# STUDY ON THE EXPRESSION OF LNCRNA H19/HOTAIR IN PATIENTS WITH PTC AND ITS RELATIONSHIP WITH TUMOR PROLIFERATION AND INVASION

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***ABSTRACT***

***Objective****: To investigate the expression of long-stranded non-coding (Lnc) RNA H19/HOTAIR in patients with papillary thyroid carcinoma (PTC) and the relationship with tumor proliferation and invasion.*

***Methods****: A total of 70 patients with PTC admitted to our hospital from January 2019 to June 2020 were included in the study, and the clinicopathological characteristics data were analyzed, and the expression levels of LncRNA H19/HOTAIR in cancer tissue, para-cancer tissue and normal thyroid tissue were detected by real-time quantitative PCR to evaluate the correlation between LncRNA H19/HOTAIR expression and clinicopathological characteristics.*

***Results****: Lnc RNA H19/HOTAIR levels in thyroid cancer tissues were significantly higher than those in both para-cancer and normal tissues (P<0.05); the differences in Lnc RNA H19/HOTAIR levels between para-cancer and normal tissues were not statistically significant (P>0.05); the results of univariate analysis showed that LncRNA H19 and LncRNA HOTAIR expression were both associated with the maximum tumor diameter, TNM stage and lymph node metastasis of patients (P<0.05).*

***Conclusion****: Both LncRNA H19/HOTAIR were highly expressed in patients with PTC, and both expressions were also associated with tumor proliferation and invasion.*

***Keywords:*** *papillary thyroid cancer, long-stranded non-coding RNA, LncRNA H19, LncRNA HOTAIR, tumor; proliferation, invasion.*

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## Introduction

Thyroid cancer is a clinically common malignancy of the endocrine system, while PTC is its main pathological histological subtype, accounting for approximately more than 85% of the total number of pa-tients; the incidence of PTC has been in-creasing year by year in the past 30 years, and its overall morbidity and mortality in the normal population are 0.40% and 0.03%, respectively[1]. Radical resection is the main treatment for PTC, and although most patients have a good prognosis, a significant number of patients still have postoperative recurrence and lead to a significant decrease in long-term survival[2]. The mechanisms of thyroid carcinogenesis and its proliferation and invasion are still controversial, and some scholars believe that molecular regulatory mechanisms play an important role in it[3]. Long non-coding RNA (LncRNA) is a special type of RNA with >200 nucleotides but no pro-tein-coding function; available evidence confirms that LncRNA is associated with various

malignant tumorigenesis, migration and apoptosis[4,5]; among them, LncRNA H19 is mainly expressed in human em-bryonic and skeletal muscle tissues and can regulate gene apoptosis in gastrointestinal and bladder tumors; while HOTAIR gene mainly regulates target gene expression through complex protein complexes in the 5' and 3' structural domain, which may be related to the development of many solid tumors and hematological malignancies[6]. However, the role of LncRNA H19 and HOTAIR expression on tumor proliferation and invasion in PTC patients is still unclear. Based on these issues, a total of 70 patients with PTC admitted to our hospital from January 2019 to June 2020 were included in this study, and the clinicopathological characteristics data, LncRNA H19 and HOTAIR expression levels were analyzed, aiming to investigate the expression of LncRNA H19/HOTAIR in PTC patients and the relationship with tumor proliferation and invasion, which are reported as follows.

## Data and methods

*Study subjects*

A total of 70 patients with PTC ad-mitted in our hospital from January 2019 to June 2020 were included in the study. In-clusion criteria: PTC confirmed by patho-ogical histology; age: 18-65 years; com-plete clinical data; exclusion criteria: distant metastasis; combined with other systemic malignancies; preoperative radiotherapy, chemotherapy or other treatments; combined with severe liver and kidney dysfunction; combined with systemic infec-tious diseases; pregnant and lactating women. The study design was approved by the hospital ethics committee, and patients or their families gave informed consent.

### Methods

***Data collection***

The cases were checked to record gender, age, disease duration, maximum tumor diameter, capsular infiltration, LN metastasis and TNM stage.

***Blood and tissue specimen collection***

5.0 ml venous blood at fasting was collected, added to EDTA anticoagulation tube, placed for 30 min and centrifuged at 1200 g for 10 min, the supernatant was aspirated into a centrifuge tube, centrifuged again at 4°C 12,000 g for 10 min and the supernatant was separated into a new centrifuge tube, 250μl plasma was taken and added to 750μl TRIzol® LS reagent and stored at -80°C for use. Fresh PTC tissue, paracancer tissue (>0.5 cm from tumor tissue) and normal thyroid tissue were collected at surgery and frozen at -80 °C until RNA extraction.

