Original paper

Up-regulated lncRNA LINC00858 facilitates proliferation and migration of gastric cancer cells

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Previous evidence has shown that the long intergenic non-protein coding RNA 858 (LINC00858) is an oncogene in non-small cell lung cancers. However, the role LINC00858 plays in gastric cancer (GC) is not clear. To illustrate the role LINC00858 plays in GC, the LINC00858 expression in GC and normal tissues was firstly detected. Then, the viability, proliferation and migration of GC BGC823 and MGC803 cells were assessed following LINC00858 knockdown by si-LINC00858 transfection. The results showed that LINC00858 had a high level of expressions in GC tissues as demonstrated by both online data and qRT-PCR assay. Also, the knockdown of LINC00858 reduced the proliferation and migration of BGC823 and MGC803 cells *in vitro*. Taken together, our data indicate that LINC00858 plays an oncogenic role in GC cells and might act as a potential therapeutic target for GC.

Key words: lncRNA, LINC00858, proliferation, migration, gastric cancer.

Introduction

Gastric cancer (GC) is one of the most common malignant tumors worldwide, causing a large number of cancer-related deaths every year [1, 2, 3]. Although much effort on diagnostic techniques and therapeutic measures has been made, the 5-year overall survival rate is still unsatisfactory [4]. Moreover, the underlying molecular mechanisms of GC progression and metastasis are unclear [5, 6]. Therefore, there is an urgent need to deeply understand the development and progression of GC, thereby finding novel diagnostic and therapeutic targets for GC.

Recently, long non-coding RNAs (lncRNAs), a class of transcripts (> 200 nt) with little or without protein capacity, have been found and reported [7, 8]. Similar to miRNAs, lncRNAs could regulate the gene expression associated with protein coding via transcription, posttranscriptional processing or epigenetic regulation, including binding with miRNA or chromatin modification and genomic imprinting. Furthermore, lncRNAs were also proved to be related to various diseases including metabolic disorders, cancers and cardiac diseases [9, 10, 11, 12].

Previous studies indicated that lncRNAs were associated with tumorigenesis and progression of GC [13, 14, 15, 16]. Specifically, the lncRNA NNT-AS1 expression markedly increased in GC cells and tissues relative to normal cells and adjacent normal tissues, and the knockdown of NNT-AS1 significantly decreased the proliferation and invasion of GC cells and led to an arrest of GC cell cycle progression at the G0/G1 phase. In addition, lncRNA PICART1 was proved to inhibit the proliferation of GC cells via

phosphoinositide-3-kinase/AKT and mitogen-activated protein kinase/extracellular-signal-regulated kinase signaling pathways [17]. Thus, this evidence suggests that lncRNA could participate in gastric cancer tumorigenesis [18]. LINC00858 is an lncRNA firstly reported in human non-small cell lung cancers, and it was highly expressed in osteosarcoma and colorectal cancers [19, 20, 21, 22]. However, its expression and related biological function in GC are still unclear. Therefore, the objective of this study was to explore the role LINC00858 plays in GC progression, thereby providing novel therapeutic targets for GC.

Material and methods

Data analysis for gene expression profile

The gene expression data about GC were obtained from The Cancer Genome Atlas (TCGA) and GEO databases. BAM and normalized probe-level intensity files were also obtained from TCGA and GEO datasets. The probe sequences were obtained from GEO or microarray manufacturers, and bowtie was employed to re-annotate probes based on the GENCODE Release 19 annotation for lncRNAs.

Table 1. Summary of clinical characteristics

Characteristics	Number of patients (%)
Total cases	24 (100)
Gender	
Male	16 (66.7)
Female	8 (33.3)
Age (years)	
< 60	6 (25.0)
≥ 60	18 (75.0)
Tumor size	
< 2 cm	7 (29.2)
≥ 2 cm	17 (70.8)
Differentiation	
Well/moderate	8 (33.3)
Poor	16 (66.7)
TNM stage	
I/II	10 (41.7)
III/IV	14 (48.3)
Lymph node metastasis	
Negative	7 (29.2)
Positive	17 (70.8)

Tissue sample collection

This trial was allowed by the Research Ethics Committee of the First People's Hospital of Huzhou (Huzhou, Zhejiang, China), and all participants provided written informed consent. 24 patients enrolled in this trial underwent resection of primary GC at the First People's Hospital of Huzhou. Subsequently, fresh tissues were snap-frozen with liquid nitrogen and then stored at –80°C for subsequent assays. The clinicopathological characteristics of all GC participants are presented in Table I.

