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Circular RNA ACVR2A promotes the progression of hepatocellular carcinoma through mir-511-5p targeting PI3K-Akt signaling pathway

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Abstract

Circular RNA (circRNA) is thought to mediate the occurrence and development of human cancer and usually acts as a tiny RNA (miRNA) sponge to regulate downstream gene expression. However, it is not clear whether and how circACVR2A (hsa_circ_0001073) is involved in the progression of HCC. The purpose of this study is to clarify the potential role and molecular mechanism of circACVR2A in regulating the progression of hepatocellular carcinoma cells (HCC). The abundance of related proteins in circACVR2A, microRNA (miR511-5p) and PI3K-Akt signaling pathway was determined by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) or Western blotting. Cell viability, invasion and apoptosis were analyzed by CCK-8, Transwell analysis and Tunel staining, respectively. The interaction between circACVR2A and microRNA was evaluated by double luciferase reporter gene assay. The results showed that circACVR2A was highly expressed in hepatocellular carcinoma cell lines. Our in vivo and in vitro data showed that circACVR2A promoted the proliferation, migration and invasion of HCC. In terms of mechanism, we found that circACVR2A can directly interact with miR511-5p and act as a miRNA sponge to regulate the expression of related proteins in PI3K-Akt signaling pathway.

In HCC, circACVR2A can mediate miR-511-5p/mRNA network to activate PI3K signal pathway. This shows that the molecular regulatory network with circACVR2A as the core is a new potential target for diagnosis and treatment of hepatocellular carcinoma.

Keywords circACVR2A, miRNA, mRNA, HCC, PI3K

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Introduction

Hepatocellular carcinoma is extremely deadly, with more than 700,000 people dying from it each year. There are about 400,000 new hepatocellular carcinoma patients in China every year, accounting for about 50% of the increase in hepatocellular carcinoma patients in the world [1, 2]. Because of the high recurrence rate and poor prognosis of patients with hepatocellular carcinoma, it is particularly necessary to find new and more effective biomarkers as targets for detection of hepatocellular carcinoma, or as targets for prevention and treatment.

circRNA is a special type of endogenous RNA molecule, which exists widely in a variety of biological cells. Unlike linear RNA, cirRNA is a closed loop without 5 'and 3' ends or polyadenosine tails [3]. It has been found that circRNA can be derived from exons or introns, or composed of both exons and introns [4]. Although the existence of circRNA has been discovered in the 1970s, it has not received enough attention for a long time [5, 6]. Until recent years, with the development of RNA deep sequencing technology and bioinformatics, a large number of endogenous circRNA have been found and expressed steadily in mammalian cells. Although many circRNA contain potential protein coding sequences, circRNA with protein coding function has not been found [7, 8], so scholars begin to think about and study the related functions of circRNA. More and more studies have confirmed that circRNA plays an important role in many diseases such as nervous system disease, cardiovascular disease, diabetes, malignant tumor and so on [9]. circRNA can play a regulatory role at the transcriptional and post-transcriptional level, and can also participate in the regulation of gene transcription or bind to RNA-binding proteins. [10, 11]. In addition, the sponge effect of circRNA on miRNA is also one of the current research hotspots [12, 13].

Studies have shown that there is a stable difference in the expression of circRNA between a variety of malignant tumors and normal tissues, so some scholars think that some circRNA can be used as ideal biomarkers of malignant tumors. circRNA is also involved in the physiological processes of tumor cell proliferation, apoptosis, invasion and metastasis [14–16]. For example, there are significant differences in the expression of hsa_circ_002059 in gastric cancer, normal tissues and blood samples, which also shows its potential diagnostic value as a biomarker [17]. The expression of circ-ITCH in esophageal squamous cell carcinoma is significantly lower than that in adjacent tissues. Overexpression of circ-ITCH can significantly inhibit the cycle progression of tumor cells and thus inhibit tumor growth [18]. However, there are few studies on whether circRNA can be used as a diagnosis and treatment target for liver cancer.

There is a molecular regulatory relationship among circRNA, miRNA and mRNA. circRNA has a sponge effect on miRNA. Some scholars have found nearly 70 miR-7 binding sites on circRNA-CDR1as [12, 13]. miRNAs are non-coding single-stranded RNA (ribonucleic acid) molecules with a length of about 22 nucleotides encoded by endogenous genes, with a size of about 21–23 bases. MiRNAs are mostly formed by the processing of Dicer enzymes (a kind of ribonuclease III) of single-stranded RNA precursors with a hairpin structure of about 70–90 bases. miRNAs have 5 'phosphate and 3' hydroxyl groups and are located at the 3'or 5 'end of the RNA precursor. It has the function of destroying the transcripts of specific target genes or inducing translation suppression [19]. mRNA is a kind of RNA (ribonucleic acid), which is transcribed by genes, carries biological genetic information and can guide protein biosynthesis by reading triad code. It consists of the upstream 5 'non-coding region (5'-UTR), the coding region and the downstream 3' non-coding region (3'-UTR), and the content accounts for about 3%-5% of the total cellular RNA. Biological genes are transcribed to form the precursor mRNA, and the precursor mRNA becomes mature mRNA after splicing modification, which carries biological genetic information into the ribosome to guide the synthesis of protein peptide chain, thus completing the expression process from gene to protein [20]. The process of translating mRNA into peptide chain is regulated by regulatory factors, and most miRNA can regulate the translation of specific corresponding mRNA into proteins. Mature miRNA binds to its complementary mRNA site in the way of base pairing, which affects the translation process of mRNA and regulates gene expression. At present, some studies have shown that the disordered expression of miRNA and mRNA caused by the imbalance of circRNA can drive the tumorigenesis and metastasis of various cancers [21]. Therefore, the molecular regulatory network with circRNA as the core may play an important role in the occurrence of hepatocellular carcinoma.

circACVR2A comes from the gene ACVR2A and is located in chr2:148653869–148,657,467 (ID: hsa_circ_0001073 in circbase database). The total length is 473 bp. In 2013, several studies detected the presence of circACVR2A in different tissue and cell samples [22, 23]. Zheng et al. detected the expression of circACVR2A in 7 cases of cancer tissues, including bladder cancer, breast cancer, colon cancer, hepatocellular carcinoma, gastric cancer, renal clear cell carcinoma and prostate cancer [24]. However, what kind of function circACVR2A has in hepatocellular carcinoma, whether it is significantly different from normal tissue, early and later stage of hepatocellular carcinoma, and its relationship with the prognosis of hepatocellular carcinoma is still under study. For this reason, we propose that "the molecular

regulatory network with circACVR2A as the core may be involved in the occurrence, development and prognosis of hepatocellular carcinoma", which provides a basis for the study of the molecular mechanism of the occurrence and development of hepatocellular carcinoma.

Phosphatidyl-inositol3-kinase (PI3K) -serine-threoninekinase (AKT) signaling pathway, as one of the important pathways of cancer signaling, can not only increase the "Warburg" effect in tumor cells, It can also promote the proliferation and spread of cancer cells [25–27]. Previous studies have shown that the PI3K signaling pathway is highly expressed in liver cancer tissues, and it has been proved that it is related to the occurrence, progression and metastasis of liver cancer [28, 29]. As one of the important signal transduction pathways in liver cancer cells, PI3K signaling pathway plays a key role in inhibiting apoptosis and promoting proliferation in cells by regulating the activation state of various downstream effector molecules [30–32]. However, whether circACVR2A-mediated miRNA/mRNA regulatory network has a direct regulatory effect on the PI3K signaling pathway needs further study.

Method

Cell culture and treatments

Human hepatoma cell lines Huh-7, HepG2 and human normal liver cell line LO2 were purchased from Hangzhou Jenker Biological Technology Co.,Ltd; Human hepatoma cell lines Hep3B and human normal hepatocyte line MIHA are provided by the Laboratory of Molecular Pharmacology, School of Pharmacology, Southwest Medical University. Huh-7, HepG2, Hep3B and HEK-293 cells were cultured in DMEM (Gibco, Shanghai, China), MIHA cells were cultured in RPMI-1640(Gibco), supplemented with 1% penicillin/streptomycin (Gibco) and 10% FBS(Gibco). Cells were grown in a humidified atmosphere of 5% CO2 at 37 °C.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

RNA was isolated with TRIzol (Life technologies, USA). To purity circRNA and explore the circular structure, RNA was treated with 3 U/μg RNase R (Geneseed) for 30 min. The RNA in nuclear and cytoplasm was isolated using Cytoplasmic & Nuclear RNA Purification Kit (Biocreative Technology, Beijing, CA). RNA was reversely transcribed to comple-mentary DNA through specific reverse transcription kit (Tiangen, Beijing, CA). Complementary DNA together with SYBR Green (Vazyme) and primers (Tsingke, Beijing, CA) was used for qRT-PCR. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or U6 served as internal reference. The relative RNA abundance was calculated according to $2^{-\Delta\Delta Ct}$ method. The sequence information of primers was listed in Table 1.

