Original Article

LINC00994 promoted invasion and proliferation of gastric cancer cell via regulating miR-765-3p

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Abstract: Emerging studies indicated that IncRNA is one crucial regulator in development and tumorigenesis. We firstly showed that LINC00994 was overexpressed in GC cells compare with GES-1. Compared to adjacent samples, the LINC00994 expression was increased in GC tissues by qRT-PCR assay. Furthermore, Elevated expression of LINC00994 induced GC cell invasion, proliferation and cycle. Luciferase reporter analysis indicated that ectopic miR-765-3p expression suppressed wild type LINC00994 luciferase activity, but not mutant LINC00994 in GC cells. Elevated expression of LINC00994 inhibited the miR-765-3p expression in GC cells. Compared to adjacent samples, the miR-765-3p expression was decreased in GC tissues by qRT-PCR assay. LINC00994 induced GC cell invasion and growth via modulating miR-765-3p. Thus, our data suggested an oncogenic role of LINC00994 in development of GC.

Keywords: LINC00994, miR-765-3p, gastric cancer, IncRNAs

Introduction

Gastric cancer is the 4th most common tumor and the 2nd leading cause of tumor-associated death worldwide [1-4]. The pathogenesis and mechanism of gastric cancer is complex and is related with several factors [5-7]. Despite achieving advances in these novel management strategies such as surgical techniques, chemotherapy and radiotherapy methods, the five year survival rate of this disease is still discontented [8-11]. Most gastric cancer patients were diagnosed at the advanced stage according to asymptomatic presentation at the early stage [12-14]. Therefore, it is useful to study molecular mechanisms and identify novel biomarkers and therapeutic targets for gastric cancer.

Long noncoding RNAs (IncRNAs) is one group of noncoding RNAs that exceed 200 nucleotides (nts) in length with no or limited protein coding capacity [15-19]. Growing studies indicated that IncRNAs participated in several cell biological processes such as stem cell pluripotency reprogramming, migration, invasion, proliferation and apoptosis [20-23]. Several IncRNAs were found to be deregulated in a variety of tumors including lung carcinoma, hepatocellular carcinoma, bladder cancer, osteosarcoma and also gastric cancer [21, 24-28]. Recently, one new IncRNA long intergenic non-protein coding RNA 994 (LINC00994) was identified and was found to be played important roles in the progression of some diseases such as type 2 diabetes (T2DM) and pancreatic cancer [29, 30]. Mansoori and their colleagues reported that LINC00994 expression level was downregulated in the T2DM cases compared to control participants [29]. Zhu and their colleagues showed that LINC00994 silencing suppressed pancreatic tumor cell proliferation, invasion and migration and induced cell cycle arrest at the G1 stage and apoptosis [30]. However, the regulatory function of LINC00994 in the gastric cancer has not been investigated.

In this study, we firstly studied that LINC00994 was overexpressed in GC cells (AGS, SGC-7901, MGC8-03, and HGC-27) compare with GES-1.
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Compared to adjacent samples, the LINC00994 expression was increased in GC tissues by qRT-PCR assay. Elevated expression of LINC00994 induced GC cell invasion, proliferation and cycle.

Materials and methods

Tissues

Surgically resected gastric tumor and their adjacent samples were obtained from gastric cancer cases that underwent surgery at our department. Surgically collected samples were immediately frozen in the liquid nitrogen until use. All patients were signed the informed consent and our research was performed following the Helsinki principles Declaration and ethics committee of Ningxia Medical University.

Cell lines cultured and transfection

Four human gastric cancer cell lines (AGS, SGC-7901, MGC8-03, and HGC-27) and gastric epithelial cell line (GES-1) were obtained from Chinese Academy of Sciences (Shanghai, China). These cell lines were cultured in RPMI-1640 supplemented with FBS (fetal bovine serum) and antibiotics. pcDNA-LINC00994 and its control vector, miR-765-3p mimic, inhibitor and control were obtained from the RiboBi company (Guangzhou, China) and then were transfected to cells by using Lipofectamine 2000 kit (Invitrogen, Carlsbad, USA).

RNA extraction and real-time quantitative PCR

Total RNA was distilled from cell or sample with Rneasy Mini (Qiagen, Germany) following the protocol of manufacture. LINC00994 and miR-765-3p expression were tested by SYBR (Takara, China) in a PCR system of Bio-Rad (VisonBio Scientific). Data were normalized to U6 or GAPDH. Primer sequences were indicated as following: The 2-ΔΔCT way was exploited for PCR data analysis. Primer information was shown as following: miR-765-3p, sense: 5'-ATCAGTGGAGGAGAAGGAGG-3' and antisense: 5'-GTGCAGGGTCCGATATTC-3'; LINC00994, sense: 5'-TCAAGGCTGGGAAAGGGA-3' and antisense: 5'-CGAATTGCTGGGAAAGGGA-3'.

