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Circ_0009910 promotes proliferation and metastasis of hepatocellular carcinoma cells through miR-335-5p/ROCK1 axis

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Abstract. – OBJECTIVE: CircRNAs serve an essential role in regulating the development and progression of various tumors. The aim of this study was to examine the role and mechanism of circ_0009910 in hepatocellular carcinoma (HCC).

MATERIALS AND METHODS: RT-qPCR was used to detect the expression of circ_0009910 and miR-335-5p in tissues and cell lines of HCC. The proliferation, migration, and invasion of HCC cells were examined using 5-ethynyl-2-deoxyuridine (EdU), colony formation, and Transwell assay, respectively. Dual-luciferase reporter gene assay was performed to verify the interaction between miR-335-5p and circ_0009910 or ROCK1. Western blot was applied to detect the protein levels. Furthermore, the antitumor effect of circ_0009910 knockdown was examined by establishing xenograft tumor model of HCC *in vivo*.

RESULTS: Circ_0009910 was upregulated in HCC tissues and cell lines. Knockdown of circ_0009910 significantly inhibited the proliferation, migration, and invasion of HepG2 cells and suppressed tumor growth and metastasis *in vivo*. Moreover, circ_0009910 directly targeted miR-335-5p, as well as for ROCK1 was a direct target gene of miR-335-5p. Mechanically, simultaneous over-expression of miR-335-5p and circ_0009910 or ROCK1 could restore the biological behaviors of HepG2 cells, which were inhibited by miR-335-5p.

CONCLUSIONS: Circ_0009910-silenced suppressed the growth and metastasis of HCC cells through upregulating the inhibitory effect of miR-335-5p on ROCK1.

Key Words.
Hepatocellular carcinoma, Circ_0009910, MiR-335-5p, ROCK1, Proliferation, Metastasis.

Introduction

Hepatocellular carcinoma (HCC), accounting for 70-80% of primary liver cancer, is one the most common causes of death worldwide because of its occult incidence, high malignant degrees, strong invasion ability and poor prognosis[1,2]. In addition, the lack of early diagnosis and treatment, as well as the lack of effective therapeutic method in advanced stage are responsible for the high mortality of HCC[3]. Therefore, to explore the molecular mechanism of the development of HCC and search for effective molecular diagnostic biomarkers is extremely important to improve the survival rate of HCC patients.

The incidence and progression of tumors is a complicated pathological process with the participation of multi-factors and multi-mechanisms[4]. Circular RNAs (circRNAs) are newly discovered endogenous non-coding RNAs featuring structural stability, high abundance, and tissues-specific expression[5]. So far, accumulating evidence have proved that circRNAs serve an important role involved in the development and progression of multiple tumors[6,7]. Current studies confirmed that circRNAs were aberrantly expressed in tumor tissues and cell lines[8], and as a novel therapeutic target of multiple tumors including leukemic[9], ovarian cancer[10], gastric cancer[8], HCC[11] and lung cancer[12]. Moreover, circRNAs can be involved in many pathophysiological processes of tumors, including cell proliferation, migration, invasion, epithelial-mesenchymal transition (EMT), and apoptosis[13,14]. In fact, upregulation of circ cRAPGEF5[15], circPLEKHM3[10], or knockdown of circ_0005276[16], circ UBAP2 significantly inhibited the growth and metastasis of cancer cells. Furthermore, the role of circRNAs targets miRNA to regulate the biological behaviors of human cancer cells through mediating downstream gene has been reported broadly[17]. Zhu et al[18] found that circRNA PVT1 promoted the progression of human HCC though downregulating inhibitory effect of miR-203 on HOXD3 expression. An et al[19] reported that knockdown of

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circRNA ZMYM2 suppressed cell proliferation and invasion of pancreatic cancer by targeting miR-335-5p to decrease the expression of JMJD2C. Kai et al[20] showed that circRNA HIPK3 promoted gallbladder cancer cell growth and invasion *via* regulating miR-124/rho-associated protein kinase 1 (ROCK1) axis. These results indicated that circRNAs/miRNAs/mRNA axis may play an important role in regulating biological behavior of cancer cell to mediate cancer progression. Interestingly, Qiu et al[21] demonstrated that the expression of circ_0009910 was overexpressed in HCC tumor tissues and cell lines, while the role and mechanism of circ_0009910 in HCC are unknown. Therefore, we aimed to uncover the underlying mechanisms of the involvement of circ_0009910/miR-335-5p/ROCK1 axis in the regulation of tumor growth and metastasis of HCC. Collectively, our findings may provide a new therapeutic biomarker for HCC treatment in clinic.

Materials and Methods

Clinical Samples

The HCC tissues and their paired normal tissues were collected from patients (n=28) at the Liaoning Cancer Hospital and Institute from 2014 to 2016; none of the patients received chemotherapy or radiotherapy prior to surgery. The clinical specimens were immediately frozen at -80°C refrigerator after resection and prepared for further experiments. Besides, all the patients have signed the informed consent and the approval was also obtained from the Ethics Committee of the Liaoning Cancer Hospital & Institute. All the involved clinical experiments were conducted in accordance with the principle of "Declaration of Helsinki".

