

Circ_0003747 promotes thyroid cancer progression by sponging miR-338-3p to upregulate PLCD3 expression

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ABSTRACT

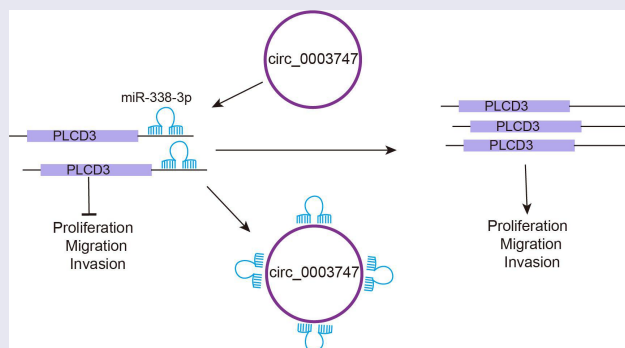
The circular RNAs (circRNAs) involved in competitive endogenous RNA (ceRNA) mechanism are critical modulators affecting pathogenesis of thyroid carcinoma (TC). The study's goal was to investigate the effects of circ_0003747 on the biological progression of papillary thyroid cancer (PTC). Normal thyroid cells Nthy-ori3-1 and TC derived cell lines were used in our study. Sanger sequencing and RNase R treatment were utilized for validating the circular structure of circ_0003747. In our work, circ_0003747 was found to be highly expressed in TC cells. Circ_0003747 knockdown reduced TC cell viability, proliferation, migration, and invasion while increasing cell apoptosis. Circ_0003747 targeted and negatively regulated miR-338-3p expression. Besides, miR-338-3p interacted with PLCD3 to repress its expression. Overexpression of miR-338-3p inhibited TC cell progression, and PLCD3 reversed these effects. Furthermore, PLCD3 overexpression reversed the effects of circ_0003747 knockdown on TC cells. Additionally, the knockdown of circ_0003747 remarkably suppressed tumour size and growth, restrained PLCD3 expression and promoted miR-338-3p expression in nude mice. In conclusion, circ_0003747 facilitated the biological progression of TC by modulating the miR-338-3p/PLCD3 axis, and it may be a new target for TC treatment.

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Abbreviations: TC: Thyroid carcinoma; PTC: Papillary thyroid carcinoma; CircRNAs: Circular RNAs; MiRNA: MicroRNA; EMT: Epithelial-mesenchymal transition; HCC: Hepatocellular carcinoma; PLCD3: Phospholipase C Delta 3; CeRNA: Competitive endogenous RNA

Introduction

Thyroid carcinoma (TC) accounts for about 1% of the systemic malignant tumour, which is characterized by enlarged thyroid nodules, dysphagia, enophthalmos, and other clinical symptoms [1]. Due to advances in radiotherapy and chemotherapy, most TCs have low malignancy and a good prognosis [2]. The common clinical treatment

methods for TC are total thyroidectomy or sub-total thyroidectomy, but they are prone to recurrence and metastasis [3]. The advanced or aggressive TC is resistant to radioactive iodine absorption or surgical resection and can lead to recurrence and even death [2]. Therefore, it is critical in clinic to investigate the related factors that contribute to the development of TC.

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As endogenous non-coding RNAs, circular RNAs (circRNAs) can interlink by covalent bonds to form a closed loop structure and regulate downstream gene expression by binding to targeted miRNAs [4,5]. As reported in the previous study, circular RNA N4BP2L2 (circN4BP2L2) could promote colorectal cancer process via miR-340-5p/CXCR4 pathway [6]. Circular RNA TAB2 (circTAB2) inhibits lung cancer progression via the miR-3142/GLIS2 axis [7]. Circular RNA DOCK1 (circDOCK1) was involved in thyroid carcinogenesis through inhibiting miR-124 expression and activating signal transduction of Janus Kinase/Signal Transducer and Activator of Transcription/Adenosine monophosphate protein kinase (JAK/STAT/AMPK) in TC cells [8]. Circ_0000144 expression was notably enhanced in TC cells and exerted a cancer-promoting effect on TC via miR-217/AKT3 signal axis [9]. The level of circ_0003747 was strikingly elevated in the Papillary Thyroid Carcinoma (PTC) [10]. However, the molecular regulatory mechanism of circ_0003747 on TC process has not been reported. Thus, the study of ceRNA in the treatment of thyroid cancer is helpful to better understand the molecular mechanism of thyroid cancer. However, the molecular regulatory mechanism of circ_0003747 on the TC process has not been reported.

MicroRNAs (miRNAs) are small non-coding RNAs that play a vital role in the post-transcriptional regulatory process [11]. It was demonstrated that dysregulation of miRNAs regulated tumour cell progression [12]. The expression changes of various miRNAs might be the main mechanism of thyroid carcinogenesis [13]. The expression of miR-338-3p was lower in gastric cancer tissues, and miR-338-3p restrained the migration and invasion of gastric cancer cells and suppressed epithelial–mesenchymal transition progress [14]. The miR-338-3p was down-regulated in hepatocellular carcinoma (HCC) tissues, and miR-338-3p could repress tumour growth and sensitize HCC cells to sorafenib via inhibiting HIF-1 α [15]. As a novel tumour suppressor, miR-338-3p restrained cell proliferation and migration, whereas accelerated apoptosis of TC by targeting AKT3 [16].

Phospholipase C (PLC) is a key enzyme in the phosphoinositol pathway and is involved in signal transduction in eukaryotes. As a member of the PLC family, phospholipase C Delta 3 (PLCD3) plays

a crucial role in multiple biological processes, such as survival of cardiomyocytes and trophoblast cells, maintenance of normal heart function, and promotion of neurite expansion [17,18]. *PLCD3* also serves as an oncogene, which has been shown to promote tumorigenesis and progression of TC [19]. In this work, a CircInteractome database analysis found that circ_0003747 had a target binding site with miR-338-3p. StarBase predicted that miR-338-3p had binding site with *PLCD3*. Our initial findings showed that circ_0003747 targeted miR-338-3p, which was negatively correlated with the target gene *PLCD3*, suggesting that circ_0003747, as a ceRNA, regulated the expression of downstream target gene *PLCD3* by directly binding to miR-338-3p.

