Long Intergenic Non-Protein-Coding RNA 01138 Accelerates Tumor Growth and Invasion in Gastric Cancer by Regulating miR-1273e

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Background:
The treatment and nursing of gastric cancer (GC) remains an enormous challenge in clinical practice. Understanding the potential mechanisms of the pathogenesis of GC would improve GC therapy. Long intergenic non-protein-coding RNA 01138 (LINC01138) was reported to promote the progression of hepatocellular carcinoma; however, whether it is involved in GC progression has been unclear.

Material/Methods:
Expressions of LINC01138 and miR-1273e in GC tissues and cell lines were measured by qRT-PCR assay. The interaction between LINC01138 and miR-1273e was predicted by the online tool miRDB, verified by dual-luciferase reporter and RNA pulldown assays. Effects of LINC01138 knockdown or miR-1273e overexpression on cell viability, proliferation, apoptosis, invasion, and migration were evaluated by MTT, colony formation assay, flow cytometry, and Transwell assays. Target genes of miR-1273e were predicted by KEGG analysis, and involvement of the mitogen-activated protein kinase (MAPK) pathway was confirmed by qRT-PCR assay.

Results:
LINC01138 was increased but miR-1273e was decreased in GC tissues and cell lines. Knockdown of LINC01138 suppressed GC cell viability, proliferation, invasion, and migration, and promoted GC cell apoptosis. We demonstrated that LINC01138 contributed to GC progression by directly sponging and inhibiting miR-1273e. Moreover, the MAPK pathway was verified to participate in the promotive effects of LINC01138 on GC progression.

Conclusions:
LINC01138 activated the MAPK signaling pathway by inhibiting miR-1273e to promote GC cell proliferation, invasion, and migration, and inhibit GC cell apoptosis, suggesting that the LINC01138/miR-1273e/MAPK axis is a promising therapeutic target for GC.

MeSH Keywords:
MicroRNAs • Mitogen-Activated Protein Kinase 1 • RNA, Long Noncoding • Stomach Neoplasms

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Background

Gastric cancer (GC), characterized by an uncontrolled proliferation of cancerous cells within the lining of the stomach, is one of the most frequently diagnosed gastrointestinal diseases, affecting many people worldwide [1,2]. Chemotherapy is usually performed for the treatment of early GC, providing an excellent survival rate for patients with early GC; however, because advanced GC is frequently resistant to chemotherapy, the primary therapeutic strategy for advanced GC is laparoscopy-assisted gastrectomy (LAG). Although LAG significantly improves the survival rate of advanced GC patients, many patients die from the postoperative complications of LAG, including anastomosis-site leakage, hemorrhage, and reflux gastritis [3–7]. In the course of clinical care of patients with gastric cancer, we found that no matter how serious and accurate the postoperative nursing work, some patients died. Thus, there is a need to elucidate the potential molecular mechanisms of GC pathogenesis, which might contribute to treatment and nursing of GC patients.

Long non-coding RNAs (lncRNAs), characterized by the lack of functional protein-coding capacity, are a type of evolutionarily conserved RNAs, belonging to the non-coding RNAs (ncRNAs) family [8]. In function, lncRNAs were demonstrated to be responsible for the regulation of multiple biological processes by controlling the transcription of corresponding genes in various tissues, such as brain, lung, liver, and skin [9,10]. Increasing evidence shows that the mechanism underlying the effects of lncRNAs might involve regulation of the expression of target genes by sponging to microRNAs (miRNAs), which subsequently influences the transcription of target genes [11]. The lncRNAs/miRNAs/mRNAs axis plays an important role in the progression of various human cancers, including GC. For instance, decreased lncRNA HOTAIRM1 was reported to inhibit GC cell progression by regulating the miR-17-5p/PTEN axis [12]. Moreover, lncRNA GAPLINC was demonstrated to promote GC cell proliferation by sponging miR-378 to release MAPK1 [13]. Thus, lncRNAs might contribute to explore novel biomarkers of GC, and also might act as potential therapeutic targets.

In this study, we revealed that long intergenic non-protein-coding RNA 1138 (LINC01138) was overexpressed in GC tissues and cell lines, and acts as an oncogenic agent. LINC01138 sponged and negatively regulated miR-1273e, which subsequently resulted in activation of the MAPK signaling pathway. Thus, in the present study, we demonstrate a critical role of the LINC01138/miR-1273e/MAPK signaling pathway in GC progression.

