Circular RNA circ_0008274 enhances the malignant progression of papillary thyroid carcinoma *via* modulating solute carrier family 7 member 11 by sponging miR-154-3p

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Abstract. CircRNAs have been implicated in the progression of human cancers, including papillary thyroid carcinoma (PTC). Although circ_0008274 has been demonstrated as a potential oncogenic circRNA in PTC, our understanding of its molecular determinants is limited. The levels of circ_0008274, miR-154-3p and *solute carrier family 7 member 11 (SLC7A11)* mRNA were determined by quantitative real-time polymerase chain reaction (qRT-PCR). SLC7A11 protein level was assessed by western blot. Cell apoptosis, migration, and adhesion capacities were examined by flow cytometry, transwell and cell adhesion assays, respectively. The targeted correlations among circ_0008274, miR-154-3p and *SLC7A11* were confirmed by a dual-luciferase reporter assay. Animal studies were performed to observe the role of circ_0008274 in tumor growth *in vivo*. Our data showed that the high levels of circ_0008274 and *SLC7A11* were associated with poor prognosis of PTC patients. The knockdown of circ_0008274 or *SLC7A11* enhanced PTC cell apoptosis and repressed cell migration and adhesion *in vitro*. Circ_0008274 knockdown suppressed tumor growth *in vivo*. Mechanistically, circ_0008274 modulated *SLC7A11* expression by acting as a sponge of miR-154-3p. *SLC7A11* was a functional mediator of circ_0008274 in regulating PTC cell apoptosis, migration and adhesion *in vitro*, and miR-154-3p overexpression repressed PTC progression *in vitro* by targeting *SLC7A11*. Our findings identified that the knockdown of circ_0008274 repressed PTC malignant progression at least in part through regulating the miR-154-3p/*SLC7A11* axis, providing a promising therapeutic opportunity for PTC treatment.

Key words: Papillary thyroid carcinoma (PTC), Circ_0008274, MiR-154-3p, Solute carrier family 7 member 11 (SLC7A11)

PAPILLARY THYROID CARCINOMA (PTC) is the most prevalent type of differentiated thyroid cancer, with a long-term survival rate of >95% in the past decades [1]. However, about 10 to 15% of PTC can de-differentiate and become more aggressive, leading to a poor outcome [2, 3]. Therefore, identifying new PTC biomarkers to develop more effective diagnostic basis and therapeutic methods is still very important.

Circular RNAs (circRNAs) are endogenous, non-coding biomolecules that have been implicated in distinct physiologic and pathologic states [4]. Evidence is emerging that some circRNAs control transcriptional

activity *via* functioning as microRNA (miRNA) sponges in various cancers, including PTC [5, 6]. For instance, Wang *et al.* identified that circRNA itchy E3 ubiquitin protein ligase (circ-ITCH) exerted an anti-tumor function in PTC *via* mediating casitas B-lineage lymphoma (CBL) expression by sponging miR-22-3p [7]. Cai *et al.* reported that circRNA BTB domain and CNC homolog 2 (circBACH2) served as a potential promoter in PTC tumorigenesis by targeting the miR-139-5p/LIM domain only 4 (LMO4) axis [8]. Jin and colleagues showed that circ_0004458 worked as a sponge of miR-885-5p to promote PTC malignant progression by mediating Rac family small GTPase 1 (RAC1) expression [9]. Moreover, previous work in the laboratory of Zhou *et al.* identified circ_0008274 as a potential oncogenic circRNA in PTC [10]. Nevertheless, the precise parts played by circ_0008274 in PTC malignant progression remain unknown.

As a unit of amino acid transporters, solute carrier family 7 member 11 (SLC7A11, also named xCT)

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enhances cystine uptake and glutathione synthesis during cancer metabolic processes, thereby contributing to the development and progression of human cancers [11, 12]. Interestingly, a recent report demonstrated that the increased expression of SLC7A11 was closely associated with the poor prognosis of patients with PTC [13]. Furthermore, it is still unclear whether *SLC7A11* is involved in the regulation of circ_0008274 in PTC malignant progression.

Growing evidence has highlighted the association between miRNAs and PTC progression [14, 15]. It was reported that miR-154-3p was down-regulated in thyroid cancer and correlated with the outcome of the disease [16]. The study also unraveled the involvement of miR-154-3p in thyroid cancer development through targeting *ras homolog family member A (RHOA)*. However, our understanding of the precise action of miR-154-3p in PTC malignant progression is limited.

