

Circular RNA Pvt1 oncogene (CircPVT1) promotes the progression of papillary thyroid carcinoma by activating the Wnt/ β -catenin signaling pathway and modulating the ratio of microRNA-195 (miR-195) to vascular endothelial growth factor A (VEGFA) expression

Linwen Zeng[§], Shaofeng Yuan[§], Pengfei Zhou, Jianming Gong, Xiangdong Kong, and Ming Wu

Department of Surgery, Tinglin Hospital of Jinshan District, Shanghai, China

ABSTRACT

Circular RNAs (circRNAs) have been reported to be involved in the progression of papillary thyroid carcinoma (PTC). However, the role of circular RNA Pvt1 oncogene (*circPVT1*) in PTC has rarely been reported. In this study, we aimed to investigate the function and mechanism of *circPVT1* in PTC. The expression level of *circPVT1*, *miR-195* and *VEGFA* were determined by reverse transcription-quantitative PCR (RT-qPCR). Fisher's exact test was used to analyze the correlation between *circPVT1* expression and PTC clinical features. Cell Counting Kit-8 (CCK-8) and 5-Ethynyl-2'-deoxyuridine (EdU) staining assay and transwell assay were conducted to evaluate the cell proliferation, migration and invasion ability. Dual-luciferase reporter and Western blot assay were conducted for evaluating the correlation between *miR-195* and *circPVT1* or *VEGFA*. The results of RT-PCR showed that the expression level of *circPVT1* was significantly upregulated in PTC tissues and cell lines. After downregulating *circPVT1* expression in PTC cells, the abilities of cell proliferation, migration, and invasion were obviously suppressed, and the Wnt/ β -catenin signaling pathway was also repressed. Besides, *miR-195* could both bind to *PVT1* and *VEGFA*, while *PVT1* could promote the expression of *VEGFA* by binding to *miR-195*. Downregulation of *VEGFA* expression in PTC cells revealed weakened cell proliferation, migration, and invasion capacities, and restrained Wnt/ β -catenin signaling pathway. Therefore, we demonstrated that *circPVT1* could promote *VEGFA* expression by sponging *miR-195*. *CircPVT1* could serve as a molecule sponge for *miR-195* and mediate the ceRNA network to promote the expression of *VEGFA*, thus contributed to the malignant progression of PTC.

ARTICLE HISTORY

Received 24 September 2021
Revised 15 November 2021
Accepted 16 November 2021

KEYWORDS



Papillary thyroid carcinoma;
circPVT1; *miR-195*; *VEGFA*;
malignant progression

Introduction

Thyroid cancer (TC) is one of most prevalent endocrine malignancy worldwide, and its incidence has doubled globally in the last 30 years [1–3], TC is one of the reasons for the high incidence of malignant tumors in women, as it is the fifth most diagnosed malignant tumor in this group [4,5]. Papillary thyroid cancer (PTC) is the most common histotype of TC, accounting for approximately 85% of all TC [6]. Although the prognosis of PTC is generally good, a high quality of life still cannot be guaranteed. This is because some PTC patients show early epidural infiltration, lymphatic metastasis, and even distant

metastasis or other high-risk complications [7]. More than 30% of PTC patients develop into lymph node metastasis [8], while 2.6% to 3.7% of patients suffer from distant metastasis [5,9]. The occurrence of distant metastasis and invasion of PTC indicates a poor prognosis, and often the disease cannot be effectively controlled [10]. Consequently, investigating the mechanism of the malignant progression of PTC has vital theoretical significance and potential clinical value.

Circular RNAs (circRNAs) were discovered approximately 40 years ago [11], but have not attracted widespread attention until recently. As circRNAs are ncRNAs, they were initially

CONTACT Ming Wu  shanghaijswm@126.com  Department of Surgery Tinglin Hospital of Jinshan District, No. 80 Siping North Road, Jinshan District, Shanghai 201505, China

[§]Both authors contributed equally to this work.

© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

thought to be byproducts of pre-mRNA splicing errors [12]. Compared with other types of non-coding RNAs (ncRNAs), circRNAs have neither 5' to 3' polarity nor a polyA tail, because they form covalently closed loops. These circRNAs are more conserved and stable than linear ncRNAs [13,14]. In addition, some studies have found that there are four basic types of circRNAs [15]: exon circRNAs, intronic circRNAs, exon-intron circRNAs, and intergenic circRNAs. With the rapid development of next-generation sequencing technology, many circRNAs have been identified in mammalian transcriptomes and various cancers [16–18]. Because circRNA is not easily degraded by exonuclease RNase R, most of them have a long half-life and are widely present in tissues and peripheral blood, and they are highly conserved and have spatial tissue and disease specificity [19,20]. These characteristics endow it with a potential function, such as acting as a miRNA molecular sponge, regulating gene expression and participating in protein translation, and may also be a potential target for disease intervention [21,22]. In addition, circRNA in peripheral blood has stable biological properties and is expected to become a molecular marker for disease diagnosis and prognosis [16,23]. In human cancers, substantial researches have demonstrated that circRNAs may play an essential role in cancer growth, metastasis, and malignant progression. For example, Li et al. find that circRNA *hsa_circ_0014130* can act as a *miR-132-3p* sponge for playing oncogenic roles in bladder cancer by upregulating potassium inwardly rectifying channel subfamily J member 12 (*KCNJ12*) expression [24]. Zhang et al demonstrate that *hsa_circ_0058106* can induce epithelial-mesenchymal transition (EMT) and metastasis in laryngeal cancer via sponging *miR-153* and inducing twist family bHLH transcription factor 1 (*TWIST1*) nuclear translocation [25]. Cai et al show that circ-NOL10 regulated by metadherin (*MTDH*)/CASC3 exon junction complex subunit (*CASC3*) can suppress breast cancer progression and metastasis via bind to multiple miRNAs and programmed cell death 4 (*PDCD4*) [26]. These studies identify that aberrant circRNAs

expression may be encouraging diagnosis biomarkers and therapeutic target for PTC.

The circRNA *PVT1* (*circPVT1*) is a recently identified circRNA situated in the known cancer susceptibility region chr8: 128902834–128903244 [27,28]. It originates from the third exon of the *PVT1* and has been identified as an oncogene [27]. Previous studies have shown that *circPVT1* may play a vital role in the initiation and progression of several tumors, including laryngeal cancer [29], non-small cell lung cancer [30], gastric cancer [31], clear cell renal cell carcinoma [32], ovarian cancer [33], cervical cancer [34] and breast cancer [35]. However, the expression pattern and biological function of *circPVT1* in PTC remain unknown. In the current study, we hypothesized that *circPVT1* might assume an essential role in the progression of PTC. The aim of this study was to reveal the biological function and potential molecular mechanism of *circPVT1* regulating the progression of PTC. We firstly measured *circPVT1* expression level in the PTC tissues and cell lines. Meanwhile, we detected the functions of *circPVT1* on the cell growth, migration, and invasiveness of PTC. Finally, we revealed the underlying mechanism that circVRK1 sponged miR-195 and accelerated the VEGFA expression, so as to regulate the Wnt/ β -catenin signaling pathway.

Materials and methods sample Collection

Fifty pairs of PTC tumor samples and matching contralateral normal samples were collected from patients with PTC in our hospital between October 2015 and March 2017. The samples were frozen in liquid nitrogen and stored at -80°C until use. We verified the cancer status of all the tissue samples by pathological examination. The Ethics Committee of the Tinglin Hospital of Jinshan District approved the study (2015–023) and informed consent was obtained from patients before surgical operation.

Cell culture

We purchased PTC cell lines (K1, IHH-4, BCPAP, and TPC-1) and human thyroid follicular epithelial cells (Nthy-ori 3–1) from the Hematology Institute (Beijing, China) of the Chinese

Academy of Chinese Medical Sciences. The K1, IHH-4 and Nthy-ori 3-1 cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Invitrogen, Grand Island, NY, USA), while TPC-1 and BCPAP cells were cultured in Dulbecco's Modified Eagle Medium (DMEM). Additional MEM Non-Essential Amino Acids Solution (NEAA 100X) (Invitrogen, 11140050) was added to the BCPAP cell line. All medium was containing 10% heat-inactivated fetal bovine serum (FBS; Invitrogen), penicillin (100 U/mL)/streptomycin (100 µg/mL). The cells were incubated in a humidified incubator at 37°C and 5% CO₂.

