Expression of lncRNA-HOTAIR in the serum of patients with lymph node metastasis of papillary thyroid carcinoma and its impact

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Abstract. The present study aimed to investigate the expression of long non-coding HOX transcript antisense RNA (lncRNA-HOTAIR) in the serum of patients with lymph node metastasis of papillary thyroid carcinoma (PTC) and the underlying mechanism. A total of 89 patients with PTC at Beijing Geriatric Hospital were recruited in this study. Based on the results of color Doppler ultrasound examination, the patients were evaluated for cervical lymph node metastases, and were thereby divided into a metastasis-negative group and a metastasis-positive group. Quantitative fluorescent PCR was used to assess the expression of HOTAIR in serum samples. The PTC cell line TPC-1 was randomly divided into a control and siRNA group. The control group was transfected with a nonsense sequence, while the siRNA group was transfected with si-HOTAIR. After transfection, cell proliferation was evaluated using the MTT assay, and cell migration and invasion were assessed using the cell scratch assay and Transwell assay. Expression levels of vimentin, E-cadherin and proteins associated with the Wnt/β-catenin signaling pathway were assessed using western blot analysis. Based on the results of the ultrasound examination, 53 patients were allocated to the metastasis-negative group, and 36 to the metastasis-positive group. The expression level of lncRNA-HOTAIR was higher in the metastasis-positive group than that in the metastasis-negative group ($P<0.05$). Compared with the control group, cell proliferation was reduced while cell migration rate and the number of migrating cells were increased in the siRNA group. Compared with the control group, the expression levels of WIF1 and E-cadherin were significantly increased, while the levels of β-catenin and vimentin were significantly decreased. In conclusion, lncRNA-HOTAIR is overexpressed in the serum of patients with lymph node metastasis of PTC. *In vitro* experiments showed that HOTAIR promoted the proliferation and metastasis of PTC cells by regulating epithelial-mesenchymal transition (EMT) mediated by the Wnt/catenin pathway. Thus, lncRNA-HOTAIR is proposed as a molecular target for the treatment of lymph node metastasis of PTC.

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Introduction

Papillary thyroid carcinoma (PTC) is the most common pathological type of thyroid carcinoma, accounting for approximately 70-80% of all cases diagnosed (1,2). Thyroid carcinomas occur mostly in women with an incidence of 1/100,000 individuals, and this number is still rising (3). A consensus has not yet been reached in terms of the pathogenesis of PTC. Studies have shown that the occurrence of PTC may be associated with life style, genetic factors, ionizing radiation exposure, iodine intake, endocrine imbalance and obesity (4-6). Some researchers believed that a variety of oncogenes, as well as tumor-suppressor genes, are involved in the progression of PTC (7). In recent years, it was found that long non-coding RNAs (lncRNAs) are abnormally expressed in cancers, suggesting that lncRNAs may be involved in cancer onset and progression. HOX transcript antisense RNA (HOTAIR) is the first example of an lncRNA that has *trans*-regulatory function (8). According to the literature, HOTAIR is closely associated with the onset of cancers such as breast cancer, liver cancer, colorectal cancer, laryngeal cancer and nasopharyngeal carcinoma (9-13). Zhu *et al* reported that HOTAIR single nucleotide polymorphisms (SNPs) are associated with the risk of PTC, and one of the SNPs was found to be a variant susceptible to PTC, which was identified only in women (14). In another study conducted through database analysis, Li *et al* reported that HOTAIR is overexpressed in PTC tissues, and patients with higher HOTAIR expression exhibit poorer prognosis in general (15).

PTC is a type of thyroid cancer with a relatively good patient prognosis. Cervical lymph node metastases often occur at the early stage of PTC, presenting in 20-50% of all PTC patients (16). Diagnosis of lymph node metastasis often depends

upon postoperative pathological tests due to the limitations of ultrasonography, resulting in a lack of specificity (17,18). Lack of accurate preoperative diagnosis of lymph node metastasis makes it difficult to decide whether a lymph node dissection is needed and where the dissection area is located. In the present study, PTC patients were divided into a metastasis-negative group and a metastasis-positive group based on ultrasound findings. The expression level of lncRNA-HOTAIR in serum was determined, and its role in regulating the PTC cell line TPC-1 was explored, to reveal the possible pathogenesis of PTC with lymph node metastasis.