Here, we identified that circ_0008274, a remarkably overexpressed circRNA in PTC, regulated PTC malignant progression *in vitro* and *in vivo*. Furthermore, we provided a molecular explanation, at least in part, for the oncogenic role of circ_0008274 in PTC.

Materials and Methods

Clinical samples and cells

A total of 60 PTC patients who had undergone surgery at the Affiliated Huaian No. 1 People's Hospital of Nanjing Medical University were enrolled in this study, from October 2011 to April 2013. Tissue samples included 60 tumor tissues and 60 matched normal tissues obtained from surgery. The clinicopathological features of these patients were provided in Table 1. The follow-up information was obtained by telephone interview every three months until October 2018. The Ethics Committee of the Affiliated Huaian No. 1 People's Hospital of Nanjing Medical University approved all protocols, and all subjects gave written informed consent.

Human PTC cell lines IHH4 and TPC1, and normal thyroid Nthy-ori 3-1 cells (all from Bnbio, Beijing, China) were propagated in RPMI-1640 medium (Thermo Fisher Scientific, Paisley, UK) supplemented with 10% fetal bovine serum (FBS, EuroClone, Milan, Italy) at 37°C, 5% CO₂. 293T cells (Bnbio) were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Thermo Fisher Scientific) under standard conditions.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted for tissues and cells using the mirVana miRNA Isolation Kit (Invitrogen, Merelbeke, Belgium) based on the protocols of manufacturers. Total

Table 1 Correlation between clinicopathological features and circ_0008274 expression in the papillary thyroid cancer cases

Variables	No. of cases	Circ_0008274 expression		p value
		High	Low	
Gender				0.602
Male	26	14	12	
Female	34	16	18	
Ages				0.755
<45	32	17	15	
≥45	28	16	12	
Tumor size(cm)				0.126
<2	23	9	14	
≥2	37	22	15	
TNM stage				0.019*
I-II	38	14	24	
III	22	15	7	
Lymph node metastasis				0.017*
Yes	29	21	8	
No	31	13	18	
Tumor infiltration				0.002**
Yes	24	19	5	
No	26	9	17	

RNA was reverse-transcribed to cDNA using an iScript cDNA Synthesis Kit (Bio-Rad, Glattbrugg, Switzerland) for circ_0008274 and *solute carrier family 7 member 11 (SLC7A11)* mRNA expression or miScript Reverse Transcription Kit (Qiagen, Manchester, UK) for miRNAs. qRT-PCR was done using SYBR-Green Master Mix (Bio-Rad) on a Bio-Rad iCycler. Primers were listed in Supplement Table 1. Data were normalized to that of internal control GAPDH or U6 with the 2^{-ΔΔCt} method [17].

Western blot

Western blot analyses were carried out as previously reported [18]. The primary antibodies, including SLC7A11 (ab37185, 1:1,000), intercellular adhesion molecule 1 (ICAM-1, ab109361, 1:3,000), Fibronectin (ab45688, 1:4,000), Vitronectin (ab46808, 1:5,000) and GAPDH (ab9485, 1:2,500) were used based on the directions of manufacturers (Abcam, Cambridge, UK). Horseradish peroxidase-conjugated anti-rabbit IgG antibody (ab205718, 1:5,000, Abcam) was used as the secondary antibody. The protein bands were detected using the Chemiluminescence Kit (GE Healthcare, Waukesha, WI, USA).

Cell transfection

For the silencing *in vitro* studies, IHH4 and TPC1 cells (1×10^5) were transiently transfected with 25 nM of siRNA targeting circ_0008274 (si-circ#1, si-circ#2 or si-circ#3, Ribobio, Guangzhou, China), *SLC7A11*-siRNA (si-*SLC7A11*#1, si-*SLC7A11*#2 or si-*SLC7A11*#3, Ribobio), or the scrambled oligonucleotide siRNA negative control (si-NC) using Lipofectamine 3000 (Invitrogen). For the alteration of miR-154-3p expression studies *in vitro*, 25 nM of miR-154-3p mimic, the inhibitor of miR-154-3p (anti-miR-154-3p) or the corresponding negative oligonucleotide controls (miR-NC mimic and anti-NC, Ribobio) were used. *SLC7A11* overexpression cells were produced using 100 ng of recombinant pcDNA-based overexpressing plasmid (*SLC7A11*, Ribobio), and non-target pcDNA plasmid was used as the negative control. For circ_0008274 overexpression *in vitro* studies, pCD5-ciR-based circ_0008274 overexpressing plasmid (circ_0008274, Geneseeed, Guangzhou, China) or negative plasmid (vector) was transfected into cells. For circ_0008274 knockdown *in vivo* studies, lentiviruses encoding shRNA specific to circ_0008274 (sh-circ_0008274) and nontarget shRNA (sh-NC) were used to transduce TPC1 cells as per the recommendation of manufacturers (HanBio, Shanghai, China). Vector-transduced cells were selected with puromycin at a final concentration of 2.5 $\mu\text{g/mL}$ over 72 h.

