

RESEARCH PAPER



Circ_0004018 suppresses cell proliferation and migration in hepatocellular carcinoma via miR-1197/PTEN/PI3K/AKT signaling pathway

He Wang^{a*}, Qiao Zhang^{ib*}, Wenyu Cui^b, Wenlan Li^b, and Jimei Zhang^c

^aDepartment of Interventional, Harbin Medical University Cancer Hospital, Harbin, Heilongjiang, China; ^bSchool of Pharmacy, Harbin University of Commerce, Harbin, Heilongjiang, China; ^cBiology College, The Ohio State University, Columbus, OH, USA

ABSTRACT

Hepatocellular carcinoma (HCC) is a common type of primary liver cancer. Circular RNAs (circRNAs) have been demonstrated to be a crucial player in multiple cancers. However, a large number of circRNAs remain to be explored. Our study focused on investigating hsa_circ_0004018 in HCC. Firstly, we conducted quantitative reverse transcription PCR (RT-qPCR) to find that circ_0004018 was down-regulated in HCC cells. Western blot analysis was performed to detect the protein levels of phosphatase and tensin homologue (PTEN) and related factors of PI3K/AKT signaling pathway. From the results of functional assays, we found that overexpression of circ_0004018 significantly inhibited the proliferative and migratory capacities of HCC cells. The regulatory mechanism of circ_0004018 in HCC was determined by RNA immunoprecipitation (RIP), RNA pull-down, and luciferase reporter assays, thereby we knew that circ_0004018 regulated PTEN by sequestering microRNA-1197 (miR-1197) to modulate PI3K/AKT signaling pathway. Finally, rescue assays verified that circ_0004018 participated in modulation of cell proliferation and migration in HCC via sponging miR-1197 and regulating PTEN. In conclusion, circ_0004018 suppresses the proliferation and migration of HCC cells via sponging miR-1197 to inactivate the PTEN/PI3K/AKT signaling pathway.

Abbreviations: HCC: Hepatocellular carcinoma; circRNAs: Circular RNAs; PTEN: Phosphatase and tensin homologue; miR-1197: microRNA-1197; ceRNA: competitive endogenous RNA; ATCC: American Type Culture Collection; EMEM: Eagle's Minimum Essential Medium; RT-qPCR: Quantitative real-time PCR; EdU: 5-ethynyl-20-deoxyuridine; FISH: Fluorescent *in situ* hybridization; RIP: RNA immunoprecipitation; 3'-UTR: 3'-untranslated region; Wt: wild-type; Mut: mutant type; gDNA: genomic DNA; Act D: Actinomycin D; PI3K: phosphatidylinositol-3-kinase; AKT: protein kinase; lncRNAs: long non-coding RNAs

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Introduction


As a common malignancy, hepatocellular carcinoma (HCC) is featured with a high incidence and mortality [1]. At the same time, many factors may contribute to its occurrence. Despite many improvements in HCC treatment, HCC remains difficult to be cured and has poor prognosis [2]. Thus, developing effective biomarkers for HCC diagnosis and treatment is urgent.

Circular RNAs (circRNAs) are recognized to be a set of noncoding RNAs, which originate from back-splicing of linear primary transcripts [3]. Increasing studies have validated that circRNAs have been identified as important molecular biomarkers for multiple cancers, such as pancreatic

ductal adenocarcinoma, colorectal cancer, and

breast cancer [4,5]. CircRNAs have also been reported to be associated with HCC in different ways [6]. For example, Huang et al. have demonstrated that circRNA-100338 participates in HCC via mTOR signaling pathway [7]. Zhu et al. have verified the role of Qki5-circZKSCAN1-FMRP-CCAR1-Wnt signaling axis in HCC stemness [8]. Moreover, based on data of the previous study, circ_0004018 has been validated to sponge miR-660-3p to modulate TEP1 expression, and thereby impedes the progression of liver fibrosis [9]. Additionally, circ_0004018 expression level in HCC was testified to be significantly lower compared with para-tumorous tissues, and its

CONTACT Qiao Zhang  zhangqiao1998@163.com  School of Pharmacy, Harbin University of Commerce, Harbin, Heilongjiang 150076, China
*Co-first author

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potential correlation with HCC was suggested [10]. However, the function of circ_0004018 involved in HCC progression remains largely unknown.

Notably, competitive endogenous RNA (ceRNA) network in which circRNAs are significant components has been identified in the pathogenesis of HCC [11]. For instance, He et al. have demonstrated that hsa_circ_0000517 acts as a ceRNA in the advancement of HCC [12]. Liu et al. have proved that circ-DENND4C sequesters miR-195-5p and enhances TCF4 expression to influence malignant phenotypes in HCC [13]. Bai et al. have confirmed that circFBLIM1 serves as an oncogene in HCC via modulating miR-346/FBLIM1 axis [14].

In the present study, we aimed to explore the biological function of circ_0004018 in HCC and unveil the related mechanism in order to find out potential therapeutic targets against HCC.

Materials and methods

Cell culture

HCC cell lines (Huh7, Hep3B, SNU-182, and SK-HEP-1) and normal epithelial cell line (THLE-3) were chosen for this study. Among them, Huh7 and Hep3B were purchased from Yaji Biotechnology Co., Ltd. (Shanghai, China) and maintained in RPMI-1640 and MEM respectively. SNU-182, SK-HEP-1 cell lines, as well as THLE-3 cell line, were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA) and separately cultured in RPMI-1640, Eagle's Minimum Essential Medium (EMEM) and BEGM. All cells were cultured in mediums containing 10% FBS with 5% CO₂ at 37°C.