Herein, the function of circ_0003747 in TC was investigated. The aim of this study was to investigate the function of circ_0003747 in TC and unveil the relationship between circ_0003747 and miR-338-3p and their roles in TC. Moreover, the potential regulatory mechanism involving the miR-338-3p/*PLCD3* axis was analysed as well. Our study provided important evidence for circ_0003747 as a promising target for TC treatment.

Materials and methods

Cell culture and treatment

The normal thyroid cells (Nthy-ori3–1) and TC derived cell lines (TPC-1, IHH4, B-CPAP, and CAL-62) were provided by the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in the DMEM/F12 medium containing 10% FBS, 100 U/mL penicillin, and 100 μ g/mL streptomycin (Gibco, USA) at 37°C in a 5% CO₂ incubator. When the cells covered about 80% of the bottom area of the culture flask, the cells were sub cultured, and the cells at logarithmic growth phase were used in the further experiments.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted according to TRIZOL reagent (Thermo Fisher Scientific, USA). The RNA levels were quantified using a Nanodrop Spectrophotometer (IMPLEN GmbH, Germany).

Table 1. Primers used for qRT-PCR in this study.

Primer names	Forward (5'-3')	Reverse (5'-3')
circ_0003747	GCCTGCTACACGCTTGATGT	TGCCATTCTGTCTGGGTAG
miR-338-3p	GCCGAGTCCAGCATCAGTGATT	GTGCAGGGTCCGAGGT
PLCD3	GAACAGCTTTGTCAAGCACA	TTGAGGTCCATCTCGTAGCC
CARF	AAGCCTGCTACACGCTTGAT	AATCCGAGCTGGACAAGTGG
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
GAPDH	GCACCGTCAAGGCTGAGAAC	GGATCTCGCTCTGGAAGATG

Reverse transcription was conducted according to instructions of reverse transcription kit (Takara, China). The relative RNA expression levels were detected by qRT-PCR assay using the SYBR Green Master PCR mix (Applied Biosystems, USA) as per the manufacturer's protocol following previous study [20]. GAPDH and U6 were used as the internal reference. The primers used in the present work are in Table 1.

Western blot assay

The Western blot assay was performed following the protocol outlined in [20]. Total proteins were extracted according to the instructions of RIPA reagent (Beyotime, China); then, the protein concentrations were measured. Equal amounts of proteins were taken to SDS-PAGE for separation, and then electrically transferred to the PVDF membranes (Invitrogen, USA). The samples were blocked in 5% non-fat milk for 60 min. Subsequently, for the detection of PLCD3 protein, the anti-PLCD3 (1/2000, Abcam, USA) primary antibody was added and incubated overnight at 4°C. Then, the goat horseradish peroxidase-conjugated anti-rabbit IgG was added. The immunoreactive bands were visualized by the electrochemical luminescence detection system following the instructions. Image J was utilized to assess grey values of target bands with GAPDH as an internal control.

Circ_0003747 identification

Sanger sequencing and RNase R treatment were utilized for validating the circular structure of circ_0003747 [21]. The total RNA was treated with the presence or absence of RNase R at 37°C for 30 min. The relative levels of RNA were

assessed using qRT-PCR assay, and the data were analysed with $2^{-\Delta\Delta C_t}$ method.

Cell transfection

For transfection [22], TC cells (1.2×10^6 cells/well) were seeded in 6-well plate. The shRNAs targeting circ_0003747 (sh-circ_0003747) and over-expression of PLCD3 (pcDNA3.1 PLCD3) were designed by GenePharma (Shanghai, China). The miR-338-3p mimics (50 nM), inhibitor (50 nM), or vectors (2 µg/mL) provided by RiboBio (Guangzhou, China) were transfected into cells with Lipofectamine 2000 (Invitrogen, USA).

MTT assay

Cells (1×10^4 cells/well) were seeded in a 96-well plate and incubated for 24 h at room temperature. Then, MTT (20 µL) was mixed into each well at 24 h, 48 h, and 72 h points, and incubated for 4 h at 37°C. Next, 150 µL dimethyl sulphoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) was also added into each well at 37°C with 5% of CO₂ for 3 h. The absorbance was measured at 490 nm [23] using a microwell reader (Analytik Jena AG, Germany) at room temperature.

Colony formation assay

Colony formation assay was conducted refer to previous study [24]. TC cells were transfected for 48 h and incubated into a six-well plate at a density of 1×10^3 cells per well. Then, the cells were cultured for 14 d at 37°C with 5% CO₂. The cells were fixed with paraformaldehyde for 30 min and stained with 0.1% crystal violet (Sigma, USA). Subsequently, excessive crystal violet was wiped off, and the cells were rinsed with PBS buffer.

The cells were photographed, and visible colonies were counted using Image J software.

Wound healing assay

Referring to the study in [25], TC cells (2×10^5 cells/mL) were incubated into a 24-well plate and cultured at 37°C in a 5% CO₂ incubator. After incubating to confluence, the cells were scratched by a sterile pipette tip. Subsequently, the plates were washed with fresh medium twice and incubated. Cells migrated from the wound edge, and the distance between the two sides of the wound was monitored at 0 h and 24 h under a microscope. The degree of cell migration was quantified by the ratio of measured gap distance.

Transwell assay

The invasion ability of the TC cells was assessed by transwell assay using a chamber (Corning Costar, Inc., USA) (8 μm pore size) with the addition of 10 μL Matrigel (BD Biosciences, USA) at 1:3 dilution. After transfection, TC cells were seeded at 5×10^5 /well on a polycarbonate membrane with a fibronectin coating inserted into the upper transwell chamber. Then, the cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet for 15 min. Subsequently, the number of invasive cells was counted under a microscope in five different fields [26].

Apoptosis detection

Cell apoptosis of TC cells was evaluated by flow cytometry using Annexin V-FITC/PI staining kit (Invitrogen, USA) following a published protocol [26]. The TC cells were trypsinized, rinsed by cold PBS buffer and stained with Annexin V-FITC/PI solution. Then, a FACScan flow cytometer (Beckman Coulter, USA) was applied, and the cell apoptosis rate was quantitatively assessed.