Cell culture

BGC-823 and MGC-803 GC cell lines were provided by the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China), grown in RPMI 1640 or DMEM medium (Gibco, USA) with 10% FBS and 100 U/ml penicillin-streptomycin at 37°C in 5% CO_2.

QRT-PCR analysis

Total RNA was firstly extracted from cells and tissues using TRIzol reagent (Invitrogen, USA). Then, cDNA was synthetized with total RNA and a Reverse Transcription kit (Takara, China). Next, qRT-PCR analysis was conducted with SYBR Green (Takara, China). The expression of target genes was normalized to GAPDH, and all primer sequences are presented in Table S1.

Cell transfection

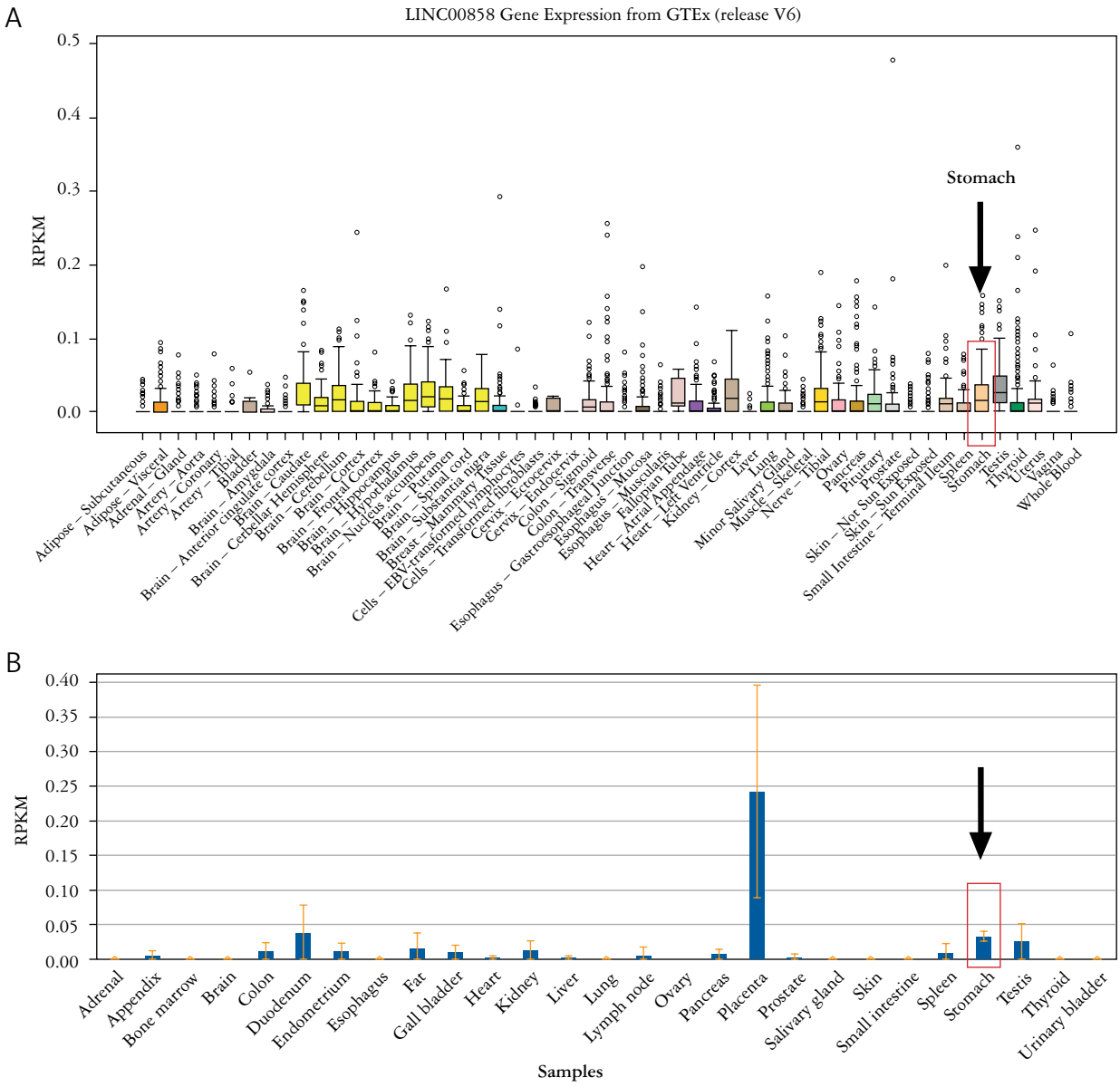
SiRNAs were transfected into BGC-823 and MGC-803 cells with Lipofectamine 2000 (Invitrogen, USA) based on the manufacturer's instructions. LINC00858-specific siRNA (siLINC00858; CCACAAGAGACAAATTGCAAGGCAT) and negative control siRNA (siNC; UUCUCCGAACGUGUCACGUTT) were provided by Invitrogen.

