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ARTICLE

Expression of ITGB1 and E-cadherin regulated by lncLSINCT5 sponging on miR-29c-3p in papillary thyroid cancer cells

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Papillary thyroid carcinoma (PTC) is the most predominant subtype of thyroid cancer, contributing to more than 80% of all thyroid or endocrine malignancies. However, the role of Long noncoding RNA long stress induced non-coding transcripts (LSINCT5) in papillary thyroid cancer remains largely unknown. In the present study, we found that the expression of LSINCT5 in PTC cell line was higher than that in human normal thyroid cell HT-ori3. The proliferation and migration ability of PTC cell lines TPC-1 and KAT-5 cells were significantly decreased after transfection of siLSINCT5-1 and siLSINCT5-2 compared with siNC transfection. The dual luciferase reporter gene and RIP confirmed that LSINCT5 is capable of specifically binding to miR-29c. Compared with the transfected LSINCT5 group, the proliferation and migration ability of TPC-1 cells in the co-transfected LSINCT5 and miR-29c groups were significantly decreased, and the expression of ITGB1 mRNA and protein were down-regulated either. Taken together, our data indicated that LSINCT5 can inhibit the tumor suppressive effect of miR-29c-ITGB1 axis and promote the proliferation and metastasis of PTC by targeting miR-29c-ITGB1 axis.

Keywords: long non-coding RNAs; LSINCT5; miR-29c; ITGB1; papillary thyroid cancer

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Papillary thyroid carcinoma (PTC) is the most predominant subtype of thyroid cancer, contributing to more than 80% of all thyroid or endocrine malignancies. Its prognosis is relatively good compared to other cancers, with more than 90% overall 10-year survival rate [1]. Treating recurrent PTCs is still a challenge and one of the important issues yet to be solved in PTC patient management is the mortality and morbidity associated with the recurrent disease. Therefore, in-depth study of the mechanism of PTC development from the molecular level has important clinical significance and value for the diagnosis and treatment of PTC.

Genetic and epigenetic changes are associated with the initiation and progression of a variety of cancers, including PTC. The regulation of long noncoding RNAs (lncRNAs) is an important type of epigenetic regulation. Recent studies have shown that a variety of lncRNAs are transcriptionally dysregulated in cancer, playing a role in promoting cancer or suppressing cancer [2-3]. For example, lncRNAs BANCR, MEG3, and PTCSC3 are transcriptionally down-regulated in PTC to function as tumor suppressors [4-6]. The lncRNAs ANRIL, HOTAIR and MALAT1 are up-regulated in PTC to play a role in cancer promotion [7-8]. However, the mechanism of action of a large number of lncRNAs in PTC is not clear. By reviewing of the evidence, the critical role of long noncoding RNA (lncRNA) LSINCT5 in a large number of human cancers is indicated. However, the mechanistic involvement of LSINCT5 in PTC is still unknown.

MicroRNAs (miRNAs) are short, endogenous noncoding

RNAs first identified in Caenorhabditis elegans which regulate gene expression by binding to the 3′ -UTR of target messenger RNA (mRNAs). Their post-transcriptional regulatory functions are involved in controlling the level of proteins involved in numerous biological processes, including embryogenesis, organogenesis, tissue homeostasis, immune system function and cell cycle control [1]. Relationship between miRNAs and tumor growth, tumor progression and metastasis has been demonstrated by many studies, indicating the ability of these molecules to be used as biomarkers for diagnosis and prognosis [9]. miRNA also plays a role as biomarker in predicting lymph nodes metastasis (LNM) in thyroid cancer [10].

This study shows that knockdown of LSCINC5 expression significantly inhibited the proliferation and metastasis of PTC cell lines TPC-1 and KAT-5. Dual luciferase reporter assay confirms that LSENC5 can compete for competitive binding to miR-29c. Co-transfection experiments further revealed that miR-29c can inhibit the proliferation and metastasis of PTC by LSINCT5 after overexpression of LSINCT5. qRT-PCR and Western blot analysis showed that miR-29c can reverse the up-regulation of ITGB1 expression by LSINCT5 after overexpression of LSINCT5, in addition to promote the expression of E-cadherin. These results indicate that LSINCT5 can function as a molecular sponge of miRNAs, inhibits the tumor suppressor of miR-29c-ITGB1 axis, and promotes proliferation and metastasis of PTC.

