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TBX3 activating PVT1 accelerates proliferation, migration, and invasion by modulating the miR-30a/LOX axis in anaplastic thyroid carcinoma

Jing Wen[1, 2]*, *Wei Zhang*[3]*, *Yan Zhou*[2], *Xiaoli Hu*[1], *Lijuan Luo*[6], *Jine Zhou*[6], *Lixin Shi*[4], *Shi Zhou*[5, 6]

[1]*Department of Ultrasound Centre, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, China*
[2]*Department of Internal Medicine, Clinical Medical College, Guizhou Medical University, Guiyang, China*
[3]*Department of Thyroid Surgery, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, China*
[4]*Department of Endocrinology, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, China*
[5]*College of Radiology Imaging, Guizhou Medical University, Guiyang, Guizhou, China*
[6]*Department of Invasive Technology, Affiliated Hospital of Guizhou Medical University, Guiyang, Guizhou, China*
**These authors contributed equally to the work*

Abstract

Introduction: Anaplastic thyroid carcinoma (ATC) is a nearly chemo-resistant malignancy with high invasion and mortality. Long non-coding RNAs (lncRNAs) have been demonstrated to be dysregulated and play a crucial role in the development and process of ATC. The present study aimed to explore the mechanism of PVT1 dysregulation in ATC.

Material and methods: The mRNA levels of PVT1 and T-box3 (TBX3), and the protein levels of TBX3 in ATC and paracancerous tissues, and FRO and Nthy-ori 3-1 cells were determined by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) and western blot, respectively. The transcriptional factor binding site was predicted and validated between TBX3 and PVT1 promoter through the JASPAR website, and ChIP and luciferase analysis. The proliferation, migration, and invasion of FRO cells were assessed by MTT, colony formation, and transwell assays.

Results: PVT1 expression was upregulated in ATC, which was positively correlative with the level of transcription factor TBX3. Downregulation of PVT1 inhibited the proliferation, migration, and invasion of FRO cells. Moreover, TBX3 targeting the promoter region of PVT1 promoted the expression level of PVT1 and modulated the downstream signalling axis of PVT1, miR-30a/LOX. Also, interference of PVT1 reversed the stimulative role of overexpression of TBX3 in the progress of FRO cells.

Conclusion: TBX3 enhanced proliferation, migration, and invasion of ATC cells via activation of PVT1 and modulation of the miR-30a/LOX signalling axis. **(Endokrynol Pol 2022; 73 (4): 690–698)**

Key words: *anaplastic thyroid carcinoma; PVT1; TBX3; proliferation; migration; invasion*

Introduction

Thyroid cancer is the most usual endocrine-related malignant tumour, which accounted for 3.4% of all new cancer cases in the United States in 2017 [1, 2]. Therein, approximately 2–3% of thyroid cancers are incurable, characterized by undifferentiation, high invasion, and near chemo-resistance, which is designated as anaplastic thyroid carcinoma (ATC) [3]. Although ATC is rare, with an incidence of 0.0009–0.0012‰, it is almost always fatal, with a median survival of only 3–4 months [4, 5]. Therefore, in-depth exploration of the mechanism of the occurrence and development of ATC is still an urgent problem to be solved in clinics.

Long non-coding RNA (lncRNA) is a non-coding transcript longer than 200 nucleotides with a variety of functions, such as sponging miRNA, splicing regulator, and chromatin remodelling, which plays a vital role in various biological and pathological processes [6]. The vital effect of lncRNA on the progression of ATC has also been reported in various studies. For instance, suppression of lncRNA H19 inhibits the proliferation and metastasis of ATC [7]. Interference of lncRNA MALAT1 restrains the processes of ATC via modulation

Lixin Shi, Department of Endocrinology, Affiliated Hospital of Guizhou Medical University, No. 28 Guiyi Street, Guiyang, Guizhou Province, China, tel: +86-18685153111; e-mail: lxshi62@163.com
Shi Zhou, Department of Invasive Technology, Affiliated Hospital of Guizhou Medical University, No. 28 Guiyi street, Guiyang, Guizhou Province, China
Shi Zhou, Institute of image, Guizhou Medical University, No. 28 Guiyi street, Guiyang, Guizhou Province, China, tel: +86-13809477708; e-mail: zhoushi@gmc.edu.cn

of the miR-200a-3p/FOXA1 signalling axis [8]. LncRNA RP11-395G23.3 regulates the proliferation and apoptosis of ATC through the miR-124-3p/ROR1 signalling axis [9]. LncRNA PVT1 is an oncogene located at the cancer risk region chromosome 8q24.21 [10], which has been demonstrated to regulate the proliferation of ATC cells via modulation of thyroid-stimulating hormone receptor (TSHR) and recruitment of EZH2 [11]. Moreover, our pre-experimental results discovered that PVT1 was dysregulated in the cancer tissues of ATC. However, the mechanism of PVT1 dysregulation in ATC is still dismal.

T-box3 (TBX3), a member of the T-box transcription factors, exerts important roles during organogenesis, embryonic development, and tissue homeostasis [12]. Furthermore, TBX3 also affects the development and progression of tumours, such as pancreatic cancer [13], breast cancer [14], and melanoma [15]. In addition, Li et al. [16] showed that the level of TBX3 is significantly increased in papillary thyroid carcinoma (PTC), which is prominently involved in the TNM stage and lymph node metastasis. Downregulation of TBX3 expression can restrain cell growth *in vitro* and tumour formation *in vivo*. More importantly, TBX3 can target the lncRNA to modulate the processes of tumours [17]. Thus, in the present study, we explored whether TBX3 could play a role in the progress of ATC by targeting PVT1. The results revealed that TBX3 activating PVT1 accelerated proliferation, migration, and invasion by regulating the miR-30a/LOX axis in ATC.