MTT assay

3×10^3 transfected cells were firstly plated in each well of 96-well plates, and then cell viability was determined every 24 h based on the manufacturer's instructions for an MTT kit (Sigma, USA).

Colony formation assay

The transfected cells were seeded in 6-well plates and cultured with appropriate media with 10% FBS for 10 to 14 days. The media were replaced every four days. Then, methanol was used to fix colonies, and 0.1% crystal violet (Sigma, USA) was employed to stain for 15 min. Finally, the stained colonies were counted to assess colony formation.



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Protein coding potential		
Metric	**Raw results**	**Interpretation**
PRIDE reprocessing 2.0	0	non-coding ?
Lee translation initation sites	0	non-coding ?
PhyloCSF score	–36.9823	non-coding ?
CPAT coding probability	9.66%	non-coding ?
Bazzini small ORFs	0	non-coding ?

Fig. 1. LINC00858 was lowly expressed in normal stomach tissues (A and B). The LINC00460 expression profile in normal tissues was obtained from UCSC (http://genome.ucsc.edu/) and NCBI (https://www.ncbi.nlm.nih.gov/gene/170425). C) LINC00858 was predicted to have no protein coding potential (https://lncipedia.org/db/transcript/LINC00858:1)

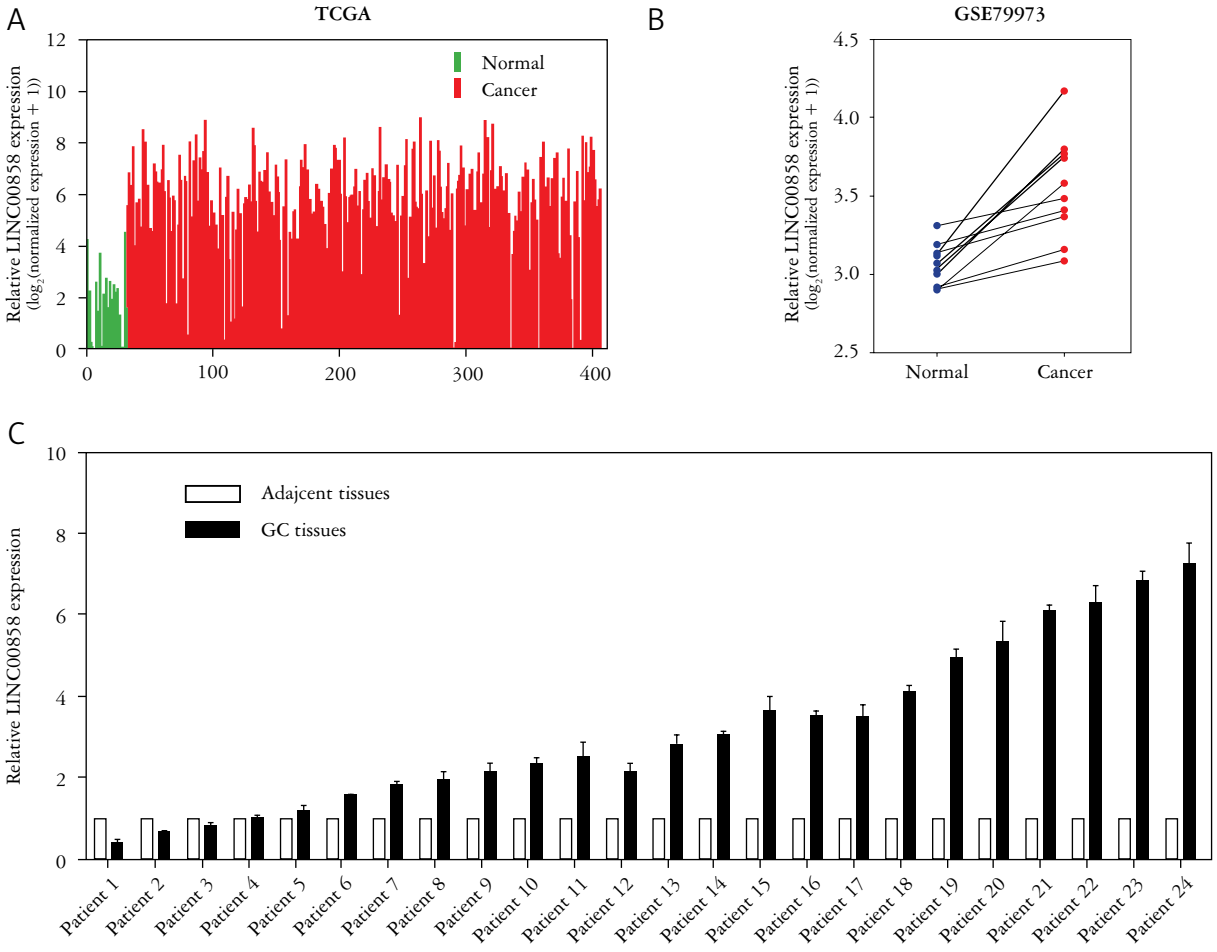

Fig. 2. LINC00858 was highly expressed in GC tissues: A) Relative expression of LINC00858 in human GC and normal tissues as demonstrated by TCGA data analysis. B) Relative expression of LINC00858 in human GC tissues relative to noncancerous tissues as demonstrated by GSE79973 data analysis. C) LINC00858 expression levels in GC (n = 24) and adjacent normal tissues (n = 24) were measured using qRT-PCR and normalized to GAPDH