Materials and methods

Cell culture

The human normal thyroid cell line HT-ori3 and the PTC cell lines NPA87, KAT-5, and TPC-1 were cultured in DMEM (HyClone) medium containing 10% fetal bovine serum (HyClone). The incubator culture temperature was set to 37 °C and the CO_2 content was 5%.

qRT-PCR detection of relative expression of lncRNA LSINCT5 and ITGB1

Total RNA in tissue and cell samples was extracted using the Trizol method and total RNA was reverse transcribed into cDNA using random primers. The upstream primers and downstream primers corresponding to the premix, cDNA template, lncRNA LSINCT5 and ITGB1 were added according to the qRT-PCR quantitative detection kit (TaKaRa) step. lncRNA LSINCT5 upstream: 5'-TAGTGGACAGAG-CCTACCCC-3', lncRNA LSINCT5 downstream: 5'-CCAGC-TACCACCCACAACAA-3'; ITGB1 upstream: 5'-AGTAGT-GCACTTTTCTCTCATGG-3', ITGB1 downstream: 5'-CCC-GTCCACCCATTTCTACC-3'; internal reference GAPDH upstream: 5'-TGGCTATTGCCGAATCGCTG-3', down-stream of GAPDH: 5'-CCTAGGCTTGAATCGGTCT-3'; reaction conditions: 95 °C for 3 min; 40 cycles of denaturation at 95 °C for 15 s; annealing at 60 °C for 20 s; 72 °C for 20 s, each One cycle collected fluorescence at 72 °C. qRT-PCR results were quantitatively analyzed using the 2-ΔΔCt method.

Detection of proliferation ability of PTC cells transfected with TPC-1 and KAT-5 by CCK-8 assay

The PTC cells TPC-1 and KAT-5 were prepared as single cell suspensions, adjusted to a cell concentration of 2×10^4/mL, and seeded into 96-well plates at a volume of 100 μL per well. After TPC-1 and KAT-5 cells were attached, siNC, siLSINCT5-1, siLSINCT5-2, Control (empty plasmid group), LSINCT5, LSINCT5-Mut, LSINCT5 and miR-29c were transfected, respectively. At 24, 48, and 72 h after transfection, 10 μL of CCK-8 reagent was added to each well and mixed. After continuing to incubate for 1 h at 37 °C, 5% CO_2, the optical density value D (450) at 450 nm of each well was measured using a Bio-Rad iMark microplate reader (the blank control value was automatically subtracted when the instrument automatically reads).

QCMTM Laminin Migration Transfer Kit for the migration of TPC-1 and KAT-5 after transfection

The QCMTM Laminin Migration Kit (Millipore, Germany) was removed from the 4 °C freezer and allowed to equilibrate for 1 h at room temperature. 0.5 mL of complete medium was added to a 12-well plate in the lower chamber of Transwell, and 200 μL of the single cell suspension that had been prepared was added to the Transwell chamber. After co-cultivation for 24 h, the upper layer of Transwell chamber was wiped off with a cotton swab and fixed in 95% ethanol for 20 min. It was placed in a clean bench and dried, then stained with 0.1% crystal violet for 15 min. Wash into the PBS solution several times. After drying again, 3 to 5 fields of view were taken on the diameter of the Transwell chamber, and the number of transmembrane cells was counted after photographing under a microscope.

LSINCT5-Wt and LSINCT5-Mut fluorescent reporter vector construction and luciferase activity assay

The LSINCT5-Wt sequence fragment was amplified by PCR and the LSINCT5-Mut sequence was obtained using the Promega site-directed mutagenesis kit (Promega, Shanghai). The luciferase reporter vector pmir-GLO was inserted after double digestion. miR-29c and the negative control miR-NC were co-transfected into HEK293 cells with the successfully constructed LSINCT5-Wt and LSINCT5-Mut reporter plas-mids. After 24 h of transfection, the corresponding fluore-scence intensity was measured and the relative activity of luciferase was calculated.