Material and Methods

Patient samples, cell lines, and transfection

Fifty-one pairs of human GC tissues and corresponding normal tissues were collected from patients who were diagnosed as having GC in the Tianjin Fifth Central Hospital during the period 2014–2018. Written consents were obtained from all subjects and this study was approved by the Ethics Committee of the Tianjin Fifth Central Hospital. The human non-malignant gastric epithelial cell line GES-1 and 4 GC cell lines (SGC-7901, MKN-28, AGS, and MGC-803) were all purchased from the Shanghai Academy of Sciences. Cells were all maintained at 37°C with 5% CO_2 and 95% O_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with penicillin (100 U/ml), streptomycin (100 µg/ml), and 10% fetal bovine serum. For the cell transfection, small interfering RNAs (siRNA) against LINC01138 (si-Lnc (Mix)), and scramble negative control siRNA (si-NC), miR-1273e mimics, and negative controls (NC) were designed and provided by GenePharma (Shanghai, China). They were transfected into GC cells through Lipofectamine 2000 Reagent (Life Technologies, Thermo Fisher Scientific, Inc., Waltham, MA).

RNA extraction and quantitative real-time PCR (qRT-PCR) assay

After being lysed with TRIzol buffer (#9109, Takara, Japan), total RNAs of GC tissues and cell lines were extracted and reverse transcribed into cDNA by using a Bestar™ qPCR RT kit (#2220, DBI Bioscience, China). qRT-PCR assay was carried out with BestarTM qPCR MasterMix (#2043, DBI Bioscience, China) on the ABI7300 system. The sequence of primers used in this study is shown in Table 1. Expression of miRNA was normalized to U6, and expression of mRNA was normalized to GAPDH.

Cell viability detection

GC cell viability was detected by MTT assay. In brief, treated GC cells were firstly cultured into 96-well plates at 37°C for 24 h, and then 30 µl of dye solution was added into each well. After incubation at 37°C for an additional 4 h, 200 µl stop solution was added and incubated for 1 h to abolish the reaction. Finally, the absorbance at 570 nm was measured at 0, 24, 48, and 72 h after transfection by Infinite® 200 PRO (FPRO-T; Tecan, Seestrasse, Switzerland).

Cell proliferation analysis

GC cell proliferation was determined through colony formation assay. Briefly, treated GC cells (1000 cells/well) were seeded into 6-well plates and maintained at 37°C for 2 weeks. Subsequently, colonies were fixed in methanol for 1 h and

Table 1. The sequences of primers in this study.

Gene	Primer sequences
GAPDH	Forward: 5'-TATGATGATATCAAGAGGGTAGT-3' Reverse: 5'-TGTATCCAAACTCATTGTCATAC-3'
LINC01138	Forward: 5'-ACATCGTGAGCACATTTGAGA-3' Reverse: 5'-TCTTGCTGTTCAGGGTGGTA-3'
EGFR	Forward: 5'-GCACATTTTGGGAAGTTGCA-3' Reverse: 5'-CTGCTCAAAGGGACAATATTCTTG-3'
FGF1	Forward: 5'-CAGCTCAGTGCGGAAAGTGCG-3' Reverse: 5'-CATTTGGTGTCTGCGAGC-3'
FGF23	Forward: 5'-GGCAACATTTTTGGATCA-3' Reverse: 5'-CCGGGGCTTCAGCACGTT-3'
MAP2K6	Forward: 5'-AGTGGAGGCTGATGACTTGG-3' Reverse: 5'-CAGACGTTTCTGCTCCTGTG-3'
MAP3K7	Forward: 5'-CCGCTCGAGCTCTGGGACCGTTACATTTTGA-3' Reverse: 5'-TCCTGCGGCCGCTCCAAGAATCACTGCAGGAAGA-3'
miR-1273e	Forward: 5'-ACACTCCAGCTGGGTTGCTTGAACCCAGGA-3' Reverse: 5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTCCACTTC-3'
U6	Forward: 5'-CGCTTCACGAATTTGCGTGTCAT-3' Reverse: 5'-GCTTCGGCAGCACATATACTAAAAT-3'

stained with Giemsa solution for 15 min. The number of colonies in the 6-well plates was determined under a microscope.

