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Expression and Function of Long Noncoding RNA NONHSAT129183 in Papillary Thyroid Cancer

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The aberrant expression of long noncoding RNAs (lncRNAs) is implicated in cancer development and progression. This study was aimed to investigate the expression and clinical significance of lncRNA NONHSAT129183 in papillary thyroid cancer (PTC), and to explore its roles in PTC cell proliferation, migration, and invasion. Our results demonstrate that lncRNA NONHSAT129183 is upregulated in human PTC tissues when compared with that in adjacent noncancerous thyroid tissue. Moreover, its expression is correlated with tumor size, lymph node metastasis, and TNM stage in PTC patients. lncRNA NONHSAT129183 silencing also significantly suppressed cell proliferation, migration, and invasion in PTC cell lines. In conclusion, our results suggest that lncRNA NONHSAT129183 plays a critical role in the regulation of PTC cell proliferation, migration, and invasion, providing new insights into PTC pathogenesis.

Key words: Papillary thyroid cancer (PTC); Long noncoding RNAs (lncRNAs); lncRNA NONHSAT129183; Proliferation; Migration; Invasion

INTRODUCTION

Thyroid cancer is the most common endocrine neoplasm. The incidence of thyroid cancer has been reported to increase in recent years[1]. The most common histological type of all thyroid cancers is papillary thyroid carcinoma (PTC), which accounts for approximately 80% of all thyroid cancers[2]. PTC is usually an indolent cancer with good prognosis and a 10-year survival rate of about 90%. However, PTC has more aggressive phenotypes that present with distant metastasis, lymph node metastasis, and dedifferentiation into more lethal thyroid cancers[3]. Although surgery, thyroid hormone therapy, isotope therapy, and radioiodine therapy are some forms of treatments for PTC, the number of advanced tumors and thyroid cancer-associated mortality is increasing[4]. Therefore, it is important to dissect the molecular mechanisms of PTC for early detection, effective treatment, and prognosis estimation.

Long noncoding RNAs (lncRNAs) are RNAs consisting of more than 200 nucleotides that are unable to translate into proteins[5]. In recent years, increasing evidence shows that lncRNAs play important functional roles in various fundamental biological processes at both the post-transcriptional and transcriptional levels[6,7]. Many studies have demonstrated that lncRNAs participate in cancer tumorigenesis, progression, cellular proliferation, metastasis, and apoptosis[8–10]. Although thousands of lncRNAs have been recently found to be differentially expressed between PTC and adjacent noncancerous samples[11], a few lncRNAs are reported to play an important role in oncogenesis and the progress of thyroid cancer. For example, BRAF-activated lncRNA (*BANCR*) increases PTC cell proliferation and activates autophagy[12]. Downregulated PTC susceptibility candidate 3 (*PTCSC3*) in thyroid and PTC tissues was found to impact PTC carcinogenesis via the S100A4 pathway[3,13].

A study performed by Wang et al. also identified hundreds of significantly differentially expressed lncRNAs in 12 PTC tissues when compared with those in paired normal adjacent tissues, and also revealed specific changes to lncRNA profiles associated with PTC, as in the case of lncRNA NONHSAT061051 and lncRNA NONHSAT129183[14]. However, their roles in PTC have not yet been revealed. Thus, the aims of this study were to detect their expression levels in human PTC tissues, analyze their clinical significance, and investigate their effects on cell proliferation, metastasis, and invasion in PTC cell lines.

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MATERIALS AND METHODS

Clinical Specimen Collection

A total of 19 pairs of PTC tissue and paired adjacent noncancerous thyroid tissue samples were obtained from patients diagnosed with thyroid cancer at the Fourth People's Hospital of Ji'nan. The diagnosis of PTC was confirmed pathologically. The tissue samples were immediately snap frozen in liquid nitrogen after surgical resection and stored at −80°C for further use. The samples used in this study were approved by the Committees for Ethical Review of Research Involving Human Subjects. None of the patients had received preoperative radiotherapy or chemotherapy. All the patients provided written informed consent before the samples were collected.