Cell transfection

We purchased the *PVT1* small interfering RNA (siRNA), *VEGFA* siRNA, miR-195 inhibitor, and their corresponding negative controls from GenePharma (Shanghai, China). Next, we mixed the cells in a 6-well plate (5×10^5 cells per well). Following the manufacturer's instructions, after the cell density reached 80%, the transfection reagent was mixed with Lipofectamine 2000 (Invitrogen) and added to the cells [36]. The mixture was incubated at room temperature for 30 min and subsequently transferred to a Petri dish. After transfection for 48 h using Lipofectamine 2000, the transfected cells were collected to assess the transfection efficiency by qRT-PCR. The sequences of primers for transfection were listed below: circPVT1 siRNA: 5'-GCAAUUGAAAGCUACCAAUTT-3'; scramble siRNA: 5'-UUCUCCG AACGUGUCACGUTT-3'; *VEGFA* siRNA: 5'-CUGGAAUUUGAUUAUCAUUGA-3'; scramble siRNA: 5'-UUCUCCGAACGUGUCACGUTT-3'; miR-195 mimics sense, 5'-UAGCAGCACAGAAUAUUGG C-3'; miR-195 mimics antisense, 5'-CAAUAUUUCUGUGCUGCUAUU-3'; mimics negative control (miR-NC) sense, 5'-UUCUCCGAACGUGUCACGUTT-3'; miR-NC antisense, 5'-ACGUGACACGUUCG GAGAATT-3'.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR)

After transfection for 24–48 h, purified total RNA was extracted from tissues and cells using TRIzol (Invitrogen). cDNA was obtained by reverse

transcription in a 20 µL reaction system using the PrimeScript RT reagent Kit (TAKARA, Code No. RR037A) according to the manufacturer's instructions. Real-time quantitative reverse transcription polymerase-chain reaction (RT-PCR) analysis was performed to determine transfection efficiency using the following conditions: 92°C for 10 min, followed by 40 cycles of 92°C for 10 s and then 60°C for 1 min [37]. GAPDH and U6 were used as the internal reference, respectively. The primer sequences used were as follows: *PVT1* (F: 5'-GGTTCCACCAGCGTTATTC-3'; R: 5'-CAACTTCCTTTGGGTCTCC-3'); miR-195 (F: 5'-ACGATGCCACGACCAAGCC-3'; R: 5'-AGCA CCATCGTCCGCAGGCA-3'); *VEGFA* (F: 5'-AACT TTCTGCTGTCTTGGGT-3'; R: 5'-TCTCGATTGG ATGGCAGTA-3'); *Wnt3a* (F: 5'-TCTACGACGTGC ACACCTG-3'; R: 5'-CCCTGCCTTCAGGTAGGAG T-3'); β -catenin (F: 5'-CAGCAGCAATTTGTGGAG GG-3'; R: 5'-GCAGCTGCACAAACAATGGA-3'); GAPDH (F: 5'-GGAATCCACTGGCGTCTTCA-3'; R: 5'-GGTTCACGCCATCACAAAC-3'); U6 (F: 5'-CTCGCTTCGGCAGCACA-3'; R: 5'-AACGCTTCA CGAATTTGCGT-3').

Cell proliferation activity assay

We used Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan) to detect cell growth ability and the method referred to the previous study [38]. Cells in the medium were digested with 0.25% trypsin and the cell suspensions were subsequently harvested and seeded into 96-well plates, with 6 wells per group. Each well contained at least 2×10^3 cells and 200 µL of the medium. The cells were incubated overnight for adherent growth and the cell supernatant was washed with phosphate-buffered saline. A mixture containing 90 µL of pure 1640 medium and 10 µL of CCK-8 solution (Beyotime Biotechnology, Shanghai, China) was added to each well. After 2 h of incubation, the absorbance of each well was measured at 450 nm using a microplate reader.

Transwell experiment

The cell invasiveness was examined with the 8-mm transwell (Millipore, Billerica, MA, USA) with (invasion assays) or without (migration assays) the coating of Matrigel. According to

previous study [39], the cells were seeded into serum-free medium and added to the upper chamber (1×10^4 cells per chamber) coated with 200 mg/mL of Matrigel. A medium containing 10% FBS was added to the lower chamber as a chemical attractant. After 24 h of incubation, the cells in the upper chamber were removed by wiping with a cotton swab. We then fixed the cells invading the lower surface of the filter in 70% ethanol for 30 min, and stained them for an additional 10 min with 0.2% crystal violet. The invading cells were photographed and counted across five random fields of view in each cell under an optical microscope at 200 \times magnification.

5-ethynyl-2'-deoxyuridine (EdU) assay

The EdU assay was conducted according to previous study [40]. The cells were pre-inoculated in a 24-well plate at a density of 5×10^3 cells per well. The plated cells were then incubated in 4% methanol for 30 min, followed by permeabilization in 0.5% TritonX-100 for 10 min, and a 30-min reaction in 400 μ L of 1 \times ApollorR. Afterward, the cells were stained with 4',6-diamidino-2-phenylindole for another 30 min. The EdU-stained cells were counted under a fluorescence microscope (CKX41-F32FL, Olympus, Tokyo, Japan) to calculate the percentage of EdU-positive cells.

Western blot analysis

The experimental method of Western blot referred to the previous literature [41]. Total protein was extracted from the transfected cells and the equal quantity proteins (30 μ g) were separated using a 10% polyacrylamide gel and transferred to a 0.22 μ m polyvinylidene fluoride membrane. The membranes were then incubated in blocking buffer (5% skim milk) for 2 h, and then incubated with primary antibodies at 4 $^{\circ}$ C overnight. After incubation with the corresponding secondary antibodies for 2 h at room temperature, the protein bars were visualized by chemiluminescence using the ECL reagent (Pierce, Rockford, IL, USA). The antibodies used were as follows: Wnt3a (ab219412, 1/1000, abcam), β -catenin (ab32572, 1/5000, abcam), β -

actin (ab8226, 1/1000, abcam), VEGFA (ab52917, 1/10000, abcam) and the anti-rabbit and anti-mouse peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology).

Dual luciferase reporter experiment

We used bioinformatics websites (starbase, <https://starbase.sysu.edu.cn/>) to predict the potential targets of *miR-195*, *PVT1*, and *VEGFA* [42]. A *PVT1* 3'-UTR or a mutant *PVT1* 3'-UTR with a predicted target site was inserted into a pGL3-promoter vector. Cells in the logarithmic growth phase were seeded into a 96-well plate at a density of 1.5×10^4 cells per well and cultured in an incubator for 24 h. After co-transfecting the cells for 48 h with a final concentration of 50 nmol/L of miRNA mimics or a non-target control, and 100 ng of the dual-reporter gene vector with a target gene 3' UTR or a mutant vector 3' UTR, the medium was aspirated and 35 μ L fresh medium added per well. Next, 35 μ L of luciferase (Promega, Madison, WI, USA) substrate was added to each well. After shaking for 10 min, the fluorescence intensity was measured. The experiment was repeated 3 times.

Statistical analysis

Data analysis was performed with the SPSS software (SPSS ver. 17.0, SPSS Inc., Chicago, IL, USA), and all the figures were processed with the GraphPad Prism software (Version X; La Jolla, CA, USA). Comparisons between two groups were analyzed using Student's t-test. One-way analysis of variance (ANOVA) followed by Tukey's test was utilized to analyze the comparisons among more than two groups. Fisher's exact test was used to analyze the correlation between *circPVT1* expression and PTC clinical features. The Kaplan–Meier method was applied to calculate the survival rate of patients. The results were displayed as mean \pm standard deviation (SD). Statistical significance was set at $p < 0.05$.

Results

This study aimed to reveal the expression pattern, biological function and potential molecular mechanism of *circPVT1* in PTC. We hypothesized that *circPVT1* might act as a significant role in the

progression of PTC. In basis of our results of in vitro experiments, we demonstrated that *circPVT1* was obviously up-regulated in osteosarcoma tissues and cell lines, and *circPVT1* could sponge *miR-195* to up-regulate *VEGFA* expression to promote PTC progression.

CircPVT1 was highly expressed in cells from PTC tissue

Through RT-PCR, we found that the level of *PVT1* expression in PTC tissue was significantly higher than that in non-cancerous tissue Figure 1(a). Meanwhile, according to the median of *circPVT1* level, we divided 50 cases into two groups, namely, *Circ PVT1* High group (n = 25) and *circPVT1* Low group (n = 25). The Kaplan–Meier curve indicated that the expression level of *circPVT1* had no significant correlation with the overall survival rate of PTC patients (cut-off = 0.225, $p > 0.05$) Figure 1(b). We also analyzed the

correlation between the expression levels of *PVT1* and special clinical pathological parameters. As shown in Table 1, the expression level of *PVT1* was significantly related to tumor size, TNM stage, and lymph node metastasis in patients with PTC. Compared to Nthy-ori 3–1 cells, the *PVT1* expression level in PTC cell lines (K1, IHH-4, BCPAP, and TPC1) increased significantly Figure 1(c). We selected TPC-1 and BCPAP cells for further experiments. First of all, we transfected *si-PVT1* into BCPAP cells and assessed the transfection efficiency. We observed a significant decrease in the *PVT1* expression levels in the BCPAP cells due to this transfection Figure 1(d).

