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Circular RNA-ABCB10 suppresses hepatocellular carcinoma progression through upregulating NRP1/ABL2 *via* sponging miR-340-5p/miR-452-5p

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Abstract. – OBJECTIVE: Hepatocellular carcinoma (HCC) is one of the most common liver malignancies worldwide with a high rate of recurrence and mortality. Circular RNA-ABCB10 (circ-ABCB10), 724 nucleotides in length, plays a pro-oncogenic role in tumor progression. However, the role of circ-ABCB10 in HCC is still unknown. Therefore, the objective of this study was to determine the role of circ-ABCB10 in HCC progression *in vitro* and *in vivo* and to elucidate the underlying mechanism.

PATIENTS AND METHODS: Tumor tissues from patients with HCC and multiple HCC cell lines were used for *in vitro* experiments and a mouse xenograft model was used for *in vivo* experiments. Quantitative Real Time-PCR, Western blots, lentivirus transfection, cell proliferation assays, cloning formation, migration, and invasion assays, flow cytometry, Luciferase reporter assays, and biotin-coupled probe pull-down assays were performed to investigate the mechanism underlying the effect of circ-ABCB10 on HCC.

RESULTS: The results revealed that the expression of circ-ABCB10 was downregulated in both HCC tissues and cell lines and was positively correlated with histological grade and tumor size. The overexpression of circ-ABCB10 exerted inhibitory effects on HCC cell proliferation, invasion, and migration. Mechanistic and functional evidence together showed that circ-ABCB10 elevated expressions of neuropilin-1 (NRP1) and ABL related gene (ABL2) by sponging miR-340-5p and miR-452-5p, which inhibited the progression of HCC. Furthermore, the *in vivo* study suggests that circ-ABCB10 inhibited tumor growth in nude mice.

CONCLUSIONS: In brief, the results demonstrate that circ-ABCB10 exerts anti-tumor roles *via* miR-340-5p/miR-452-5p-NRP1/ABL2 signaling axis, providing a promising biomarker and therapeutic target for HCC.

Key Words:
Hepatocellular carcinoma, Circ-ABCB10, MiR-340-5p, MiR-452-5p, NRP1, ABL2.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common liver malignancy globally and a leading cause of cancer-related death[1], along with less than 5 percent of overall 5-year survival rate[2]. Due to the low efficacy of traditional chemotherapeutic intervention, surgical resection/liver transplantation has gradually become one of the few effective treatments for patients with HCC[3]. However, only about 40 percent of HCC-patients are suitable for such surgical operation thanks to the high heterogeneity and high rate of recurrence and metastasis in HCC[3,4]. Thus, it is essential and urgent to investigate the mechanism underlying HCC development and progression and explore novel targets or biomarkers for developing effective early diagnosis and treatments of HCC.

From the perspective of etiology, HCC is associated with a series of complicated etiological factors, including chromosomal instability, disrupted epigenetic regulation, and abnormal expression of both coding or noncoding genes[4-6]. Among these factors, aberrant expressions of circular RNAs (circRNAs), a group of endogenous noncoding RNAs characterized by covalently closed loops[7], have been demonstrated to be highly relevant to the development of HCC[8-10]. Qin et al[8] reported that the downregulation of circ-0001649 in HCC may serve as a biomarker related to tumorigenesis of HCC. Han et al[9] reported that circ-MTO1 exerts

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an inhibitory effect on HCC progression through the microRNA-9/p21 signaling pathway. Also, it has demonstrated that the high expression of circ-ciRS-7 is closely correlated with HCC progression and it may be a potential predictive factor of hepatic microvascular invasion in HCC[10]. Together, increasing studies in recent years demonstrate that circ-RNAs play essential roles in the progression and pathogenesis of HCC.

Circ-ABCB10, also named hsa_circ_0008717, is found at chr1:229665945-229678118 with 724 nucleotides in length in gene symbol ATP Binding Cassette Subfamily B Member 10 (ABCB10)[11]. In breast cancer, highly expressed circ-ABCB10 plays an important role in apoptosis and proliferation in cancer cells by silencing microRNA-1271[11]. Also, circ-ABCB10 acts as a pro-oncogenic factor to promote tumor cell proliferation, invasion, migration, and inhibits apoptosis in osteosarcoma[12]. Together, these researches demonstrate the potential function of circ-ABCB10 in tumorigenesis. Since the function of circ-ABCB10 in HCC has not been reported, the aim of the present study was to investigate the function of circ-ABCB10 in HCC progression *in vitro* and *in vivo* and elucidate the underlying mechanisms.

Patients and Methods

Patients and Samples

The study protocols were approved by the Ethics Committee of China-Japan Friendship Hospital of Jilin University (Approved No. of Ethic Committee: 2017110615) and all experimental procedures were conducted based on the Declaration of Helsinki Principles. In this study, all patients were informed before enrollment and consent was given before the study. All 25 pairs of adjacent normal and HCC tumor tissues were collected from patients who were diagnosed with HCC and undergone surgery in the China-Japan Friendship Hospital of Jilin University between 2015-2017. The 25 patients included 14 males and 11 females aged 34 to 66 years (median age: 51.4 years). All tissue samples were evaluated by two professional pathologists. Adjacent normal and HCC tissues were stored at -80°C before processing. The clinicopathological information was also collected from the same patients.

