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ORIGINAL RESEARCH

circ_0003418 Inhibits Tumorigenesis And Cisplatin Chemoresistance Through Wnt/β-Catenin Pathway In Hepatocellular Carcinoma

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Hang Chen[1]
Shan Liu[1]
Molin Li[2]
Ping Huang[2]
Xiaoping Li[1]

[1]Department of Oncology and Hematology, The People's Hospital of Tongliang District, Chongqing 402560, People's Republic of China; [2]Department of Hepatobiliary Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing Medical University, Chongqing 400000, People's Republic of China

Background: Accumulating evidences indicate that circRNAs play important roles in the progression and chemoresistance of human cancers. The present study is designated for researching the roles of circ_0003418 in hepatocellular carcinoma (HCC).

Methods: We detected the expression profile of circ_0003418 in human HCC tissues and cell lines by quantitative real-time-PCR assays. CCK-8 assay, transwell migration assay, transwell invasion assay and drug-sensitivity analysis were carried out to estimate the effects of circ_0003418 on HCC cells' proliferation, migration, invasion and resistance to cisplatin, respectively. Mouse xenograft model was conducted to monitor the role of circ_0003418 in cisplatin resistance in vivo. Western blotting was performed to explore the changes of the Wnt/β-catenin pathway after knockdown of circ_0003418. The rescue experiment was carried out to explore circ_0003418-activated biological functions through Wnt/β-catenin pathway.

Results: The expression level of circ_0003418 was downregulated in HCC tissues and cell lines, and the level correlated with tumor size, TNM stage and HBsAg level in HCC patients. circ_0003418 knockdown promoted HCC cells' proliferation, migration, and invasion. Additionally, suppression of circ_0003418 enhanced cisplatin resistance of HCC cells in vivo and vitro. Knockdown of circ_0003418 activated the Wnt/β-catenin signalling pathway in HCC cells. The effect of circ-0003418 on sensitivity of HCC cells to cisplatin was reversed after inhibition of Wnt/β-catenin pathway.

Conclusion: circ-0003418 exerts an antitumorigenic role in HCC and advances the sensitivity of HCC cells to cisplatin by restraining the Wnt/β-catenin pathway. Thus, circ-0003418 may represent a novel biomarker and provide us a new strategy for the treatment of HCC.

Keywords: circRNA, circ-0003418, hepatocellular carcinoma, cisplatin resistance, Wnt/β-catenin

Correspondence: Xiaoping Li
Department of Oncology and Hematology, The People's Hospital of Tongliang District, 528# Zhongxing East Road, Dongcheng Street, Tongliang District, Chongqing 402560, People's Republic of China
Tel +86 189 8394 5611
Email 1983978571@qq.com

Ping Huang
National Key Clinical Department, Department of Hepatobiliary Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing Medical University, Chongqing 400000, People's Republic of China
Tel +86 186 2331 7078
Email 202041@cqmu.edu.cn

Introduction

Liver cancer is one of the most common malignancies; hepatocellular carcinoma (HCC) is the most common classification of liver cancers, which is the second cause of cancer-related deaths worldwide.[1,2] Surgical resection, liver transplantation and chemotherapy are the major therapeutic strategies for HCC.[3] Cisplatin is the first-line chemotherapeutic agent, an efficient-spectrum antitumor agent, which leads to inhibition of cancer cells split-up and induces apoptosis by binding to and cross-linking DNA to inhibit replication and transcription.[3,4] However, owing to cisplatin resistance that occurs during chemotherapy, the overall 5-year survival rate of HCC patients is worrying.[5] Therefore, it is necessary to identify tumor initiation, progression and the chemoresistance mechanism to improve clinical outcomes.

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Non-coding RNAs, including miRNAs, lncRNAs and circRNAs, play important roles in physiological and pathological processes such as proliferation, invasion, apoptosis and chemoresistance.[6–8] CircRNAs are a new group of steady, endogenous and evolutionary conservative noncoding RNAs that are alien from linear RNA and are RNA molecules with 3′ and 5′ ends covalently linked in a circular structure.[9] One of the main biological functions of circRNAs is serving as a sponge to combine and sequester miRNAs in a sequence-specific manner.[10] Recent studies have shown that upregulation of miR-7 enhances the sensitivity of lung adenocarcinoma cells to cisplatin via induction of apoptosis by targeting Bcl-2, and miR-383 inhibits chemoresistance in HCC cells by targeting EIF5A2.[11,12] In addition, bioinformatics research has indicated that circ-0003418 may interplay with miR-7 and miR-383. Therefore, we hypothesized that circ-0003418 affects the biological behavior of HCC cells and participates in the regulation of cisplatin resistance.

In the present study, we aimed to elucidate the relative expression levels of circ-0003418 in HCC tissues and cells, its effects on biological behavior of HCC cells and the potential role of circ-0003418 in cisplatin resistance. Our results indicated that circ-0003418 inhibited HCC proliferation, migration, and invasion and advanced sensitivity of HCC cells to cisplatin by restraining the Wnt/β-catenin pathway. Therefore, circ-0003418 may be a biomarker and therapeutic target for HCC.

Materials And Methods

Patients And Specimens

A total of 46 pairs of HCC and matched adjacent antitumor tissues were obtained from HCC patients undergoing surgery at the First Affiliated Hospital of Chongqing Medical University between August 2015 and December 2017. All patients did not receive chemotherapy or radiotherapy before surgery and HCC was diagnosed by pathological examination. All tissue specimens were stored at −80°C until detection.