RNA Extraction and Real-Time Quantitative PCR (qPCR)

Total RNA was extracted from the tissues and cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The concentration of RNA was measured using the NanoDrop ND-1000 Spectrophotometer (Agilent, CA, USA). Total RNA (1 μg) was then reverse transcribed to cDNA using a PrimeScript RT reagent kit (Takara, Dalian, China). lncRNA NONHSAT061051 and lncRNA NONHSAT129183 expressions were determined by performing qPCR with the SYBR Premix Ex Taq (Takara) and 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). Sequences of primers used to perform qPCR are as follows: lncRNA NONHSAT061051, 5′-ACAGAGGG AACAGAGACCCC-3′ (forward) and 5′-CTCAACTGG TGTCGTGGA-3′ (reverse); lncRNA NONHSAT129183, 5′-CTCCTGGGGCAAGAGAAGAA-3′ (forward) and 5′-CTCAACTGGTGTCGTGGA-3′ (reverse); 18*S* rRNA, 5′-CCTGGATACCGCAGCTAGGA-3′ (forward) and 5′-GCGGCGCAATACGAATGCCCC-3′ (reverse). lncRNA NONHSAT061051 and lncRNA NONHSAT129183 mRNA expressions were normalized to 18S rRNA (internal control). The levels of relative lncRNA NONH SAT061051 and lncRNA NONHSAT129183 expressions were calculated using the $2^{-\Delta\Delta}$Ct method.

Cell Culture and siRNA Transfection

Human PTC cell lines (SW579 and TT) were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cell lines were cultured in RPMI-1640 (Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 U/ml penicillin, and 100 mg/ml streptomycin (Invitrogen) at 37°C in a humid atmosphere containing 5% CO_2. Three siRNAs were initially designed and used in our study. The siRNA-1, siRNA-2, and siRNA-3 sequences were purchased from RiboBio (Guangzhou, Guangdong, China) and are as follows: siRNA-1 (Si-1, 5′-AGAAAGUGUUGUUGAAUAACU-3′), siRNA-2 (Si-2, ′-GGAUGGUCAAGUUCUAGAAC A-3′), siRNA-3 (Si-3, 5′-UUCUAGAACUUGACCAUC CUU-3′), and negative control siRNA (si-NC; 5′- UUC UCCGAACGUGUCACGUTT-3′). The TT and SW579 cells were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. After 48 h of transfection, lncRNA NONHSAT129183 expression was determined using qPCR. Preliminary qPCR results indicated that siRNA-2 had a significantly higher silencing efficiency than siRNA-1 and siRNA-3. Thus, siRNA-2 was chosen for the cellular physiological function assays.

Cell Proliferation Assay

Cell proliferation was assessed by an MTT assay kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. Cells (1×10^4 per well) in the si-NC and si-NONHSAT129183 groups were cultured in 96-well plates. After culturing for 0, 24, 48, and 72 h, 10 μl of MTT reagent was added to each well. After 4 h of incubation, 100 μl of formazan solvent was added to each well and further incubated until the formazan dissolved. The optical density (OD) of each well was measured at 570 nm using a microplate reader. All assays were performed in triplicate.

Cell Migration and Invasion Assays

Cell migration and invasion assays were performed using Transwell chambers (Corning Co., Corning, NY, USA) with or without Matrigel (BD Biosciences, San Jose, CA, USA). After 48 h of transfection, cells were harvested and suspended in a serum-free medium and then placed into the upper chamber with 10 μg/ml Matrigel or without Matrigel, while the lower chamber was filled with medium containing 10% bovine calf serum. After incubation for 48 h at 37°C in a humid atmosphere containing 5% CO_2, nonmigrating cells on the upper side of the filter were removed by wiping with a cotton swab, and cells that migrated across the membrane were fixed with methanol for 15 min, stained with 0.1% crystal violet in PBS for 15 min, and counted under a microscope (Olympus, Tokyo, Japan) at a magnification of 200×. Three independent experiments were carried out.

