Long Noncoding RNA H19 Inhibits Cell Viability, Migration, and Invasion Via Downregulation of IRS-1 in Thyroid Cancer Cells

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Abstract
Thyroid cancer is a common endocrine gland malignancy which exhibited rapid increased incidence worldwide in recent decades. This study was aimed to investigate the role of long noncoding RNA H19 in thyroid cancer. Long noncoding RNA H19 was overexpressed or knockdown in thyroid cancer cells SW579 and TPC-1, and the expression of long noncoding RNA H19 was detected by real-time polymerase chain reaction. The cell viability, migration, and invasion were determined by 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide assay, Transwell assay, and wound healing assay, respectively. Furthermore, cell apoptosis was analyzed by flow cytometry, and expressions of some factors that were related to phosphatidylinositol 3-kinases/protein kinase B and nuclear factor κB signal pathway were measured by Western blotting. This study revealed that cell viability and migration/invasion of SW579 and TPC-1 were significantly decreased by long noncoding RNA H19 over-expression compared with the control group (P < .05), whereas cell apoptosis was statistically increased (P < .001). Meanwhile, cell viability and migration/invasion were significantly increased after long noncoding RNA H19 knockdown (P < .05). Furthermore, long noncoding RNA H19 negatively regulated the expression of insulin receptor substrate 1 and thus effect on cell proliferation and apoptosis. Insulin receptor substrate 1 regulated the activation of phosphatidylinositol 3-kinases/AKT and nuclear factor κB signal pathways. In conclusion, long noncoding RNA H19 could suppress cell viability, migration, and invasion via downregulation of insulin receptor substrate 1 in SW579 and TPC-1 cells. These results suggested the important role of long noncoding RNA H19 in thyroid cancer, and long noncoding RNA H19 might be a potential target of thyroid cancer treatment.

Keywords
thyroid cancer, long noncoding RNA H19, cell proliferation, apoptosis, insulin receptor substrate 1

Abbreviations
ATC, anaplastic thyroid carcinomas; Bcl-2, B-cell lymphoma 2; FBS, fetal bovine serum; IRS-1, insulin receptor substrate 1; lncRNA, long noncoding RNA; mRNA, messenger RNA; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide; NF-κB, nuclear factor κB; PI, prodium iodide; PTC, papillary thyroid carcinomas; RT-PCR, real-time polymerase chain reaction; shNC, small hairpin RNA negative control; shRNA, small hairpin RNA.

Introduction
Thyroid cancer is the most common endocrine gland malignancy, and the incidence of thyroid cancer has continuously and sharply increased worldwide with uncertain reasons. Thyroid cancer is composed of follicular cells, parafollicular cells, and connective tissue with occasional lymphocytes. Thyroid cancer is generally divided into papillary thyroid...
carcinomas (PTCs), follicular carcinomas, medullary thyroid carcinomas, anaplastic thyroid carcinomas (ATCs), primary thyroid lymphomas, and primary thyroid sarcomas. More than 80% of all thyroid cancers are PTCs. Follicular carcinoma is the second most common thyroid cancer, accounting for approximately 10% of cases. Additionally, ATC is the most aggressive form of thyroid cancer with a poor prognosis that might cause acute airway compromise which could lead death of patients with ATC. Even if benign thyroid disease is relatively common and patients with thyroid cancers generally have a favorable prognosis compared with many other solid tumors, treatment of patients with thyroid malignancy is still a very complicated process that requires a multidisciplinary approach involving endocrinology, thyroid surgery, radiology, and on occasion medical and radiation oncology. Therefore, in-depth understanding of the pathogenesis of thyroid cancer may contribute to the clinical treatment.

