Overexpression of long intergenic noncoding RNA LINC00312 inhibits the invasion and migration of thyroid cancer cells by down-regulating microRNA-197-3p

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The study evaluated the ability of long intergenic noncoding RNA LINC00312 (LINC00312) to influence the proliferation, invasion, and migration of thyroid cancer (TC) cells by regulating miRNA-197-3p. TC tissues and adjacent normal tissues were collected from 211 TC patients. K1 (papillary TC), SW579 (squamous TC), and 8505C (anaplastic TC) cell lines were assigned into a blank, negative control (NC), LINC00312 overexpression, miR-197-3p inhibitors, and LINC00312 overexpression + miR-197-3p mimics group. The expression of LINC00312, miR-197-3p, and p120 were measured using quantitative real-time PCR (qRT-PCR) and Western blotting. Cell proliferation was assessed via CCK8 assay, cell invasion through the scratch test, and cell migration via Transwell assay. In comparison with adjacent normal tissues, the expression of LINC00312 is down-regulated and the expression of miR-197-3p is up-regulated in TC tissues. The dual luciferase reporter gene assay confirmed that P120 is a target of miR-197-3p. The expression of LINC00312 and p120 was higher in the LINC00312 overexpression group than in the blank and NV groups. However, the expression of miR-197-3p was lower in the LINC00312 overexpression group than in the blank and NC groups. The miR-197-3p inhibitors group had a higher expression of miR-197-3p, but a lower expression of p120 than the blank and NC groups. The LINC00312 overexpression and miR-197-3p inhibitor groups had reduced cell proliferation, invasion and migration than the blank and NC groups. These results indicate that a LINC00312 overexpression inhibits the proliferation, invasion, and migration of TC cells and that this can be achieved by down-regulating miR-197-3p.

Introduction

Thyroid cancer (TC) accounts for only 1% of all the malignancies and is a common endocrine malignancy, which is derived from follicular thyroid or parafollicular C cells [1]. In China in 2000, the incremental incidence and mortality rates of TC in patients below 74 years were 0.32 and 0.03%, respectively [2]. The overall 10-year survival rate of differentiated TC is above 80%, 5–20% of patients develop local or regional recurrences and 10–15% develop distant metastases [3]. Conventional surgery such as adjuvant ablation by radioiodine treatment has been the main therapy for TC. In most cases, however, it is often incurable [1]. Identifying genetic factors can facilitate the treatment and prevention of TC, but the ideal genetic marker for TC detection has not yet been identified [4]. Recently, researchers have attempted to
explore long noncoding RNAs (lncRNAs) as a possible regulator in tumorigenesis and tumor development. To date, only a few lncRNAs such as lncRNA PTSC3, lncRNA NAMA, and lncRNA BANCR have been proven to be involved in TC development and progression [5-7].

lncRNAs have little to no protein coding ability, transcripts which exhibit cell-type specific expression, localization of subcellular compartments, or correlation with human diseases. Many lncRNAs are processed to yield small RNAs [8]. Long intergenic noncoding RNA LINC00312 (LINC00312), also known as NAG7, is a long intergenic noncoding RNA located on 3p25.3 and has been demonstrated to be a novel putative tumor suppressor gene [9]. Subsequently, it has been reported that LINC00312 may act as a potential biomarker for metastasis, progression, and prognosis in nasopharyngeal carcinoma [10]. In addition, miRNAs are small noncoding RNAs which can affect tumorigenesis through different mechanisms [11]. miR-197-3p belongs to miR-197 family and has been demonstrated to have a low expression in primary biliary cirrhosis [12]. In basal cell carcinoma, miR-197-3p is usually overexpressed, and can inhibit keratinocyte proliferation and migration [13]. LINC00312 has been reported to have a negative correlation with miR-197-3p in bladder cancer. It has also been reported that LINC00312 can inhibit the invasion and metastasis of bladder cancer cell by down-regulating miR-197-3p [14]. lncRNAs and miRNAs have been found to be significantly associated with TC. For example, lncRNA H19 regulates YES1 expression by binding miR-17-5p and miR-146a polymorphism predisposing patients to TC [15,16]. However, the effects of LINC00312 and miR-197-3p have not been proven on TC. Therefore, this research was conducted to investigate the involvement of LINC00312 and miR-197-3p in TC and demonstrate their effect on the proliferation, invasion, and migration ability of TC cells.