Statistical Analysis

The SPSS 19.0 software (IBM Corporation, Armonk, NY, USA) was used to carry out statistical analysis. Data are presented as mean±standard deviation (SD). Independent *t*-tests were used to analyze the differences in lncRNA expression between human PTC tissues and matched adjacent normal tissues, as well as the differences

between si-NC and si-NONHSAT129183 treatments. A value of $p<0.05$ was considered to indicate statistically significant differences.

RESULTS

Correlation Between lncRNA NONHSAT061051 and lncRNA NONHSAT129183 Expression and Clinicopathological Characteristics in Patients With PTC

To validate whether the levels of lncRNA NONHSAT 061051 and lncRNA NONHSAT129183 increased in human PTC tissues, we measured their expression in 19 pairs of human PTC tissues and matched adjacent normal tissue using qPCR. lncRNA NONHSAT129183 expression levels were observed to have significantly increased in PTC tissues when compared with those in the matched adjacent normal tissue (Fig. 1A). Its expression levels in PTC tissues were also higher than those in adjacent noncancerous thyroid tissue, which accounted for 89.4% (17/19) (Fig. 1B). However, lncRNA NONHSAT061051 expression levels were not significantly increased in PTC tissues (Fig. 1C). lncRNA NONHSAT061051 expression levels were higher than those in adjacent noncancerous thyroid tissue, which accounted for 63.1% (12/19) (Fig. 1D). Thus, lncRNA NONHSAT129183 was chosen for the cellular physiological function assays.

To further understand the significance of lncRNA NONHSAT129183 expression in PTC, we examined the relationship between lncRNA NONHSAT129183 expression and the clinicopathological characteristics in patients with PTC. We found that its expression significantly correlated with tumor size, TNM stage, and lymph node metastasis (Fig. 2A–C), but not with age and gender (Fig. 2D and E).

Silencing lncRNA NONHSAT129183 Inhibits SW579 and TT Cell Proliferation

Considering the high expression of lncRNA NONH SAT129183 in PTC tissues when compared with that in the adjacent nontumor tissues, we investigated whether silencing lncRNA NONHSAT129183 inhibited the in vitro proliferation of PTC cell lines SW579 and TT. Three siRNAs were initially designed and used in our study. However, qPCR results showed that siRNA-2 had a significantly higher silencing efficiency than siRNA-1 and siRNA-3 in the SW579 and TT cell lines (Fig. 3A and B). Thus, siRNA-2 was chosen for the cellular physiological function assays. Our MTT assay data indicated that the proliferation rate of SW579 and TT cells transfected with si-NONHSAT129183 significantly decreased when compared with that of cells transfected with si-NC after 72 h of transfection (Fig. 3C and D).