Cell Culture

The HCC cell lines (97H, 97L, BEL-7402, Hep3B, HepG2, SMMC-7721, and SNU423) and the human normal liver cell line (L-02) were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Xuhui District, Shanghai, China). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA) at 37°C with 5% CO_2.

Cell Transfection

A sequence of 724 bp of circ-ABCB10 cDNA was synthesized and cloned into PLCDH-cir vector to overexpress circ-ABCB10 (GenePharma, Shanghai, China). The miR-340-5p/miR-452-5p mimics were obtained from GenePharma Co., Ltd (Pudong New Area, Shanghai, China). The transfection procedures were performed according to the manufacturer's instructions of the Lipofectamine™ 3000 Transfection Reagent (Invitrogen, Waltham, MA, USA). After forty-eight hours of transfection, the treated cells were used for subsequent experiments.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

The total RNA was isolated from tissues and cells following the manufacturer's instructions for the RNeasy Mini kit (Qiagen, Hilden, Germany). Then, cDNAs were synthesized using the RNA PCR Core Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. Real Time-PCR reactions were conducted on a Lightcycler platform (Roche, Mannheim, Germany) in 10 µl reactions containing: 0.20 µL of cDNA, 4.50 µL of ddH2O, 0.3 µl of each primer, and 5 µL of 2×Fast Start Universal SYBR Green Master (Thermo Fisher Scientific, Tampa, FL, USA). The primers were designed with Primer Express 3.0 software (Applied Biosystems, Foster City, CA, USA) and synthesized by GenePharma Co., Ltd (Pudong New Area, Shanghai, China). Primer information was summarized in Table I. The data were analyzed using the $\Delta\Delta^{CT}$ method[13] and U6 and β-actin were used as constitutive controls for miRNA and mRNA analysis, respectively.

Western Blots

Cell protein was extracted using the cell lysis buffer (Beyotime Institute of Biotechnology, Songjiang District, Shanghai, China). The protein extracts were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electrically transferred to polyvinylidene difluoride (PVDF) membranes,

which were blocked with 5% nonfat dry milk in Phosphate-Buffered Saline 0.05% Tween-20 (PBST) for 1 hour. Next, the primary antibodies for neuropilin-1 (NRP1; 1:1000), ABL related gene (ABL2; 1:1000), and β-actin (1:2000) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were incubated at 4°C overnight. Then, the membranes were treated with the secondary antibody (1:5000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 hour at 37°C. Afterward, the protein abundances were quantified by enhanced chemiluminescence (ECL) assays (Thermo Fisher Scientific, Rockford, IL, USA) and the Uvitec Alliance software (Eppendorf, Hamburg, Germany)[14].

Cell Proliferation Assays

HCC cells were placed in 96-well plates (3000 cells per well). After 0, 24, 48, 72, and 96 hours of cell culture, cell viability was evaluated using the Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Kumamoto, Japan) according to the manufacturer's instructions. Quantification was performed using the microplate reader (Tecan, Männedorf, Switzerland) at 450 nm absorbance.

Cell Cloning Formation Assays

HCC cells were placed in 6-well plates (600 cells per well) and cultured in DMEM containing 10% FBS (Gibco, Grand Island, NY, USA). After 14 days, HCC cells were fixed with methanol and stained with 0.1% crystal violet. Cell colonies were imaged and quantified.

Migration and Invasion Assays

HCC cells (1×10^4) were subjected to the invasion assay (BD Biosciences, Franklin Lakes, NJ, USA) and the transwell assay (migration; BD Biosciences, Franklin Lakes, NJ, USA). The detailed protocols were consistent with the previous report[15]. There were 3-5 replicates in each treatment group.

Flow Cytometry Assays

HCC cell apoptosis was evaluated using the annexin V-APC/7AAD apoptosis kit (eBiosciences, San Diego, CA, USA) according to the manufacturer's instructions and quantified using flow cytometry (Attune NxT, Thermo Fisher, Waltham, MA, USA).

Luciferase Reporter Assays

The cells were co-transfected with miR-340-5p/miR-452-5p mimics and plasmids containing 3'-UTR of wild or mutant sequences of miRNA binding site in NRP1/ABL2 using the Lipofectamine™ 3000 Transfection Reagent (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. After 24 hours of transfection, the relative Luciferase activities were determined using the Dual-Luciferase reporter assay kit (Promega, Madison, WI, USA).

Biotin-Coupled Probe Pull-Down Assays

The biotinylated probe was synthesized to specifically bind to circ-ABCB10, and the oligo probe was used as a control. After washing with PBS, the cells (1×10^7) were lysed in lysis buffer and incubated with biotinylated probe (3 μg) for two hours. Next, the magnetic beads coated with streptavidin (Life Technologies, Carlsbad, CA, USA) were applied to pull-down the biotin-coupled RNA complex and were washed with lysis

Table I. Real time-PCR primer sequence.