Transwell assay

Cell migration was evaluated using a Transwell assay as previously described [23]. In brief, 24-well chambers were firstly placed into the upper chamber of an insert with an 8-μm pore size polycarbonate membrane (Millipore, USA). Then, the media with 15% FBS were added into the lower chamber. Following incubating for 24 h, a cotton swab was employed to remove the cells on the upper membrane. Next, 0.1% crystal violet was used to stain the cells that migrated through the polycarbonate membrane. Finally, the numbers of migrated cells were counted at 200 × magnification from five random views in each chamber. This trial was conducted in triplicate.

Statistical analysis

All data were presented as means ±SD. Statistical analysis was carried out with SPSS 20.0. Student's t-test was employed to compare the differences between control and experimental groups. P values < 0.05 were regarded as statistically significant.

Results

LINC00858 was highly expressed in GC tissues but rarely expressed in normal stomach tissues

According to the annotations of NCBI and University of California, Santa Cruz (UCSC) databases, LINC00858 is located on chr10: 84279980-84294659 and transcribed into a 2685 nt long lncRNA. As the UCSC database described, LINC00858 was lowly expressed in normal stomach tissues from 570 healthy participants (Fig. 1A, B). Moreover, as the LNCipedia database predicted, LINC00858 did not exhibit protein coding potential (Fig. 1C). To explore whether LINC00858 is involved in gastric tumorigenesis, the difference in LINC00858 expression between GC and normal