Downregulation of PVT1 could inhibit the proliferation, migration, and invasion of PTC cells

The effect of the *PVT1* expression level on the PTC cells was detected using a CCK-8 test. After the

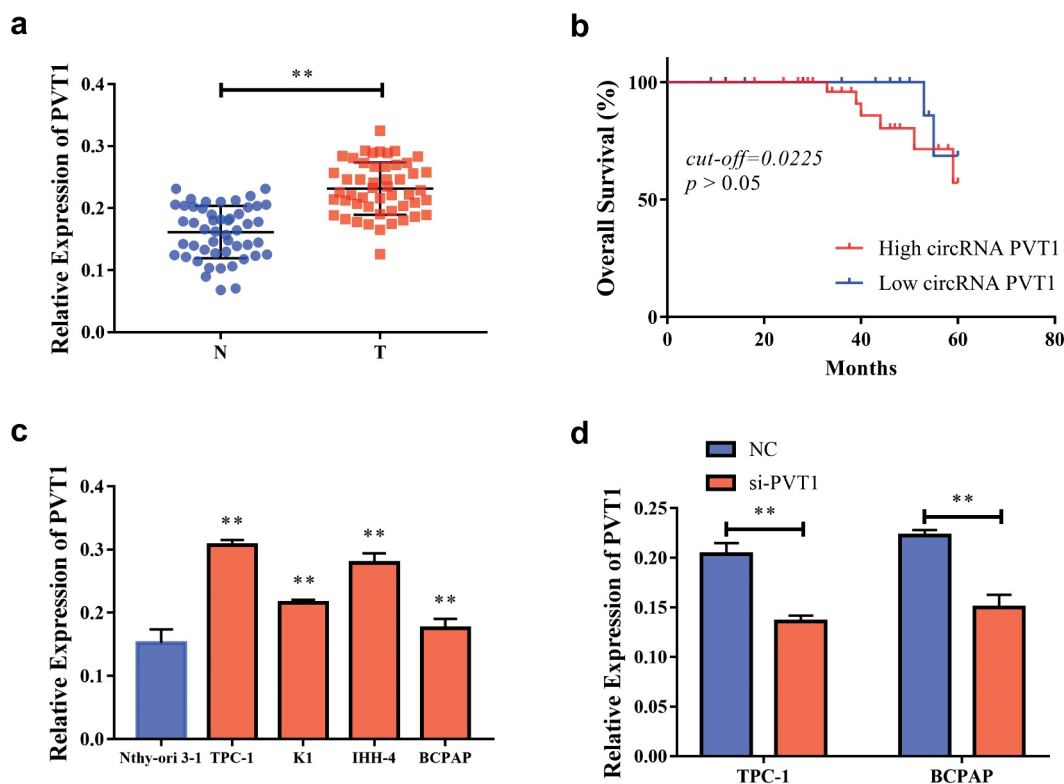


Figure 1. CircPVT1 had high expression in PTC tissue. a. Compared to cancer-side tissue, the level of *PVT1* expression in thyroid papillary carcinoma tissue was significantly increased; b. Statistically analyzed the expression level of *PVT1* had no significant correlation with PTC patients; c. Compared to human thyroid follicle epithelial cells (Nthy-ori 3–1), *PVT1* was significantly high in thyroid papillary cell lines (K1, IHH-4, BCPAP, TPC1); d. *PVT1* expression was significantly reduced after TPC-1 and BCPAP cells transfected *si-PVT1*. (** $P < 0.01$, the expression level of *PVT1* was normalized to U6).

Table 1. Relationship between circPVT1 expression and the clinical pathological characteristics of PTC patients (n = 50).

Clinic pathological features	NO. of cases	CircPVT1 (n, %)		p-value
		Low	High	
Gender	Male	29	14	$P > 0.05$
	Female	21	11	
Age	≤45	34	15	$P > 0.05$
	> 45	16	7	
Extra thyroidal extension	Negative	17	7	$P > 0.05$
	Positive	33	14	
Tumor size	≤1	36	25	$P < 0.05$
	>1	14	4	
TNM stage	I/II	28	20	$P < 0.05$
	III/IV	22	7	
	IV	22	15	
Lymph node metastasis	≤45	23	16	$P < 0.05$
	>45	27	9	
Nodular Goiter	Negative	31	15	$P > 0.05$
	Positive	19	9	

reduction of *PVT1* expression in TPC-1 and BCPAP cells, the cell proliferation capacity was significantly inhibited [Figure 2\(a,b\)](#). Similar results were observed in the EdU experiment [Figure 2\(c,d\)](#). The transwell assay showed that after the downregulation of *PVT1* expression, cell migration [Figure 2\(e,f\)](#), and invasiveness were also significantly reduced [Figure 2\(g,h\)](#). Based on the above observations, we concluded that reducing *PVT1* expression can significantly inhibit the proliferation, migration, and invasion of PTC cells.

Downregulation of *PVT1* could inhibit the Wnt/β-catenin signaling pathway

To further explore the specific role of *PVT1* in PTC development, we lowered the expression of *PVT1* in the PTC cells and detected the expression of genes related to the Wnt/β-catenin signaling pathway using RT-PCR. The results showed that following the inhibition of *PVT1* expression, the mRNA expression of these genes was significantly decreased [Figure 3\(a,b\)](#). Furthermore, the level of proteins related to Wnt/β-catenin signaling pathway-related genes was also significantly reduced [Figure 3\(c\)](#). Thus, we found that following the inhibition of *PVT1* expression, the expression of the Wnt/β-catenin signaling pathway was also significantly inhibited. This indicated that *PVT1* could play a role in activating the Wnt/β-catenin signaling pathway.

***PVT1* could target *miR-195* and regulate its expression**

We used bioinformatics to predict potential binding targets for *circPVT1* and *miR-195* [Figure 4\(a\)](#), and then constructed wild-type and mutant sequences of *PVT1* (*PVT1*-WT and *PVT1*-MUT, respectively). Using dual-luciferase reporter gene experiments, we discovered that after the transfection of *miR-195* mimics into the TPC-1 and BCPAP cells, the luciferase level in the *PVT1*-WT 3'UTR group was reduced, while there was no significant difference in the luciferase level in the *PVT1*-MUT 3'UTR group [Figure 4\(b,c\)](#). This result implied that *PVT1* could target *miR-195*. To further study the regulatory relationship between the two, we transfected the cells with an *miR-195* inhibitor. Using RT-PCR, we found that inhibition resulted in significantly decreased *miR-195* expression levels after transfection [Figure 4\(d,e\)](#), and significantly increased *PVT1* expression levels [Figure 4\(f\)](#). In addition, we found that *miR-195* expression was significantly increased after *PVT1* expression was downregulated [Figure 4\(d\)](#). Thus, we found that *PVT1* could target *miR-195* and inhibit its expression.

PVT1* could regulate *VEGFA* expression through *miR-195

We also used bioinformatics to predict potential binding targets for *miR-195* and *VEGFA* [Figure 5\(a\)](#) and then constructed *VEGFA* wild-type and mutant sequences (*VEGFA*-WT and *VEGFA*-MUT, respectively). Using a dual luciferase reporter gene experiment we found that, after transfection of *miR-195* mimics into TPC-1 and BCPAP cells, the luciferase levels in the *VEGFA*-WT 3'UTR group decreased, while those in the *VEGFA*-MUT 3'UTR showed no significant difference [Figure 5\(b,c\)](#). This suggested that *miR-195* can target *VEGFA*. To further study the regulatory relationship between the two, we transfected cells with *si-VEGFA* to inhibit its expression. RT-PCR showed that following transfection, the *VEGFA* expression level in the cells was significantly reduced [Figure 5\(d\)](#), while the *miR-195* expression level significantly increased [Figure 5\(e\)](#), and the