Gene name	Primer sequence
NRP1_forward	5′-AAATGCGAATGGCTGATTCAG-3′
NRP1_reverse	5′-CTCCATCGAAGACTTCCACGTAGT-3′
ABL2_forward	5′-TAGCCACCATATCACAATGT-3′
ABL2_reverse	5′-GGCATCTTGATTATTATGTGG-3′
circ-ABCB10_forward	5′-CTAAGGAGTCACAGGAAGACATC-3′
circ-ABCB10_reverse	5′-GTAGAATCTCTCAGACTCAAGGTTG-3′
miR-340-5p_forward	5′-GCGGTTATAAAGCAATGAGA-3′
miR-340-5p_reverse	5′- GTGCGTGTCGTGGAGTCG-3′
miR-452-5p_forward	5′-AAGAGGGCATGGAAACACTG-3′
miR-452-5p_reverse	5′-ACTCACCCCATTCTTCAAGG-3′
U6_forward	5′-CGCTTCGGCAGCACATATACTAAAATTGGAAC-3′
U6_reverse	5′-GCTTCACGAATTTGCGTGTCATCCTTGC-3′
β-actin_forward	5′-CGCTTCGGCAGCACATATACTAAAATTGGAAC-3′
β-actin_reverse	5′-GCTTCACGAATTTGCGTGTCATCCTTGC-3′

buffer. MiRNAs in the pull-down complex were isolated using the TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) and detected by qRT-PCR.

Mouse Xenograft Model

All animal experimental protocols were approved by the Institutional Animal Care and Use Committee of China-Japan Friendship Hospital of Jilin University and the experimental procedures were performed following the approved guidelines. Hep3B cells were transfected with the GFP vector (control) and circ-ABCB10. Female BALB/c athymic nude mice (n=6, 21±1.32 g, 6 weeks old) were subcutaneously injected with transfected cells (1×10^7). Tumor growth was monitored weekly and the tumor volume (V) was quantified by measuring the length (L) and width (W) with a caliper and calculated with the formula $V = (L \times W^2) \times 0.5$. Five weeks post-injection, all mice were euthanized, and tumor volume and weight were measured.

Statistical Analysis

Statistical analysis was performed using SPSS v.19.0 software (SPSS, Chicago, IL, USA). Data were presented as means ± SD. The comparison between the two groups was analyzed by two-tailed *t*-tests and the multiple comparisons analysis was conducted by F-test ANOVA and the post-hoc Tukey test. Pearson's correlation analysis was applied to analyze the correlations. The differences were considered to be significant at $p<0.05$. For each treatment group, there were at least three replicates.

Results

Downregulation of Circ-ABCB10 was Correlated with the Progression of HCC

In this study, the expression of circ-ABCB10 in HCC tissues and the adjacent normal tissues were determined. The results showed that circ-ABCB10 was significantly downregulated in HCC tissues compared to normal tissues (Figure 1A). Next, the expression of circ-ABCB10 in multiple HCC cell lines was detected and circ-ABCB10 was decreased in all HCC cell lines including 97H, 97L, BEL-7402, Hep3B, HepG2, SMMC-7721, and SNU423, compared to the normal liver cell line L-02 (Figure 1B). The correlation between the expression of circ-ABCB10 and the clinicopathological parameters of patients with HCC was analyzed and the results revealed that the expression of circ-ABCB10 was positively correlated with tumor size and histological grade (Figure 1C), suggesting that circ-ABCB10 may be associated with the progression of HCC.

Overexpression of Circ-ABCB10 Inhibited the Progression of HCC

To determine the effect of circ-ABCB10 in HCC progression, PLCDH-ABCB10 vectors were applied to overexpress circ-ABCB10 in 97H and Hep3B cell lines. The overexpression efficiency of vectors was determined by qRT-PCR (Figure 1D). In functional experiments, the overexpression of circ-ABCB10 decreased cell viability in both 97H and Hep3B cell lines, as determined by CCK-8 assays (Figure 1E). Also, the upregulation of circ-ABCB10 was associated with reduced migration and invasion capabilities in 97H and Hep3B cells, as shown in transwell migration and invasion assays (Figure 1F, 1G). Wound healing assays revealed that the cells with overexpression of circ-ABCB10 showed a slower closing of scratch wounds (Figure 1I). Moreover, the number of cell colonies in PLCDH-ABCB10-transfected 97H and Hep3B cells was less than those of the control cells, as determined by the colony formation assays (Figure 1J). Furthermore, flow cytometry assays demonstrated that the overexpression of circ-ABCB10 promoted apoptosis in both 97H and Hep3B cell lines (Figure 1H). Taken together, the results indicated that circ-ABCB10 may play an inhibitory effect on the progression of HCC.

Circ-ABCB10 Acted as Sponge for MiR-340-5p/MiR-452-5p

The multiple bioinformatics databases, such as TargetScan (www.targetscan.org), MiRanda (www.microrna.org), and RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/), were used to find the putative circ-ABCB10 binding site in miR-340-5p and miR-452-5p. Next, biotin-coupled probe pull-down assays were performed to confirm the binding sites. The results showed that the expressions of circ-ABCB10, miR-340-5p, and miR-452-5p were detected in the complex pull downed by a special circ-ABCB10 probe in Hep3B cells (Figure 2A). Then, the expressions of miR-340-5p and miR-452-5p were detected in HCC tissues and the results showed that both miR-340-5p and miR-452-5p were increased in HCC tumor tissues compared to normal tissues (Figure 2B). Pearson's correlation analysis indicated a moderate negative correlation between the level