Flow cytometry analysis

Twenty-four hours after transfection, GC cells were collected and centrifuged for 10 min at 2000 rpm. Cells were then washed with pre-cold phosphate-buffered saline 3 times, and fixed with 4% paraformaldehyde for 1 h, followed by staining with an apoptosis detection kit (Biolegend, San Diego, CA, USA) for 10 min at room temperature. Finally, treated GC cells were subjected to apoptosis and cell cycle analysis with a flow cytometer (FACSCanto™ II, BD Biosciences).

Migration and invasion analysis

GC cell invasion and migration abilities were examined by Transwell assay by using 8-μm pore chambers (Corning Incorporated, Corning, NY, USA) with or without Matrigel matrix (BD Biosciences, Franklin Lakes, NJ, USA), respectively. Treated GC cells were harvested and re-suspended in culture medium, making the final concentration 1×10^4 cells/ml. Subsequently, 0.2 ml of the GC cell suspension solution was added into the upper chamber, and 0.5 ml of culture medium containing 20% FBS was added to the lower chamber. After incubation at 37°C for 24 h, the invaded and migrated GC cells were stained with 0.5% crystal violet (Beyotime Institute of Biotechnology, Haimen, China) and counted under a microscope.

Plasmid construction and dual-luciferase activity assay

For plasmid establishment, the sequence of LINC01138 containing wild-type (WT) or mutated (MUT) potential binding site for miR-1273e was inserted downstream of the luciferase gene in a luciferase vector psi-CHECK (Promega, Madison, USA). For dual-luciferase activity assay, MGC-803 and SGC-7901 cells were cultured into 24-well plates and then transfected with recombinant plasmid and miR-1273e mimics or its negative control using Lipofectamine 2000 Reagent (Life Technologies, Thermo Fisher Scientific, Inc., Waltham, MA). Forty-eight hour after transfection, relative luciferase activity was detected by a Dual-Luciferase Assay Kit (Promega, Madison, WI). Renilla luciferase activity was normalized to Firefly luciferase activity.

Pathway enrichment analysis

The target genes of miR-1273e was analyzed by Kyoto Encyclopedia of Genes and Genomes (KEGG) via the Database for Annotation, Visualization and Integrated Discovery (DAVID, https://david.ncifcrf.gov/).

