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Effect and Molecular Mechanism of MiR-181a-5p on the Proliferation and Invasion of Papillary Thyroid Cancer Cells

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Keywords

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Abstract

Objective: To investigate the effect and regulation mechanism of miR-181a-5p on papillary thyroid carcinoma (PTC) cells. **Methods:** The expressions of miR-181a-5p and thyrotropin receptor (TSHR) in PTC tissues, and the targeting relationship between these two genes were analyzed using bioinformatics tools, and their targeting relationship was verified by dual-luciferase reporter assay. After TPC-1 cells were transfected with miR-181a-5p inhibitor and shTSHR, the expression levels of miR-181a-5p, TSHR, E-cadherin, N-cadherin and Vimentin were detected by quantitative reverse transcription polymerase chain reaction or Western blot assay. The effects of miR-181a-5p and TSHR on the viability and invasion of TPC-1 cells were detected by MTT and Transwell assay. **Results:** The expression of miR-181a-5p was upregulated in PTC tissues and cells ($p < 0.001$), and the expression of TSHR was lowered in PC tissues. MiR-181a-5p could directly target TSHR. Downregulation of miR-181a-5p inhibited viability and invasion, and the expressions of N-cadherin and Vimentin ($p < 0.01$), but promoted the expression of E-cadherin in TPC-1 cells ($p < 0.001$). TSHR knockdown produced the opposite results ($p < 0.01$), and partially reversed the effect of miR-181a-5p downregulation on TPC-1 cells ($p < 0.01$). Conversely, down-regulation of miR-181a-5p could offset the effect of TSHR knockdown ($p < 0.05$). **Conclusion:** Downregulation of miR-181a-5p suppressed the malignant progression of PTC cells by promoting TSHR expression.

1 Introduction

Thyroid cancer is the most common malignant tumor in the endocrine system, and its incidence rate has increased annually in recent years [1,2]. Papillary thyroid cancer (PTC) accounts for about 80% of thyroid cancers [3]. Although some patients can have good prognosis through thyroidectomy, radioactive iodine therapy (RT), and thyroid stimulating hormone suppression, 20% and 10% of PTC patients still experience local recurrence and distant metastasis [3]. At present, there is still a lack of understanding on the pathogenesis of these more invasive PTC, and the treatment outcomes for these patients are suboptimal, with lower survival rates [4]. Therefore, it is necessary to explore the molecular mechanisms driving the high invasiveness of PTC and customize treatment strategies to improve prognosis.

Epithelial mesenchymal transition (EMT) is an evolutionarily conserved cellular process associated with the occurrence and development of cancer. It can enhance the vitality, invasion, and resistance to apoptosis of cancer cells, thereby endowing cells with metastatic properties [5]. Previous studies have confirmed that targeting EMT is an effective pathway for inhibiting PTC cell invasion and migration [6]. Therefore, studying the molecular mechanism of targeted EMT is of great significance for formulating effective strategies to inhibit the progression of PTC.

MicroRNA (miRNA; miR) is a type of endogenous non-coding RNA with a length of approximately 22 nucleotides [7]. MiRNA can interact with various target genes to alter various biological processes of cells (including EMT), thereby affecting the pathological processes of various diseases, such as cancer [8,9]. Previous findings have confirmed that the expression of miR-181a-5p is higher in PTC tissues than in normal tissues, and it can reduce the efficacy of RT by directly regulating the expressions of target genes [10], revealing the oncogenic role of miR-181a-5p in PTC. Existing reports have shown that miR-181a-5p, as an oncogene, can promote the proliferation, cell cycle progression, invasion, migration, and EMT of gastric cancer cells [11]; however, the specific regulatory mechanisms of miR-181a-5p in the progression of PTC still need to be explored.