Material and methods

Tissue samples

Forty-two pairs of ATC and adjacent paracancerous tissues were collected from ATC patients at the Affiliated Hospital of Guizhou Medical University. All the patients had not received any preoperative radiation or chemotherapy. All the studies were approved by the Ethics Committee of the Affiliated Hospital of Guizhou Medical University, and written informed consent was authorized from all subjects. The sample tissues were promptly frozen in liquid nitrogen after surgery and subsequently stored at –80°C for further experiments.

Cells culture

Human ATC cell line FRO and human normal thyroid cell line Nthy-ori 3-1 were bought from Punosai (Wuhan, China). Cells were maintained in DMEM (Solarbio, Beijing, China) provided with 0.1% FBS (Gibco, Rockville, MD, USA) and 1% streptomycin and penicillin (Solarbio) and cultured at 37°C with 5% carbon dioxide (CO_2).

Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR)

Total RNA was isolated from cell or tissue samples by TRIzol reagent (TaKaRa Biotechnology Co., Ltd., Dalian, China) based on the manufacturer's instructions. cDNA was reversely transcribed with a PrimeScript RT reagent Kit (Takara, RR047A) based on the manufacturer's specifications. qRT-PCR was performed with the Bio-Rad ScripTM cDNA Synthesis Kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The primer sequences of PVT1 (Forward primer: 5′-GCCCCTTCTATGGGAATCACTA-3′, Reverse primer: 5′-GGGGCAGAGATGAAATCGTAAT-3′), TBX3 (Forward primer: 5′-AGCCTGTTCCCTTACCCCTAC-3′, Reverse primer: 5′-GTTCAGAGCCCGAGTCCACT-3′), miR-30a (Forward primer: 5′-GCAACTGTAAACATCCTCGACTGGA-3′, Reverse primer: 5′-TGAAAGCCCATTTGTGGCTTCACAG-3′), LOX (Forward primer: 5′-GATACGGCACTGGCTACTTCCA-3′, Reverse primer: 5′-GCCAGACAGTTTTCCTCCGCC-3′), GAPDH (Forward primer: 5′-TATCGGACGCCTGGTTAC-3′, Reverse primer: 5′-CGTTCAAGTTGCCGTGTC-3′) and U6 (Forward primer: 5′-ACAGAGAAGATTAGCATGGCC-3′, Reverse primer: 5′-GACCAATTCTCGATTTGTGCG-3′) were designed and synthesized in Sangon Biotech Co., Ltd. (Shanghai, China). The qRT-PCR amplification conditions were as follows: 94°C for 5 min, 94°C for 15 s, and 60°C for 30 s, for 40 cycles. GAPDH acted as the endogenous normalization controls. The relative expression level of genes was determined by the $2^{-\Delta\Delta CT}$ method.

Western blot

Total protein from cell or tissue samples was separated by RIPA lysis buffer (Beyotime, Shanghai, China). Protein concentration was quantified with the BCA Protein Assay kit (Boster Biological Technology, Ltd., Wuhan, China). Protein samples were isolated with 10% SDS-PAGE and electrically transferred onto a PVDF membrane (EMD Millipore, Boston, MA, USA). The membranes were sealed with TBST (Sigma, St. Louis, MO, USA) including 5% skimmed milk powder (Anchor, New Zealand) and then hatched with primary antibodies (rabbit polyclonal antibody TBX3, ab99302, 1:1000; rabbit polyclonal antibody LOX, ab31238, 1:1000; rabbit polyclonal antibody GAPDH, ab9485, 1:2500; Abcam, Cambridge, UK) at 4°C overnight. The membrane was hatched with the HRP-conjugated goat anti-rabbit IgG secondary antibody (ab6721, 1:20,000, Abcam) for one hour at room temperature after being washed three times with TBST. The protein bands were visualized with the DAB kits (Sigma) and quantified by a ChemiDoc™ MP imaging system (Bio Rad, Hercules, CA, USA).

Chromatin immunoprecipitation (ChIP)

Chromatin immunoprecipitation assay was carried out using a ChIP Assay Kit (ab500, Abcam) based on the manufacturer's protocols. Chromatin was splintered into 200–1000 bp fragments by sonication, and FRO cells were cross-linked with formaldehyde. Then, TBX3 antibody and IgG antibody were incubated with DNA fragments overnight at 4°C. The DNA cross-linking was reversed with NaCl. The level of PVT1 was determined by qRT-PCR.

Luciferase assay

The TBX3 binding sites of PVT1 promoter was constructed into pGL3-Basic luciferase vector (Promega Corporation, Madison, WI, USA). Subsequently, the empty vector or constructed vector was transfected into FRO cells using Lipofectamine® 2000 (Invitrogen, Carlsbad, CA, USA). Luciferase activity was examined with a Dual-Luciferase Reporter Assay kit (E1910; Promega Corporation) following transfection for 48 h.