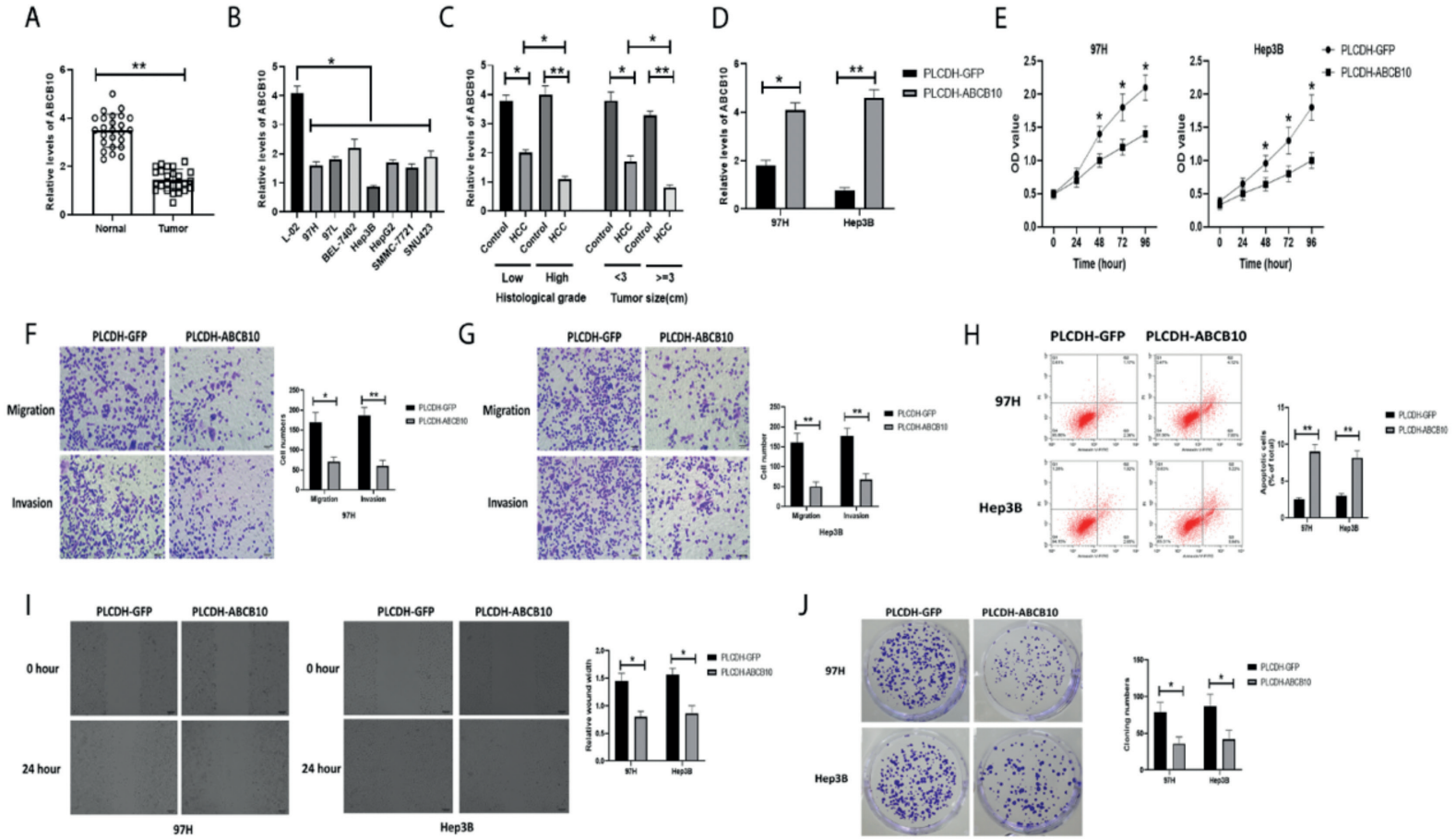

Figure 1. Circ-ABCB10 regulates tumor cell migration, invasion, growth, and colony formation in hepatocellular carcinoma. **A,** Circ-ABCB10 was decreased in HCC tumor tissue compared to adjacent normal tissues. **B,** Circ-ABCB10 was decreased in multiple HCC cell lines. **C,** Expression of circ-ABCB10 was correlated with histological grade and tumor size. **D,** Efficacy of PLCDH-vectors in overexpressing circ-ABCB10 in 97H and Hep3B cell lines. **E,** Overexpression of circ-ABCB10 inhibited cell proliferation in 97H and Hep3B cell lines. **F-G,** Overexpression of circ-ABCB10 inhibited cell migration and invasion in 97H and Hep3B cell lines (scale bar: 50 μM). **H,** Overexpression of circ-ABCB10 promoted apoptosis in 97H and Hep3B cell lines. **I,** Overexpression of circ-ABCB10 in 97H and Hep3B cell lines displayed a slower closing in wound healing assays (scale bar: 100 μM). **J,** Overexpression of circ-ABCB10 inhibited cell colony formation in 97H and Hep3B cell lines (magnification, × 100). Values are means ± SD. *p<0.05, **p<0.01, ***p<0.001.