RNA pulldown assay

The interaction between LINC01138 and miR-1273e was further confirmed by RNA pulldown assay based on MS2-MBP protein, which was purified from *E. coli*. MS2 binding sites were inserted into the LINC01138 by site-directed mutagenesis to form LINC01138-WT-MS2 or LINC01138-MUT-MS2 recombinant
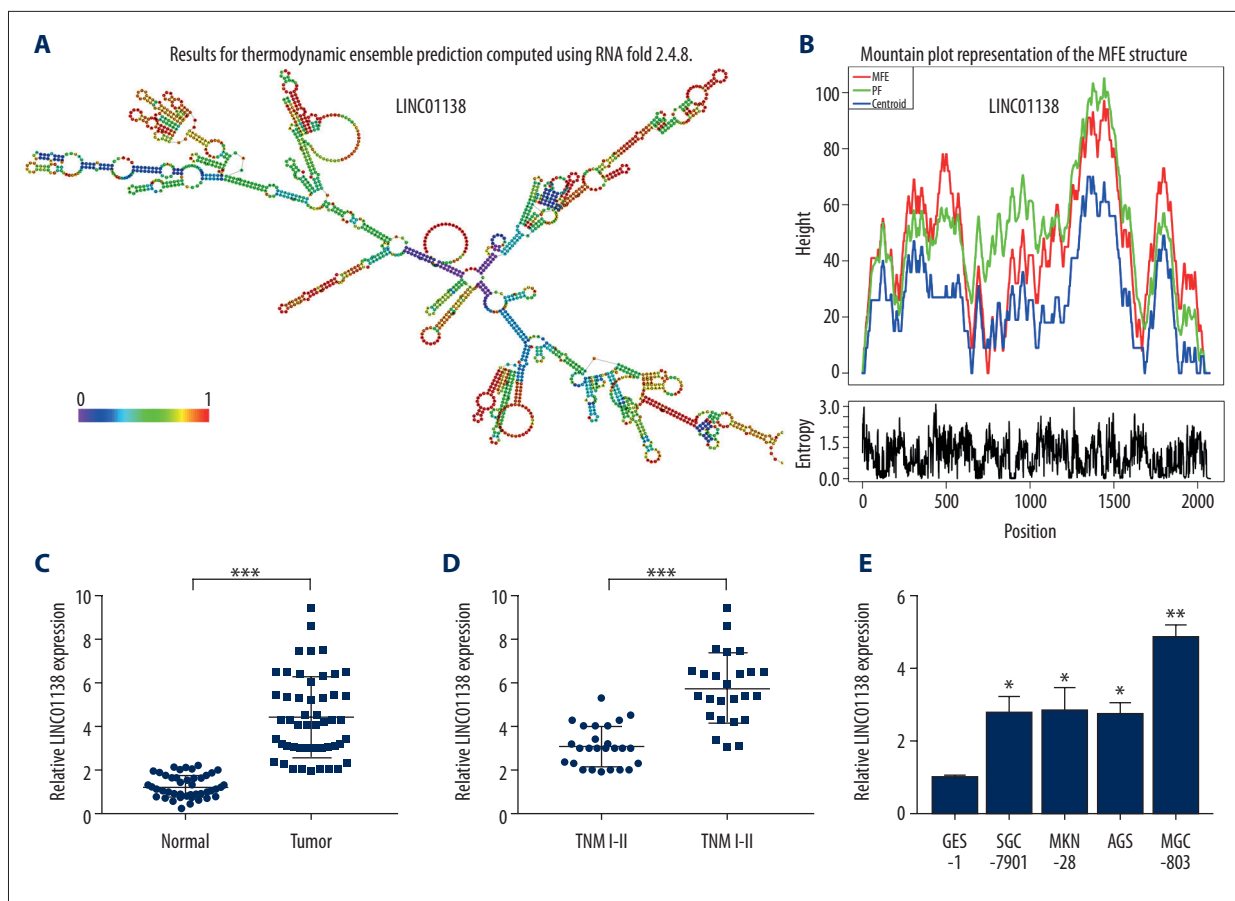
Figure 1. LINC01138 was overexpressed in GC tissues and cell lines. (**A**) Results for thermodynamic ensemble prediction of LINC01138 computed using RNAfold 2.4.8. (**B**) Mountain plot representation of the MFE structure of LINC01138. (**C, D**) Expression of LINC01138 in 51 paired GC tissues (26 TNM I–II, 25 TNM III–IV) and corresponding normal tissues was assessed by qRT-PCR assay (*** *P*<0.001). (**E**) Expression of LINC01138 in four GC cell lines (SGC-7901, MKN-28, AGS, and MGC-803) and a non-malignant gastric epithelial cell line GES-1 was evaluated by using qRT-PCR assay (* *P*<0.05, ** *P*<0.01).

plasmids. MGC-803 and SGC-7901 cells were transfected with empty vector (MS2), LINC01138-WT-MS2, or LINC01138-MUT-MS2 recombinant plasmid and harvested 48 h after transfection. After RNA pulldown analysis, the expression of miR-1273e was confirmed by qRT-PCR assay.

Statistical analysis

Experimental data are all presented as mean ± SEM, and were analyzed with GraphPad (Ver. Prism 7, GraphPad Prism Software, La Jolla, CA, USA). P values were analyzed using the t test and one-way analysis of variance analysis, as appropriate, and P values less than 0.05 were considered statistically significant.

Results

LINC01138 was overexpressed in GC tissues and cell lines

The LINC01138 secondary structure and volcano plot representation of the minimum free energy (MFE) structure were predicted by RNAfold (Figure 1A, 1B). The free energy of the thermodynamic ensemble is -632.85 kcal/mol and the ensemble diversity is 560.15. To explore the roles of LIN01138 in GC, we examined its expression in GC tissues and cell lines by qRT-PCR assay. Results showed that LINC01138 expression was significantly higher in GC tissues than in matched normal tissues (*P*<0.001 *vs.* normal tissues, Figure 1C), and its expression gradually increased with clinical stage (*P*<0.001 *vs.* TNM I–II, Figure 1D). Moreover, LINC01138 was found to be significantly upregulated in GC cell lines SGC-7901, MKN-28, AGS, and MGC-803 compared with the non-malignant gastric epithelial cell line GES-1 (*P*<0.05, *P*<0.01 *vs.* GES-1, Figure 1E). In addition, the 51 GC patients were divided into a high LINC01138

Table 2. Correlations between LINC01138 expression and clinicopathologic characteristics in GC.