Cell apoptosis assay

Apoptosis of cells after 24 h transfection was tested by double-staining with Annexin V-coupled fluorescein isothiocyanate (FITC) and propidium iodide (PI) as recommended by the manufacturers (Invitrogen), and data were analyzed by a FACScan flow cytometer (BD Biosciences, Oxford, UK).

Transwell migration assay

Transfected cells were plated at 5×10^4 per well into the top chamber with Matrigel-precoated membrane inserts (8 μm pores, Corning, Rochester, NY, USA), and the lower chambers were filled with 15% FBS medium as a chemoattractant. After 24 h culture at 37°C, the migrated cells through the membrane inserts were determined under a 100 $\times$ magnification microscope after being stained with 1% crystal violet.

Cell adhesion assay

Cell adhesion capacity was gauged as previously described [19] with minor modifications. Briefly, transfected cells (1×10^5) were seeded into 6-well plates in growth medium and maintained at 37°C for 6 h. Following the wash with PBS, the adhesion cells were fixed with 3.7% formaldehyde for 10 min and stained with

0.1% crystal violet for 30 min before adding 10% acetic acid for 10 min. Data were read at 570 nm with a microplate reader.

Bioinformatics and dual-luciferase reporter assay

The targeted miRNAs of circ_0008274 and the molecular targets of miR-154-3p were predicted using the starBase software (<http://starbase.sysu.edu.cn/>). The fragment of circ_0008274 harboring the miR-154-3p-pairing sites and the full-length 3'UTR of *SLC7A11* were individually cloned into the pmirGLO vector (Promega, Charbonnières, France) to construct wild-type reporter plasmids (WT-circ_0008274 and WT-*SLC7A11* 3'UTR). Site-directed mutations (MUT-circ_0008274 and MUT-*SLC7A11* 3'UTR) in the target region were produced using the QuikChange Mutagenesis kit (Stratagene, Marcy L'Etoile, France). 100 ng of pmirGLO vector or WT-*SLC7A11* 3'UTR was transfected into 293T cells were transfected with together with miRNAs mimics or miR-NC mimic. 100 ng of WT-circ_0008274, MUT-circ_0008274, WT-*SLC7A11* 3'UTR or MUT-*SLC7A11* 3'UTR was cotransfected into IHH4 and TPC1 cells and miR-NC mimic or miR-154-3p mimic using Lipofectamine 3000. Twenty-four h post-transfection, cells were harvested for the luciferase activity assays based on the manufacturing guidance (Dual-luciferase Assay System, Promega).

Animal studies

All animal studies were done under the guidelines approved by the Animal Research Ethics Committee at The Affiliated Huaian No. 1 People's Hospital of Nanjing Medical University. About 1×10^7 sh-circ_0008274-transduced or sh-NC-infected TPC1 cells were subcutaneously implanted into the male BALB/c nude mice aged 6 weeks (Henan Laboratory Animal Center, Zhengzhou, China, $n = 5$ per group). The tumor growth was monitored every 4 days by measuring tumor size and calculating tumor volume by the formula: $(\text{length} \times \text{width}^2) \times 0.5$. Twenty-eight days later, all mice were euthanized and tumor tissues were harvested.

Statistical analysis

Data were expressed as the mean $\pm$ standard deviation (SD). A Student's *t*-test or analyses of variance (ANOVA) was used to compare the measured parameters. The correlation between circ_0008274 and *SLC7A11* or E2F8 expression levels in PTC tissues was determined by the Spearman correlation analysis. For survival analysis, the Kaplan-Meier method and log-rank test were used. The association between circ_0008274 and the clinicopathologic features of PTC patients was analyzed by χ^2 test. Significance values were presented as * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$.