**Materials and methods**

**Ethical statement**

The study was approved by the ethical committee of the First Affiliated Hospital of Nanchang University. All research tissues were obtained from patients who had signed informed consent forms.

**Study subjects**

The study included 211 TC tissues and 70 adjacent normal tissues (2 cm away from the tumor site) obtained from 211 TC patients (99 females and 112 females) who were diagnosed with TC. All patients received primary surgical resection at the First Affiliated Hospital of Nanchang University between October 2013 and August 2015. All the samples were confirmed via pathological examination, all patients had not received any previous treatment and had no severe systemic diseases such as malignant tumors or severe systemic infections. The average age of patients was 46.43 ± 14.27 years (ranging from 20 to 75 years). According to the tumor node metastasis (TNM) staging standards [17] published by the Union for International Cancer Control (UICC), there were 190 patients in phase I/II and 21 patients in phase III/IV [17]. Sixty-nine patients had lymph node metastasis and 142 patients did not show lymph node metastasis. Seventy-two patients had tumor diameter \(≥ 1.0 \) cm and 139 patients had tumor diameter \(< 1.0 \) cm. One hundred and eight patients had papillary TC, 54 patients had follicular TC, 36 patients had squamous TC, and 13 patients had anaplastic TC. The samples were preserved at \(-70^\circ C\) for further use.

**Cell culture**

K1 (papillary TC), SW579 (squamous TC), and 8505C (anaplastic TC) cell lines (Chinese Academy of Sciences, Shanghai, China) were used in our study. Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) (Promega, Madison, WI, U.S.A.) containing 15% FBS (HyClone, Logan, Utah, U.S.A.) and 1% streptomycin at 37°C with 95% relative humidity and 5% CO₂. Cells with 80% adherence were used for subculturing. Cells were then rinsed twice with PBS and digested with trypsin (Gibco Company, Grand Island, NY, U.S.A.). The trypsin was removed when the intercellular space was enlarged. Cells were routinely passaged without suspension cells in the above-mentioned culture medium.

**Luciferase reporter gene assay**

The potential target gene and fragment sequences containing miR-197-3p reaction sites were analyzed using microRNA.org. The DNA was extracted in strict accordance with the instructions of the TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing, China). The p120 3’-UTR wild-type (WT) sequence named p120-3’-UTR-WT was 5’-CACCCTTTATTTTTTTTGTTGGAAT-3’ and the mutant sequence of p120 3’-UTR missing the binding site with miR-197-3p named p120-3’-UTR-Mut was 5’-CACCCTTTATTTTTTTTGACAGTCCT-3’. The luciferase reporter gene vector was constructed and TC cells were transfected. Luciferase reporter gene assay kits (Promega, Madison, WI, U.S.A.) were used to detect the luciferase activity of samples. At 48 h after transfection, the culture medium was
Table 1 Primer sequences for NCs, miR-197-3p mimics, and miR-197-3p inhibitors

| Gene          | Sequence                      |
|---------------|-------------------------------|
| NCs           | 5′-CAGUACUUJULJUGUGUAGUACAA-3′|
| miR-197-3p mimics | 5′-UUCCACCUUUCUGCCACCCAGC-3′ |
| miR-197-3p inhibitors | 5′-GCUUGGUGAACAGUGUGUGA-3′ |