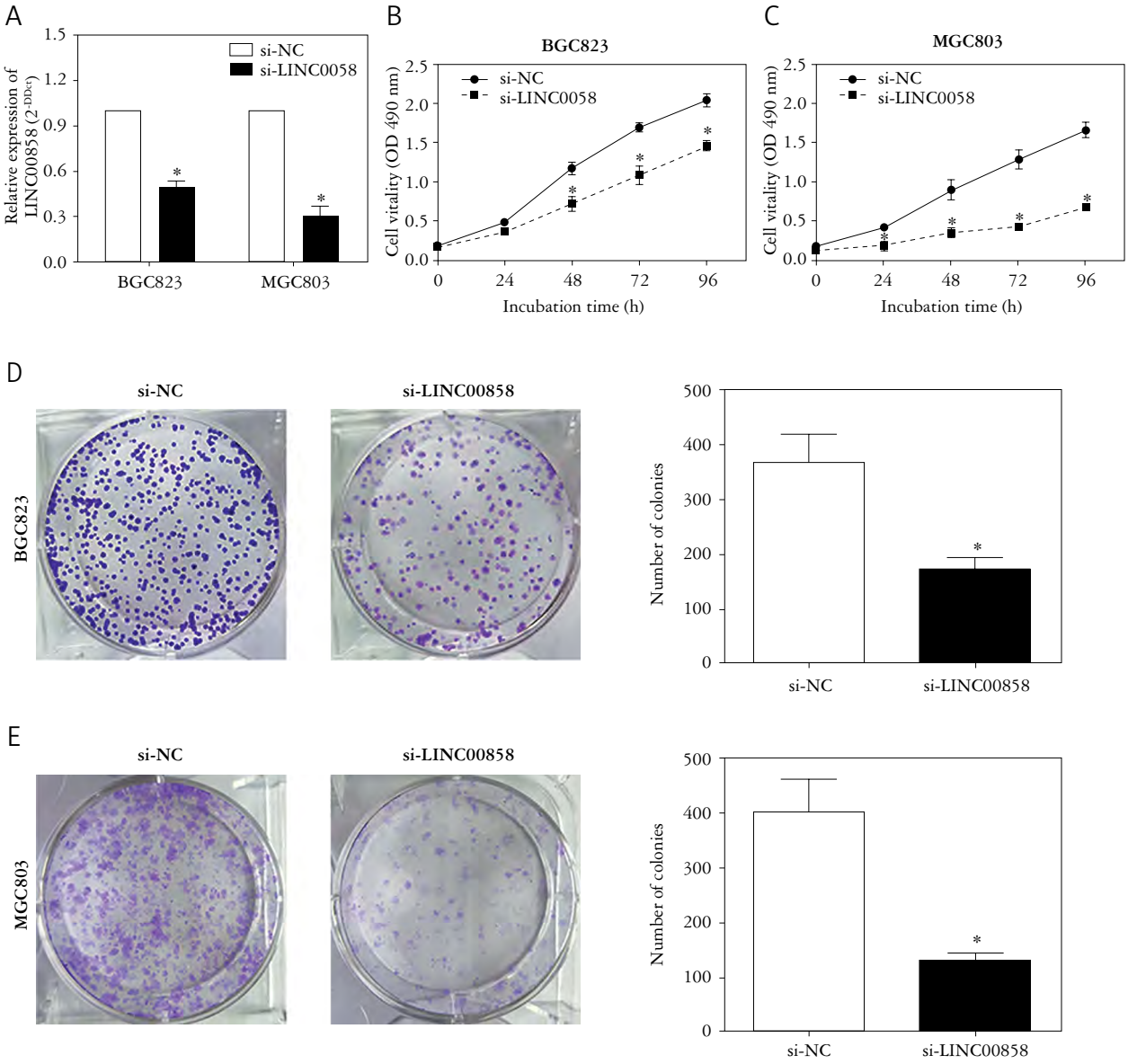

Fig. 3. LINC00858 knockdown suppressed GC cell proliferation: A) The expression of LINC00858 in siNC or siLINC00858-transfected BGC823 and MGC803 cells was determined using qRT-PCR. B) and C) MTT assay was carried out to measure the proliferation of BGC823 and MGC803 cells. D) Representative results from colony formation assay in the siNC or siLINC00858-transfected BGC823 and MGC803 cells. Data are expressed as mean values and SD from 3 representative experiments, * $p < 0.05$

stomach tissues was analyzed with the RNA sequencing data from TCGA and GSE79973 databases. The results showed that LINC00858 significantly increased in GC tissues relative to normal tissues, suggesting that LINC00858 could have potential oncogenic effects on GC (Fig. 2A, B). To further verity the microarray result, qRT-PCR was employed to measure the LINC00858 expression in 24 paired GC and adjacent normal tissues. As Fig. 2C demonstrates, LINC00858 was highly expressed in 79.2% of GC tissues (19 of 24). Together, the LINC00858 expression level markedly increased in GC tissues relative to normal stomach tissues.