Results

High levels of circ_0008274 and SLC7A11 were associated with poor prognosis of PTC patients

To observe the clinical significance of circ_0008274 and *SLC7A11*, we investigated their correlations with the prognosis of these patients. According to the median of circ_0008274 or *SLC7A11* expression, these patients were divided into two groups: high expression group ($n = 30$) and low expression group ($n = 30$). The data showed that the patients in the high circ_0008274 level group had a shorter survival time than those in the low circ_0008274 level group (Fig. 1A). Moreover, the patients with high *SLC7A11* level had a shorter survival time than those with low *SLC7A11* expression (Fig. 1B). Additionally, a strong positive correlation between circ_0008274 and *SLC7A11* mRNA expression levels in PTC tissues was discovered (Fig. 1C). Furthermore, circ_0008274 expression was closely associated with the TNM stage, lymph node metastasis and tumor infiltration of these tumors (Table 1).

Knockdown of circ_0008274 enhanced PTC cell apoptosis and suppressed cell migration and adhesion in vitro

To determine the detailed function of circ_0008274 in PTC progression, we carried out *in vitro* loss-of-function analyses using circ_0008274-siRNAs (si-circ#1, si-circ#2 and si-circ#3). Transient transfection of circ_0008274-siRNAs, but not the scrambled oligonucleotide sequence, significantly reduced the level of circ_0008274 in both IHH4 and TPC1 cells (Fig. 2A). The transfection of si-circ#2 caused the most significant down-regulation of circ_0008274, so we selected it for further analyses (Fig. 2A). Functional experiments showed that the knockdown of circ_0008274 significantly promoted cell apoptosis (Fig. 2B), and repressed cell migration (Fig. 2C), as well as inhibited cell adhe-

sion (Fig. 2D). Moreover, circ_0008274 silencing led to a significant decrease in the levels of ICAM-1, Fibronectin and Vitronectin in the two cell lines (Fig. 2E), demonstrating the suppression of circ_0008274 knockdown on cell adhesion.

Knockdown of SLC7A11 enhanced PTC cell apoptosis and weakened cell migration and adhesion in vitro

To directly test the biological function of *SLC7A11* in PTC progression, we reduced its level using siRNAs targeting *SLC7A11* (si-*SLC7A11*#1/2/3) in both IHH4 and TPC1 cells. Transient introduction of si-*SLC7A11* remarkably decreased *SLC7A11* expression in the two PTC cell lines (Fig. 3A). Owing to the most significant reduction of si-*SLC7A11*#3 in *SLC7A11* level (Fig. 3A), we selected it for further research. Functional analyses showed that the silencing of *SLC7A11* remarkably enhanced cell apoptosis (Fig. 3B), and suppressed cell migration (Fig. 3C) and adhesion (Fig. 3D). Furthermore, *SLC7A11* depletion resulted in decreased levels of ICAM-1, Fibronectin and Vitronectin in both IHH4 and TPC1 cells (Fig. 3E), supporting the repressive effect of *SLC7A11* silencing on cell adhesion.

SLC7A11 was a functionally important mediator of circ_0008274 in regulating PTC cell apoptosis, migration and adhesion in vitro

Our above findings demonstrated that circ_0008274 and *SLC7A11* negatively regulated PTC malignant progression *in vitro*. These resemblances prompted us to examine whether *SLC7A11* could work as a molecular mediator of circ_0008274 in PTC progression. By contrast, circ_0008274 knockdown led to a significant decrease in the expression of *SLC7A11* protein, and this effect was strongly abolished by the transfection of *SLC7A11* overexpressing plasmid (Fig. 4A). Further functional experiments revealed that the restored expression

Fig. 1 High levels of circ_0008274 and *SLC7A11* were associated with poor prognosis of PTC patients. (A and B) Survival analysis of PTC patients divided into two groups according to the median of circ_0008274 or *SLC7A11* expression in PTC tissues. (C) Correlation between circ_0008274 and *SLC7A11* expression levels in PTC tissues by the Spearman test.

Fig. 2 Circ_0008274 silencing enhanced PTC cell apoptosis and suppressed cell migration and adhesion *in vitro*. (A) Circ_0008274 expression by qRT-PCR in IHH4 and TPC1 cells transfected with si-NC, si-circ#1, si-circ#2 or si-circ#3. IHH4 and TPC1 cells were transfected with si-NC or si-circ#2, followed by the determination of cell apoptosis by flow cytometry (B), cell migration by transwell assay (C), cell adhesion by the adhesion assay (D), ICAM-1, Fibronectin and Vitronectin levels by western blot (E). *** $p < 0.001$.