Cell transfection

FRO cells were plated into 6-well plates at a density of 1×10^5 cells/well and cultured with 5% CO_2 at 37°C overnight. TBX3 sequences were generated and inserted into pcDNA vector plasmids (Vigene Biosciences, Rockville, MD, USA) to upregulate the expression of TBX3. Two TBX3 siRNAs (si-TBX3) including si-TBX3 1# and si-TBX3 2# (50 pmol/mL), two PVT1 siRNAs (si-PVT1) including si-PVT1 1# and si-PVT1 2# (50 pmol/mL), and their corresponding negative control (NC) siRNAs were acquired from RiboBio (Guangzhou, China). The plasmids or siRNAs were transfected into FRO cells by Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific) based on the operating instructions. Cells were collected and analysed after transfection for 24 h.

MTT assay

FRO cells were inoculated into 96-well plates with a density of 4×10^3 cells/well and cultured at 37°C with 5% CO_2 overnight. Then

the cells were hatched with 10 μL MTT solution (Sigma) at 0, 24, 48, and 72 h for 4 h. Next, the supernatant fluid was abandoned, and 100 μL DMSO was appended to each well to dissolve the crystals. The absorbance was detected at 570 nm by the microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Colony formation assay

FRO cells were seeded in 6-well plates with a density of 1×10^4 cells/well and maintained with 5% CO_2 at 37°C for 14 days. Subsequently, the colonies were immobilized with 4% paraformaldehyde and stained with 0.1% crystal violet (Solarbio) for 30 min, and then counted manually.

Transwell assay

The migration and invasion of FRO cells were detected by transwell assays. Following transfection for 24 h, FRO cells were digested and suspended with phosphate buffer saline (PBS, Solarbio) at a density of 1×10^6 cells/mL. For the migration assay, 600 μL of DMEM culture medium with 10% serum and 100 μL of FRO cell suspension were placed into the lower chamber and the upper chamber, respectively. For the invasion assay, the Matrigel matrixes with serum-free medium dilution were dispersed with the transwell chamber, and subsequently appended with the FRO cell suspension. FRO cells were maintained with 5% CO_2 at 37°C. Following cultivation for 24 h, FRO cells were immobilized and stained with 4% paraformaldehyde and 0.1% crystal violet for 15 min, respectively. The images were captured and counted by microscope (Olympus, Tokyo, Japan).

Statistical analysis

Statistical differences between 2 groups were examined by the Student's t-test, whereas the differences between multiple groups were tested with the one-way analysis of variance (ANOVA) followed by *post hoc* Bonferroni test by SPSS 20.0 software (IBM, Armonk, New York, USA). $p < 0.05$ was considered as a significant difference.

Results

The expression of PVT1 was upregulated in ATC and positively correlative with TBX3 level

qRT-PCR results showed that the relative mRNA expression of PVT1 was memorably upregulated in ATC tumour tissues relative to that in adjacent normal tissues (Fig. 1A). Also, the relative mRNA level of TBX3 in ATC