Materials and methods

Reagents. The following reagents were purchased from commercial sources: RPMI-1640 medium and fetal bovine serum (FBS) from Gibco; Thermo Fisher Scientific, Inc. TRIzol reagent, Lipofectamine 2000 reagent and Opti-MEM medium were purchased from Invitrogen; Thermo Fisher Scientific, Inc. Fluorescent dyes for quantitative PCR were obtained from Bio-Rad Laboratories, Inc. A reverse transcription kit was obtained from Toyobo Co., Ltd. and a BCA kit (cat. no. P0009) was from Beyotime Biotechnology. Monoclonal antibodies for E-cadherin (cat. no. 3195), vimentin (cat. no. 5741), β-catenin (cat. no. 8480), and Wnt inhibitory factor 1 (WIF1, cat. no. 2064) were purchased from Cell Signaling Technology, Inc. Internal reference β-actin antibody (cat. no. 20536-1-AP) and horseradish peroxidase (HRP)-labeled goat anti-rabbit secondary antibody (cat. no. SA00001-2) were from Proteintech Group, Inc. and the siRNA was obtained from RiboBio.

Subjects. A total of 89 patients with PTC who were admitted to Beijing Geriatric Hospital from February 2018 to March 2019 were recruited in this study. Patients who met the following criteria were eligible for this study: i) patients who were diagnosed with PTC regardless of cervical lymph node metastasis through preoperative color Doppler ultrasound examination; ii) patients who had surgical indications; iii) patients who were confirmed to have PTC by postoperative pathological tests. Patients who presented with the following criteria were excluded from this study: i) patients who had other conditions such as cardiovascular and cerebrovascular diseases, endocrine diseases, genetic diseases, and other cancers; and ii) patients who had previous history of chemotherapy, radiotherapy and surgery. All patients were informed of the study and signed informed consent forms. This study was approved by the Medical Ethics Committee of Beijing Geriatric Hospital (Beijing, China). Peripheral blood was collected before surgery, and thyroid cancer tissues were collected after surgery for pathological examination to confirm the diagnosis of PTC.

Ultrasonography. Color Doppler ultrasonography was performed before surgery on a GE Logiq P5 ultrasound machine equipped with a 7.5-10 MHz frequency probe (General Electric Co.). The patient was placed in the supine position with the neck fully exposed. The sonographers were doctors who had been engaged in color Doppler ultrasound examination for more than 5 years and had extensive experience in thyroid ultrasound examination. Lymph nodes in areas covering the bilateral thyroid gland, isthmus and cervix were carefully checked for size, morphology, location, internal echo, blood flow and calcification. After evaluating the sonographic findings, those lymph nodes presenting two or more of the following ultrasound signs were regarded as metastasis-positive (18): i) longest diameter of the central lymph node >5 mm and longest diameter of the lateral lymph node >8 mm; ii) longitudinal nodal diameter to transverse diameter ratio (L/T) <2; iii) eccentric or absent hilum; and iv) hyperechoic mass with hypoechoic rim accompanied by cystic formation and calcification. The subjects were divided into a metastasis-positive group and a metastasis-negative group based on the ultrasonographic findings and metastatic assessment. There were 36 patients allocated to the metastasis-positive group with a mean age of 47.8±4.9 years. There were 53 patients allocated to the metastasis-negative group with a mean age of 48.3±5.8 years.

Whole blood total RNA extraction and quantitative fluorescent PCR. Whole blood total RNA was extracted using the RNAprep Pure high-efficiency blood total RNA extraction kit (cat. no. DP443, Tiangen Biotech) in accordance with the kit user manual. The obtained RNA concentration was measured using a spectrophotometer. Complementary DNA (cDNA) was synthesized from 1 μg of RNA via reverse transcription in accordance with the manual provided in the reverse transcription kit. *U6* was used as the internal reference gene. The primer sequences were as follows: U6 forward, GACAAG CCCTACCTACAG, and U6 reverse, GATGATACAGTACAA GTCGC; HOTAIR forward, GGAGTGAGTCCCATCCAT CT, and HOTAIR reverse, TGCCCAATGGTACTACAGAAG AT. The PCR amplification reaction was performed as follows: Pre-denaturation at 95˚C for 5 min, 40 cycles of 95˚C for 5 sec (denaturation), 60˚C for 30 sec (annealing), and 72˚C for 30 sec (extension), followed by extension at 72˚C for 5 min at the end of the cycles. All samples were run in triplicate. The relative expression level of the target gene was calculated using the $2^{-\Delta\Delta Ct}$ method (19), where $\Delta Ct=Ct_{\text{target gene}}-Ct_{\text{internal reference}}$ and $\Delta\Delta Ct=\Delta Ct_{\text{experimental group}}-\Delta Ct_{\text{control group}}$.