MiRNAs negatively regulate gene transcription by base pairing with the 3′ untranslated regions (3′ UTRs) of protein-coding genes. The misexpression or dysfunction of miRNAs can interfere with the expressions of oncogenic or tumor-suppressive target genes, which is closely related to the pathogenesis of cancer [12]. The thyrotropin receptor (TSHR) is a G protein coupled receptor that is expressed in thyroid cells and binds to thyroid stimulating hormone, thereby stimulating the production and release of thyroid hormones to regulate thyroid function [13]. According to previous studies, TSHR expression is an independent factor affecting the prognosis of PTC patients. High expression of TSHR can promote apoptosis of PTC cells, inhibit their metastasis, and hinder the development of PTC [14]. In addition, TSHR can inhibit the EMT of cancer cells and the distant metastasis of PTC [15]. In this study, bioinformatics analysis data revealed that miR-181a-5p can bind to the 3′ UTR of TSHR. Therefore, we hypothesized that miR-181a-5p may mediate EMT via targeting TSHR, thereby influencing the progression of PTC. This study explored the regulatory effects of miR-181a-5p and TSHR on the malignant progression of PTC cells through cell functional and molecular biological experiments.

2 Materials and methods

2.1 Bioinformatics analysis

The expressions of miR-181a-5p and TSHR in thyroid cancer tissues were analyzed using the dbDEMC website (https://www.biosino.org/dbDEMC/index) and UALCAN website (https://ualcan.path.uab.edu/), respectively. The complementary binding sites

between miR-181a-5p and TSHR were obtained from the TargetScan website (https://www.targetscan.org/vert_80/).

2.2 Cell culture

Human PTC cells (TPC-1 cells) and normal human thyroid cells (Nthy-ori 3-1 cells) were purchased from Meisen Cell (CTCC-400-0084; CTCC-0407-Luc2, Hangzhou, China). TPC-1 and Nthy-ori 3-1 cells were cultured in RPMI-1640 medium (11875119, ThermoFisher, USA) containing 10% fetal bovine serum (FBS; 12106C, Sigma-Aldrich, St. Louis, Missouri, USA) and 1% penicillin/streptomycin (15140148, ThermoFisher, Waltham, Massachusetts, USA) in a humidified incubator with 5% CO_2 at 37 ℃.

2.3 Cell transfection

MiR-181a-5p inhibitor (I, miR20000256-1-5), mimics (M, miR10000256-1-5), and their controls (inhibitor/mimic control (IC/MC), miR2N0000001-1-5/miR1N0000001-1-5) were purchased from Ribobio (Guangzhou, China). Short hairpin RNAs targeting TSHR (shTSHR) and their negative controls (shNC) were obtained from OriGene (TR308613; TR20003, Rockville, Maryland, USA). TPC-1 cells were seeded in 96-well plates (1×10^4 cells/well). When the cells reached 80% confluence, they were transfected with Lipofectamine™ 3000 transfection reagent (L3000015, ThermoFisher, USA) according to the manufacturer's instructions. TPC-1 cells were transfected with M/MC (used only for dual-luciferase reporter assays), I/IC, IC + shNC, I + shNC, IC + shTSHR, or IC + shTSHR. After incubation for 48 h, the transfection efficiency was detected by quantitative reverse transcription polymerase chain reaction (qRT-PCR).

2.4 QRT-PCR

Total RNA was extracted from transfected/untransfected TPC-1 cells and normal Nthy-ori 3-1 cells using Trizol reagent, and reverse-transcribed into cDNA using SuperScript IV reverse transcriptase (18090010, ThermoFisher, USA). Subsequently, cDNA was amplified using PowerUp SYBR Green Master Mix (A25742, ThermoFisher, USA) and the primers listed in Table 1 in real-time fluorescence quantitative PCR detection system (CFX Connect, Bio-Rad, Philadelphia, Pennsylvania, USA). The amplification conditions were as follows: initial denaturation at 95 ℃ for 10 min, followed by 40 cycles of denaturation at 95 ℃ for 15 s, annealing at 95 ℃ for 15 s, and extension at 60 ℃ for 60 s. The relative expression levels of miR-181a-5p and TSHR were calculated using the $2^{-\Delta\Delta Ct}$ method [16], with U6 or GAPDH as an internal control.

Table 1 Primers of genes tested in quantitative reverse transcription polymerase chain reaction.