Plasmid transfection

PcDNA3.1 (+) circRNA Mini Vectors were sub-cloned with circ_0004018 to construct pcDNA3.1-circ_0004018 for overexpression and empty pcDNA3.1 (+) circRNA Mini Vector (hereafter termed as pcDNA3.1) was regarded as the negative control. MiR-1197 mimics and control mimics were purchased from GenePharma (Shanghai, China). In addition, the short hairpin

RNAs (shRNAs) targeting PTEN (sh-PTEN#1, sh-PTEN#2), as well as control shRNA (sh-NC) were generated by Genechem (Shanghai, China). Lipofectamine 3000 (2.0 µl/50 µl; Invitrogen) was used for cell transfection.

Quantitative reverse transcription PCR (RT-qPCR) analysis

In accordance with the protocols of suppliers, total RNA was isolated with 1 ml TRIzol Reagent (Introgen, Carlsbad, CA, USA) and reverse transcribed into cDNA by means of RevertAid First Strand cDNA Synthesis Kit (Thermo fisher, IL, USA). SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) was applied to conduct PCR analysis. The relative expression of genes was determined by 2^{-ΔΔCt} method. GAPDH and U6 served as internal controls of cytoplasm and nucleus, respectively. Primer sequences were clearly listed in Table 1.

5-ethynyl-20-deoxyuridine (EdU) staining assay

EdU assay was performed as previously described [15]. Cells were put on sterile coverslips in 96-well plates with 5 × 10⁴ cells in each well. EdU kit from

Table 1. Primer sequences used in RT-qPCR were listed.

Sequences for RT-qPCR primers	
Gene	Primer sequence
linear-SMYD4	F GGGGTACCAGAGACCTGTGC
	R AGCAACCAGACGCACATTCT
circ_0004018	F AGCAGCAGTGACACCTGAAT
	R GACACGTCTGTGTGTATGG
PTEN	F CAGCAGCTTCTGCCATCTCT
	R TGCTTTGAATCCAAAACCTTACT
miR-1197	Sequence: taggacacatggtctacttct
	Reverse transcription stem loop primer
	CTCAACTGGTGTCTGGAGTCGGCAATTGAGC
	agaagt
miR-378 g	Stem loop universal reverse primer
	CTCAACTGGTGTCTGGAGTCGGCAATTGAGC
	cttctg
	Stem loop universal reverse primer
GAPDH	F TCGCAactgggcttgagtg
	R GCGCCAATACGACCAATC
U6	F TCCCTTCGGGGACATCCG
	R AATTTGGACATTCTCGATTGT

RiboBio (Guangzhou, China) was used following guidebook. Cell nucleus was double-stained with 50 μ M EdU solution and 500 μ l DAPI dye (Beyotime, Shanghai, China). Images were acquired by fluorescence microscope (Olympus, Tokyo, Japan).

Colony formation assay

HCC cells were incubated in 6-well plates at a density of 200 cells at 37°C for 14 days to detect colony formation ability. Subsequent to fixation with 4% paraformaldehyde, colonies were stained with 0.1% crystal violet. The stained cell colonies were counted manually.

Transwell migration assay

Cell samples were planted to the upper chamber for migration assay. The lower chamber was supplemented with 1% FBS. The cultivation process lasted 24 hours. Migrated cells into bottom were then fixed with 4% paraformaldehyde and dyed with 0.1% crystal violet. Finally, the migrated cells were observed and photographed under a microscope.

Subcellular fractionation

Subcellular fractionation was conducted with the employment of Cytoplasmic and Nuclear RNA Purification Kit (NORGEN, Thorold, ON, Canada). After cell cytoplasm and nucleus were separated by centrifugation, the expression of circ_0004018 in different fragments was detected by RT-qPCR. GAPDH or U6 was separately treated as the cytoplasmic control or the nuclear control.

Fluorescent in situ hybridization (FISH)

FISH assay was performed as previously described [16]. After fixation in 4% PFA for 15 min at 37°C and then permeabilized with 0.5% Triton X-100, HCC cells were hybridized with circ_0004018-specific FISH probe in buffer and counterstained with 500 μ l Hoechst solution. Images were obtained with a confocal laser microscope (Olympus).

RNA pull down assay

With the aim to investigate the interaction between circ_0004018 and miR-1197, RNA pull down assay was adopted. Biotin-labeled circ_0004018 (Bio-circ_0004018), with a biotinylated nonsense sequence as the negative control (Bio-NC), was mixed with the cell lysates overnight, and then magnetic beads were added. The bound RNAs were purified for RT-qPCR analysis.

RNA immunoprecipitation (RIP)

With the aim to explore the relationship among circ_0004018, miR-1197 and PTEN in SNU-182 and SK-HEP-1 cells, RNA-binding protein immunoprecipitation kit (Millipore, Burlington, MA, USA) was used for RIP assay. HCC cell lysates were immunoprecipitated with anti-Ago2 (Abcam) or with the negative control anti-IgG (Abcam). After protein digestion, the precipitation of RNA was isolated. Finally, RT-qPCR was performed to analyze the extracted RNA precipitates.

Luciferase reporter assay

Circ_0004018 or PTEN mRNA 3'-untranslated region (3'-UTR) sequence of wild-type (Wt) and mutant type (Mut) of miR-1197 with luciferase reporter vector was generated as pmirGLO-circ_0004018-Wt/Mut and pmirGLO-PTEN 3'-UTR-Wt/Mut respectively. Later, the reporter plasmids were co-transfected with control mimics and miR-1197 mimics into HCC cells. After 48 h, the Dual-Luciferase Reporter Gene Assay Kit (Beyotime, Shanghai, China) was applied to measure luciferase activity. The luciferase activity of the experimental reporter was normalized to Renilla luciferase (internal control).

