Circular RNA hsa_circ_0006421 inhibits hepatocellular carcinoma by acting as a ceRNA targeting miR-134-5p/CELF2 pathway

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Abstract

Background: Hepatocellular carcinoma (HCC) has ranked sixth most common cancer in number of malignancies in the worldwide. There is plenty of evidence indicated that circular RNAs (circRNAs) exert vitally important roles in the progression of many malignancies. Nevertheless, the molecular mechanism and role of hsa_circ_0006421 in HCC are still unclear. The aim of our research is to verify the molecular mechanism and effects of hsa_circ_0006421 in HCC.

Methods: First, we collected 34 paired HCC tissues and paraneoplastic tissues surgically resected from patients and analyzed the expression of hsa_circ_0006421 in HCC tissues and its relationship with clinicopathological characteristics. Then, CCK8, colony formation, cell migration assay, transwell invasion assay and Annexin-V/PI staining were used to assess the effects of hsa_circ_0006421 on the growth, migration, invasion and apoptosis of HCC cells. Next, quantitative real-time PCR (qRT-PCR) and Western blots were used to detect the expression of miR-134-5p, CELF2 and hsa_circ_0006421. In the end, the targeting interactions of miR-134-5p and hsa_circ_0006421， CELF2 and miR-134-5p were explored by the dual-luciferase reporter assay.

Results: Hsa_circ_0006421 was diminished in HCC tissues and its down-regulation was related to cirrhosis history. Knocking down hsa_circ_0006421 promoted HCC cells growth, migration, invasion and inhibited apoptosis, whereas overexpressing

hsa_circ_0006421 had the opposite effects. Moreover, hsa_circ_0006421 served as the competing endogenous RNA of miR-134-5p. Subsequently, there was a reciprocal relationship between CELF2 and miR-134-5p. Hsa_circ_0006421 could positively regulate the protein level of CELF2 in HCC.

Conclusion: hsa_circ_0006421 inhibits liver cancer by regulating miR-134-5p/CELF2 axis.

Key Words： hsa_circ_0006421; miR-134-5p; CELF2; hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is an incurable disease with high morbidity and mortality rates [1]. According to GLOBOCAN cancer statistics, in 2020, HCC was the sixth most frequently diagnosed malignancy and the number of deaths occupied the third place [2]. China accounts for 47.1% and 45.6% of new deaths and new cases of HCC worldwide, respectively, which seriously threatened the lives and health of Chinese residents [3]. Therefore, promising biomarkers for early detection and prediction of HCC prognosis need to be identified. The up-regulation or down-regulation of non-coding RNAs (ncRNAs) is related to the pathogenesis of HCC [4]. Therefore, these ncRNAs have potential to be predictive markers for HCC patients.

CircRNAs , a new class of ncRNAs, were first identified in eukaryotic cells in 1979 [5].Unfortunately， they received less attention and were deemed as by-products of erroneous splicing in the past [6]. Compared with linear RNAs, circRNAs are stable RNAs because of their closed loop structure [7]. The evidence is mounting that the dysregulation of circRNAs expression is related to multiple malignant tumors, such as cervix cancer [8],

breast tumor [9, 10], colorectal cancer [11], lymphoma [12], GC [13] and HCC [14]. Currently, growing evidence revealed that circRNAs could function as sponges of miRNAs or modulate RNA-binding proteins (RBPs). For example, hsa_circ_0003410 facilitates cell proliferation via the miR-139-3p/CCL5 in HCC [15]. Circular RNA circGLIS3 plays an oncogenic role in bladder cancer through miR-1273f/SKP1/Cyclin D1 axis [16].The differentially expressed circRNAs were selected by bioinformatics, after consulting the literature, we found that hsa_circ_0006421 decelerates cell proliferation in stomach cancer through miR-134-5p/CELF2 axis [17], but the biological effects of hsa_circ_0006421 in HCC remains elusive. Therefore, we finally chose hsa_circ_0006421 as the target, and then further proved whether hsa_circ_0006421/miR-134-5p/CELF2 pathway also has inhibitory effect in HCC.

In this research, hsa_circ_0006421 was diminished in HCC tissues and its down-regulation was related to cirrhosis history. Then, vitro experiments revealed that hsa_circ_0006421 is an oncogene suppressor in HCC. Furthermore, the data demonstrated that hsa_circ_0006421 decelerated the growth and invasion of HCC via miR-134-5p/CELF2 axis. For these reasons, hsa_circ_0006421/ miR-134-5p/CELF2 axis is expected to provide a basis for early HCC diagnosis.

Materials and Methods

Patients and sample collection

A total of 34 fresh HCC tissue specimens and para cancer tissues were obtained from patients who underwent surgery. This research was approved by the Medical Ethics Committee of the Affiliated Hospital of Youjiang Medical University for Nationalities (protocol code: YYFY-LL-2022-13). All of the patients were informed consent. Samples

receiving chemoradiotherapy prior to surgery before collections were excluded. These patients were pathologically diagnosed as hepatocellular carcinoma and signed the in-formed consents. After surgical excision of the fresh tissues, they were deep frozen with liquid nitrogen and stored at −80ºC for subsequent use.

Cell lines and cultures

HepG2、MHCC97H cells and human embryotic kidney cells (HEK293T) were purchased from Chinese Academy of Sciences (Beijing, China). All of the cells were cultured in DMEM high sugar liquid medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Beyotime, Nantong, China), in a 37°C cell incubator containing 5% CO_2.

Cell transfection

For sh-hsa_circ_0006421 (sh-circ) and its negative control transfection, lentiviral transfection of HepG2 and MHCC97H cells with a multiplicity of infestation (MOI) of 100. And then, purinomycin with concentration of 4g/mL was added to complete culture medium to screen lentivirus stably transformed cell lines. For hsa_circ_0006421 overexpression (over-circ), hsa_circ_0006421 cDNA was synthesized and cloned into pcDNA3.1. MiR-134-5p mimics, si-hsa_circ_0006421 (si-circ) were obtained from GenePharma Co., Ltd. (Suzhou, China). They are shown in Table 1. Lipofectamine 3000 (Invitrogen, ThermoFisher, USA) was utilized to transfect.