Cell Culture

The human HCC cell lines (HepG2, HCCLM3, MHCC97L, and Hep3B), human normal liver cell line L02 and HEK-293 cells were purchased from Shanghai Institutes for Biological Sciences of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in the Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific) in the incubator with humidified atmosphere containing 5% CO_2 at 37°C.

Transfection

The pcDNA-circ_0009910, circ_0009910 shRNA, miR-335-5p mimic, pcDNA-ROCK1 and scrambled negative control RNA were designed and constructed by Shanghai GenePharma Co., Ltd (Shanghai, China). All the above vectors were transfected into the HepG2 cells using the Lipofectamine 3000 kit purchased from Invitrogen (Thermo Fisher Scientific) according to the manufacturer's instruction.

RT-qPCR

The total RNA of tissues and cell lines was extracted using TRIzol reagent purchased from Life Technologies (Camarillo, CA, USA). After that, the TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, USA) were employed to reversely transcribed the total RNA to complementary DNA (cDNA). The SYBR Green PCR Master Mix (TaKaRa, Otsu, Shiga, Japan) was next used to quantify the expression levels of targeted genes at transcriptional levels. The relative mRNA and miRNA levels were normalized to β-actin and U6. The sequences of the involved primers were designed according to the previous studies[21, 22] and listed in Table I.

Western Blot

The polyvinylidene difluoride (PVDF) membranes were incubated overnight at 4°C with the primary antibodies ROCK1 (1:1500, Cell Signaling Technology, Danvers, MA, USA) and β-actin (1:1000, Cell Signaling Technology). The horseradish peroxidase (HRP)-coupled secondary antibody (1:2000, Cell Signaling Technology) was incubated with the membranes for 1 h at room temperature. The ECL western blot detection kit (Bio-Rad, Hercules, CA, USA) was employed to detect the optical density of the protein bands to evaluate the expression levels of the protein proteins.

Table I. Name and sequences of the primers.

Name	Primer sequences
circ_0009910	F: 5'-CAGGTTCTGGACGTCAAAGG-3' R: 5'-TCACCTCAGCCATGTGTCTC-3'
miR-335-5p	F: 5'-TCAAGAGCAATAACGAAAAATGT-3' R: 5'-GCTGTCAACGATACGCTACGT-3'
ROCK1	F: 5'-AACATGCTGCTGGATAAATCTGG-3' R: 5'-TGTATCACATCGTACCATGCCT-3'
U6	F: 5'-GGTCGGGCAGGAAAGAGGGC-3' R: 5'-GCTAATCTTCTCTGTATCGTTCC-3'
β-actin	F: 5'-AGAAAATCTGGCACCACACC-3' R: 5'-CAGAGGCGTACAGGGATAGC-3'

F: Forward primer; R: Reverse primer.

5-Ethynyl-2-Deoxyuridine (EdU) Staining

HepG2 cells were inoculated into 96-well plates with 1×10^4 cells per well and cultured in a 37°C incubator with 5% CO_2 for 1 h to adhere cells. After that, cells were incubated with 10 μM EdU for 2 h according to the manufacturer' s instructions. After enzymatic digestion and collection of cells, the percentage of EdU-positive cells was calculated and photographed with a scamming microscope (Olympus, Tokyo, Japan).

Colony Formation Assay

The HCC cell lines were transfected with different vectors. The cells were cultured under the standard conditions and collected until the cell confluency reached about 70-80%. After that, the cells were diluted and seeded into the 6-well plates at the density of 1000 cells per well for 14 days. The cells were then stained with crystal violet (Beyotime, Shanghai, China) for 45 min. The colony number was counted by using an inverted microscope produced by Thermo Fisher Scientific (Waltham, MA, USA).

Transwell Migration and Invasion Assay

The HepG2 cells were transfected with different vectors and collected until the cell confluency reached about 70-80%. 2×10^4 cells were paced on the surface of the Transwell upper chamber. For invasion assay, Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) was coated the upper surface of polycarbonate filters. After 24 h, the invaded and migrated cells were fixed with 4% paraformaldehyde and rinsed three times with PBS, then stained with 0.1% crystal violet for 10 min and rinsed three times with PBS. Next, a random selection of 5 fields of vision for cell count, observation and photography, was carried out.

Dual-Luciferase Reporter Gene System

The circ_0009910 or ROCK1 fragments containing miR-335-5p binding sites were synthesized to generate wild-type (WT)-circ_0009910 or WT-ROCK1 and mutant to produce mutant (MUT)-circ_0009910 or MUT-ROCK1. The WT-circ_0009910 or WT-ROCK1 and MUT-circ_0009910 or MUT-ROCK1 fragments were subcloned into the Renilla luciferase gene pGL3-Luciferase reporter vectors (Promega, Madison, WI, USA) to generated pGL3-WT (circ_0009910 or ROCK1) and pGL3-MUT (circ_0009910 or ROCK1) vectors, respectively. Besides, the miR-335-5p mimic and miR-NC were designed and synthesized by Shanghai GenePharma Co., Ltd (Shanghai, China). The above vectors were co-transfected into the HEK-293T cells for 24 h at 37°C. After that, the cells were lysed using the Dual-luciferase Assay Kit (Promega), and the luciferase activities were examined by luminescence plate reader (Molecular Devices Inc., San Jose, CA, USA).