PTC cell culture. The PTC cell lines TPC-1, HTori-3 and FTC-133 were purchased from the US ATCC Cell Bank. The cells were cultured in RPMI-1640 medium supplemented with 10% FBS in a humidified incubator supplied with 5% CO_2 and maintained at 37˚C. The medium was changed every other day, and the cells were passaged at about 80-90% confluency.

HOTAIR knockdown using siRNA. Cells were digested with 0.25% trypsin at about 80-90% confluency. The cells were seeded in 6-well plates at $3x10^5$ cells/well, and randomly divided into a control group, an siRNA group and an siRNA+LiCl group. Cell transfection was performed when the cells were approximately 30-50% confluent. Cells in the control group were transfected with a nonsense sequence, and cells in the siRNA group were transfected with the siHOTAIR sequence. The transfection protocol is briefly described below. Ten microliters of siRNA (10 nM) was added to 250 μl of Opti-MEM medium. Lipofectamine 2000 (5 μl) was added to another 250 μl of Opti-MEM medium. After incubation at room temperature for 5 min, the two solutions were combined, mixed and incubated for another 5 min. This transfection

mixture was added to the cells in the 6-well plates. After 4-6 h of incubation, the medium was replaced with complete medium. The group with LiCl was incubated with 20 mM LiCl and siRNA for 24 h. LiCl can activate the Wnt/β-catenin pathway and subsequent experiments were carried out 72 h after transfection. The forward and reverse sequences of the nonsense sequence were 5'-UUCUCCGAACGUGUCACG UTT-3' and 5'-ACGUGACACGUUCGGAGAATT-3', respectively. The forward and reverse sequences of the siHOTAIR sequence were: 5'-GCACAGAGAAAUGGCAAAUU-3' and 5'-UUUGCCAUUAUCUCUGUGCUU-3', respectively.

MTT assay. The transfected TPC-1 cells were digested and seeded in a 96-well plate at $3x10^3$ cells/200 μl/well. Six replicate wells were set for each group. The cells were cultured for 5 consecutive days, during which cell proliferation was measured each day in accordance with a protocol described below. Twenty microliters of MTT (5 mg/ml) was added to each well, followed by incubation at 37˚C for 4 h. The medium was aspirated carefully and replaced with 150 μl of DMSO. The plate was shaken in the dark for 10 min at room temperature, followed by measurement of the absorbance in each well at a wavelength of 450 nm using a microplate reader. A cell proliferation curve was plotted using days as the abscissa and the absorbance at 450 nm as the ordinate.

Cell scratch assay. TPC-1 cells were seeded in 6-well plates at $3x10^5$ cells/well. When the cells were 100% confluent, the medium was removed and replaced with serum-free medium. After starvation for 24 h, at least three even scratches were created in the bottom of each well using a pipette tip in a perpendicular way. The plate was rinsed three times with PBS to remove detached cells, followed by adding serum-free medium and incubation for 24 h. Images were captured at 0 and 24 h under an inverted microscope at 10x10 times to measure the widths of the scratches. Cell migration rate=(Scratch width at 0 h-Scratch width at 24 h)/Scratch width at 0 h.

Transwell assay. TPC-1 cells were seeded in Transwell chambers with an 8.0-μm pore membrane at $1x10^4$ cells/300 μl/well. When the cells were attached, the medium was switched to serum-free medium. After starvation for 24 h, the Transwell chambers were inserted into a 24-well plate containing 600 μl serum-free medium per well. After incubation for 24 h, a cotton swab was used to gently rub the area inside the chamber to remove non-migrated cells. The migrated cells were fixed by immersing the chambers in 4% paraformaldehyde for 10 min. The fixed cells were stained with 0.1% crystal violet, followed by washing under running water to rinse off the excessive dye and drying at room temperature. Images were captured and the migrated cells were counted under an inverted microscope at 10x40 times.