Table 2 Primer sequences of LINC00312, miR-197-3p, p120, U6, and β-actin for quantitative real-time PCR

| Gene      | Sequence                                                                 |
|-----------|---------------------------------------------------------------------------|
| LINC00312 | F: 5′-AAGCGAACCAAGCACAATA-3′                                               |
|           | R: 5′-CAAATCCCTGAAAACCTG-3′                                               |
| miR-197-3p| F: 5′-AGTTGTCCAGCACCTCTCCAC-3′                                           |
|           | R: 5′-TACGGTTGTACTCAGGCGATCA-3′                                          |
| p120      | F: 5′-GGACACCCCTCAGACCCCTG-3′                                            |
|           | R: 5′-GGTTGCTAAGACCTCTAGCTC-3′                                           |
| U6        | F: 5′-GTCGTCGTTCAAGCAAGCATA-3′                                           |
|           | R: 5′-AAAAATAGGACACGCTAGAATTG-3′                                          |
| β-actin   | F: 5′-CACGCAAGCAGACTATGAC-3′                                             |
|           | R: 5′-GAAAGGATGTAACGCAAACTA-3′                                           |

Abbreviations: F, forward; R, reverse.

removed and the samples were washed twice with 0.1 M PBS (8 g NaCl, 0.2 g KCl, 3.58 g Na₂HPO₄·12H₂O, and 0.24 g KH₂PO₄ mixed and dissolved with double distilled water to 100 ml, pH 7.4). Passive lysis buffer (100 µl) was added into each well. Samples were slightly oscillated at room temperature for 15 min and then the cell lysis buffer was collected. Two seconds of prereading was conducted before 10 s of reading. The sample volume of Luciferase Assay Reagent II (LARII) and Stop & Glo® Reagent was 100 µl. The luminotron or luminous plate (20 µl per sample) which had been added with LARII and Stop & Glo® Reagent was placed into the biological luminous detector (type Modulus™, Turner BioSystems, Inc., Sunnyvale, CA, U.S.A.).

Cell transfection and grouping
The primer sequences of the negative control (NC) plasmid, miR-197-3p inhibitors plasmid, and LINC00312 overexpression + miR-197-3p mimics plasmid were constructed by Sangon Biotech, Shanghai, China (Table 1) based on the miR-197-3p sequence issued by National Center for Biotechnology Information. After trypsin digestion, the second-generation cells were seeded into a 24-well plate and the culture medium was abandoned after the cells displayed monolayer growth. The cells were divided into a blank (no transfection), NC (cells transfected with NC plasmids), LINC00312 overexpression (cells transfected with LINC00312 overexpression plasmids), miR-197-3p inhibitors (cells transfected with miR-197-3p inhibitor plasmids), and LINC00312 overexpression + miR-197-3p mimics group (cells transfected with LINC00312 overexpression + miR-197-3p mimic plasmids).

Quantitative real-time PCR
Total RNA was extracted from cancer tissues and adjacent normal tissues using the TRIzol standard method (Invitrogen, Carlsbad, CA, U.S.A.). Isolated RNA was dissolved in 0.1% diethylpyrocarbonate water (Fermentas, Glen Burnie, MD, U.S.A.) and then optical density (OD) at 260/280 nm and RNA quality were determined. Primer sequences design is shown in Table 2. Reverse transcription and PCR were also conducted in accordance with the manufacturer’s instructions. Reverse transcription was carried out three times as follows: 10 min at 74°C, 2 min of ice bathing, 60 min at 42°C, and 10 min at 70°C. PCR was performed three times in the following sequence: predenaturation at 95°C for 1 min, followed by 40 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. The relative expression of target genes were calculated using the 2⁻ΔΔCt method with U6 as the internal reference for miR-197-3p and β-actin for other target genes. Each experiment was repeated three times.