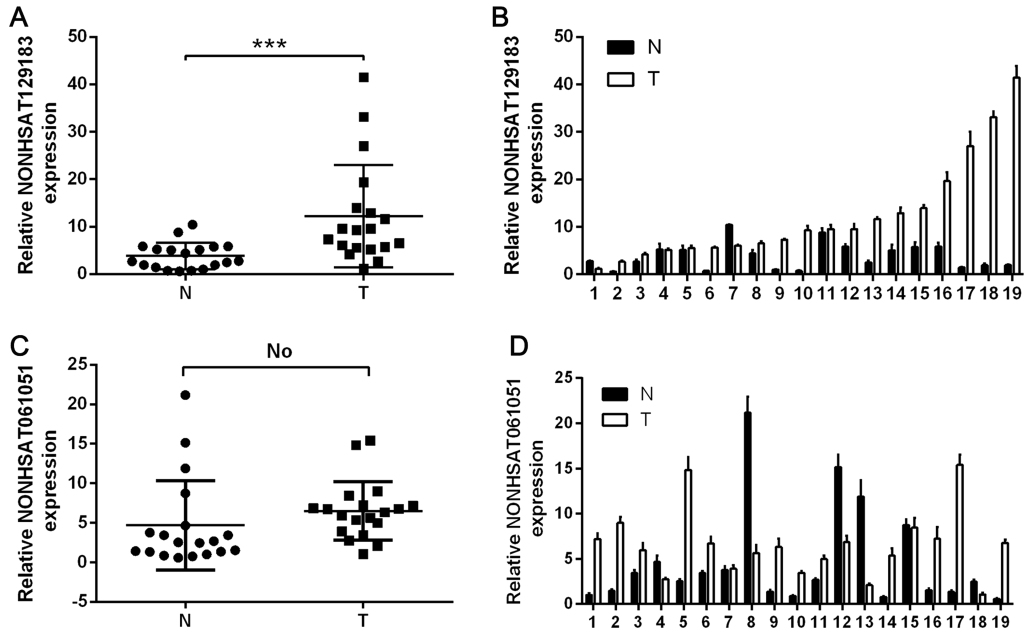

Figure 1. Relative expression of long noncoding RNA (lncRNA) in normal (N) and tumor (T) tissues. (A) Relative expression of lncRNA NONHSAT129183 in papillary thyroid cancer (PTC) tissues compared with that in paired noncancerous thyroid tissues. (B) lncRNA NONHSAT129183 expression in 19 pairs of PTC and adjacent noncancerous thyroid tissues. (C) Relative expression of lncRNA NONHSAT061051 in PTC tissues compared with that in paired noncancerous thyroid tissues. (D) lncRNA NONHSAT061051 expression in 19 pairs of PTC and adjacent noncancerous thyroid tissues. ***$p<0.001$.