RNA extraction and qRT-PCR analysis

Total RNA in HCC specimens and cultured cells was extracted by using Trizol rea-gent (Invitrogen, USA). RNA samples were converted into cDNA by using the RevertAid Master Mix (Thermo Fisher Scientific, USA). For miRNA analysis, RT-PCR

was completed using a miRNA First Strand cDNA Synthesis (Sangon Biotech, China). The synthesized cDNA was detected by qRT-PCR by using SYBR Green (Hieff®, Shanghai, China). The amplification reaction conditions were as follows: 95°C for 10 s and 60°C for 30 s. We used U6 and GAPDH as reference genes for data normalization. The primer sequences are showed in **Table 1**.

Table 1 Sequence of small interfering RNA, short hairpin RNA, mimic, or polymerase chain reaction (PCR) primers.

Name	Sequence (5'-3')
si-circ_0006421:	Sense: 5'AGAGGAGUGGAAGCAGAACTT3'
	Antisense: 5'GUUCUGCUUCCACUCCUCUTT3'
sh-circ_0006421:	Sense: 5'-CAGAGGAGTGGAAGCAGAA-3'
miR-134-5p mimic:	Sense: 5'-UGUGACUGGUUGACCAGAGGGG-3'
	Antisense: 5'-CCUCUGGUCAACCAGUCACAUU-3'
hsa_circ_0006421:	Forward: 5'-ACTGGCTTCACGTGGATATGG-3'
	Reverse: 5'-CCAGTTCTGCTTCCACTCCTC-3'
miR-134-5p:	Forward: 5'- CCTCTATTCTGTGACTGGTTGACC -3'
	Reverse: 5'- TATGGTTTTGACGACTGTGTGAT -3'
CELF2:	Forward: 5'-CTGGCGGGAAACAAACTCTG-3'
	Reverse: 5'-TCTAAGCCCTTGGCCTCCTC-3'
GAPDH:	Forward: 5'-CAGGAGGCATTGCTGATGAT-3'
	Reverse: 5'-GAAGGCTGGGGCTCATTT-3'
U6:	Forward:5'-CAGCACATATACTAAAATTGGAACG-3'
	Reverse: 5'-ACGAATTTGCGTGTCATCC-3'

Cell proliferation assay

About three thousand HCC cells (HepG2、MHCC97H) were cultured in 96-well plates. PBS was added to the other wells around the cells. Before detecting the OD value, we added 10 microliter of Cell Counting Kit-8 (CCK8) (MedChemExpress, China) to each

well. After 2 hours, the absorbance value was detected by using an enzyme immunoassay analyzer at 450 nm.

Colony formation assay

Lentiviral stable transfected cell lines were uniformly inoculated with 1.7×10^3 cells in a 6-well plate. DMEM medium containing 20% FBS was changed every three days. On the ninth day, visible colonies were formed, and cell culture was terminated. Carefully add 4% paraformaldehyde along the inner wall to a 6-well plate and fix for 20 minutes, stained by the crystal violet solution for about fifteen minutes. After washing, the numbers of clones were then counted and analyzed.

Cell migration assay

HCC cells (HepG2 and MHCC97H) were seeded into 6-well plates and starved for 24h. They were incubated in cell incubator until the cells reached about 95% confluence. Next, we draw several scratches in the cell using a clean 100 μl pipette tip. Finally, after cultured for 12h and 24h, the moving distances of cells were measured and the cell healing rates were calculated.

Transwell invasion assay

After 24 hours of transfection, the HCC cells (HepG2 and MHCC97H) were digested and detected by transwell invasion assay. The Matrigel (BD, USA) diluted 10 times with pre-cooled serum-free DMEM medium to obtain a suitable concentration of Matrigel solution. We added 100μL of the solution to the upper chamber and incubated in the incubator for 1h. After the cells were washed with PBS, they were added to the serum-free medium and mixed evenly to prepare a cell suspension of 2×10^5 cells/mL, 200 μL of cell suspension was seeded into each well of the upper chamber. 600 μL of DMEM complete

medium was supplied in the lower chamber. After cultured for 24h, the cells in the upper chamber were eliminated. The transmembrane cells were fixed for 15 minutes with 4% paraformaldehyde and 0.5% crystal violet was added to stain. The number of HCC cell invasions was counted under a microscope in five random visual fields.

Western blot

After transfection for 48 hours, HCC cells were washed with pre-chilled PBS. Protein samples were obtained by the RIPA Lysis and Extraction Buffer (Solarbio, China). The protein concentrations were detected using a Pierce BCA kit (Beyotime, Shanghai, China). The cell lysates (30μg/lane) were electrophoresed on 10% SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and then transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA).After blocking with 5% skim milk for 2h, the membranes were incubated with anti-β-actin (1:3000; ab227387, Abcam), anti-CELF2 (1:3000, ab179447, Abcam) at 4℃ overnight. Subsequently, washed these bands with TBST solution and secondary antibodies (1:5000; Invitrogen, USA) were incubated for one hour.

Cell apoptosis analysis

After transfection, HCC cells (HepG2 and MHCC97H) were collected and centrifuged for 3 mins with 800 rpm. After washing the HCC cells with pre-chilled PBS, they were stained with an Annexin V-FITC/ PI (Invitrogen, ThermoFisher). Finally, HCC cells were subjected to apoptosis and detected by using a flow cytometer (FACSCanto™ II, BD Biosciences).

Luciferase reporter assay

We obtained the dual-luciferase plasmid from GenePharma Co., Ltd. (Suzhou, China).