Cell proliferation, cell cycle and migration assays

Cell growth was carried out using CCK-8 assay (Dojin, Japan) at different time point. Cells were plated in the 96-well and CCK-8 fluid was added into each plate well. After 2 hours incubation, the absorbance at 450 nm was checked by Multiplate Reader (Thermo Scientific). Cell cycle was fixed by ice-cold 70% ethanol overnight and then stained with PI (propidium iodide, Beyotime) supplement with RNase A in dark environment. Cell cycle was carried out on flow cytometer (Biosciences, USA). Transwell analysis was made to evaluate cell invasion. Cells were kept in upper chamber and FBS was added to lower chamber. After 48 h, invasive cells were fixed, stained and counted.

Luciferase reporter assay

miR-765-3p binding sites on LINC00994 were sought from starBase (http://starbase.sysu.edu.cn/index.php). Wild-type (Wt) binding type and mutant binding type of LINC00994 were synthesized and produced LINC00994-wt and LINC00994-mut. Cells were transfected with miR-765-3p mimic and scramble, LINC00994-wt or LINC00994-mut. Luciferase activity was detected by Luciferase Reporter analysis kit (Promega, Madison, USA) after transfection with 48 h.

Statistical analysis

Data were shown as the mean value and the standard deviation (SD) was presented the
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Results

LINC00994 expression was examined by qRT-PCR in the GC cell lines. As indicated in Figure 1A, LINC00994 was overexpressed in GC cells (AGS, SGC-7901, MGC8-03, and HGC-27) compared with GES-1. In line with this, the expression of LINC00994 was upregulated in GC cells (AGS, SGC-7901, MGC8-03, and HGC-27) compared to GES-1 by PCR (Figure 1B).

LINC00994 was upregulated in GC samples

Compared to adjacent samples, the LINC00994 expression was increased in GC tissues by qRT-PCR assay (Figure 2A). Furthermore, LINC00994 was overexpression in 31 GC cases (31/40; 77.5%) compared to adjacent specimens (Figure 2B).

Elevated expression of LINC00994 induced GC cell proliferation and cycle

Then, we discussed the cell function of LINC00994 in GC cell, we established a LINC00994 overexpression plasmid. We verified that the LINC00994 expression was upregulated both in the SGC-7901 (Figure 3A) and HGC-27 cells (Figure 3B). CCK-8 analysis data indicated that LINC00994 overexpression promoted SGC-7901 cell growth (Figure 3C) and elevated expression of LINC00994 increased HGC-27 cell proliferation (Figure 3D). Moreover, ectopic
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LINC00994 expression enhanced the cell cycle both in the SGC-7901 cell (Figure 3E) and HGC-27 cells (Figure 3F).

**Ectopic LINC00994 expression enhanced GC cell invasion**

Furthermore, the function of LINC00994 overexpression on cell invasion was measured using invasion transwell analysis. It was shown that elevated expression of LINC00994 induced the SGC-7901 cell invasion (Figure 4A and 4B). Overexpression of LINC00994 increased cell invasion in the SGC-7901 cell (Figure 4C and 4D).

**LINC00994 can sponge miR-765-3p expression in GC cell**

As indicated in Figure 5A, miR-765-3p was downregulated in GC cells (AGS, SGC-7901, MGC8-03, and HGC-27) compare with GES-1. B. miR-765-3p expression was upregulated both in the SGC-7901 cells after transfection with miR-765-3p mimic. C. Seeking from starBase (http://starbase.sysu.edu.cn/index.php), miR-765-3p harbored binding sites with LINC00994. D. Ectopic miR-765-3p expression suppressed wild type LINC00994 luciferase activity, but not mutant LINC00994 in SGC-7901 cells. E. Elevated expression of LINC00994 inhibited the miR-765-3p expression in SGC-7901 cells. **P<0.01.
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miR-765-3p was downregulated in GC samples

Compared to adjacent samples, the miR-765-3p expression was decreased in GC tissues by qRT-PCR assay (Figure 6A). Furthermore, miR-765-3p was downregulated in 29 GC cases (29/40; 72.5%) compared to adjacent specimens (Figure 6B). Pearson’s correlation was done to measure correlation between the expression of miR-765-3p and LINC00994. Data indicated that LINC00994 level was inversely correlated with miR-765-3p in cases with GC (Figure 6C).

LINC00994 induced GC cell invasion and growth via modulating miR-765-3p

We performed rescue analysis to study role of LINC00994/miR-765-3p in modulating GC cell invasion and proliferation. As shown in Figure 7A, cell growth enhanced by transfection with pcDNA-LINC00994 was partly rescued by miR-765-3p mimic introduction. In addition, we found that miR-765-3p overexpression decreased the cell cycle in the LINC00994-overexpressing SGC-7901 cells (Figure 7B). Additionally, our data indicated that ectopic miR-765-3p expression suppressed cell invasion in the LINC00994-overexpressing SGC-7901 cells (Figure 7C and 7D).