Western blotting
Cell samples were washed with precooled 0.1 M PBS and then lysed with cell lysis solution (RIPA). Ice bathing was conducted for 30 min, followed by centrifugation at 12000 rpm at 4°C for 10 min. The supernatant was collected
and preserved at −20°C for further use. BSA (2 μg/μl) was diluted with 0.1 M PBS to the following concentrations: 20, 15, 10, 5, 2.5, and 0 μg/ml. Protein concentration was detected using BCA (Thermo Fisher Scientific Inc., MA, U.S.A.) according to the instructions and sample numbers. The protein was electrophoresed in a 4°C chromography cabinet with 80 V of compressive gel and 120 V of separation gel. The electrophoresed proteins were transferred on to a PVDF membrane, blocked in TBST (25 mM Tris, 140 mM NaCl, and 0.1% Tween 20, pH 7.5) containing 5% skimmed milk and incubated for 2 h. The proteins were combined with the primary antibody of p120 (1:500; article number ab10297; Abcam Inc., Cambridge, MA, U.S.A.) and β-actin (1:10000; article number ab8226; Abcam Inc., Cambridge, MA, U.S.A.) and incubated at 4°C overnight. After being washed (3 × 10 min) with TBST, the secondary antibody was added and incubated at room temperature for 1 h. Subsequently, the cells were rewashed with TBST (3 × 10 min) and chemiluminescence was conducted. The results were analyzed according to the developed and fixed X-ray films.

**CCK8 assay**
Cells in the logarithmic phase of each group were digested with trypsin to regulate cell concentration. Cells were seeded into six-well plates (1 × 10^5 cells/well), cultured in an incubator (Heal Force HF90, HealForce Biomedical Technology Holding Group, Hongkong, China) for 24 h and then transfected. Subsequently, the cells were digested with trypsin and the cell culture medium was collected and placed into centrifuge tubes. Once the cell concentration reached 3 × 10^3/ml, cells were seeded in four 96-well plates (3000 cells/well). Four groups were set in each plate, and four replicates were set in each group. The samples were incubated in culture plates with 5% CO_2 at 37°C. The culture plates were placed in an incubator and were taken out after 24, 48, 72, and 96 h. CCK8 solution (10 μl, Sigma, SF, U.S.A.) was added into each well (no bubbles were observed when the mixture was combined). The culture plates were placed in an incubator at 37°C with 5% CO_2 for 2 h. The absorption value was measured at 450 nm using a microplate reader (Bio-Rad, Cal, U.S.A.). The experiment was repeated three times.

**Transwell assay**
The wells in the upper chamber were coated with 30 μl of Matrigel (dissolved overnight, diluted with FBS-free DMEM in triplicate volume and added at a 15-min interval). Each well in the upper chamber was inoculated with 2 × 10^4 cells. DMEM (0.5 ml) containing 10% FBS was added into each well of the lower chamber. After 24 h of incubation, the cells were fixed with parafomaldehyde and stained with 0.1% Crystal Violet for 0.5 h to remove any uninfected cells from the upper chamber. Next, cells were washed with 0.1 M PBS, counted and photographed in randomly selected fields with a light microscope. The experiment was repeated three times. The mean value of cells which penetrated through the Matrigel was analyzed to determine the cells’ migration ability.

**Scratch test**
Cells were seed into a 12-well plate with 1.0 × 10^4 cells in each well. Three parallel lines were made on the back of each well. After the well was completely covered with cells, the confluent monolayer of each well was scratched with a pipette tip. Cells were then washed with 0.1 M PBS and cultured in a medium. Cells were photographed under a light microscope after 0 and 48 h. The cell position at 0 h was used as the reference point. The relative scratch width of cancer cells was recorded and analyzed to detect the cancer cells invasion ability. The experiment was repeated at least three times.

**Tumor xenograft in nude mice**
TC cells in the logarithmic phase were digested with trypsin to obtain cell suspension. The number of cells was observed with an optical microscope and then cells were collected and preserved at −20°C for further use. Fifty 6–8 week old healthy nude mice (Hunan SIA Laboratory Animal Co. Ltd, Changsha, China) were randomly divided into three groups with five mice in each group. The mice were injected with the transfected cell suspension (1 × 10^5 cells) subcutaneously and then fed conventionally. After the tumor formation, its size (length, width, and height) was roughly calculated every 2 days. After 3 weeks, the mice were killed via cervical dislocation and photos were taken.