The sequences of hsa_circ_0006421 and CELF2 3′UTR containing miR-134-5p binding sites were inserted into the pmirGLO promoter vector. HEK293T cells were cultured in 24-well plates overnight and were cotransfected with 1μg of WT or MUT plasmids and 1.5μL of miR-134-5p mimics or miR-134-5p NC using the lipofectamine 3000 transfection reagent. After 48 hours of transfection, the luciferase activity was quantified using a dual-luciferase reporter assay kit (Promega, USA).

Statistical analysis

Each experiment was repeated triplicate. All values are expressed as means ± SD. We performed the statistical analysis using R 4.0.2 software. Wilcoxon pair test was used to analyze the relative expression of hsa_circ_0006421 in liver cancer and paracancerous tissue. Student's t-tests were used to calculate statistical significance. Categorical data were analyzed using Fisher's exact test. $p<0.05$ was considered statistically significant.

Results

Hsa_circ_0006421 is abnormally low expressed in HCC tissues

We collected 34 clinical specimens of hepatocellular carcinoma ， as shown by qRT-PCR, judged side by side with paracancerous tissues, hsa_circ_0006421 was down-regulated in 22 cases and upregulated in 12 cases. After statistical analysis, the expression level of hsa_circ_0006421 was lower in hepatocellular carcinoma compared to paraneoplastic tissues (Fig. 1A). What's more, we analyzed the clinicopathological features further of 34 patients and found that the hsa_circ_0006421 expression was associated with cirrhosis history (p= 0.042) (**Table 2**). To sum up, hsa_circ_0006421 is abnormally low expressed in HCC and its low expression is a red flag for patients with cirrhosis, implying that hsa_circ_0006421 may be an anti-oncogene in HCC.

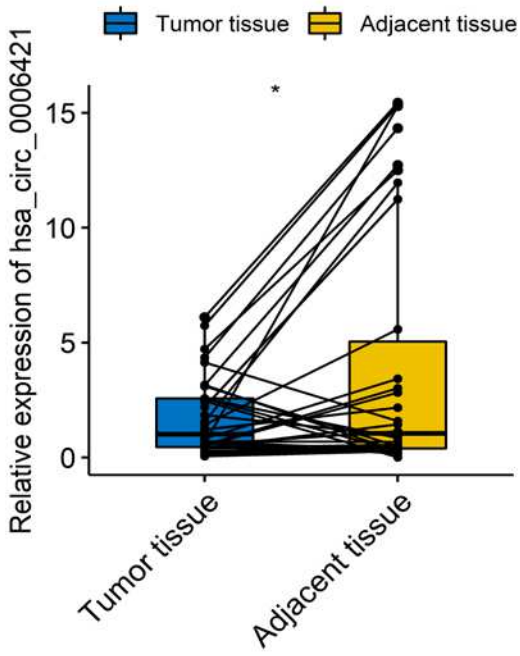

Figure1. Hsa_circ_0006421 is lowly expressed in HCC tissues. (A)The expression of hsa_circ_0006421 was examined in 34 HCC samples and normal tissues. *p < 0.05, **p < 0.01 and ***p < 0.001.

To further verify the function of hsa_circ_0006421 in HCC cells, we used small interfering RNA (si-circ) and lentiviral (sh-circ) prominently reduced the expression of hsa_circ_0006421 in HepG2 and MHCC97H cell lines. Moreover, the hsa_circ_0006421 plasmid (over-circ) was constructed to overexpress hsa_circ_0006421. The hsa_circ_0006421 knockdown and overexpression efficiency in HepG2 and MHCC97H cells were confirmed by qRT-PCR (**Fig.2A**). After successful construction of hsa_circ_0006421 knockdown and overexpression cell lines, CCK8 assay and clone formation assay were used to verify cell viability. Migration assay and transwell invasion assay were used to detect cell migration and invasion, respectively. Apoptosis assay was used to detect cell apoptosis. Results showed that the overexpression of hsa_circ_0006421 substantially suppressed cell viability (**Fig. 2B, 2C**), cell migration (**Fig. 2D**), cell invasion

(**Fig. 2E**) and promoted apoptosis (**Fig. 2F**), whereas knocking down hsa_circ_0006421 had the opposite effects in HepG2 and MHCC97H cells. To sum up, We came to the conclusion that hsa_circ_0006421 inhibits invasion and proliferation of hepatocellular carcinoma cells.

Table 2 Correlation between hsa_circ_0006421 expression and clinicopathological characteristics in HCC

Characteristics	**N=34**	**Expression of hsa_circ_0006421**		***p***
		Low（n=22）	**High(n=12)**	
Age				0.462
≧50y	13	7	6	
＜50y	21	15	6	
Gender				1.00
Female	5	3	2	
Male	29	19	10	
HBsAg				0.225
Positive	29	20	9	
Negative	5	2	3	
Cirrhosis history				0.042
Positive	29	21	8	
Negative	5	1	4	
Tumor size				0.128
≧5cm	20	15	5	
＜5cm	14	7	7	
AFP				0.315
≧20ng/mL	23	16	7	
＜20ng/mL	11	6	5	
T classification				0.08
T3-T4	21	16	5	
T1-T2	13	6	7	
Tumor number				0.610
≧2	11	7	4	
1	23	15	8	

