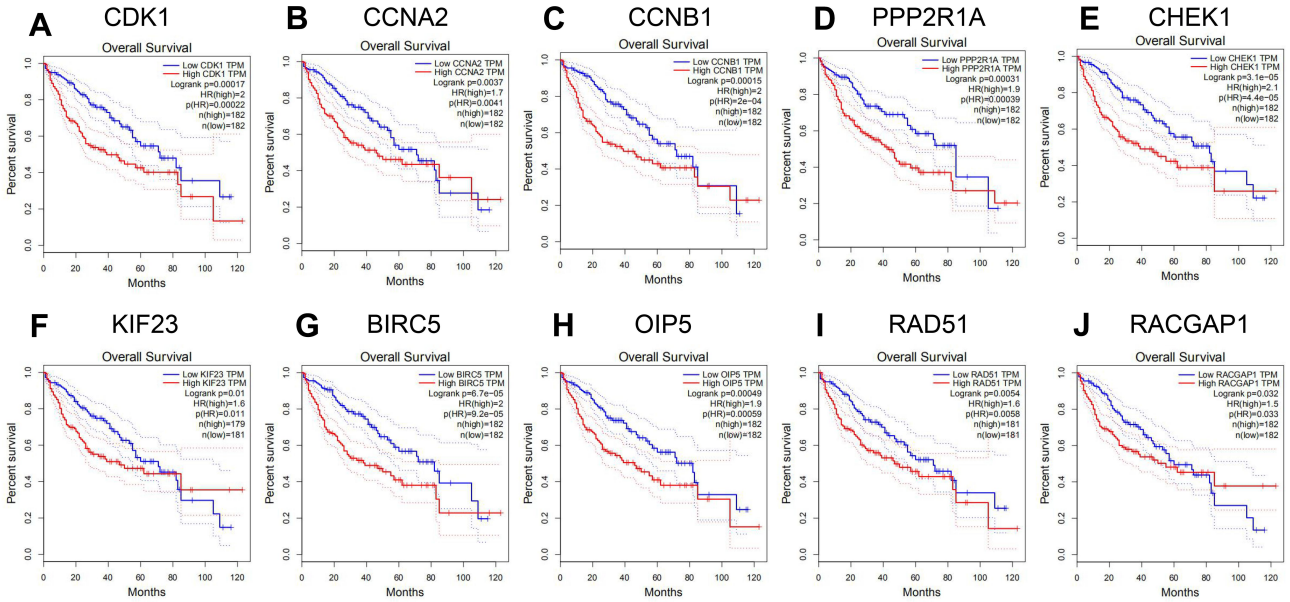

Figure 9 Association of expressions among 10 upregulated Hub genes and the disease-free survival of patients with HCC. (**A**) *CDK1*. (**B**) *CCNA2*. (**C**) *CCNB1*. (**D**) *PPP2R1A*. (**E**) *CHEK1*. (**F**) *KIF23*. (**G**) *BIRC5*. (**H**) *OIP5*. (**I**) *RAD51*. (**J**) *RACGAP1*.

Based on qRT-PCR results, *circ_0130911* was relatively and highly expressed in HCC tissues and was closely related to the poor prognosis of HCC. Importantly, the biological function of *circ_0130911* in tumors is largely understudied. Therefore, our research is of great significance for further understanding the mechanism of circRNA in HCC.

By predicting the miRNAs bound to circRNA, 7 downregulated DEmiRNAs were identified, including: *hsa-miR-590-3p, hsa-miR-15a-5p, hsa-miR-23c, hsa-miR-29a-3p, hsa- miR-424-5p, hsa-miR-497-5p*, and *hsa-miR-592*. In addition, several lines of evidence have shown that these 7 DEmiRNAs are related to the development and metastasis of cancer and that the expression of *miR-590-3p* is