Long noncoding RNA (lncRNA) is a group of endogenous transcripts that are longer than 200 nucleotides. A large number of lncRNAs have been identified in mammalian transcriptomes over the past few years. To date, studies about lncRNAs were mostly focused on regulation effect on gene expression at transcriptional and posttranscriptional levels. Long noncoding RNA was previously regarded as “noise” of the genome owing to the lack of protein-encoding capacity. However, more and more emerging evidences indicated that the lncRNAs had multiple biological functions that were involved in cell proliferation, survival, differentiation, and chromatin remodeling. Numerous of lncRNAs have been reported to be involved in different types of cancers. Among that, LncRNA H19 was considered as a vital player in cancer development. Aberrant alterations in LncRNA H19 expression have been demonstrated in various tumors, and it also actively participated in almost all stages of tumorigenesis. Long noncoding RNA H19 was one of the first identified imprinted lncRNA with a high expression level in embryogenesis, and it implicated a crucial role of tumor suppressor in cancer progression. Recent study showed that LncRNA H19 might be an oncogenic lncRNA in several tumors. The role of LncRNA H19 in tumor is still controversial. Some researchers suggested that the role of LncRNA H19 might depend on the type of cancer, development stage, or molecular background. However, the role of LncRNA H19 in thyroid cancer was still unclear. Thus, in this study, human thyroid cancer cell line SW579 and TPC-1 cells were used to investigate the role of LncRNA H19 in thyroid cancer cells in vitro, as well as explore the potential mechanisms of LncRNA H19 on thyroid cancer.

Materials and Methods

Cell Culture

The human thyroid squamous cell carcinoma cell line SW579 and human thyroid papilloma cancer cell line TPC-1 were obtained from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). The cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Thermo Fisher Scientific, Waltham, Massachusetts) supplemented with 10% (vol/vol) fetal bovine serum (FBS; Thermo Fisher Scientific), 100 U/mL penicillin, and 100 μg/mL streptomycin (Thermo Fisher Scientific). All of the cells were cultured in an incubator with humidified atmosphere containing 5% CO₂, at 37°C. For subculture, cells at the logarithmic growth phase were treated by using trypsin-EDTA (Thermo Fisher Scientific), followed by centrifugation at 300g for 5 minutes and resuspension in RPMI-1640 medium containing 10% FBS for the next culture progress.

Cell Transfection

For overexpression transfection of LncRNA H19 or insulin receptor substrate 1 (IRS-1) in SW579 and TPC-1 cells, the constructed LncRNA H19-pcDNA3.1 vectors (pcDNA-H19), pcDNA-IRS-1, and pcDNA3.1 empty vectors (pcDNA3.1; Invitrogen, California, USA) were transiently transfectioned into cells, respectively. Meanwhile, small hairpin RNA (shRNA) vector of PTP1b (inducible), pGIPz (stable) shRNA vector and TransLenti Viral Packaging systems were obtained from Thermo Scientific. Viral particles with shRNA vectors (Invitrogen) special for knockdown of LncRNA H19 or IRS-1 were synthesized, respectively, according to the manufacturer’s protocol. Then cells were transfected with LncRNA H19 shRNA (sh-H19) and IRS-1 shRNA (sh-IRS-1) by using Lipofectamine 2000 (Invitrogen), according to the manufacturer’s instructions. Forty-eight hours posttransfection, the cells were selected with 400 μg/mL G418 (Geneticin, Life Technologies, Carlsbad, CA, USA) for 4 to 5 weeks, and stable cultured clones were isolated and selected.

Real-Time Polymerase Chain Reaction

Total RNA of cells after transfection was extracted by using TRIzol (Life Technologies), according to the manufacturer’s instructions, and been purified by RNasy Mini kit (Qiagen, Hilden, Germany). Then the reverse transcription was performed by using Superscript III kit (Life Technologies), according to the manufacturer’s instructions. The complementary DNAs were subsequently analyzed by quantitative real-time PCR. The primers of LncRNA H19 were as follows: F: 5’-ACCACCTGCACATCGACCT-3’; R: 5’-CCGCAGGGGGTGCATGAA-3’. And relative messenger RNA (mRNA) expressions were quantified and analyzed by real-time polymerase chain reaction (RT-PCR) using SYBR Green PCR Supermix kit (Bio-Rad Laboratories, Hercules, CA, USA). The reaction was performed in triplicate for each sample at least 3 independent runs. The expression levels were analyzed by Real-Time StatMiner (Integromics, Madrid, Spain), and data were calculated by using 2^-ΔΔCt method.