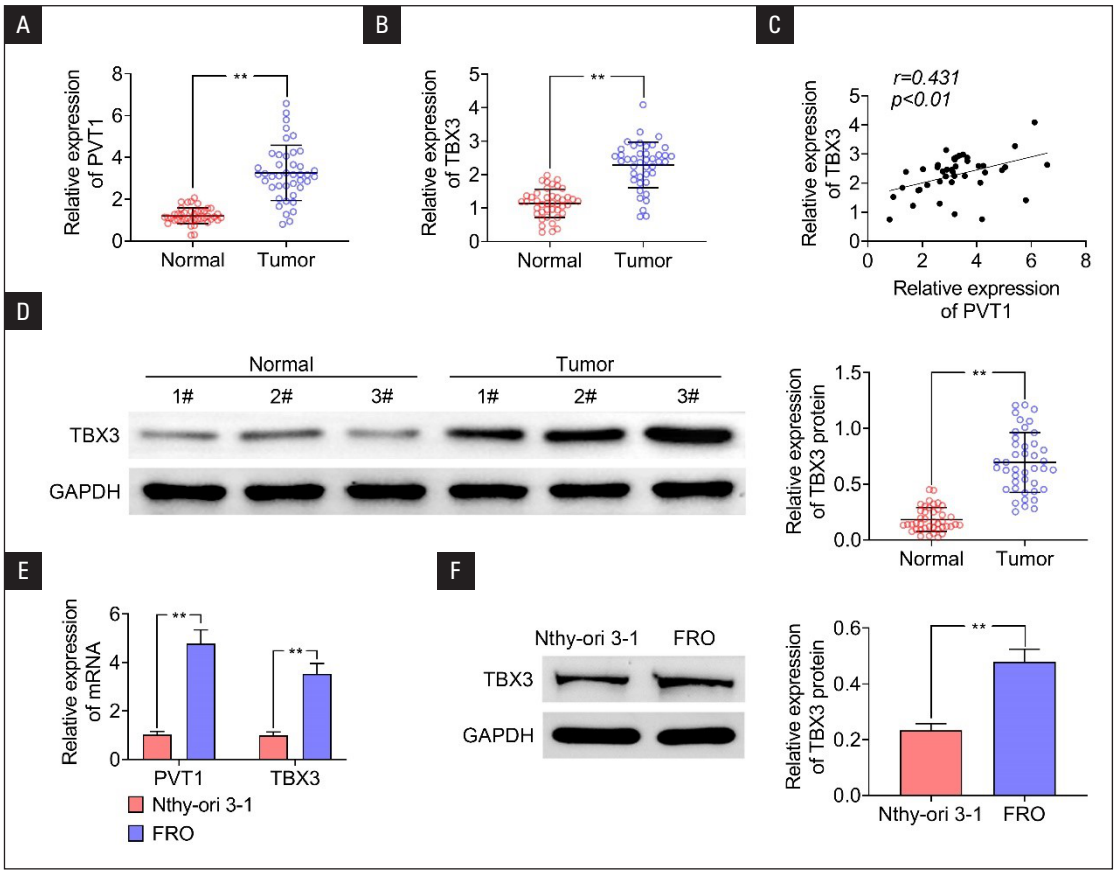

Figure 1. *The level of PVT1 was upregulated in anaplastic thyroid carcinoma (ATC) and positively associated with T-box3 (TBX3) level.* **AB.** *The relative mRNA expressions of both PVT1 (***A***) and TBX3 (***B***) in ATC tumour and adjacent normal tissues were examined by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). The data were shown after being normalized to GAPDH;* **C.** *The correlation analysis between PVT1 and TBX3 levels;* **D.** *The relative protein levels of TBX3 in ATC tumour and adjacent normal tissues were determined by western blot. The data were shown after being normalized to GAPDH;* **E.** *The relative mRNA expressions of both PVT1 (***A***) and TBX3 (***B***) in FRO and Nthy-ori 3-1 cells were examined by qRT-PCR. The data were shown after being normalized to GAPDH;* **F.** *The relative protein levels of TBX3 FRO and Nthy-ori 3-1 cells were measured by western blot. The data were shown after being normalized to GAPDH.* $^{**}p < 0.01$

tumour tissues was significantly higher than that in adjacent normal tissues (Fig. 1B). Expectedly, the level of PVT1 was positively correlated with the TBX3 level (Fig. 1C). Also, the relative protein levels of TBX3 in ATC tumour tissues were obviously enhanced relative to that in adjacent normal tissues (Fig. 1D). Furthermore, the relative mRNA expressions of both PVT1 and TBX3 in FRO cells were notably elevated compared with those in Nthy-ori 3-1 cells (Fig. 1E). Consistently, the relative protein levels of TBX3 in FRO cells were markedly augmented relative to that in adjacent normal tissues (Fig. 1f). Thus, these findings indicated that the level of PVT1 was upregulated in ATC and positively associated with the TBX3 level.

TBX3 modulated the expression of PVT1 via binding to its promoter region

To investigate the interaction between PVT1 and TBX3, we first used the JASPAR website to predict the binding sites between PVT1 and TBX3 (Fig. 2A). To mechanistically confirm the binding sites between PVT1 and TBX3, a ChIP experiment was conducted by hatching the DNA fragments with TBX3 antibody or IgG antibody. The results showed that TBX3 antibody but not IgG antibody gathered TBX3 promoter DNA fragments via PCR amplification analysis, which indicated that PVT1 assuredly targeted the TBX3 promoter (Fig. 2B). To further verify the binding sites between PVT1 and TBX3, the TBX3 overexpression vector or empty vector combined with the PVT1 promoter were co-transfected into FRO cells, and then the relative luciferase activity of PVT1 promoter was detected by the luciferase assay. The results revealed that TBX3 overexpression vector, but not empty vector, notably enhanced the relative luciferase activity, which further verified the binding sites between PVT1 and TBX3 (Fig. 2C). In addition, two siRNAs of TBX3 (si-TBX3 1# and si-TBX3 2#) were constructed and used to restrain the expression of TBX3, of which si-TBX3 1# was more effective, although both si-TBX3 1# and si-TBX3 2# visibly inhibited the relative protein expression of TBX3. Consequently, only si-TBX3 1# prominently reduced the relative mRNA expression of PVT1 compared with that in the control or si-NC group. Thus, si-TBX3 1# was also selected for subsequent assays. Taken together, these data illustrated that TBX3 modulated the expression of PVT1 via binding to its promoter region.

TBX3 targeting PVT1 modulated the miR-30a/LOX signalling axis

The role of TBX3/PVT1 complex was further investigated. Because one of the major functions of lncRNA is as a competing endogenous RNA (ceRNA) to target underlying miRNA finally regulating the expression of downstream genes, we measured the relative mRNA expression of miR-30a and LOX after FRO cells were trans-