Animal Model

All animal experiments were performed in accordance with the Laboratory Animal Care guidelines of Animal Ethics Committee of Liaoning Cancer Hospital & Institute. HepG2 cells were pre-transfected with circ_0009910 shRNA for 24 h. Afterward, 200 μL cells (1×10^7) were injected subcutaneously into the flanks of 6-week-old female nude mice (8 mice per group) and allowed to form tumors. Tumors length and width were measured every three days. After 4 weeks incubation, the tumor tissues were collected for further experiments, such as the immunohistochemistry staining was performed to detect the expression of Ki-67 (1:1500, Abcam, Cambridge, MA, USA), E-cadherin (1:1000, Abcam), and Vimentin (1:1500, Abcam) according to the previous studies[23].

Statistical Analysis

The data in this study are presented as mean ± standard deviation (SD) of 3 or 8 individual experiments and analyzed by one-way ANOVA test, and the difference between two groups was determined using the Student *t*-test, which was considered statistically significantly when $p<0.01$ or $p<0.001$. The Pearson Correlation analysis was performed using the GraphPad Prism (Version 8.0; La Jolla, CA, USA).

Results

Circ_0009910 Was Upregulated in Tissues and Cell Lines of HCC

Previous studies confirmed that circRNAs play an important role involved in the progression of multiples tumors including HCC[24]. In this study, we detected the expression of circ_0009910 in tissues and cell lines of HCC. As shown in Figure 1A, the expression level of circ_0009910 in HCC tissues was higher than the adjacent tissues ($p<0.001$). Moreover, circ_0009910 was significantly overexpressed in HCC cells compared with the human normal liver cell line L02 ($p<0.001$, Figure 1B), especially the HepG2 cells ($p<0.01$). Taken together, circ_0009910 was markedly overexpressed in HCC tissues and cell lines.

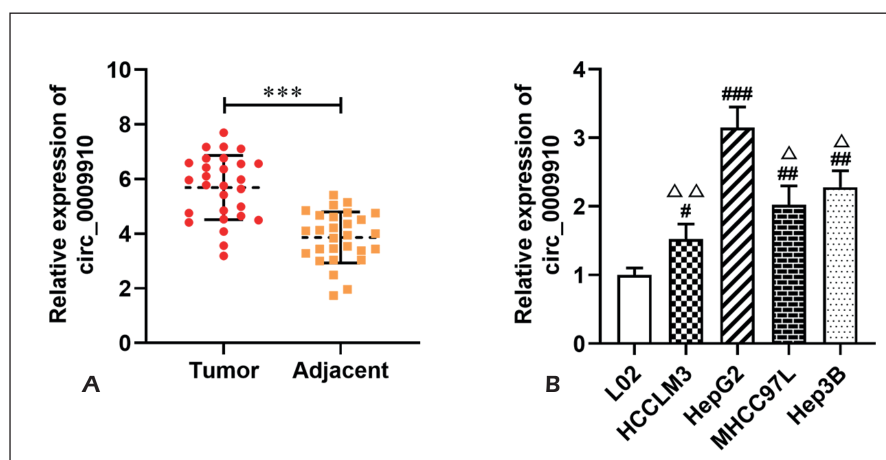

Figure 1. The expression of circ_0009910 in tissues and cell lines of hepatocellular carcinoma (HCC). **A**, RT-qPCR was used to detect the expression level of circ_0009910 in HCC tissues and paired adjacent tissues; **B**, RT-qPCR was applied to measure the expression of circ_0009910 in HCC cell lines and human normal liver cell line L02. Data were presented as mean ± standard error of the mean. $^{***}p<0.001$, compared with the adjacent tissues; $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, compared with the L02 cells; $^{\Delta}p<0.05$, $^{\Delta\Delta}p<0.01$, compared with the HepG2 cells.

Knockdown of Circ_0009910 Suppressed the Proliferation, Invasion and Migration of HCC Cells

To explore the effect of circ_0009910 on the biological behavior of HCC cells *in vitro*, we transfected with circ_0009910 shRNA or control into HepG2 cells and determined its expression using RT-qPCR ($p<0.001$, Figure 2A). EdU assay and colony formation results showed that knockdown of circ_0009910 significantly inhibited cell proliferation compared with the sh-NC group (both $p<0.01$, Figure 2B, C). Moreover, the results of Transwell showed that knockdown of circ_0009910 decreased the migration and invasion capacity of HepG2 cells (both $p<0.001$, Figure 2D, E). Taken together, knockdown of circ_0009910 significantly inhibited cell proliferation and metastasis of HCC cells.

Circ_0009910 Is Targeted by miR-335-5p

Previous studies confirmed that circRNAs regulated the development and progression of tumor through targeting miRNA[25]. In this study, bioinformatics tools (http://starbase.sysu.edu.cn/) were applied to analyze the potential interaction between circ_0009910 and miRNAs. The result showed that miR-335-5p was selected to the potential target of circ_0009910, and the potential binding site between miR-335-5p and circ_0009910 was shown in Figure 3A. Meanwhile, previous studies have been showed that miR-335-5p was correlated with the progression of multiple tumors[26]. As expected, RT-qPCR showed that miR-335-5p was lowly expressed in the HCC tumor tissues compared with the adjacent tissues ($p<0.001$, Figure 3B). Similarly, Spearman's correlation analysis revealed a remarkably negative correlation between the expression of circ_0009910 and miR-335-5p in HCC tumor tissues (r=-0.594, $p<0.001$, Figure 3C). Moreover, the dual-luciferase reporter gene analysis results showed that overexpression of miR-335-5p decreased the luciferase activity of the WT reporter compared with the NC group ($p<0.01$, Figure 3D), but had no effect on the luciferase activity of the MUT report vector. Furthermore, knockdown of circ_0009910 significantly enhanced the expression of miR-335-5p in HepG2 cells ($p<0.001$, Figure 3E). The above results showed that the target gene of circ_0009910 was miR-335-5p, and negatively regulated the expression of miR-335-5p.