Western blotting

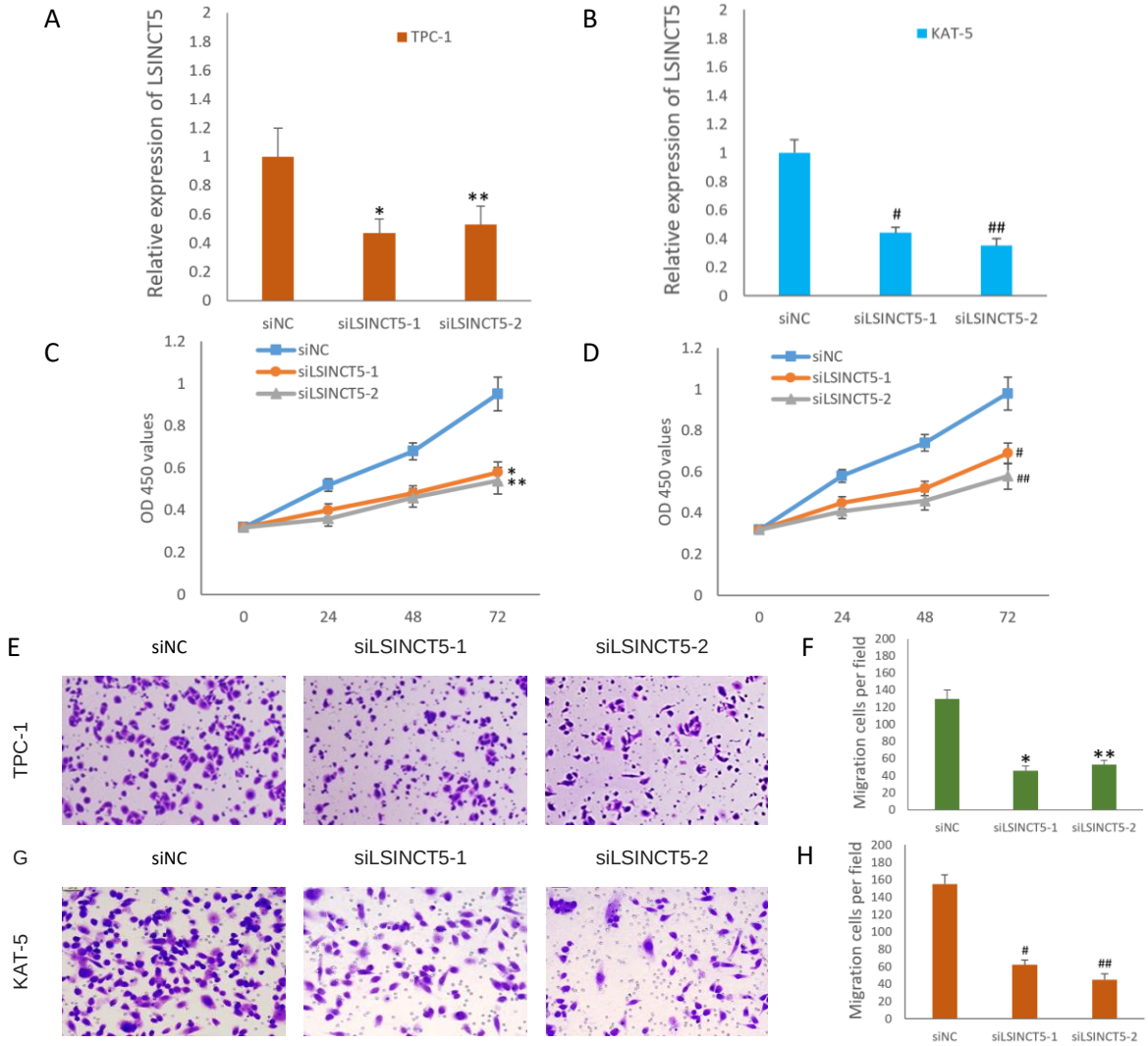

Figure 1. Knockdown of LSINCT5 expression inhibits proliferation and metastasis of PTC cell lines TPC-1 and KAT-5. A, B: qRT-PCR detects the expression level of LSIPT5 in TPC-1 and KAT-5 after transfection of siLSINCT5 (n= 3,x±s); C, D: CCK-8 detects the proliferation of TPC-1 and KAT-5 after transfection of siLSINCT5; E, F: Transwell migration assay detects the change of TPC-1 transfer ability after transfection of siLSINCT5 (n=5, x±s); G, H: Transwell migration assay detected changes in KAT-5 metastasis after transfection of siLSINCT5 (n=5, x±s); a: $P < 0.05$, compared with siNC group.