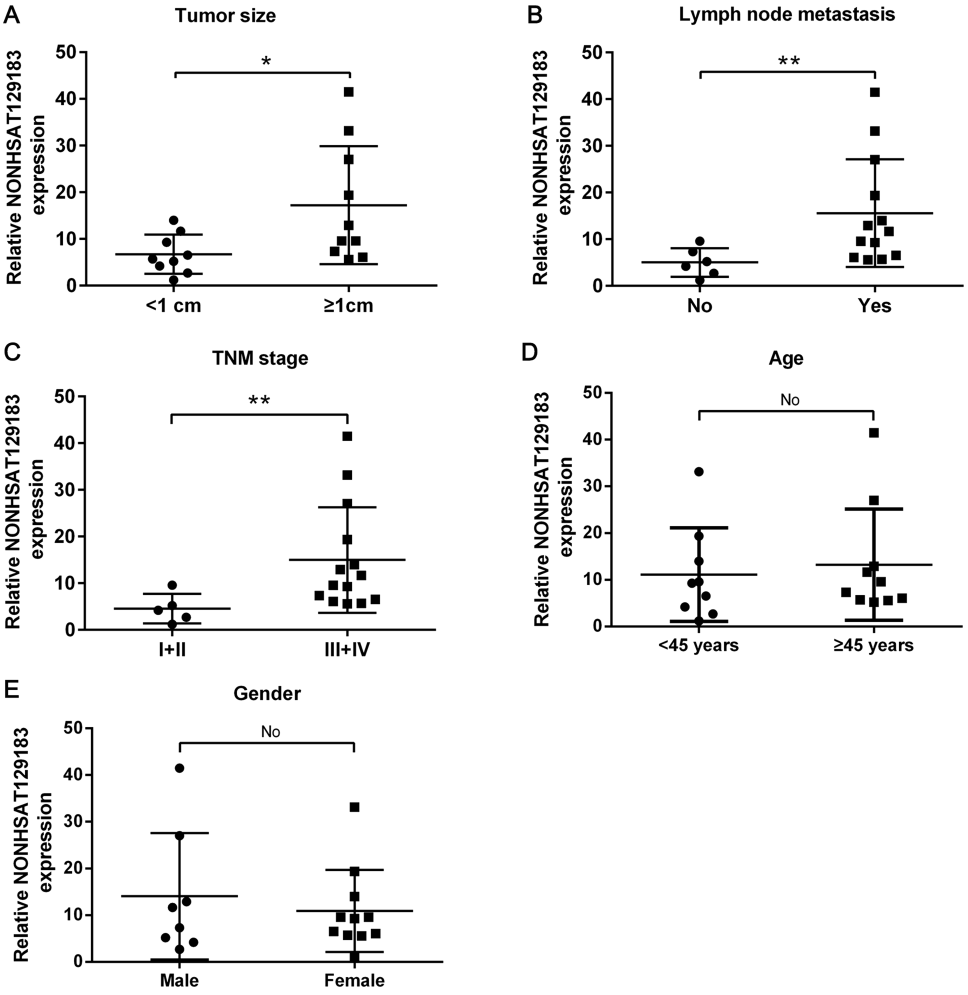

Figure 2. Correlation between lncRNA NONHSAT129183 expression and clinicopathological features in PTC. (A) Correlation between lncRNA NONHSAT129183 expression and tumor size. (B) Correlation between lncRNA NONHSAT129183 expression and lymph node metastasis. (C) Correlation between lncRNA NONHSAT129183 expression and TNM stage. (D) Correlation between lncRNA NONHSAT129183 expression and age. (E) Correlation between lncRNA NONHSAT129183 expression and gender. *$p<0.05$, **$p<0.01$.

Silencing lncRNA NONHSAT129183 Inhibits SW579 and TT Cell Migration and Invasion

We performed the Transwell migration and invasion assays to investigate the effect of lncRNA NON HSAT129183 in the PTC cell lines SW579 and TT. The results showed that SW579 and TT cells transfected with si-NONHSAT129183 had a slower migration rate than the si-NC group (Fig. 4A and B). Similarly, the Matrigel invasion Transwell assay revealed the weakened metastasis capacity of SW579 and TT cells transfected with si-NONHSAT129183 when compared with that of the si-NC group (Fig. 4C and D).

DISCUSSION

Although most PTC patients have favorable prognosis, PTC patients with lymph node or distant metastases may still have fatal outcomes[15]. Therefore, it is clinically significant to identify biomarkers for the early prediction of metastatic PTC. Increasing evidence confirms the advantages of lncRNAs as diagnostic and prognostic biomarkers in cancers[16]. Although the mechanisms of lncRNAs associated with tumorigenesis have not yet been elucidated, a few studies have initiated research on the diagnostic significance of lncRNAs in cancer. For example, a study performed by Tong et al. reported that lncRNA POU3F3 had a high diagnostic value in esophageal squamous cell carcinoma[17]. Emerging evidence has implicated aberrant expression of lncRNAs in cancer development and progression[18]. lncRNAs in PTC are still an emerging field, and therefore our knowledge is still limited[19].

Aberrant expression of lncRNAs in PTC motivated us to explore the effects of lncRNA NONHSAT061051 and