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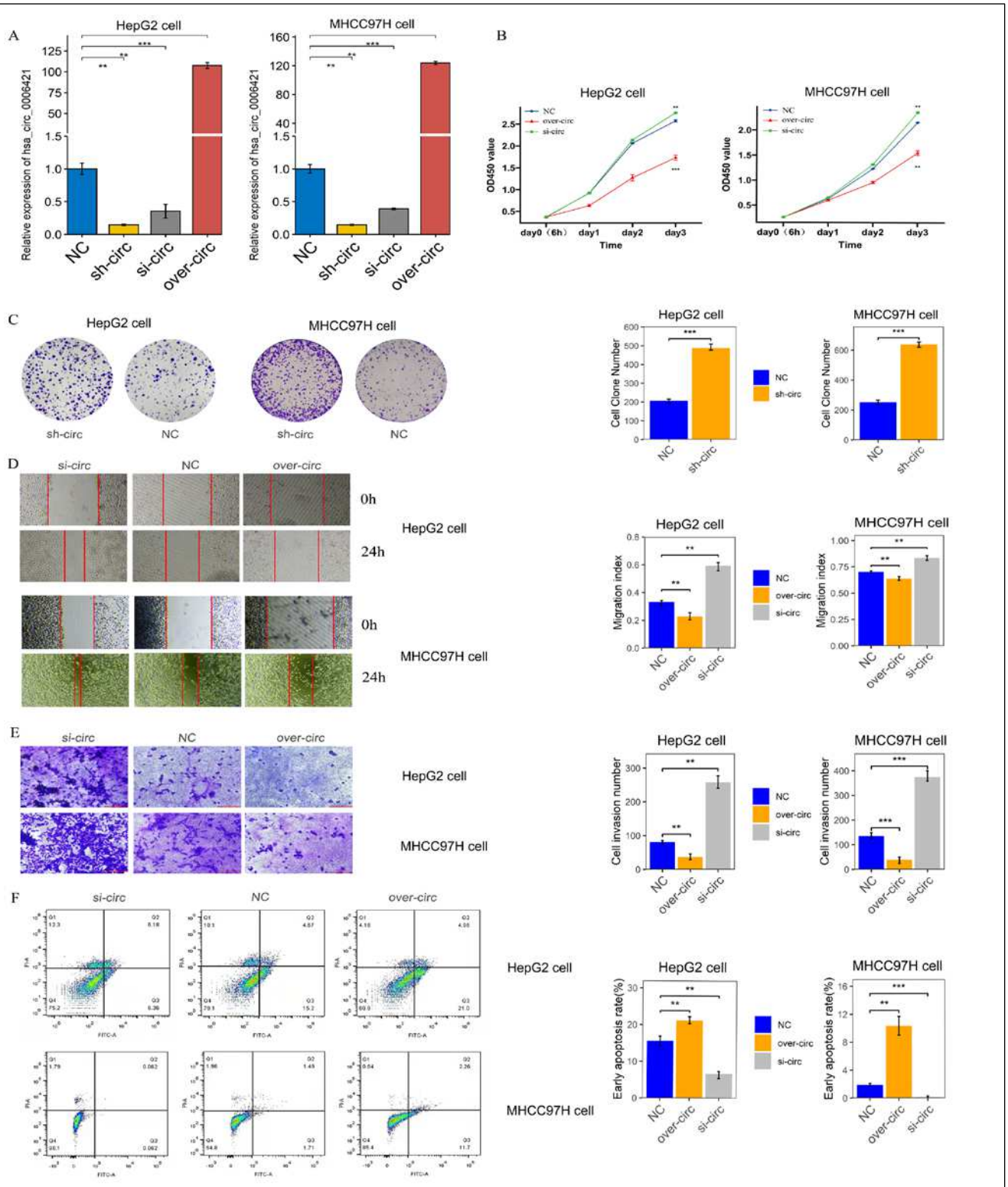

Figure2. Knocking down hsa_circ_0006421 promoted HCC cells growth, migration, invasion and inhibited apoptosis, whereas overexpressing hsa_circ_0006421 had the opposite effects. (A)The expression of hsa_circ_0006421 was determined by qRT-PCR in hsa_circ_0006421 overexpression or knockdown HCC cells. The effect of hsa_circ_0006421 on HCC cell proliferation was determined by CCK-8(B) and colony formation（C）.（D）The effect of hsa_circ_0006421 on HCC cell migration was evaluated by migration assay .(E) The effect of hsa_circ_0006421 on HCC cell invasion was evaluated by transwell assays. (F) The apoptosis of HCC cells was measured by staining with AnnexinV/PI, followed by FACS analysis. The scale bars were 100 μm. Data were expressed as mean ± SD.

Hsa_circ_0006421 inhibits HCC progression via miR-134-5p/CELF2 pathway

A new study reveals that hsa_circ_0006421 inhibits stomach cancer through regulating miR-134-5p/CELF2 pathway [17]. To investigate whether hsa_circ_0006421 could inhibit hepatocellular carcinoma through this pathway, we overexpressed hsa_circ_0006421 in HepG2 and MHC97H cell lines, the result of qRT-PCR showed that miR-134-5p was significantly downregulated (**Fig. 3A**), implying that they were negatively regulated. Then, the interaction relationship between hsa_circ_0006421 and miR-134-5p was further verified by luciferase reporter assay, the binding sites between miR-134-5p and hsa_circ_0006421 are shown in (**Fig. 3B**). Results showed that the luciferase intensities were reduced in miR-134-5p mimics and WT-hsa_circ_0006421 co-transfected group in HEK293T cell lines. However, the luciferase intensities of MUT-hsa_circ_0006421 were nearly unchanged (**Fig. 3C**). Thus， hsa_circ_0006421 and miR-134-5p have a targeting relationship in hepatocellular carcinoma.

Next, we validated the targeting relationship between miR-134-5p and CELF2 in HCC and we found that the overexpression of miR-134-5p significantly reduced CELF2 expression in HCC cells according to the qRT-PCR results (**Fig. 3D**). We demonstrated a binding target between CELF2 and miR-134-5p shown in **Fig. 3E**. Dual-luciferase assays revealed that the luciferase activity of WT CELF2 3'UTR reduced in miR-134-5p mimics group, but the activity of the MUT CELF2 3'-UTR remained unchanged (**Fig. 3F**). Therefore, CELF2 and miR-134-5p have a targeting relationship in hepatocellular carcinoma.