Oligonuleotide transfection

Overexpression plasmids, interference RNA (shRNA), miRNA mimics, miRNA inhibitors and negative control oligonucleotides were purchased from Hanbio Biotechnology (Shanghai, China). Lipofectamine RNAiMax (Life Technologies) was used for cell transfection. pCD3.1-CMV-ciR vector (Hanbio Biotechnology, Shanghai, CA) was used to generate circACVR2A overexpression vector, and pCD3.1-CMV-ciR vector was used as negative control. pCDNA3.1-U6-CMV-ZsGreen vector (Hanbio Biotechnology) was used to generate shRNA interference vector, and pCDNA3.1-U6-CMV-ZsGree vector was used as negative control. 2 μ g vector oligodeoxynucleotides were transfected into cells using Lipofectamine 3000 (Thermo Fisher, Wilmington, Deutschland).

Cell proliferation, wound healing, migration, invasion assays and apoptosis

For the detection of cell survival, about 2×10^4Huh-7 and Hep3B cells were inoculated into a 96-well plate and

Table 1 The sequence information of primers

Gene	Sequence	
	Forward (5′-3′)	Reverse (5′-3′)
circACVR2A	ATTTTTGTTGCTGTGAGGG	GTGGCACCAAGGAATAGAG
COL4A4	CCTTCCCGTATTTAGCACGC	GTCCTCAGAACACACCCATGA
CREB1	GCCACATTAGCCCAGGTATCTAT	TGTTTGGACTTGTGGAGACTGAA
ERBB4	GGACAACACTCTTCAGCACAATC	GCCATTCTCAAACTCCCGAAATT
FN1	GAGGGCAGAAGAGACAACATGAA	CCCTTCATTGGTTGTGCAGATTT
KRAS	AGGGACTAGGGCAGTTTGGA	AATGTCTTGGCACACCACCA
NTRK2	TCTGCTCACTTCATGGGCTG	AGACCGAGAGATGTTCCCGA
PIK3R3	TGACAGAATCCCGCTCTTCC	CCTCCCTTCCGCAAAGTCAA
PPP2R5E	GAACGCTGAGGTTCGGGT	GAGGAGTAGTTGGTGCTGAGG
GAPDH	CAAGGCTGAGAACGGGAAG	TGAAGACGCCAGTGGACTC
miR511-5p	CGCGGTGTCTTTTGCTCTG	
U6	GCTTCGGCAGCACATATACT	

maintained for 24–96 h. Subsequently, 10 μ L CCK-8 (Beyotime, Shanghai, CA) is added to each hole. After 2 h of culture, the optical density level at 450 nm was detected by enzyme labeling instrument (Bio-Rad, USA). The cell viability was analyzed by normalizing to the control group.

In the colony formation experiment, the transfected cells were inoculated in 6-well plates with the density of 1×10^3 cells per well. After about 10 days, the clones were washed with PBS, fixed with 4% paraformaldehyde fixation solution (Beyotime, Shanghai, CA) and stained with 0.1% crystal violet. Then the results were imaged and quantified.

For the wound healing experiment, the wound was made in the middle of the six-hole plate with the suction head of 200 μ L liquid transfer gun (time 0 h), and then the cells were cultured in serum-free medium immediately. After 24 h and 48 h of culture, the cell migration photos were taken, the cell migration distance was measured, and normalized to the relative mobility of 0 h control for comparison.

For Transwell experiment, when the 24-well chamber (Corning, Corning, USA) was pre-coated with Matrigel (Solarbio, Beijing, CA), about 5×10^4 Huh-7 or Hep3B cells were placed in serum-free DMEM medium. Add 500 μ L culture medium and 10% serum in the low room. After 24 h of culture, the invasive cells were washed with PBS, fixed with 4% paraformaldehyde fixation solution (Beyotime) and stained with 0.1% crystal violet, and counted under microscope.

For the detection of apoptosis, using terminal deoxynucleotidyl transferase-mediated Nick end labeling (TUNEL), TdT enzyme and DAPI enzyme (Elabscience, Wuhan, CA) were used to stain the cell slides fixed with 4% paraformaldehyde fixation solution (Beyotime), and detected by fluorescence microscope.

Western blot analysis

Proteins were extracted with RIPA lysis buffer supplemented with 1% protease inhibitor and quantified by a BCA kit (Beyotime). Equal amounts of proteins (40 μ g) were separated by 10%or12% SDS-PAGE and transferred to PVDF membranes (Merckmillipore, DE). After blocking with 5% skim milk powder at room temperature for 1 h, the membrane were incubated with primmary antibodies specific to PI3K (1:1000, CST, USA), p-PI3K (1:1000, CST, USA), Akt (1:1000, CST, USA), p-Akt(1:1000, CST, USA), Foxo1 (1:1000, CST, USA), p-Foxo1 (1:1000, CST, USA), Bcl2 (1:800, Beyotime, CA), Bax (1:800, Beyotime, CA), GAPDH (1:1000, Abcam, UK) and β-Actin (1:1000, Beyotime, CA) at 4 ℃ overnight. Then the goat anti-mouse or goat anti-rabbit secondary antibodies coupled with horseradish peroxidase was incubated and developed with chemiluminescence HRP substrate (BIO-RAD).

Luciferase reporter assay

HEK293T cells (1×10^5) were inoculated in a 12-well plate and co-transfected with corresponding pSI-Check2 vectors and MicroRNA mimics. After incubation for 48 h, according to the instructions of the manufacturer, the activities of fireflies and Renilla luciferase were detected by dual luciferase reporter gene detection system (Beyotime). Independent experiment in triplicate.

Animal experiments

BALB/c nude mice (5–6 weeks old) were purchased from Beijing HFK Bio-Technology. All animal care and experimental procedures are approved by the Institutional Animal Care and use Committee of Southwest Medical University and carried out in accordance with established guidelines. For tumor growth study, each group included 5 mice. Hep3B cells stably expressing or silencing circACVR2A (2×10^6 cells per mouse) were subcutaneously injected into the right back. After a week, the size of the tumor was measured every three days. After 21 days, the mice were killed and the weight of the tumor was examined. The tumor samples were fixed and embedded in paraffin wax, and then the samples were cut into 5 μ m thick paraffin sections for HE staining.

Statistical analysis

Three independent experiments were carried out. GraphPad Prism8.0 (GraphPad Prism, Inc., La Jolla, California, USA) was used for statistical analysis. The difference between the two groups was evaluated by Student's t test (two-tailed) or analysis of variance (ANOVA). The data are shown as mean ± SD. The experimental results were repeated more than three times, $P<0.05$ indicates that the difference is statistically significant.

Result

circACVR2A is highly expressed in HCC and has sponge effect on miR-511-5p

RT-qPCR assay detected the expression of circACVR2A in two human normal liver cell lines (LO2 and MIHA) and three human hepatocellular carcinoma cell lines (HepG2, Huh-7 and Hep). The results showed that compared with normal hepatocytes, the expression of circACVR2A was up-regulated in hepatocellular carcinoma cell lines (Fig. 1A). In order to rule out the possibility that extreme expression levels in hepatocellular carcinoma cells could affect the efficiency of overexpression or knockdown of circACVR2A, two hepatocellular carcinoma cell lines with intermediate circACVR2A expression levels, Huh-7 and Hep3B, were selected. By means of biological information analysis, the expression difference