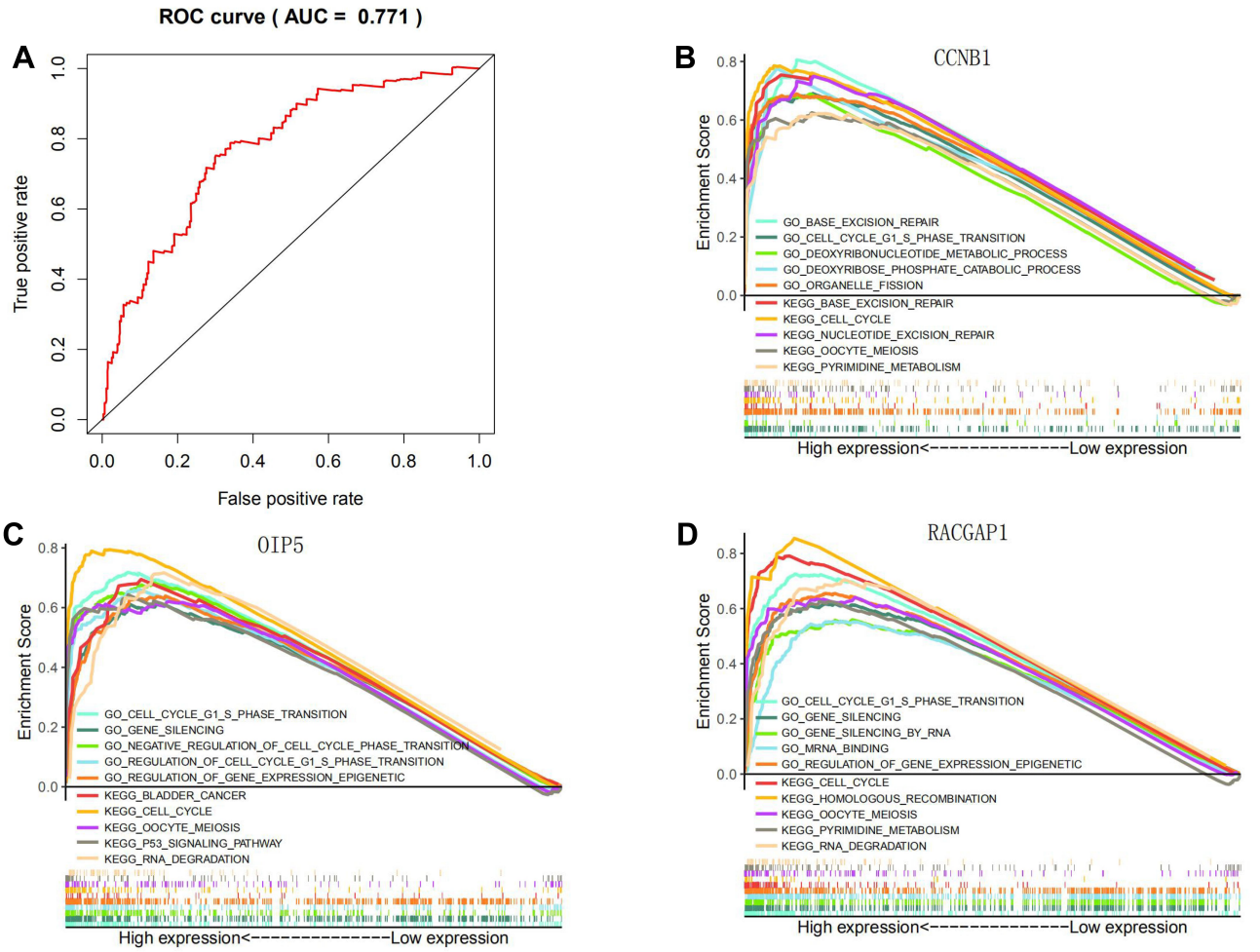

Figure 10 (**A**) ROC analysis of 3 Hub genes in TCGA-LIHC database. The Enrichment plots from GSEA analysis of Hub genes in the TCGA-LIHC dataset. (**B**) *CCNB1*. (**C**) *OIP5*. (**D**) *RACGAP1*.

downregulated in triple-negative breast cancer. Besides, *miR-590-3p* directly inhibits the invasion and migration ability of triple-negative breast cancer by sponge slugs.[27] A research by He et al found that *circZNF609* activates the Hedgehog pathway through the sponge *miR-15a-5p/15b-5p* and promotes the expression of *GLI2* protein, thereby enhancing the proliferation and metastatic capability of HCC cells.[28] In bladder cancer, *lncRNA CASC9* acted as a *miR-497-5p* sponge to up-regulate the expression of *FZD6,* activate the*Wnt/β-catenin*signaling pathway, as well as promote the growth and metastasis of bladder cancer.[29] Additional studies have revealed that *circFGFR3* participates in the *miR-29a-3p/E2F1* axis by inducing the EMT process of ovarian cancer cells and promote the initiation of ovarian cancer.[30]

In total, we found 113 DEmRNAs in the established ceRNA network. KEGG pathway enrichment analysis suggests that the up-regulated genes were primarily implicated in the following pathways: *PI3K-Akt* signaling and cell cycle and *p53* signaling pathways. Reports indicate that changes in cell cycle rhythm are closely related to the occurrence and development of HCC.[31] The imbalance of the *p53* signaling pathway promotes the proliferation of HCC cells, thereby contributing to the occurrence of HCC.[32]