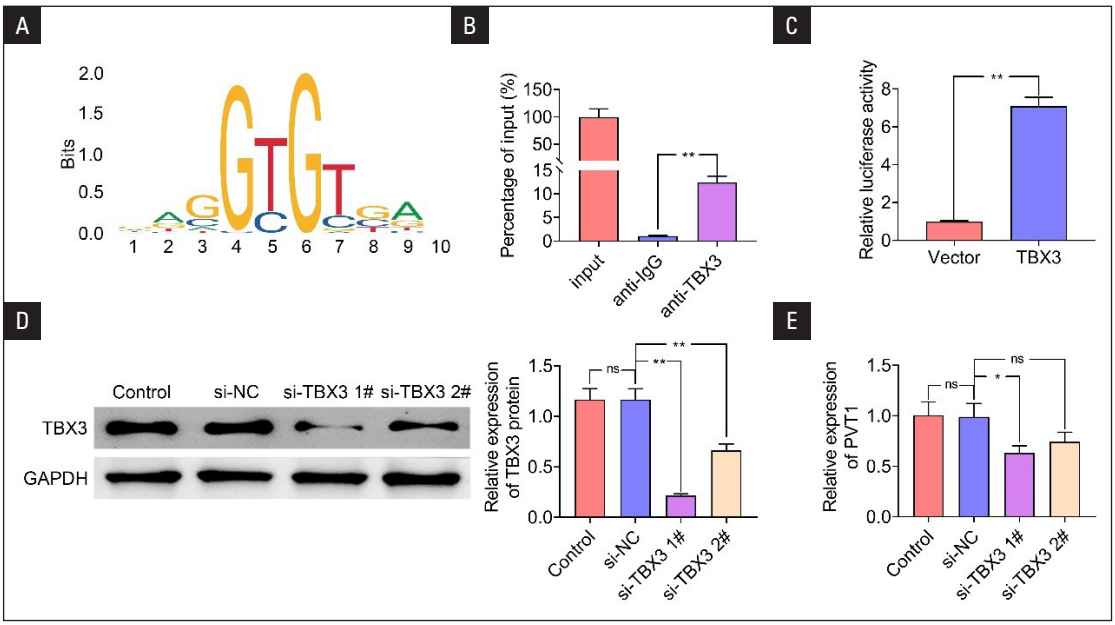

Figure 2. *T-box3 (TBX3) modulated the level of PVT1 via targeting its promoter region.* **A.** *The binding sites between PVT1 and TBX3 were forecasted by the JASPAR website;* **BC.** *The binding sites between PVT1 and TBX3 were confirmed by ChIP (***B***) and luciferase (***C***) assays, respectively;* **D.** *The relative protein level of TBX3 was detected by western blot after TBX3 levels were downregulated by si-RNAs. The data are shown after being normalized to GAPDH;* **E.** *The relative mRNA expression of PVT1 was examined by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) after TBX3 levels were interfered by si-RNAs. The data are shown after being normalized to GAPDH. *p < 0.05, **p < 0.01, and ns means no significance.*

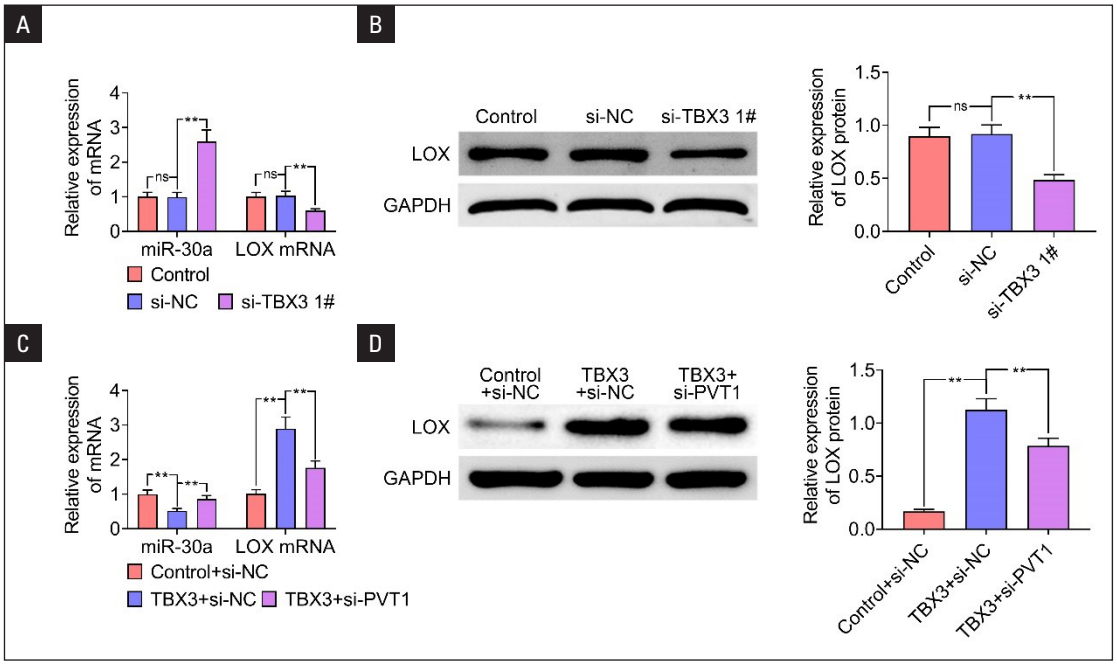

Figure 3. *T-box3 (TBX3) binding to PVT1 modulated the miR-30a/LOX signalling axis;* **A.** *The relative mRNA expressions of miR-30a and LOX were analysed by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) after TBX3 levels were interfered by si-RNAs. The data are shown after being normalized to GAPDH.* **B.** *The relative protein expression of LOX was measured by western blot after TBX3 levels were inhibited by si-RNAs. The data are shown after being normalized to GAPDH;* **C.** *The relative mRNA expressions of miR-30a and LOX were examined by qRT-PCR after FRO cells were transfected with TBX3 overexpressed plasmids or TBX3 overexpressed plasmids combined with si-PVT1. The data were shown after being normalized to GAPDH;* **D.** *The relative protein expression of LOX was determined by western blot after FRO cells were transfected with TBX3 overexpressed plasmids or TBX3 overexpressed plasmids combined with si-PVT1. The data are shown after being normalized to GAPDH.* $^{**}p < 0.01$, *and ns represents no significance*