Cell Viability Assay

The 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) assay was performed to determine
cell viability. Cells were seeded in the 96-well plates at a density of $1 \times 10^5$ cells/mL and then were cultured in humidified atmosphere incubator with 5% CO$_2$ at 37°C. Forty-eight hours after transfections, MTT assay was performed and cell viability was measured by adding 10 μL MTT into each well on the day of determination (1 day, 2 days, 3 days, and 4 days) and then cells were incubated for 4 hours at 37°C. The detection was performed by using microplate reader at 492 nm (Thermo Scientific). Three independent experiments were repeated.

**Apoptosis Assay**

The relative apoptotic cells were measured by using annexin V-fluorescein isothiocyanate (FITC)/prodium iodide (PI) apoptosis detection kit (Shanghai Kaifeng Biotechnology, Shanghai, China) followed by flow cytometry analysis. In brief, cells were seeded in 6-well plates ($1 \times 10^5$ cells/well), then 100 μL annexin V was added in to each well. The plates were incubated in the dark for 15 minutes at room temperature. Then 4 μL of PI that has been diluted 1:10 in 1× annexin V binding buffer was added, and cells were incubated in the dark for 15 minutes at room temperature. Treated cells were washed twice by using cold phosphate-buffered saline (Sigma) and then been measured by flow cytometer (BD Biosciences, San Jose, CA, USA) to identify apoptotic cells.

**Migration and Invasion Assay**

Cell migration assay was performed by using Transwell chamber (8-μm pore size polycarbonate filters; BD Biosciences). Briefly, 48 hours after transfection, cells ($2 \times 10^5$ cells/well) were seeded in the upper compartments of chambers and 600 μL of complete medium was added into the lower compartments and been incubated at 37°C. Nontraversed cells were removed from the upper surface of the filter with a cotton swab. Then traversed cells on the lower side of the filter were fixed by 100 μL methanol (Thermo Fisher Scientific) and been stained with crystal violet solution for microscopic counting.

**Wound Healing Assay**

The transfection cells were cultured in standard conditions until 80% to 90% confluence and treated with 10 μg/mL of mitomycin C during the wound healing assay. The cell migration was assessed by measuring the movement of cells into the acellular area created by a sterile insert. The wound closure was observed after 48 hours. The scratch wound closure percentage was calculated as: (the scratch area before incubation — the scratch area after incubation)/(the scratch area before incubation) $\times$ 100%.

**Western Blot**

The protein from treated cells used for Western blotting was extracted by radio immunoprecipitation assay lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitor (Roche, Guangzhou, China). The protein samples were quantified by using the Bicinchoninic Acid Protein Assay Kit (Pierce, Appleton, Wisconsin). Equal amounts of samples were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and been transferred to polyvinylidene fluoride membranes. Primary antibodies were prepared in 5% blocking buffer at a dilution of 1:1000 and incubated at 37°C overnight, and the membranes were washed and incubated with secondary antibodies marked by horseradish peroxidase (Abcam, Cambridge, UK) for 1 hour at room temperature. The bands were visualized by using diaminobenzidine kit (CW0125 M, 100 mL; CWBio, China) and were analyzed by Image Lab Software (Bio-Rad, Shanghai, China). Primary antibodies used in this experiments were as follows: B-cell lymphoma 2 (Bcl-2, sc509); Bax (sc20067); caspase 3 (sc7272); caspase 9 (sc17784); p65(sc109); phosphorylated (p-) p65(sc101749); nuclear factor of kappa light polypeptide gene enhancer in B-cell inhibitor, alpha (IκBα, sc847); phosphorylated nuclear factor of kappa light polypeptide gene enhancer in B-cell inhibitor, alpha (p-IκBα, sc101713); Santa Cruz, CA, USA); IRS-1(ab201644); protein kinase B (AKT, ab54752); phosphorylated protein kinase B.
(p-AKT, ab38449); phosphatidylinositol 3-kinases (ab86714); phosphorylated phosphatidylinositol 3-kinases (p-PI3K, ab182651); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, ab8245), all purchased from Abcam.