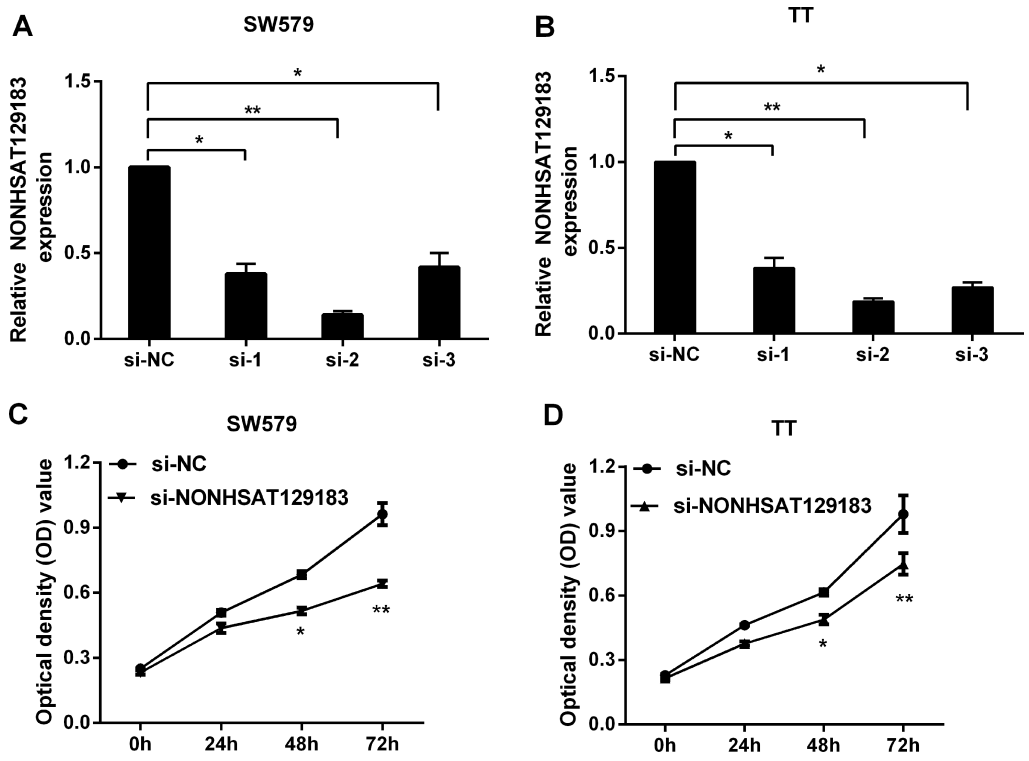

Figure 3. Relative expression of lncRNA NONHSAT129183 in SW579 and TT cell lines and cell proliferation curves. siRNA-NC (si-NC), siRNA-1 (si-1), siRNA-2 (si-2), siRNA-3 (si-3), and siRNA-NONHSAT129183 (si-NONHSAT129183). (A) Relative expression of lncRNA NONHSAT129183 in SW579 cells with transfection. (B) Relative expression of lncRNA NONHSAT129183 in TT cells with transfection. (C) The proliferation curve of SW579 cells after being transfected with si-NC and si-NONHSAT129183. (D) The proliferation curve of TT cells after being transfected with si-NC and si-NONHSAT129183. $*p<0.05$, $**p<0.01$.

lncRNA NONHSAT129183. In this study, we verified the aberrant expression of lncRNA NONHSAT129183 in human PTC tissues when compared with that in adjacent noncancerous thyroid tissue. Our results revealed that lncRNA NONHSAT129183 was upregulated in PTC tissues, which was consistent with the results of a previous study[14]. However, lncRNA NONHSAT061051 expression was not significantly increased in PTC tissues. To further understand the significance of lncRNA NONHSAT129183 expression in PTC, we examined the relationship between lncRNA NONHSAT129183 expression and the clinicopathological characteristics of PTC patients. The results showed that high expression of lncRNA NONHSAT129183 correlated with tumor size, lymph node metastasis, and TNM stage in PTC patients, suggesting that lncRNA NONHSAT129183 plays an important role in PTC tumorigenesis and metastasis.

Recent research indicated that lncRNAs could affect cell proliferation in cancer[20]. We investigated the effect of lncRNA NONHSAT129183 in PTC cells by applying the loss-of-function approach. Our results demonstrated that silencing lncRNA NONHSAT129183 expression inhibited the proliferation of the PTC cell lines SW579 and TT. Moreover, several studies have confirmed that lncRNAs could impact cell motility[21]. We performed the Transwell migration and invasion assays to investigate the effect of lncRNA NONHSAT129183 on the SW579 and TT cell lines. Our results showed that lncRNA NONHSAT129183 played a positive role in cancerous cell migration and invasion. Silencing lncRNA NONHSAT129183 expression could inhibit the migration and invasion abilities of the PTC cell lines SW579 and TT. Cumulatively, these findings suggest that lncRNA NONHSAT129183 could function as a tumor promoter by regulating cell proliferation, migration, and invasion, and could function as a new potential biomarker for PTC.