**Statistical analysis**
The SPSS 21.0 software (SPSS Inc., Chicago, IL, U.S.A.) was utilized for data analysis. Measurement data are expressed as mean ± S.D. and the comparison between the two groups was measured using the independent t test. One-way ANOVA was used to compare multiple groups and LSD-t was adopted for pairwise comparisons. A P-value less than 0.05 is considered statistically significant.
Results

Expression levels of LINC00312 and \textit{miR-197-3p} in TC and adjacent normal tissues

The expression levels of LINC00312 and \textit{miR-197-3p} in TC tissues and adjacent normal tissues were detected via quantitative real-time PCR (qRT-PCR). Figure 1A demonstrates that the expression level of LINC00312 in TC tissues (1.02 ± 0.33) is significantly lower than that of the adjacent normal tissues (3.89 ± 0.58) (\(P<0.05\)). Figure 1B displays that \textit{miR-197-3p} is significantly higher in TC tissues (2.94 ± 0.65) (\(P<0.05\)) than in adjacent normal tissues (1.41 ± 0.36).

Correlation between the expression level of LINC00312 and \textit{miR-197-3p} and the clinicopathological characteristics of TC patients

Table 3 highlights that LINC00312 expression is significantly lower in patients with anaplastic TC, lymph node metastasis, tumors in stage III/IV, or have tumor diameter \(\geq 1.0\) cm (all \(P<0.05\)). The expression level of \textit{miR-197-3p} is
p120 was confirmed to be a target of miR-197-3p

The targetted site at which p120 combined with miR-197-3p was predicted using the online software TargetScan. Figure 2A shows the binding sequences of 3′-UTR (where the p120 mRNA combines with miR-197-3p). TC cells were transfected with miR-197-3p mimics and WT-miR-197-3p/p120 or Mut-miR-197-3p/p120 recombinant plasmids. The results indicate that miR-197-3p mimics have no significant effect on the luciferase activity of Mut-miR-197-3p/p120 plasmids (0.93 ± 0.03) (P < 0.05). However, they decreased the luciferase activity of the WT-miR-197-3p/p120 plasmid (0.35 ± 0.09) by 65% (P < 0.05) (Figure 2B).

Expression levels of LINC00312, miR-197-3p, p120 in K1, SW579, and 8505C cells after transfection

The expression levels of LINC00312, miR-197-3p, p120 in the K1, SW579, and 8505C cell lines were detected and are displayed in Figure 3. In the 8505C cell line, the expression level of LINC00312 was 0.78 ± 0.13, the mRNA level of p120 was 0.73 ± 0.17 and the protein level of p120 was 1.09 ± 0.12. In the K1 cell line, the expression level of LINC00312 was 1.62 ± 0.18, the mRNA level of p120 was 1.27 ± 0.12, and the protein level of p120 was 2.44 ± 0.36. In the SW579 cell line, the expression level of LINC00312 was 1.21 ± 0.12, the mRNA level of p120 was 1.01 ± 0.11, and the protein level of p120 was 2.01 ± 0.21 (all P < 0.05). The expression level of miR-197-3p in the 8505C, K1, and SW579 cell lines was 1.93 ± 0.11, 0.84 ± 0.09, and 1.21 ± 0.10, respectively (all P < 0.05). Results of the three cell lines in the other groups were similar. For example, in the K1 cell line, the expression levels of LINC00312, miR-197-3p, and the mRNA level of p120 in the blank group was 1.08 ± 0.12, 1.74 ± 0.19, and 0.86 ± 0.09, respectively. In the NC group, the expression levels of LINC00312, miR-197-3p, and the mRNA level of p120 was 1.02 ± 0.08, 1.78 ± 0.12, and 0.84 ± 0.08, respectively. There was no significant difference between the two groups (all P > 0.05). The expression level of LINC00312 in the LINC00312 overexpression group was 1.62 ± 0.18. In the INC00312 + miR-197-3p mimics group, it was 1.64 ± 0.11. Both these results were higher than the blank group (1.08 ± 0.12) (all P < 0.05). There were no significant differences in the expression level of LINC00312 between blank and miR-197-3p inhibitor groups. The expression level of miR-197-3p in the LINC00312 overexpression (0.84 ± 0.09) and miR-197-3p inhibitors group (0.88 ± 0.08) was significantly lower (all P < 0.05) than the blank (1.74 ± 0.19) and NC group (1.78 ± 0.12). There was no significant difference in the expression of miR-197-3p between the
The expression levels of LINC00312, miR-197-3p, and p120 amongst different TC cells detected by qRT-PCR and Western blotting (mean ± S.D.)