ROCK1 Was a Target Gene of MiR-335-5p

Furthermore, we discovered that miR-335-5p may target at ROCK1 directly from the starBase database (Figure 4A). To further confirm that, the luciferase activity in ROCK1-WT+ miR-335-5p mimics group was lower than ROCK1-WT+miR-NC group ($p<0.01$, Figure 4B), while there was no significant difference between miR-335-5p mimics+ ROCK1-MUT group and NC+ ROCK1-MUT group. Moreover, upregulation of miR-335-5p significantly decreased the level of ROCK1 protein compared with the NC group ($p<0.01$, Figure 4C). Taken together, our data suggested that ROCK1 was the direct target gene of miR-335-5p.

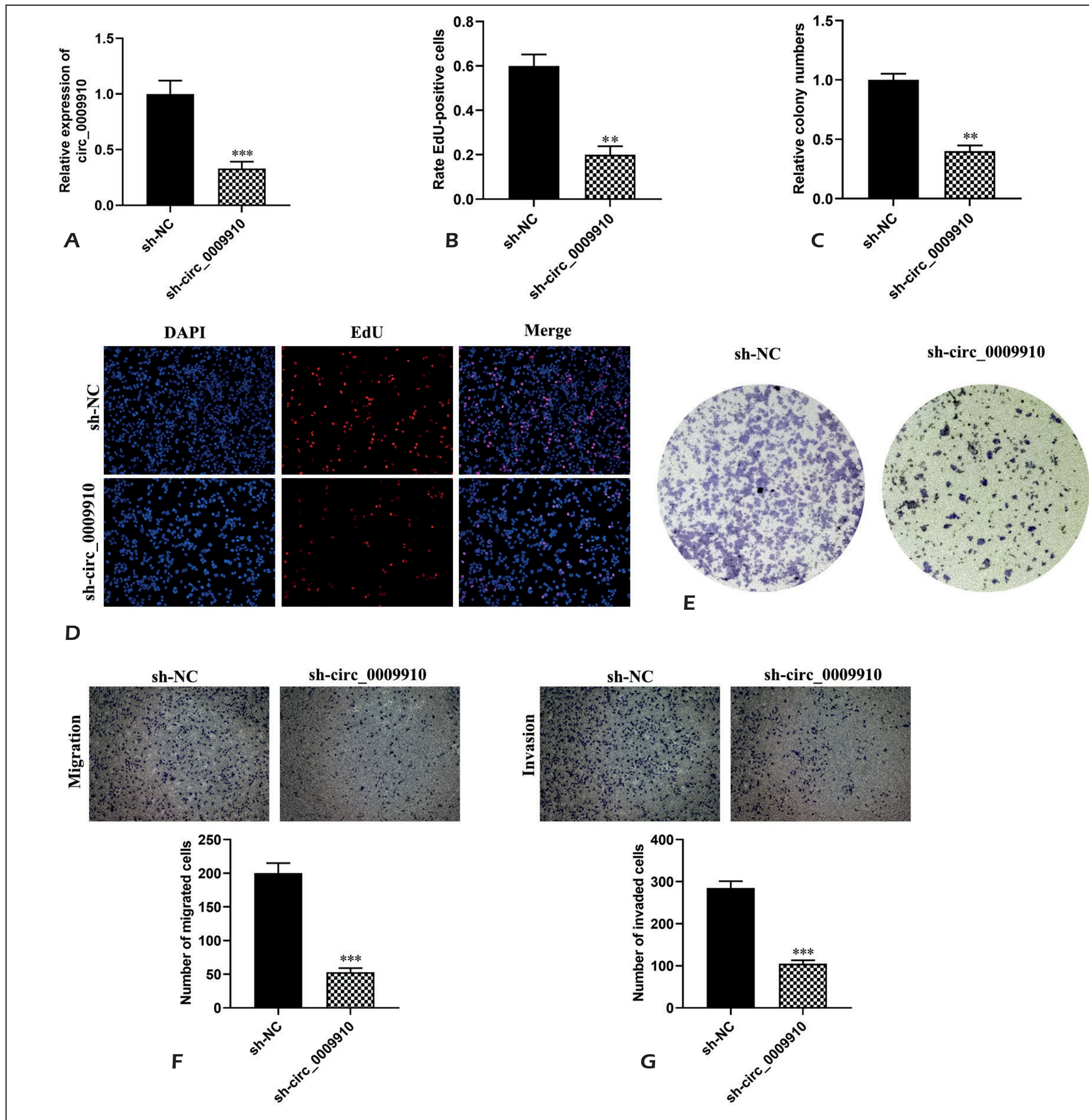

Figure 2. The effect of circ_0009910 knockdown on the proliferation, migration and invasion of HepG2 cells. **A**, The expression of circ_0009910 in HEpaG2 cells transfected with sh-circ_0009910 and control was measured using RT-qPCR; **B** and **D**, The proliferation ability of HepG2 cells was detected using EdU assay; **C** and **E**, Colony formation experiment was used to evaluate the number of colony formation of HepG2 cells; **F** and **G**, Transwell assay was performed to examine the migration and invasion capacity of HepG2 cells. Data were presented as mean ± standard error of the mean. ***p*<0.01, ****p*<0.001, compared with the sh-NC group.

