Linc01555 promotes proliferation, migration and invasion of gastric carcinoma cells by interacting with Notch signaling pathway

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Summary

Purpose: To detect the expression level of long non-coding ribonucleic acid 01555 (linc01555) in gastric cancer (GC) tissues and cells, and its effects on the biological functions of GC cells.

Methods: The relative expression of linc01555 in 61 cases of GC and para-carcinoma tissues and GC cells was detected via quantitative reverse transcription-polymerase chain reaction (qRT-PCR). GC cells were divided into experimental group (si-linc01555) and control group (si-NC), and the interference efficiency was detected through qRT-PCR. The effects of interference in linc01555 expression on GC cell proliferation, colony formation ability, migration and invasion were determined using cell counting kit-8 (CCK-8) assay, colony formation assay, wound healing assay and Transwell assay. Moreover, the expressions of molecular markers in the downstream Notch pathway were detected using western blotting.

Results: The results of qRT-PCR showed that the expression of linc01555 was upregulated in GC tissues and cells. The results of CCK-8 assay revealed that the proliferative activity of GC cells declined after interference in linc01555 expression. It was found in colony formation assay that the proliferation ability of GC cells declined after interference in linc01555 expression, and it was observed in wound healing assay that the cell migration ability in the experimental group was weakened compared with that in the control group. According to the results of transwell assay, both migration and invasion ability of GC cells declined after interference in linc01555 expression. Finally, the western blotting showed that there were changes in the expressions of molecular markers in the Notch signaling pathway after interference in linc01555 expression.

Conclusions: The expression of linc01555 is upregulated in GC tissues and cells, and the highly-expressed linc01555 promotes the proliferation, invasion and metastasis of GC cells through the Notch signaling pathway.

Key words: gastric cancer, linc01555, biological function, Notch signaling pathway

Introduction

Gastric cancer (GC) is the fourth major malignant tumor in the world, ranking 2nd among cancer-related deaths [1]. Although the morbidity rate of GC has slightly declined in recent years, its morbidity and mortality rates are still the second highest in China [2]. A better curative effect can be achieved with early diagnosis and treatment of GC, but GC patients in China have been mostly in locally advanced stage at the initial diagnosis, with a poor prognosis [3]. The tumor invasion and metastasis are the most important causes of poor prognosis [4]. In 2014, an international research group jointly proposed the molecular typing theory of GC [5], which has important clinical significance for further searching for related predictors for the prognosis of GC.
With the completion of the Human Genome Project in 2003, it was found in subsequent studies that only 2% of genes are protein-coding genes, while the remaining 90% of genes are transcribed into non-coding ribonucleic acids (ncRNAs). According to a large number of studies, these ncRNAs play an important regulatory role in complex organisms [6,7]. There is a kind of long ncRNAs (lncRNAs) with more than 200 nucleotides in length, which are widely involved in the life processes, such as chromosomal reconstruction, cell differentiation and immune response [8-10]. In addition, a large amount of tumor-related research has demonstrated that lncRNAs play an important regulatory role in the occurrence and development of tumors [11,12]. However, the pathophysiological effect of lncRNAs in GC remains unclear.

In the present study, it was found for the first time that the relative expression of linc01555 was upregulated in GC tissues and cells, in vitro experiments confirmed that linc01555 promoted the proliferation, migration and metastasis of GC cells, and the results of western blotting further revealed that linc01555 facilitated the malignant phenotype of GC through regulating the Notch signaling pathway.

**Methods**

**Tissue specimens**

A total of 61 cases of GC tissues and para-carcinoma tissues obtained from January 2013 to December 2015 were selected. The patients were diagnosed as GC and underwent radical resection in our hospital. The para-carcinoma tissue specimens were taken from normal gastric tissues at more than 5 cm away from the tumor edge. The specimens were taken, placed in the frozen pipe and immediately stored in liquid nitrogen for subsequent experiments. Before enrollment, the patients received no radiotherapy and chemotherapy, and they had no history of major organ diseases.