Cell Culture, Infection And Transfection

Human HCC cell lines (Hep-3B, Huh-7, Sk-hep-1, SMMC-7721 and PLC) and normal human hepatocyte line (HL-77O2) were purchased from the China Center for Type Culture Collection (Wuhan, China). Hep-3B, Huh-7 and Sk-hep-1 cells were routinely cultured in DMEM (Gibco, Carlsbad, CA, USA), while SMMC-7721, PLC and HL-77O2 cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco). Both mediums contained 10% fetal bovine serum (PAN, Bavaria, Germany). All cells were cultured in a 5% CO_2 humidified incubator with a temperature of 37°C.

LV3-has_circ_0003418 (LV3-circ_0003418) and LV3-NC were synthesized by GenePharma (Shanghai, China). Lentivirus was transinfected into cells using polybrene (Hanbio Biotechnology Co., Ltd., Shanghai, China) according to the manufacturer's instructions. Puromycin (2 μg/mL) was used to remove uninfected cells for 2 weeks. β-Catenin/TCF-mediated transcription inhibitor ICG-001 was purchased from Lianmai Biological Engineering Co., Ltd (Shanghai, China).

Quantitative Real-Time PCR (qRT-PCR)

The TRIzol reagent (Invitrogen, USA) was applied to extract total RNA from tissues or cells according to the manufacturer's manual. circRNA reverse transcription kit (Jisai Biotechnology Co., Ltd., Guangzhou, China) was used to synthesize cDNA. The qRT-PCR was carried out with circRNA real-time PCR detection kit on an ABI7500 Real-time PCR system (Applied Biosystems, Foster City, CA, USA). The RNA primers were circ_0003418, 5′-CGTGG ACTCCGACAG CAA3′ (forward), 5′-GACATCATCACTC ATGCGGA A-3′ (reverse). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), CAGCTAGCCGCATCTTCTTT T (forward), GTGACCAGGCGCCCAATAC (reverse). GAP DH was used as the internal control for circ-0003418 expression. The relative circRNA expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.[13]

Cell Proliferation Assay

Cell Counting Kit-8 assay (CCK-8, Hanbio Biotechnology Co., Ltd., Shanghai, China) was used to evaluate cell viability. Briefly, HCC cells (4×10^3 cells per well) were seeded into 96-well plates. Subsequently, CCK-8 reagent (10 μl) was put into each well at different timepoints and incubated at 37°C for 2 hrs. After incubation, the Multi-Mode Microplate Reader (Thermo Fisher Scientific Inc., USA) was used to determine the absorbance at 450 nm.

Chemotherapy Sensitivity Assay

The infected cells (4×10^3 cells per well) were seeded into 96-well plates. After incubation for 24 hrs, the cells were treated with various concentrations of cisplatin (0, 1, 2, 4, 8, 16, 32, 64 and 128 mg/L). The cell viability was determined by CCK-8 assay after 24 hrs. The Multi-Mode Microplate Reader (Thermo Fisher Scientific Inc.) was used to determine the absorbance at 450 nm. The dose–response curve was charted and the half-maximal inhibitory concentration (IC_{50}) was calculated according to these data.[6]

Transwell Migration And Invasion Assays

Cell migration and invasion abilities were measured using transwell chambers (8.0 µm pore size; EMD Millipore, Billerica, MA, USA) and Matrigel (diluted 1:9) (Corning Inc., USA), respectively. Infected Huh-7 and Hep-3B cells (4×10^5 cells) were resuspended in 200 µl of serum-free MEM medium and were added into the upper chamber of the insert without or with 10 µl of Matrigel, and the bottom chamber was filled with 500 µl of complete medium containing 10% FBS. After incubation for 24 hrs, the cells remaining on the upper membrane surface were removed. The cells on the bottom surface were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet (Beyotime, Jiangsu, China). The cells were photographed and counted under an upright microscope (Nikon, Japan).

Western Blot Analysis

Cells were lysed with lysis buffer (Beyotime, Shanghai, China) containing 1 nM phenylmethylsulfonyl fluoride. Protein concentration was determined with a BCA Protein Assay kit (Beyotime). Protein separation was determined by SDS-PAGE (Beyotime) and transferred onto polyvinylidene difluoride membrane (Millipore). The membranes were incubated with anti-β-catenin (1:800, Ruiyingbio, Suzhou, China), anti-c-Myc (1:500, Ruiyingbio) and anti-GAPDH (1:500, Ruiyingbio) at 4°C overnight, and then hybridized with mouse antirabbit IgG secondary antibody conjugated to horseradish peroxidase (1:5000, Ruiyingbio) at room temperature for 2 hrs. Protein bands were visualized with a WestrenBright ECL Kit (Advansta, USA). Finally, signal intensity of bands was analyzed using the Image Lab software (Bio-Rad, Hercules, CA, USA).

Xenograft Tumor Model

Female BALB/c nude mice aged 4 weeks were obtained from the Laboratory Animal Center Chongqing Medical University (Chongqing, China). Stably transfected cells (5×10^6 cells) resuspended in 150 µL of PBS were injected subcutaneously into the right flank of nude mice. Twelve days later, cisplatin (5 mg/kg) or vehicle in 50 µL of physiological saline were intraperitoneally injected twice a week. Tumor volumes were measured with digital calipers, and the size of the tumors was calculated by length × width^2/2 (mm^3). All mice were euthanized on the 36th day, and tumors were removed, weighed and photographed.

Statistical Analysis

GraphPad software 6.0 (GraphPad Inc., San Diego, CA, USA) and SPSS software (version 24.0 SPSS, Chicago, IL, USA) were used to analyze the results. All experiments were repeated at least three times. Data were shown as mean±SD (standard deviation). $P < 0.05$ was considered to demonstrate statistical significance. The Student's *t*-test and one-way ANOVA were used to compare statistical differences of two groups and multiple groups, respectively. The χ^2 test was used to evaluate the connection between circ_0003418 expression and the clinicopathological features of patients with HCC.