To further determine the key genes in the ceRNA network, we considered the top 10 genes in the PPI network as Hub genes including, *CDK1, CCNA2, CCNB1, PPP2R1A, CHEK1, KIF23, BIRC5, OIP5, RAD51,* and *RACGAP1*. Then, the circRNA-miRNA-Hub mRNA network was constructed. The Oncomine and HPA databases were used to verify the true expression of Hub genes in HCC at the transcription and translation levels. The results indicated that the mRNA levels and immunohistochemical staining of these genes in HCC tissues were significantly higher compared to those in normal tissues, suggesting that these Hub genes regulate the development of HCC. To identify the

potential prognostic markers in HCC, the GEPIA database was used to analyze the OS and DFS of Hub genes in the prognosis of HCC. We found that the high expression of Hub genes was significantly related to the poor prognosis of HCC patients. Moreover, as demonstrated by the results of the COX regression model, the 3 genes (*CCNB1, OIP5,* and *RACGAP1*) were the most significant genes influencing the prognosis of HCC. The ROC curve of these 3 Hub genes was used to analyze the relationship with the prognosis of HCC, and the AUC value was calculated as 0.771. Therefore, these 3 Hub genes were predicted as the potential biomarkers for the prognosis of HCC.

We used the TCGA database to perform GESA enrichment analysis and discovered that when these 10 Hub genes were overexpressed in HCC, they were primarily enriched in Cell cycle, Regulation of gene expression epigenetic, Gene silencing, Cell cycle g1 s phase transition, etc. Reports have confirmed that the dysregulation of cell cycle pathways regulates tumor development and prognosis. For example, *TRIM59* regulates the cell cycle by degrading protein phosphatase 1B, thereby promoting the development of HCC.[33] Based on various sources of evidence, the high expression of *p38 MAPKγ* acts as a CDK-like kinase, which can act synergistically with CDK in regulating cell cycle and promotes the occurrence of liver tumors.[34] Also, it has been widely reported that the Hub gene discovered in this work can affect tumor development through the cell cycle pathway. Notably, *CCNB1* was found to be highly expressed in HCC. *LINC00346* regulates the expression of *CCNB1* through sponge *miR-199a-3p*, which influences the *p53* signaling pathway and ultimately regulates the cell cycle process of HCC cells.[35] He et al found that *OIP5* was up-regulated in patients with glioblastoma, and its expression was positively correlated with the poor prognosis of patients. Further studies highlighted that *OIP5* promotes cell cycle progression, and knocking down *OIP5* will trigger G2/M cell cycle arrest.[36] *RACGAP1* is highly expressed in HCC, gastric cancer, and other cancers.[37,38] For instance, knocking down *RACGAP1* in basal-like breast cancer inhibits the growth of cancer cells through the failure of cytokinesis.[39]

Here, we propose a novel circRNA–miRNA–mRNA network related to the poor prognosis of HCC. This network might promote the progress of HCC through cell cycle and other pathways, hence it is expected to become a novel biomarker for predicting the poor prognosis of HCC. Nevertheless, our findings primarily relied on qRT-PCR and bioinformatics predictions. Thus, additional experiments are necessary to validate our hypothesis.

Conclusion

In conclusion, this work used qRT-PCR to validate that the expression level of *circ_0130911* in HCC tissues is significantly different from that in adjacent tissues. As a consequence, we effectively established a complete circRNA-miRNA- Hub genes network by the analysis of bioinformatics research strategies of multiple databases. Moreover, it was found that the *CCNB1, OIP5,* and *RACGAP1* in the network might influence the poor prognosis of HCC patients by regulating the cell cycle and other pathways. Eventually, we uncovered that *circ_0130911* acts as a ceRNA in regulating the cell cycle geared towards promoting the progress of HCC and providing novel biomarkers for the prognosis of HCC. Nevertheless, the specific mechanism by which this ceRNA network regulates the development of HCC merits further research.

Abbreviations

BP, biological process; CC, cellular component; CI, confidential interval; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, molecular function; HCC, hepatocellular carcinoma; PPI, protein–protein interaction network; qRT-PCR, quantitative real time PCR; GSEA, Gene Set Enrichment Analysis.

Ethics and Consent Statement

This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Subjects have given their written informed consent and the study protocol regarding human specimen were approved by the Ethics Committee of the Affiliated Tumor Hospital of Guangxi Medical University.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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