Genes	Speciesc	Forward (5′- -3′)	Reverse (5′- -3′)
miR-181a-5p	human	GAACATTCAACGCTGTCGGTG	ATCCAGTGCAGGGTCCGAGGTA
TSHR	human	GGAATGGGGTGTTCGTCTCC	GCGTTGAATATCCTTGCAGGT
GAPDH	human	GAGAAGGCTGGGGCTCATTT	AGTGATGGCATGGACTGTGG
U6	human	CTCGCTCGGCAGAACA	AACGCTTCACGAATTTGCGT

2.5 Dual-luciferase reporter assay

The wild-type sequence of the 3′ UTR of TSHR containing the binding site for miR-181a-5p was cloned into the pmirGLO vector (E1330, Promega, Madison, Wisconsin, USA), thus constructing the pmirGLO-TSHR-WT reporter plasmid. The mutant sequence of the TSHR 3′ UTR was generated using the GeneTailor Site-Directed Mutagenesis System (12397-014, Invitrogen, Carlsbad, California, USA) and cloned into the vector to obtain the pmirGLO-TSHR-MUT reporter plasmid. The reporter plasmids were co-transfected with MC/M into TPC-1

cells. After incubation for 48 h, the cells were collected and lysed, and the luciferase activity in the lysates was analyzed using the Dual-Luciferase Reporter Assay System (E1980, Promega, USA). The luciferase activity values were measured using a luminometer (GloMax® 20/20, Promega, USA). The firefly luciferase activity was normalized to the Renilla luciferase activity to assess the binding of miR-181a-5p to TSHR.

2.6 MTT assay

Transfected/untransfected TPC-1 cells were planted in 96-well plates (1×10^4 cells/well). After incubation for 24, 48, and 72 h, 10 μL of MTT solution (5 mg/mL, V900888, Sigma-Aldrich, St. Louis, Missouri, USA) was added to each well and incubated for an additional 4 h in the cell culture incubator. Next, 150 μL of dimethyl sulfoxide (D2650, Sigma-Aldrich, USA) was added to each well, mixed thoroughly, and incubated until all formazan crystals were dissolved. Finally, the optical density was measured at 570 nm using a microplate reader (ELx808, BioTek, Winooski, Vermont, USA).

2.7 Transwell assay

The density of transfected/untransfected TPC-1 cells was adjusted to 2×10^4 cells/mL in serum-free medium. The invasive ability of the cells was detected using Transwell chambers (3422, Corning, USA). Matrigel (356234, 50 μL, 1 mg/mL, Corning, New York, USA) was pre-coated on the bottom of the upper chamber of the Transwell insert with an 8 μm pore size polycarbonate membrane. 200 μL of cell suspension was added to the upper chamber, while 600 μL of medium containing 20% FBS was added to the lower chamber. The Transwell chambers were incubated at 37 ℃ for 48 h. Non-adherent cells in the upper chamber were removed with a cotton swab, and the adherent cells on the lower membrane surface were fixed with 4% paraformaldehyde (P6148, Sigma-Aldrich, USA) for 10 min. The cells were then stained with 0.5% crystal violet for 30 min. Finally, the invasive cells were counted under an inverted microscope at ×250 magnification.

2.8 Western blot

Total protein was extracted from transfected/untransfected TPC-1 cells using RIPA lysis buffer containing protease inhibitors (P1005, Beyotime, Shanghai, China) (V900854, Sigma-Aldrich, USA). The protein concentration was measured using a BCA assay kit (A53227, ThermoFisher, USA). The extracted proteins were separated by SDS-PAGE gel (1615100, BIO-RAD, Hercules, CA, USA) and then transferred to PVDF membranes (1620256, BIO-RAD, USA). The membranes were blocked with 5% bovine serum albumin (A801320, Macklin, Shanghai, China) at room temperature for 1 h, then incubated with primary antibodies against E-cadherin (ab40772, 97 kDa, 1:1000, Abcam, UK), N-cadherin (ab76011, 100 kDa, 1:5000, Abcam, UK), Vimentin (ab92547, 54 kDa, 1:1000, Abcam, UK), and GAPDH (ab8245, 36 kDa, 1:10000, Abcam, UK) overnight at 4 ℃. The membranes were washed three times with PBST buffer (28352, ThermoFisher, USA) and then cultured with secondary antibodies (goat anti-rabbit/mouse IgG, ab97051/ab205719, Abcam, UK) at room temperature for 2 h. Protein signals were detected using Clarity Western ECL substrate (1705060, BIO-RAD, USA) and visualized on a chemiluminescent imager (GE-ImageQuant LAS 4000 mini, Cytiva, Tokyo, Japan). Protein density was analyzed using ImageJ software (version 3.0, National Institutes of Health, Bethesda, Maryland, USA).