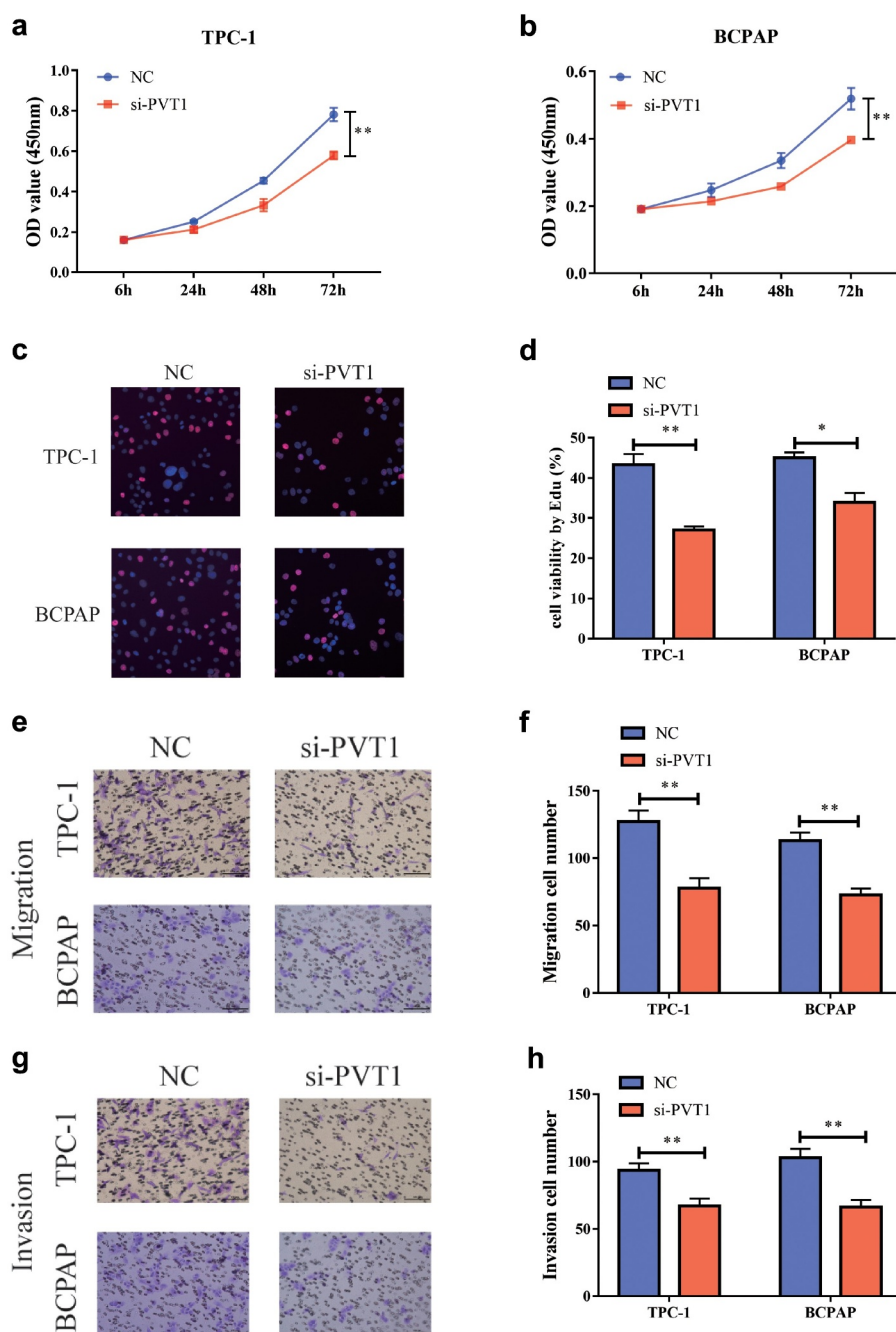


Figure 2. Down-regulation of circPVT1 could restrain the multiplication, migration as well as aggression of thyroid papillary carcinoma cells. **a**, CCK-8 test showed that when reducing PVT1 expression in TPC-1 cells, cell proliferation was reduced; **b**, CCK-8 experiments showed that when PVT1 expression was reduced in BCPAP cells, cell multiplication was reduced; **c**, **d**, Flat-panel cloning experiments showed that in TPC-1 as well as BCPAP cells, when reducing PVT1 expression, cell multiplication ability decreased; **e**, **f**, Transwell experiments showed that the reduction of PVT1 expression in TPC-1 and BCPAP cells, and the ability to migrate cells decreased; **g**, **h**, Transwell experiments showed that the cell attack ability decreased when reducing the expression of PVT1 in TPC-1 as well as BCPAP cells. (** $P < 0.01$).

PVT1 expression level decreased significantly **Figure 5 (f)**. To further explore this regulatory behavior, we used RT-PCR to show that *VEGFA* expression levels increased after downregulation of *miR-195* expression **Figure 5(g)**, and also that *VEGFA* expression was

significantly suppressed after *PVT1* expression was suppressed **Figure 5(h)**. Western blot analysis also showed the same results at the protein level **Figure 5 (i)**. Based on the above results, we concluded that *PVT1* could regulate the expression of *VEGFA*

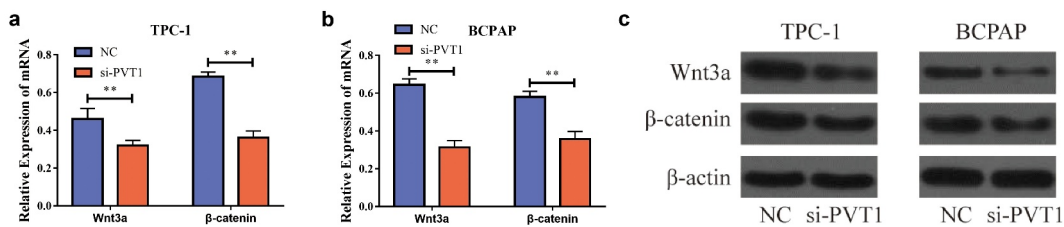


Figure 3. circPVT1 could activate the Wnt/ β -catenin signal pathway. a. Down-regulation of PVT1 expression in TPC-1 cells inhibits mRNA expression of Wnt/ β -catenin signal pathway-related genes; b. Lower PVT1 expression in BCPAP cells inhibits the mRNA expression of Wnt/ β -catenin signal pathway-related genes; c. Western blot showed that the level of gene proteins related to the Wnt/ β -catenin signal pathway decreased when PVT1 expression was lowered in TPC-1 as well as BCPAP cells. (** $P < 0.01$).

through targeted binding with miR-195 in the PTC cells.

Lowering VEGFA restrained the growth, migration, and invasion of PTC cells

In order to further explore the specific role of VEGFA in the development of PTC, we tested its influence on cell proliferation capacity via a CCK-8 test. The results suggested that the cell proliferation capacity was significantly inhibited Figure 6(a, b) after the reduction of VEGFA expression in the TPC-1 and BCPAP cells. The EdU experiment revealed the same outcome Figure 6(c,d). We used the transwell assay to detect cell migration and invasion and found that, after reducing VEGFA expression, the cell migration capacity was significantly reduced Figure 6(e,f) and the invasion capacity was also significantly inhibited Figure 6(g,h). Thus, we concluded that reduced VEGFA expression can significantly inhibit the proliferation, migration, and invasion of PTC cells.

Downregulation of VEGFA inhibited the Wnt/ β -catenin signaling pathway

In order to further elucidate the mechanism by which VEGFA is involved in the development of PTC, we tested the expression of Wnt/ β -catenin signaling pathway-related genes through RT-PCR after lowering VEGFA expression in the cells. Upon lowering the VEGFA expression, the level of mRNA expression for the Wnt/ β -catenin signaling pathway-related genes decreased significantly Figure 7(a,b), and the Western blot also showed significantly lower Wnt/ β -catenin signaling

pathway-related protein levels Figure 7(c). Taken together, we found that the Wnt/ β -catenin signaling pathway-related gene expression also significantly inhibited VEGFA expression, and that PVT1 activated the Wnt/ β -catenin signaling pathway by targeting miR-195 to upregulate VEGFA expression.

Discussion

In recent decades, the incidence of TC has been increasing, and it is the most common incretory tumor [43,44]. PTC is the main type of TC, accounting for approximately 90% of cases and with an overall 5-year survival rate of approximately 97% [45,46]. However, the clinical prognosis of late PTC is poor, partly because the molecular mechanism of PTC occurrence and development is not adequately understood. Therefore, finding new biomarkers and therapeutic targets to improve the diagnosis and therapeutic outcomes of PTC is a clinical problem.

Many ncRNAs have been shown to regulate the expression of genes in cancer cells and are expected to become molecular biomarkers of cancer [12,47]. The structure of circRNAs has been shown to be highly stable and resistant to RNA degradation pathways. This suggests that circRNAs may be more technically suitable as molecular biomarkers for human cancer. In recent years, many studies have shown that circRNAs are involved in the inniation and development of PTC. Luo et al. identify that *hsa_circ_0001018* can promote PTC by facilitating cell survival invasion, G/S cell cycle progression, and repress cell apoptosis via crosstalk with miR-338-3p and SRY-box transcription factor 4 (SOX4) [48]. Chu et al