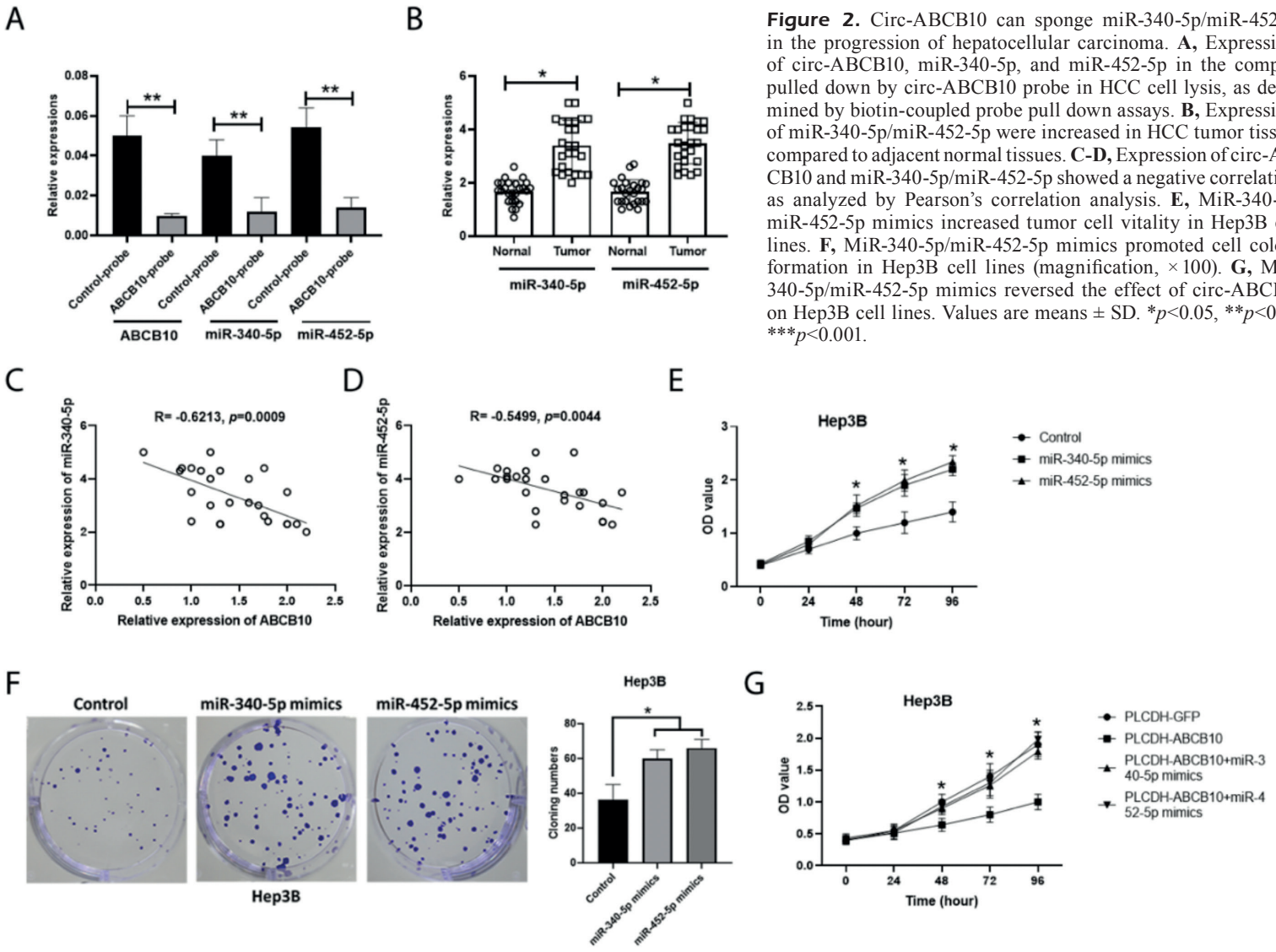

Figure 2. Circ-ABCB10 can sponge miR-340-5p/miR-452-5p in the progression of hepatocellular carcinoma. **A,** Expressions of circ-ABCB10, miR-340-5p, and miR-452-5p in the complex pulled down by circ-ABCB10 probe in HCC cell lysis, as determined by biotin-coupled probe pull down assays. **B,** Expressions of miR-340-5p/miR-452-5p were increased in HCC tumor tissues compared to adjacent normal tissues. **C-D,** Expression of circ-ABCB10 and miR-340-5p/miR-452-5p showed a negative correlation, as analyzed by Pearson's correlation analysis. **E,** MiR-340-5p/miR-452-5p mimics increased tumor cell vitality in Hep3B cell lines. **F,** MiR-340-5p/miR-452-5p mimics promoted cell colony formation in Hep3B cell lines (magnification, × 100). **G,** MiR-340-5p/miR-452-5p mimics reversed the effect of circ-ABCB10 on Hep3B cell lines. Values are means ± SD. *p<0.05, **p<0.01, ***p<0.001.

of circ-ABCB10 and miR-340-5p or miR-452-5p (Figure 2C, 2D). These results together suggested that circ-ABCB10 could sponge miR-340-5p and miR-452-5p. In addition, the overexpression of miR-340-5p/miR-452-5p could significantly increase cell viability and colony formation ability in Hep3B cells (Figure 2E, 2F), suggesting that miR-340-5p/miR-452-5p may be involved in the regulation of HCC progression. To further verify this, rescue experiments were performed by co-transfecting circ-ABCB10 and miR-340-5p/miR-452-5p mimics in Hep3B cells (Figure 2G).