2.9 Statistical methods

Continuous data were described as mean ± standard deviation. Comparisons between two groups were performed using independent sample *t*-tests, while comparisons among multiple groups were conducted using One-way ANOVA. All statistical analyses were completed using GraphPad Prism 8.0 software. $p <$

0.05 was considered statistically significant.

3 Results

3.1 Expression of mir-181a-5p in human thyroid cancer tissues and cells and its effects on PTC cell vitality and invasion

Bioinformatics analysis based on the dbDEMC website revealed that the expression of miR-181a-5p was higher in human thyroid cancer tissues than in normal tissues (Figure 1A). Subsequently, we detected the expression of miR-181a-5p in TPC-1 cells and found it was significantly upregulated compared to that in Nthy-ori 3-1 cells (Figure 1B, $p < 0.001$). To elucidate the specific role of miR-181a-5p in thyroid cancer development, we transfected TPC-1 cells with I to reduce the expression of miR-181a-5p and then examined its effects on cell activity and invasion. QRT-PCR results showed that the expression of miR-181a-5p was evidently decreased in TPC-1 cells transfected with I (Figure 1C, $p < 0.001$). MTT assays indicated that cell viability was markedly diminished at 24, 48, and 72 h after transfection with I (Figure 1D, $p < 0.001$). Additionally, the Transwell assay results showed that miR-181a-5p downregulation remarkably inhibited the invasion of TPC-1 cells (Figure 1E-F, $p < 0.001$). After transfection with I, the expression level of the epithelial marker E-cadherin was greatly elevated, while those of the mesenchymal markers N-cadherin and Vimentin were significantly decreased in TPC-1 cells (Figure 1G-H, $p < 0.01$).