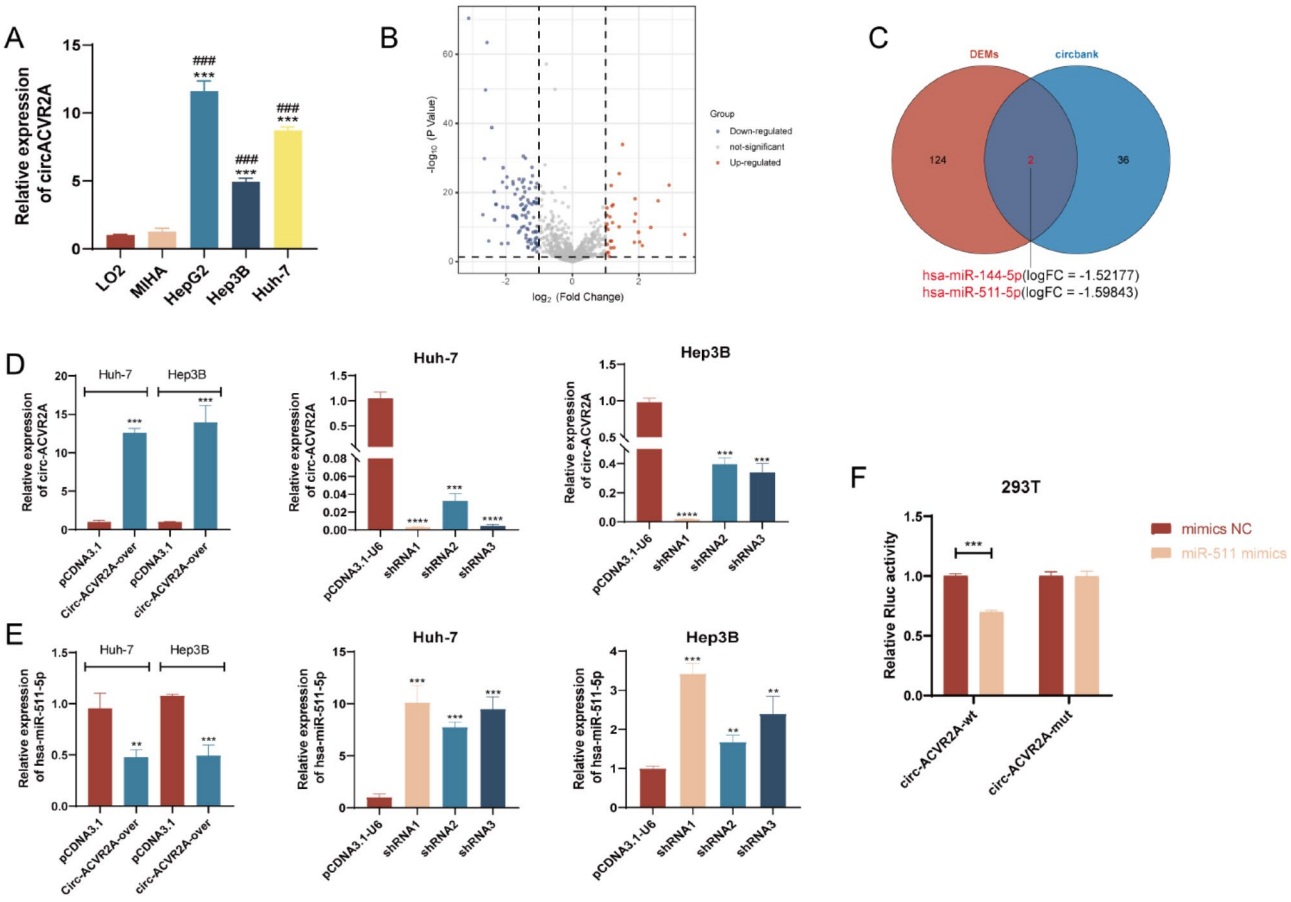

Fig. 1 circACVR2A was highly expressed in hepatoma cells and was used as a sponge for miR-511-5p (**A**) RT-qPCR to detect the expression of circACVR2A in normal liver cells and liver cancer cells. (**B**) Volcano map of miRNAs differential expression. (**C**) Intersection the differentiated miRNAs with the circACVR2A-miRNAs predicted in the circbank database. (**D**) RT-qPCR assay to verify the efficiency of overexpression and knockdown of circACVR2A in hepatocellular carcinoma cells. (**E**) RT-qPCR assay verified the expression of miR-511-5p after overexpression and knockdown of circACVR2A in hepatoma cells. (**F**) HEK293T cotransfected with miRNA mimics, Psi-Check2-wild-type circACVR2A (circACVR2A-WT) or Psi-Check2-mutant circACVR2A (circACVR2A-MUT) plasmid Luciferase reporter gene was detected in cells. All experiments were repeated three times, expressed as mean ± SD, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. In Fig. 1A * LO2 serves as the control group; # MIHA serves as the control group compared with pcDNA3.1 and PCDNA3.1-U6 in Fig. 1D and E, compared with mimics NC in Fig. 1F

of miRNA in liver cancer was analyzed using gene expression mapping (GEO) database, and 126 miRNAs with differential expression were obtained (Fig. 1B). Then, we intersect the differential miRNAs with the predicted circACVR2A-miRNAs in the circbank database to get two down-regulated miRNA, miR144-5p and miR511-5p. (Fig. 1C). We selected miR-511-5p, which has a larger difference, for the follow-up experiment.

The circACVR2A overexpressed and knockdown plasmid was constructed, and the circACVR2A overexpressed and knockdown HCC cell lines Huh-7 and Hep3B were constructed using transient and lentiviral infection methods. Next, we verified the efficiency of overexpression and knockdown of circACVR2A in hepatocellular carcinoma cells by RT-qPCR assay, and demonstrated successful overexpression and knockdown of circACVR2A in hepatocellular carcinoma cell lines Huh-7 and Hep3B (Fig. 1D). At the same time, the expression levels of miR511-5p were detected using the same batch of RNA extracted. The results showed that overexpression of circACVR2A in hepatoma cell lines Huh-7 and Hep3B could decrease the expression of miR511-5p. In contrast, knockdown of circACVR2A could up-regulate miR-511-5p expression (Fig. 1E). This suggests that circACVR2A may have a regulatory effect on miR-511-5p. To verify whether circACVR2A has a spongy effect on miR511-5p, we designed a dual luciferase assay to construct a circACVR2A sequence of wild-type (WT) or a sequence with mutant (MUT) miR511-5p binding site. Constructed reporter plasmids or plasmids carrying the luciferase gene were co-transfected into HEK293T cells with miR511-5p or miR-NC mimics. After 48 h, firefly luciferase activity and sea kidney luciferase activity were detected by double luciferase kit. The results showed that the miR511-5p co-transfection group with circACVR2A-WT had lower relative Rluc activity

than the control group, demonstrating that circACVR2A can exert a spongy effect on miR-511-5p (Fig. 1F).

circACVR2A can promote the proliferation of HCC

CCK-8 assay and cell clone formation assay were used to explore whether circACVR2A can affect the proliferation of hepatocellular carcinoma cells. In hepatoma cell lines Huh-7 and Hep3B which overexpressed or knocked down circACVR2A, the cell viability was measured by CCK8 at 0 h, 24 h, 48 h, 72 h and 96 h, respectively. Compared with the control group, it was found that overexpression of circACVR2A could significantly enhance the cell viability of hepatoma cells Huh-7 and Hep3B, while knocking down circACVR2A would lead to a significant decrease in the viability of Huh-7 and Hep3B cells (Fig. 2A, B). In the experiment of cell clone formation, we found that overexpression of circACVR2A increased the number of cell clones, while silence circACVR2A decreased the number of cell clones, which indicated that circACVR2A could promote the tumorigenesis of Huh-7 and Hep3B cells in vitro (Fig. 2C, D).

circACVR2A promotes invasion and migration of HCC

circACVR2A was overexpressed or knocked down in hepatocellular carcinoma cell lines Huh-7 and Hep3B. The effect of circACVR2A expression on the invasion and migration of hepatocellular carcinoma cells was detected by cell scratch test and transwell cell invasion test in vitro. The results showed that in the migration experiment, compared with the control group, the growth rate of overexpressed circACVR2A cells was significantly faster than that of knock-down circACVR2A cells (Fig. 3A, B). It is suggested that circACVR2A can promote the migration of Huh-7 and Hep3B in hepatocellular carcinoma