Western blot analysis

The cell lysis buffer (100 μ l) was utilized firstly to abstract total protein from cells. After that, protein samples were separated with SDS-PAGE and later transferred to PVDF membranes (Millipore, Billerica, MA, USA). Afterward, 5% nonfat milk was exploited for membrane sealing-off and

primary antibodies were used to treat membranes at 4°C overnight. After washed with PBS (1 ml) ×3, the blots were then incubated with secondary antibody for 1 h at dark room. The antibodies against PTEN, PI3K, p-PI3K, AKT, p-AKT and GAPDH were all obtained from Abcam. GAPDH served as the internal control. The protein concentrations were quantified by Chemiluminescence system (GE Healthcare, Chicago, USA).

Statistical analysis

SPSS 22.0 statistical software package was used to analyze experimental data. All data were expressed as mean ± standard error of the mean (SEM). Differences between two groups or more groups were compared by Student's t-test or analysis of variance (ANOVA). Each independent experiment was conducted at least three times. Differences were indicated to be statistically significant when $P < 0.05$.

Results

Circ_0004018 is obviously down-regulated in HCC cells

In accordance with the results on circRNADisease (<http://cgga.org.cn:9091/circRNADisease/>), circ_0004018 expression was down-regulated in HCC (Figure 1(a)). Besides, there are several reports demonstrating the anti-tumor effect of circ_0004018 in HCC [17,18]. In addition, from the results of RT-qPCR analysis, we found that compared with normal cell line (THLE-3), circ_0004018 was obviously down-regulated in HCC cell lines (Huh7, Hep3B, SNU-182, and SK-HEP-1) (Figure 1(b)). Furthermore, RT-qPCR confirmed that linear-SMYD4 level was observably reduced after adding RNase R while circ_0004018 was resistant to RNase R digestion, testifying the circular characteristics of circ_0004018 (Figure 1(c)). In addition, agarose gel electrophoresis (AGE) was conducted to further corroborate the circular structure of circ_0004018. It turned out cDNA of circ_0004018 could be detected both in divergent and convergent primers, suggesting its ring form (Figure 1(d)). The expression level of circ_0004018 and linear-SMYD4 was detected in SNU-182 and

SK-HEP-1 cells treated with the transcription inhibitor, Actinomycin D (Act D) to examine the stability of circ_0004018. It was displayed in the results that after Act D treatment, the half-life of circ_0004018 was much longer than that of linear SMYD4, suggesting that circ_0004018 was much more stable than linear-SMYD4 (Figure 1(e)). All data testified the presence of circ_0004018 in HCC cells.

Up-regulation of circ_0004018 suppresses cell proliferation and migration in HCC

Functional assays were implemented with the aim to reveal circ_0004018 impact on HCC cell proliferation and migration. Firstly, we examined the overexpression efficiency of pcDNA3.1-circ_0004018 (Figure 2(a)). Then gain-of-function experiments were performed in cells with or without pcDNA3.1-circ_0004018 transfection. EdU and colony formation assays were carried out for observation on HCC cell proliferation. An overt reduction in number of EdU positive cells and cell colonies could be discovered in response to the transfection of pcDNA3.1-circ_0004018, which suggested that circ_0004018 up-regulation significantly hindered cell proliferation in HCC (Figure 2(b–c)). In addition, cell migratory capacity was assessed by transwell assay. Similarly, we found that relative to the control group, the number of migrated cells was much smaller after circ_0004018 was up-regulated, indicating that overexpression of circ_0004018 inhibited HCC cell migration (Figure 2(d)). In a word, circ_0004018 negatively modulated HCC cell proliferation and migration.

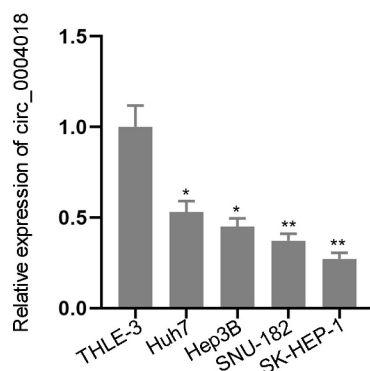
Circ_0004018 serves as miR-1197 sponge in HCC cells

Subcellular fractionation and FISH assays were conducted to observe the distribution of circ_0004018 in HCC cells and we discovered that circ_0004018 was preferentially enriched in the cytoplasm (Figure 3(a–b)). Previous reports have confirmed that circRNAs can modulate gene expression dependent on sponging miRNAs in the cytoplasm [19]. In this case, we conjectured that circ_0004018 could also serve as miRNA sponge.