fected with si-TBX3. The results showed that downregulation of TBX3 significantly enhanced and decreased the relative mRNA expressions of miR-30a and LOX compared with that in the si-NC group, respectively, while no statistical differences were discovered in the relative mRNA expression of miR-30a and LOX between the si-NC and control group (Fig. 3A). Interference of LOX also markedly reduced the relative protein level of LOX relative to that in the si-NC and control group (Fig. 3B). Additionally, overexpression of TBX3 significantly diminished the relative mRNA expression of miR-30a, but notably elevated the mRNA and protein expressions of LOX compared with those in the control group (Fig. 3C). However, knockdown of PVT1 reversed the changes of the relative mRNA expression of miR-30a and LOX as well as the relative protein expressions of LOX caused by upregulation of TBX3 (Fig. 3D). Therefore, these findings showed that TBX3 binding to PVT1 regulated the miR-30a/LOX signalling axis.

Downregulation of PVT1 repressed the proliferation, migration, and invasion of FRO cells

Repression of PVT1 markedly attenuated the cell viability (Fig. 4A) and numbers of colonies of FRO cells (Fig. 4B) relative to those in the si-NC and control group, with no statistical differences between the si-NC and control group, which indicates that suppression of PVT1 notably inhibited the proliferation of FRO cells. Also, transwell assays showed that knockdown of PVT1 visibly enhanced the wound width (Fig. 4C) but dramatically dampened the numbers of invasion cells relative to those in the si-NC and control group (Fig. 4D). Thus, all these findings clarify that attenuation of PVT1 restrained the proliferation, migration, and invasion of FRO cells.

Interference of PVT1 antagonized the promoted role of TBX3 in the progress of FRO cells

In addition, overexpression of TBX3 increased the cell viability (Fig. 5A) and numbers of colonies of FRO cells (Fig. 5B) relative to those in the control group, as expected, which suggests that the upregulation of TBX3 accelerated the proliferation of FRO cells. Overexpression of TBX3 memorably decreased the wound width (Fig. 5C), while notably enhancing the numbers of invasion cells (Fig. 5D) compared with those in the control group. However, downregulation of PVT1 significantly inverted the results of the cell viability, numbers of