**Figure 2.** The effects of long noncoding (LncRNA) H19 on cell viability, migration/invasion, and apoptosis of SW579 and TPC-1 cells. Cells were transfected with recombined LncRNA H19 overexpression vector (pcDNA-H19), pcDNA3.1 empty vector small hairpin RNA (shRNA) specifically for LncRNA H19 (sh-H19), shRNA negative control (shNC), respectively. A, Cell viability was measured by 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) assay. B, Cell migration was assessed by using Transwell migration assay. C, Relative cell invasion was assessed by Transwell assay. D, After treatment with 10 μg/mL of mitomycin C, cell viability was measured by MTT assay. E, The ability of migration was detected by wound healing assay. E, Relative number of apoptotic cells was measured by flow cytometry. F-J, The expression of Bcl-2, Bax, caspase 3, and caspase 9 in SW579 cells and TPC cells were measured by real-time polymerase chain reaction (RT-PCR) and Western blotting. GAPDH acted as internal control. **P < .01 or ***P < .001 compared with the corresponding negative control.

**Statistical Analysis**

All data of 3 independent experiments were expressed as mean (standard deviation). Statistical analyses were performed using GraphPad Prism 6.0 statistical software (GraphPad Software...
Inc, La Jolla, California). The P values were calculated by using 2-way analysis of variance followed by Bonferroni test (more than 2 groups) or Student t test for 2 groups. P value of <.05 was considered to indicate a statistically significant result.

Results

Long noncoding RNA H19 Was Overexpressed or Knockdown in SW579 and TPC-1 Cells

To determine whether LncRNA H19 was involved in the development of thyroid cancer, LncRNA H19 was overexpressed or knockdown in SW579 and TPC-1 cells by transfection, respectively. The expression of LncRNA H19 was detected by RT-PCR. As shown in Figure 1, the expression level of LncRNA H19 in SW579 and TPC-1 cells was increased after LncRNA H19 overexpression vector transfection compared to the empty control (P < .001), and the expression of LncRNA H19 was decreased in sh-LncRNA-H19-transfected group compared with small hairpin RNA negative control (shNC) group (P < .05). These results showed that LncRNA H19 was effectively overexpressed or knockdown in SW579 and TPC-1 cells.

Long noncoding RNA H19 Overexpression Inhibited Cell Viability, Migration, and Invasion and Promoted Cell Apoptosis

The results of cell viability assay in Figure 2A showed that in both SW579 and TPC-1 cells, the cell viability was observed to be increased by 23% (0.05%) and 26% (0.05%) in SW579 and TPC-1 cells by suppression of H19, whereas cell viability was observed to be decreased by 25% (0.04%) and 27% (0.07%) in both two cell lines by overexpression of H19. Cell migration and invasion assay results shown in Figure 2B and C suggested that both migration and invasion of cells were increased by 22% (0.04%) and 23% (0.05%), respectively, in sh-H19 group compared with shNC group, and they were decreased by 26% (0.06%) and 24% (0.07%) in pcDNA-H19 group compared with the pcDNA3.1 group in SW579 cells. Similarly, cell migration and invasion were observed to be increased by 30% (0.07%) and 28% (0.07%) in TPC-1 cells by suppression of H19, but they were decreased by 32% (0.04%) and 25% (0.06%) in TPC-1 cells by overexpression of H19. In addition, to exclude the effects of cell proliferation on migration or invasion, we detected the migration ability by using wound healing assay after adding mitomycin C. As results revealed in Figure 2D and 2E, the cell viability of SW579 and TPC-1 was significantly suppressed after treatment by mitomycin C (P < .01). Wound healing assay results showed that migration distances in sh-H19 were markedly narrower than its control after treatment by mitomycin C in both SW579 and TPC-1 (P < .05), indicating the promotive effect of H19 knockdown on cell migration.