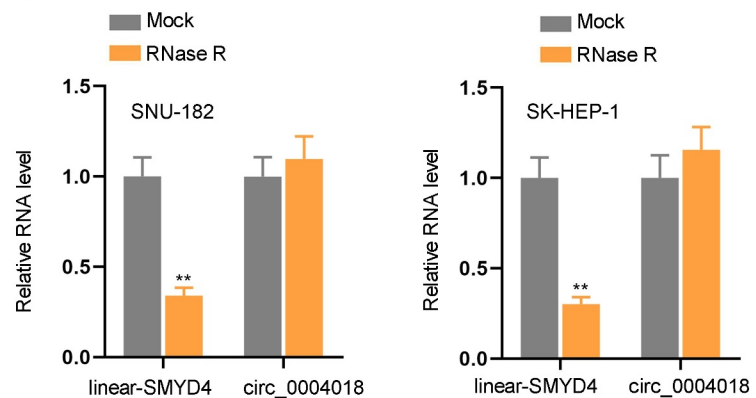
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circRNA_id	circRNA_name	disease	host gene	method	expression pattern	pubmed	details
hsa_circ_0007874	circMTO1, hsa_circ_104135	hepatocellular carcinoma	MTO1	qRT-PCR	down-regulated	28520103	more...
-	circ-ITCH	hepatocellular carcinoma	ITCH	qRT-PCR	down-regulated	28636993	more...
-	hsa_circRNA_100338	hepatocellular carcinoma	SNX27	qRT-PCR, microarray	up-regulated	28710406	more...
-	hsa_circRNA_104075	hepatocellular carcinoma	NUP153	qRT-PCR, microarray	up-regulated	28710406	more...
hsa_circ_0005986	-	hepatocellular carcinoma	PRDM2	qRT-PCR	down-regulated	28410211	more...
-	circRNA_000839	hepatocellular carcinoma	-	qRT-PCR	up-regulated	28695771	more...
hsa_circ_0003570	-	hepatocellular carcinoma	FAM53B	qRT-PCR, microarray	down-regulated	28493512	more...
hsa_circ_0004018	-	hepatocellular carcinoma	SMYD4	qRT-PCR, microarray	down-regulated	28938566	more...
hsa_circ_0001727	circZKSCAN1	hepatocellular carcinoma	ZKSCAN1	RNA-seq	down-regulated	28211215	more...
hsa_circ_0005075	-	hepatocellular carcinoma	EIF4G3	qRT-PCR, microarray	up-regulated	27258521	more...
hsa_circ_0000284	circHIPK3	hepatocellular carcinoma	HIPK3	qRT-PCR, RNA-seq	up-regulated	27050392	more...
hsa_circ_0001649	-	hepatocellular carcinoma	SHPRH	qRT-PCR	down-regulated	26600397	more...

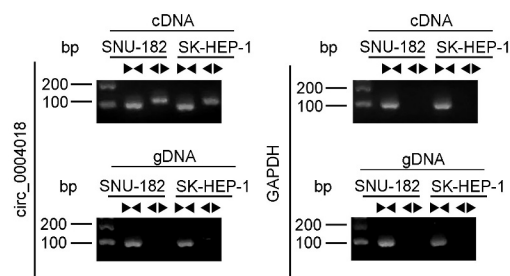
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d



e

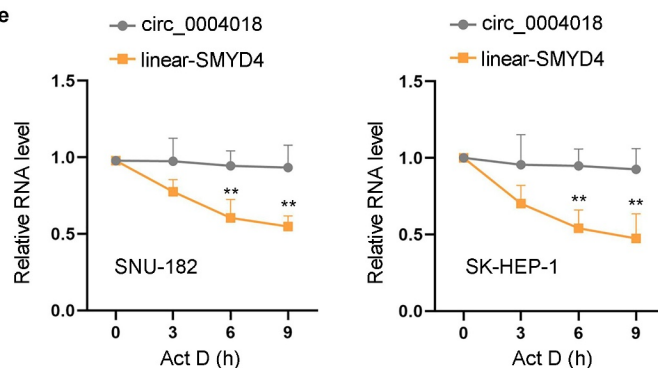


Figure 1. Circ_0004018 is obviously down-regulated in HCC cells. (a) Down-regulation of circ_0004018 in HCC was demonstrated in circRNADisease database. (b). Measurement of circ_0004018 expression in different cell lines was performed via RT-qPCR. (c). RNA levels of circ_0004018 and linear-SMYD4 were detected after RNase R treatment in SNU-182 and SK-HEP-1. (d). AGE was conducted to test the expression of circ_0004018. (e). ActD was added to examine the stability of circ_0004018 and linear-SMYD4 in SNU-182 and SK-HEP-1 cells. * $P < 0.05$, ** $P < 0.01$.

Based on the condition of CLIP Data ≥ 3 , starBase (<http://starbase.sysu.edu.cn/>) predicted 2 potential miRNAs (miR-1197 and miR-378 g). RNA pull down assay indicated that miR-1197, instead of miR-378 g, was pulled down by Bio-circ_0004018 probe (Figure 3(c)). Hence, we selected miR-1197 for further study. It can be understood from

a recent literature that miR-1197 is sponged by circ_0000429 in Non-small-cell lung carcinoma [20]. As shown in Figure 3(d), the binding site between circ_0004018 and miR-1197 was predicted by starBase. SNU-182 and SK-HEP-1 cells were transfected with miR-1197 mimics to enhance the expression of miR-1197 (Figure 3