***Real-time quantitative PCR***

Total RNA extraction and cDNA first strand synthesis: Tumor tissues and plasma total RNA extraction was performed strictly according to the instructions of TRIzol® LS reagent (Invitrogen, USA), and the extracted RNA was dissolved in DEPC-treated water. The reverse tran-scription kit (TaKaRa, Dalian, China) was used for cDNA first strand synthesis, and the synthesized cDNA first strand was stored at -20°C for use. (3) Real-time quantitative PCR assay Based on the se-quences of H19, HOTAIR and the internal reference GAPDH in Genebank, primers were designed using Primer 5.0 software, and primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. The Ct values were obtained after PCR amplification us-ing SYBR® Advantage® qPCR Premix (TaKaRa, Dalian), and the expression lev-els of H19 and HOTAIR in tumor tissues and plasma were calculated using the 2-ΔΔCt method.

***Statistical methods***

SPSS20.0 software was selected to process the data; t-test was used to compare the ratio between groups of measurement data, expressed as $\bar{x}\pm s$; $\chi^2$ test was used to compare the ratio of count data, expressed as %; $P<0.05$ was considered statistically significant difference.

## Results

***Analysis of LncRNA H19/HOTAIR expression levels in cancer tissues, pa-ra-cancer tissues and normal thyroid tissues***

Lnc RNA H19/HOTAIR levels in thyroid cancer tissues were significantly higher than those in para-cancer and nor-mal tissues ($P<0.05$); no statistically sig-nificant differences were found in the comparison of Lnc RNA H19/HOTAIR levels in para-cancer and normal tissues ($P>0.05$); see Table 1.

***Correlation analysis between LncRNA H19 expression and clinicopathological features***

The results of univariate analysis showed that LncRNA H19 expression was correlated with the

maximum tumor di-ameter, TNM stage and lymph node me-tastasis of patients (P<0.05); see Table 2.

| Indicators | LncRNA H19 | LncRNA HOTAIR |
|---|---|---|
| Cancer tissues | 4.12±0.73 | 29.76±4.39 |
| Para-cancer tissues | 3.43±0.54 | 16.54±2.14 |
| Normal tissues | 3.27±0.49 | 15.70±2.07 |
| *F* | 5.12 | 7.81 |
| *P* | 0.00 | 0.00 |

**Table 1:** Analysis of LncRNA H19/HOTAIR expression levels in cancer tissue, para-cancer tissue and normal thyroid tissue.

| Indicators | Number of cases | LncRNA H19 level | *P* |
|---|---|---|---|
| Sex | | | 0.20 |
| male | 21 | 4.20±0.86 | |
| female | 49 | 4.09±0.79 | |
| Age | | | 0.87 |
| <55 years | 32 | 4.07±6.90 | |
| ≥55 years | 38 | 4.15±0.50 | |
| Maximum tumor diameter | | | 0.00 |
| ≤1cm | 16 | 3.27±0.70 | |
| >1cm | 54 | 5.52±1.23 | |
| capsular infiltration | | | 0.20 |
| yes | 31 | 4.99±0.97 | |
| no | 39 | 3.60±0.80 | |
| TNM stage | | | 0.01 |
| stage I~II | 29 | 3.75±0.72 | |
| stage III~IV | 41 | 4.83±0.94 | |
| Lymph node metastasis | | | 0.01 |
| yes | 37 | 6.40±0.98 | |
| no | 33 | 2.84±0.53 | |

**Table 2:** Correlation analysis between LncRNA H19 expression and clinicopathological features (n=70).

***Correlation analysis between LncRNA HOTAIR expression and clinicopatholog-ical features***

The results of univariate analysis showed that LncRNA HOTAIR expression was correlated with the maximum tumor diameter, TNM stage and lymph node metastasis of patients (P<0.05); see Table 3.

## Discussion

Thyroid carcinoma is the most common malignant tumor of the thyroid gland, accounting for about 1% of systemic malignancies[7]; in recent years, the incidence rate in China is increasing year by year, among which papillary thyroid car-cinoma (PTC) is the most common, and the prognosis of PTC has been shown to be closely related to clinicopathological pa-rameters such as age, gender, and lymph node metastasis[8,9]. It is generally ac-cepted that patients with PTC have a better prognosis after thyroidectomy and a lower mortality rate[10]. However, there are still some aggressive PTC with poor prognosis, so further search for PTC-related tumor markers is important for the accurate diagnosis of PTC and the elucidation of the pathogenesis of PTC.