Ethics Statement

This study was approved by the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University in Chongqing, Chongqing Province, China, and written informed consent was obtained from all patients. For animal experiments, before the commencement of the study, the protocols of animal experiments were approved by the Animal Ethical Committee of the First Affiliated Hospital of Chongqing Medical University, and were in compliance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Results

circ_0003418 Downregulation In HCC Is Associated With HCC Patient

To explore the role of circ_0003418 in HCC, we first performed qRT-PCR to assess circ_0003418 expression in HCC and adjacent noncancerous tissues. We found that the expression of circ_0003418 was lower in HCC tissues than that in adjacent tumor-free tissues (Figure 1A). As expected, circ_0003418 was downregulated in HCC cell lines (Huh-7, PLC, Sk-hep-1, SMMC-7721 and Hep-3B) compared with normal hepatocyte cell line (HL-77O2) (Figure 1B). Given its deregulation in HCC tissues and cells, we analyzed the connection between the level of circ_0003418 and clinical clinicopathological features of HCC patients. The result indicated that circ_0003418 expression was associated with tumor size, TNM stage and HBsAg level (Table 1). These data suggested that circ_0003418 may act as a biomarker for diagnosis of HCC and prediction for the outcome of HCC.

To explore the regulatory role of circ_0003418 in HCC, we first infected LV3-circ_0003418 and LV3-NC into Huh-7 and Hep-3B cells. The knockdown efficiency of lentivirus was confirmed using qRT-PCR. The results demonstrated that compared with cells infected with LV3-NC, circ_0003418

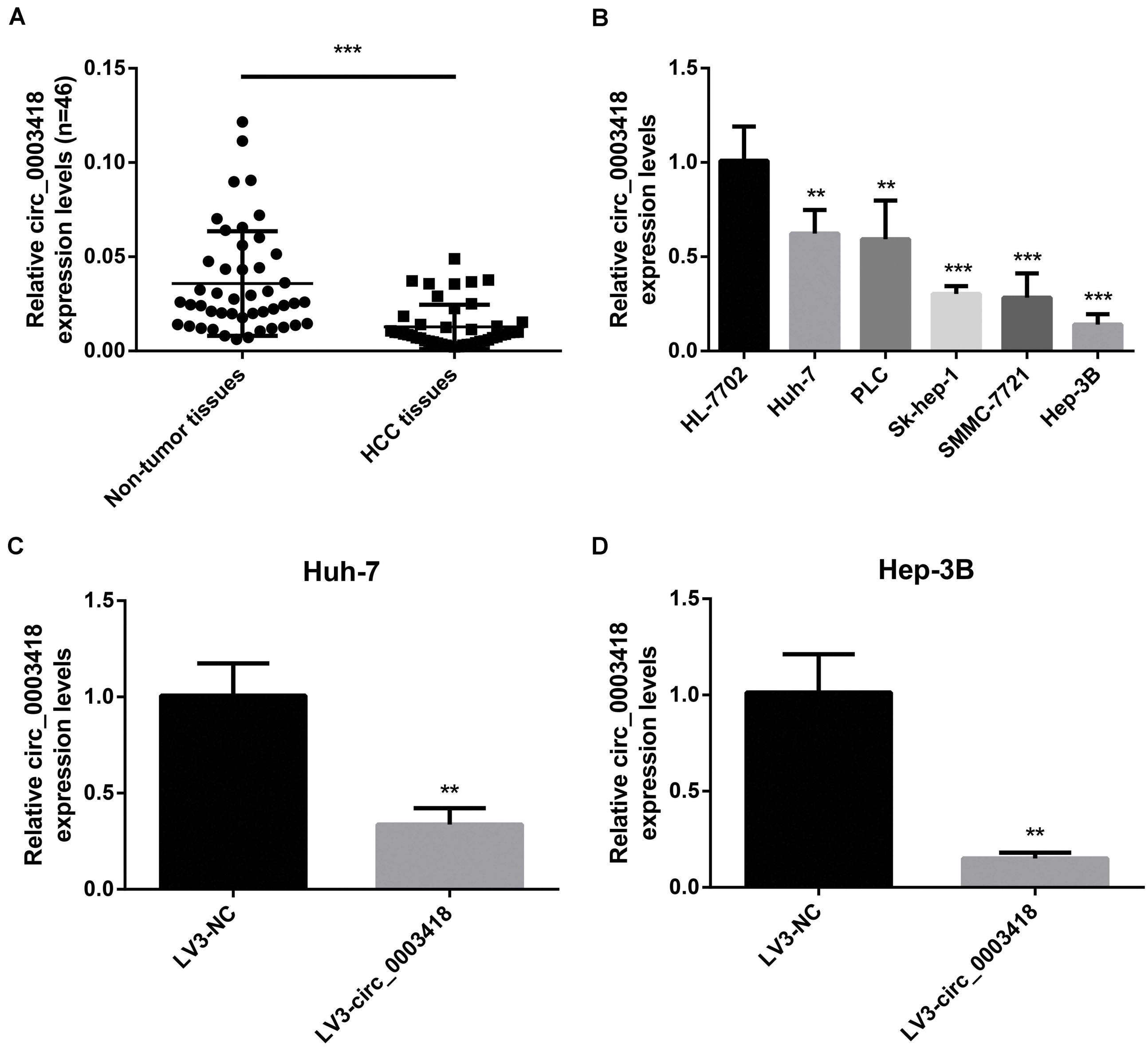

Figure 1 circ_0003418 downregulation in HCC tissues and cells.
Notes: (**A**) The expression profile of circ_0003418 in HCC tissues and adjacent noncancerous tissues were detected by qRT-PCR assays. (**B**) qRT-PCR was used to analyze the level of circ_0003418 in five HCC cell lines and one normal human hepatocyte cell line. (**C** and **D**) The knockdown efficiency of LV3-circ_0003418 on circ_0003418 in Huh-7 and Hep-3B cells was verified via qRT-PCR. ** P<0.01 and *** P<0.001 compared to control group.
Abbreviations: HCC, hepatocellular carcinoma; NC, negative control.

expression was significantly downregulated in cells infected with LV3-circ_0003418 (Figure 1C and D).