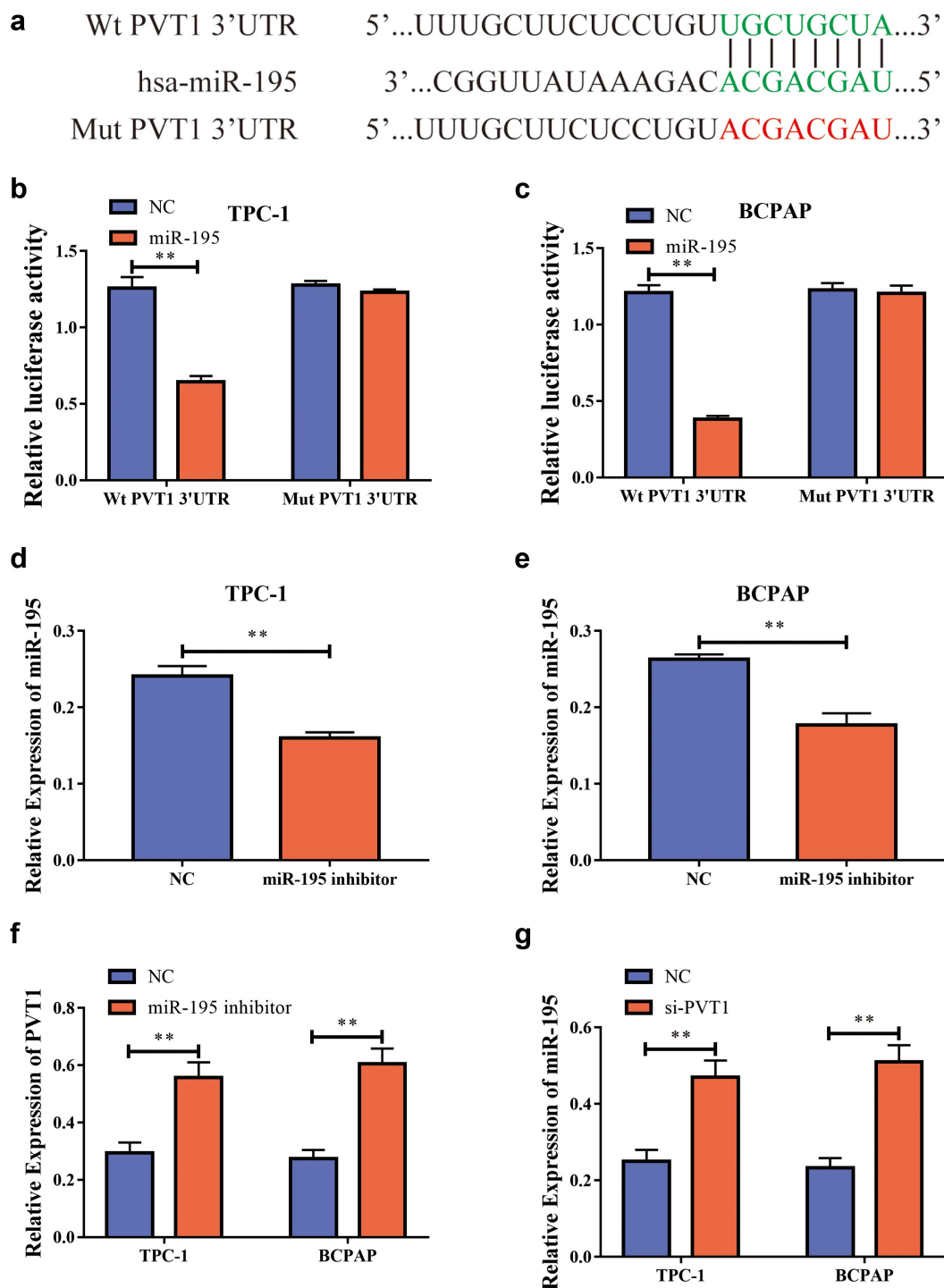


Figure 4 circPVT1 could target miR-195. a. Bioinformatics predicts potential binding sites of PVT1 and miR-195; b. Dual luciferase reporting genetic experiment of TPC-1 cells shows that PVT1 can target miR-195; c. Dual luciferase reporting genetic experiment in BCPAP cells shows that PVT1 can target miR-195; d. After transfecting the miR-195 inhibitor into the TPC-1 cell, the expression level of miR-195 decreased; e. BCPAP cells have reduced the level of miR-195 expression after transiting miR-195 pointon; f. PVT1 expression levels rises after down-regulation of miR-195 expression in TPC-1 as well as BCPAP cells; g. MiR-195 expression levels rises after the expression of PVT1 was reduced in TPC-1 and BCPAP cells. (** P < 0.01, the expression level of PVT1 was normalized to U6).

show that circular RNA *circRUNX1* can promote PTC progression and metastasis by sponging *miR-296-3p* and regulate DHD domain containing 2

(*DDHD2*) expression [49]. Ye and colleagues have demonstrated that circular RNA *circFOXMI1* may play a vital role in PTC by sponging *miR-1179*

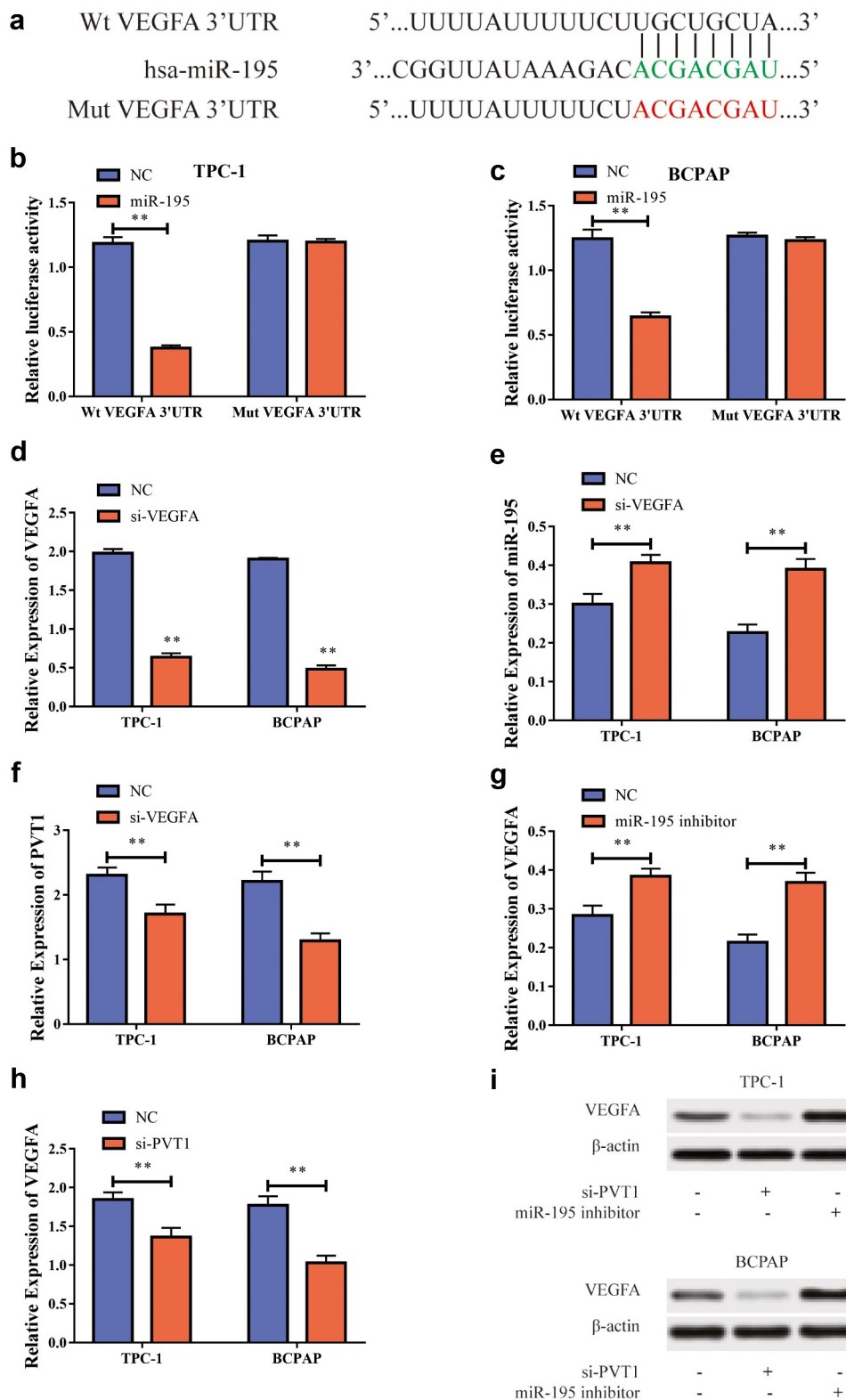


Figure 5 miR-195 could target VEGFA. **a**. Bioinformatics predicts the potential binding sites between miR-195 and VEGFA; **b**. Dual luciferase reporting gene experiment in TPC-1 cells shows that miR-195 can target VEGFA; **c**. Dual luciferase reporting gene experiment in BCPAP cells shows that miR-195 can target VEGFA; **d**. After transfecting si-VEGFA into TPC-1 as well as BCPAP cells, VEGFA expression was reduced; **e**. MiR-195 expression levels rises after down-regulating expression of VEGFA in TPC-1 and BCPAP cells; **f**. PVT1 expression level declines after VEGFA expression is lowered in TPC-1 and BCPAP cells; **g**. VEGFA expression levels rose after downward expression in TPC-1 and BCPAP cells; **h**. The expression of VEGFA is reduced after PVT1 expression is reduced in TPC-1 and BCPAP cells; **i**. Western blot shows that when lowering PVT1 expression in TPC-1 as well as BCPAP cells, VEGFA protein levels decrease; when miR-195 is downgraded, VEGFA protein levels increase. (** $P < 0.01$, the expression level of VEGFA was normalized to GAPDH).