Knockdown of LINC00858 expression in GC cells

Next, to further explore the potential biological effects of LINC00858 on GC cells, LINC00858 knockdown was performed through transfecting siLINC00858 into BGC823 and MGC803 cells. Subsequently, qRT-PCR was conducted to determine LINC00858 mRNA expression after 24 h of transfection. Our results demonstrated that LINC00858 expression markedly decreased in BGC823 and MGC803 cells after siLINC00858 transfection (Fig. 3A). Therefore, the siLINC00858-transfected BGC823 and MGC803 cells were used in subsequent trials.

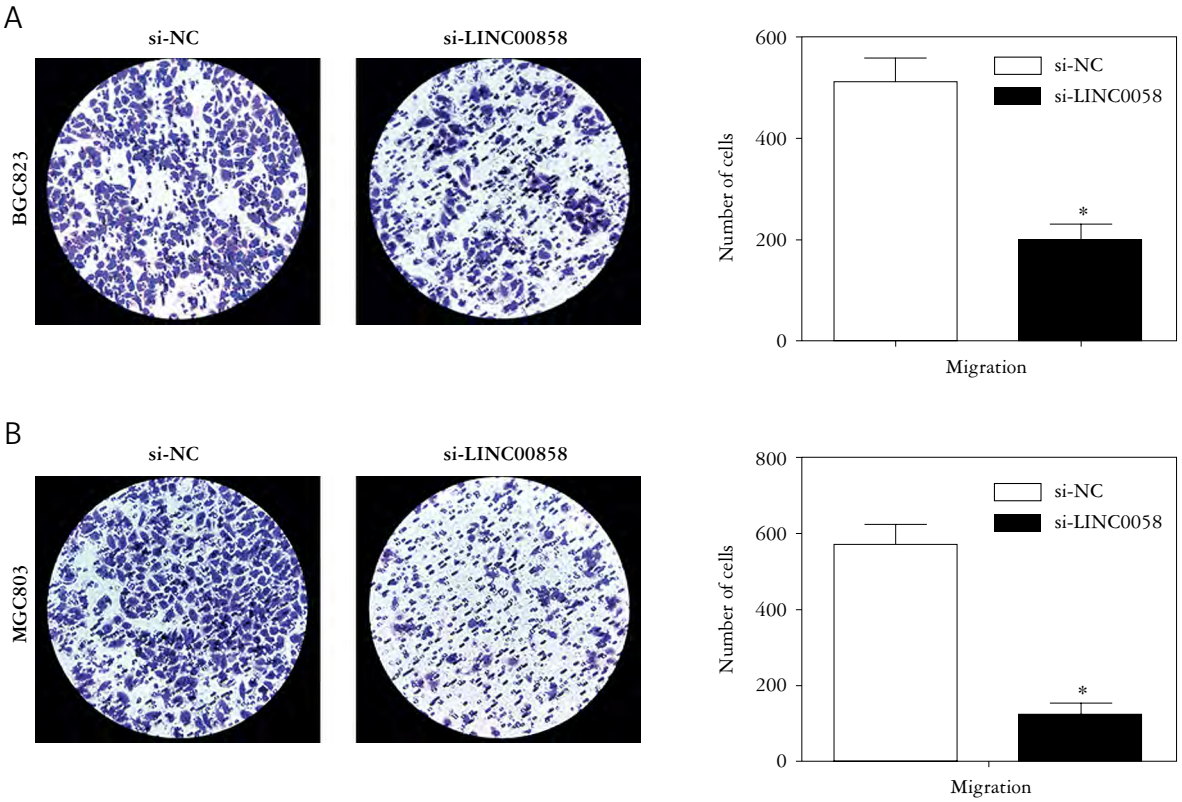

Fig. 4. LINC00858 knockdown suppressed GC cell migration. A, B) Transwell assay was employed to evaluate the migratory abilities of GC cells. Representative images from the transwell assay; the numbers of migrated cells were counted at 200 × magnification from five random views. Data were expressed as mean values and SD from 3 representative experiments, * $p < 0.05$