Silencing of Circ_0009910 Inhibited Cell Proliferation, Invasion and Migration in HCC Cells Via Targeting miR-335-5p to Decrease the Expression of ROCK1

To further examined whether circ_0009910 regulated the malignant biological behavior through miR-335-5p/ROCK1 axis. RT-qPCR analysis results showed that the expression of miR-335-5p in HepG2 cell was significantly increased after transfecting with miR-335-5p mimic (p<0.001, Figure 5A), while co-transfected pcDNA-circ_0009910 could abolish this effect (p<0.001). Moreover, EdU and colony formation assay revealed that overexpression of miR-335-5p significantly suppressed HepG2 cell proliferation compared with the control group (both p<0.001,

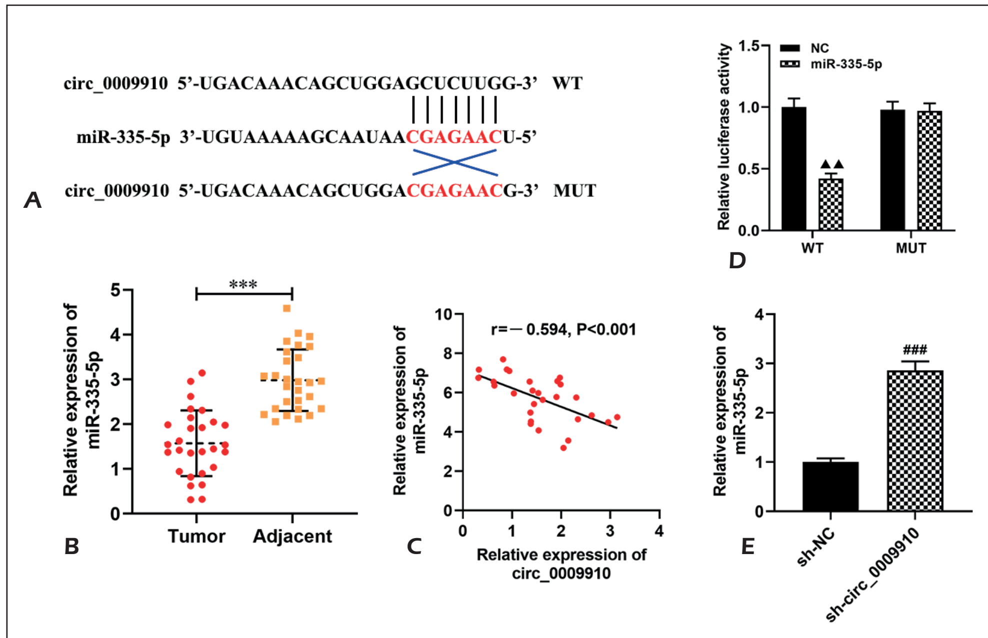

Figure 3. circ_0009910 can be a direct target of miR-335-5p. **A**, Binding sites of miR-335-5p on circ_0009910 were predicted; **B** and **C**, RT-qPCR was used to examine the expression of miR-335-5p in HCC tissues and cell lines, **D**, Luciferase reporter assays was applied to verify the interaction between miR-335-5p and circ_0009910; E, RT-qPCR was performed to detect the expression of miR-335-5p in HepG2 cell transfected with sh-circ_0009910 and control. Data were presented as mean ± standard error of the mean. ***p<0.001, compared with the adjacent tissues; $^{\Delta\Delta}p$<0.01, compared with the L02 cells; $^{\#\#\#}p$<0.001, compared with the sh-NC group.

Figure 5B-E). Furthermore, Transwell assay results showed that upregulation of miR-335-5p decreased the number of migrated and invaded HepG2 cells (both p<0.001, Figure 5F-I). Of note, HepG2 cells co-transfected with miR-335-5p mimic+pcDNA-ROCK1 or miR-335-5p mimic+pcDNA-circ_0009910 repressed the inhibitory effect of miR-335-5p on the proliferation,