Finally, we verified whether hsa_circ_0006421 could positively regulate the expression of CELF2. We used small interfering RNA and plasmid to knock down and

overexpress hsa_circ_0006421 in HepG2 and MHCC97H cells respectively, and then detected the expression of CELF2 protein by Western blot. The results display that hsa_circ_0006421 could significantly positively regulate the expression of CELF2 protein in HCC cells (Fig. 3G). These data are sufficient to support our view that hsa_circ_0006421 inhibits HCC progression via miR-134-5p/CELF2 pathway.

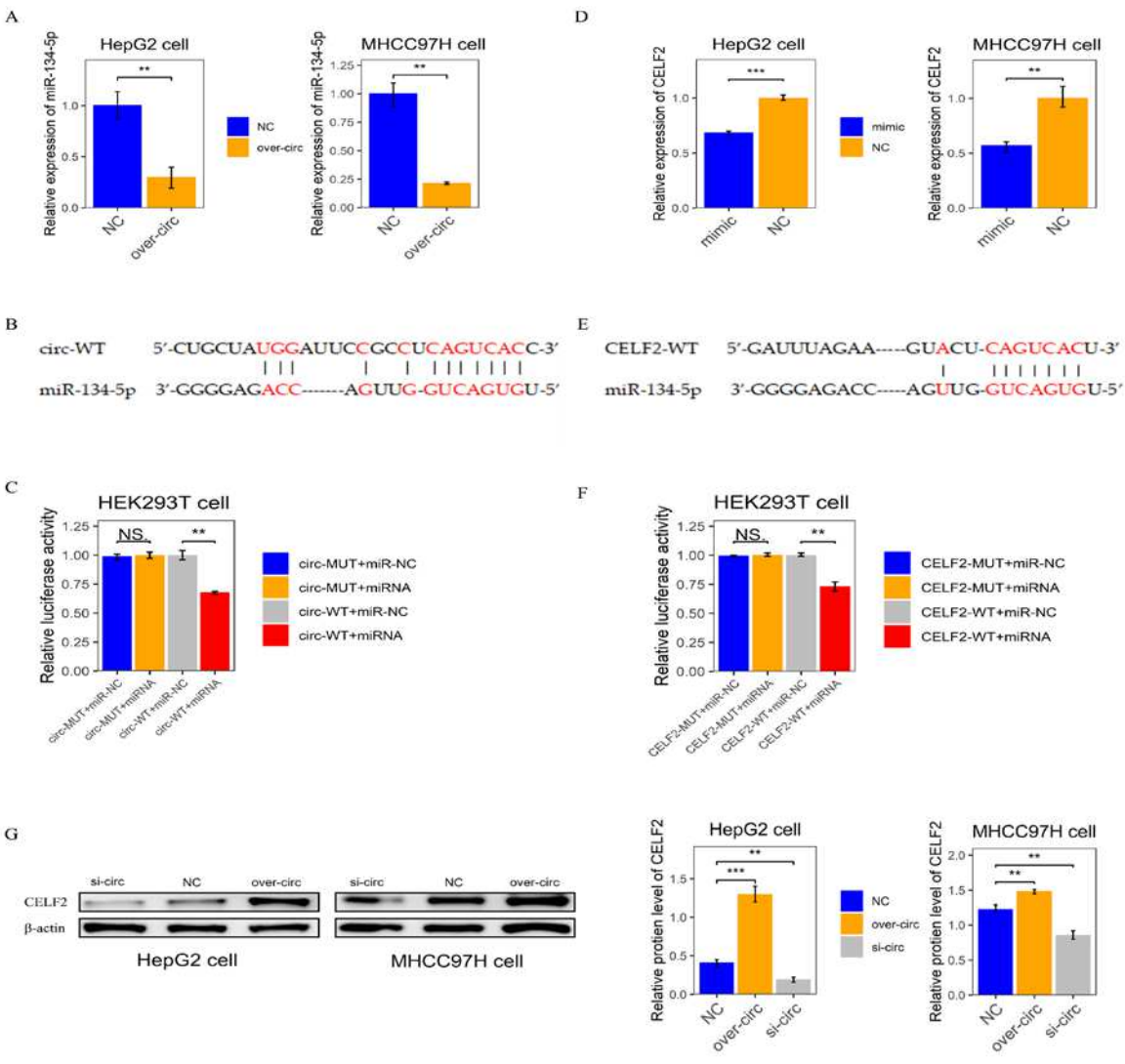

Figure3. Hsa_circ_0006421 inhibits HCC by regulating miR-134-5p/CELF2 axis. (A)The qRT-PCR results showed that the overexpression of hsa_circ_00006421 significantly reduced miR-134-5p expression in HepG2 cells and MHCC97H cells. (B) The potential binding site of hsa_circ_0006421 and miR-134-5p.(C) Dual-luciferase assays revealed that the miR-134-5p mimics could reduce the luciferase activity of WT hsa_circ_0006421 3'UTR. (D) The qRT-PCR results showed that miR-134-5p could negatively regulate the expression of CELF2 in HepG2 cells and MHCC97H cells. (E) The potential binding site of miR-134-5p and CELF2. (F) Dual-luciferase assays revealed that the miR-134-5p mimics could reduce the luciferase activity of WT CELF2 3'UTR. (G) Western blot analysis showed that hsa_circ_00006421 could positively regulate the expression of CELF2 protein in HCC cells.

Discussion

Hepatocellular carcinoma has become one of the most refractory diseases in the world，due to its complexity and high recurrence [18]. With the high burden of hepatocellular carcinoma in China, it is an urgent thing to find specific biomarkers and more effective treatments for the diagnosis and treatment of early stage HCC to reduce patient mortality and improve their prognosis [19]. HCC occurs because the genes of liver cells have changed, and the normal regulation of hepatocyte proliferation and apoptosis is lost [20].