Furthermore, apoptosis assay results shown in Figure 2F revealed that relative apoptotic cells were increased in pcDNA-H19 group compared with the pcDNA3.1 group (P < .001). The expression levels of apoptosis-related factors were also been measured by RT-PCR and Western blotting. Figure 2G to J shows that the expression level of Bcl-2 was
decreased, whereas the expression level of Bax, caspase 3, and caspase 9 was increased in pcDNA-H19 group compared with the pcDNA3.1 empty control group \((P < .001)\). All the above results suggested that LncRNA H19 overexpression inhibited cell viability, migration, and invasion and promoted cell apoptosis in SW579 and TPC-1 cells.

**Long noncoding RNA H19 Negatively Regulated the Expression of IRS-1**

Thereafter, the expression level of IRS-1 was detected in SW579 and TPC-1 cells after corresponding transfections. The results in Figure 3A and 3B showed that the mRNA expression level of IRS-1 was increased in sh-H19 group compared with the shNC group in SW579 and TPC-1 cells \((P < .01)\) and IRS-1 mRNA expression was decreased in pcDNA-H19 group compared with the control group in SW579 and TPC-1 cells \((P < .05)\). In addition, the protein expression levels of IRS-1 were also been measured by Western blotting. The results shown in Figure 3C and D suggested that the expression level of IRS-1 in SW579 and TPC-1 cells was increased by LncRNA H19 knockdown, and IRS-1 expression was decreased in LncRNA H19 overexpression group. These results suggested that LncRNA H19 could negatively regulate the IRS-1 expression in SW579 and TPC-1 cells.

**Effect of LncRNA H19 on Cell Viability, Migration/Invasion, and Apoptosis Might Be Via Regulating Expression of IRS-1**

To verify that LncRNA H19 negatively regulated the expression of IRS-1 might affect SW579 and TPC-1 cell proliferation...
and apoptosis, LncRNA H19 and IRS-1 were knockdown by shRNA transfections. Cell viability in sh-H19 + sh-IRS-1 transfection group was observed to be decreased by 57% (0.06%) and 53% (0.07%) compared with sh-H19 group in SW579 and TPC-1 cells (Figure 4A). The migration of cells in sh-H19 + sh-IRS-1 group was observed to be decreased by 63% (0.08%) and 50% (0.06%) compared with sh-H19 group in SW579 and TPC-1 cells (Figure 4B). In addition, cell invasive ability of sh-H19 + sh-IRS-1 group was observed to be decreased by 60% (0.09%) and 53% (0.09%) compared with sh-H19 group in SW579 and TPC-1 cells (Figure 4C). Meanwhile, the results of apoptosis assay showed that the relative number of apoptotic cells in sh-H19 + sh-IRS-1 group was observed to be increased by 150% (0.06%) and 203% (0.07%) compared with sh-H19 group (Figure 4D). The RT-PCR and Western blotting assay results showed that the expression level of Bcl-2 was decreased, whereas the expression level of Bax, caspase 3, and caspase 9 was increased in sh-H19 + sh-IRS-1 group compared with sh-H19 group (Figure 4E and F). Therefore, combining with the above results, it is suggested that LncRNA H19–affected cell proliferation and apoptosis might be via regulating the expression of IRS-1 in SW579 and TPC-1 cells.

**Insulin receptor substrate 1 Knockdown Inhibited Cell Viability, Migration, and Invasion While Promoted Cell Apoptosis**

The corresponding transfections were performed in both SW579 and TPC-1 cells to overexpression or knockdown of IRS-1, respectively. As shown in Figure 5A, the mRNA expressions of IRS-1 were increased after pcDNA-IRS-1 transfection compared with pcDNA 3.1 control (P < .01), and the mRNA expression of IRS-1 in sh-IRS-1 groups was decreased compared with that in shNC group in both SW579 and TPC-1 cells (P < .01), suggesting that IRS-1 was effectively overexpressed or knockdown. The protein expression levels of IRS-1 in SW579 and TPC-1 cells were also been measured by Western blotting. Results in Figure 5B and C showed that the protein expressions of IRS-1 were increased in pcDNA-IRS-1-transfected group compared with pcDNA3.1 group in SW579 and TPC-1 cells, while it was decreased in sh-IRS-1 groups compared with that in shNC group.