Silencing circ_0003418 Facilitates Proliferation, Migration And Invasion In HCC Cells

The results of CCK-8 assays showed that silencing circ_0003418 increased cell proliferation (Figure 2A and B). Subsequently, migration and invasion abilities were evaluated using transwell migration and invasion assays, respectively. We found that the migration and invasion abilities of Huh-7 and Hep-3B cells infected with LV3-circ_0003418 significantly advanced compared with the control group (Figure 2C–F). These results indicated that circ_0003418 attenuated HCC cells' proliferation, migration and invasion.

Silencing circ_0003418 Enhances The Cisplatin Resistance In HCC Cells

Previous studies showed that overexpression of miR-7 advanced the cisplatin chemosensitivity of lung

Table 1 Association Between Circ_0003418 Expression And Clinicopathological Features In HCC (n=46)

Clinicopathological Factors	Number (n=46)	circ_0003418 Expression		P-value
		High	Low	
Gender				0.583
Male	38	15	23	
Female	8	4	4	
Age (years)				0.108
≥50	25	13	12	
<50	21	6	15	
Tumor size (cm)				0.002
≥5	29	7	22	
<5	17	12	5	
TNM stage				0.029
III–IV	28	8	20	
I–II	18	11	7	
HBsAg				0.037
+	36	12	24	
-	10	7	3	
HBV DNA				0.79
+	28	12	16	
-	18	7	11	
ALT (U/L)				0.685
≥40	25	11	14	
<40	21	8	13	
AST (U/L)				0.337
≥40	28	10	18	
<40	18	9	9	
AFP (μg/L)				0.901
≥400	15	6	9	
<400	31	13	18	

Abbreviations: HBsAg, Australia antigen; HBV, hepatitis B virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

adenocarcinoma cells by targeting Bcl-2, and miR-383 is significantly correlated with chemoresistance in HCC cells.[10,11] Moreover, bioinformatics research showed that circ-0003418 may function by interacting with miR-7 and miR-383. Hence, we investigated whether circ_0003418 has function in regulating HCC cisplatin resistance, we examined the cell viability in HCC cells treated with different concentrations of cisplatin and calculated the IC_{50} value. Compared with cells infected with LV3-NC, cells infected with LV3-circ_0003418 had higher cell viability and greater cisplatin IC50 values (Figure 3A and B). Huh-7 and Hep-3B cells infected with LV3-NC or LV3-circ_0003418 were incubated with cisplatin for 24 hrs. Migration and invasion abilities were enhanced in cells infected with LV3-circ_0003418 compared to cells infected with LV3-NC (Figure 3C–F). These results showed that circ_0003418 enhanced the sensitivity of HCC cells to cisplatin.

Silencing circ_0003418 Facilitates HCC Growth And Cisplatin Chemoresistance In Vivo

To further explore the regulatory role of circ_0003418 in tumor growth and cisplatin resistance in HCC, we constructed the mouse xenograft model to assess the effect of circ_0003418 on tumor growth and cisplatin sensitivity. Silencing circ_0003418 promoted tumor growth in mice. Treatment with cisplatin resulted in distinct tumor-inhibitory effects on the implanted tumors. Notably, LV3-circ_0003418 plus cisplatin treatment led to smaller tumor inhibition of the growth than LV3-NC plus cisplatin treatment (Figure 4A–D). These findings suggested that circ-0003418 silencing promoted tumor growth and cisplatin resistance in HCC cells.

circ-0003418 Exerts Biological Functions In HCC Via The Wnt/β-Catenin Pathway

Accumulating evidence has suggested that the Wnt/β-catenin signal pathway involves in the chemoresistance of various cancers.[15] Hereby, we detected the protein expression levels of β-catenin and c-Myc in HCC cells with different treatments. Silencing circ_0003418 increased the protein expression levels of β-catenin and c-Myc (Figure 5A and B). Compared with the combination of LV3-NC with cisplatin treatment, the combination of LV3-circ_0003418 with cisplatin treatment increased β-catenin and c-Myc expression in Huh-7 and Hep-3B cells. ICG-001 significantly inhibited the protein level of β-catenin in the Huh-7 and Hep-3B cells (Figure 5C and D). Cell proliferation assay showed that cell proliferation was significantly dropped in the group of LV3-circ_0003418 plus ICG-001 compared with the group of LV3-circ_0003418 (Figure 5E and F). Chemotherapy sensitivity assay showed that sensitivity of HCC cells to cisplatin was increased after ICG-001 treatment in cells infected with LV3-circ_0003418 (Figure 5G and H). The aforementioned results implied that circ-0003418 affected the expression of β-catenin and c-Myc. In addition, the effect of circ-0003418 on sensitivity of HCC cells to cisplatin was reversed after inhibition of Wnt/β-catenin pathway.