Western blot assay. After digestion, the cells were centrifuged at 3,445 x g for 5 min, followed by removal of the supernatant. Two hundred microliters of RIPA lysis buffer and 4 μl of protease inhibitor were added to the cell pellet, followed by sonication on ice for 5 min. After complete lysis, the mixture was centrifuged at 12,000 x g for 15 min in a low temperature centrifuge. The supernatant was collected, and 10 μl was used for total protein concentration measurement using the BCA assay. Loading buffer 5X was added to the remaining supernatant, followed by boiling at 100˚C for 10 min. Equal amounts of 30 μg total proteins from the different samples were loaded on gels containing 5% stacking gel and 10% separation gel for electrophoretic analysis. The gels were run at a constant voltage of 80 V until bromophenol blue entered the stacking gel with minimal distortion of the bands, when the voltage was changed to 120 V until the target bands were separated. The protein bands were transferred from gel to PVDF membranes by a wet transfer method under a constant current of 275 mA for 80 min. After the membrane was blocked with 5% milk at room temperature for 2 h, the corresponding diluted primary antibody (dilution factor, 1:1,000) was added, including antibodies for E-cadherin, vimentin, β-catenin and Wnt inhibitory factor 1 (WIF1). After incubation at 4˚C overnight and subsequent washing, the HRP-labeled goat anti-rabbit secondary antibody supplemented with 2% milk (dilution factor: 1:10,000) was added, followed by incubation at room temperature for 1 h. After development, the images were analyzed using Image J software (1.52a) (National Institutes of Health, Bethesda, MD, USA) for gray values of the bands. β-actin was used as an internal reference. The ratio of the gray value of the target protein to the gray value of β-actin was regarded as the expression level of that protein.

Statistical analysis. SPSS 23 software (IBM Corp.) was used for statistical analysis. Each test was repeated more than three times. Data are expressed as mean ± standard deviation. The unpaired t-test was used for PCR. Other data were firstly analyzed by one-way ANOVA to determine the overall difference, and then the Bonferroni pairwise comparison was performed. $P<0.05$ was indicative of a statistically significant difference.

Results

Serum expression of lncRNA-HOTAIR in lymph node metastasis-positive patients. Ultrasonograms of PTC nodules and normal thyroid nodules are shown in Fig. 1A.

Serum levels of lncRNA-HOTAIR in both metastasis-positive and metastasis-negative PTC patients were determined. As shown in Fig. 1B, the relative expression level of lncRNA-HOTAIR in the lymph node metastasis-positive group was 1.01±0.52 and 0.74±0.65 in the metastasis-negative group, The difference was statistically significant, P=0.0399. The relative expression of lncRNA-HOTAIR in lymph node metastasis-positive PTC tumor tissues was significantly higher than that in the PTC paracancerous tissues (0.69±0.73), HOTAIR was significantly overexpressed in PTC tissues (P=0.038) and the difference was statistically significant as shown in Fig. 1C.

Modulating effect of HOTAIR on PTC cell proliferation. We assessed HOTAIR levels in the TPC-1, HTori-3 and FTC-133 cell lines and found that the HOTAIR level in the TPC-1 cell line was the highest (Fig. 2A), which was then selected for subsequent experiments. After knockdown of lncRNA-HOTAIR expression with small interfering RNA, the expression of HOTAIR in the transfected TPC-1 cells was