Clinicopathologic characteristics	No. of patients	LINC01138		P value
		High	Low	
Age (year)				
>60	24	11 (45.8%)	13 (54.2%)	0.340
≤60	27	15 (55.6%)	12 (44.4%)	
Gender				
Male	27	17 (63.0%)	10 (37.0%)	0.362
Female	24	13 (54.2%)	11 (45.8%)	
Tumor size (cm)				
<3	34	18 (52.9%)	16 (47.1%)	0.310
≥3	17	11 (64.7%)	6 (35.3%)	
Differentiation grade				
Well/moderately	28	10 (35.7%)	18 (64.3%)	0.065
Poorly/undifferentiated	23	14 (60.9%)	9 (31.9%)	
Invasion				
T0–T2	29	9 (31.0%)	20 (69.0%)	0.021*
T3–T4	22	14 (63.6%)	8 (36.4%)	
Lymphatic metastasis				
N0	23	7 (30.4%)	16 (69.6%)	0.016*
N1–N4	28	18 (64.3%)	10 (35.7%)	
Distal metastasis				
M0	38	13 (34.2%)	25 (65.8%)	0.030*
M1	13	9 (69.2%)	4 (30.8%)	
TNM stage				
0 & I & II	25	9 (36.0%)	16 (64.0%)	0.008**
III & IV	26	19 (73.1%)	7 (26.9%)	

* $P<0.05$; ** $P<0.01$, TNM stage: pathologic tumor, node, metastasis stage.

group (n=26) and a low LINC01138 group (n=25), and statistical results indicated that LINC01138 expression was closely correlated with invasion, lymphatic metastasis, distant metastasis, and TNM stage, but not age, gender, tumor size, or differentiation grade (Table 2).

LINC01138 knockdown inhibited GC cell proliferation and promoted cell apoptosis

Subsequently, we analyzed the effects of LINC01138 on GC cell viability, proliferation, apoptosis, and cycle in LINC01138-silenced MGC-803 and SGC-7901 cells. The knockdown efficiency of LINC01138 in MGC-803 and SGC-7901 cells was examined by qRT-PCR assay, showing LINC01138 was significantly down-regulated in si-Lnc(Mix)-treated MGC-803 and SGC-7901 cells compared with those cells treated with si-NC ($P<0.05$ *vs.* si-NC,

Figure 2A). Compared to the si-NC group, LINC01138 knockdown remarkably reduced cell viability ($P<0.05$, Figure 2B), inhibited cell proliferation ($P<0.05$, Figure 2C), and promoted cell apoptosis ($P<0.05$, Figure 2D) of MGC-803 and SGC-7901 cells. Moreover, LINC01138 deficiency significantly decreased the MGC-803 and SGC-7901 cell numbers in S phase ($P<0.05$, Figure 2E).