Fig. 3 SLC7A11 depletion accelerated PTC cell apoptosis and mitigated cell migration and adhesion. (A) SLC7A11 protein level by western blot in IHH4 and TPC1 cells transfected with si-*SLC7A11*#1, si-*SLC7A11*#2, si-*SLC7A11*#3, or si-NC. Cell apoptosis by flow cytometry (B), cell migration by transwell assay (C), cell adhesion by cell adhesion assay (D), ICAM-1, Fibronectin and Vitronectin levels by western blot (E) in IHH4 and TPC1 cells transfected with si-NC or si-*SLC7A11*#3. ** $p < 0.01$ or *** $p < 0.001$.

of SLC7A11 dramatically abrogated circ_0008274 knockdown-mediated pro-apoptosis (Fig. 4B), anti-migration (Fig. 4C) and anti-adhesion (Fig. 4D and 4E).

MiR-154-3p directly interacted with circ_0008274 and SLC7A11 3'UTR

Using the starBase software (<http://starbase.sysu.edu.cn/>), the data revealed that circ_0008274 and *SLC7A11* 3'UTR harbored putative target sequences for miR-487a-3p, miR-656-3p, miR-154-3p, miR-660-5p

and miR-140-3p (Fig. 5A). By contrast, circ_0008274 silencing led to a significant increase in the levels of miR-154-3p and miR-140-3p in TPC1 cells (Fig. 5B). To preliminarily confirm whether the five miRNAs could directly target *SLC7A11*, we constructed *SLC7A11* 3'UTR wild-type luciferase reporter (WT-*SLC7A11* 3'UTR) and performed dual-luciferase assays. Interestingly, the transfection of miR-656-3p or miR-154-3p mimic remarkably reduced the luciferase activity of WT-*SLC7A11* 3'UTR (Fig. 5C). We therefore selected

Fig. 4 *SLC7A11* was a functionally important mediator of circ_0008274 in regulating PTC cell apoptosis, migration and adhesion *in vitro*. IHH4 and TPC1 cells were transfected with si-NC, si-circ#2, si-circ#2+pcDNA or si-circ#2+SLC7A11, followed by the determination of SLC7A11 protein level by western blot (A), cell apoptosis by flow cytometry (B), cell migration by transwell assay (C), cell adhesion by the adhesion assay (D), ICAM-1, Fibronectin and Vitronectin levels by western blot (E). pcDNA: negative control plasmid, SLC7A11: SLC7A11 overexpressing plasmid. ** $p < 0.01$ or *** $p < 0.001$.

Fig. 5 MiR-154-3p directly interacted with circ_0008274 and *SLC7A11* 3'UTR. (A) Venn diagram showing the putative targeted miRNAs of circ_0008274 and *SLC7A11* identified by starBase software. Red circle: miRNA binding to circ_0008274, purple circle: miRNA binding to *SLC7A11*. (B) The levels of miR-487a-3p, miR-656-3p, miR-154-3p, miR-660-5p and miR-140-3p by qRT-PCR in si-NC- or si-circ#2-transfected TPC1 cells. (C) Dual-luciferase reporter assays in 293T cells cotransfected with pmirGLO vector or WT-*SLC7A11* 3'UTR and miR-487a-3p, miR-656-3p, miR-154-3p, miR-660-5p, miR-140-3p or miR-NC mimic. Relative luciferase activity in IHH4 and TPC1 cells cotransfected with miR-NC mimic or miR-154-3p mimic and WT-circ_0008274 or MUT-circ_0008274 (D), WT-*SLC7A11* 3'UTR or MUT-*SLC7A11* 3'UTR (E). * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$.

miR-154-3p for further investigation. When we used circ_0008274 wild-type (WT-circ_0008274) or mutant-type (MUT-circ_0008274) luciferase reporter in dual-luciferase assays, the cotransfection of WT-circ_0008274 and miR-154-3p mimic into the two PTC cell lines produced lower luciferase activity than cells cotransfected with the miR-NC control but MUT-circ_0008274 significantly abrogated the suppressive effect of miR-154-3p (Fig. 5D). Furthermore, the transfection of WT-*SLC7A11* 3'UTR in the presence of miR-154-3p mimic caused a significant down-regulation of luciferase activity, and this effect was dramatically abolished by the mutation of the target region (MUT-*SLC7A11* 3'UTR) (Fig. 5E), demonstrating the validity of the target sequences for interaction.