Dual-luciferase reporter gene detection

The targeted binding site between circ_0003747 and miR-338-3p was predicted through the online website CircInteractome (<https://circinteractome.irp.nia.nih.gov/>). Meanwhile, the StarBase online

website (<http://starbase.sysu.edu.cn/index.php>) was utilized for predicting the binding site between miR-338-3p and PLCD3. The wild-type and mutant-type full-length sequences involving the predicted binding sequence of circ_0003747 (circ_0003747-WT/circ_0003747-MUT) and PLCD3 3'-UTR (PLCD3-WT/PLCD3-MUT) were cloned into the XhoI I-Not I restriction sites of pmirGLO firefly luciferase plasmid. All constructs were sequenced to verify the integrity. Lipofectamine 3000 (Invitrogen, USA) was used for Circ_0003747-WT/circ_0003747-MUT and miR-338-3p mimics (GenePharma, China) co-transfection. Luciferase assay was then carried out using a dual-luciferase reporter assay kit according to the manufacturer's protocol (Promega) 48 h after transfection [25]. Data were normalized to Renilla Luciferase activity and the relative activities of Luciferase were calculated. Besides, the correlation between miR-338-3p and PLCD3 was validated using the same method.

Mice xenograft assay

BALB/c female nude mice (4 weeks) were provided by the Animal Center of the Chinese Academy of Science and housed under controlled laboratory conditions. The mice were randomly divided into sh-circ_0003747 group and sh-NC group, with 6 mice in each group. TPC-1 cells (5×10^6) were treated with sh-NC or sh-circ_0003747 before being injected subcutaneously into the dorsal sides of the mice, referring to a previous study [25]. The length and width of the tumour were measured every 3 d, and the tumour volume was calculated using $(\text{length} \times \text{width}^2)/2$. After euthanizing on the 28th d, the tumour tissues were imaged and prepared for further experimental use. Our protocol was approved by the Ethics Committee of Xiangya Hospital, Central South University.

Immunohistochemistry assay

The tumour tissues were fixed, embedded in paraffin, and then cut into sections of 4 μm. Specific rabbit anti-PLCD3 antibody (1/100, Santa Cruz Biotechnology, USA) was added and incubated at 4°C overnight. Then, the tissues were incubated with goat anti-rabbit secondary antibody. The

complexes were detected by HRP–streptavidin conjugate and visualized using diaminobenzidine (DAB) [25].

Statistical analysis

Each experimental group repeated three times and obtained three independent data. Statistical data was analysed by GraphPad Prism 8, and the values were shown as means \pm standards deviation (SD). For the normally distributed data, the Student's *t*-test was adopted for pairwise comparison, and

multi-group comparison was conducted using one-way ANOVA. *P* value <0.05 indicates statistically significant. * Indicates $p < 0.05$; ** indicates $p < 0.01$ and *** indicates $p < 0.001$.

Results

Relative expressions of circ_0003747, miR-338-3p, and PLCD3 in TC cells

As shown in Figure 1(a,c) the transcription levels of circ_0003747 and PLCD3 in TC cells were higher than those in Nthy-ori3-1 cells. In contrast,