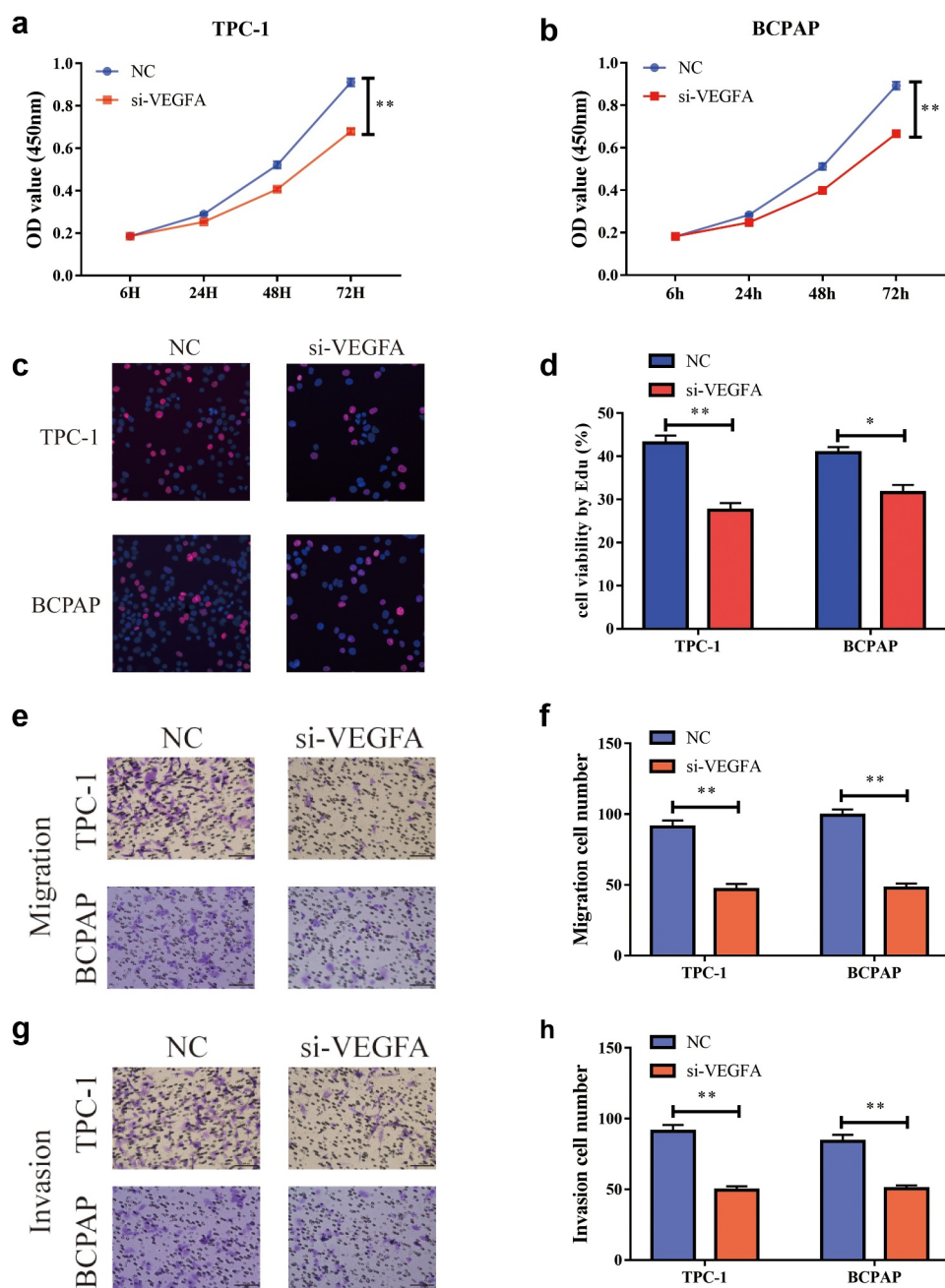


Figure 6. Down-regulation of VEGFA could restrain the multiplication, migration as well as aggression of thyroid papillary carcinoma cells. a. CCK-8 test shows that cell proliferation is reduced when reducing VEGFA expression in TPC-1 cells; b. CCK-8 test shows that VEGFA expression is reduced in BCPAP cells, cell multiplication is reduced; c, d. Flat-panel cloning experiment shows that when VEGFA expression is reduced, cell proliferation is reduced in TPC-1 as well as BCPAP cells; e, f. Transwell experiments shows that the cell migration capacity is reduced when VEGFA expression is reduced in BCPAP and TPC-1 cells; g, h. Transwell experiments shows that cell aggression is reduced when VEGFA expression is reduced in BCPAP and TPC-1 cells. (** $P < 0.01$).

and regulate high mobility group box 1 (*HMGB1*) expression [50].

In this study, we found that *circPVT1* had significantly increased expression in PTC tissue. Specifically, *PVT1* expression level had a significant correlation with tumor size, TNM

stage, and lymphatic metastasis in patients with PTC. In addition, we also found that the *circPVT1* expression level was significantly increased in the PTC cell lines compared to the Nthy-ori 3-1 cells. However, due to the limited number of specimens we collected and the short

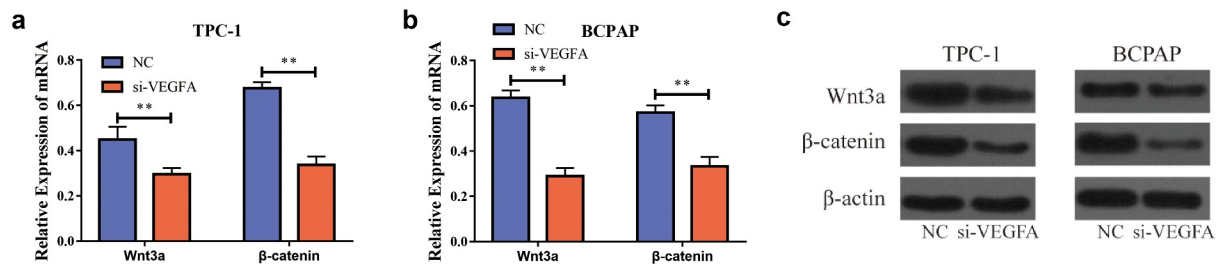


Figure 7. VEGFA could activate the Wnt/ β -catenin signal pathway. a. Down-regulation of VEGFA expression in TPC-1 cells inhibits the mRNA expression of Wnt/ β -catenin signal pathway-related genes; b. In BCPAP cells, when VEGFA expression is downregulated, the mRNA expression of Wnt/ β -catenin signal pathway-related genes is inhibited; c. Western blot shows that when reducing VEGFA expression in BCPAP as well as TPC-1 cells, the protein levels of Wnt/ β -catenin signal pathway-related genes decrease. (** $P < 0.01$).

follow-up time, we have not yet observed the correlation between the expression level of *CircPVT1* and the prognosis of PTC patients. At present, we have collected more specimens, and follow-up information will be improved. We will further verify the potential of *circPVT1* as a diagnostic marker for PTC.

Then, we detected the effect of *circPVT1* on cell proliferation, migration and invasion. After reducing the expression level of *circPVT1* in PTC cells, the ability of cell proliferation, migration, and invasion were significantly inhibited, and the Wnt/ β -catenin signaling pathway was also inhibited. The Wnt/ β -catenin signaling pathway, also known as the classic Wnt signaling pathway, has been studied in-depth [51,52]. Activation of the classic Wnt signaling pathway can contribute to extracellular Wnt combining with the transmembrane receptors *LRP5/6* and *Fzd* to form a complex. These receptors then become activated, which in turn activates the intracellular Disheveled protein, causing the inhibition of glycogen synthase enzyme-3 activity and subsequent detachment from the axin protein. This prevents formation of the β -catenin degradation complex, which in turn, prevents phosphorylation and degradation of β -catenin, thereby increasing the intracellular β -catenin expression. When β -catenin reaches a certain level, free β -catenin undergoes nuclear transfer and binds to the transcription factor *TCF/LEF* to form a transcription-activated complex. This complex eventually enhances or weakens the expression of certain genes. The Wnt/ β -catenin signaling pathway has also been proved to be involved in the progression of PTC [53–55].

Previous studies documented that circRNAs were involved in regulating diverse biological processes via functioning as competing endogenous RNAs for miRNAs [37,38]. *CircPVT1* has been proved to locate mainly in cytoplasm, which meant that it might act as microRNA sponges to regulate downstream gene expression. *MiR-195* is dysregulated in several tumors, such as colon cancer [56], non-small lung cancer [57], laryngeal cancer [58,59], gastric cancer [60], cervical cancer [61], colorectal cancer [62], breast cancer [63], lung adenocarcinoma [64] and osteosarcoma [65]. Moreover, miR-195 has been demonstrated that play a significant role in PTC [66–69]. *VEGFA* is a powerful and specific endothelial cell fissure agent that, in combination with receptors, can participate in promoting the division and proliferation of vascular endothelial cells and increase microvascular permeability [70,71]. *VEGFA* has been shown to play an important role in tumor development. *VEGFA* is not only important to endothelial cell survival but also helps circulating endothelial cells reach new blood vessels. This in turn can promote tumor growth and angiogenesis, and thus *VEGFA* expression may become a new tumor biomarker [72,73]. *VEGFA* also participates in the development of PTC [74]. In this work, by using the dual luciferase reporter gene and Western blotting, we found that *circPVT1* expression can be increased by targeting *miR-195* to increase *VEGFA* expression. We have also shown in numerous experiments that the Wnt/ β -catenin signaling pathway was inhibited and the cell proliferation, migration, and invasion abilities decreased after the reduction of *VEGFA*

expression in cells. In summary, we found that *circPVT1* could activate the Wnt/ β -catenin signaling pathway to promote the development of PTC.

Of course, this article still has many shortcomings. First of all, we have not conducted in vivo experiments to further verify the biological effects and molecular mechanisms of *circPVT1*. Secondly, there are multiple binding sites for miRNAs on *circPVT1*. It remains to be explored whether *circPVT1* binds to which miRNA plays the most important role. Thirdly, many studies have reported that ncRNAs have the potential to be developed as reagents for clinical treatment [75,76], whether *circPVT1* can be used as a therapeutic target for corresponding research and development still needs a lot of experimental proof. Moreover, we also need to collect more clinical specimens to explore the clinical application potential of *circPVT1*.