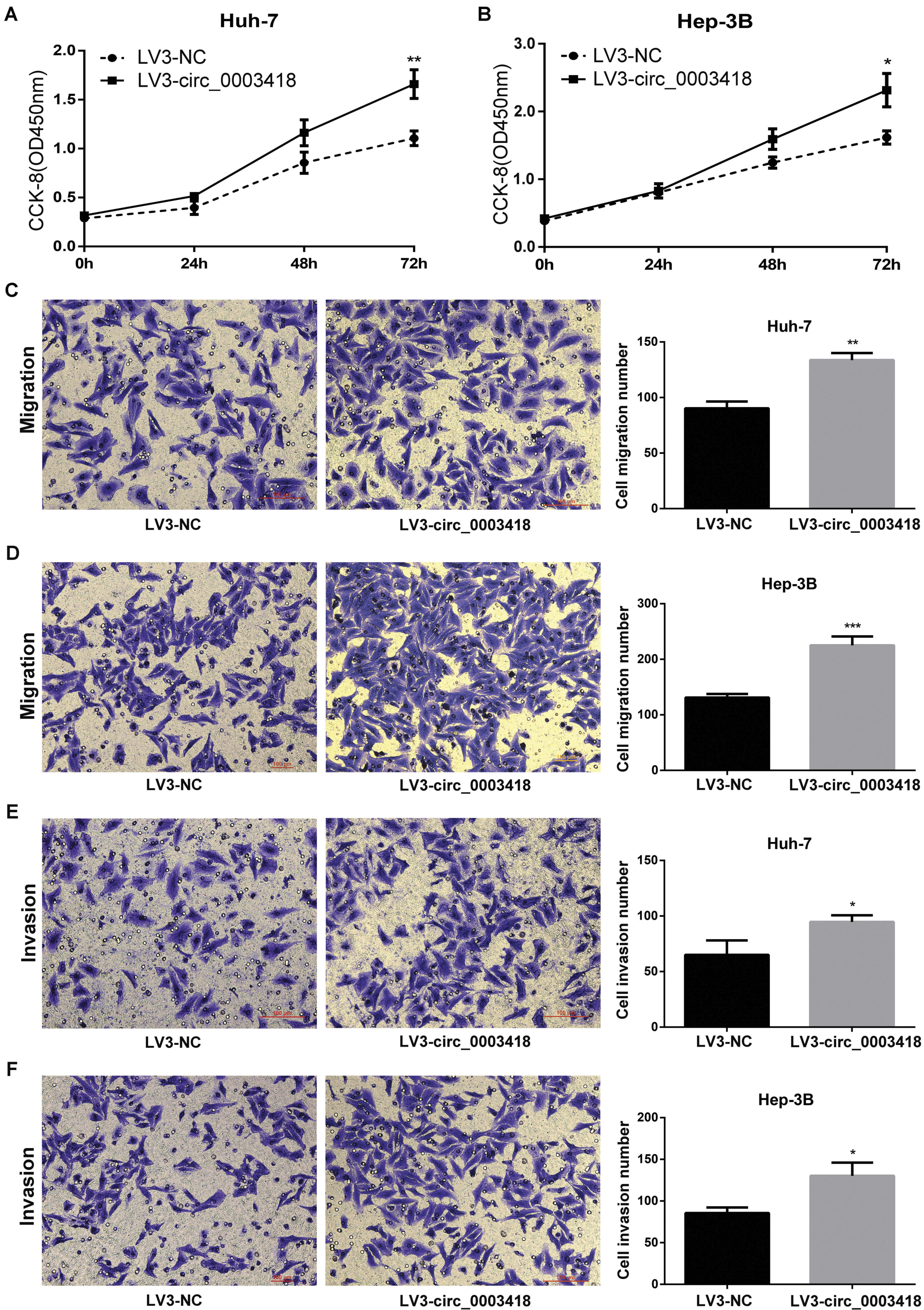

Figure 2 circ_0003418 suppresses proliferation, migration, and invasion and promotes apoptosis in HCC cells.

Notes: (**A** and **B**) CCK-8 assays were performed to measure the effect of silencing circ_0003418 on the proliferation in Huh-7 and Hep-3B cells. (**C–F**) Effect of silencing circ_0003418 on cell migration (**C** and **D**) and invasion (**E** and **F**) were analyzed by transwell migration and invasion assays, respectively. * P<0.05, ** P<0.01 and *** P<0.001 compared to control group.

Abbreviations: CCK-8, cell counting kit 8; NC, negative control.