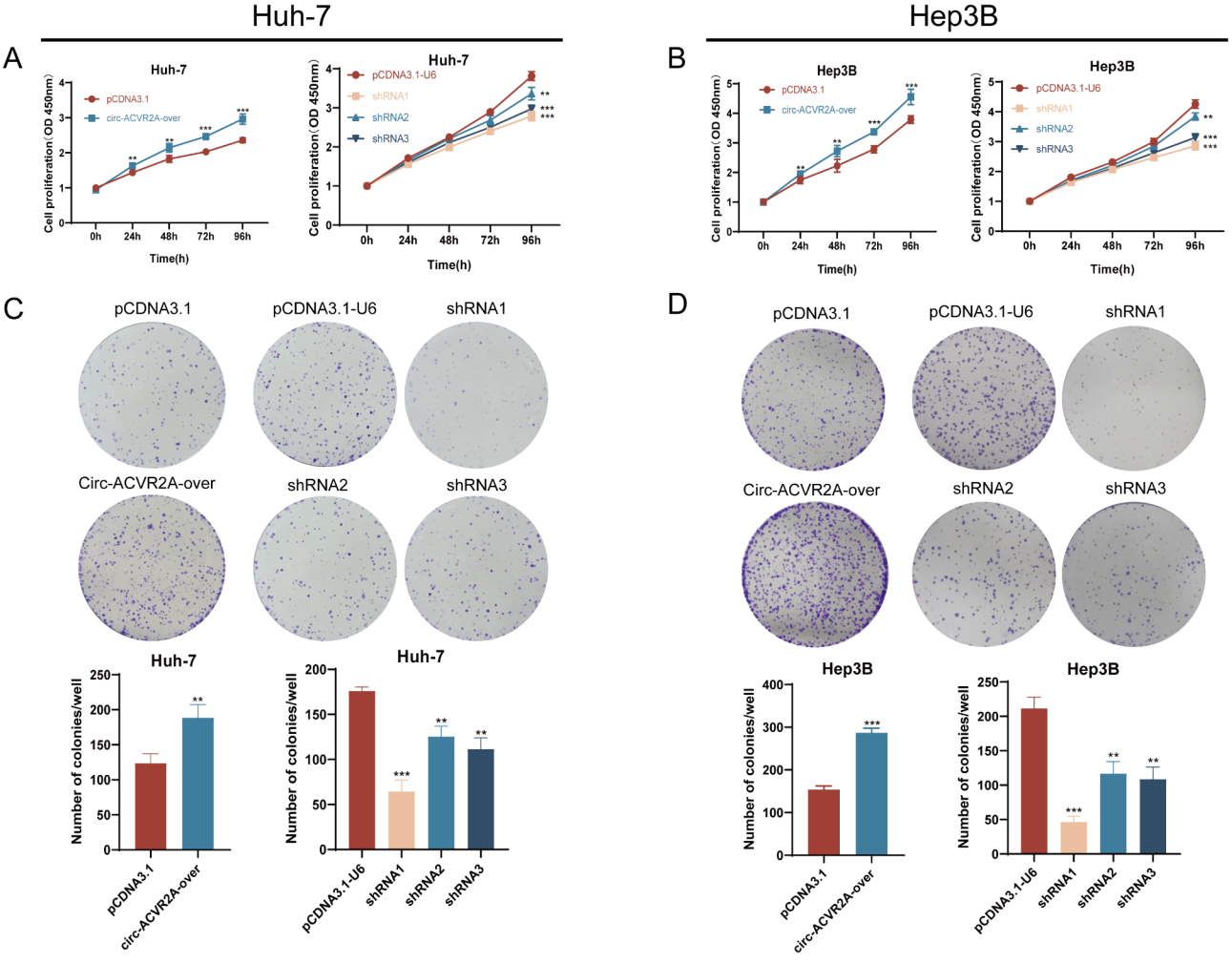

Fig. 2 circACVR2A can promote the proliferation of hepatoma cells in vitro. (**A**) The effect of circACVR2A on the proliferation of hepatoma cell line Huh-7 was detected by CCK8 assay. (**B**) The effect of circACVR2A on the proliferation of hepatoma cell line Hep3B cells was detected by CCK8 assay. (**C**) The effect of circACVR2A on the clonal formation of hepatoma cells Huh 7 cells were detected by cell cloning assay. (**D**) The effect of circACVR2A on the clonal formation of hepatoma cells Hep3B cells was detected by cell cloning assay. All experiments were repeated three times, expressed as mean ± SD, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with pCDNA3.1 or pCDNA3.1-U6 in Fig. 2

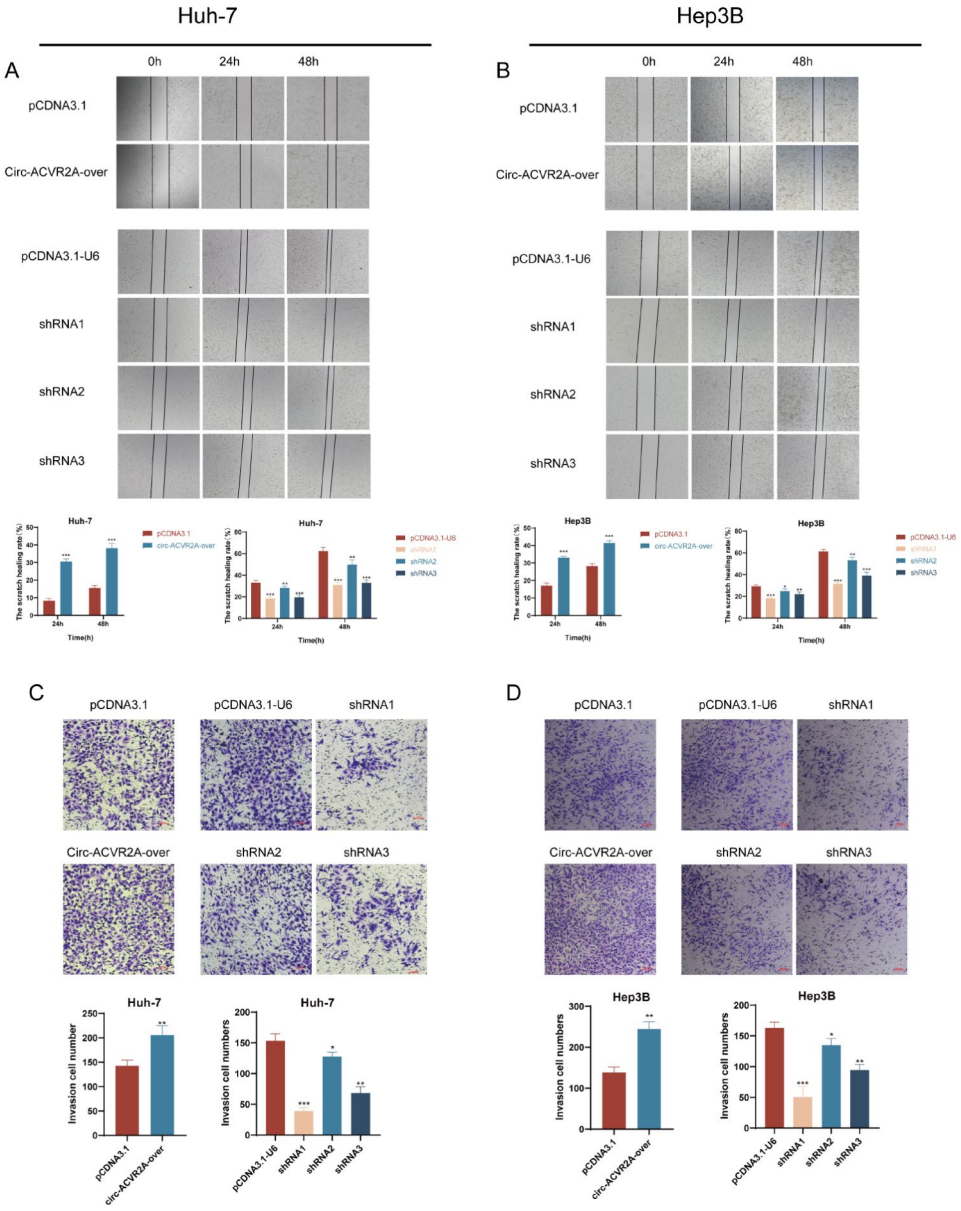

Fig. 3 circACVR2A can promote the migration and invasion of hepatoma cells. **(A**, **C)** Cell migration and invasion test: to detect the migration and invasion ability of hepatoma cell Huh-7 in vitro; **(B**, **D)** Cell migration and invasion test: to detect the migration and invasion ability of hepatoma cell Hep3B in vitro. All the experiments were repeated three times, expressed as mean ± SD, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with pCDNA3.1 or pCDNA3.1-U6

cells; in the invasion experiment, overexpression of circACVR2A can promote more Huh-7 and Hep3 cells to pass through the transwell chamber than the control group, while knocking down circACVR2A will cause fewer cells to penetrate the chamber (Fig. 3C, D). It is suggested that circACVR2A can promote the invade of Huh-7 and Hep3B in hepatocellular carcinoma cells.

circACVR2A can inhibit the apoptosis of hepatocellular carcinoma cells

TUNEL staining test was used to detect whether circACVR2A could affect the apoptosis of hepatocellular carcinoma cells. After Huh-7 and Hep3B were overexpressed or silenced by circACVR2A, the prepared cell slides were stained with TUNEL staining kit, TdT enzyme staining solution and DAPI enzyme staining solution. Then, it was observed and photographed by fluorescence microscope. According to the results of TUNEL staining (Fig. 4A, B). The positive TUNEL staining was red and varied in shape and size, and distributed randomly among normal cells in a dispersed form. There were almost no positive cells in the control group, and almost no red positive cells indicating apoptosis in the overexpressed circACVR2A group, and there