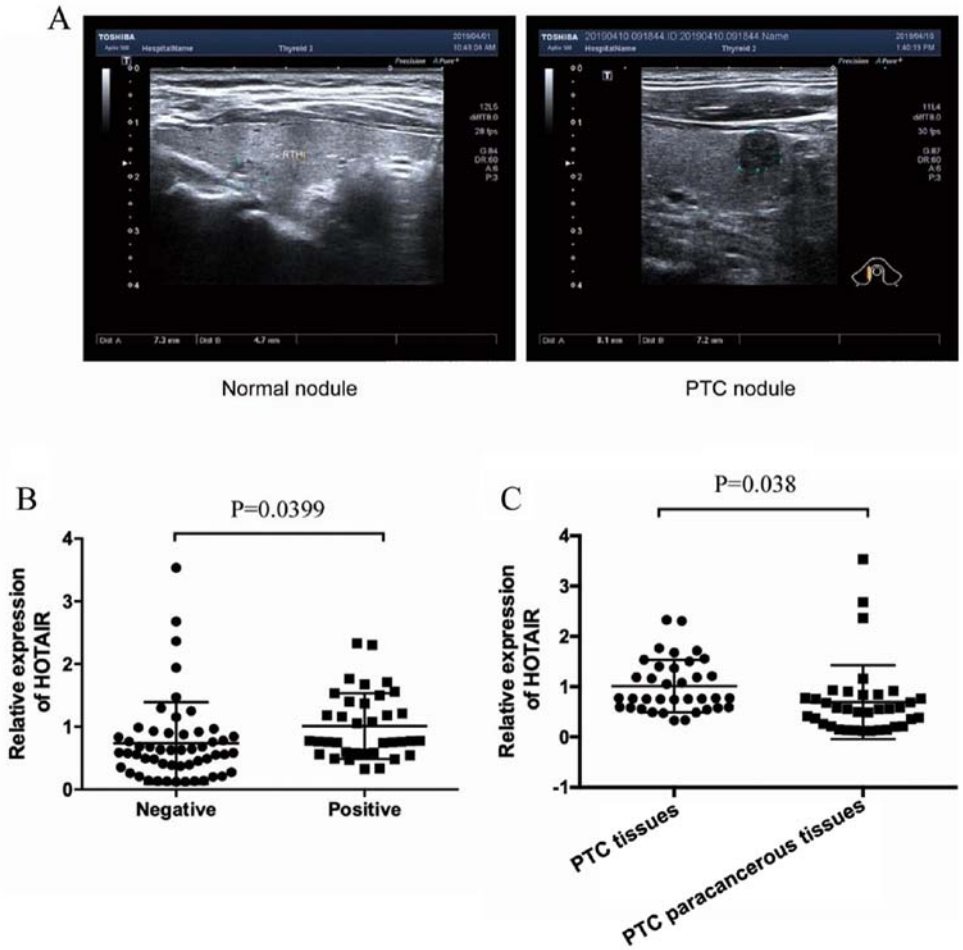

Figure 1. Ultrasonogram of PTC and HOTAIR expression in PTC patients. (A) Ultrasonograms of benign thyroid nodules and PTC nodules. (B) Relative expression levels of the HOTAIR gene in lymph node metastasis-positive and metastasis-negative patients. (C) Expression of HOTAIR gene in lymph node metastasis-positive PTC tissues and PTC paracancerous tissues.

decreased by 3.45 times (Fig. 2B). The difference was statistically significant (P=0.0041). The MTT assay showed that after siRNA interference on TPC-1 cells, TPC-1 cell proliferation was significantly slower than that of the control group, but the difference did not reach a statistical difference (Fig. 2C).

Effect of HOTAIR on PTC cell migration. Cell migration rates in the siRNA group, the control group and the siRNA+LiCl group were 20.11±5.46, 28.95±5.23 and 26.96±3.20%, respectively, as measured by a scratch assay indicating that the migration rate in the siRNA group was significantly lower than that in the control group (P=0.004). The migration rate in the siRNA group was significantly lower than that in the siRNA+LiCl group (P=0.021). Migrated cell numbers in the siRNA group, the control group and siRNA+LiCl group were 157.86±25.99, 249.42±39.79 and 228.00±36.11, respectively, as measured by the Transwell assay. The siRNA group was significantly lower than that in the control group, P<0.001. The siRNA group was significantly lower than that in the siRNA+LiCl group, P=0.002 (Fig. 2D-G).

Knockdown of HOTAIR and its impact on the Wnt/β-catenin signaling pathway. Compared with the control group, knockdown of HOTAIR significantly increased the relative expression of WIF1 (0.54±0.08, P=0.018), while knockdown of HOTAIR significantly decreased the expression of β-catenin (0.43±0.09, P=0.0318) (Fig. 3A and C). The relative expression levels of E-cadherin and vimentin were 0.53±0.12 and 1.12±0.23, respectively, in the control groups, while the levels of the two proteins were 1.00±0.23 and 0.75±0.09, respectively, in the siRNA groups. Apparently, HOTAIR knockdown increased the expression of E-cadherin (P=0.0123), but decreased the expression of vimentin (P=0.0088) (Fig. 3B and D). In order to further confirm that HOTAIR affects the proliferation and migration of PTC cells through the Wnt/β-catenin pathway, we added the agonist (LiCl) of Wnt/β-catenin after siRNA interference, and found that the proliferation and migration of PTC cells were increased compared with that of the siRNA group, almost similar to that of the control cell group (Fig. 2C-G).