LINC01138 knockdown suppressed GC cell invasion

As we had already demonstrated that LINC01138 participates in the regulation of GC cell viability, proliferation, and apoptosis, we wondered whether it is involved in GC cell invasion and migration. As expected, LINC01138 knockdown significantly reduced the migratory and invasive cell numbers of MGC-803 and SGC-7901 cells ($P<0.05$ *vs.* si-NC, Figure 3A, 3B).
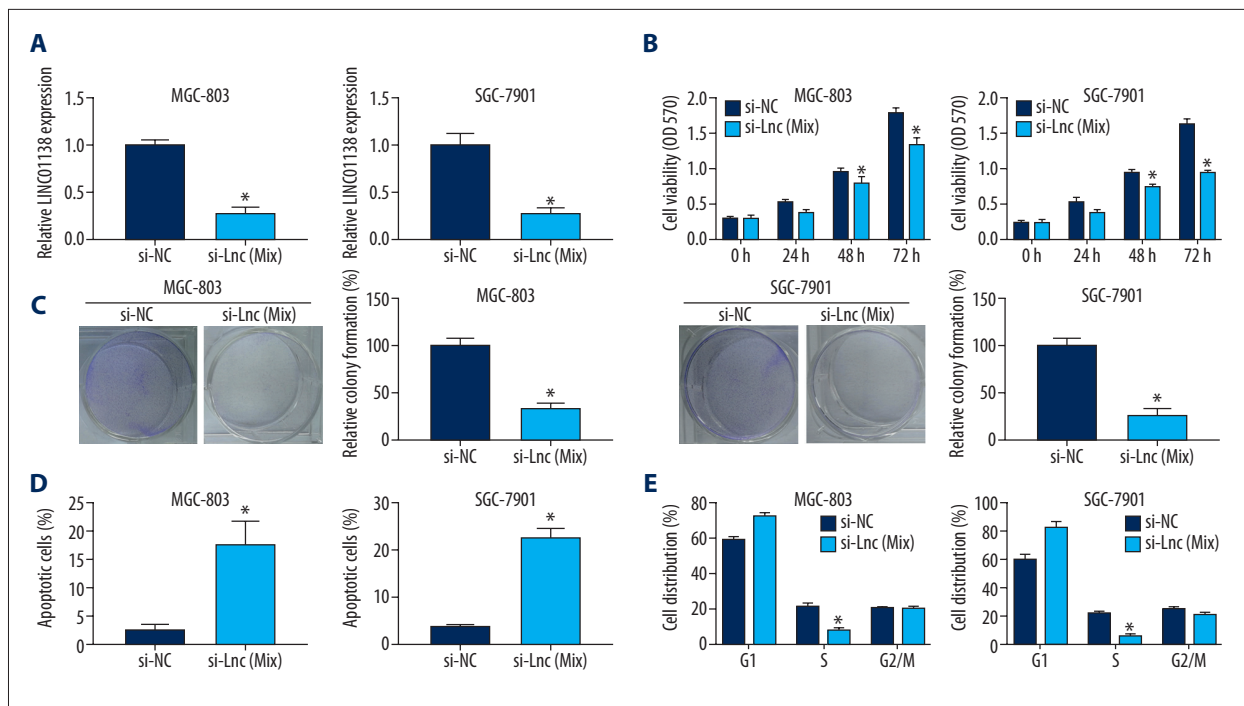
Figure 2. LINC01138 knockdown inhibited GC cell proliferation and promoted cell apoptosis. (**A**) Expression of LINC01138 in MGC-803 and SGC-7901 cells transfected with si-NC or si-Lnc(Mix) (* *P*<0.05). (**B**) MTT assay was performed in MGC-803 and SGC-7901 cells transfected with si-NC or si-Lnc(Mix) to evaluate cell viability (* P<0.05). (**C**) Colony formation assay was carried out in MGC-803 and SGC-7901 cells transfected with si-NC or si-Lnc(Mix) to detect the effects of LINC01138 knockdown on cell proliferation (* *P*<0.05). (**D**) Cell apoptosis of MGC-803 and SGC-7901 cells transfected with si-NC or si-Lnc(Mix) was detected by flow cytometry analysis (* *P*<0.05). (**E**) Cell cycle distribution of MGC-803 and SGC-7901 cells transfected with si-NC or si-Lnc(Mix) was assessed via flow cytometry (* *P*<0.05).

Moreover, we evaluated the epithelial-mesenchymal transition (EMT) markers (E-cadherin and N-cadherin) by qRT-PCR assay in MGC-803 and SGC-7901 cells transfected with si-NC or si-Lnc(Mix). Results showed that LINC01138 knockdown significantly decreased the N-cadherin expression, whereas it increased the E-cadherin expression in MGC-803 and SGC-7901 cells (*P*<0.05 *vs.* si-NC, Figure 3C).

LINC01138 sponged miR-1273e

To further explore the potential molecular mechanisms of LINC01138 in GC progression, we predicted the binding partners of LINC01138 by using the miRDB online tool (*http://mirdb. org/miRDB/index.html*). Results indicated that LINC01138 potentially binds to miR-1273e (Figure 4A) and its expression was significantly downregulated in GC tumor tissues compared with corresponding normal tissues (*P*<0.001 *vs.* normal group, Figure 4B). Subsequently, dual-luciferase activity was assessed to verify the binding of LINC01138 and miR-1273e. Results showed that miR-1273e transfection significantly suppressed the luciferase activity driven by the wild-type (WT) LINC01138 binding sequence, but it had no obvious effects on luciferase activity when the binding sequence was mutated in MGC-803

and SGC-7901 cells (*P*<0.05 *vs.* NC group, Figure 4C). RNA pull-down assay was carried out to further confirm the interaction between LINC01138 and miR-1273e in MGC-803 and SGC-7901 cells. Results indicated that the MS2-tagged LINC01138-WT remarkably enriched miR-1273e in MGC-803 and SGC-7901 cells, while mutation of the binding sequence abolished this effect (*P*<0.05 *vs.* MS2 group, Figure 4D). In addition, results from qRT-PCR assay showed that overexpression of miR-1273e led to decreased LINC01138 expression in MGC-803 and SGC-7901 cells (*P*<0.05 *vs.* NC, Figure 4E), while LINC01138 knockdown resulted in increased miR-1273e expression in MGC-803 and SGC-7901 cells (*P*<0.05 *vs.* si-NC, Figure 4F).