The TPC-1 cells after lysis with RIPA lysis buffer were centrifuged at 12 000 × g for 15 min, and the supernatant was collected and the protein concentration was determined. Protein loading buffer (5 × SDS) was added in a ratio of 4:1 and boiled in a metal bath for further use. The appropriate amount of the sample was separated by 12% SDS-PAGE gel, wet transferred onto a PVDF membrane, and blocked with 3% skim milk powder, respectively, using mouse-derived anti-ITGB1 primary antibody (Abcam, USA) (1:1 000 dilution), mouse-derived anti-E-cadherin primary antibody (Abcam, USA) (1:800 dilution), mouse-derived anti-GAPDH primary antibody (Abcam, USA) (1:2) Incubate for 1000 overnight, and then incubate with HRP-labeled goat anti-mouse IgG for 1 h. The PVDF membrane after washing the membrane was placed in a dark room exposure imaging apparatus, and a chemiluminescent chromogenic substrate was added to detect the chemiluminescence of the target protein band.

Statistics

Data processing was performed using statistical software SPSS 22.0. Measurement data were expressed as x±s, and differential analysis was performed using t test.

Results

Knockdown of LSINCT5 expression inhibits proliferation and metastasis of PTC cell lines TPC-1 and KAT-5

The expression level of LSENCT5 in TPC-1 and KAT-5 cells after transfection of siLSINCT5 was first examined. The results showed that the expression levels of LSINCT5 in TPC-1 and KAT-5 cells were significantly decreased after transfection of siLSINCT5-1 and siLSINCT5-2 ($P < 0.05$, Fig. 1 A and B). To evaluate the effect of LSINCT5 on the proliferation of TPC-1 and KAT-5 cells, CCK-8 assay was used to detect the proliferation of cells in each group, as shown in Figure 2C and D. Compared with the siNC group, siLSINCT5-1, and siLSINCT5-2 group significantly inhibited the proliferation of TPC-1 and KAT-5 cells at 72 h after transfection ($P < 0.05$). As shown in Fig. 1E~H, Transwell migration experiments showed that the invasive ability of TPC-1 and KAT-5 was significantly inhibited in the siLSINCT5-1 and siLSINCT5-2 groups compared with the siNC group ($P < 0.05$).

lncRNA LSINCT5 targeting and binding to miR-29c

To confirm whether LSISCT5 binds to miR-29c, a dual luciferase reporter assay was performed. As a result, as shown in Fig. 2, the relative luciferase activity values of the co-transfected miR-NC and pmirGLO groups were not significantly different from the relative luciferase activity values of the co-transfected miR-29c and pmirGLO groups. The relative luciferase activity values of the co-transfected miR-NC and LSINCT5-Mut groups were not significantly different from the relative luciferase activity values of the co-transfected miR-29c and LSINCT5-Mut groups. The relative luciferase activity values of the co-transfected miR-NC and LSINCT5 groups were significantly lower than those of the co-transfected miR-29c and LSINCT5 groups, and the total transfection of miR-29c and LSINCT5 groups was significantly lower ($P < 0.05$). The above results indicate that LSINCT5 contains a binding site of miR-29c and is capable of targeting and binding to miR-29c. This result suggests that LSINCT5 may participate in the occurrence and progression of PTC by exerting molecular sponge adsorption function and binding to miR-29c to play a post-transcriptional regulation.

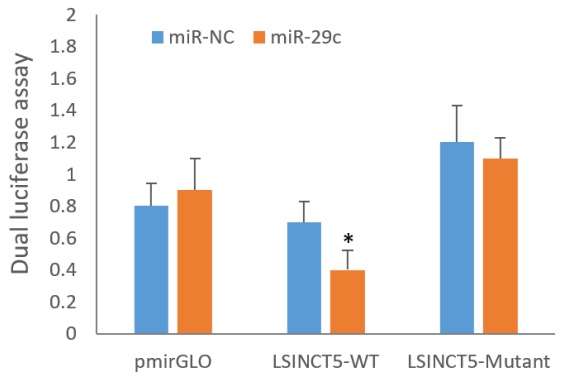

Figure 2. Dual luciferase reporter assay for LSINCT5 binding to miR-29c. *P < 0.05, compared with miR-NC and LSINCT5-Mutant group.