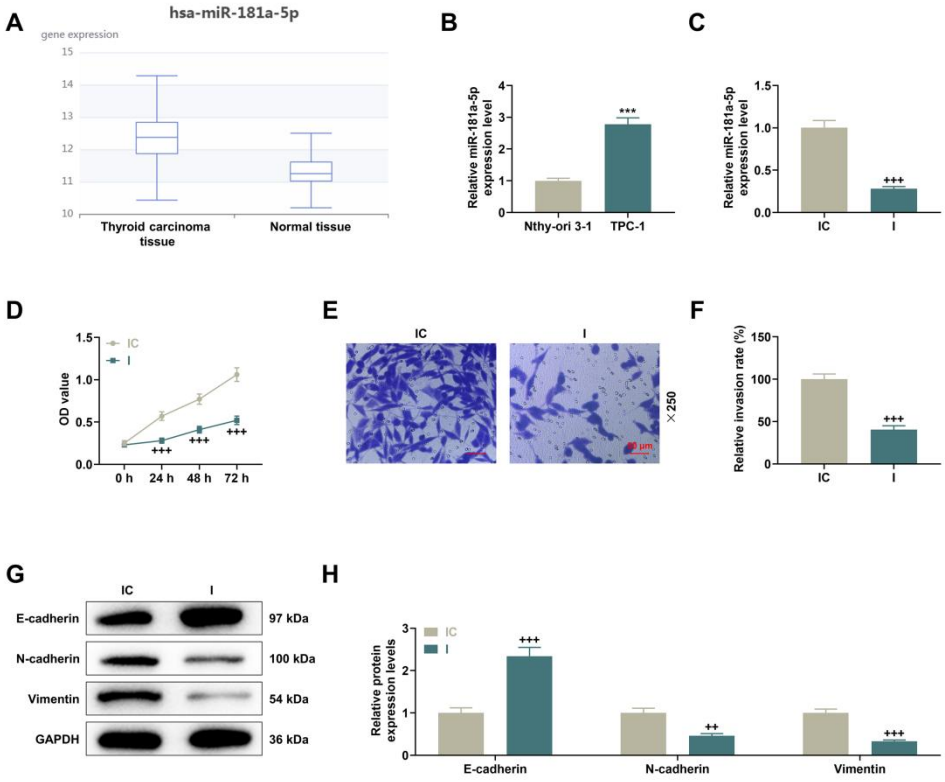

Figure 1 Expression of miR-181a-5p in human thyroid carcinoma tissues and cells and its effect on the viability and invasion of PTC cells. (A) The expression of miR-181a-5p in thyroid cancer tissues was analyzed in dbDEMC database. (B) The expression of miR-181a-5p in Nthy-ori 3-1 and TPC-1 cells was determined by qRT-PCR. (C) The transfection efficiency was determined by qRT-PCR after TPC-1 cells transfection with I or IC. (D) The regulation effect of I on the viability of TPC-1 cells was tested by MTT assay. (E-F) Transwell assay was used to test the effect of I on the invasion of TPC-1 cells. (G-H) The regulatory effect of I on the expressions of epithelial mesenchymal transition-related proteins (E-cadherin, N-cadherin and Vimentin) in TPC-1 cells was determined by

Western blot. $^{++}p < 0.01$; *** p or $^{+++}p < 0.001$; * vs. Nthy-ori 3-1; $^{+}$ vs. IC; (PTC: papillary thyroid carcinoma; qRT-PCR: quantitative reverse transcription polymerase chain reaction; I: miR-181a-5p inhibitor; IC: inhibitor control).

3.2 TSHR was lowly expressed in human thyroid cancer tissues, and knockdown of TSHR reversed the effect of miR-181a-5p downregulation on the viability of PTC cells

Bioinformatics analysis based on the UALCAN website unveiled that TSHR expression was lower in thyroid cancer samples (n = 505) than in normal tissue samples (n = 59) (Figure 2A). Using the TargetScan website, we identified the complementary binding sites between TSHR and miR-181a-5p (Figure 2B). Furthermore, we validated this interaction through dual-luciferase reporter assays. We co-transfected TPC-1 cells with MC or M along with pmirGLO-TSHR-WT or pmirGLO-TSHR-MUT reporter plasmids. Subsequently, the dual-luciferase reporter gene detection system was used for reporter gene detection. The results showed that transfection with M significantly reduced the luciferase activity of pmirGLO-TSHR-WT compared to MC, but had no effect on pmirGLO-TSHR-MUT (Figure 2C, $p < 0.001$). This indicated that miR-181a-5p can directly target TSHR.

To further investigate how miR-181a-5p affects thyroid cancer cells through TSHR, we transfected TPC-1 cells with shTSHR. The results revealed that shTSHR transfection successfully knocked down TSHR in TPC-1 cells (Figure 2D, $p < 0.001$). Additionally, shTSHR promoted viability in TPC-1 cells and reversed the inhibitory effect of I on cell viability (Figure 2E, $p < 0.05$).