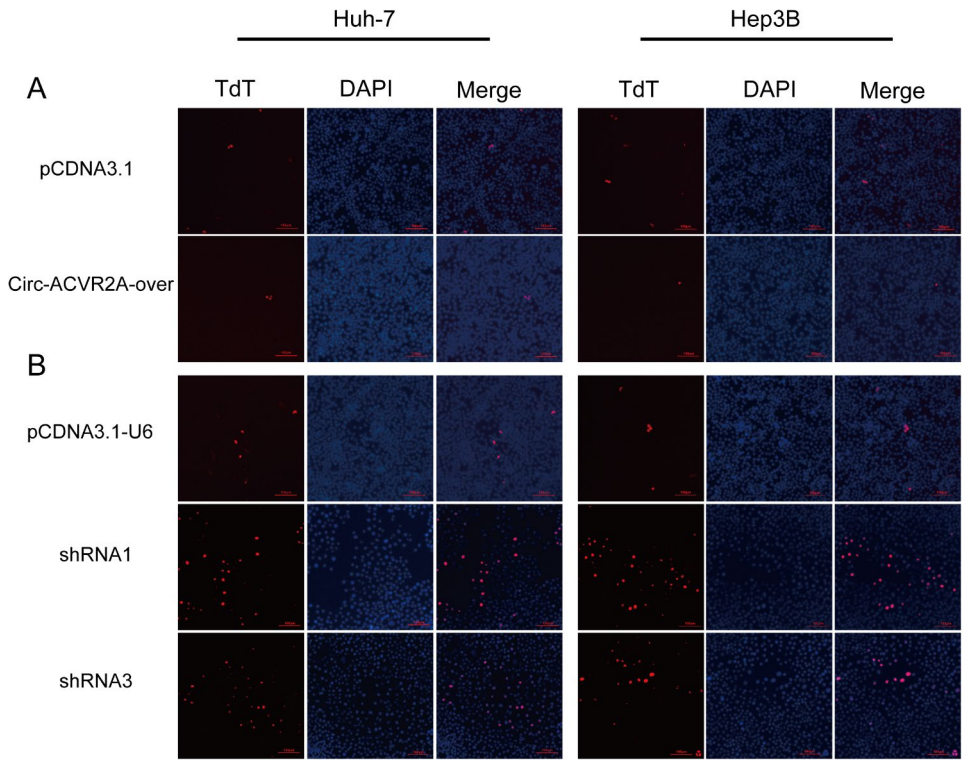

Fig. 4 circACVR2A can inhibit the apoptosis of hepatoma cells in vitro. (**A**) In Huh-7 and Hep3B cells overexpressing circACVR2A, the climbing slices fixed with 4% paraformaldehyde were stained with TdT enzyme and DAPI enzyme respectively. (**B**) In the silent circACVR2A hepatoma cells Huh-7 and Hep3B, the climbing slices fixed with 4% paraformaldehyde were stained with TdT enzyme and DAPI enzyme, respectively

was no significant difference between the two groups of cells. However, in the knock-down circACVR2A group (shRNA1 and shRNA3), the positive cells increased significantly and were higher than those in the respective control groups. This suggests that inhibition of circACVR2A can promote the apoptosis of Huh-7 and Hep3B cells.

miR511-5p can modulate the abundance of mRNAs on the PI3K signaling pathway

In order to construct the regulatory network of circACVR2A-miRNA-mRNA, we use three databases (TargetScan, mirDIP, miRWalk) to predict the intersection of has-miR511-5p/mRNAs to get 160 mRNAs (Fig. 5A). After KEGG enrichment analysis, it was found that these mRNAs could affect multiple signal pathways respectively (Fig. 5B). The classical signal pathway PI3K was selected and the enriched mRNA on its pathway was analyzed. Nine mRNAs enriched in PI3K pathway were obtained, including COL4A4, CREB1, ERBB4, FN1, KRAS, FGF9, NTRK2, PIK3R3 and PPP2R5E. Among them, except that there was no difference in the expression of FGF9, the other 8 mRNAs showed significant differences in the comparison between normal tissues and hepatocellular carcinoma tissues, and these 8 mRNAs showed a trend of high expression in tumors (Fig. 5C). In order to verify that circACVR2A can regulate these mRNAs through miR511-5p, we overexpressed and silenced circACVR2A in hepatocellular carcinoma cell lines Huh-7 and Hep3B, and detected the abundance changes of COL4A4, CREB1, ERBB4 and other 8 mRNAs in hepatocellular carcinoma cells by RT-qPCR assay. The results showed that circACVR2A could regulate these mRNAs through miR511-5p (Fig. 5D-K).

circACVR2A can activate the PI3K signaling pathway

In order to further verify that circACVR2A can play a carcinogenic role through PI3K signal pathway, we used Western Blot assay to detect the expression of proteins related to PI3K signal pathway after overexpression and silence of circACVR2A in Huh-7 and Hep3B cell lines. The results showed that overexpression of circACVR2 significantly enhanced the expression of PI3K, p-PI3K, p-AKT and p-FoxO1, and significantly inhibited the expression of FoxO1, while silencing circACVR2A showed the opposite result (Fig. 6A). In addition, we also detected the apoptosis-related protein Bax/Bcl2 in hepatocellular carcinoma cell lines Huh-7 and Hep3B. It was found that overexpression of circACVR2 in hepatoma cell lines Huh-7 and Hep3B could significantly inhibit the expression of Bax and enhance the expression of Bcl2, while the opposite result was found after silencing

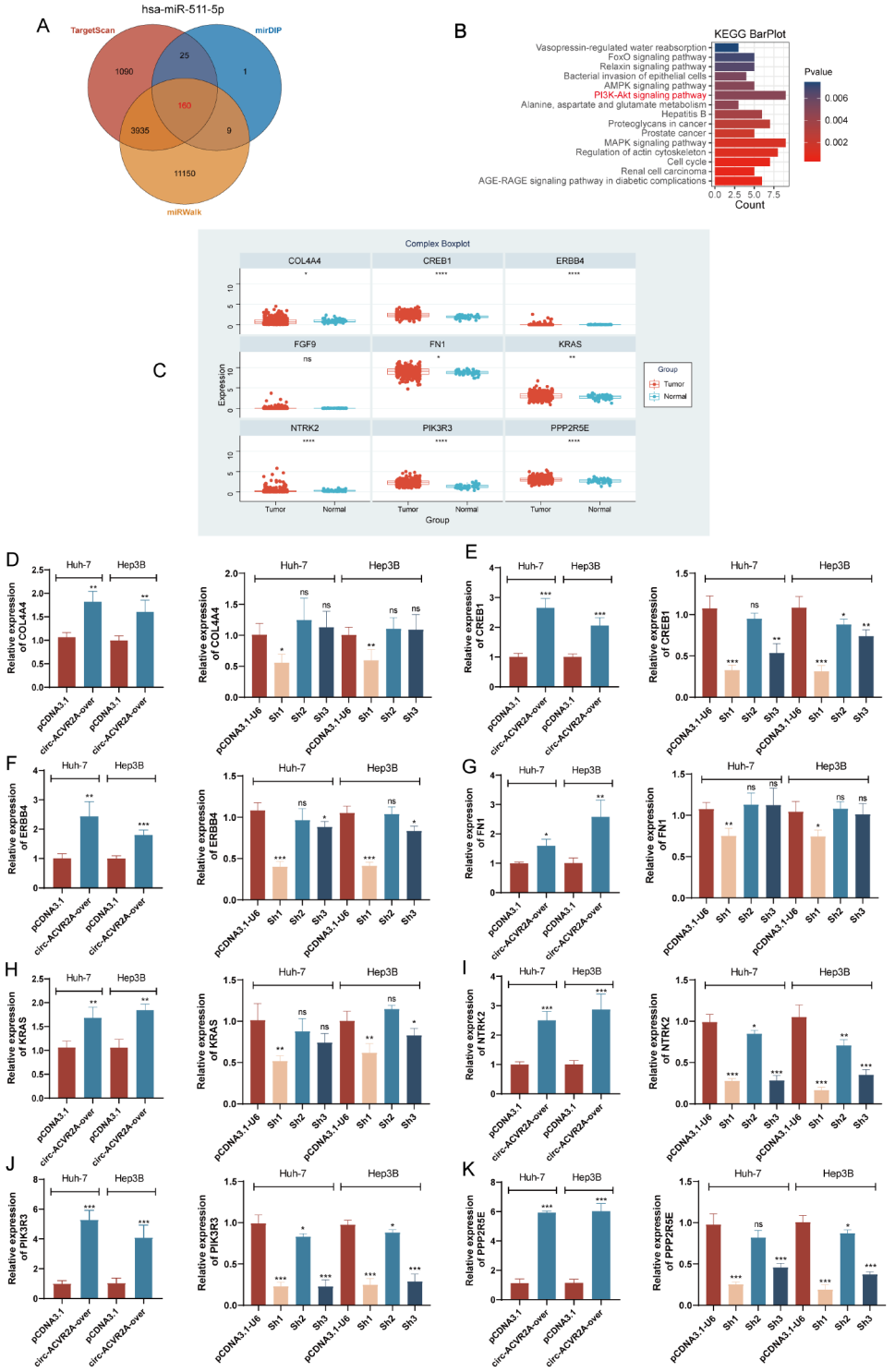

Fig. 5 circACVR2A can regulate the abundance of mRNAs related to PI3K signal pathway through miR-511-5p. **(A)** Predict hsa-miR511-5p/mRNAs intersection through three databases (TargetScan, mirDIP, miRWalk); **(B)** KEGG enrichment analysis of overlapping mRNAs; **(C)** Difference analysis of 9 mRNAs enriched to PI3K signal pathway; **(D-K)** RT-qPCR verification of 8 mRNAs with differences. All the experiments were repeated three times, expressed as mean ± SD, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and compared with pCDNA3.1 or pCDNA3.1-U6