Circ_0008274 mediated SLC7A11 expression by sponging miR-154-3p

We then determined whether circ_0008274 could modulate miR-154-3p expression in both IHH4 and TPC1 cells. The transfection efficiency of circ_0008274 overexpressing plasmid was gauged by qRT-PCR (Fig. 6A). As expected, miR-154-3p level was significantly decreased by circ_0008274 overexpression, and it was

dramatically increased as a result of circ_0008274 knockdown in the two PTC cell lines (Fig. 6B). To confirm whether miR-154-3p regulated *SLC7A11* expression, we manipulated miR-154-3p level in the two PTC cell lines. The transfection efficiencies of miR-154-3p mimic and anti-miR-154-3p were gauged by qRT-PCR (Fig. 6C). By construct, *SLC7A11* protein level was notably reduced by miR-154-3p overexpression, and it was strikingly elevated by miR-154-3p depletion in both cell lines (Fig. 6D).

We next asked whether circ_0008274 regulated *SLC7A11* expression by miR-154-3p. As expected, the transfection of anti-miR-154-3p prominently abolished the inhibition of *SLC7A11* protein expression of circ_0008274 silencing in the two PTC cell lines (Fig. 6E). Furthermore, the enforced expression of circ_0008274 led to a significant increase in the level of *SLC7A11* protein, and this effect was abrogated by miR-154-3p up-regulation (Fig. 6F).

Enforced expression of miR-154-3p promoted PTC cell apoptosis and repressed migration and adhesion by down-regulating SLC7A11 in vitro

To determine whether *SLC7A11* was a functional

Fig. 6 Circ_0008274 mediated *SLC7A11* expression by sponging miR-154-3p. (A) Relative circ_0008274 level by qRT-PCR in IHH4 and TPC1 cells transfected with vector or circ_0008274. (B) MiR-154-3p expression by qRT-PCR in IHH4 and TPC1 cells transfected with vector, circ_0008274, si-NC or si-circ#2. MiR-154-3p expression by qRT-PCR (C) and *SLC7A11* protein level by western blot (D) in IHH4 and TPC1 cells transfected with miR-NC mimic, miR-154-3p mimic, anti-NC or anti-miR-154-3p. (E and F) *SLC7A11* protein level by western blot in IHH4 and TPC1 cells transfected with si-NC, si-circ#2, si-circ#2+anti-NC, si-circ#2+anti-miR-154-3p, vector, circ_0008274, circ_0008274+miR-NC mimic or circ_0008274+miR-154-3p mimic. vector: negative control plasmid, circ_0008274: circ_0008274 overexpressing plasmid. *** $p < 0.001$.

target of miR-154-3p in regulating PTC malignant progression *in vitro*, we transfected miR-154-3p mimic alone or together with SLC7A11 overexpressing plasmid into both IHH4 and TCP1 cells. By contrast, the transfection of SLC7A11 overexpressing plasmid significantly abolished the reduction of miR-154-3p overexpression on SLC7A11 level in the two PTC cell lines (Fig. 7A). Functional analyses revealed that the increased expression of miR-154-3p triggered a strong enhancement in cell apoptosis (Fig. 7B) and a striking inhibition in cell migration (Fig. 7C) and adhesion (Fig. 7D and 7E). Nevertheless, these functional regulatory effects of miR-154-3p were dramatically abrogated by the restored SLC7A11 expression in the two PTC cell lines (Fig. 7B–7E).

Knockdown of circ_0008274 hampered tumor growth *in vivo*

An important question was whether circ_0008274 could influence tumor growth *in vivo*. To address this, we subcutaneously implanted sh-circ_0008274-transduced or sh-NC-infected TPC1 cells into the nude mice to generate the xenograft model. By contrast, the transduction of sh-circ_0008274 remarkably restrained tumor growth *in vivo* (Fig. 8A and 8B). Moreover, circ_0008274 and SLC7A11 levels were significantly decreased and miR-154-3p was highly increased in the tumor tissues derived from sh-circ_0008274-transduced TPC1 cells (Fig. 8C–8E). Additionally, the silencing of circ_0008274

resulted in reduced expression of ICAM-1, Fibronectin and Vitronectin in the sh-circ_0008274-transduced TPC1 tumors (Fig. 8E).