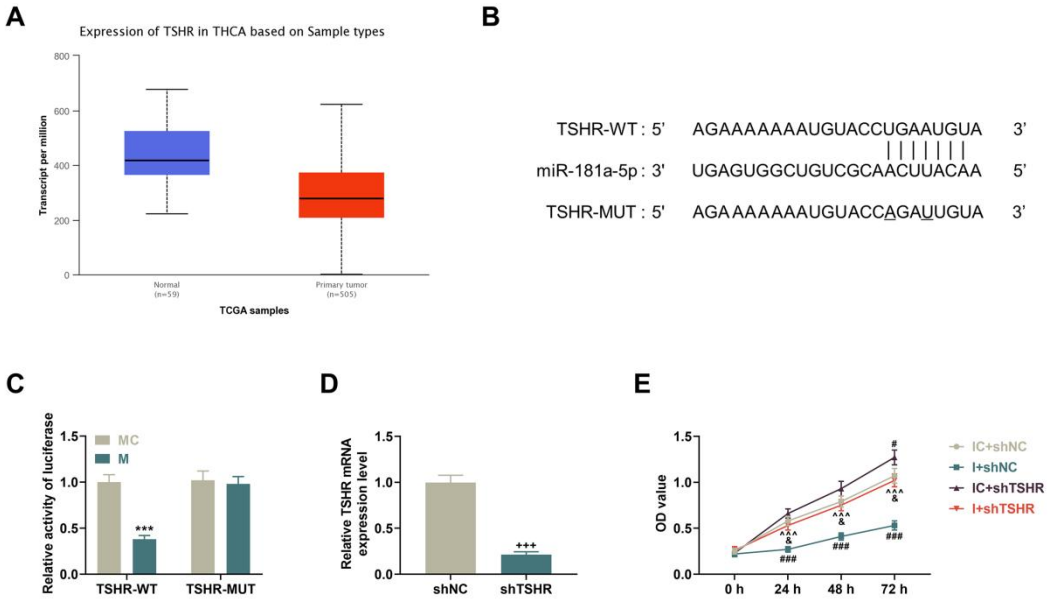

Figure 2 TSHR was lowly expressed in human thyroid cancer tissues, and knockdown of TSHR reversed the effect of miR-181a-5p downregulation on the viability of PTC cells. (A) The expression of TSHR in thyroid cancer tissues was analyzed in UALCAN database. (B) The complementary binding sites between miR-181a-5p and TSHR were analyzed using TargetScan. (C) The targeting relation between miR-181a-5p and TSHR was verified by dual-luciferase reporter gene assay. (D) TPC-1 cells were transfected with shTSHR or shNC, and the transfection efficiency was detected by qRT-PCR. (E) After TPC-1 cells were transfected with shTSHR or I, cell viability was detected by MTT assay. $^{\#}p$ or $^{\&}p$ <0.05; *** p or ^{+++}p or $^{\#\#\#}p$ or $^{\wedge\wedge\wedge}p$ <0.001; * vs. MC; $^{+}$ vs. shNC; $^{\#}$ vs.

IC+shNC; ^ vs. I+shNC; & vs. IC+shTSHR (PTC: Papillary thyroid cancer; qRT-PCR: quantitative reverse transcription polymerase chain reaction; I: miR-181a-5p inhibitor; IC: inhibitor control; TSHR, thyrotropin receptor; shTSHR: short hairpin RNA for TSHR; shNC: short hairpin RNA control).

3.3 MiR-181a-5p played a regulatory role in PTC cell invasion via mediating TSHR expression

Transwell assay results showed that downregulation of miR-181a-5p signally inhibited the invasion of TPC-1 cells, while shTSHR significantly enhanced the invasive ability of TPC-1 cells and partially reversed the inhibitory effect of miR-181a-5p knockdown on cell invasion (Figure 3A-B, $p < 0.01$). Next, Western blot analysis data further revealed that in TPC-1 cells transfected with shTSHR, the expression of E-cadherin was apparently decreased, while those of N-cadherin and Vimentin were significantly increased. Conversely, in TPC-1 cells transfected with I, the expressions of these proteins present opposite trends. Additionally, shTSHR partially offset the effects of miR-181a-5p downregulation on the expressions of E-cadherin, N-cadherin, and Vimentin (Figure 3C-D, $p < 0.001$).