Discussion

LncRNAs are found to act as critical regulator in cell processes through controlling gene expression at epigenetic, post-transcriptional and transcriptional level [31-34]. In this research, we firstly showed that LINC00994 was overexpressed in GC cells (AGS, SGC-7901, MGC8-03, and HGC-27) compared with GES-1. Compared to adjacent samples, the LINC00994 expression was increased in GC tissues by qRT-PCR assay. Furthermore, LINC00994 was overexpression in 31 GC cases (31/40; 77.5%) compared to adjacent specimens. Elevated expression of LINC00994 induced GC cell invasion, proliferation and cycle. Luciferase reporter analysis indicated that ectopic miR-765-3p expression suppressed wild type LINC00994 luciferase activity, but not mutant LINC00994 in GC cells. Elevated expression of LINC00994 inhibited the miR-765-3p expression in GC cells. Compared to adjacent samples, the miR-765-3p expression was decreased in GC tissues by qRT-PCR assay. Additional, miR-765-3p was upregulated both in the SGC-7901 cells (Figure 5B). Seeking from starBase (http://starbase.sysu.edu.cn/index.php), miR-765-3p harbored binding sites with LINC00994 (Figure 5C). Luciferase reporter analysis indicated that ectopic miR-765-3p expression suppressed wild type LINC00994 luciferase activity, but not mutant LINC00994 in SGC-7901 cells (Figure 5D). Elevated expression of LINC00994 inhibited the miR-765-3p expression in SGC-7901 cells (Figure 5E).

Figure 6. MiR-765-3p was downregulated in GC samples. A. Compared to adjacent samples, the miR-765-3p expression was decreased in GC tissues by qRT-PCR assay. B. miR-765-3p was downregulated in 29 GC cases (29/40; 72.5%) compared to adjacent specimens. C. LINC00994 level was inversely correlated with miR-765-3p in cases with GC.
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was downregulated in 29 GC cases (29/40; 72.5%) compared to adjacent specimens. LINC00994 induced GC cell invasion and growth via modulating miR-765-3p. Thus, our data suggested an oncogenic role of LINC00994 in development of GC.

LINC00994 was found to be downregulated in type 2 diabetes in the Iranian cohort [29]. Recently, Zhu et al [30]. showed that LINC00994 expression was overexpressed in pancreatic cancer samples and knockdown LINC00994 expression suppressed cell migration, growth and invasion and induced cell apoptosis and cycle arrest at G1 stage. Nevertheless, function role of LINC00994 in GC remains almost unknown. We firstly detected the LINC00994 expression in GC cells (AGS, SGC-7901, MGC8-03, and HGC-27) and GES-1. Our data indicated that LINC00994 was overexpressed in GC cells compare with GES-1. Compared to adjacent samples, the LINC00994 expression was increased in GC tissues by qRT-PCR assay. Furthermore, LINC00994 was overexpression in 31 GC cases (31/40; 77.5%) compared to adjacent specimens. Ectopic expression of LINC00994 induced GC cell invasion, proliferation and cycle.

Growing references suggested that LncRNAs play as miRNA sponges to inhibit the modulatory function of miRNAs, which cause post-transcriptional regulation on potential mRNAs [35, 36]. For instance, Wang et al [37]. demonstrated that UCA1 induced migration and growth and suppressed cell apoptosis via sponging miRNAs. Guo et al [38]. indicated that TUBA4B decreased GC cell progression via regulating miR-216b/a and miR-214. Dong et al [39]. found that GAS5 overexpression decreased
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development and tumorigenesis of GC via sponging miR-106a-5p. Huang et al [40], demonstrated that TRPM2-AS induced GC progression through modulating miR-195. Zhu et al [30], showed that LINC00994 inhibition inhibited pancreatic tumor cell progression via regulating miR-765-3p. Seeking from starBase (http://starbase.sysu.edu.cn/index.php), miR-765-3p harbored binding sites with LINC00994. Luciferase reporter analysis indicated that ectopic miR-765-3p expression suppressed wild type LINC00994 luciferase activity, but not mutant LINC00994 in SGC-7901 cells. Elevated expression of LINC00994 inhibited the miR-765-3p expression in SGC-7901 cells. Compared to adjacent samples, the miR-765-3p expression was decreased in GC tissues. Pearson’s correlation data indicated that LINC00994 level was inversely correlated with miR-765-3p in cases with GC.

Our data suggested that LINC00994 expression was overexpressed in GC cells and samples and ectopic LINC00994 expression promoted GC cell cycle, invasion and proliferation through modulating miR-765-3p. Thus, our data suggested an oncogenic role of LINC00994 in development of GC.

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Disclosure of conflict of interest

None.

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