Thereafter, cell viability assay, migration/invasion assay, and apoptosis assay were performed to detect the effect of IRS-1 on thyroid cancer cells in vitro. The results of cell viability assay in Figure 6A showed that the cell viability was observed to be increased by 34% (0.03%) and 29% (0.04%) in both SW579 and TPC-1 cells by IRS-1 overexpression compared with control groups, while cell viability was observed to be decreased by 28% (0.03%) and 43% (0.03%) by IRS-1 knockdown compared with shNC groups. Results of cell migration/invasion assay shown in Figure 6B and C revealed that cell migration and invasion were increased by 22% (0.04%) and 27% (0.03%), as well as decreased by 24% (0.03%) and 22% (0.02%), respectively, by IRS-1 overexpression or IRS-1 knockdown in SW579 cells. In TPC-1 cells, cell migration and invasion were increased by 30% (0.04%) and 29% (0.04%) and decreased by 33% (0.03%) and 28% (0.03%) by IRS-1 overexpression or IRS-1 knockdown. In addition, cell apoptosis assay results in Figure 6D showed that cell apoptosis was observed to be increased by 401% (0.07%) and 235%
(0.07%) in SW579 and TPC-1 cells by suppression of IRS-1. Real-time polymerase chain reaction and Western blotting assay results in Figure 6E and F showed that expression level of Bel-2 was decreased and the expression level of Bax, caspase 3, and caspase 9 was increased in sh-IRS-1 group compared with the negative control group. All these results suggested that IRS-1 might be related to the proliferation or viability and apoptosis of SW579 and TPC-1 cells.

Insulin receptor substrate 1 Was Related to Activation of PI3k/AKT and Nuclear Factor κB Signal Pathways

To investigate the mechanism that IRS-1 overexpression promoting cell viability, migration/invasion, and inhibited apoptosis, we detected the expression levels of activation-dependent factors in PI3K/AKT and nuclear factor κB (NF-κB) signal pathways in both SW579 and TPC-1 cells by RT-PCR and Western blotting. For PI3K/AKT signal pathways, IRS-1 knockdown decreased the expression level of phosphorylated (p-) PI3K and p-AKT in SW579 and TPC-1 cells (P < .001; Figure 7A and B). In NF-κB signal pathway, IRS-1 knockdown decreased the expression level of p-p65 and p-IκBα in SW579 and TPC-1 cells (P < .01 or P < .001; Figure 7C and D). Therefore, these results suggested that IRS-1 expression in SW579 and TPC-1 cells might be related to activation of PI3K/AKT and NF-κB signal pathways.