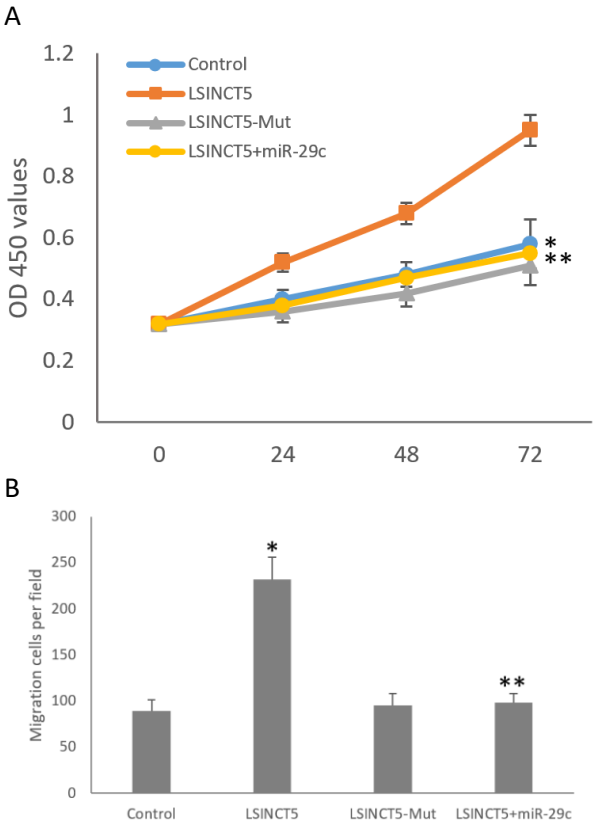

Figure 3. Co-transfection of miR-29c inhibits the effect of LSINCT5 on PTC pro-proliferation and metastasis (n=5, x±s). A: Co-transfection of miR-29c inhibits LS-promoting effect of LSINCT5; B: co-transfection miR-29c inhibited LSINCT5 on PTC-induced metastasis. *P < 0.05, compared with Control group; **P < 0.05, compared with the LSINCT5 group.

Co-transfection of miR-29c inhibits the proliferation of PTC by LSINCT5

The results of the proliferation assay are shown in Figure 3A: compared with the Control (empty plasmid) group, the cell proliferation ability of the LSINCT5 group was significantly increased 72 h after transfection ($P < 0.05$); compared with the LSINCT5 group, the proliferation of TPC-1 cells of the LSISTIN5+miR-29c group was significantly decreased at 72 h after transfection ($P < 0.05$). Compared with the Control group (Fig. 3), the cell migration ability of the LSINCT5 group was significantly increased 72 h after transfection ($P < 0.05$); compared with the LSINCT5 group, the LSCINC5+miR-29c group of TPC-1 cells Cell migration ability decreased significantly at 72 h after transfection ($P < 0.05$). Those results further suggests the binding of LSINCT5 to miR-29c, while overexpression of miR-29c antagonizes the role of LSINCT5 in promoting PTC proliferation and metastasis after binding to endogenous miR-29c.