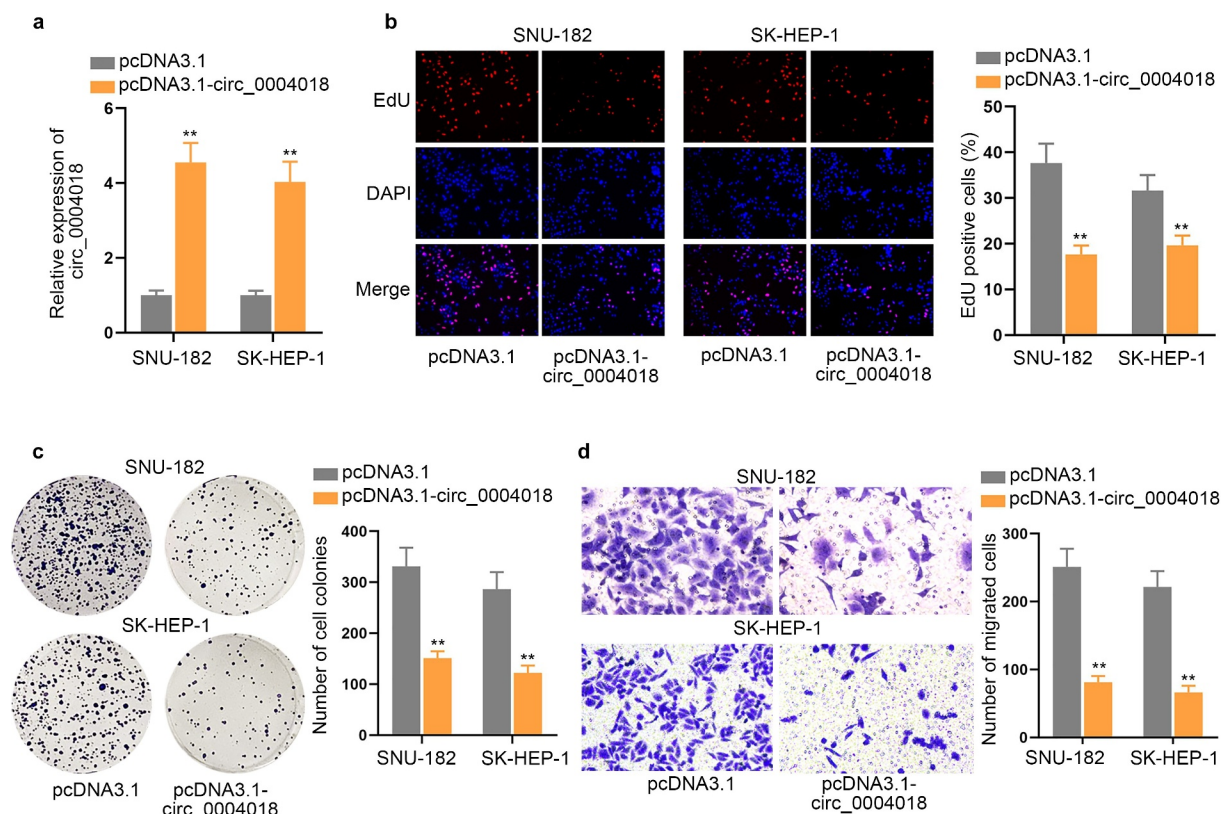


Figure 2. Up-regulation of circ_0004018 suppresses cell proliferation and migration in HCC. (a) The expression of circ_0004018 was analyzed after pcDNA3.1-circ_0004018 transfection into cells via RT-qPCR. (b-c). Cell proliferation was observed by means of EdU and colony formation assays in response to an augment in circ_0004018 expression. (d). Transwell migration assay was implemented with the aim to assess HCC cell migration under the influence of circ_0004018 overexpression. **P < 0.01.

(e). Luciferase reporter assay displayed that miR-1197 mimics dramatically decreased the luciferase activity of circ_0004018-Wt rather than that of circ_0004018-Mut in HCC cells (Figure 3(f)). The finding certified the targeted binding between circ_0004018 and miR-1197. To be concluded, miR-1197 was sequestered by circ_0004018 in HCC cells.

Circ_0004018 positively modulates PTEN expression to inactivate PI3K/AKT signaling pathway by sequestering miR-1197

PTEN has been reported to be a tumor suppressor in HCC [21,22]. Additionally, PTEN has been defined as a negative regulator of the phosphatidylinositol-3-kinase (PI3K)/protein kinase (AKT) pathway [23]. Intriguingly, through starBase, we found PTEN was a target gene of miR-1197. Thus,

we speculated that circ_0004018 might regulate PTEN expression to inactivate PI3K/AKT signaling pathway via sponging miR-1197. From western blot analysis, we discovered that overexpression of circ_0004018 exactly enhanced PTEN protein level while cutting down the protein levels of p-PI3K and p-AKT (Figure 4(a)), suggesting that circ_0004018 could inactivate PI3K and AKT signaling pathway via modulating PTEN expression. The binding sites for PTEN 3'-UTR on miR-1197 were demonstrated in Figure 4(b). RIP assay manifested that circ_0004018, miR-1197 and PTEN were all highly precipitated in Ago2 antibody (Figure 4(c)). Luciferase reporter assays unveiled that miR-1197 mimics effectively lessened the luciferase activity of PTEN 3'-UTR-Wt, while PTEN 3'-UTR-Mut group had no significant change (Figure 4(d)). More interestingly, the protein level of PTEN was prominently enhanced by