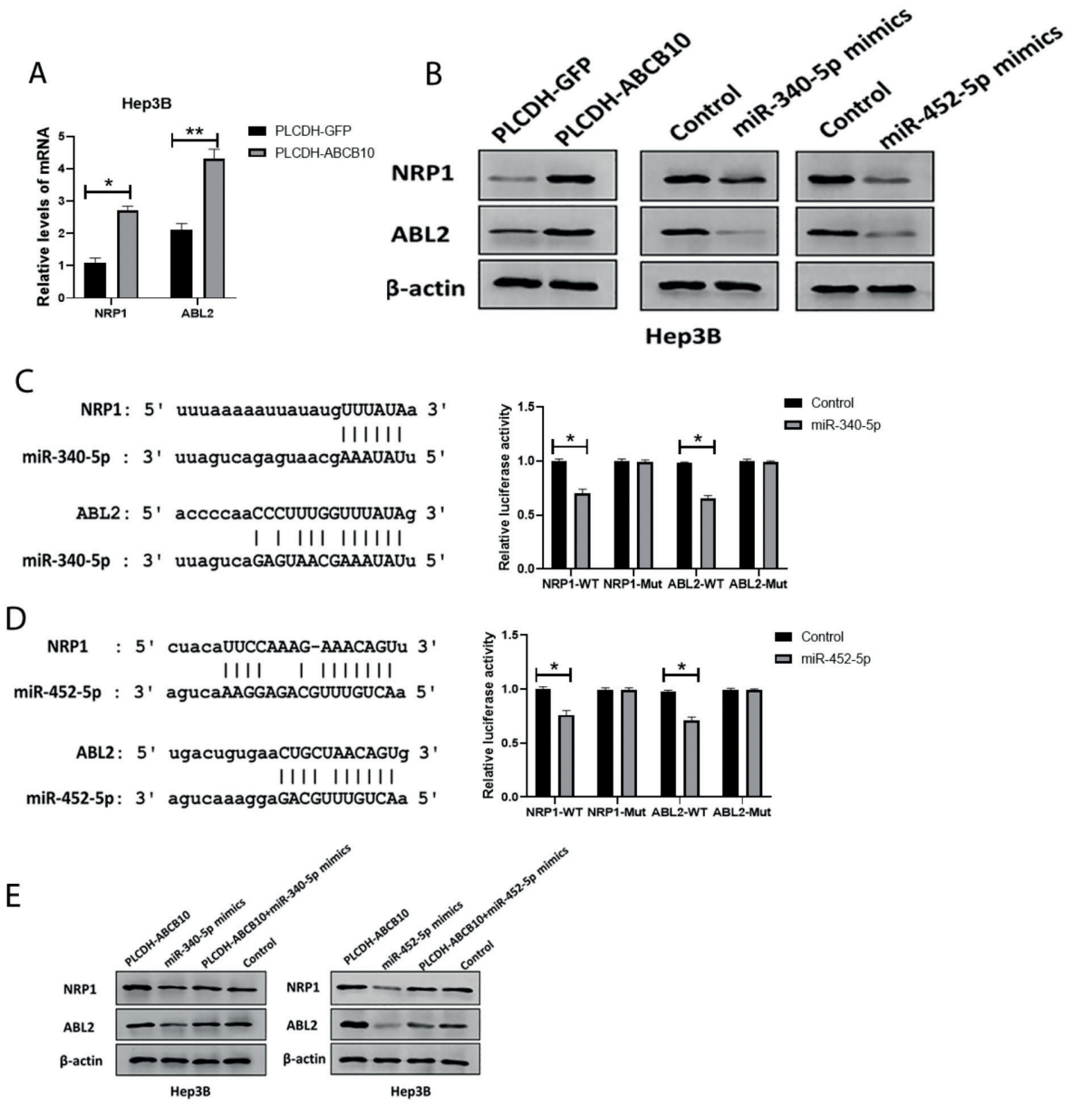

Figure 3. Circ-ABCB10 regulated the expressions of NRP1/ABL2 *via* sponging miR-340-5p/miR-452-5p. **A,** mRNA expressions of NRP1/ABL2 were increased in Hep3B cells with overexpression of circ-ABCB10. **B,** Protein expressions of NRP1/ABL2 were increased in Hep3B cells with overexpression of circ-ABCB10 whereas decreased in Hep3B cells with overexpression of miR-340-5p/miR-452-5p. **C,** Putative miR-340-5p binding sequence in the 3′UTR of NRP1/ABL2 and luciferase reporter assays. **D,** putative miR-452-5p binding sequence in the 3′UTR of NRP1/ABL2 and luciferase reporter assays. **E,** MiR-340-5p/miR-452-5p mimics reversed the effect of overexpression of circ-ABCB10 on protein expressions of NRP1/ABL2. Values are means ± SD. *p<0.05, **p<0.01, ***p<0.001.