miR-1273e inhibited GC cell proliferation, migration, and invasion

Effects of miR-1273e on GC progression were subsequently investigated by MTT, colony formation, and Transwell assays in miR-1273e-overexpressed MGC-803 and SGC-7901 cells. The efficiency of miR-1273e overexpression in MGC-803 and SGC-7901 cells was examined by qRT-PCR assay (*P*<0.05 *vs.* NC, Figure 5A). MTT assay indicated that miR-1273e overexpression significantly reduced the MGC-803 and SGC-7901
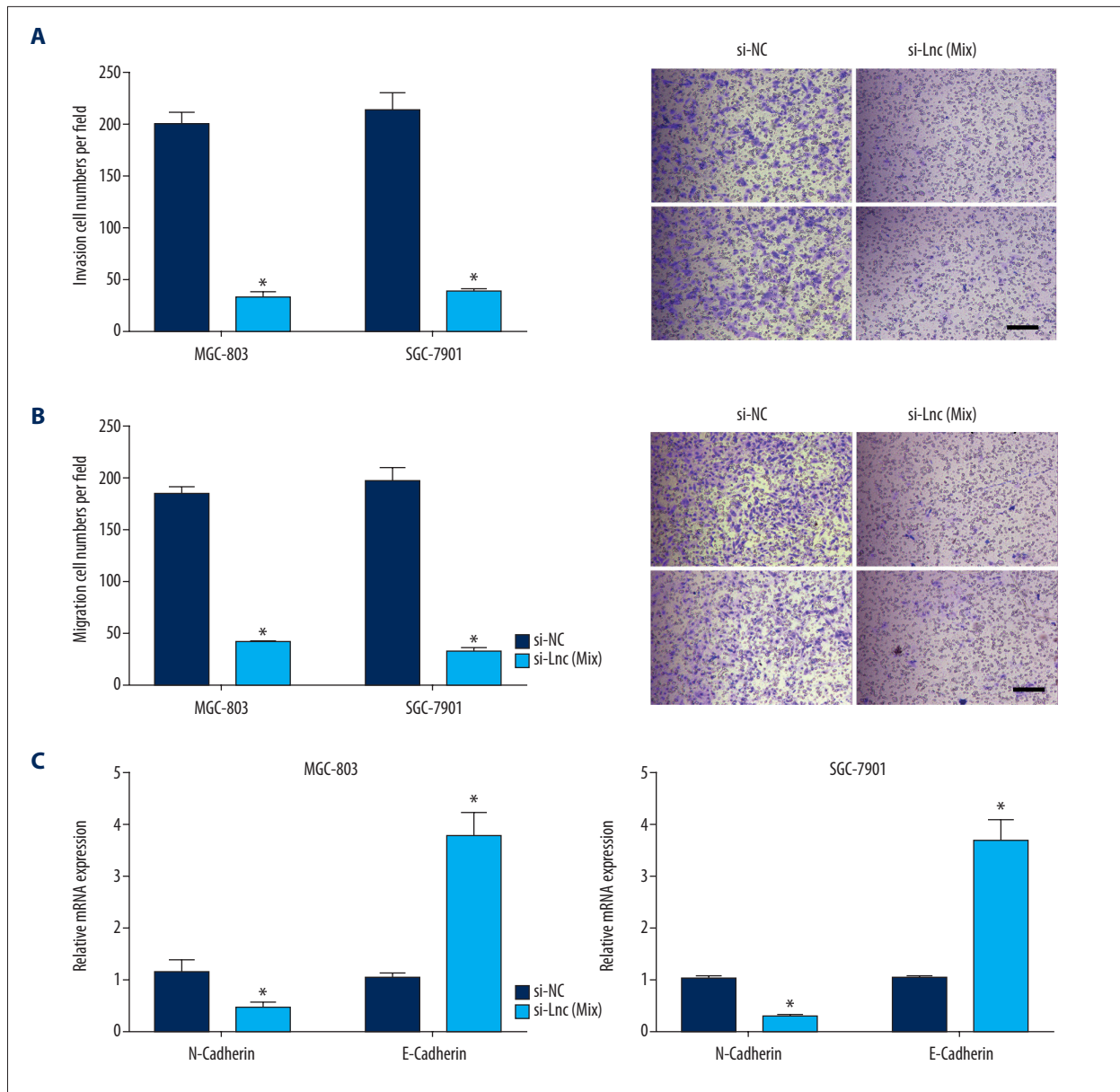
Figure 3. LINC01138 knockdown suppressed GC cell invasion. (**A, B**) MGC-803 and SGC-7901 cells transfected with si-NC or si-Lnc(Mix) were subjected to the analysis of invasive capacity by transwell assay (* *P*<0.05). (**C**) Expressions of N-Cadherin and E-Cadherin in si-NC- or si-Lnc(Mix)-treated MGC-803 and SGC-7901 cells were measured by qRT-PCR assay (* *P*<0.05).