Conclusion

Overall, our study demonstrated that *circPVT1* expression was frequently increased in PTC and high expression level of *circPVT1* was related to tumor growth and metastasis and poor prognosis. From the perspective of mechanism, *circPVT1* could function as a molecule sponge for *miR-195* and mediated the ceRNA network to promote the expression of *VEGFA*, finally promoted the malignant progression of PTC. Therefore, the *circPVT1/miR-195/VEGFA* axis might be considered as a novel biomarker for prognosis and a promising therapeutic target for PTC treatment. Nonetheless, the current study only presents a theoretical basis for the mechanism of *circPVT1* involvement in PTC. Further research is required to explore the specific mechanisms involving various other molecular pathways involved in PTC carcinogenesis.

Disclosure statement

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

Funding

The author(s) reported there is no funding associated with the work featured in this article.

Data Availability Statement

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

References

- [1] Wang TS, Sosa JA. Thyroid surgery for differentiated thyroid cancer - recent advances and future directions. *Nat Rev Endocrinol.* 2018;14(11):670–683.
- [2] Leboulleux S, Tuttle RM, Pacini F, et al. Papillary thyroid microcarcinoma: time to shift from surgery to active surveillance? *Lancet Diabetes Endocrinol.* 2016;4(11):933–942.
- [3] Lim AM, Solomon BJ. Immunotherapy for anaplastic thyroid carcinoma. *J Clin Oncol.* 2020;38(23):2603–2604.
- [4] Kitahara CM, Sosa JA. The changing incidence of thyroid cancer. *Nat Rev Endocrinol.* 2016;12(11):646–653.
- [5] Tiedje V, Fagin JA. Therapeutic breakthroughs for metastatic thyroid cancer. *Nat Rev Endocrinol.* 2020;16(2):77–78.
- [6] Fagin JA, Wells SA Jr. Biologic and clinical perspectives on thyroid cancer. *N Engl J Med.* 2016;375(11):1054–1067.
- [7] Lin RX, Yang SL, Jia Y, et al. Epigenetic regulation of papillary thyroid carcinoma by long non-coding RNAs. *Semin Cancer Biol.* 2021. DOI:10.1016/j.semcancer.2021.03.027
- [8] Vaccarella S, Lortet-Tieulent J, Colombet M, et al. Global patterns and trends in incidence and mortality of thyroid cancer in children and adolescents: a population-based study. *Lancet Diabetes Endocrinol.* 2021;9(3):144–152.
- [9] Fallahi P, Ferrari SM, Elia G, et al. Primary cell cultures for the personalized therapy in aggressive thyroid cancer of follicular origin. *Semin Cancer Biol.* 2020; DOI:10.1016/j.semcancer.2020.06.013.
- [10] How CW, Ong YS, Low SS, et al. How far have we explored fungi to fight cancer? *Semin Cancer Biol.* 2021. DOI:10.1016/j.semcancer.2021.03.009
- [11] He L, Man C, Xiang S, et al. Circular RNAs' cap-independent translation protein and its roles in carcinomas. *Mol Cancer.* 2021;20(1):119.
- [12] Shen H, Liu B, Xu J, et al. Circular RNAs: characteristics, biogenesis, mechanisms and functions in liver cancer. *J Hematol Oncol.* 2021;14(1):134.
- [13] Yang Y, Lei W, Jiang S, et al. CircRNAs: decrypting the novel targets of fibrosis and aging. *Ageing Res Rev.* 2021;70:101390.
- [14] van Zonneveld AJ, Kolling M, Bijkerk R, et al. Circular RNAs in kidney disease and cancer. *Nat Rev Nephrol.* 2021;17(12):814–826.

- [15] Zhou WY, Cai ZR, Liu J, et al. Circular RNA: metabolism, functions and interactions with proteins. *Mol Cancer*. 2020;19(1):172.
- [16] Wang S, Zhang K, Tan S, et al. Circular RNAs in body fluids as cancer biomarkers: the new frontier of liquid biopsies. *Mol Cancer*. 2021;20(1):13.
- [17] Tan KL, Chia WC, How CW, et al. Benchtop isolation and characterisation of small extracellular vesicles from human mesenchymal stem cells. *Mol Biotechnol*. 2021;63(9):780–791.
- [18] Long F, Lin Z, Li L, et al. Comprehensive landscape and future perspectives of circular RNAs in colorectal cancer. *Mol Cancer*. 2021;20(1):26.
- [19] Goodall GJ, Wickramasinghe VO. RNA in cancer. *Nat Rev Cancer*. 2020;21(1):22–36.
- [20] Gokool A, Loy CT, Halliday GM, et al. Circular RNAs: the brain transcriptome comes full circle. *Trends Neurosci*. 2020;43(10):752–766.
- [21] Ma S, Kong S, Wang F, et al. CircRNAs: biogenesis, functions, and role in drug-resistant Tumours. *Mol Cancer*. 2020;19(1):119.
- [22] Chen LL. The expanding regulatory mechanisms and cellular functions of circular RNAs. *Nat Rev Mol Cell Biol*. 2020;21(8):475–490.
- [23] Wen G, Zhou T, Gu W. The potential of using blood circular RNA as liquid biopsy biomarker for human diseases. *Protein Cell*. 2020. DOI:10.1007/s13238-020-00799-3
- [24] Li G, Guo BY, Wang HD, et al. CircRNA hsa_circ_0014130 function as a miR-132-3p sponge for playing oncogenic roles in bladder cancer via upregulating KCNJ12 expression. *Cell Biol Toxicol*. 2021. DOI:10.1007/s10565-021-09668-z
- [25] Zhang X, Wu N, Wang J. Hsa-circ_0058106 induces EMT and metastasis in laryngeal cancer via sponging miR-153 and inducing Twist1 nuclear translocation. *Cell Oncol (Dordr)*. 2021. DOI:10.1007/s13402-021-00644-7
- [26] Cai Y, Zhao X, Chen D, et al. circ-NOL10 regulated by MTDH/CASC3 inhibits breast cancer progression and metastasis via multiple miRNAs and PDCD4. *Mol Ther Nucleic Acids*. 2021;26:773–786.
- [27] Ghetti M, Vannini I, Storlazzi CT, et al. Linear and circular PVT1 in hematological malignancies and immune response: two faces of the same coin. *Mol Cancer*. 2020;19(1):69.
- [28] Traversa D, Simonetti G, Tolomeo D, et al. Unravelling similarities and differences in the role of circular and linear PVT1 in cancer and human disease. *Br J Cancer*. 2021. DOI:10.1038/s41416-021-01584-7
- [29] Yu F, Lin Y, Ai MM, et al. Knockdown of circular RNA hsa_circ_PVT1 inhibited laryngeal cancer progression via preventing wnt4/beta-Catenin signaling pathway activation. *Front Cell Dev Biol*. 2021;9:658115.
- [30] Li X, Zhang Z, Jiang H, et al. Circular RNA circPVT1 promotes proliferation and invasion through sponging miR-125b and activating E2F2 signaling in non-small cell lung cancer. *Cell Physiol Biochem*. 2018;51(5):2324–2340.
- [31] Chen J, Li Y, Zheng Q, et al. Circular RNA profile identifies circPVT1 as a proliferative factor and prognostic marker in gastric cancer. *Cancer Lett*. 2017;388:208–219.
- [32] Zheng Z, Chen Z, Zhong Q, et al. CircPVT1 promotes progression in clear cell renal cell carcinoma by sponging miR-145-5p and regulating TBX15 expression. *Cancer Sci*. 2021;112(4):1443–1456.
- [33] Li M, Chi C, Zhou L, et al. Circular PVT1 regulates cell proliferation and invasion via miR-149-5p/FOXM1 axis in ovarian cancer. *J Cancer*. 2021;12(2):611–621.
- [34] Wang H, Wei M, Kang Y, et al. Circular RNA circ_PVT1 induces epithelial-mesenchymal transition to promote metastasis of cervical cancer. *Aging (Albany NY)*. 2020;12(20):20139–20151.
- [35] Bian Q. Circular RNA PVT1 promotes the invasion and epithelial-mesenchymal transition of breast cancer cells through serving as a competing endogenous RNA for miR-204-5p. *Onco Targets Ther*. 2019;12:11817–11826.
- [36] Ishola AA, Chien CS, Yang YP, et al. Oncogenic circRNA hsa_circ_0000190 modulates EGFR/ERK pathway in promoting NSCLC. *Cancer Res*. 2021; DOI:10.1158/0008-5472.CAN-21-1473.
- [37] Chen SW, Zhu SQ, Pei X, et al. Cancer cell-derived exosomal circUSP7 induces CD8(+) T cell dysfunction and anti-PD1 resistance by regulating the miR-934/SHP2 axis in NSCLC. *Mol Cancer*. 2021;20(1):144.
- [38] Li J, Gao X, Zhang Z, et al. CircCD44 plays oncogenic roles in triple-negative breast cancer by modulating the miR-502-5p/KRAS and IGF2BP2/Myc axes. *Mol Cancer*. 2021;20(1):138.
- [39] Mao W, Wang K, Xu B, et al. ciRS-7 is a prognostic biomarker and potential gene therapy target for renal cell carcinoma. *Mol Cancer*. 2021;20(1):142.
- [40] Rong D, Wu F, Lu C, et al. m6A modification of circHPS5 and hepatocellular carcinoma progression through HMGA2 expression. *Mol Ther Nucleic Acids*. 2021;26:637–648.
- [41] Yin H, Chen L, Piao S, et al. M6A RNA methylation-mediated RMRP stability renders proliferation and progression of non-small cell lung cancer through regulating TGFBR1/SMAD2/SMAD3 pathway. *Cell Death Differ*. 2021. DOI:10.1038/s41418-021-00888-8
- [42] Li JH, Liu S, Zhou H, et al. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res*. 2014;42(D1):D92–7.
- [43] Salvatore D, Santoro M, Schlumberger M. The importance of the RET gene in thyroid cancer and therapeutic implications. *Nat Rev Endocrinol*. 2021;17(5):296–306.
- [44] Fallahi P, Ferrari SM, Galdiero MR, et al. Molecular targets of tyrosine kinase inhibitors in thyroid cancer. *Semin Cancer Biol*. 2020. DOI:10.1016/j.semcancer.2020.11.013