Discussion

Thyroid cancer is one of the most common endocrine malignancies in the world, and papillary thyroid carcinoma (PTC) has the highest incidence (1). Despite multiple treatment strategies for PTC, 30% of patients with PTC show a high tendency for lymph node metastasis and recurrence within 10 years (19). Long non-coding RNA HOX transcript antisense RNA (lncRNA-HOTAIR) has been reported to promote PTC proliferation, invasion and migration, while high levels of

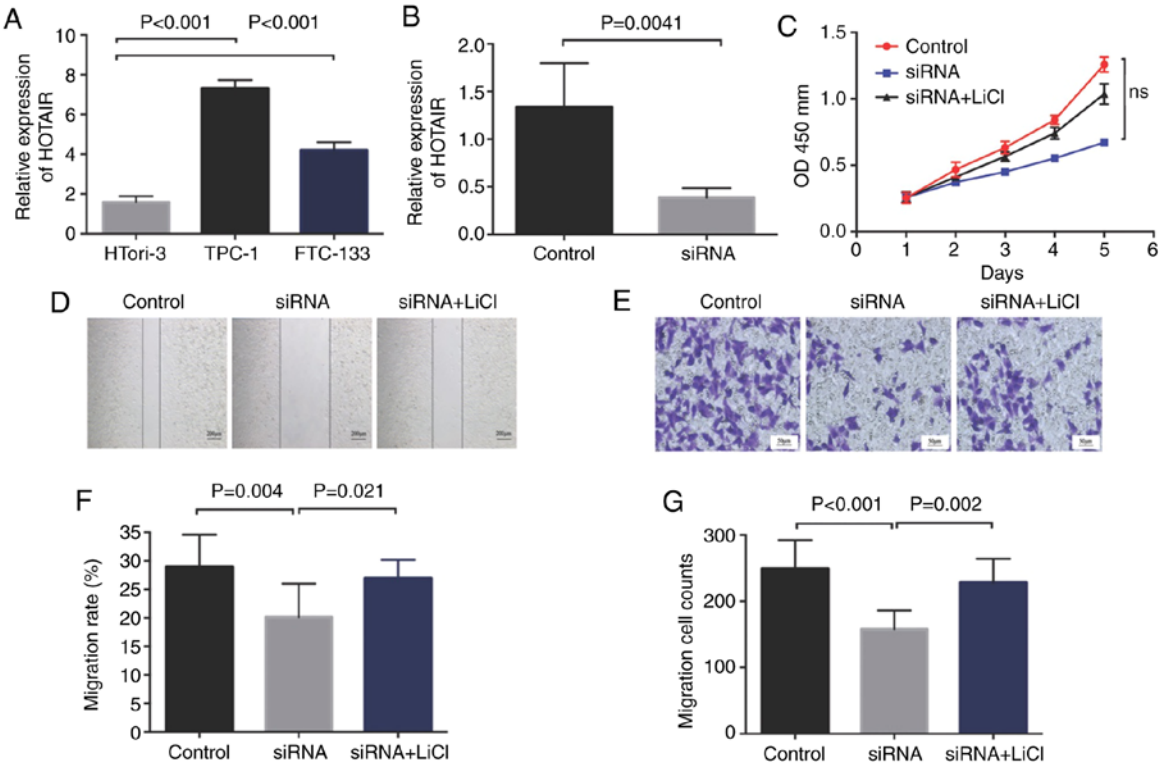

Figure 2. Effect of HOTAIR on TPC-1 cell proliferation, migration and invasion. (A) Expression levels of HOTAIR in the PTC HTori-3, TPC-1 and FTC-133 cell lines. (B) mRNA expression level of the HOTAIR gene in the TPC-1 cells after silencing with siRNA. (C) Cell proliferation after HOTAIR gene silencing (absorbance at a wavelength of 490 nm represents the number of cells). (D and F) Cell migration (scale bar, 200 μm) and histogram of cell migration rate after HOTAIR silencing. (E and G) Transwell image (scale bar, 50 μm) and histogram of the migrated cell number after HOTAIR gene silencing. HOTAIR, HOX transcript antisense RNA; PTC, papillary thyroid carcinoma.