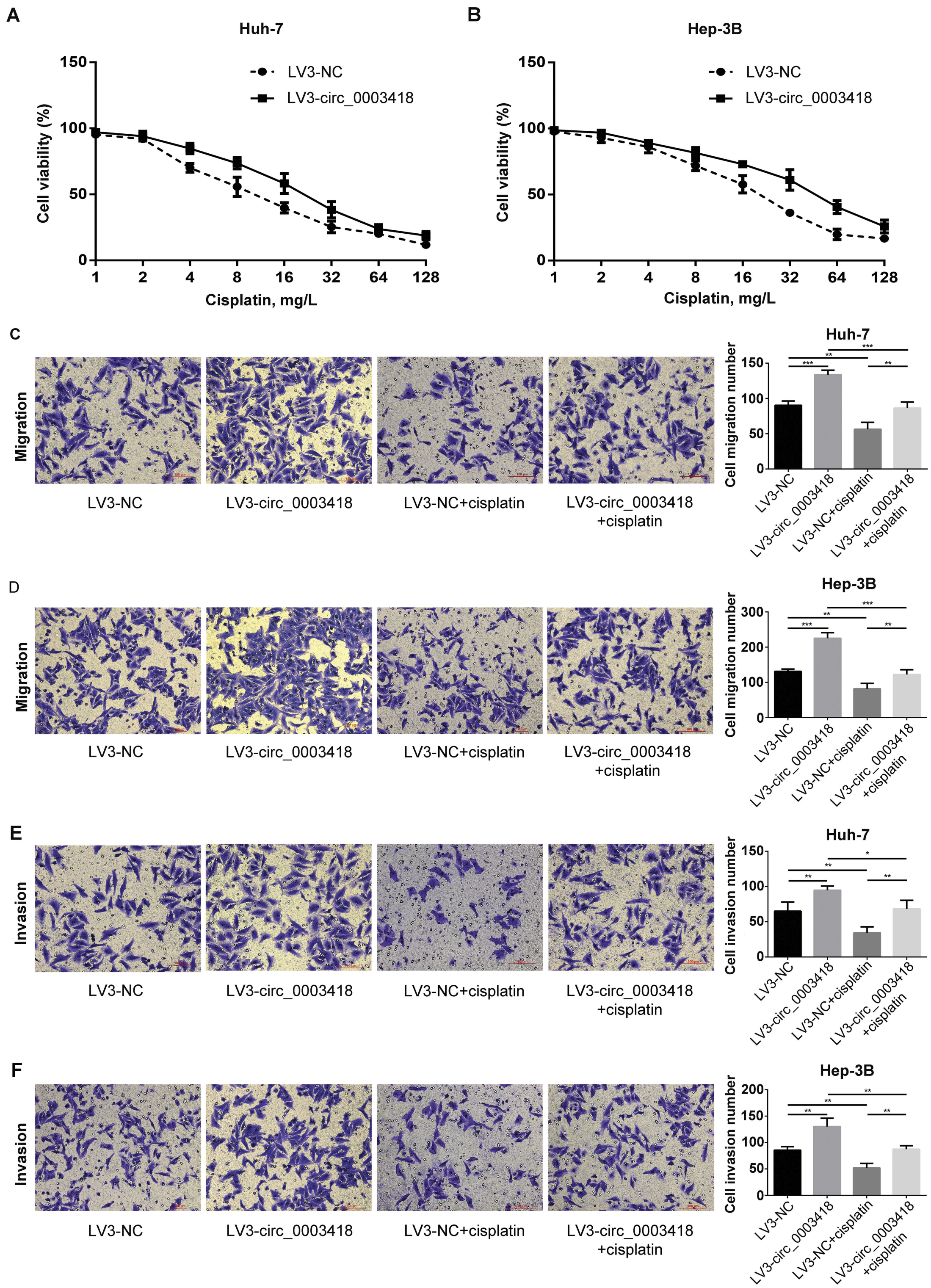

Figure 3 circ-0003418 sensitizes HCC cells to cisplatin in vitro.

Notes: (**A** and **B**) Huh-7 and Hep-3B cells infected with LV3-NC or LV3-circ_0003418 were treated with different doses of cisplatin (1, 2, 4, 8, 16, 32, 64 and 128 mg/L) for 24 hrs, and then cell viability was determined by CCK-8 assays. (**C–F**) Huh-7 cells were treated with cisplatin (11.39 mg/L) for 24 hrs as well as Hep-3B cells were treated with cisplatin (20.18 mg/L) for 24 hrs, and then cell migration and invasion were detected by transwell migration (**C** and **D**) and invasion (**E** and **F**) assays, respectively. * P<0.05, ** P<0.01 and *** P<0.001 compared to control group.

Abbreviation: NC, negative control.