Discussion

Accumulating evidence is pointing towards circRNAs as essential regulators in cancer biology, opening up a novel field of cancer diagnostic and therapeutic opportunities [5]. CircRNAs have been implicated in the tumorigenesis and progression of PTC [20, 21]. Here, we sought to identify the detailed role of circ_0008274 in PTC malignant progression and the mechanistic insight of the circRNA-miRNA-mRNA network mediated by circ_0008274 in PTC.

Here, we validated that the up-regulation of circ_0008274 predicted poor prognosis of PTC patients, suggesting its role as a potential prognosis marker in PTC. Cell adhesion to the extracellular matrix (ECM) is an important step of cancer tumorigenesis and metastasis [22]. ICAM-1 is an Ig-like cell adhesion molecule, and Fibronectin and Vitronectin are two major extracellular matrix proteins, which play essential roles in tumor cell adhesion to ECM [23–25]. Our results reported here demonstrated that circ_0008274 silencing suppressed PTC malignant progression *in vitro* and *in vivo*, in agreement with previous work [10]. On the contrary, Liang *et al.* reported the down-regulated expression of circ_0008274 in lung adenocarcinoma [26], which

Fig. 7 MiR-154-3p overexpression promoted cell apoptosis and repressed migration and adhesion *in vitro* by down-regulating SLC7A11. IHH4 and TPC1 cells were transfected with miR-NC mimic, miR-154-3p mimic, miR-154-3p mimic+pcDNA or miR-154-3p mimic+SLC7A11. (A) Western blot for SLC7A11 protein expression in transfected cells. (B) Flow cytometry for cell apoptosis. (C) Transwell assay for cell migration. (D) Cell adhesion assay for cell adhesion capacity. (E) Western blot for ICAM-1, Fibronectin and Vitronectin levels. pcDNA: negative control plasmid, SLC7A11: SLC7A11 overexpressing plasmid. ** $p < 0.01$ or *** $p < 0.001$.

Fig. 8 Circ_0008274 silencing hindered tumor growth *in vivo*. sh-circ_0008274-transduced or sh-NC-infected TPC1 cells were subcutaneously implanted into the nude mice ($n = 5$ per group). Twenty-eight days later, all mice were euthanized. (A) Tumor volume measurement every 4 days. (B) Tumor average weight of the excised tumors. Circ_0008274 (C) and miR-154-3p (D) levels by qRT-PCR, and SLC7A11, ICAM-1, Fibronectin and Vitronectin levels by western blot (E) in the excised tumors. * $p < 0.05$ or *** $p < 0.001$.

possibly was due to the different types of tumors and diversified tumor microenvironment.

SLC7A11 has been identified as a tumor promoter in various cancers [11, 12]. For example, Robert *et al.* demonstrated that SLC7A11 expression was elevated in glioma and predicted poor prognosis of these patients [27]. Ji and colleagues found that the increased expression of SLC7A11 enhanced the malignant progression of non-small cell lung cancer [28]. Moreover, *SLC7A11* mRNA was found to be associated with the poor prognosis outcome of PTC patients [13]. Our data first demonstrated the oncogenic role of SLC7A11 in PTC. More interestingly, we showed the regulation of circ_0008274 in PTC progression by *SLC7A11*.

It is widely accepted that some circRNAs exert regulatory function by working as miRNA sponges [4]. Here, we first confirmed that circ_0008274 mediated SLC7A11 expression by sponging miR-154-3p. MiR-154-3p has been demonstrated to be involved in the pathogenesis of lung cancer, breast cancer and PTC [16, 29, 30]. Here, we first identified that *SLC7A11* was a functional target of miR-154-3p in regulating PTC progression *in vitro*. Previous reports showed that several other miRNAs, such as miR-27a and miR-375, modula-

ted human carcinogenesis by targeting *SLC7A11* [31, 32]. The direct evidence of the novel regulatory axis in PTC progression *in vivo* was lacked, which was expected to be performed in further work. Future work should build on the findings by determining precisely how the novel circRNA-miRNA-mRNA regulatory network modulates PTC progression *in vitro* and *in vivo*.

In conclusion, the current report identified that the knockdown of circ_0008274 hampered PTC malignant progression by the miR-154-3p/*SLC7A11* axis. Our findings highlighted a new circRNA-miRNA-mRNA network in PTC, providing a promising therapeutic opportunity for PTC treatment.

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Disclosure of Interest

The authors declare that they have no conflicts of interest.

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