**Cell culture**

GC cell lines MGC803, MKN45, BSG823, SGC7901 and normal human gastric mucosal epithelial cell line GES-1 were purchased from the Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China), and were cultured in Roswell Park Memorial Institute 1640 (RPMI 1640) medium (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) in an incubator with 50 ml/L CO$_2$ at 37°C. The medium was replaced once every 2 days. The total cell lysate was extracted using radioimmunoprecipitation assay (RIPA) buffer (Yeasen, Shanghai, China). Then, the protein was separated via 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to a membrane. The membrane was blocked with 5% non-fat milk and incubated with primary antibodies at 4°C overnight. After being washed with phosphate buffered saline (PBS), the membrane was incubated with the corresponding secondary antibodies at room temperature for 1 h. Finally, the protein bands were visualized by enhanced chemiluminescence (ECL). Western blotting

**Quantitative real-time polymerase chain reaction (qRT-PCR)**

The total RNA was extracted from tissue specimens and cells using TRIzol (Invitrogen, Carlsbad, CA, USA), and the purity and concentration of RNA were detected. RT and PRC were performed in strict accordance with the manufacturer’s instructions (TaKaRa, Tokyo, Japan), with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal reference for relative quantification. Three replicates were set in each group. The expressions were detected using ROCH3 qRT-PCR instrument. Primer and interference sequences were as follows: linc01555 F: 5'-CATTCCA TTACATTCTTTCTCTA-3', R: 5'-GGCTAGGCCAGGGGATCG-3', GAPDH F: 5'-ATTTAACCGGATGGATTAGC-3', R: 5'-TATTCGATGGAAACACT-3'.

The relative expression levels were calculated via 2$^{-ΔΔCt}$.

**Colony formation assay**

The cells transfected with si-linc01555 and si-NC were inoculated into 96-well plates (3000 cells/well), with 5 replicates in each group, and cultured in the incubator, followed by CCK-8 assay (Dojindo, Kumamoto, Japan) at 0, 24, 48, 72 and 96 h. Ten μL of CCK-8 reagent were added into each well for incubation for 2 h, and the absorbance at 450 nm was measured using a microplate reader.

**Transwell assay**

The cells were inoculated into 6-well plates (1000 cells/well), with 3 replicates in each group. The cells were cultured in the incubator for 10-14 days, the RPMI 1640 medium was replaced once every 2-3 days, and the cell status was observed. The second generation cells in good growth status were harvested, and were washed twice with phosphate buffered saline (PBS), fixed with methanol at 4°C for 15 min, and stained with crystal violet dye (1 mL/well) for 20 min.

**Wound healing assay**

The cells in the experimental group and control group were inoculated into the 6-well plates. When more than 70% of cells were in confluence, each well was scratched in parallel using a 10 μL spearhead, and the shedding cells were washed away with PBS. Then, 2 mL of serum-free medium was added into each well for culture in the incubator. The scratch width was photographed and recorded at 0 and 48 h.

**Western blotting**

The total cell lysate was extracted using radioimmunoprecipitation assay (RIPA) buffer (Yeasen, Shanghai, China). Then, the protein was separated via 12% sodium dodecyl sulfate polyacrylamide gel electropho-
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resis, transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA), sealed with 5% skim milk powder, incubated with the primary antibody (1:1000) at 4°C overnight, and incubated again with the secondary antibody. Finally, the protein band was detected using the electrochemiluminescence (ECL) substrate kit, and the experiment was repeated for 5 times.

Statistics

SPSS 21.0 software (IBM, Armonk, NY, USA) was used for statistical analyses. The measurement data were expressed as mean ± standard deviation (mean ± SD). Student’s t-test was performed for the comparison of means between two groups. P<0.05 suggested that the difference was statistically significant.

Results

Linc01555 was upregulated in GC tissues and cells

First, qRT-PCR was used to detect the relative expression of linc01555 in GC tissues. The results showed that the relative expression of linc01555 was upregulated in GC tissues compared with that in para-carcinoma tissues (Figure 1A). It was found that linc01555 was also upregulated in GC cells (Figure 1B). To study the biological function of linc01555 in GC cells, specific interference sequences of linc01555 were designed, with MKN45 and SGC7901 cells as model cells, and the interference sequences were transiently transfected into GC cells using lip2000. Then, the interference efficiency was detected after 48 h (Figure 1C and 1D).

Interference in linc01555 expression inhibited GC cell proliferation

To study the effect of linc01555 on the proliferation of GC cells, CCK-8 assay was performed. The results revealed that the proliferative activity of cells in the experimental group declined compared with that in the control group (Figure 2A and 2B). Then, colony formation assay was performed.