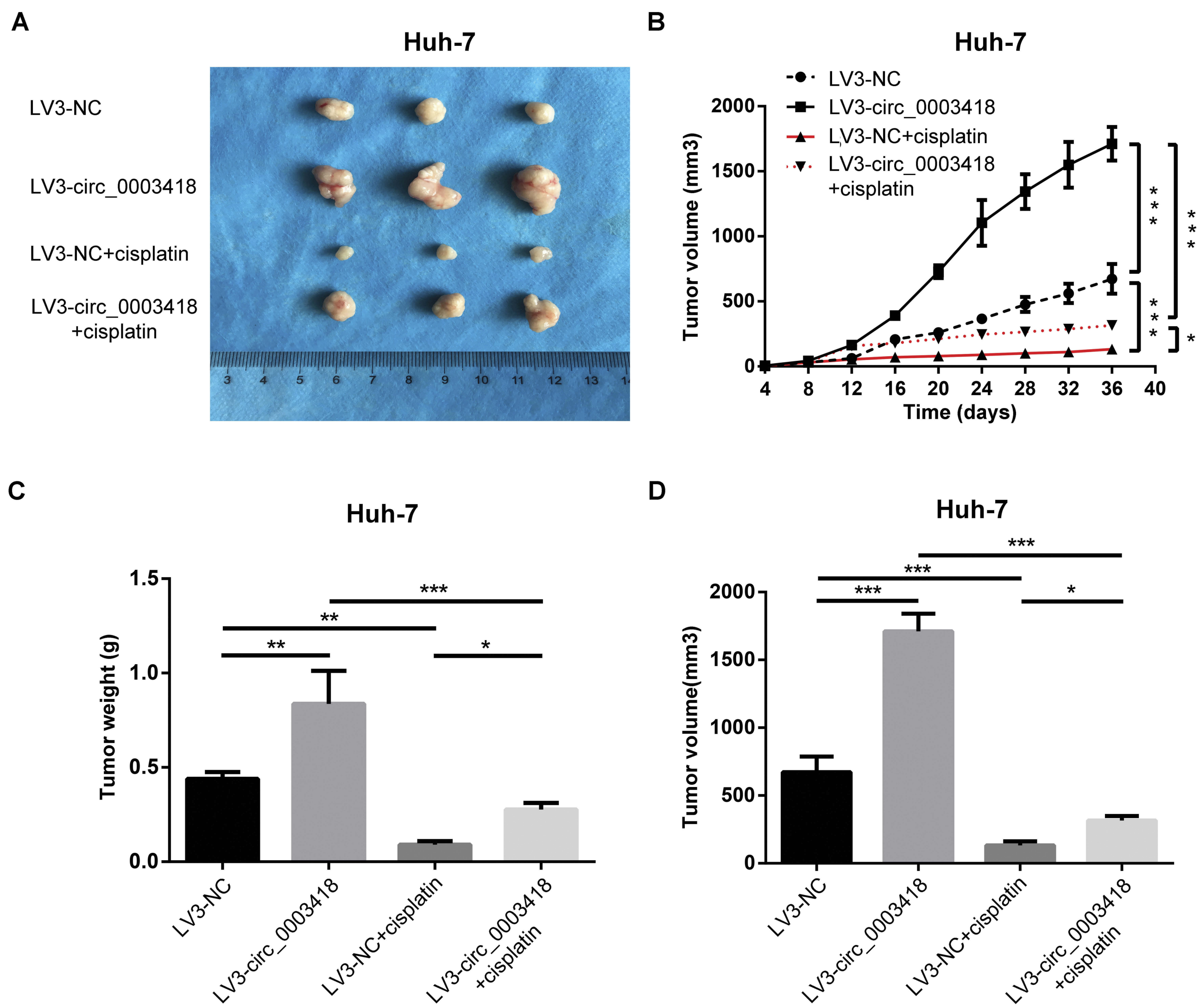

Figure 4 circ-0003418 enhances sensitivity of HCC cells to cisplatin in vivo. Female BALB/c nude mice were implanted subcutaneously with Huh-7 cells infected with LV3-NC or LV3-circ_0003418. Twelve days later, the LV3-NC tumor-bearing mice were treated with saline or cisplatin (5 mg/kg) by intraperitoneal injection twice a week up to 36 days posttreatment.[14] The mice bearing LV3-circ_0003418 tumors received the same treatment.

Notes: (**A**) Image of the tumors in the nude mice. (**B**) The tumors growth curve of xenograft model mice. (**C** and **D**) The weight and volume of the tumors in the nude mice. * P<0.05, ** P<0.01 and *** P<0.001 compared to control group.

Abbreviation: NC, negative control.

Discussion

Liver cancer is one of the most common malignancies.[16] The International Agency for Research on Cancer estimates that 841,000 cases and 782,000 deaths occurred worldwide during 2018, and HCC accounts for 75–85% of primary liver cancers.[2] Unfortunately, we lack the effective methods to treat HCC, especially for advanced HCC. The extensive use of systemic chemotherapy and transarterial chemoembolization (TACE) make HCC treatment to step into a new stage.[17] Cisplatin is considered as an important antitumour agent for HCC.[4] Like other tumors, HCC may initially be sensitive to cisplatin, but soon turn to resistance. Therefore, it is urgent to explore suitable biomarkers and clarify the molecular mechanisms of cisplatin chemoresistance.

circRNAs and miRNAs involve in important cancer phenotypes, such as proliferation, invasion, apoptosis and chemoresistance.[18–20] circRNAs function biologically via various molecular mechanisms. The most common molecular mechanism is that circRNAs regulate cell phenotypes by sponging miRNAs.[21] Given that overexpression of miR-7 advances the cisplatin chemosensitivity of lung adenocarcinoma cells by targeting Bcl-2, and miR-383 is significantly correlated with chemoresistance in HCC cells.[10,11] We conducted a bioinformatics research and found that circ-0003418