- [45] Dsa M, Zhang L, Durante C, et al. Contemporary debates in adult papillary thyroid cancer management. *Endocr Rev.* **2019**;40(6):1481–1499.
- [46] Pstrag N, Ziemnicka K, Bluysen H, et al. Thyroid cancers of follicular origin in a genomic light: in-depth overview of common and unique molecular marker candidates. *Mol Cancer.* **2018**;17(1):116.
- [47] Lauretti E, Dabrowski K, Pratico D. The neurobiology of non-coding RNAs and alzheimer's disease pathogenesis: pathways, mechanisms and translational opportunities. *Ageing Res Rev.* **2021**;71:101425.
- [48] Luo Q, Guo F, Fu Q, et al. hsa_circ_0001018 promotes papillary thyroid cancer by facilitating cell survival invasion, G1/S cell cycle progression, and repressing cell apoptosis via crosstalk with miR-338-3p and SOX4. *Mol Ther Nucleic Acids.* **2021**;24:591–609.
- [49] Chu, J, Tao, L, and Yao, T. Circular RNA circRUNX1 promotes papillary thyroid cancer progression and metastasis by sponging MiR-296-3p and regulating DDHD2 expression. *Cell Death Dis.* **2021**;122: 112.
- [50] Ye M, Hou H, Shen M, et al. Circular RNA circFOXO1 plays a role in papillary thyroid carcinoma by sponging miR-1179 and regulating HMGB1 expression. *Mol Ther Nucleic Acids.* **2020**;19:741–750.
- [51] Yu F, Yu C, Li F, et al. Wnt/beta-catenin signaling in cancers and targeted therapies. *Signal Transduct Target Ther.* **2021**;6(1):307.
- [52] Tewari D, Bawari S, Sharma S, et al. Targeting the crosstalk between canonical Wnt/beta-catenin and inflammatory signaling cascades: a novel strategy for cancer prevention and therapy. *Pharmacol Ther.* **2021**;227:107876.
- [53] Dai W, Jin X, Han L, et al. Exosomal lncRNA DOCK9-AS2 derived from cancer stem cell-like cells activated Wnt/beta-catenin pathway to aggravate stemness, proliferation, migration, and invasion in papillary thyroid carcinoma. *Cell Death Dis.* **2020**;11(9):743.
- [54] Ma J, Huang X, Li Z, et al. FOXE1 supports the tumor promotion of Gli2 on papillary thyroid carcinoma by the Wnt/ β -catenin pathway. *J Cell Physiol.* **2019**;234(10):17739–17748.
- [55] Bi W, Huang J, Nie C, et al. CircRNA circRNA_102171 promotes papillary thyroid cancer progression through modulating CTNNBIP1-dependent activation of beta-catenin pathway. *J Exp Clin Cancer Res.* **2018**;37(1):275.
- [56] Gu H, Xu Z, Zhang J, et al. circ_0038718 promotes colon cancer cell malignant progression via the miR-195-5p/Axin2 signaling axis and also effect Wnt/beta-catenin signal pathway. *BMC Genomics.* **2021**;22(1):768.
- [57] Xi J, Xi Y, Zhang Z, et al. Hsa_circ_0060937 accelerates non-small cell lung cancer progression via modulating miR-195-5p/HMGB3 pathway. *Cell Cycle.* **2021**;20(19):2040–2052.
- [58] Zhou M, Wang Y, Zhang C, et al. MicroRNA-195-5p suppresses the proliferation, migration, invasion and epithelial-mesenchymal transition of laryngeal cancer cells in vitro by targeting E2F3. *Exp Ther Med.* **2021**;22(4):1078.
- [59] Shen D, Li J, Tao K, et al. Long non-coding RNA MCM3AP antisense RNA 1 promotes non-small cell lung cancer progression through targeting microRNA-195-5p. *Bioengineered.* **2021**;12(1):3525–3538.
- [60] Yan B, Ren Z, Sun J, et al. IGF2-AS knockdown inhibits glycolysis and accelerates apoptosis of gastric cancer cells through targeting miR-195/CREB1 axis. *Biomed Pharmacother.* **2020**;130:110600.
- [61] Liu S, Li B, Li Y, et al. Circular RNA circ_0000228 promotes the malignancy of cervical cancer via microRNA-195-5p/ lysyl oxidase-like protein 2 axis. *Bioengineered.* **2021**;12(1):4397–4406.
- [62] Li S, Lv C, Li J, et al. LncRNA LINC00473 promoted colorectal cancer cell proliferation and invasion by targeting miR-195 expression. *Am J Transl Res.* **2021**;13(6):6066–6075.
- [63] Wu J, Xu W, Ma L, et al. Formononetin relieves the facilitating effect of lncRNA AFAP1-AS1-miR-195/miR-545 axis on progression and chemo-resistance of triple-negative breast cancer. *Aging (Albany NY).* **2021**;13(14):18191–18222.
- [64] Wang A, Zhang T, Wei W, et al. The Long Noncoding RNA LINC00665 facilitates c-myc transcriptional activity via the miR-195-5p MYCBP axis to promote progression of lung adenocarcinoma. *Front Oncol.* **2021**;11:666551.
- [65] Yang B, Li L, Tong G, et al. Circular RNA circ_001422 promotes the progression and metastasis of osteosarcoma via the miR-195-5p/FGF2/PI3K/Akt axis. *J Exp Clin Cancer Res.* **2021**;40(1):235.
- [66] Li C, Zhu L, Fu L, et al. CircRNA NRIP1 promotes papillary thyroid carcinoma progression by sponging mir-195-5p and modulating the P38 MAPK and JAK/STAT pathways. *Diagn Pathol.* **2021**;16(1):93.
- [67] Xu J, Li Z, Su Q, et al. Suppression of long noncoding RNA LINC00324 restricts cell proliferation and invasion of papillary thyroid carcinoma through down-regulation of TRIM29 via upregulating microRNA-195-5p. *Aging (Albany NY).* **2020**;12(24):26000–26011.
- [68] Gui X, Li Y, Zhang X, et al. Circ_LDLR promoted the development of papillary thyroid carcinoma via regulating miR-195-5p/LIPH axis. *Cancer Cell Int.* **2020**;20(1):241.
- [69] Yin Y, Hong S, Yu S, et al. MiR-195 inhibits tumor growth and metastasis in papillary thyroid carcinoma cell lines by targeting CCND1 and FGF2. *Int J Endocrinol.* **2017**;2017:6180425.
- [70] Guryanov I, Tennikova T, and Urtti A. Peptide inhibitors of vascular endothelial growth factor A: current situation and perspectives. *Pharmaceutics.* **2021**; 13(9) ;1337.
- [71] Guo X, Yi H, and Li TC, et al. Role of Vascular Endothelial Growth Factor (VEGF) in human embryo implantation: clinical Implications. *Biomolecules.* **2021**;11(2) ;253.

- [72] Ren S, Tan X, Fu MZ, et al. Downregulation of miR-375 contributes to ERBB2-mediated VEGFA over-expression in esophageal cancer. *J Cancer*. 2021;12(23):7138–7146.
- [73] Ciccarese C, Iacovelli R, Porta C, et al. Efficacy of VEGFR-TKIs plus immune checkpoint inhibitors in metastatic renal cell carcinoma patients with favorable IMDC prognosis. *Cancer Treat Rev*. 2021;100:102295.
- [74] Wang R, Ma Q, Ji L, et al. miR-622 suppresses tumor formation by directly targeting VEGFA in papillary thyroid carcinoma. *Onco Targets Ther*. 2018;11:1501–1509.
- [75] Looi QH, Foo JB, Lim MT, et al. How far have we reached in development of effective influenza vaccine? *Int Rev Immunol*. 2018;37(5):266–276.
- [76] Yu AM, Tu MJ. Deliver the promise: rNAs as a new class of molecular entities for therapy and vaccination. *Pharmacol Ther*. 2021;107967. doi:10.1016/j.pharmthera.2021.107967