Co-transfection of miR-29c inhibits the upregulation of ITGB1 by LSINCT5

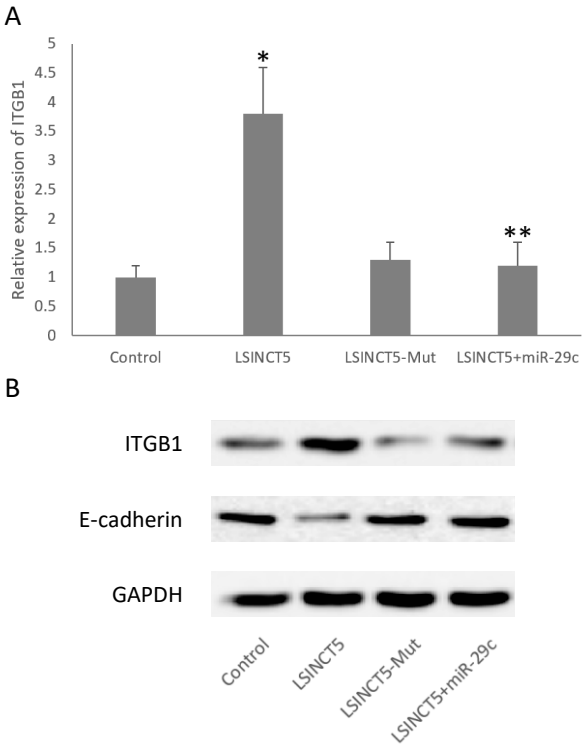

Figure 4. Co-transfection of miR-29c inhibits the upregulation of ITGB1 by LSINCT5. A: qRT-PCR detects the expression level of ITGB1 in each group after transfection (n=3, x±s); B: Western blot detection The expression of ITGB1 and E-cadherin in the cells after staining. *P < 0.05, compared with Control group; **P < 0.05, compared with the LSINCT5 group.

Compared with the Control group (Fig. 4A), the expression level of ITGB1 was significantly increased at 72 h after transfection in the LSINCT5 group (P < 0.05); compared with the LSINCT5 group, the expression level of ITGB1 in the TPCTIN5+miR-29c group was significantly decreased at 72 h after transfection (P < 0.05). The expression level of ITGB1 protein is consistent with the qRT-PCR results (Fig. 4B). At the same time, compared with the Control group, the expression level of E-cadherin was decreased at 72 h after transfection in the LSINCT5 group. Compared with the LSINCT5 group, the expression level of E-cadherin in the LSCINC5+miR-29c group at 72 h after transfection is up-regulated. These results indicate that co-transfection of miR-29c can reverse the up-regulation of ITGB1 expression by LSINCT5 and promote the expression of E-cadherin after overexpression of LSINCT5. Moreover, the result suggests that LSINCT5 inhibits the anti-cancer effect of miR-29c-ITGB1 axis by targeting and binding to miR-29c, and further promotes the proliferation and metastasis of PTC.

Discussion

Papillary thyroid carcinoma is the most predominant subtype of thyroid cancer, contributing to more than 80% of all thyroid or endocrine malignancies. Its prognosis is relatively good compared to other cancers, with more than 90% overall 10-year survival rate [1]. However, despite the favorable prognosis, some cases exhibit aggressive phenotype. On average, 50% of the patients are presented with lymph node metastases (LNM) at diagnosis [11]. LNM in PTC confers various poor prognostic indicators; it increases recurrence risk and decreases long term survival predominantly in patients older than 45 years old [12, 13]. Treating recurrent PTCs is still a challenge [14] and one of the important issues yet to be solved in PTC patient management is the mortality and morbidity associated with the recurrent disease [15].

MicroRNAs (miRNA) are small non-coding RNAs of approximately 19-25 nucleotides in length that are responsible for the posttranscriptional regulation of gene expression in both eukaryotic and prokaryotic cells. Over the past decade, many studies have shown that miRNA dysregulation plays an important role in cancer development through the regulation of essential roles in tumor cell differentiation, migration, apoptosis and proliferation [16-22]. These regulatory processes are controlled through the over and under expression of specific miRNAs, leading to amplified expression of oncogenic and tumour suppressing genes. The unique miRNA profiles have shown potential in the development of cancer biomarkers and novel drugs. The alterations in miRNA also translated to the clinical and pathological impacts of patients with cancer.

Recent studies have shown that epigenetic regulation is one of the most important regulatory approaches in the body. lncRNAs can participate in epigenetic regulation of the body through chromatin remodeling, transcriptional regulation, and post-transcriptional. It is involved in cell differentiation, proliferation, apoptosis, neurodevelopment, immune homeostasis, tumorigenesis and other life processes.

This study shows that knockdown of LSCINC5 expression significantly inhibited the proliferation and metastasis of PTC cell lines TPC-1 and KAT-5. Dual luciferase reporter assay confirms that LSENC5 can compete for competitive binding to miR-29c. Co-transfection experiments further revealed that miR-29c can inhibit the proliferation and metastasis of PTC by LSINCT5 after overexpression of LSINCT5. qRT-PCR and Western blot analysis showed that miR-29c can reverse the up-regulation of ITGB1 expression by LSINCT5 after overexpression of LSINCT5, in addition to promote the expression of E-cadherin. These results indicate that LSINCT5 can function as a molecular sponge of miRNAs, inhibits the tumor suppressor of miR-29c-ITGB1 axis, and promotes proliferation and metastasis of PTC.

Conflicting interests

The authors have declared that no conflict of interests exist.

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