![Figure 1](image-url)

Figure 1. Expression of linc01555 in GC tissues and cells. A: Expression of linc01555 in GC tissues and para-carcinoma tissues detected via qRT-PCR. The results show that the expression of linc01555 is upregulated in GC tissues, with GAPDH as an internal reference. B: Expression of linc01555 in GC cells detected via qRT-PCR. The expression of linc01555 is upregulated in GC cells compared with that in GES-1 cells. C & D: Interference efficiency of si-linc01555 detected via qRT-PCR. *p<0.05, **p<0.01.
and it was observed that si-linc01555 reduced the proliferation of GC cells compared with si-NC (Figure 2C and 2D).

Linc01555 promoted GC cell migration and invasion through Notch signaling pathway

Furthermore, the effect of linc01555 on the migration ability of GC cells was explored using wound healing assay. The results showed that the migration ability of GC cells declined after interference in the linc01555 expression (Figure 3A and 3B). The results of transwell assay showed that both migration and invasion of GC cells were decreased after interference in the linc01555 expression (Figure 3C and 3D). To study the downstream regulatory mechanism of linc01555, the changes in molecular expressions in Notch signaling pathway were detected through western blotting. The results manifested that, compared with those in the control group, the expressions of Notch1, Notch2, DLL3 and Hes1 in the Notch signaling pathway were changed after interference in the linc01555 expression (Figure 3E and 3F).

Discussion

GC is the second major cause of cancer death [1], as well as the most common malignant tumor of gastrointestinal tract in some regions in East Asia, Eastern Europe and Central and South America [13]. Despite the rapid development of diagnostic techniques and new molecular targeted drugs, the overall 5-year survival rate of GC patients is still low [14]. Therefore, it is particularly important to understand the molecular mechanism of occurrence and development of GC and search for effective biological targets.

According to a large number of reports, IncRNAs are closely related to the occurrence and development of tumors. For example, IncRNA MALAT1 can promote the movement of lung adenocarcinoma cells and indicates the poor prognosis of lung cancer [15]. PCGEM1 is overexpressed in prostate cancer and closely related to the occurrence and development of prostate cancer [16]. Recent studies have also shown that many IncRNAs also play an important role in the occurrence and develop-

Figure 2. Effect of linc01555 on the proliferation of GC cells. A & B: Si-linc01555 and si-NC are transiently transfected into GC cells, and the changes in cell proliferative activity in the experimental group and control group are explored via CCK-8 assay. C & D: Effect of si-linc01555 on GC cell proliferation explored via colony formation assay. *p<0.05 vs si-NC.
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Mental of GC: MALAT1 and HOTAIR facilitate the peritoneal metastasis of GC cells [17]. Xu et al. [18] found that lncRNA FENDER inhibits invasion and metastasis of GC cells. LncRNA TINCR accelerates the degradation of KLF2 mRNA, thus promoting proliferation of GC [19]. Therefore, identifying GC-related lncRNAs may provide new ideas for network regulation of known oncogenes and cancer suppressor genes.

Currently, there are few reports on linc01555. Wang et al [20] found that linc01555 is upregulated in colorectal cancer and promotes invasion and metastasis of colorectal cancer cells through regulating neuromedin-U. The role of linc01555 in the occurrence and development of GC has not been reported. In this study, it was found for the first time that linc01555 was upregulated in GC tissues and cells, and interference in the expression of linc01555 inhibited cell proliferation, migration, and invasion. The Notch signaling pathway is evolutionarily conserved and involved in many cellular processes, including proliferation, differ-

Figure 3. Effect of linc01555 on GC cell migration and invasion. A & B: The effect of si-linc01555 on GC cell migration is detected via wound healing assay. The results show that the cell migration ability in the experimental group declines compared with that in the control group. C & D: The results of transwell assay reveal that the migration and invasion of GC cells decline after interference in the expression of linc01555. E & F: The results of western blotting manifest that there are changes in the expressions of molecular markers in the Notch signaling pathway after interference in the expression of linc01555.
entiation, apoptosis and stem cell maintenance. The activation and inhibition of Notch signaling pathway are closely related to the occurrence and development of tumor [21,22]. Moreover, IncRNAs can promote the occurrence and development of tumors through regulating the Notch signaling pathway [23]. In this study, it was confirmed through in vitro experiments that linc01555 promoted the proliferation, migration and invasion of GC cells through regulating the Notch signaling pathway.

**Conclusions**

The expression of linc01555 is upregulated in GC tissues and cells, and the highly-expressed linc01555 promotes the proliferation, invasion and metastasis of GC cells through the Notch signaling pathway.

**Conflict of interests**

The authors declare no conflict of interests.

**References**