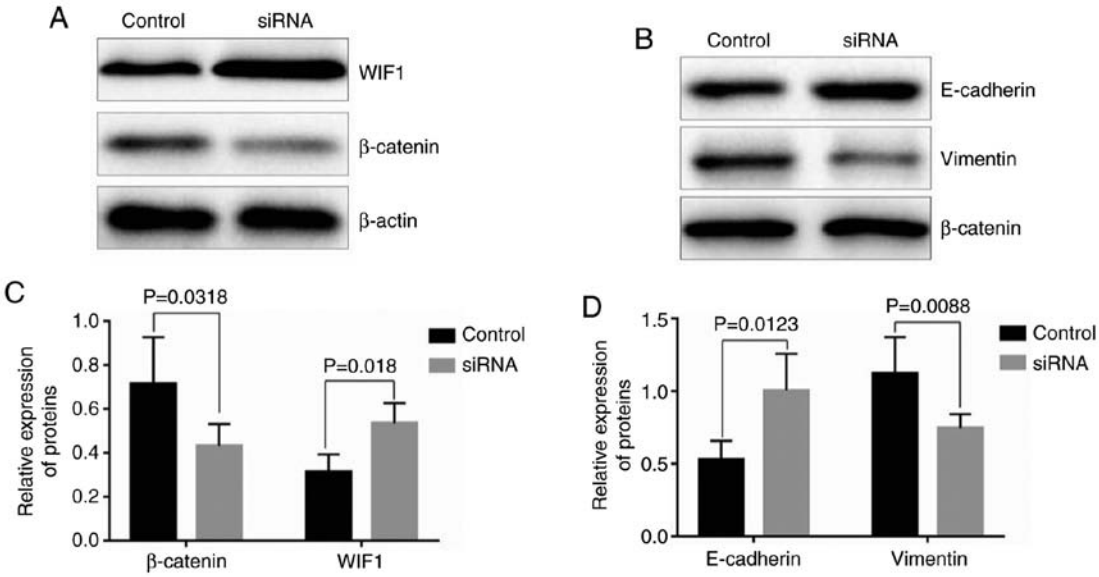

Figure 3. Impact of HOTAIR knockdown on the Wnt/β-catenin signaling pathway and cell phenotype of PTC TPC-1 cells. (A and C) Western blot analysis of WIF1 and β-catenin and histogram of the relative expression levels of the two target proteins. (B and D) Western blot analysis of E-cadherin and vimentin and the histogram of the relative expression levels of the two target proteins. Relative expression of a protein is the ratio of the target protein expression to the internal reference protein (β-actin) expression. HOTAIR, HOX transcript antisense RNA; PTC, papillary thyroid carcinoma; WIF1, Wnt inhibitor 1.

lncRNA-HOTAIR are potential biomarkers for PTC patients and are associated with poor prognosis (15,20,21). However, previous studies did not include the presence or absence of cervical lymph node metastasis. In addition, we further explored the specific mechanism of the involvement of lncRNA-HOTAIR in cervical lymph node metastasis and focused on the role of epithelial-mesenchymal transition (EMT). Therefore, the present study has innovative and clinical application value. It was found that the expression of lncRNA-HOTAIR was upregulated in the lymph node metastasis-positive group compared

with the metastasis-negative group among the PTC patients. These findings suggest that HOTAIR overexpression in PTC patients may be closely associated with lymph node metastasis. To further investigate the relationship between HOTAIR overexpression and PTC lymph node metastasis, the *HOTAIR* gene in the PTC cell line TPC-1 was knocked down using siRNA, followed by assessment of the proliferation and migration of the transfected cells. Impacts on the Wnt/β-catenin pathway and cell phenotype were also assessed using western blot analysis to explore the mechanism underlying the HOTAIR-mediated promotion of cell migration.

HOX transcript antisense RNA (HOTAIR), which was first discovered in a study on homeotic genes in *Drosophila melanogaster* (8), demonstrates abnormal expression in cancer tissues. Researchers have reported that HOTAIR is overexpressed in PTC and peripheral blood (15,20,21). The results of this study were consistent with those of previous studies. We also found that the proliferation of TPC-1 cells was significantly inhibited after HOTAIR knockdown using siRNA. The findings suggest that HOTAIR may be closely associated with the growth and metastasis of PTC, and HOTAIR overexpression may promote the occurrence and metastasis of PTC through a certain mechanism. This result is consistent with the findings of Di *et al* (20). HOTAIR overexpression can also downregulate the expression of tumor-suppressor gene miR-1, thereby promoting the onset of thyroid cancers through the HOTAIR/ miR-1/CCND2 axis (22,23). In addition, PTC susceptibility, onset and progression were found to be closely associated with the three SNP loci of HOTAIR (14). This evidence indicates that lncRNA-HOTAIR plays an important role in PTC onset and progression.