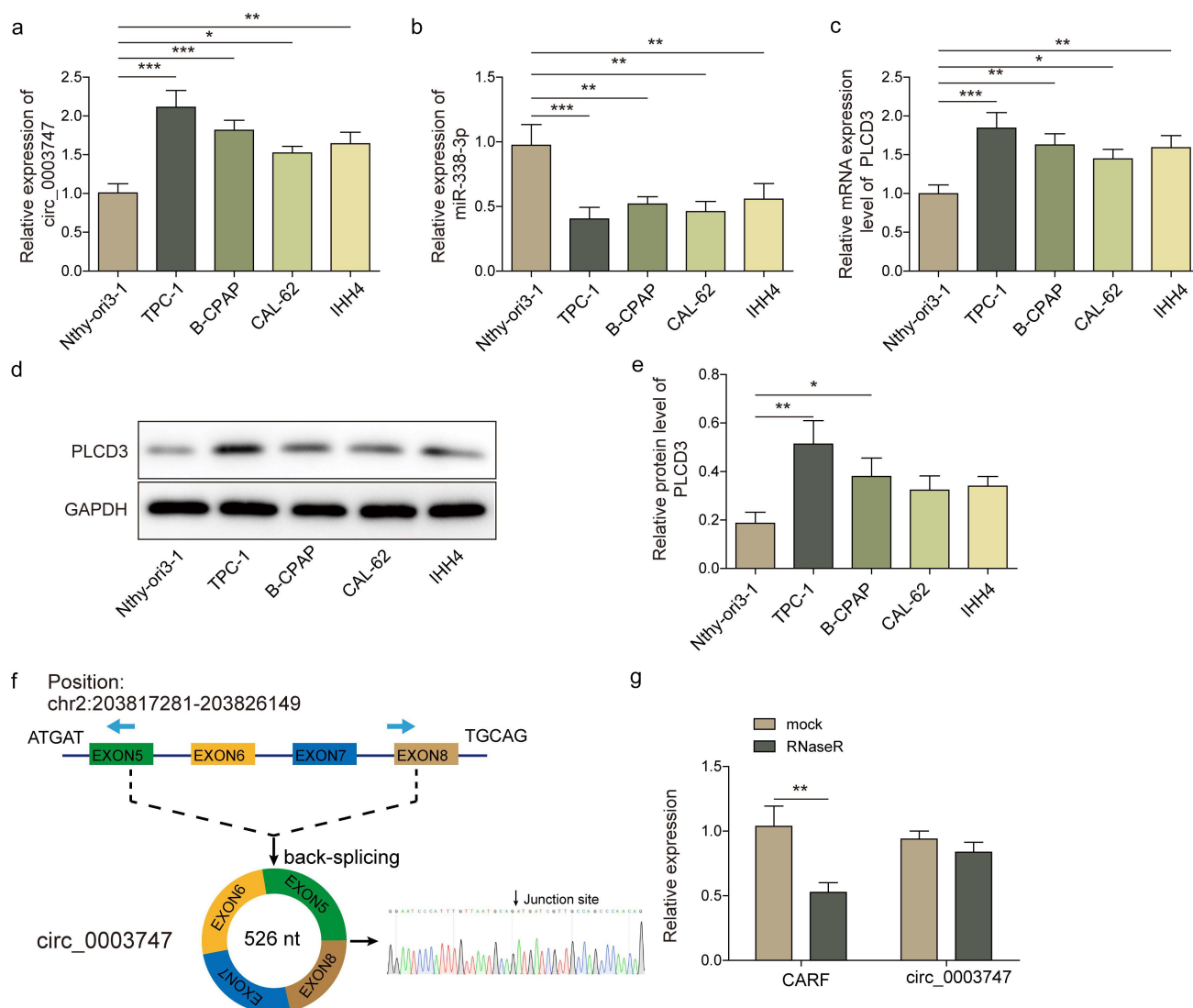


Figure 1. The expressions of circ_0003747 and PLCD3 are increased, miR-338-3p expression is reduced in TC cells.

(a-c) The levels of circ_0003747, miR-338-3p, and PLCD3 were measured using qRT-PCR. (d, e) PLCD3 protein expression was assessed by western blot. (f) The molecular characteristic structure of circ_0003747 was determined by Sanger sequencing. (g) The stability of circ_0003747 was detected by qRT-PCR after the RNase R treatment. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

miR-338-3p level was reduced in TC cells than that in Nthy-ori3-1 cells (Figure 1b). Moreover, PLCD3 protein level was up-regulated in TC cells (Figure 1d, e). Circ_0003747 was located on exon 5–8 of CARF gene of chromosome 2, with a total length of 526 nt, and its molecular characteristic structure was determined by Sanger sequencing (Figure 1f). After treatment with RNase R, the relative expression level of CARF was dramatically reduced, and circ_0003747 level was basically unchanged, indicating that circ_0003747 was a stable circular structure (Figure 1g).

Knockdown of circ_0003747 represses TC cell proliferation and migration but promotes apoptosis

To investigate the effect of circ_0003747 on TC progression, sh-circ_0003747 was transfected into TPC-1 and B-CPAP cells. As shown in Figure 2(a,b) the levels of circ_0003747 and PLCD3 were reduced in TC cells after sh-circ_0003747 transfection. Besides, PLCD3 protein expression was strikingly inhibited in transfected TC cells (Figure 2c). Knockdown of circ_0003747 inhibited viability and proliferation of TC cells (Figure 2d,e). In addition, the downregulation of circ_0003747 significantly depleted the migration and invasion of TC cells (Figure 2f,g). Additionally, the apoptosis rate was notably elevated in TC cells after knockdown of circ_0003747 (Figure 2h). The above results illuminated that circ_0003747 could positively regulate PLCD3 expression and circ_0003747 knockdown restrained progression of TC cells.

Circ_0003747 serves as a miR-338-3p sponge in TC cells

CircInteractome database analysis found that circ_0003747 had a target-binding site with miR-338-3p (Figure 3a). miR-338-3p mimics restrained luciferase activities in circ_0003747-WT cells, but there were no significant changes in circ_0003747-MUT cells (Figure 3b). The expression of miR-338-3p in TC cells was elevated after circ_0003747 was knocked down and enormously declined after miR-338-3p inhibitor reintroduction (Figure 3c). The cell viability, proliferation ability, migration, and invasion activity

were drastically restrained in sh-circ_0003747 transfected TC cells, but these effects were counteracted due to the miR-338-3p inhibitor reintroduction (Figures 3d-f and S1). Additionally, sh-circ_0003747 transfection promoted apoptosis of TC cells, while miR-338-3p inhibitor weakened this effect (Figure 3b). Taken together, circ_0003747 acted as a sponge of miR-338-3p and regulated the progression of TC cells.

miR-338-3p targets PLCD3 and inhibits TC process

StarBase predicted that miR-338-3p had binding site with PLCD3 (Figure 4a), and the potential regulatory mechanism of miR-338-3p and PLCD3 in TC was explored. The luciferase activities were remarkably suppressed after miR-338-3p mimics transfection in PLCD3-WT cells, but there were no significant changes in PLCD3-MUT cells (Figure 4b). Meanwhile, miR-338-3p mimic transfection could repressed the mRNA expression level of PLCD3 in TC cells (Figure 4c). Furthermore, the overexpression of PLCD3 reversed the downregulation effect of miR-338-3p on PLCD3 gene expression (Figure 4d,e). Cell viability, proliferation ability, migration, and invasion activity were markedly restrained in TC cells with miR-338-3p mimic transfection, but the effect was abrogated due to PLCD3 overexpression (Figures 4f-h and Fig. S2). In addition, miR-338-3p mimic transfection promoted apoptosis of TC cells, while simultaneous upregulation of PLCD3 attenuated this effect (Figure 4i). Collectively, it was illustrated that miR-338-3p targeted PLCD3 to negatively regulate its expression and repressed TC cell progression.

Overexpression of PLCD3 eliminates the effects of circ_0003747 knockdown on TC cells

To further address whether circ_0003747 involved in TC cell malignant phenotypes via regulating PLCD3 expression, TC cells were co-transfected with sh-circ_0003747 and PLCD3. PLCD3 overexpression attenuated the inhibition effect of sh-circ_0003747 on its gene expression (Figure 5a,b). In addition, upregulation of PLCD3 eliminated the