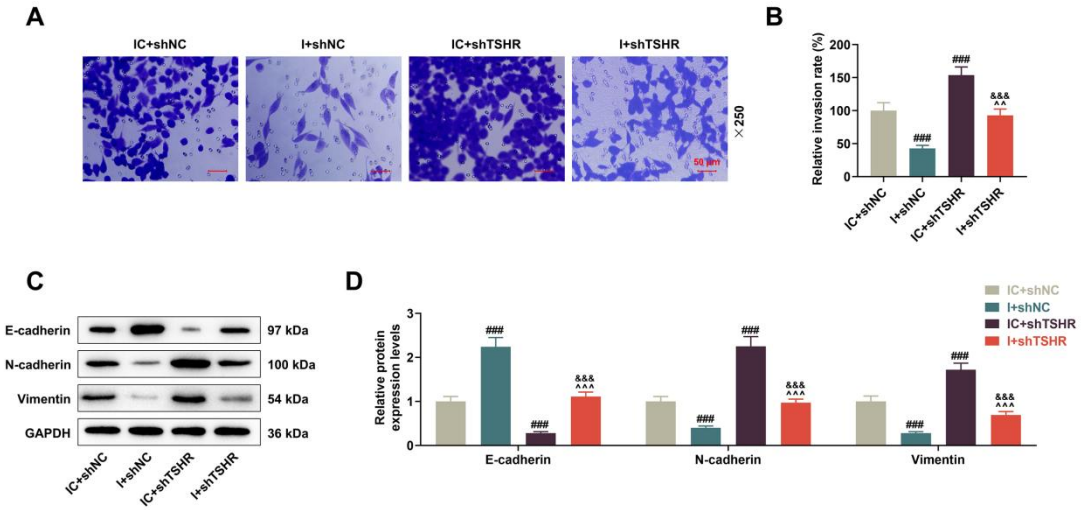

Figure 3 MiR-181a-5p plays a regulatory role in PTC cell invasion by regulating TSHR expression. (A-B) After TPC-1 cells were transfected with shTSHR and I, the invasion ability of TPC-1 cells was detected by Transwell assay. (C-D) After TPC-1 cells were transfected with shTSHR and I, the expression levels of epithelial mesenchymal transformation-related proteins (E-cadherin, N-cadherin and Vimantin) in TPC-1 cells were determined by Western blot. ^^ $p < 0.01$; ### p or ^^^ p or &&& p <0.001; # vs. IC+shNC; ^ vs. I+shNC; & vs. IC+shTSHR (PTC: Papillary thyroid cancer; I: miR-181a-5p inhibitor; IC: inhibitor control; TSHR, thyrotropin receptor; shTSHR: short hairpin RNA for TSHR; shNC: short hairpin RNA control).

4 Discussion

There are a small number of tumor variants in PTC that have stronger invasiveness, higher recurrence and metastasis rates, and in some cases lack affinity for RT, resulting in lower survival rates for PTC patients [4]. Therefore, exploring and diagnosing factors that can promote transformation of PTC into more malignant variants are of great significance for the treatment and prognosis of patients. MiRNA spectra and individual miRNA have shown certain importance in the diagnosis, development, treatment, and prognosis of PTC tumors [17]. This study found through in vitro experiments that downregulating miR-181a-5p can inhibit the EMT process of cancer cells via boosting the expression of TSHR, thereby preventing the malignant development of PTC cells.

PTC is mainly caused by BRAF mutations, RAS mutations, and RET/PTC rearrangements, which can

subsequently lead to refractory radioactive iodine resulting from activation of the mitogen activated protein kinase signaling pathway [3]. Radioactive iodine plays an important role in the treatment of PTC; however, some cases gradually begin to dedifferentiate under natural conditions or during treatment, losing the ability to ingest iodine and ultimately leading to resistance to RT. Previous studies have found that miR-181a-5p is highly expressed in PTC tissues and can reduce the efficacy of RT by binding to the 3' UTR of the sodium/iodide symporter SLC5A5, thereby decreasing radioiodine uptake [10], denoting that miR-181a-5p may act as an oncogenic factor in the development of PTC. Numerous studies have identified the oncogenic role of miR-181a-5p. For example, miR-181a-5p can promote the proliferation and invasion of breast cancer cells to facilitate tumor growth [19]; miR-181a-5p acts as an oncogenic miRNA that drives the EMT process, enhancing the invasiveness of prostate cancer cells and promoting tumor growth [20]; moreover, miR-181a-5p can boost the proliferation and invasion of cervical cancer cells while inhibiting apoptosis [21]; miR-181a-5p is highly expressed in gastric cancer tissues and is associated with lymph node invasion, neural invasion, and vascular invasion, promoting the proliferation of gastric cancer cells [22]. This study re-confirmed that miR-181a-5p was highly expressed in PTC cells and found that inhibiting its expression can reduce the activity and invasiveness of PTC cells, suggesting that miR-181a-5p knockdown can hinder the progression of PTC.

EMT is a pathological process that leads to tumor progression [23]. During the EMT process, the interactions between cells and between cells and the extracellular matrix are reshaped, which induces the separation of epithelial cells from each other and from the underlying basement membrane, and activates new transcription programs to promote the fate of mesenchymal phenotype evolution, thereby endowing cancer cells with the potential to increase tumor growth and metastasis [23]. E-cadherin is a classical cadherin and a typical marker of the epithelial cell state, maintaining cell-to-cell connections and apical-basal polarity. Its complete loss of expression triggers extensive cancer cell metastasis, and its expression is downregulated during EMT [25]. In contrast, N-cadherin is another classical cadherin that, together with the cytoplasmic intermediate filament protein Vimentin, defines the mesenchymal phenotype. The expressions of N-cadherin and Vimentin are elevated during EMT, which is associated with enhanced migration and invasiveness, and is the main reason for the decrease in survival rate of cancer patients [26,27]. Previous studies have found that miR-181a-5p can promote the malignant development of prostate cancer and advanced retinoblastoma by driving the EMT process [20,24]. In this study, after downregulating the expression of miR-181a-5p in PTC cells, we observed upregulation of E-cadherin and downregulation of N-cadherin and Vimentin, suggesting that miR-181a-5p knockdown can suppress the EMT process and thereby impede the progression of PTC.