However, there are some limitations to this study. Although we analyzed the in vitro effects of lncRNA NONHSAT129183 on the proliferation, migration, and invasion of the PTC cell lines, the relationship between lncRNA NONHSAT129183 and cell cycle, apoptosis, and xenograft carcinogenesis was not investigated. Although silencing lncRNA NONHSAT129183 expression drastically inhibited the in vitro proliferation, migration, and invasion of the PTC cell lines, we did not further explore its specific mechanism. Despite these drawbacks, our study highlights the potential role of lncRNAs in improving clinical prognosis prediction in PTC patients.

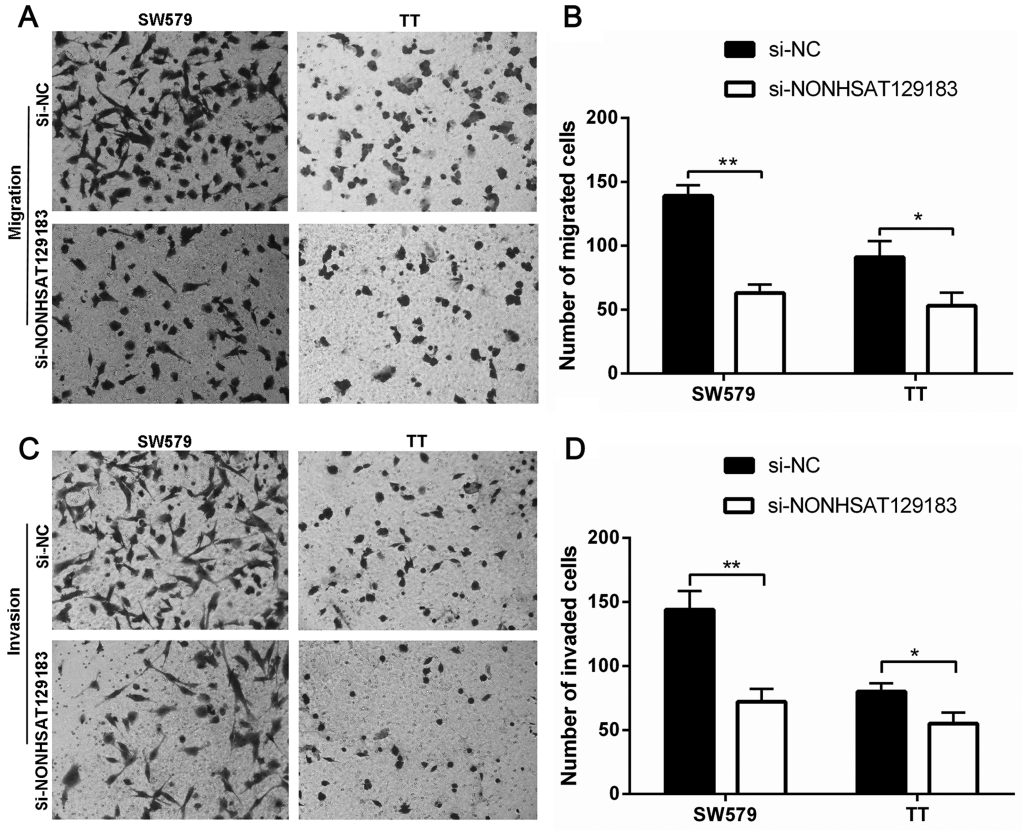

Figure 4. Migration and invasion capacities determined by Transwell assays in PTC cell lines SW579 and TT. (A, B) Effects of the si-NC and si-NONHSAT129183 transfections on the migration abilities of the SW579 and TT cell lines. (C, D) Effects of the si-NC and si-NONHSAT129183 transfections on invasion abilities of the SW579 and TT cell lines. *$p<0.05$, **$p<0.01$.

In conclusion, the current study demonstrates that lncRNA NONHSAT129183 is upregulated in PTC tissues. Silencing lncRNA NONHSAT129183 expression could drastically inhibit PTC cell proliferation, migration, and invasion in vitro. We also observed that lncRNA NONHSAT129183 could serve as a new promising diagnostic biomarker and therapeutic target for PTC.

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