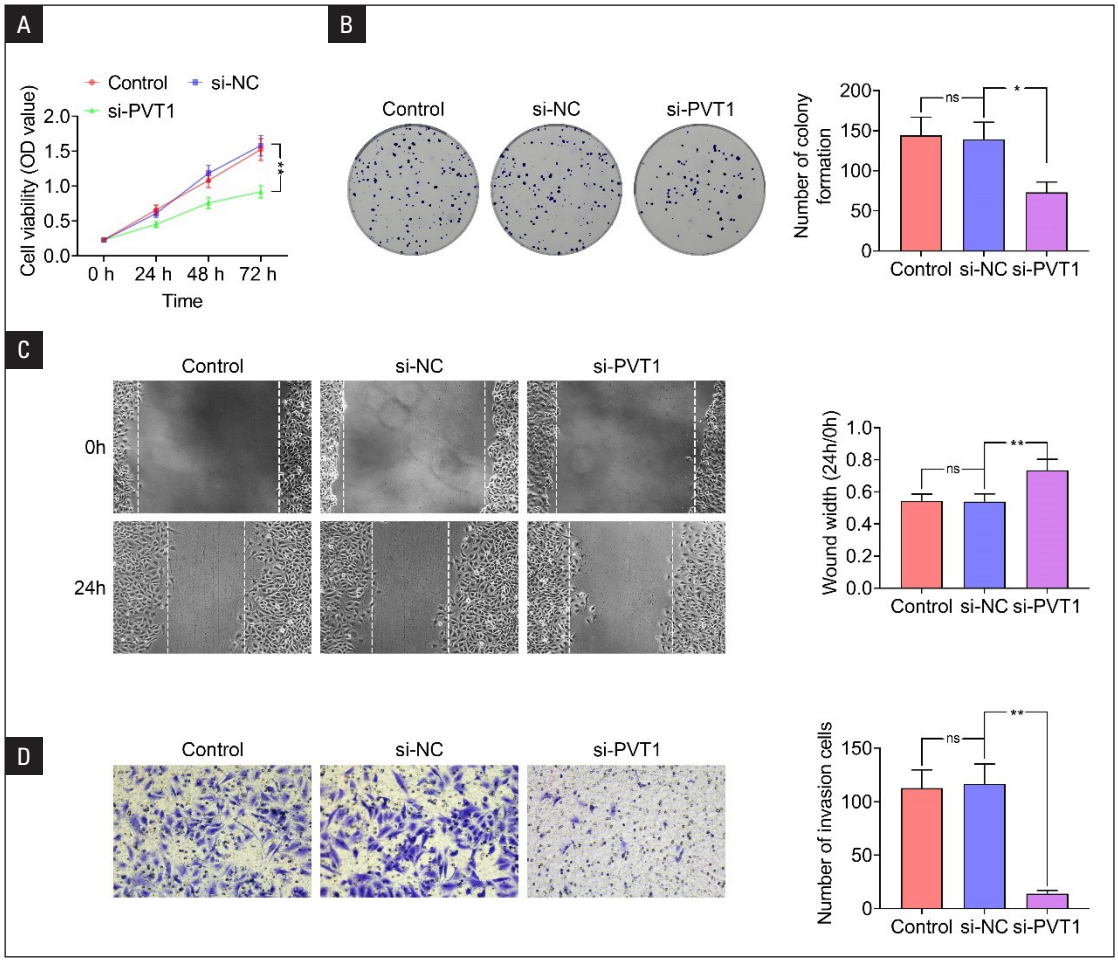

Figure 4. *Knockdown of PVT1 restrained the proliferation, migration, and invasion of FRO cells.* **A.** *The cell viability was assessed by MTT assay;* **B.** *The numbers of colonies of FRO cells were measured by the colony formation assay;* **CD.** *The ability of migration (***C***) and invasion (***D***) of FRO cells was determined by transwell assays. *p < 0.05, **p < 0.01, and ns represents no significance*

colonies, wound width, and numbers of invasion cells of FRO cells induced by upregulation of TBX3. Therefore, these results indicated that interference of PVT1 reversed the stimulative role of overexpression of TBX3 in the progress of FRO cells.

Discussion

ATC is derived from thyroid follicular cells, which are generally believed to be mutated from pre-existing differentiated carcinomas, such as papillary carcinoma, follicular carcinoma, or poorly differentiated carcinoma [18]. LncRNAs play an important role in the development and process of various malignancies; thus, a series of lncRNAs have been identified to be dysregulated in diverse types of tumours, including ATC [19, 20]. Here, we found that lncRNA PVT1 level was upregulated in ATC, which was positively correlated with the level of transcription factor TBX3. Knockdown of PVT1 restrained the proliferation, migration, and invasion of FRO cells. Moreover, TBX3 targeting the promoter region of PVT1 promoted the expression level of PVT1 and modulated one of the downstream signalling axes of PVT1, miR-30a/LOX. Also, interference of PVT1 reversed stimulative role of overexpression of TBX3 in the progress of FRO cells.

The expression of PVT1 has been demonstrated to be upregulated in various cancers, such as colorectal cancer [21], oesophageal carcinoma [22], and laryngeal squamous cell carcinoma [23]. Also, the level of PVT1 was upregulated in papillary thyroid carcinoma [24] and thyroid cancer [11, 25]. In line with these findings, our results showed that the level of PVT1 was notably enhanced in ATC tissues and FRO cells. Moreover, PVT1 regulated the progression of different types of tumours, including thyroid cancer. For instance, PVT1 enhances cell growth and invasion through upregulation of miRNA-214-3p in colorectal cancer [21]. PVT1 promoted the proliferation and invasion via modulation of the miRNA-30a/IGF1R signalling axis in papillary