CircRNA is a non-coding RNAs that have attracted attention for their extensive involvement in tumor development [21]. Many surveys show that the up-regulation and down-regulation of circRNA expression were closely related to many types of incurable diseases. For example, hsa_circ_0002232 suppresses malignant progression of colon cancer through inhibition of TGF-β/Smad signaling [22]. CircKDM4B inhibits the invasion of breast cancer through miR-675/NEDD4L pathway [23]. The circROBO1/KLF5/FUS feedback loop promotes malignant progression and liver metastasis of breast cancer [24]. CircVAMP3 inhibits the progression of HCC by suppressing c-Myc translation [25]. In the present study, our results indicated that hsa_circ_0006421 is decreased in HCC tissues, and the down-regulation of hsa_circ_0006421 was associated with cirrhosis history, which means that hsa_circ_0006421 is a tumor suppressor in hepatocellular carcinoma. Actually, we demonstrated that hsa_circ_0006421 overexpression decelerated the growth and migration of HCC in vitro. Furthermore, we identified hsa_circ_0006421 suppressed miR-134-5p expression in HCC cells. In addition, we also proved their interaction by luciferase

reporter assay. A previous study showed that high expression of miR-134-5p promotes the relapse of early-stage lung adenocarcinoma, leading to poor survival [26]. Other studies also indicated that miR-134-5p suppresses the aggressiveness and growth of AML cells [27]. However, the role of miR-134-5p in HCC remains elusive. In this work, we found that miR-134-5p is a potential cancer-promoting factor in HCC.

CELF2 belongs to the CUGBP Elav-like family and is a RNA-binding protein [28]. High expression of CELF2 means a better prognosis in various tumors [29]. For example, CELF2 inhibits the progression of breast cancer by downregulating NFATc1 [30]. CELF2 inhibits ovarian cancer cell invasion by Stabilizing FAM198B [31]. LncRNA CRNDE promotes hepatocellular carcinoma development by facilitating epigenetic repression of CELF2 and LATS2 [32]. STYXL1 promotes hepatocellular carcinoma cell invasion by downregulating CELF2 through the PI3K/Akt axis [33]. We found that miR-134-5p negatively regulates CELF2 in HCC. Moreover, the dual-luciferase activity showed that the luciferase activity of WT CELF2 3'UTR reduced in miR-134-5p mimics group, which means that there was a targeting relationship between CELF2 and miR-134-5p. Mean-while, overexpression of hsa_circ_0006421 promotes the CELF2 protein levels in HCC cells, whereas knocking down hsa_circ_0006421 has the opposite effects. For these rea-sons, hsa_circ_00006421 could positively regulate the expression of CELF2 protein in HepG2 and MHCC97H cells. Last but not least, hsa_circ_0006421 inhibits HCC by regu-lating miR-134-5p/CELF2 pathway.

In conclusion，we first found that hsa_circ_0006421 acts as an anti-oncogene in HCC, and its low expression may increase the possibility of canceration for patients with cirrhosis of liver. Our studies indicated hsa_circ_0006421 inhibits to HCC progression via

increasing CELF2 expression by sponging miR-134-5p, suggesting that hsa_circ_0006421 might be a promising target for HCC therapy.

Conclusions

In summary, our results indicate that hsa_circ_0006421 inhibits hepatocellular carcinoma by acting as a ceRNA targeting miR-134-5p/CELF2 pathway, which may represent a vital strategy for inhibiting the proliferation of liver cancer.

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Author Contributions

writing—review and editing, project administration：Y.D.；data curation, writing—original draft preparation, formal analysis: L.Z.; validation, supervision: X.C.; methodology: J.P.; software: J.S.; All authors have read and agreed to the published version of the manuscript.

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Declarations

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the Affiliated Hospital of Youjiang Medical University for Nationalities (protocol code: YYFY-LL-2022-13 and date of approval:27 June 2022).

Consent for publication

Not applicable in the declarations section.

Competing interests

The authors declare that they have no competing interests.