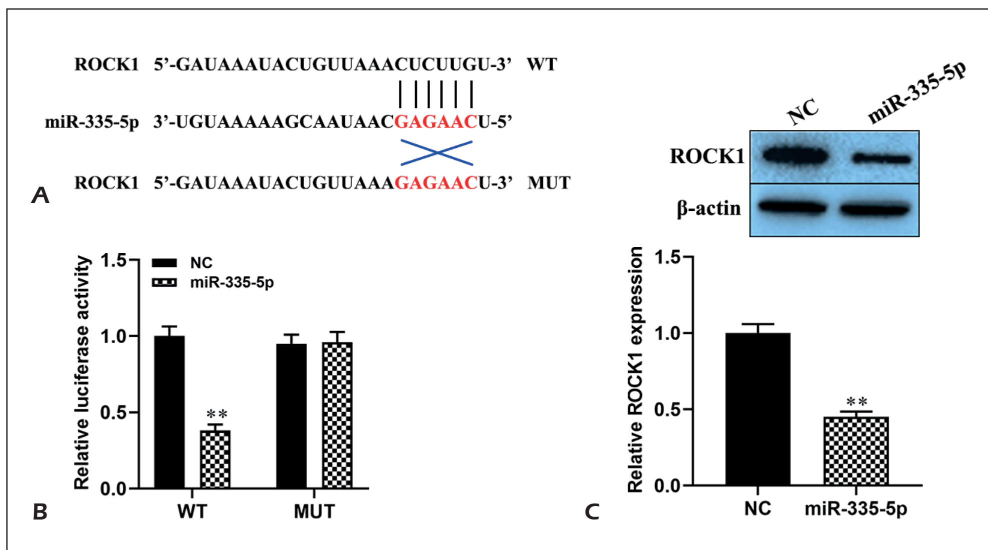

Figure 4. ROCK1 was a target gene of miR-335-5p. **A**, Binding sites of miR-335-5p on ROCK1 were predicted; **B**, Luciferase reporter assays was applied to verify the interaction between miR-335-5p and circ_0009910; **C**, Western blot was performed to detect the protein level of ROCK1 in HepG2 cell transfected with miR-335-5p mimic and control. Data were presented as mean ± standard error of the mean. **p<0.01, ***p<0.001, compared with the NC group.