| Indicators | Number of cases | LncRNA HOTAIR level | *P* |
|---|---|---|---|
| Sex | | | 0.38 |
| male | 21 | 29.20±4.86 | |
| female | 49 | 30.09±4.79 | |
| Age | | | 0.19 |
| <55 years | 32 | 28.69±4.31 | |
| ≥55 years | 38 | 31.46±4.27 | |
| Maximum tumor diameter | | | 0.72 |
| ≤1cm | 16 | 29.25±4.65 | |
| >1cm | 54 | 29.90±4.32 | |
| capsular infiltration | | | 0.46 |
| yes | 31 | 29.42±4.69 | |
| no | 39 | 28.60±4.20 | |
| TNM stage | | | 0.00 |
| stage I~II | 29 | 26.86±3.47 | |
| stage III~IV | 41 | 34.12±5.18 | |
| Lymph node metastasis | | | 0.00 |
| yes | 37 | 37.13±6.78 | |
| no | 33 | 22.49±4.81 | |

**Table 3:** Correlation analysis between LncRNA HOTAIR expression and clinicopathological features.

LncRNAs are a class of RNA mole-cules with transcripts longer than 200nt that regulate gene expression at multiple levels (epigenetic regulation, transcriptional reg-ulation, and post-transcriptional regulation, etc.) but do not encode proteins[11]. LncRNA was initially thought to be the 'noise' of genome transcription, a by-product of RNA polymerase II transcription that has no biological function. As bioinformatics and molecular biology technologies (RNA-seq, microarray, RNAi, RIP, etc.) have been applied to the study of lncRNA,

more and more studies have confirmed that lncRNA has complex biological functions and play important roles in regulating embryonic development, stem cell directed differentiation, subcellular structure distribution, etc. and abnormal expression of LncRNA may lead to disease occurrence[12,13].

Recent studies have revealed that LncRNA may function mainly by affecting downstream gene expression, interfering with mRNA shearing, acting as a prerequisite for small molecule RNAs to produce endogenous siRNAs, regulating the activity of binding proteins, and sub-cellular localization[14]. There is growing evidence that LncRNAs influence the oc-currence, progression and prognosis of a wide range of tumors by mediating the biological properties of proliferation, mi-gration, invasion, and apoptosis[15]. Several genes associated with thyroid cancer lncRNA BANCR has been identified to be up-regulated in thyroid cancer tissues, while PTCSC3, NAMA, and Ak023948 are down-regulated in expression[16].

H19 is a lncRNA molecule located on human chromosome 11p15.5 with a length of 2,300 bp and can interact with insu-lin-like growth factor 2 (IGF-2), exhibiting paternal imprinting[17]. Recent studies have found that H19 is aberrantly ex-pressed in a variety of tumors and is strongly associated with poor patient prognosis, and that H19 can interact with a variety of oncogenes or tumor suppressor genes (e.g., c-Myc, p53) and microRNAs, thus playing an important function in tumor growth, proliferation, differentiation apoptosis, and invasion and metastasis[18]. While HOTAIR is the first lncRNA with transtranscriptional regulation identified by Rinn et al. The human HOTAIR gene is localized in the 12q13 region, and the gene (RefSeq NR_003716) is 2364 bp long and consists of one long exon and five short exons[19]. Several studies have found that HOTAIR is highly expressed in hematological malignancies such as lymphoma and acute leukemia, in addition to solid tumors such as breast, esophageal, gastric, liver, colon, kidney, cervical, ovarian, pancreatic and lung cancers[20,21]. Do-mestic and foreign scholars found that the expression level of H19 was down-regulated in thyroid cancer cell lines and tumor tissues, which was associated with prognosis, while the expression of HOTAIR was increased in thyroid cancer cell lines and tumor tissues, which was associated with tumor cell invasion, migration and prognosis[22].

In the results of the current study, Lnc RNA H19/HOTAIR levels in thyroid cancer tissues were significantly higher than those in both paracancer and normal tissues ($P<0.05$); the difference in Lnc RNA H19/HOTAIR levels between paraneoplastic and normal tissues was not statistically significant ($P>0.05$), indirectly suggesting that the elevated expression level of LncRNA H19/HOTAIR may be involved in the process of PTC disease development. In vitro experiments con-firmed that LncRNA H19 and LncRNA HOTAIR could promote cancer cell proliferation; meanwhile both were involved in the epithelialmesenchymal transition process, indirectly suggesting an important role of both in promoting malignant tumor metastasis[23]. Another study showed that LncRNA H19 could positively regulate miR-675 expression and inhibit P53 gene activation[24]; while LncRNA HOTAIR was thought to inhibit ubiquitin protein-linked enzyme E3 family expression in breast cancer cell lines, increasing the risk of tumor cell shedding and metastasis. And the results of univariate analysis in this study showed that both LncRNA H19 and LncRNA HOTAIR expression were asso-ciated with the maximum tumor diameter, TNM stage and lymph node metastasis in patients ($P<0.05$), indicating that high ex-pression of LncRNA H19 and LncRNA HOTAIR were associated with PTC proliferation and invasion, further supporting the above view.

In conclusion, both LncRNA H19/HOTAIR were highly expressed in PTC patients, while both expressions were also associated with tumor proliferation and invasion.

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