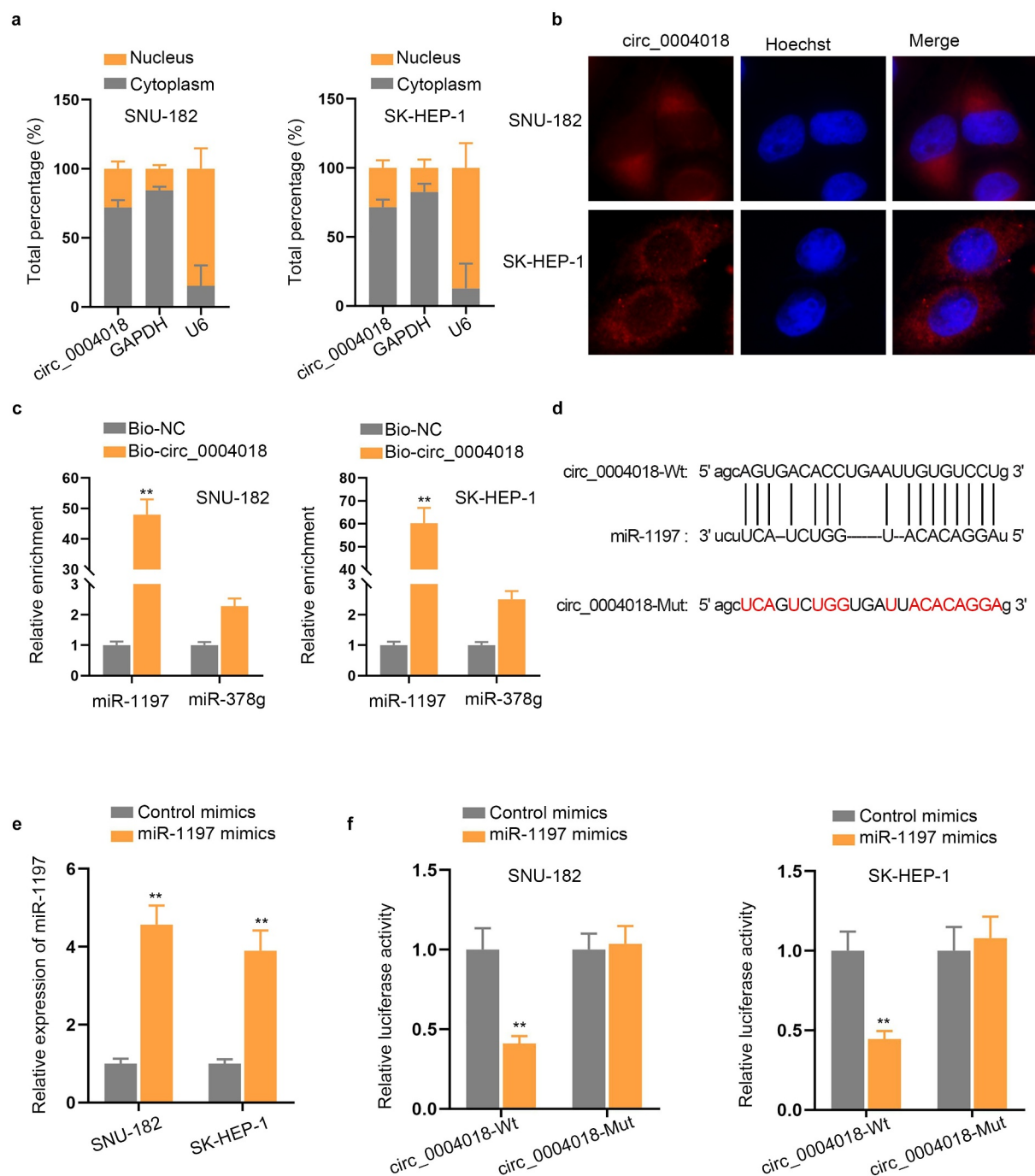


Figure 3. Circ_0004018 serves as miR-1197 sponge in HCC cells. (a-b). The distribution of circ_0004018 in the nucleus and cytoplasm of HCC cells was examined through subcellular fractionation and FISH assays. (c). RNA pull down verified the strong affinity of circ_0004018 and miR-1197. (d). The predicted binding site of miR-1197 within the sequence of circ_0004018 was presented. (e). Enhanced miR-1197 expression level was observed in miR-1197 mimics-transfected HCC cells based on RT-qPCR results. (f). The binding of circ_0004018 and miR-1197 was further verified with the help of luciferase reporter assay. ** $P < 0.01$.