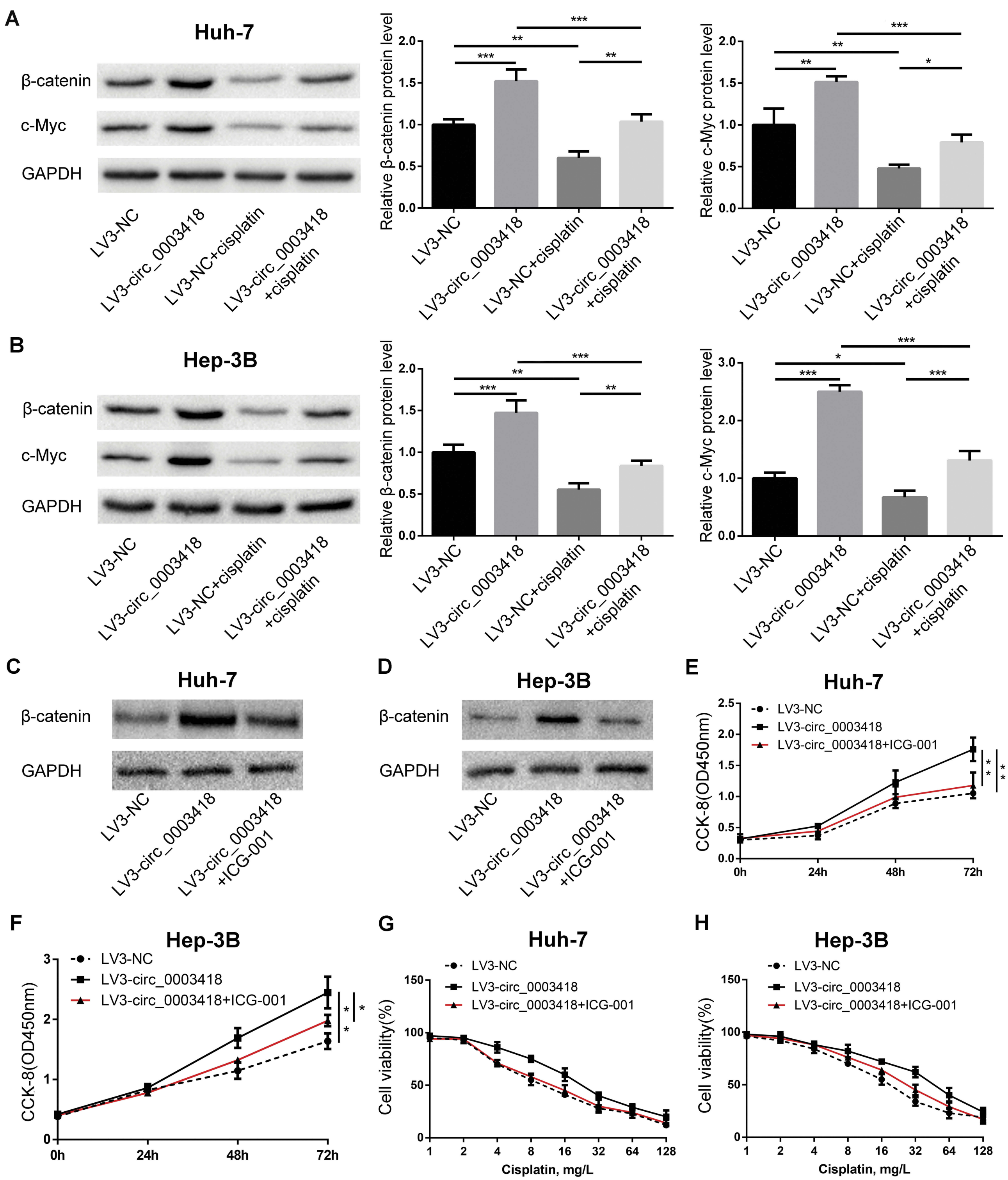

Figure 5 Silencing circ_0003418 induces cisplatin resistance of HCC cells through activating the Wnt/β-catenin pathway.

Notes: (**A** and **B**) Huh-7 cells were treated with cisplatin (11.39 mg/L) for 24 hrs as well as Hep-3B cells were treated with cisplatin (20.18 mg/L) for 24 hrs, and then the protein levels of β-catenin and c-Myc in the Huh-7 and Hep-3B cells were detected by Western blotting. (**C** and **D**) The inhibition efficiency of ICG-001 was detected by Western blotting. (**E** and **F**) Cell proliferation assay showed that inhibition of Wnt/β-catenin pathway in cells infected with LV3-circ_0003418 inhibited cell proliferation. (**G** and **H**) Chemotherapy sensitivity assay showed that inhibition of Wnt/β-catenin pathway in cells infected with LV3-circ_0003418 enhanced sensitivity of HCC cells to cisplatin. * P<0.05, ** P<0.01 and *** P<0.001 compared to control group.

Abbreviation: NC, negative control.

may target miR-7 and miR-383. Therefore, we hypothesized that circ-0003418 affects the biological behavior of HCC cells and participates in the regulation of cisplatin resistance. According to circBase, circ-0003418 is located on chromosome 6 (31860183–31860687).[22] First, we detected the expression of circ_0003418 in HCC tissues and cells, as well as explored the effect of circ_0003418 on proliferation, migration and invasion in HCC cells. We found that the expression of circ_0003418 was downregulated in HCC tissues and cell lines. circ_0003418 expression was negatively associated with tumor size, TNM stage and HBsAg level. circ_0003418 suppressed proliferation, migration and invasion in HCC cells. Subsequently, we explored the role of circ_0003418 in drug resistance. We found that circ-0003418 sensitized HCC cells to cisplatin in vitro and vivo.

Wnt/β-catenin signal pathway plays an indispensable role in embryonic development, the occurrence of tumors and the chemoresistance of cancers.[23,24] Inactivation of Wnt/β-catenin signaling, β-catenin is phosphorylated by GSK-3b. In the situation of Wnt-activating signals, phosphorylation of β-catenin by GSK-3b is inhibited.[25] β-Catenin is transferred into the nucleus where it engages DNA-bound TCF transcription factors and then influences gene transcription, such as c-Myc, survivin and cyclin D1[26] c-Myc, survivin and cyclin D1 can control cell cycle progress.[27,28] GSK-3b, a multifunctional serine–threonine protein kinase, is regulated positively by the phosphorylation of tyrosine 216 (pGSK-3b-tyr-216) and negatively by the phosphorylation of serine 9 (pGSK-3b-ser-9).[29,30] Recently, the roles for GSK-3b in regulating cisplatin resistance in non-small cell lung cancer and HCC were reported.[31,32] Cisplatin enhanced cytoplasmic GSK-3b activity in tumor cells by reducing the level of p-GSK-3bser9 and increasing the level of p-GSK-3btyr216, thereby reducing the expression of β-catenin and its downstream genes.[30] Given that Wnt/β-catenin pathway is important and intricate, we explored the effect of silencing circ_0003418 plus β-catenin inhibitor on proliferation and drug sensitivity to cisplatin in HCC cells. Our result of Western blotting assay revealed that β-catenin and c-Myc were affected by circ_0003418 and participated in the regulation of circ_0003418 mediated cisplatin sensitivity. Lastly, further research on the exhaustive mechanism about circ-0003418 advancing sensitivity of HCC cells to cisplatin is essential. In addition, whether circ-0003418 can combine with miRNAs or proteins in HCC requires further study.

Conclusions

In summary, circ_0003418, an anti-tumorigenic circRNA, was downregulated in HCC tissues and cells; circ_0003418 inhibited tumorigenesis and cisplatin chemoresistance through the Wnt/β-catenin pathway in HCC. These indicated that circ_0003418 may be a useful biomarker in HCC and a hopeful therapeutic target for the treatments of HCC.

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Disclosure

The authors report no conflicts of interest in this work.

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