(A) The expression levels of LINC00312 and miR-197-3p, and the mRNA level of p120; (B) protein band patterns of p120 amongst different TC cells; the first row from top to bottom, the gray value of p120 protein band in K1 cells; the second row, the gray value of β-actin band in K1 cells; the third row, the gray value of p120 protein band in SW579 cells; the fourth row, the gray value of β-actin band in SW579 cells; the fifth row, the gray value of p120 protein band in 8505C cells; the sixth row, the gray value of β-actin band in 8505C cells; (C) the protein level of p120 by Western blotting; *, P<0.05, compared with the blank group; †, P<0.05, compared with the NC group; ‡, P<0.05, compared with the LINC00312 overexpression + miR-197-3p mimics group; four wells of cells in each group were repeated, and the experiment was repeated for three times.

Proliferation abilities of K1, SW579, and 8505C cells amongst five groups (mean ± S.D.)

*, P<0.05, compared with the blank group; †, P<0.05, compared with the NC group; ‡, P<0.05, compared with the LINC00312 overexpression + miR-197-3p mimics group; four wells of cells in each group were repeated, and the experiment was repeated for three times.

blank (1.74 ± 0.19) and LINC00312 overexpression + miR-197-3p mimics groups (1.71 ± 0.11). In comparison with the blank (0.86 ± 0.09 and 1.93 ± 0.29) and NC groups (0.84 ± 0.09 and 1.76 ± 0.28), the expression level of p120 was higher in the LINC00312 overexpression (1.27 ± 0.12 and 2.44 ± 0.36) and miR-197-3p inhibitors groups (1.35 ± 0.20 and 2.48 ± 0.38) (all P<0.05). There was no significant difference in the expression of p120 in the blank and LINC00312 overexpression + miR-197-3p mimic groups (0.79 ± 0.08 and 1.85 ± 0.31). The expression level of K1 cell line in the LINC00312 group was much higher than that of the LINC00312 + miR-197-3p mimics group. The results of the other two cell lines were in-line with the results of the K1 cell line.

LINC00312 inhibits K1, SW579, and 8505C cell proliferation by down-regulating miR-197-3p

The proliferation of K1, SW579, and 8505C cells was measured via CCK8 assay (Figure 4). The OD value of the TC cell lines at 48, 72, and 96 h after transfection demonstrate that 8505C cells have the strongest proliferation ability (0.38 ± 0.02, 0.60 ± 0.03, and 0.70 ± 0.03). The proliferation ability of K1 cells was the weakest (0.13 ± 0.02, 0.25 ±
miR-197-3p down-regulating LINC00312 suppresses K1, SW579, and 8505C cell invasion by
increasing the number of invasive cells, the number of invasive cells was higher in the blank group than in the LINC00312 overexpression + miR-197-3p mimics group (all P<0.05). These results indicate that LINC00312 inhibits K1, SW579, and 8505C cell proliferation by down-regulating miR-197-3p.