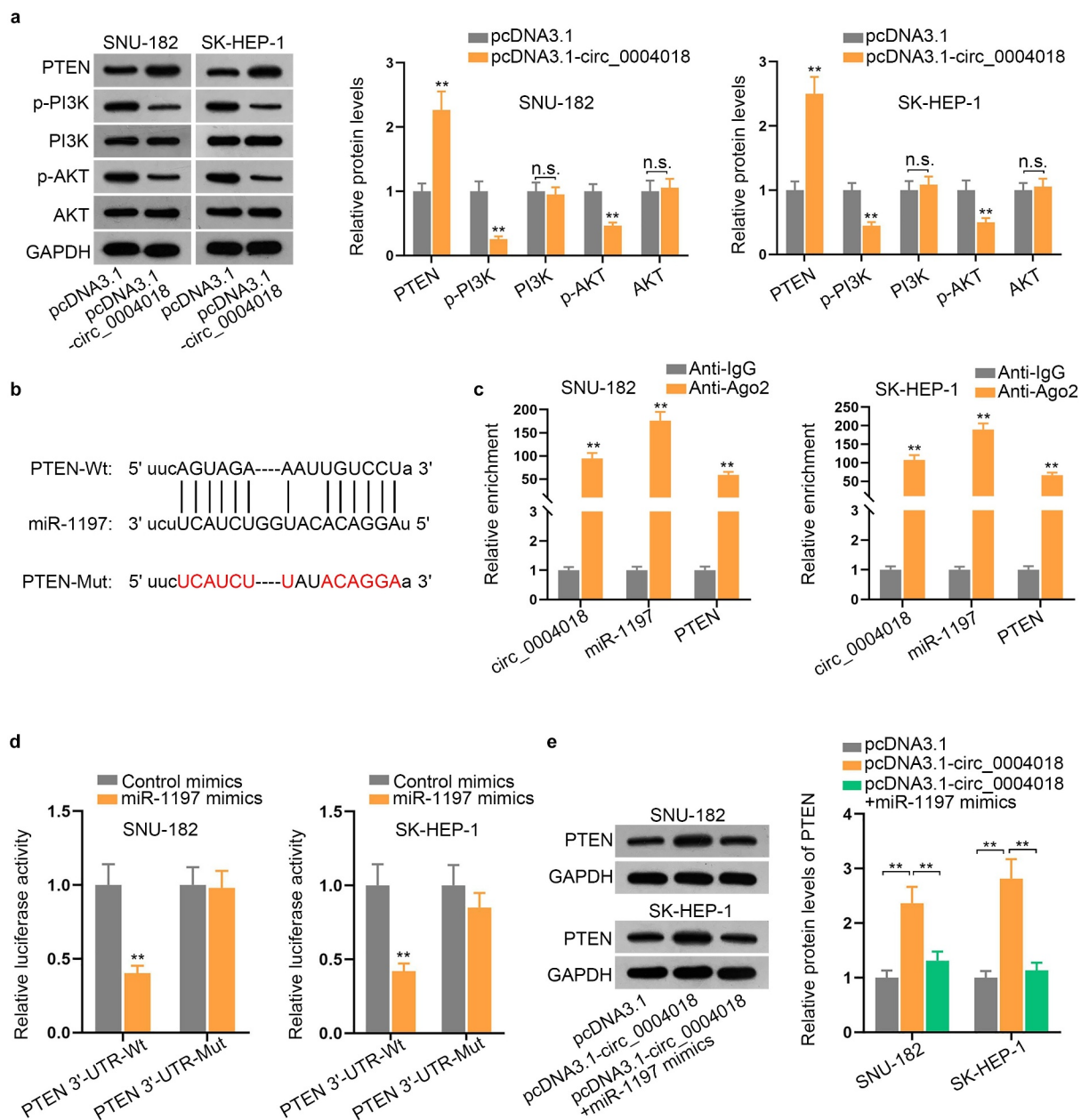


Figure 4. Circ_0004018 sequesters miR-1197 and modulates PTEN expression to inactivate PI3K/AKT signaling pathway. (a) The protein levels of PTEN and related factors of PI3K/AKT pathway were analyzed by western blot after circ_0004018 was over-expressed. (b) The binding sequence of miR-1197 and PTEN was shown. (c) RIP assay exhibited the enrichment of circ_0004018, miR-1197, as well as PTEN in Ago2 group. (d) The binding between PTEN and miR-1197 was revealed out with the help of luciferase reporter assay. (e) PTEN protein level was tested in cells with the transfection of different plasmids, including pcDNA3.1, pcDNA3.1-circ_0004018 and pcDNA3.1-circ_0004018+ miR-1197 mimics. ** $P < 0.01$. n.s.: no significance.