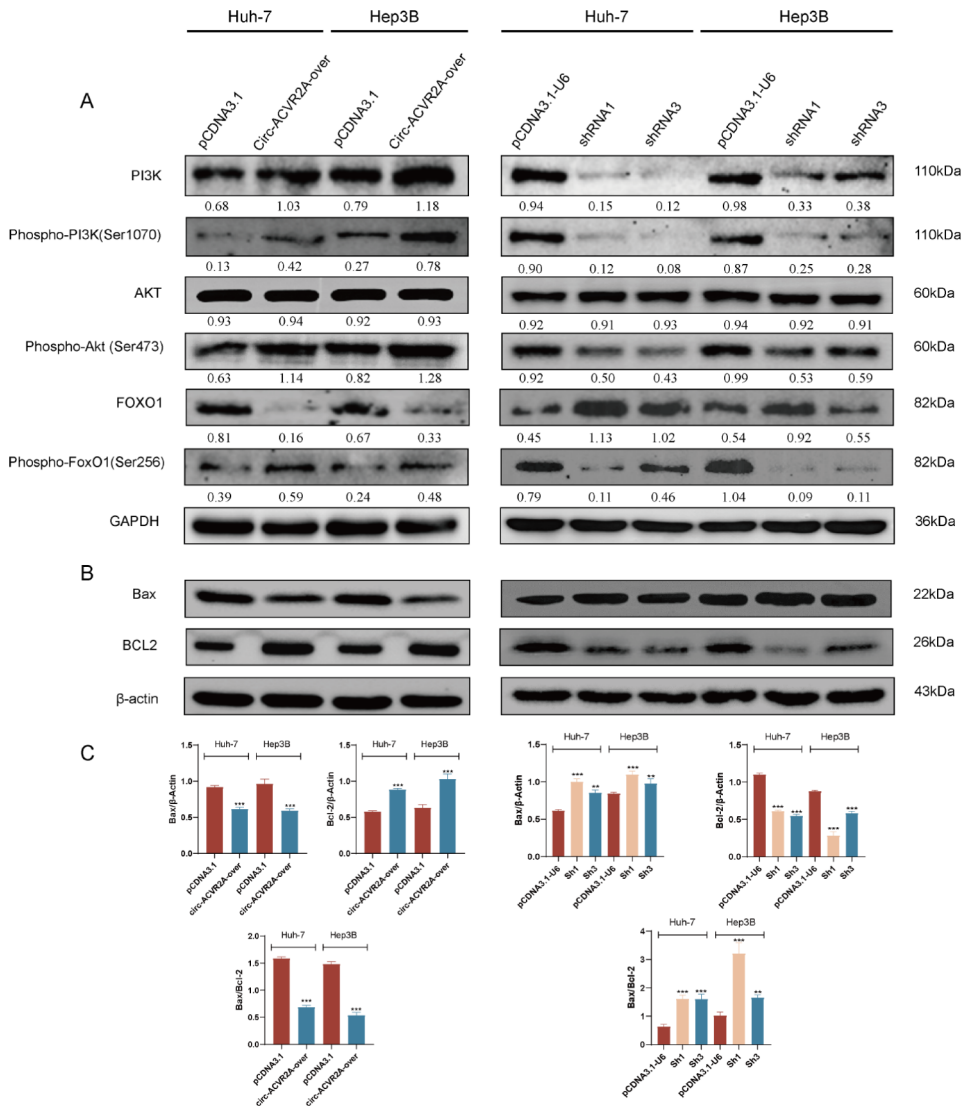

Fig. 6 circACVR2A can regulate the PI3K/AKT/FOXO1 signaling pathway. **(A)** After overexpression and silencing of circACVR2A in Huh-7 and Hep3B, the abundance of PI3K, Akt, FOXO1 and their phosphorylated proteins, with GAPDH as the internal reference protein; **(B, C)** After overexpression and silencing of circ ACVR2A in Huh-7 and Hep3B, the abundance of Bax and BCL2, with β-Actin as the internal reference protein. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and compared with pCDNA3.1 or pCDNA3.1-U6

circACVR2A (Fig. 6B, C). This indicates that the regulatory network mediated by circACVR2A can up-regulate the PI3K-AKT signal pathway and play a carcinogenic role.

circACVR2A promotes the growth of HCC in vivo

In order to further verify the effect of circACVR2A on tumor growth in vivo, Hep3B cells with stable expression of circACVR2A and its control vector were constructed, and circACVR2A with shRNA1 silencing Hep3B cells was selected according to the results of cell experiments. BALB/c nude mice aged about 5–6 weeks were selected and the cells of overexpression or knockdown of circACVR2A and their control groups were inoculated in the right axilla of mice. One week after cell inoculation, tumors grew in all groups, and the tumor volume was measured and recorded every 3 days. After 21 days of inoculation, the mice were killed, the tumor tissue was stripped and the weight was examined. The results showed that compared with the control group, the tumor tissue of tumor-bearing mice in circACVR2A overexpression group showed obvious necrosis (Fig. 7A). In addition, the tumor volume and weight of circACVR2A overexpression group increased significantly, while the tumor volume and weight of knock down group decreased significantly compared with the control group (Fig. 7B-D). HE staining was used to detect the development of tumors in each group. The cells with light red cytoplasm and light blue nucleus or blue disappearance were defined as tumor necrotic cells. The necrosis of

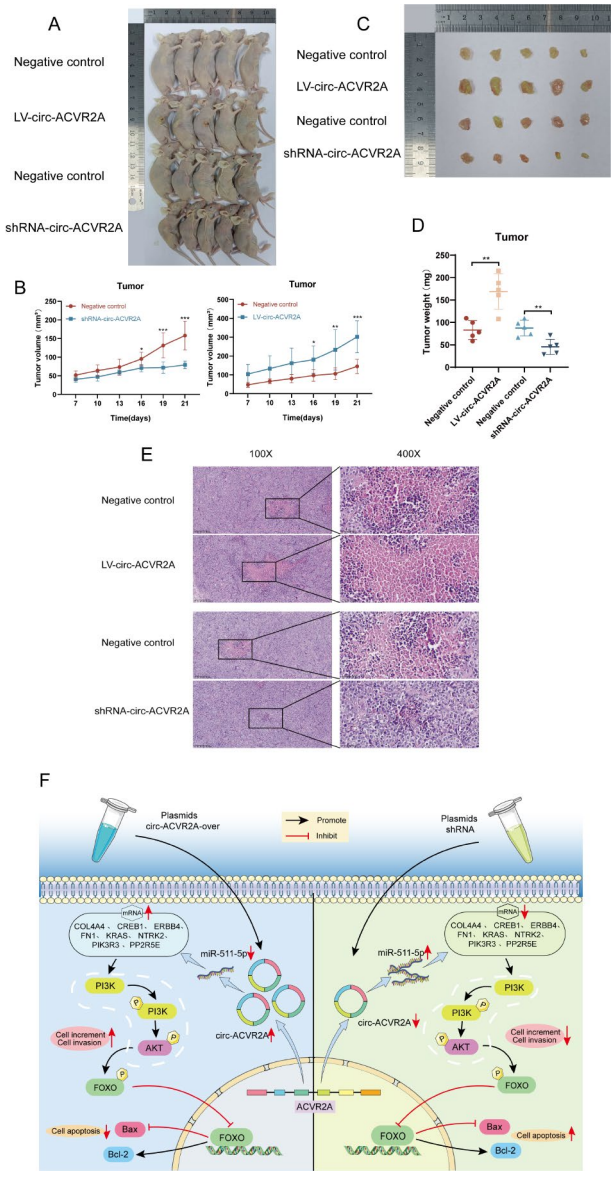

Fig. 7 Overexpression or silencing of circACVR2A promoted or inhibited the growth of hepatoma cells in vivo. **(A, B)** Hep3B cells stably expressing circACVR2 or control vector and Hep3B cells stably silencing circ-ACVR2 or control vector were inoculated into the right axilla of BALB/c nude mice. **(C, D)** The tumor volume and weight of circACVR2A overexpression group were significantly increased, while those of silent group were significantly decreased. **(E)** The representative images of HE staining analysis of tumors in each group. **(F)** The schematic diagram shows that circACVR2A inhibits the proliferation, metastasis and apoptosis of hepatocellular carcinoma cells through the miR-511/PI3K axis. $*P<0.05$, $**P<0.01$, $***P<0.001$, and compared with pCDNA3.1 or pCDNA3.1-U6

tumor cells is usually closely related to the rapid growth of tumor and insufficient blood supply. The results showed that the area of tumor necrotic cells in the circACVR2A overexpression group was significantly larger than that in the control group, while the tumor necrotic cell area in the circACVR2A knockdown group was significantly smaller than that in the control group (Fig. 7E). This shows that down-regulating the expression of circACVR2A in hepatocellular carcinoma can inhibit the growth of tumor in vivo.

Discussion

In recent years, circRNAs have been identified as an important class of non-coding RNA molecules, many of which play key roles in the origin and development of cancer through different pathways. They are usually expressed in tissue-specific and cancer-specific ways, and with the emergence of more and more research data, we are gradually discovering that these molecules may have potential clinical value and application prospects [33, 34]. In particular, it has great potential as a target for diagnosis and therapy [15, 35, 36].