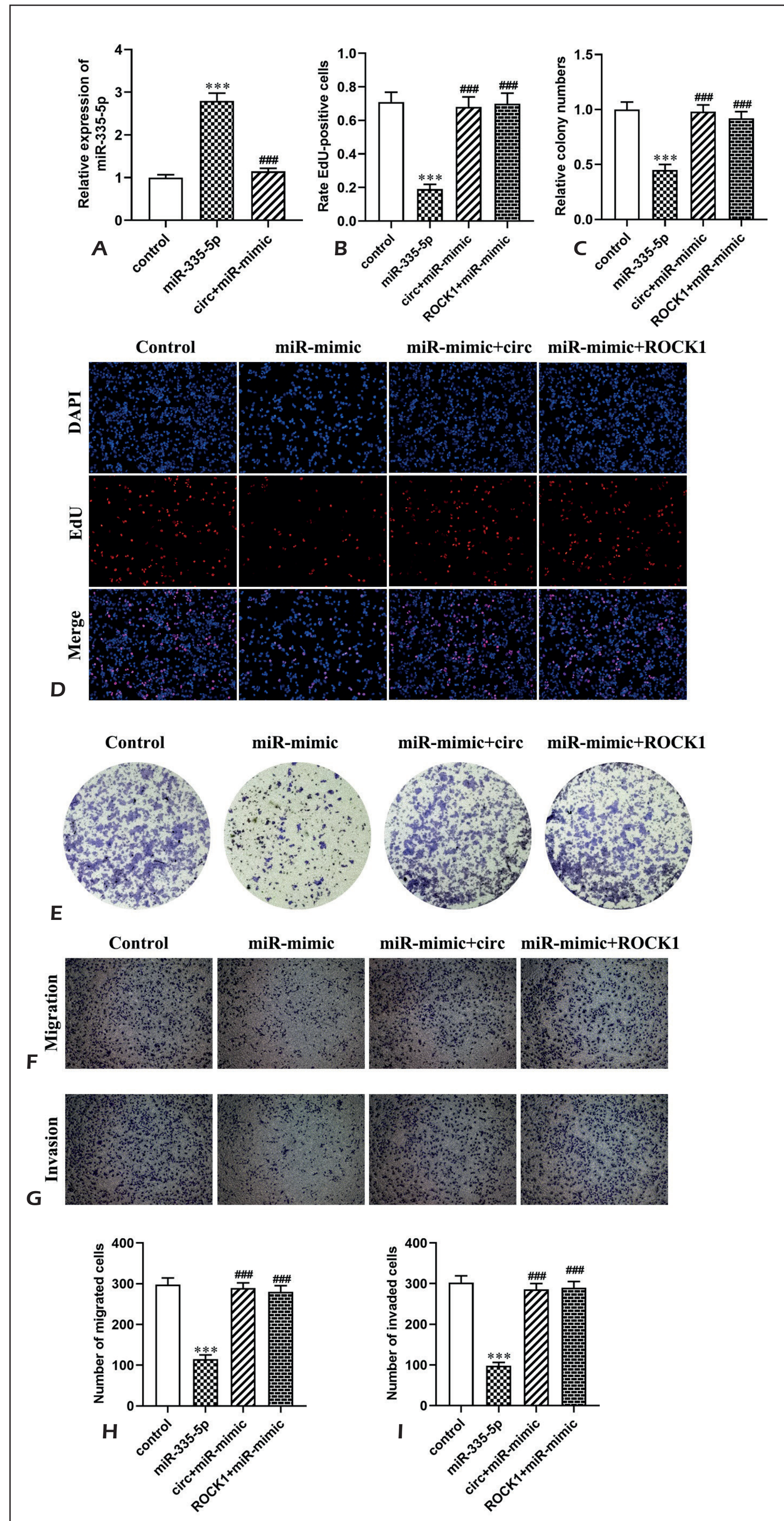

Figure 5. The effect of circ_0009910/miR-335-5p/ROCK1 axis on the biological behavior of HepG2 cells. **A**, The expression of circ_0009910 in HepG2 cells transfected with miR-335-5p mimic, miR-335-5p mimic+pcDNA-circ_0009910 and control; **B** and **D**, The proliferation ability of HepG2 cells was detected using EdU assay; **C** and **E**, Colony formation experiment was used to evaluate the number of colony formation of HepG2 cells; **F-I**, The Transwell assay was performed to examine the migration and invasion capacity of HepG2 cells. Data were presented as mean ± standard error of the mean. ***p<0.001, compared with the control group; ###p<0.001, compared with the miR-335-5p group. MiR-335-5p: miR-335-5p mimic; miR-mimic+circ: miR-335-5p mimic+pcDNA-circ_0009910; miR-mimic+ROCK1: miR-335-5p mimic+ROCK1.

migration and invasion of HCC cells ($p<0.001$). However, there was no significant difference between the control group and the miR-335-5p mimic+pcDNA-ROCK1 group or miR-335-5p mimic+pcDNA-circ_0009910 group. Taken together, overexpression of miR-335-5p by circ_0009910 inhibition suppressed the malignant biological behavior of HCC cells through targeting ROCK1.

Circ_0009910 Knockdown Inhibited HCC Tumorigenesis In Vivo

We further examined the role of circ_0009910 in tumor growth and metastasis of HCC *in vivo*. As shown in Figure 6A, knockdown of circ_0009910 remarkably decreased the tumor growth ($p<0.01$) and tumor weight ($p<0.001$) compared with the control group. Moreover, immunohistochemical staining showed that the expression of Vimentin and Ki-67, a proliferative marker, was decreased in tumor tissues after circ_0009910 knockdown (Figure 6C) but enhanced the expression of E-cadherin. Furthermore, RT-qPCR analysis results showed that the expression of miR-335-5p was enhanced in tumor tissues when inhibition of circ_0009910 ($p<0.001$, Figure 6D), as well as decreased the mRNA level of ROCK1 ($p<0.001$, Figure 6E). In conclusion, knockdown of circ_0009910 suppresses the tumor growth and metastasis of HCC *in vivo*.

Discussion

HCC is the main type of primary hepatic carcinoma and one of the most popular malignancies around the world. Though various treat-