MiRNA can exert a sponge-like effect to inhibit the expression of target genes, thus performing oncogenic or anticancer functions [12]. A large number of differentially expressed genes in PTC have been identified as target genes of miR-181a-5p [28]. Therefore, we further explored which target gene is regulated by miR-181a-5p to affect the progression of PTC. According to previous studies, TSHR expression was lowered in differentiated thyroid cancer, and the lack of TSHR expression is associated with distant metastasis and low survival rate. Upregulation of TSHR can inhibit the invasion and metastasis of thyroid cancer cells by repressing the EMT process [15]. Recent reports revealed that in PTC, TSHR preferentially couples with G-α proteins upon TSH binding, leading to the activation of adenylate cyclase

and increased cAMP levels [29]. cAMP further stimulates the signaling pathway of the upstream enhancer of SLC5A5, inducing SLC5A5 transcription [30]. Then, SLC5A5 actively transports iodine ions into cells to potentiate the therapeutic effect of RT and exert anticancer effects [31]. In addition, the CpG islands of gene promoters in healthy cells are usually protected from high methylation, and the methylation of TSHR promoters causes silencing of epigenetic level. The methylation level of TSHR is higher in PTC tissues compared to adjacent normal tissues [32], suggesting low expression of TSHR in PTC tissues. Also, high TSHR methylation levels are pertinent with larger tumors (>2 cm), lymph node metastasis, lymphovascular invasion, and multifocality, while TSHR methylation levels are decreased after anti-cancer treatment [32]. These studies revealed that TSHR acts as a tumor suppressor gene in PTC. Conversely, some reports have identified TSHR as an oncogenic factor in PTC, with high expression in 90.8% of PTC tissues, 78.2% of cervical lymph node metastasis cases, and 86.7% of radioiodine-resistant cases, and can serve as a potential target antigen for developing anti-TSHR CAR-T therapy for differentiated thyroid cancer, to treat patients with local-regional recurrence or distant metastasis [33]. In fact, there are studies proving that excessive activation of TSHR can lead to hyperfunction of follicular thyroid adenomas, but these tumors are rarely malignant, hinting that TSHR may have a dual function: preventing thyroid tissue from transforming into malignant tumors, or promoting carcinogenesis when activated by other carcinogenic factors [34]. Using bioinformatics analysis, we found that TSHR level was downregulated in PTC samples and confirmed that miR-181a-5p can directly target the 3′ UTR of TSHR. Since miRNA forms complementary binding with the 3′ UTR of mRNA, which leads to translation inhibition and/or mRNA degradation [12], we proposed that high expression of miR-181a-5p was one of the reasons for the low expression of TSHR in PTC. Through shTSHR transfection and cellular phenotype detection experiments, we obtained results similar to previous findings that TSHR acted as a tumor suppressor gene in PTC. We found that knocking down TSHR enhanced PTC cell activity, invasion, and the EMT process. Additionally, we discovered that knocking down TSHR in PTC cells can partially reverse the regulatory effects of miR-181a-5p downregulation on PTC cells.

In conclusion, we demonstrated that miR-181a-5p acts as an oncogenic miRNA in PTC, which can directly suppress the tumor suppressor gene TSHR. Downregulation of miR-181a-5p can inhibit PTC cell activity, invasion, and the EMT process via promoting TSHR expression, thereby suppressing the progression of PTC. Our findings provide new therapeutic targets for the treatment of PTC, but currently our research is limited to *in vitro* cell experiments and lacks animal model studies, which needs further research for verification in the future. In addition, the dearth of miR-181a-5p quantification in clinical tumor tissues is also a limitation of this study.

Acknowledgements

Not applicable.

Conflicts of Interest

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

J.H. conceptualized the trial with support from co-authors. W.H. participated in creating the study design. S.L. made the first draft of the manuscript. S.L. participated in creating the statistical analysis plan. All authors reviewed and revised the manuscript critically for important intellectual content. All authors reviewed

the final manuscript as submitted. All authors read and approved the final manuscript.

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Availability of Data and Materials

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

Supplementary Materials

Not applicable.

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