The inhibitory effect of circ-ABCB10 on cell viability was attenuated by the overexpression of miR-340-5p/miR-452-5p, implying that circ-ABCB10 may inhibit HCC progression partly, at least, through miR-340-5p/miR-452-5p signaling.

Circ-ABCB10 Modulated the Expressions of NRP1/ABL2 Via Sponging MiR-340-5p/MiR-452-5p

Since miRNAs can also act as molecule sponges for genes by inhibiting gene expression[16], the downstream genes of circ-ABCB10/miR-340-5p/miR-452-5p signaling were investigated. Through analysis in the bioinformatic databases mentioned above, NRP1 and ABL2 contained common binding sites of miR-340-5p/miR-452-5p in their 3'UTR (Figure 3C, 3D). Thus, the levels of NRP1 and ABL2 in Hep3B cells transfected with PLCDH-ABCB10 were detected and the results revealed that mRNA expressions of NRP1 and ABL2 were increased (Figure 3A). Also, the protein expressions of NRP1 and ABL2 were elevated in Hep3B cells with the overexpression of circ-ABCB10, whereas were reduced in the cells with the overexpression of miR-340-5p/miR-452-5p (Figure 3B). Moreover, the Luciferase reporter assays revealed that the co-transfection of miR-340-5p/miR-452-5p mimics and reporter plasmids containing wild-type 3'UTR sequence of NRP1/ABL2 led to reduced relative Luciferase activity in Hep3B cells. On the contrary, co-transfection of miRNAs mimics and mutant-sequence containing plasmids could not affect Luciferase activity (Figure 3C, 3D), indicating that miR-340-5p/miR-452-5p can directly target NRP1 and ABL2. Furthermore, the overexpression of circ-ABCB10 could attenuate the inhibitory effect of miR-340-5p/miR-452-5p on the expressions of NRP1 and ABL2 (Figure 3E). Collectively, the results demonstrated that circ-ABCB10 may inhibit HCC progression through miR-340-5p/miR-452-5p-NRP1/ABL2 signaling axis.

Circ-ABCB10 Inhibited Tumor Growth in the Mouse Xenograft Model In Vivo

To investigate the effect of circ-ABCB10 *in vivo*, Hep3B cells transfected with PLCDH-ABCB10 vector were injected into nude mice and the tumor growth was monitored weekly by measuring the tumor volume (Figure 4A, 4B). The results revealed that the overexpression of circ-ABCB10 significantly decreased tumor volume (Figure 4C) and tumor weight (Figure 4D) in PLCDH-ABCB10 vector-injected mice, compared to the control group. Also, the protein expressions of NRP1 and ABL2 were increased in tumors of mice with overexpression of circ-ABCB10 (Figure 4E). Thus, the *in vivo* results demonstrate that circ-ABCB10 may act as an anti-oncogenic factor in HCC.

Discussion

Hepatocellular carcinoma, resulting in high mortality, is one of the most common liver malignancies worldwide[1]. Despite the existing clinical interventions, including surgery and chemoembolization, the high level of recurrence and metastasis are still challenging for both basic and clinical fields. Therefore, exploring the mechanism underlying the hepatocarcinogenesis is becoming increasingly urgent. We found that circ-ABCB10 may act as a tumor-inhibitor in HCC by sponging miR-340-5p/miR-452-5p and promoting NRP1/ABL2, indicating an important regulatory function of circ-ABCB10 in HCC.

CircRNAs, covalently closed single-stranded transcripts, are widely distributed in eukaryotic cells, most likely in the cytoplasm[17]. CircRNAs are essential for the regulation of gene and protein expression at both transcriptional and post-transcriptional levels, thereby playing critical roles in various biological and pathological processes[18]. CircRNAs are associated with the modulation of cancer development through diverse mechanisms, such as epithelial-mesenchymal transition and sponging miRNAs[18]. To date, previous researches reported the essential involvement of circRNAs in the development and progression of HCC. The downregulation of circ-0001649 may promote tumor growth and tumor embolus *via* the matrix metalloproteinase-9, 10, and 13 pathway[8]. The enhanced expression of circ-0005075 is associated with tumor size, which may act as a potential biomarker in HCC[19]. The present investigation showed a significant decrease of circ-ABCB10 expression in both HCC tissues and several HCC cell lines and a positive correlation between circ-ABCB10 expression and the histological grade and tumor size. The upregulation of circ-ABCB10 enhanced apoptosis and inhibited cell proliferation, thereby suppressing invasion and migration in HCC cells. Furthermore, the *in vivo* study revealed that circ-ABCB10 had inhibitory roles in tumor growth. Collectively, the results demonstrated an anti-oncogenic effect of circ-ABCB10 in HCC.