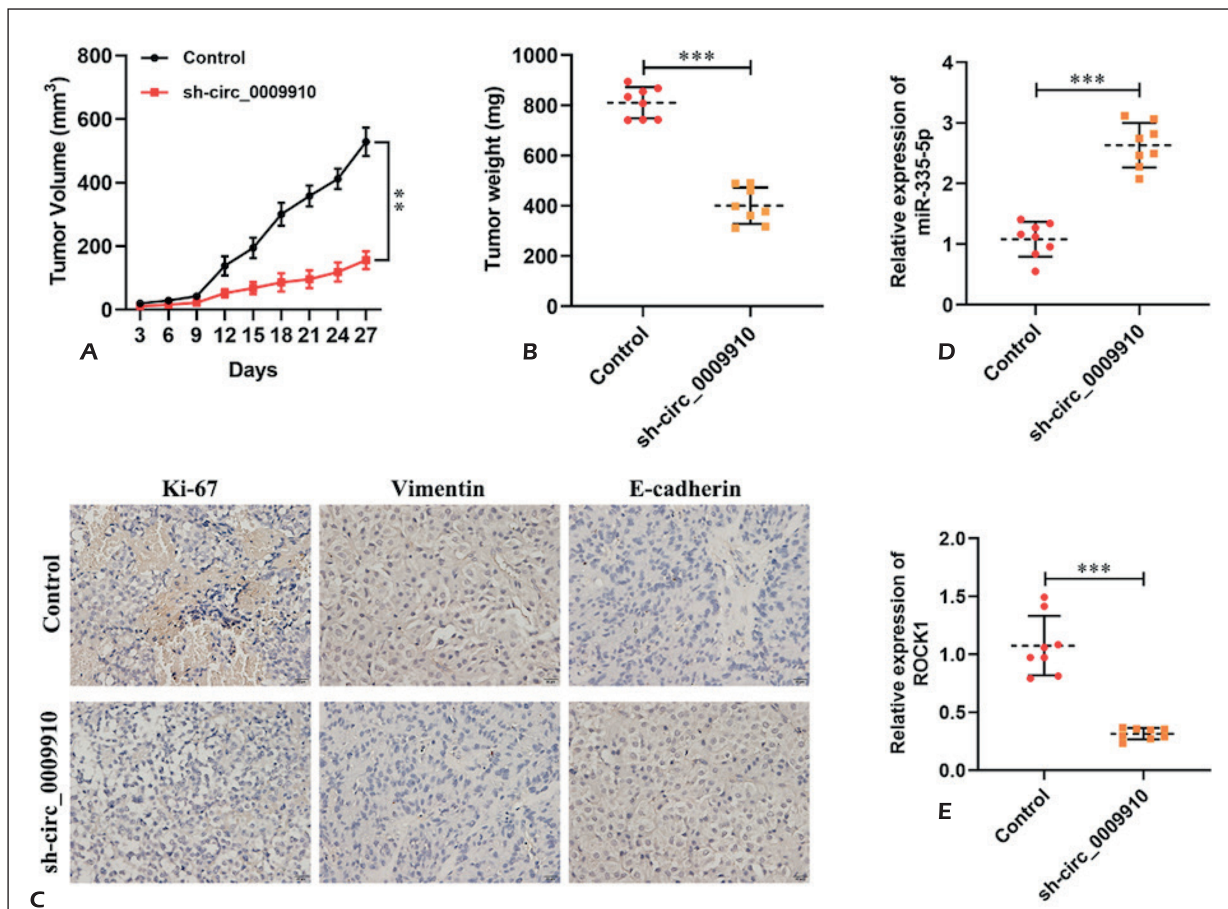

Figure 6. The effect of circ_0009910 knockdown on tumor proliferation and metastasis of HCC *in vivo*. **A**, The tumor volume curve of nude mice; **B**, The tumor weight in sh-circ_0009910 group and control group was measured; **C**, The expression of Ki-67, Vimentin and E-cadherin was detected in tumor tissues by immunohistochemistry staining. Scale bar=20 µM; **D** and **E**, RT-qPCR was used to measure the expression of miR-335-5p and ROCK1 in tumor tissues. Data were presented as mean ± standard error of the mean. **$p<0.01$, ***$p<0.001$, compared with the control group.

ment strategies, HCC is still the second leading cause of cancer-associated mortality worldwide, especially in China, where the morbidity and mortality rates of HCC are particularly high[27]. However, the reliable biomarkers for tumorigenesis and effective treatment methods of HCC are limited. In the present study, we demonstrated that circ_0009910/miR-335-5p/ROCK1 axis play a vital role in the development and progression of HCC. Inhibition of circ_0009910 significantly inhibited cell proliferation, migration and invasion of HCC. Mechanically, circ_0009910 knockdown exhibits anti-proliferation and anti-metastasis activities through upregulating the inhibitory effect of miR-335-5p on the expression of ROCK1 in HCC.

CircRNAs, as novel potential biomarkers for cancer[28], were aberrantly expressed in tumor tissues, cell lines and plasma exosomes[29,30]. Xu et al[31] found that circRNAs were abnormally expressed in colorectal cancer though the secondary sequencing. Similarly, Qiu et al[21] also found that 560 circRNAs were differentially expressed in HCC tissues and circ_0009910 was one of the most upregulated circRNAs. Of note, circRNAs are closely related to the development and progression of various tumors trough regulating malignant biological behavior of cancer cells. For example, circ_0000144 was upregulated in gastric cancer tissues and cell lines, and knockdown of circ_0000144 inhibited cell proliferation[8]. Downregulation of circRNA cRAPGEF5 was correlated positively with poor overall survival and aggressive clinical characteristics, and overexpression of circRNA cRAPGEF5 suppressed the growth and metastasis of renal cell carcinoma[15]. In line with previous studies, we explored the role of circ_0009910 in the proliferation and metastasis of HCC for the first time. As expected, we found that circ-0009910 was overexpressed in HCC tissues and cell lines and silencing of circ_0009910 markedly inhibited HCC cell proliferation, migration and invasion. It has been already demonstrated that circRNA regulates cancer cell proliferation and metastasis by binding miRNAs to mediate mRNA degradation and post-transcriptional translation inhibition. Guan et al[32] reported that circ_0016788 promoted the proliferation, invasion and decreased apoptosis of HCC cell through targeting miR-486 to enhance CDK4 expression. In the present study, our data showed that circ_000910 knockdown suppressed tumor growth and metastasis by regulating miR-335-5p/ROCK1 axis in HCC.

As a new type of regulatory factor, miRNAs play an important role in biological and pathological process in many human solid cancer[33]. Currently, several researches indicated that miR-335-5p, as tumor suppressor gene[34], was related to the occurrence and development of multiple tumors including non-small cell lung cancer[35], colorectal cancer[36], and HCC[37]. Moreover, miR-335-5p may serve as a candidate miRNA biomarker in HBV-related HCC[38], and hepatic stellate cell derived exosome miR-335-5p as a novel therapeutic strategy in HCC[39]. Further studies found that miR-335-5p targets downstream gene to regulate cancer cell biological behavior, such as miR-335-5p overexpression significantly inhibited the proliferation, migration and invasion of colorectal cancer cells by targeting LDHB[40]. Du et al[35] demonstrated that miR-335-5p inhibits cell proliferation and the process of epithelial-mesenchymal transition in non-small cell lung cancer by targeting ROCK1. Similarly, our studies revealed that miR-335-5p suppressed HCC cell proliferation, migration and invasion by targeting ROCK1. In addition, ROCK1 reported a positive correlation with high invasiveness of cancer cells; for example, the upregulation of ROCK1 promoted cell migration and invasion of non-small cell lung cancer[41], melanoma[42], and pancreatic adenocarcinoma[43]. The above results suggested that the inhibition of ROCK may be an effective treatment method of HCC clinical.

Conclusions

Based on the above data, this study found that knockdown of circ_0009910 suppressed cell proliferation and metastasis of HCC by upregulating the inhibitory effect of miR-335-5p on ROCK1. Our work will provide new therapeutic target for diagnosis and prognosis in HCC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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