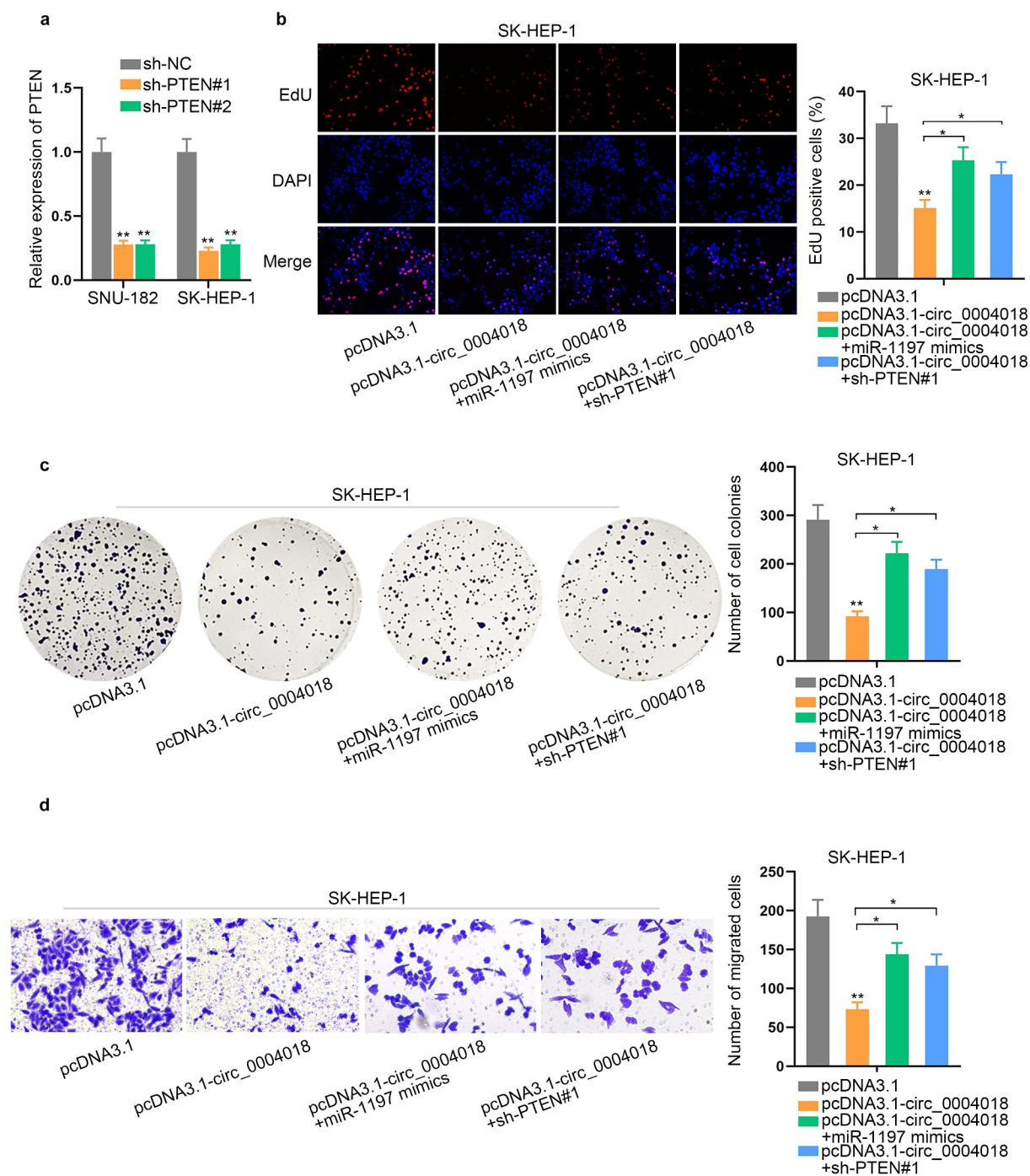


Figure 5. Up-regulation of miR-1197 or down-regulation of PTEN rescues the suppressive impacts of circ_0004018 up-regulation on HCC cell proliferation and migration. (a) Quantification and analysis of PTEN expression in SNU-182 and SK-HEP-1 cells transfected with sh-PTEN#1/2 were achieved via RT-qPCR. (b-c). Cell proliferative capacity was ascertained in different groups by conducting EdU and colony formation assays. (d). With the help of transwell assay, assessment in HCC cell migration was conducted under different conditions. * $P < 0.05$, ** $P < 0.01$.

circ_0004018 overexpression and then restored by miR-1197 mimics transfection (Figure 4(e)). In summary, circ_0004018 inactivated PI3K/AKT signaling pathway via enhancing PTEN expression.

Up-regulation of miR-1197 or down-regulation of PTEN rescues the suppressive impacts of circ_0004018 overexpression on HCC cell proliferation and migration

To elucidate the validity of ceRNA mechanism among circ_0004018, miR-1197 and PTEN, rescue assays were implemented in SK-HEP-1 cells. We firstly knocked down the expression of PTEN (Figure 5(a)). The results of cell proliferation assays disclosed that circ_0004018 overexpression restrained cell proliferation in HCC and this effect was rescued by miR-1197 up-regulation or PTEN down-regulation (Figure 5(b–c)). Transwell migration assay also revealed that miR-1197 augment or PTEN depletion rescued the repressed cell migratory capacity caused by upregulation of circ_0004018 (Figure 5(d)). Overall, upregulation of miR-1197 or downregulation of PTEN recovered the suppressed cell proliferation and migration caused by circ_0004018 overexpression in HCC.

Discussion

HCC is an appalling disease all over the world due to high incidence, frequent recurrence and poor survival rate. CircRNAs have been recognized as important participants in the occurrence and progression of HCC [24]. Circ_0004018 has been found to be significantly downregulated in liver fibrosis and suppresses the progression of liver fibrosis via modulating miR-660-3p/TEP1¹⁸, [25]. In accordance with past research, circ_0004018 presents overtly low expression in HCC tissues than in their paired non-tumor tissues from HCC patients, and its link with miR-626 and the Wnt/ β -catenin signaling pathway is also revealed [26]. Fu et al. have also discovered that circ_0004018 is down-regulated in HCC tissues and cell lines [10]. In our study, the primary object was the deep investigation into circ_0004018 role in HCC cell proliferation and migration.

Consistent with the above reports, our experimental findings manifested that circ_0004018 expression in HCC cells was obviously lower than in THLE-3, and overexpression of circ_0004018 remarkably weakened the proliferative and migratory capacities of HCC cells.

Recent evidence has demonstrated the importance of ceRNA mechanism in diverse cancers. In brief, circRNAs or long non-coding RNAs (lncRNAs) have the capacity to regulate mRNA expression by competing for common miRNAs [27]. The interaction between circ_0004018 and several miRNAs in HCC has been demonstrated by Fu et al. [10]. Similarly, in this paper, we found that circ_0004018 was mainly accumulated in the cytoplasm of HCC cells, suggesting that circ_0004018 might exert its roles at post-transcriptional level. With the help of bioinformatics analysis and RNA pull down assay, miR-1197 was screened out as the candidate miRNA of circ_0004018. Based on previous literature, miR-1197 could be sponged by circ_0000429 and thereby involved in the specific regulatory mechanism of circ_0000429 in Non-small-cell lung carcinoma [20]. Consistently, the interaction between circ_0004018 and miR-1197 was verified based on luciferase reporter assays, s. Furthermore, PTEN was discovered as a target gene of miR-1197. More importantly, the protein level of PTEN was enhanced by circ_0004018 upregulation while counteracted by up-regulation of miR-1197.

As one of the most crucial oncogenic pathways, PI3K/AKT signaling pathway influences cancer development and progression via regulation of cell survival, cell death, and cell growth [28,29]. PTEN is a crucial component involved in PI3K/AKT signaling pathway. To be more specific, as a negative regulator of PI3K/AKT pathway, PTEN may lead to inactivation of PI3K/AKT signaling network [30]. In addition, PTEN is determined to be a valuable predictor for HCC recurrence and overall survival [31]. In this research, we found that circ_0004018 inactivated PI3K/AKT pathway to inhibit cell proliferation and migration in HCC via sponging miR-1197 and modulating PTEN expression.

Overall, circ_0004018 was demonstrated to be significantly downregulated in HCC cells. Functionally, circ_0004018 restrained cell

proliferation and migration in HCC. Moreover, circ_0004018 sponged miR-1197 and regulated the expression of PTEN, thereby inactivating the PI3K/AKT signaling pathway in HCC cells. Finally, rescue experiments substantiated that up-regulation of miR-1197 or down-regulation of PTEN rescued the suppressive impact of circ_0004018 on cell proliferation and migration in HCC, which further verified the circ_0004018/miR-1197/PTEN/PI3K/AKT pathway and might provide a potential biomarker for HCC diagnosis and treatment in the future.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Qiao Zhang  <http://orcid.org/0000-0001-5307-7317>

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