At present, some scholars have detected the expression of circACVR2A in seven types of cancer tissues, including bladder cancer, breast cancer, colon cancer, hepatocellular carcinoma, gastric cancer, renal clear cell carcinoma, and prostate cancer by high-throughput sequencing of different types of cancer [24]. Dong et al. proposed that circACVR2A inhibited the proliferation and metastasis of bladder cancer cells through the miR-626/EYA4 axis, and low expression of circACVR2A was detected in bladder cancer cells [37]. Liu et al. also proposed that circACVR2A targeted miR-626/LIFR axis to inhibit lung cancer progression, and also detected low expression of circACVR2A in lung cancer cells [38]. However, the function of circACVR2A in liver cancer remains largely unknown and requires further exploration. In this study, we found that circACVR2A acts as a tumor suppressor in bladder and lung cancer, but promotes tumor development in liver cancer. circACVR2A is up-regulated in Huh-7 and Hep3B cells, and overexpression of circACVR2A significantly promotes the proliferation, migration and invasion of hepatoma cells, while SHRNA-mediated circACVR2A silencing has opposite effects on hepatoma cells.

With the continuous improvement of high-throughput RNA sequencing techniques and new bioinformatics tools, researchers have discovered thousands of circRNA s in a variety of organisms [39–41]. These circRNAs have abundant miRNA binding sites, and by binding to specific miRNAs, the inhibition of miRNAs on their target genes can be lifted [42, 43]. circRNA adsorbs miRNA by sponge, affects mRNA transcription and translation, and thus regulates gene expression [44–46]. In our study, we verified that circACVR2A can interact with miR-511-5p in liver cancer cells through bioinformatics analysis combined with dual luciferase reporter gene assay. However, in previous studies, it has been proved that miR-511-5p is low expressed in liver cancer and plays a cancer-suppressing role in liver cancer [47], which is consistent with the results obtained in this study. In addition, the

expression of circACVR2A mediated the expression of miR-511-5p by RT-qPCR. These results suggest that circACVR2A can act as a miRNA sponge for miR-511-5p.

Previous studies have shown that miRNAs can disrupt transcription products of target-specific genes or induce translation inhibition by binding to specific mRNAs [19]. Recent studies have shown that circRNA s can directly bind to miRNAs to regulate gene expression, thereby preventing them from interacting with target genes [21, 44–46]. In our study, candidate target genes of miR-511-5p were predicted by TargetScan, mirDIP and miRWalk, and multiple tumor-related signaling pathways were obtained by KEGG enrichment analysis. As one of the major signaling pathways in hepatocellular carcinoma, PI3K-Akt signaling pathway is widely involved in the regulation of cell cycle, cell proliferation, apoptosis, metabolism and angiogenesis [28, 48].In our study, nine candidate target genes of miR-511-5p were found to be enriched in the PI3K-Akt signaling pathway, among which eight mRNAs (COL4A4, CREB1, ERBB4, FN1, KRAS, NTRK2, PIK3R3 and PPP2R5E) showed significant differences compared with the normal group. Subsequently, the regulatory relationship of miR-511-5p on these 8 target genes was verified by RT-qPCR. Although there have been studies showing that five mRNAs, including PIK3R3, ERBB and FN1, can regulate the PI3K-Akt signaling pathway in hepatocellular carcinoma and act as tumor-promoting genes [49–52], their association with miR-511-5p and circACVR2A has not been described. Therefore, this study provides more information for understanding the biological function of CIRcacVR2A-mediated miR-511-5p/mRNA regulatory network in hepatoma cells. In addition to detecting PI3K and Akt expression, we also detected FoxO1 expression, which has been identified as a negatively regulated downstream gene of Akt. Previous studies have shown that the change of FoxO1 expression is related to cell proliferation and apoptosis, which is consistent with our experimental results.

Although circRNA has received a lot of attention in recent years and circRNA has been shown to play a crucial role in the progression and prognosis of human liver cancer [33, 53, 54], the study of circRNA, including circACVR2A, for clinical application is just beginning. On the one hand, there is no research on the function and possible mechanism of circACVR2A in hepatocellular carcinoma, which is worthy of further study. On the other hand, it is due to the lack of preclinical studies that include a sufficient number of patients. Although it has been proved by in vitro and in vivo experiments that the expression of circACVR2A is closely related to the occurrence and progression of hepatocellular carcinoma cells, due to the lack of clinical tissue samples of primary liver cancer, the differential expression of circACVR2A in normal subjects and patients with liver cancer cannot be directly verified, so there are still some limitations. In the future research, clinical samples will be collected and sequenced to verify the difference of circACVR2A expression between normal people and patients with liver cancer. In addition, the molecular mechanism of liver cancer mediated by circACVR2A regulatory network will be further explored.

Conclusions

To sum up, studies have shown that the expression of circACVR2A is up-regulated in hepatocellular carcinoma cell lines, and its high expression is closely related to the progression of hepatocellular carcinoma. In terms of mechanism, circACVR2A significantly inhibits the proliferation and metastasis of hepatocellular carcinoma by directly binding to miR-511-5p, thus reducing the inhibitory ability of miR-511-5p on PI3K signal pathway (Fig. 7F). Studies have proved that the molecular regulatory network with circACVR2A as the core may be a new therapeutic target for liver cancer.

Abbreviations

ACVR2A	Activin A receptor type 2 A
HCC	Hepatocellular Carcinoma Cells
circRNAs	circular RNAs
PI3K	Phosphoinositide 3-Kinase
FoxO1	Forkhead box O1
RT-qPCR	Real-time quantitative polymerase chain reaction
WB	Western Blot
CCK-8	Cell Counting Kit-8
TUNEL	Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling assay

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Not applicable.