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Figures

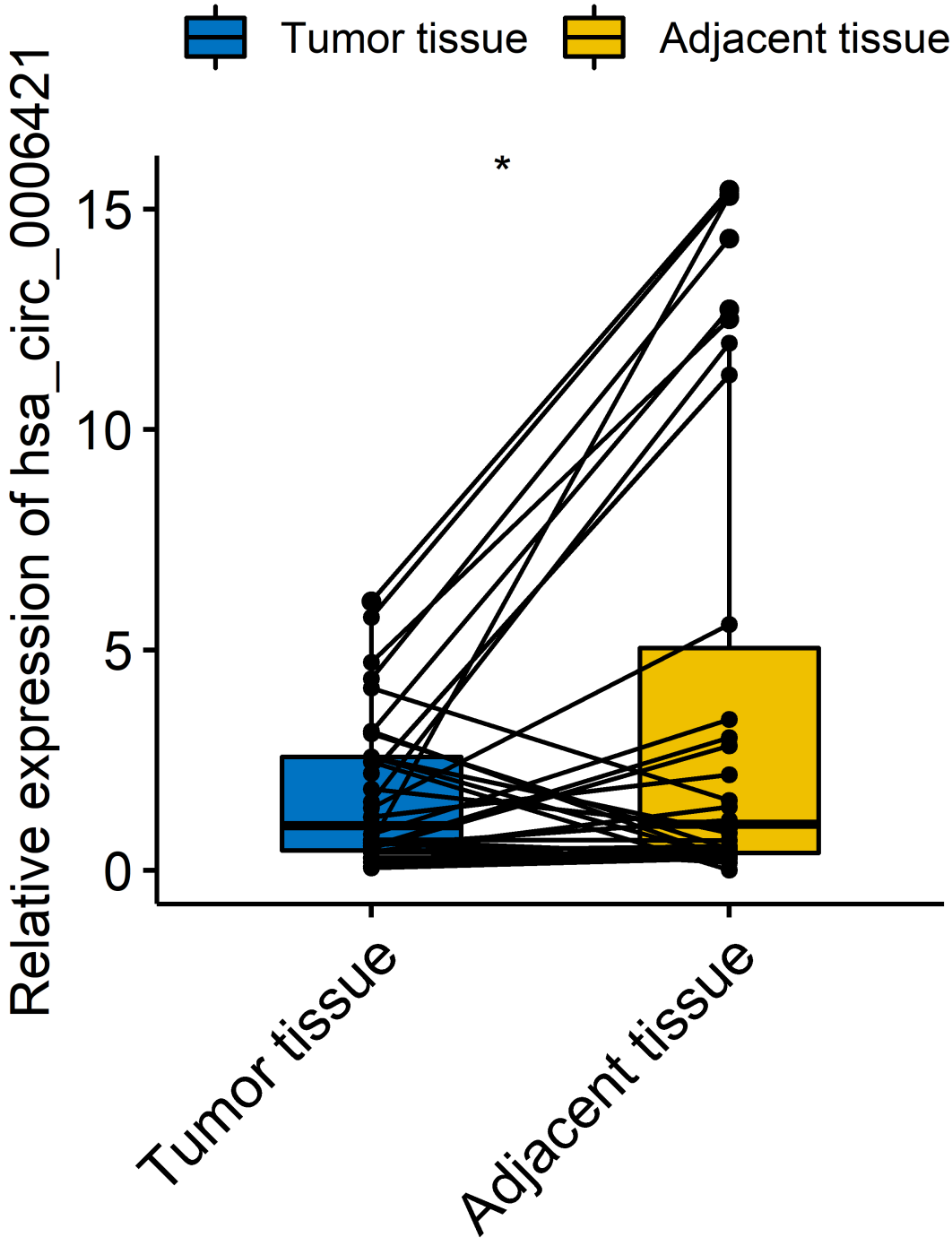

Figure 1

Hsa_circ_0006421 is lowly expressed in HCC tissues. (A)The expression of hsa_circ_0006421 was examined in 34 HCC samples and normal tissues. *p < 0.05, **p < 0.01 and ***p < 0.001.

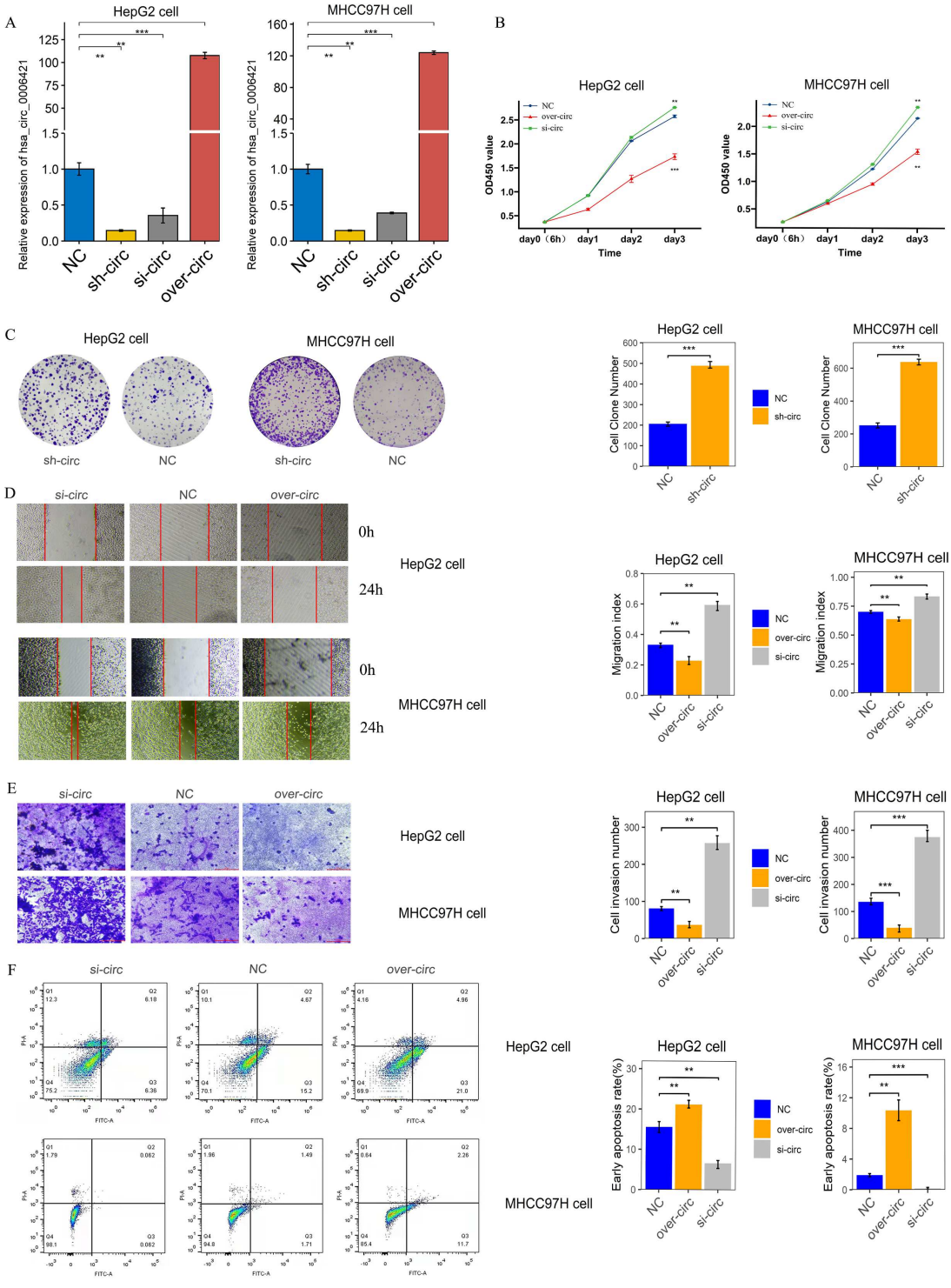

Figure 2

Knocking down hsa_circ_0006421 promoted HCC cells growth, migration, invasion and inhibited apoptosis, whereas overexpressing hsa_circ_0006421 had the opposite effects. (A)The expression of hsa_circ_0006421 was determined by qRT-PCR in hsa_circ_0006421 overexpression or knockdown HCC cells. The effect of hsa_circ_0006421 on HCC cell proliferation was determined by CCK-8(B) and colony formation（C）. （D）The effect of hsa_circ_0006421 on HCC cell migration was evaluated by migration