LINC00312 reduces K1, SW579, and 8505C cell migration by down-regulating miR-197-3p

Figure 6 presents the scratch width percentages of each cell line 48 h after transfection in the blank, NC, LINC00312 overexpression, miR-197-3p inhibitors, and LINC00312 overexpression + miR-197-3p mimics groups (the same...
The tumor xenograft results in nude mice are presented in Figure 7. In the LINC00312 overexpression group, tumor size was largest in 8505C cells (689.05 ± 65.61), smallest in K1 cells (239.05 ± 47.91) and SW579 cells were in between (451.82 ± 79.08) (all P<0.05). The overexpression of LINC00312 and low expression of miR-197-3p significantly weakened the thyroid tumorigenesis of K1, SW579, and 8505C cells in nude mice. There was no significant difference in the tumor size of nude mice in the blank (627.21 ± 113.29), NC (682.21 ± 79.08), and LINC00312 overexpression + miR-197-3p mimic groups (649.35 ± 70.54) in the K1, SW579, and 8505C cell lines (all P>0.05). In the K1, SW579, and 8505C cells, tumor size in the LINC00312 overexpression (239.05 ± 49.71) and miR-197-3p inhibitor groups (221.82 ± 54.37) were significantly smaller than the blank (27.21 ± 113.29) and the NC groups (682.21 ± 79.08) (all P<0.05). Tumor size in the LINC00312 overexpression group (239.05 ± 49.71) was significantly smaller than in the LINC00312 overexpression + miR-197-3p mimics group (649.35 ± 70.54) (P<0.05). The results demonstrate that LINC00312 inhibits the in vivo tumorigenesis of K1, SW579, and 8505C cells by down-regulating miR-197-3p. The results also reveal that miR-197-3p mimics reverse the tumorigenic ability of TC cells.

Discussion

TC is an endocrine malignancy, which usually forms as a result of ionizing radiations [18]. Although death rates associated with TC have declined in most parts of the world, TC incidence has been continuously rising over the recent decades [19]. It has been recently revealed that a relationship exists between TC and IncRNAs such as NR_036575.1 and PVT1. The expression of these IncRNAs contribute to the biological changes in TC cells [5,20]. Our study aims to detect the effect of LINC00312 on TC by regulating miR-197-3p. The results demonstrate that LINC00312 overexpression can inhibit the proliferation, migration and invasion of TC cells by down-regulating the expression of miR-197-3p.
Figure 7. Effects of LINC00312 on thyroid tumorigenesis by down-regulating miR-197-3p (mean ± S.D.)

(A) The tumor measurement of nude mice in each group; (B) the tumor size of nude mice in each group; *, P<0.05, compared with the blank group; †, P<0.05, compared with the NC group; ‡, P<0.05, compared with LINC00312 overexpression + miR-197-3p mimics group; four wells of cells in each group were repeated, and the experiment was repeated for three times.

Most importantly, the present study indicated that TC tissues have a lower expression of LINC00312 and a higher expression of miR-197-3p than the adjacent normal tissues. The role of miRNAs and lncRNAs during the transcription and post-transcriptional stages has been widely acknowledged [21]. LncRNAs aberrant expression in human cancer tissues may be correlated with tumor metastasis and disease prognosis [22]. In addition, Yang et al. [23] revealed that the altered expression of lncRNAs can induce gene aberrant expression and tumorigenesis in human papillary TC. Zhang et al. [10] advocated that there is a low LINC00312 expression in nasopharyngeal carcinoma. A recent study conducted by Wang et al. elucidated that LINC00312 is negatively associated with miR-197-3p in bladder cancer [14]. Our study also found a relatively high expression of LINC00312 and low expression of miR-197-3p in normal tissues [14]. In TC specimens, the up-regulation of miR-197-3p may be regulated by LINC00312 through its endogenous RNA or miRNA sponges [22].