EMT is a biological process that plays an important role in tumor metastasis. EMT is excessively activated in tumor tissues, during which mesenchymal cell phenotypes increase, such as vimentin and N-cadherin; while epithelial cell phenotypes decrease, such as E-cadherin (24). Liu *et al* (25) reported that cell invasion was reduced after HOTAIR silencing in gastric cancer cells, and the cell phenotypes were altered as well, indicating that HOTAIR can promote tumor invasion by activating the process of EMT. In addition, HOTAIR was found to be able to modulate cell invasion and metastasis in breast cancer MCF-7 cells as well through the p53/Akt/JNK pathway (26). The above-mentioned reports and our findings indicate that HOTAIR may play an important role in tumor metastasis.

The EMT process in tumor cells is modulated by the Wnt/β-catenin signaling pathway. Activation of the Wnt/β-catenin signaling pathway upregulates the expression of Snail and ZEB1 which are known to play a significant role in EMT, leading to tumor metastasis (27,28). In the present study, the impact of HOTAIR silencing on the Wnt/β-catenin signaling pathway was evaluated. It was found that the expression of Wnt inhibitor 1 (WIF1) was significantly increased, while the expression of β-catenin was significantly reduced after HOTAIR silencing. In addition, the expression of the epithelial cell phenotype E-cadherin was increased, while the expression of the mesenchymal cell phenotype vimentin was reduced. The findings suggest that HOTAIR can promote TPC-1 cell migration and invasion through the Wnt/β-catenin pathway. The Wnt/β-catenin signaling pathway is a type of canonical Wnt signaling pathway. When it is in an inactive state, β-catenin is phosphorylated by the β-catenin destruction complex assembled by APC/GSK-3β/CKIα/Axin, leading to its degradation by ubiquitination. When the pathway is activated, the β-catenin destruction complex is unstable, leading to accumulation of β-catenin in the cytoplasm. Excessive β-catenin then translocates into the nucleus where it causes transcription of downstream target genes (29). Therefore, activation of the Wnt/β-catenin signaling pathway plays an important role in proliferation, apoptosis, cell cycle and onset/progression of cancer cells. WIF1 is a protein that antagonizes Wnt activity. It binds to Wnt in the extracellular space, therefore blocking the Wnt signal transduction, leading to inhibition of the Wnt/β-catenin pathway (30), and the weakening of WIF1 can result in the overactivation of Wnt/β-catenin. In addition, to further demonstrate that HOTAIR regulates the Wnt pathway and promotes tumor growth and metastasis, LiCl was used in a rescue assay. Studies have reported that LiCl can activate the Wnt/β-catenin pathway. After adding LiCl to activate Wnt/β-catenin, we observed that the migration, invasion and proliferation of the TPC-1 cells was not inhibited by HOTAIR siRNA and was reversed similar to levels of the control. Therefore, it was further confirmed that HOTAIR regulated the proliferation and invasion of TPC-1 cells through Wnt/β-catenin (31). These results demonstrate that HOTAIR may cause activation of the Wnt/β-catenin pathway by inhibiting WIF1, resulting in PTC cell proliferation and metastasis, and EMT may also be involved.

In summary, in the present study it was found that lncRNA-HOTAIR was overexpressed in the serum of patients with lymph node metastasis of PTC. In *in vitro* experiments, HOTAIR was found to promote cell growth and migration of PTC cells by modulating the Wnt/β-catenin pathway. This evidence indicates the correlation between HOTAIR expression and lymph node metastasis of PTC. Moreover, the results of this study suggest that high levels of lncRNA-HOTAIR in the blood of patients with clinical PTC can predict the possibility of lymph node metastasis, which is of referential significance for clinical diagnosis and treatment. It is undeniable that there are shortcomings in this study. Firstly, ultrasonography lacks specific indicators for lymph node metastases, and sonographers may have subjective judgments or lack experience. Although efforts were made to reduce the impact of these factors, missed diagnosis or even misdiagnosis are still possible. Secondly, the sample size of this study was small, lacking tumor-related indicators and patient prognosis data, and the relationship between HOTAIR and clinical and pathological features were not further analyzed. It is necessary to further expand the sample size to improve the study.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

LW and YS conceived and designed the study, and drafted the manuscript. LW, BL and MZ collected, analyzed and interpreted the experiment data, and revised the manuscript critically for important intellectual content. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Beijing Geriatric Hospital (Beijing, China). Signed written informed consents were obtained from all of the patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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