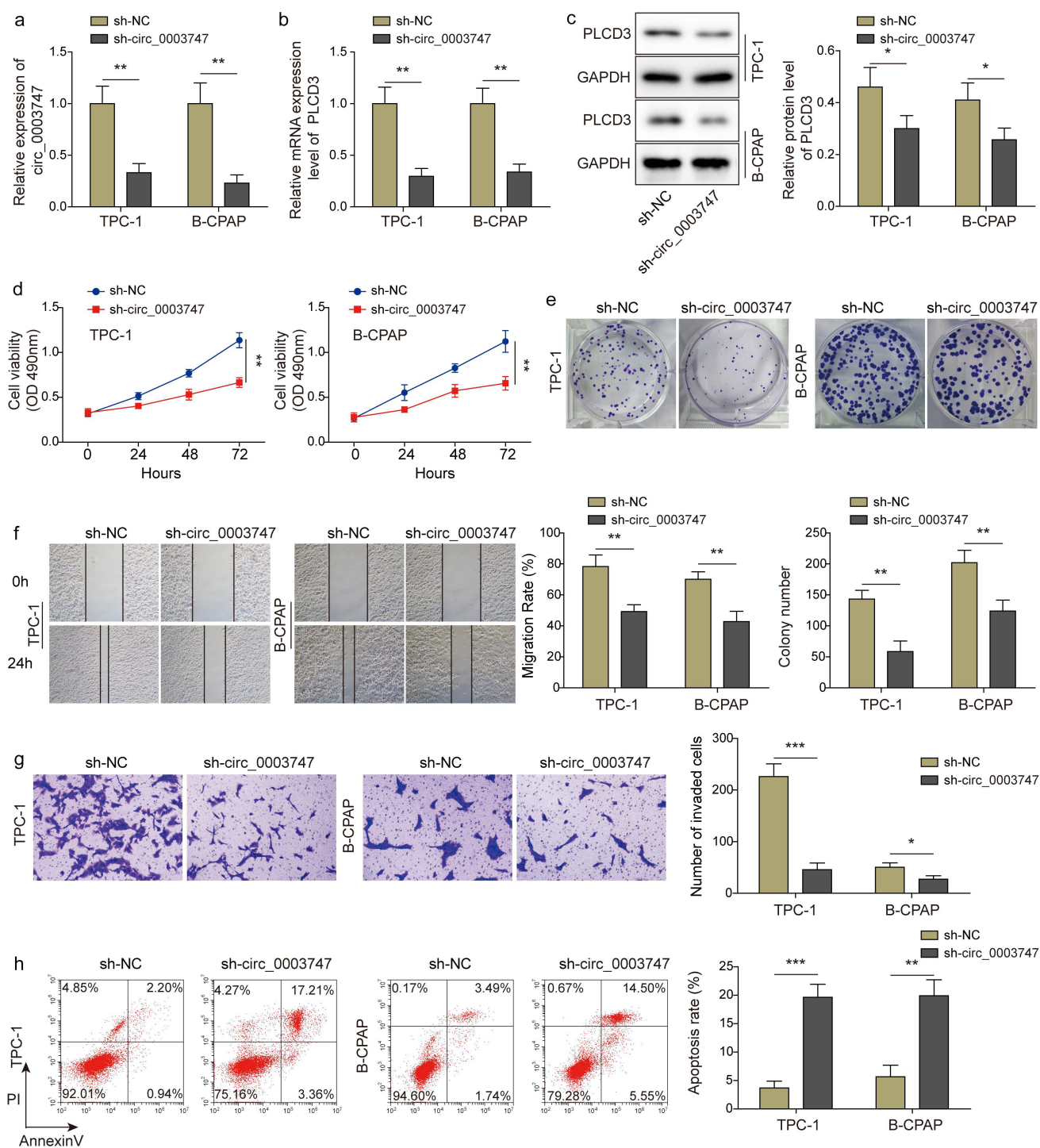


Figure 2. Circ_0003747 knockdown suppresses TC cells progression.

(a, b) The levels of circ_0003747 and PLCD3 after transfection with sh-circ_0003747 were assessed using qRT-PCR. (c) PLCD3 protein level in TC cells with circ_0003747 knockdown was detected by western blot. (d, e) Cell viability and proliferation were evaluated by MTT and colony formation assays. (f) The wound healing assay was conducted to detect cell migration activity. (g) Cell invasion activity was measured by transwell assay. (h) Cell apoptosis after transfection was monitored by flow cytometry. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