Discussion

Regardless of the reported increase in small tumors, the patients with thyroid cancer have long been thought to need undergo surgery, and many patients also require a radioactive iodine treatment to kill remaining cancer cells. Experts are starting to rethink how they approach the rapidly increasing number of thyroid cancer and improve the prognosis.21 Research about the pathogenesis of cancer from the molecular
Figure 7. The expression of factors that were related to phosphatidylinositol 3-kinases (PI3K)/AKT and nuclear factor κB (NF-κB) signal pathways in insulin receptor substrate 1 (IRS-1)-transfected SW579 and TPC-1 cells. Cells were transfected with recombined IRS-1 overexpression vector (pcDNA-IRS-1), pcDNA3.1 empty vector (pcDNA 3.1), small hairpin RNA (shRNA) specifically for IRS-1 (sh-IRS-1), and shRNA negative control (shNC), respectively. Protein expressions were measured by real-time polymerase chain reaction (RT-PCR) and Western blotting. A and B, The expression level of phosphatidylinositol 3-kinases (PI3K), phosphorylated (p-) PI3K, AKT, and p-AKT in SW579 and TPC-1 cells. C and D, Protein expression levels of p65, p-p65, IκBα, and p-IκBα in SW579 and TPC-1 cells. GAPDH acted as internal control. **P < .01 or ***P < .001 compared with the corresponding control.
level in recent years has become a hot spot. Long noncoding RNA H19 play critical roles in cellular proliferation, apoptosis, differentiation, and invasion. Long noncoding RNA H19 was involved in bladder cancer metastasis through association with EZH2 that lead to the activation of Wnt/β-catenin signaling and the downregulation of E-cadherin. Furthermore, LncRNA H19 also served as a precursor of microRNA-675 and regulated tumorigenesis, progression, and metastasis of multiply cancers. In the present study, we investigated the role of LncRNA H19 in SW579 and TPC-1 cells and found that LncRNA H19 was negatively related to cell viability, migration, and invasion. And LncRNA H19 could promote cell apoptosis, suggesting that LncRNA H19 might be a potential therapeutic target of thyroid cancer clinical treatment. Then we also found that the expression of IRS-1 was negatively regulated by LncRNA H19 in SW579 and TPC-1 cells in vitro. Insulin receptor substrate proteins are cytoplasmic adapters that function as downstream signaling intermediates of cell surface receptors. It is well known that phosphorylated IRS could act as docking protein. It could activate a series of downstream signaling effectors with SH2 domain, including PI3K C-terminal Src kinase, and several growth factor–binding proteins, such as growth factor receptor-bound protein-2 and the transcription factor NF-κB. Both IRS-1 and IRS-2, as cytoplasmic adapters and insulin-like growth factor-1 receptor, play the major role in determining the cellular response to stimulation. Meanwhile, IRSs are required for the activation AKT and mechanistic target of rapamycin to promote cell proliferation, survival, motility, protein synthesis, and glucose metabolism. In this study, we found that LncRNA H19 negatively regulated the expression of IRS-1 and thus affect cell proliferation or viability and apoptosis. Furthermore, we also found that the cell viability, migration / invasion, and apoptosis were all regulated by IRS-1 overexpression or knockdown, suggesting that LncRNA H19 might regulate thyroid cancer cells activity via regulating IRS-1 expression in vitro.

Phosphatidyl inositol 3-kinase/AKT pathway plays an important role in tumorigenesis. It was proved to promote tumor malignant transformation, including elevated cancer cell survival, proliferation, and metabolism. Lu et al found that LncRNA highly up-regulated in liver cancer promoted cell proliferation via regulating PI3K/AKT signaling pathway in chronic myeloid leukemia. In addition, Chen et al suggested that LncRNA melanoma highly expressed non-coding RNA promoted melanoma progression via regulating miR-425/489-mediated PI3K/AKT pathway, suggesting the important roles of PI3K/AKT signaling pathway in malignant tumor. And previous studies also suggested that NF-κB signaling could cross talk with PI3K/AKT pathway. Therefore, to better understand how IRS-1 affected thyroid cancer cells, we detected the phosphorylation level of several factors in PI3K/AKT and NF-κB signal pathway. The expression levels of p-PI3K, p-AKT, p-p65, p-IkBz were increased by IRS-1 overexpression and been decreased after IRS-1 knockdown. The results suggested that IRS-1 might be related to activation of PI3K/AKT and NF-κB signal pathways and thus affect LncRNA H19 effect on thyroid cancer cells in vitro.

In summary, our findings suggested that the overexpression of LncRNA H19 could inhibit cell proliferation and promoted apoptosis via downregulation of IRS-1 in thyroid cancer cells in vitro. These findings might provide important clues for the establishment of LncRNA H19 functional network in thyroid cancer. Long noncoding RNA H19 might be a potential new target for antitumor therapy of thyroid cancer.

Authors’ Note
Peng Wang and Guoqing Liu were co-first authors.

Declaration of Conflicting Interests
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