As one of the most important mechanisms of circRNAs in cancer regulation, circRNAs behave as miRNAs sponge molecules, modulating the expression of genes *via* silencing miRNAs[20]. We observed that circ-ABCB10 may physically interact with miR-340-5p/miR-452-5p and negatively correlate with the two miRNAs. According to several reported studies, miR-340-5p and miR-452-5p may be involved in the regulation of cancer progression. Upregulation of miR-340-5p promotes tumor cell proliferation by suppressing bone morphogenetic protein 4 (BMP4) in thyroid cancer[21]. Hepatitis B virus increases cell migration by inhibiting miR-340-5p in liver cancer[22]. Moreover, miR-452-5p regulates the tumor cell cycle by targeting cyclin-dependent kinase inhibitor 1B (CDKN1B) in lung squamous cell carcinoma[23]. Long noncoding RNA LINC00052 inhibits cancer cell migration and invasion by silencing miR-452-5p in HCC[24]. We found that the overexpression of miR-340-5p and miR-452-5p promoted cell proliferation and cloning ability in HCC cell lines. Thus, miR-340-5p and miR-452-5p may act as essential regulators in HCC development and progression.

Our results revealed that enforced expression of circ-ABCB10 is associated with the upregulation of NRP1 and ABL2, suggesting that there is a regulatory interaction between circ-ABCB10 and NRP1/ABL2. Since miRNAs can also act as molecule sponges for genes by inhibiting gene expression[16], circ-ABCB10 may regulate the expressions of NRP1/ABL2 by sponging miR-340-5p/miR-452-5p in the regulation of HCC development and progression. According to the Luciferase reporter assays and functional studies, miR-340-5p/miR-452-5p could promote HCC progression by targeting NRP1 and ABL2 in HCC. Jubb et al[25] reported that NRP1 acts as an anti-angiogenic target in various tumor types and plays an important role in vascular endothelial growth factor-associated angiogenesis in cancer progression. Meanwhile, the regulatory role of ABL2 is demonstrated in multiple cancer types, including prostate cancer[26], cervical carcinoma[27], and breast cancer[28]. Taken together, a regulatory signaling axis including circ-ABCB10, miR-340-5p/miR-452-5p, and NRP1/ABL2, was found in HCC and was related to tumor cell apoptosis and viability.

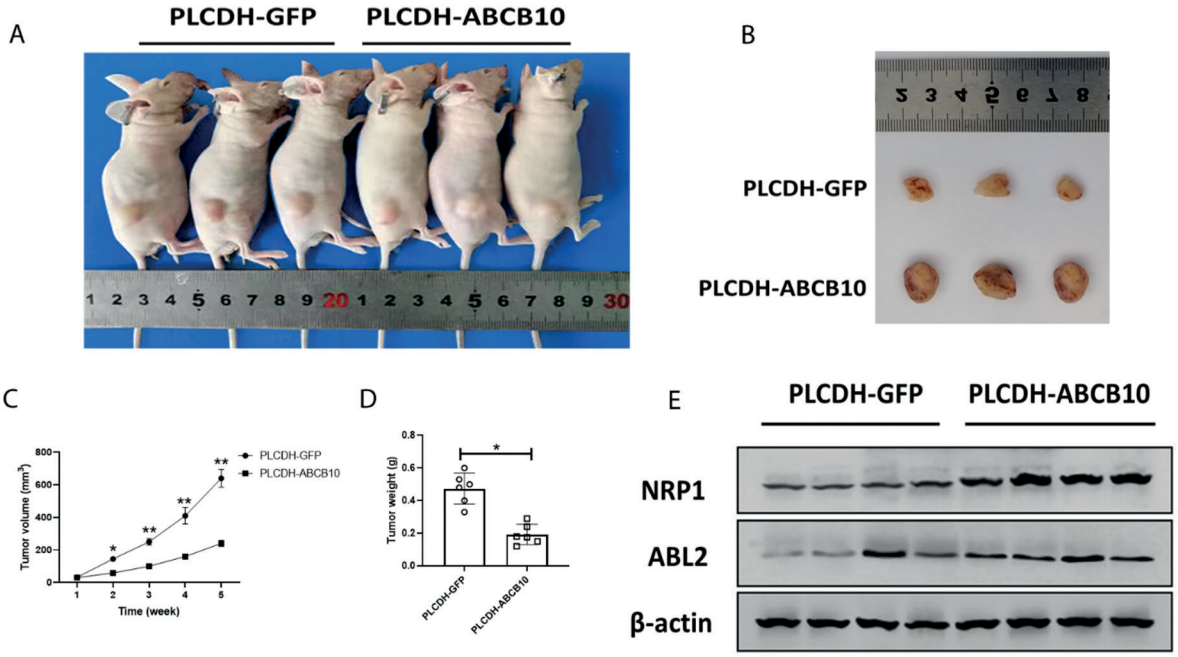

Figure 4. Circ-ABCB10 promoted tumor progression in the mouse xenograft model. **A,** Representative image of mice injected with Hep3B cells transfected with PLCDH-ABCB10. **B,** Representative image of tumor of mice injected with Hep3B cells transfected with PLCDH-ABCB10. **C-D,** xenograft mice with overexpression of circ-ABCB10 showed larger tumor volume and weight. **E,** protein expressions of NRP1/ABL2 were increased in xenograft mice with overexpression of circ-ABCB10. Values are means ± SD. *p<0.05, **p<0.01, ***p<0.001.

Conclusions

In summary, the results demonstrated that the downregulation of circ-ABCB10 is positively correlated with histological grade and tumor size. In addition, circ-ABCB10 plays anti-oncogenic roles in HCC by upregulating NRP1/ABL2 *via* sponging miR-340-5p/miR-452-5p. This study may provide a promising biomarker and therapeutic target for HCC.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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