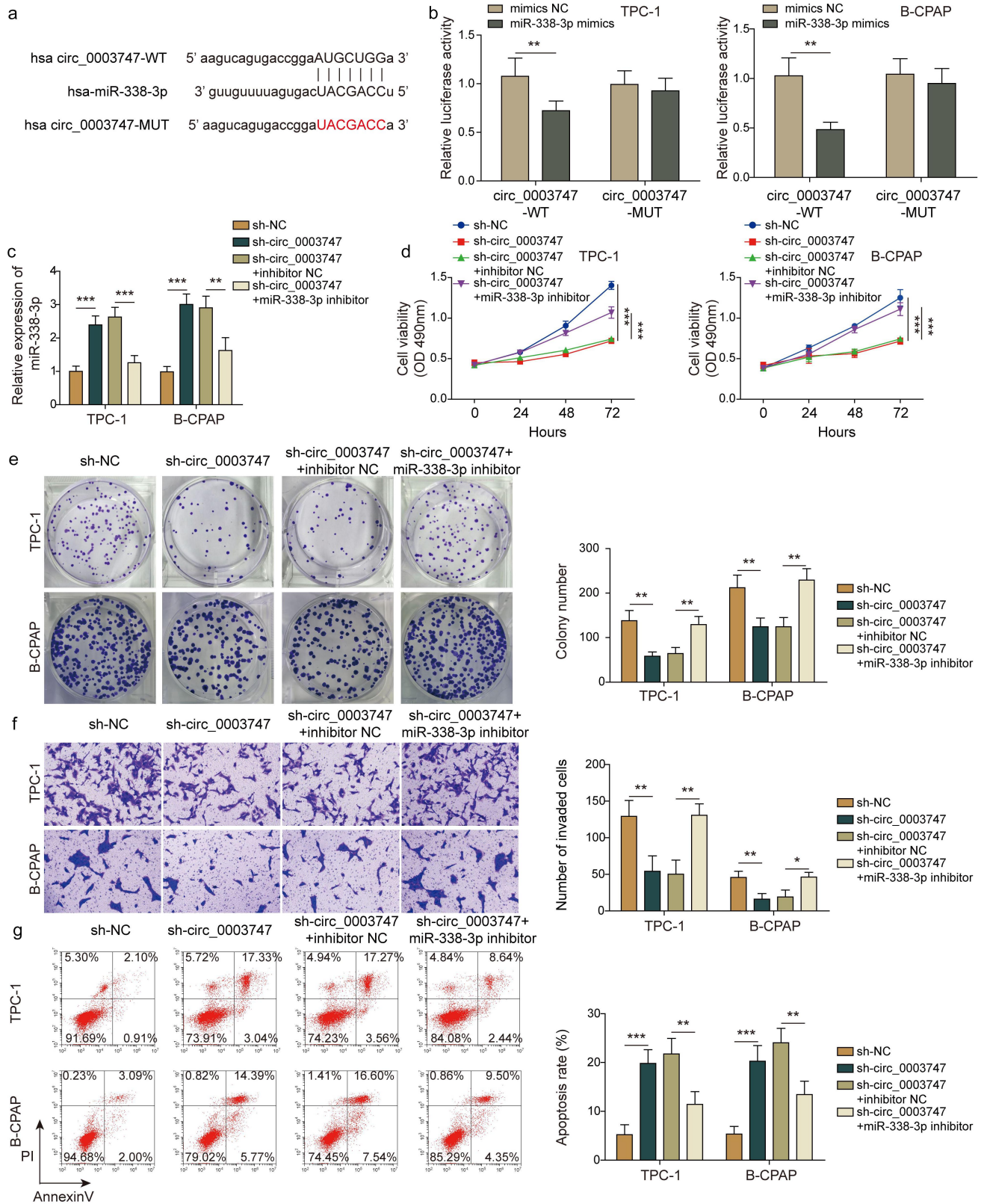
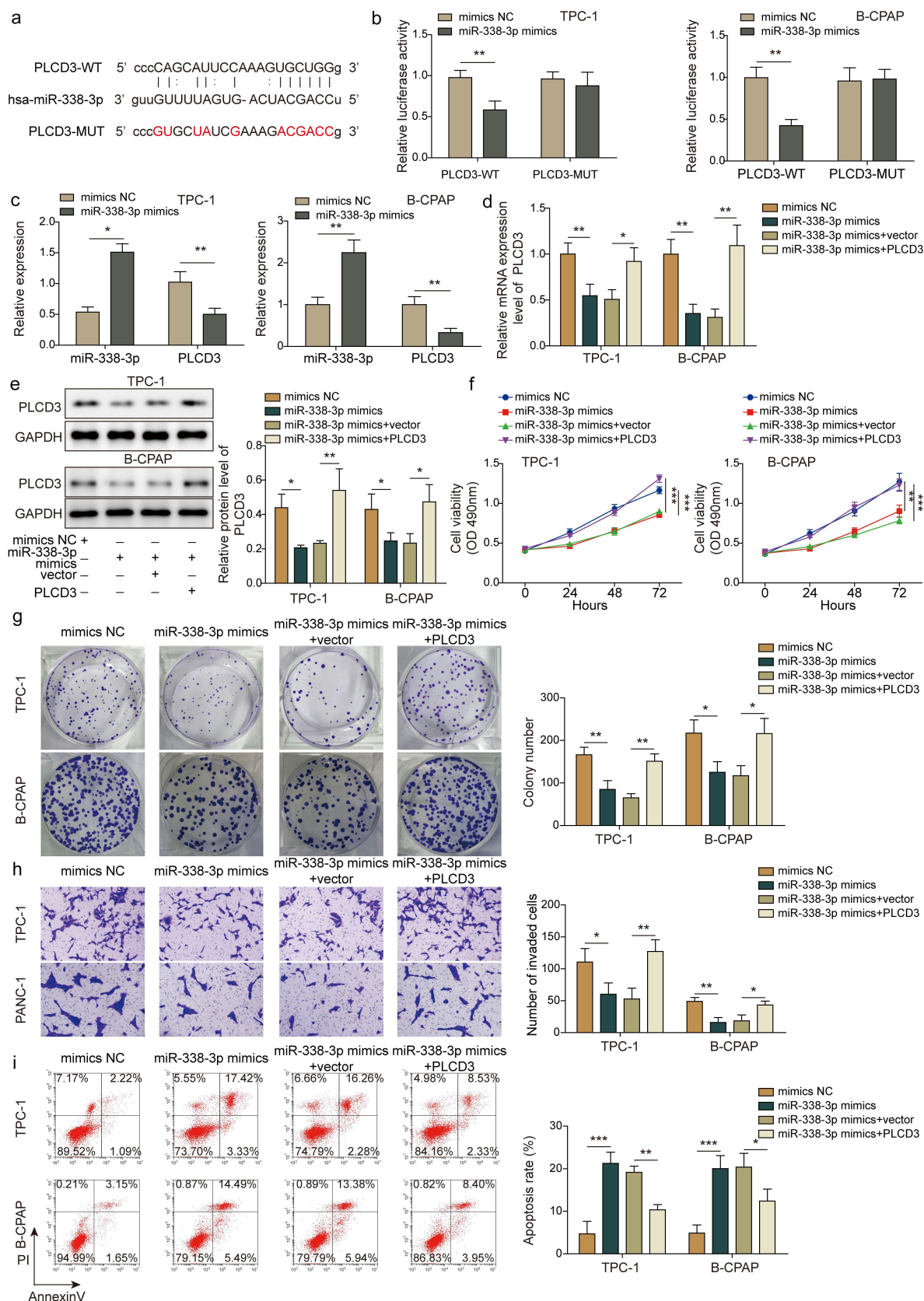
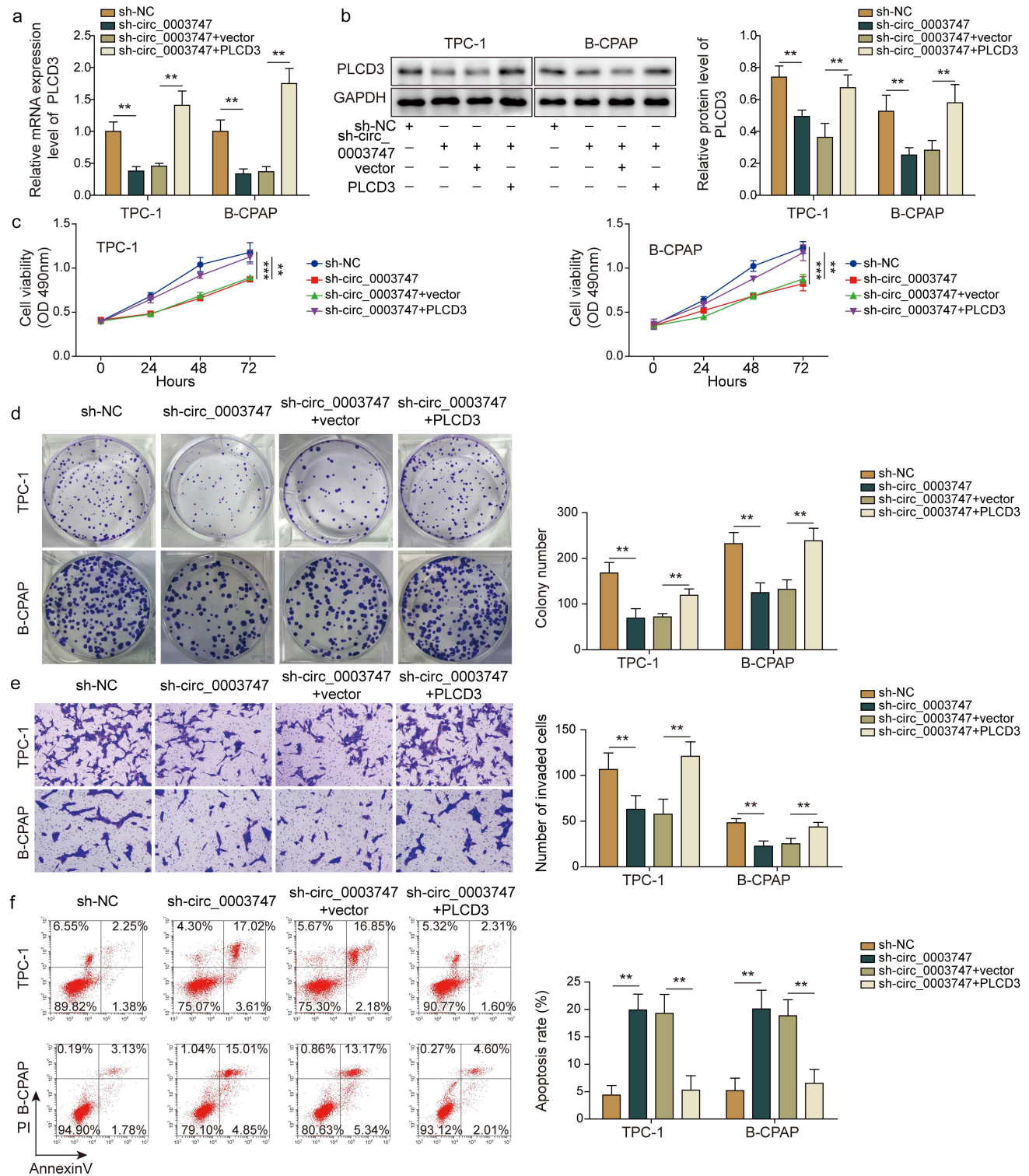