In addition, the present study highlights that a low expression of LINC00312 may appear in patients with lymphatic metastasis, in TNM stage III/IV or have larger tumor diameters. Conversely, miR-197-3p is expressed highly in patients with such clinicopathological characteristics. In nasopharyngeal carcinoma, LINC00312 was revealed to be positively correlated with lymphatic metastasis and negatively correlated with tumor diameter [10]. Moreover, according to Sun et al. [20], IncRNA NR_036575.1 is significantly correlated with tumor diameter and extrathyroidal extension in papillary TC. Furthermore, the results indicate that the expression levels of LINC00312 and miR-197-3p are related to TC tissue-type. Therefore, LINC00312 and miR-197-3p may be assistant proofs for detecting TC clinicopathological characteristics.

Moreover, it was demonstrated that LINC00312 overexpression can weaken the migration, invasion, and tumorigenesis ability (in vivo) of TC cells by down-regulating miR-197-3p. Additionally, the expression level of p120 was proved to be negatively correlated with miR-197-3p expression and positively correlated with LINC00312 expression. This further validates the role of LINC00312 and miR-197-3p in TC cells. We confirmed that p120 is a direct target gene of miR-197-3p. MiR-197-3p is a mature body of miR-197, and miR-197 is associated with the expression of various tumor-suppressor genes such as FUS1 and NLK [24,25]. Hamada et al. [26] stated that miR-197 directly targets p120 through the transient expression of miR-197 and the 3’-UTR assay of p120 mRNA. Additionally, a lack of p120 is likely to increase the risk of cancer cell migration and invasion in the buccal cavity, gullet, and fore stomach. This is due to the fact that p120 mediates cadherin constancy and turnover [27]. Moreover, miR-197-3p overexpression in ovarian cancer can stimulate cancer cell (A2780) proliferation and invasion [25]. MiR-197-3p has also been shown to facilitate pancreatic cancer cells invasion and migration [26]. A recent study indicated that LINC00312 can suppress the invasion and metastasis of bladder cancer cell by directly mediating miR-197-3p [14]. Therefore, we hypothesize
that LINC00312 can inhibit the migration, invasion, and tumorigenesis abilities of TC cells indirectly by regulating p120 and miR-197-3p.

According to the transwell and scratch test, the 8505C cell line (anaplastic TC) had the strongest invasion and migration abilities, K1 (papillary TC) had the weakest, and SW579 (squamous TC) was in between. Anaplastic TC is a highly aggressive malignancy [28], whereas K1 papillary TC is well differentiated and has a good prognosis [29]. Squamous TC is an aggressive neoplasm which has a bad prognosis [30]. In terms of differentiation, 8505C is undifferentiated, K1 is well differentiated, and SW579 is poorly differentiated. The results observed may be caused by the negative correlation between the invasion and migration ability, and the degree of cell differentiation.

Overall, we demonstrated that the migration and invasion of TC cells can be suppressed by LINC00312 overexpression by down-regulating miR-197-3p. Our study also indicated that LINC00312 and miR-197-3p may be potential therapeutic targets for TC. However, TC is influenced by various genes through a complex mechanism. Additionally, the sample size of research which studied the clinicopathological features of TC was limited. Therefore, the exact monocular mechanism behind LINC00312 effect on miR-197-3p is still awaiting further specific research.

Acknowledgements
We thank the reviewers for their critical comments on this article.

Funding
The authors declare that there are no funding associated with the manuscript.

Competing interests
The authors declare that there are no competing interests associated with the manuscript.

Author contribution
W.H. designed the study. K.L. and X.M. collated the data, designed and developed the database, carried out data analyses, and produced the initial draft of the manuscript. D.-Q.Y. and Q.L. contributed to drafting of the manuscript. All authors have read and approved the final submitted manuscript.

Abbreviations
DMEM, Dulbecco’s modified Eagle’s medium; LARI, luciferase assay reagent II; LINC00312, long intergenic noncoding RNA LINC00312; IncRNA, long noncoding RNA; LSD-t, least significant difference; NC, negative control; OD, optical density; qRT-PCR, quantitative real-time PCR; TBST, Tribs-buffered saline Tween-20; TC, thyroid cancer; TNM, tumor node metastasis; WT, wild-type.

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