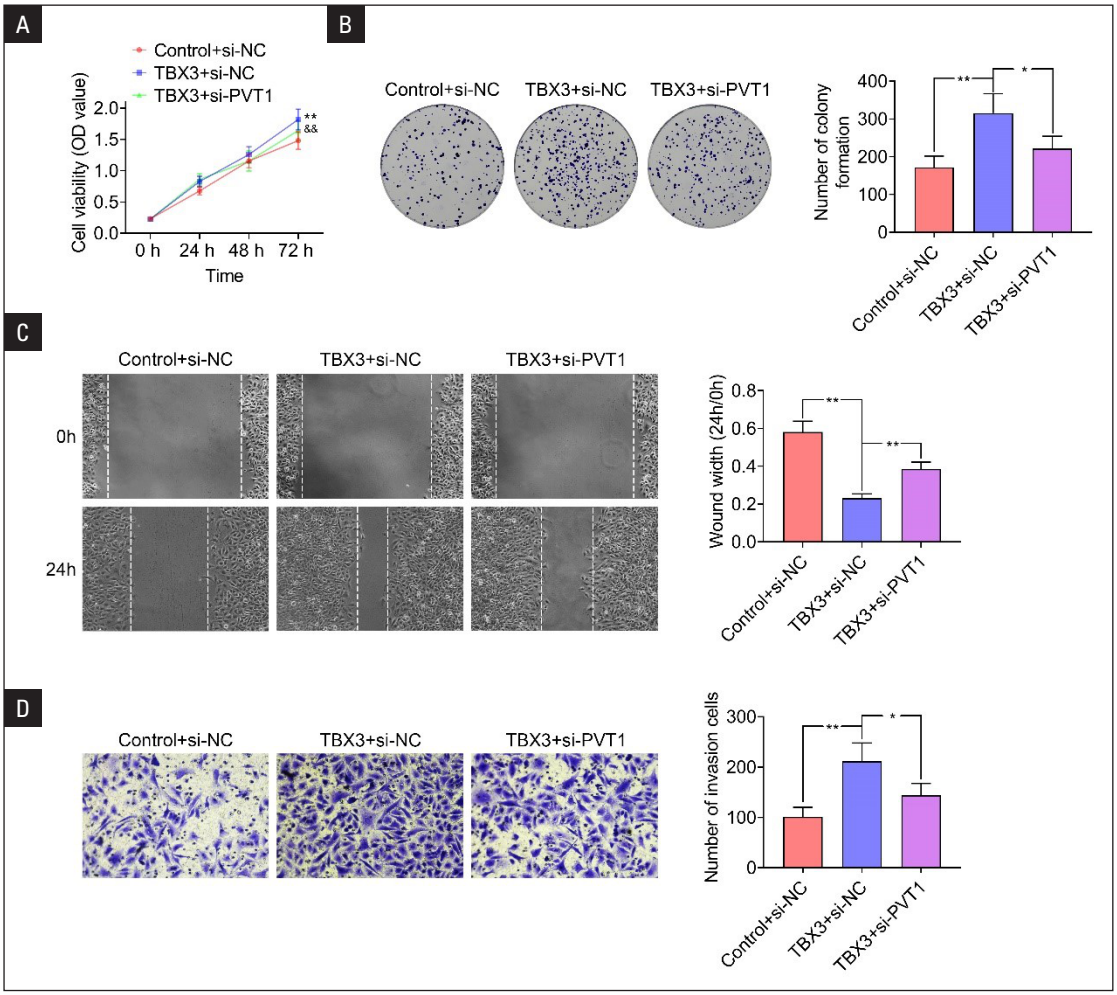

Figure 5. *Knockdown of PVT1 antagonized the stimulative role of overexpression of T-box3 (TBX3) in the progress of FRO cells.* **A.** *The cell viability was evaluated by MTT assay.* $^{**}p < 0.01$ *compared with Control+si-NC and* $^{\&\&}p < 0.01$ *compared with TBX3+si-NC. (b) The numbers of colonies of FRO cells were examined by the colony formation assay.* $^{*}p < 0.05$, *and* $^{**}p < 0.01$; **CD.** *The ability of migration (***C***) and invasion (***D***) of FRO cells was assessed by transwell assays.* $^{*}p < 0.05$, *and* $^{**}p < 0.01$

thyroid carcinoma cells [24]. PVT1 accelerated the proliferation and invasion but inhibited the apoptosis of thyroid cancer via the miR-423-5p/PAK3 signalling axis [25]. Our results consistently showed that repression of PVT1 markedly attenuated the cell viability, numbers of colonies, and numbers of invasion cells and increased the wound width of FRO cells, which suggests that knockdown of PVT1 suppressed the proliferation, migration, and invasion of FRO cells. Therefore, these findings suggest that upregulation of PVT1 modulated the progression of FRO cells.

A lot of research has shown that the level of lncRNA can be modulated by transcription factors via targeting the upstream promoter region [26–28]. Wang et al. showed the binding site between PVT1 promoter and the transcriptional factor SOX2, which was further confirmed by the ChIP assay [29]. In our study, the transcriptional factor binding site was predicted between TBX3 and PVT1 promoter by the JASPAR website, which was also verified via ChIP and luciferase analysis. Moreover, the expression of TBX3 was positively correlated with the level of PVT1, which was further confirmed by the result that knockdown of TBX3 visibly reduced the level of PVT1. Thus, these results indicate that transcription factor TBX3 promoted the transcriptional expression of PVT1.The dysregulation of TBX3 has been displayed in breast cancer [30], gastric cancer [31], hypopharyngeal carcinoma [32], and papillary thyroid carcinoma [16]. The present results also showed that the transcriptional and translational expressions of TBX3 were prominently highly expressed in both ATC tissues and FRO cells. One of the most important functions of lncRNA is as a ceRNA to target underlying miRNA, subsequently modulating the expression of downstream genes [33]. PVT1 functions as a ceRNA of miR-30a that has been demonstrated in papillary thyroid carcinoma [24] and gastric cancer [34]. Also, miR-30a targeting LOX has been shown in

previous studies [35, 36]. Thus, a PVT1/miR-30a/LOX signalling axis exists in different tumours. In the present study, knockdown of TBX3 elevated the relative mRNA expression of miR-30a, and reduced the relative mRNA and protein expression of LOX. Moreover, overexpression of TBX3 reduced the transcriptional level of miR-30a and enhanced the transcriptional and translational levels of LOX, which was antagonized by knockdown of PVT1. Taken together, these findings show that TBX3 regulated the transcriptional expression of PVT1, which then may modulate its downstream signalling axis miR-30a/LOX by targeting the promoter region of PVT1.

Transcription factors targeting lncRNAs can modulate the progress of diverse tumours. For example, the transcription factor USF1 activates the expression of lncRNA HAS2-AS1 that enhances the migration and invasion of glioma cells [26]. The transcription factor SOX2 facilitates the proliferation and invasion of breast cancer cells via activation of PVT1 [29]. In the present study, knockdown of PVT1 notably reversed the promoted results of the cell viability, numbers of colonies, wound width, and numbers of invasion cells of FRO cells induced by upregulation of TBX3, which indicated that interference of PVT1 antagonized the stimulative role of overexpression of TBX3 in the progress of FRO cells. Therefore, these findings indicate that TBX3 regulated the transcriptional expression of PVT1, which may then modulate its downstream signalling axis miR-30a/LOX by targeting the promoter region of PVT1, eventually affecting the progress of ATC.

In conclusion, the results in this study showed that TBX3 activating PVT1 promoted proliferation, migration, and invasion by regulating the miR-30a/LOX axis in ATC. We hope our findings can lay a foundation for the development of ATC therapy.

Conflict of interests

The authors state that there are no conflicts of interest to disclose.

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Not applicable.

Ethics approval

Ethical approval was obtained from the Ethics Committee of the Affiliated Hospital of Guizhou Medical University.

Informed consent

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

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