Author contributions

ZZ, XJ, FD, and FW contributed to the conception and design of the study. YW performed the statistical analysis. FD wrote the first draft of the manuscript. JC, SC, LF, LY, QR, SD, FD, HL, JZ, JS, YZ, XW, ML, ZX, ZW and YL wrote sections of the manuscript. All authors contributed to the manuscript revision, and read, and approved the submitted version.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The current study was approved by the Institutional Animal Care and Use Committee of Southwest Medical University (swmu20230041).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Xing M, Wang X, Kiken RA, He L, Zhang J-Y. Immunodiagnostic biomarkers for Hepatocellular Carcinoma (HCC): the first step in detection and treatment. Int J Mol Sci 2021; 22.
2. Bond MJG, Bolhuis K, Loosveld OJL, de Groot JWB, Droogendijk H, Helgason HH, et al. First-line systemic treatment strategies in patients with initially unresectable colorectal cancer liver metastases (CAIRO5): an open-label, multicentre, randomised, controlled, phase 3 study from the Dutch Colorectal Cancer Group. Lancet Oncol. 2023;24:757–71.
3. Chen L, Wang C, Sun H, Wang J, Liang Y, Wang Y, et al. The bioinformatics toolbox for circRNA discovery and analysis. Brief Bioinform. 2021;22:1706–28.
4. Du WW, Zhang C, Yang W, Yong T, Awan FM, Yang BB. Identifying and characterizing circRNA-Protein Interaction. Theranostics. 2017;7:4183–91.
5. Sanger HL, Klotz G, Riesner D, Gross HJ, Kleinschmidt AK. Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures. Proc Natl Acad Sci U S A. 1976;73:3852–6.
6. Zaitseva GN, Kolesnikov AA, Shirshov AT. The genetic system of kinetoplasts in trypanosomatides. Mol Cell Biochem. 1977;14:47–54.
7. Zang J, Lu D, Xu A. The interaction of circRNAs and RNA binding proteins: an important part of circRNA maintenance and function. J Neurosci Res. 2020;98:87–97.
8. Shi Y, Jia X, Xu J. The new function of circRNA: translation. Clin Transl Oncol. 2020;22:2162–9.
9. Li R, Jiang J, Shi H, Qian H, Zhang X, Xu W. CircRNA: a rising star in gastric cancer. Cell Mol Life Sci. 2020;77:1661–80.
10. Chen C-K, Cheng R, Demeter J, Chen J, Weingarten-Gabbay S, Jiang L et al. Structured elements drive extensive circular RNA translation. Mol Cell 2021; 81.
11. Stagsted LVW, O'Leary ET, Ebbesen KK, Hansen TB. The RNA-binding protein SFPQ preserves long-intron splicing and regulates circRNA biogenesis in mammals. Elife 2021; 10.
12. Shao Y, Li M, Yu Q, Gong M, Wang Y, Yang X, et al. CircRNA CDR1as promotes cardiomyocyte apoptosis through activating hippo signaling pathway in diabetic cardiomyopathy. Eur J Pharmacol. 2022;922:174915.
13. Zhang J, Liu Y, Shi G. The circRNA-miRNA-mRNA regulatory network in systemic lupus erythematosus. Clin Rheumatol. 2021;40:331–9.
14. Xue C, Li G, Zheng Q, Gu X, Bao Z, Lu J, et al. The functional roles of the circRNA/Wnt axis in cancer. Mol Cancer. 2022;21:108.
15. Kristensen LS, Jakobsen T, Hager H, Kjems J. The emerging roles of circRNAs in cancer and oncology. Nat Rev Clin Oncol. 2022;19:188–206.
16. Zhu G, Chang X, Kang Y, Zhao X, Tang X, Ma C et al. CircRNA: a novel potential strategy to treat thyroid cancer (review). Int J Mol Med 2021; 48.
17. Zhang H, Shen Y, Li Z, Ruan Y, Li T, Xiao B, et al. The biogenesis and biological functions of circular RNAs and their molecular diagnostic values in cancers. J Clin Lab Anal. 2020;34:e23049.
18. Su K, Yi Q, Dai X, Liu O. Circular RNA ITCH: an Emerging Multifunctional Regulator. Biomolecules 2022; 12.
19. Hill M, Tran N. miRNA interplay: mechanisms and consequences in cancer. Dis Model Mech 2021; 14.
20. Pardi N, Hogan MJ, Weissman D. Recent advances in mRNA vaccine technology. Curr Opin Immunol. 2020;65:14–20.
21. Pisignano G, Michael DC, Visal TH, Pirlog R, Ladomery M, Calin GA. Going circular: history, present, and future of circRNAs in cancer. Oncogene. 2023;42:2783–800.
22. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, Rybak A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature. 2013;495:333–8.
23. Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, et al. Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA. 2013;19:141–57.
24. Zheng Q, Bao C, Guo W, Li S, Chen J, Chen B, et al. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. Nat Commun. 2016;7:11215.
25. Xu K, Yin N, Peng M, Stamatiades EG, Shyu A, Li P, et al. Glycolysis fuels phosphoinositide 3-kinase signaling to bolster T cell immunity. Science. 2021;371:405–10.
26. Xu K, Yin N, Peng M, Stamatiades EG, Chhangawala S, Shyu A et al. Glycolytic ATP fuels phosphoinositide 3-kinase signaling to support effector T helper 17 cell responses. Immunity 2021; 54.
27. Pan T, Sun S, Chen Y, Tian R, Chen E, Tan R, et al. Immune effects of PI3K/Akt/HIF-1α-regulated glycolysis in polymorphonuclear neutrophils during sepsis. Crit Care. 2022;26:29.
28. Paskeh MDA, Ghadyani F, Hashemi M, Abbaspour A, Zabolian A, Javanshir S, et al. Biological impact and therapeutic perspective of targeting PI3K/Akt signaling in hepatocellular carcinoma: promises and challenges. Pharmacol Res. 2023;187:106553.
29. Sun Y, Zhang H, Meng J, Guo F, Ren D, Wu H, et al. S-palmitoylation of PCSK9 induces sorafenib resistance in liver cancer by activating the PI3K/AKT pathway. Cell Rep. 2022;40:111194.
30. Abdel-Megeed RM, Abd El-Alim SH, Arafa AF, Matloub AA, Farrag AERH, Darwish AB, et al. Crosslink among phosphatidylinositol-3 kinase/Akt, PTEN and STAT-5A signaling pathways post liposomal galactomannan hepatocellular carcinoma therapy. Toxicol Rep. 2020;7:1531–41.
31. Shi H, Fang R, Li Y, Li L, Zhang W, Wang H, et al. The oncoprotein HBXIP suppresses gluconeogenesis through modulating PCK1 to enhance the growth of hepatoma cells. Cancer Lett. 2016;382:147–56.
32. Lu M, Hartmann D, Braren R, Gupta A, Wang B, Wang Y, et al. Oncogenic Akt-FOXO3 loop favors tumor-promoting modes and enhances oxidative damage-associated hepatocellular carcinogenesis. BMC Cancer. 2019;19:887.
33. Song R, Ma S, Xu J, Ren X, Guo P, Liu H, et al. A novel polypeptide encoded by the circular RNA ZKSCAN1 suppresses HCC via degradation of mTOR. Mol Cancer. 2023;22:16.
34. Li J, Hu Z-Q, Yu S-Y, Mao L, Zhou Z-J, Wang P-C, et al. CircRPN2 inhibits aerobic glycolysis and metastasis in Hepatocellular Carcinoma. Cancer Res. 2022;82:1055–69.
35. Yu T, Wang Y, Fan Y, Fang N, Wang T, Xu T, et al. CircRNAs in cancer metabolism: a review. J Hematol Oncol. 2019;12:90.
36. Zhang F, Jiang J, Qian H, Yan Y, Xu W. Exosomal circRNA: emerging insights into cancer progression and clinical application potential. J Hematol Oncol. 2023;16:67.
37. Dong W, Bi J, Liu H, Yan D, He Q, Zhou Q, et al. Circular RNA ACVR2A suppresses bladder cancer cells proliferation and metastasis through miR-626/EYA4 axis. Mol Cancer. 2019;18:95.
38. Liu Q, Cao G, Wan Y, Xu C, He Y, Li G. Hsa_circ_0001073 targets miR-626/LIFR axis to inhibit lung cancer progression. Environ Toxicol. 2021;36:1052–60.
39. Goodall GJ, Wickramasinghe VO. RNA in cancer. Nat Rev Cancer. 2021;21:22–36.
40. Liu C-X, Chen L-L. Circular RNAs: characterization, cellular roles, and applications. Cell. 2022;185:2016–34.
41. Li X, Yang L, Chen L-L. The Biogenesis, functions, and challenges of Circular RNAs. Mol Cell. 2018;71:428–42.
42. Yang Y, Yujiao W, Fang W, Linhui Y, Ziqi G, Zhichen W, et al. The roles of miRNA, lncRNA and circRNA in the development of osteoporosis. Biol Res. 2020;53:40.
43. Wu W, Cao L, Jia Y, Xiao Y, Zhang X, Gui S. Emerging roles of miRNA, lncRNA, circRNA, and their cross-talk in Pituitary Adenoma. Cells 2022; 11.
44. Ma B, Wang S, Wu W, Shan P, Chen Y, Meng J, et al. Mechanisms of circRNA/lncRNA-miRNA interactions and applications in disease and drug research. Biomed Pharmacother. 2023;162:114672.
45. Mohanapriya R, Akshaya RL, Selvamurugan N. A regulatory role of circRNA-miRNA-mRNA network in osteoblast differentiation. Biochimie. 2022;193:137–47.
46. Tong K-L, Tan K-E, Lim Y-Y, Tien X-Y, Wong P-F. CircRNA-miRNA interactions in atherogenesis. Mol Cell Biochem. 2022;477:2703–33.
47. Xu Y, Ke S, Lu S, Wang C, Li Z, Feng Z, et al. HLTF promotes hepatocellular carcinoma progression by enhancing SRSF1 stability and activating ERK/MAPK pathway. Oncogenesis. 2023;12:2.

48. Du D, Liu C, Qin M, Zhang X, Xi T, Yuan S, et al. Metabolic dysregulation and emerging therapeutical targets for hepatocellular carcinoma. Acta Pharm Sin B. 2022;12:558–80.
49. Guo W, Yao X, Lan S, Zhang C, Li H, Chen Z, et al. Metabolomics and integrated network pharmacology analysis reveal SNKAF decoction suppresses cell proliferation and induced cell apoptisis in hepatocellular carcinoma via PI3K/Akt/P53/FoxO signaling axis. Chin Med. 2022;17:76.
50. Lee WY, Bachtiar M, Choo CCS, Lee CG. Comprehensive review of Hepatitis B Virus-associated hepatocellular carcinoma research through text mining and big data analytics. Biol Rev Camb Philos Soc. 2019;94:353–67.
51. Dietrich P, Koch A, Fritz V, Hartmann A, Bosserhoff AK, Hellerbrand C. Wild type Kirsten rat sarcoma is a novel microRNA-622-regulated therapeutic target for hepatocellular carcinoma and contributes to sorafenib resistance. Gut. 2018;67:1328–41.
52. Ni Q, Chen Z, Zheng Q, Xie D, Li J-J, Cheng S, et al. Epithelial V-like antigen 1 promotes hepatocellular carcinoma growth and metastasis via the ERBB-PI3K-AKT pathway. Cancer Sci. 2020;111:1500–13.
53. Liu L, Gu M, Ma J, Wang Y, Li M, Wang H, et al. CircGPR137B/miR-4739/FTO feedback loop suppresses tumorigenesis and metastasis of hepatocellular carcinoma. Mol Cancer. 2022;21:149.
54. Sunagawa Y, Yamada S, Sonohara F, Kurimoto K, Tanaka N, Suzuki Y, et al. Genome-wide identification and characterization of circular RNA in resected hepatocellular carcinoma and background liver tissue. Sci Rep. 2021;11:6016.

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