LINC00858 knockdown suppressed GC cell proliferation

In this trial, an MTT assay was conducted to assess the role LINC00858 plays in cell viability. As Fig. 3B and C show, cell proliferation significantly decreased in the siLINC00858-transfected GC cells relative to the siNC-transfected cells. In addition, as the colony formation assay showed, clonogenic survival also decreased after LINC00858 knockdown (Fig. 3D, F). The data together suggested that LINC00858 promoted GC cell proliferation.

LINC00858 knockdown suppressed GC cell migration

Following siLINC00858 transfection, a Transwell assay was performed to further evaluate the role LINC00858 plays in the metastasis of GC cells. The results showed that LINC00858 knockdown markedly decreased the migration of both BGC823 and MGC803 cells (Fig. 4A, B), indicating that LINC00858 might be closely involved the migration of GC cells.

Discussion

With the development of high-throughput RNA sequencing technology, numerous lncRNAs have been found [24, 25] and reported to be related to kinds of diseases including cancers [26, 27]. Some lncRNAs were proved to be able to modulate nearby gene expression through RNA-protein interactions, and other lncRNAs can be regarded as local regulators [28, 29]. Moreover, lncRNAs were reported to have important effects on genome stability, which is considered important in carcinogenesis [30]. That is to say, these lncRNAs can act as oncogenes or tumor suppressors [25]. Specifically, lncRNA HOXA-AS2 knockdown significantly suppressed cell proliferation via inhibiting the cell cycle and caused pancreatic cancer cell apoptosis [31]; LINC00337 promoted GC cell proliferation via epigenetically suppressing the EZH2-mediated p21 [32]; and LincRNA-p21 acted as a tumor suppressor in esophageal squamous cell carcinoma and induced G1 arrest via the p53 signaling pathway [33]. Therefore, investigation of the role dysregulated lncRNAs play might provide a novel perspective for cancer therapy.

Many studies have reported that lncRNAs play key roles in the development and progression of GC [34, 35],

but their specific roles and underlying mechanisms of action are still unclear. Also, the dysregulation of lncRNAs including HOTAIR, LINC00460, DUXAP10, MEG3 and SNHG15 was proved to affect the proliferation, apoptosis, migration, invasion and tumorigenicity of GC cells [36, 37, 38]. However, it is not easy to identify the abnormally expressed lncRNAs in GC and deeply investigate their roles and mechanisms due to the functional diversity of lncRNAs. In this study, according to the TCGA and GEO databases, we selected LINC00858 to further study GC, and found that LINC00858 was highly expressed in 24 GC tissues. Therefore, we supposed that LINC00858 might act as an oncogene in GC. As expected, LINC00858 promoted the proliferation and migration of GC cells.

Meanwhile, there were some limitations of this study. At first, the study population was not large enough to further verify the importance of LINC00858 in the progression of GC. More importantly, the possible targets and underlying regulatory mechanisms of LINC00858 in GC need to be further investigated.

In conclusion, our study showed that LINC00858 was up-regulated in GC tissues. Furthermore, the knockdown of LINC00858 markedly suppressed the proliferation and migration of GC cells, suggesting that LINC00858 could be involved in the progression of GC.

Learning points

LINC00858 was lowly expressed in normal stomach tissues. Additionally, the difference in LINC00858 expression between GC and normal stomach tissues was identified with the RNA sequencing data from TCGA and GSE79973 databases, and LINC00858 expression markedly increased in GC tissues relative to the corresponding noncancerous tissues. As functional experiments demonstrated, LINC00858 knockdown markedly diminished the proliferation and migration of GC cells. Together, our data indicated that LINC00858 could act as an 'onco-lncRNA' in GC and be a potential therapeutic target for GC.

The authors declare no conflict of interest.

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