Figure 3. Circ_0003747 negatively regulates miR-338-3p expression in TC cells.

(a) The predicted correlation between circ_0003747 and miR-338-3p was analysed using the CirInteractome database. (b) The binding relationship between circ_0003747 and miR-338-3p was verified by a dual-luciferase reporter assay. (c) The expression of miR-338-3p in TC cells was detected by qRT-PCR. (d, e) Cell viability and proliferation were assessed. (f) Cell invasion activity was measured by transwell assay. (g) Cell apoptosis of TC cells after transfection was monitored using flow cytometry. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.





inhibition effects of cell viability, proliferation ability, migration, and invasion activity by sh-circ_0003747 transfections in TC cells (Figures 5c-e and S3). Moreover, the promoted apoptosis of TC cells by sh-circ_0003747 transfections were weakened after PLCD3 reintroduction (Figure 5f). These findings indicated that circ_0003747 affected TC cell progression by regulating PLCD3 expression.

Circ_0003747 facilitates tumour growth *in vivo* through miR-338-3p/PLCD3 signalling axis

To validate the effects of circ_0003747 on tumorigenesis *in vivo*, TC cells were subcutaneously injected into the nude mice. Knockdown of circ_0003747 dramatically suppressed the tumour size and growth *in vivo* (Figure 6a,b). Circ_0003747 knockdown restrained the mRNA expression levels of PLCD3 and promoted miR-338-3p expression (Figure 6c). Furthermore, western blot and immunohistochemistry assays showed that PLCD3 protein expression was inhibited in circ_0003747 knockdown group in comparison with that in the NC group (Figure 6d,e). Based on these data, circ_0003747 plays a crucial role in TC cell progression by modulating the miR-338-3p/PLCD3 axis.

Discussion

TC is the most common malignancy of the endocrine system, and the prognosis and treatment at diagnosis depends on the type and stage of the tumour. The treatment strategies for TC can improve survival rate and reduce recurrence rate in patients with TC [27]. However, the molecular regulatory mechanism of TC is complex, and the exploration of specific biomarkers will be beneficial to the diagnosis and treatment of TC in clinic. Therefore, it is critical to investigate the signal cascade in TC process. In our study, it was proved for the first time that circ_0003747-modulated miR-338-3p/PLCD3 axis was responsible for regulating the progress of TC cells. This work provides a new insight into the possible pathogenesis of TC.