assay .(E) The effect of hsa_circ_0006421 on HCC cell invasion was evaluated by transwell assays. (F) The apoptosis of HCC cells was measured by staining with AnnexinV/PI, followed by FACS analysis. The scale bars were 100 μm. Data were expressed as mean ± SD.

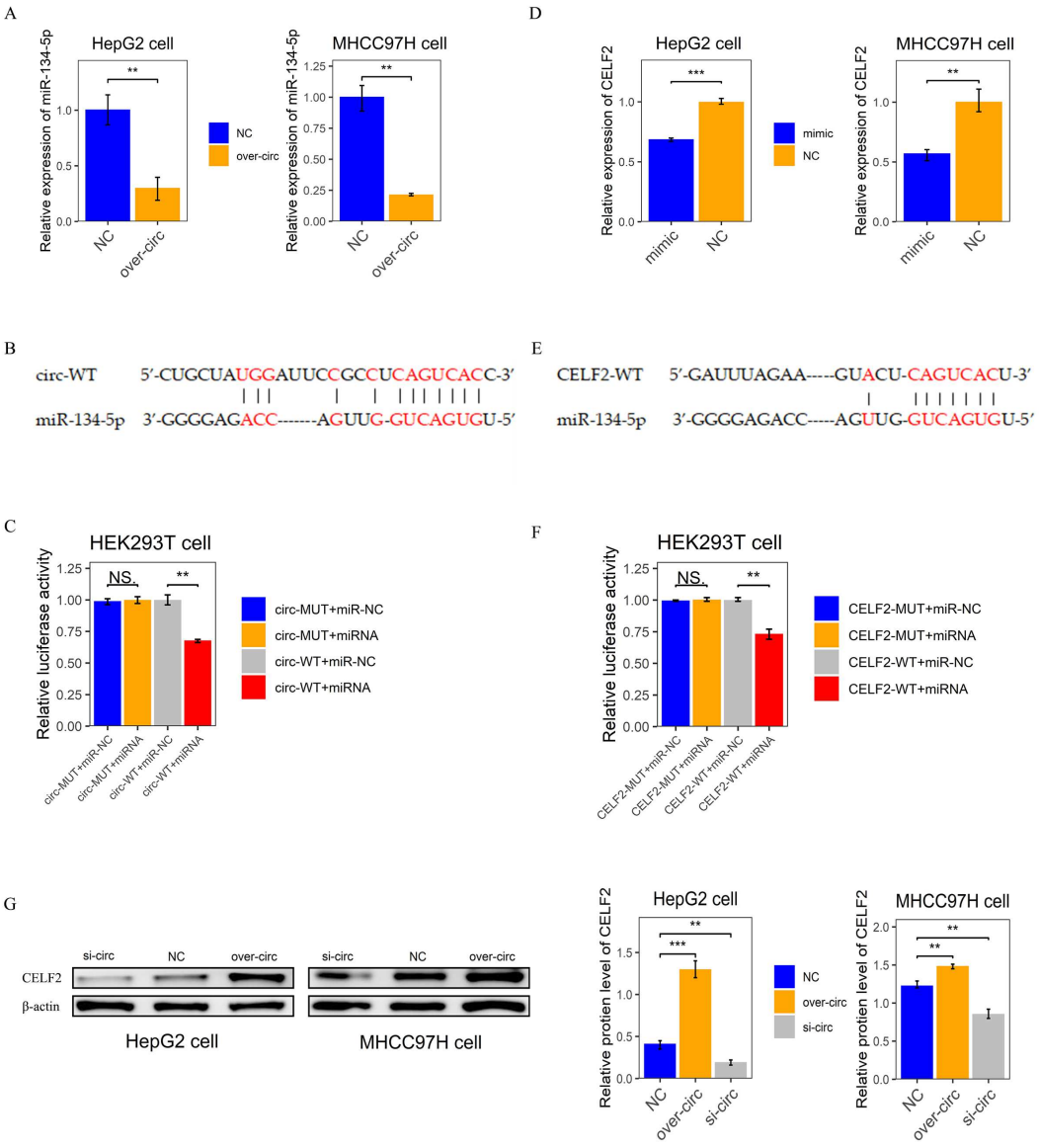

Figure 3

Hsa_circ_0006421 inhibits HCC by regulating miR-134-5p/CELF2 axis. (A)The qRT-PCR results showed that the overexpression of hsa_circ_00006421 significantly reduced miR-134-5p expression in HepG2 cells and MHCC97H cells. (B) The potential binding site of hsa_circ_0006421 and miR-134-5p.(C) Dual-luciferase assays revealed that the miR-134-5p mimics could reduce the luciferase activity of WT hsa_circ_0006421 3’UTR. (D) The qRT-PCR results showed that miR-134-5p could negatively regulate the expression of CELF2 in HepG2 cells and MHCC97H cells. (E) The potential binding site of miR-134-5p and CELF2. (F) Dual-luciferase assays revealed that the miR-134-5p mimics could reduce the luciferase activity of WT CELF2 3’UTR. (G) Western blot analysis showed that hsa_circ_00006421 could positively regulate the expression of CELF2 protein in HCC cells.