CircRNAs are produced by back-splicing and regulate the expression of downstream genes through binding to miRNAs [28]. The dysregulation of the circRNA-miRNA-mRNA regulatory network may contribute to multiple disease progression, including the development of cancer [29]. As demonstrated by Chu et al., circRNA runx family transcription factor 1 (circRUNX1) regulated the progression of PTC cells through the miR-296-3p/DDHD2 axis [30]. The level of circ_0003747 was strikingly elevated in Papillary Thyroid Carcinoma (PTC) [10]. Therefore, we paid attention to the role of circ_0003747 in

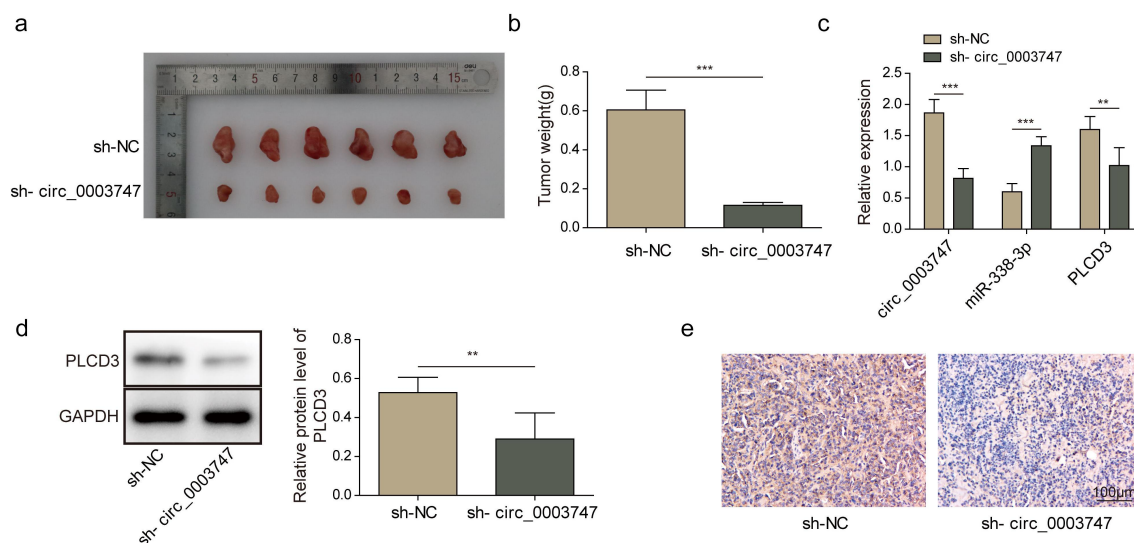


Figure 6. Circ_0003747 knockdown inhibits tumour growth in nude mice.

(a, b) The tumour growth was assessed by detecting the tumour size and weight. (c) The expressions of miR-338-3p and PLCD3 were detected using qRT-PCR assay. (d) The protein level of PLCD3 *in vivo* was measured using western blot. (e) The PLCD3 level was determined by immunohistochemistry assay. **P < 0.01, ***P < 0.001.

TC. In our research, it was found that circ_0003747 was highly expressed in TC cells and circ_0003747 knockdown inhibited TC cell progression.

MiRNAs play a vital role in human cancer and can be used as biomarkers for the diagnosis, prognosis, and treatment of cancer [31]. miR-199a-3p impaired cell proliferation and migration in PTC-derived cell lines [32]. In our research, miR-338-3p was found to be lowly expressed in TC, and the overexpression of miR-338-3p inhibited TC cell progression, implying that miR-338-3p may be a suppressor of biological behaviours at least in the TC process. A large number of evidences have shown that circRNAs can perform their biological function as ceRNAs by competitively reducing the contents of active miRNAs, which may be prognostic and predictive factors for clinical assessment of disease condition and outcome [33,34]. Circ_0009294 was reported to promote tumour cell proliferation, migration, and invasion via binding to miR-451a in Papillary Thyroid Carcinoma [35]. As reported, in hepatocellular carcinoma cells, circ_104566 promoted cell proliferation, migration, and invasion, and knockdown of circ_104566 restrained HCC progression via miR-338-3p/FOXP1 axis [36]. Consistent with the above studies, in the present work, miR-338-3p was verified as target of circ_0003747, and miR-338-3p inhibitor abolished the effects of circ_0003747 knockdown on TC cell progression.

PLCD3 plays vital roles in many biological processes, such as promoting neurite extension, cardiomyocyte survival, and normal heart function, regulating tumorigenesis and progression of TC. To investigate the function of miR-338-3p in TC, we focused on PLCD3 because PLCD3 has been proved to be an oncogene in TC cell lines [19]. Our findings discovered that miR-338-3p directly targeted PLCD3 to negatively regulate its expression. To further verify whether circ_0003747 could function by miR-338-3p, we co-transfected sh-circ_0003747 and PLCD3 in TC cells. We found that interference with circ_0003747 and then overexpression of PLCD3 might reverse the role of circ_0003747 in TC cell progression. Moreover, the knockdown of circ_0003747 remarkably suppressed tumour size and growth, restrained PLCD3 expression and promoted miR-338-3p expression in nude mice. Existing reports have

suggested that circRNAs served mainly as an miRNA molecular sponge and contained numbers and types of miRNA response elements at varying degrees [37]. For example, a previous report by Zhang W, et al. [38] demonstrated that circ_102002 might promote PTC process by sponging miR-488-3p and upregulating HAS2 expression. The same results were obtained in our study, where we reported for the first time that circ_0003747 could directly bind to miR-338-3p and increase PLCD3 expression, thus promoting TC cell progression.

In summary, circ_0003747 was overexpressed in PTC cells. The proliferation and migration of TC cells were repressed, and apoptosis was promoted by circ_0003747 knockdown. Circ_0003747 mainly sponged miR-338-3p and negatively regulated its expression. In addition, PLCD3 was identified as a target of miR-338-3p in PTC. Circ_0003747 promoted the TC process in a miR-338-3p/PLCD3-dependent manner. These findings about the signal axis of circ_0003747/miR-338-3p/PLCD3 may help to understand the PTC pathogenesis and improve clinical treatment. However, it is still unknown whether circ_0003747 can regulate other miRNAs in TC. Future study should conduct to investigate the in-depth mechanisms of circ_0003747.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Authors' contribution

XLD: Conceptualization; Writing-original draft; Methodology; Formal analysis; FDX: Investigation; Funding acquisition; Project administration; Writing-review & editing;

XYL: Investigation; Funding acquisition; Project administration; Writing-review & editing.

All authors have read and approved the final version of this manuscript to be published.

Availability of data and material

All data generated or analysed during this study are included in this article. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable. This article does not contain any studies with human participants performed by any of the authors.

Ethical approval

Our protocol was approved by the Ethics Committee of Xiangya Hospital, Central South University.

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