cell viability compared to the NC group (*P*<0.05 *vs.* NC, Figure 5B). Meanwhile, miR-1273e overexpression resulted in attenuated colony formation capacity of MGC-803 and SGC-7901 cells (*P*<0.05 *vs.* NC, Figure 5C). Moreover, the invasive and migratory abilities were also remarkably inhibited in the miR-1273e-silenced MGC-803 and SGC-7901 cells (*P*<0.05 *vs.* NC, Figure 5D, 5E).

LINC01138 knockdown inhibited the MAPK signaling pathway

To investigated the downstream targets of miR-1273e, we performed KEGG analysis of miR-1273e to predict its targets genes, showing that the MAPK signaling pathway was the most correlated pathway (Figure 6A). Next, we verified the effects of LINC01138 knockdown on the MAPK signaling pathway by examining the expression of several key factors in the MAPK pathway in MGC-803 cells via qRT-PCR assay. Results indicated that the expression levels of epidermal growth factor
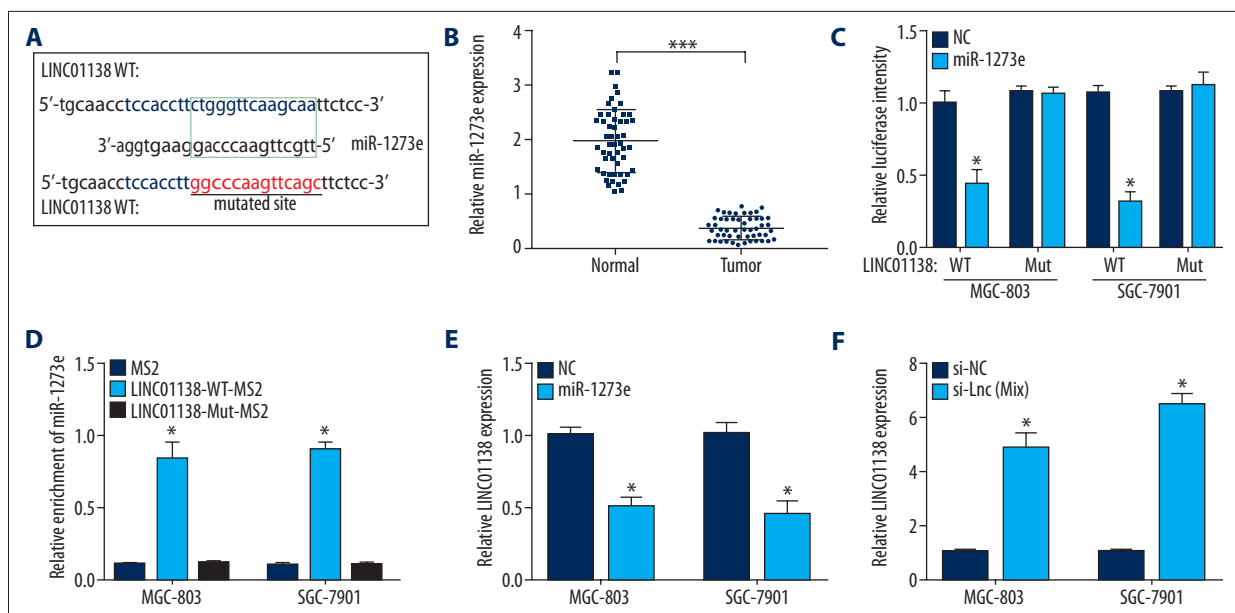
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Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System]
[ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica]
[Chemical Abstracts/CAS]

Figure 4. LINC01138 sponged miR-1273e. (**A**) Putative interacting site with miR-1273e in LINC01138. (**B**) Expression of miR-1273e in 51 paired GC and normal tissues was measured by qRT-PCR assay (*** P<0.001). (**C**) Interaction between miR-1273e and LINC01138 was verified by dual-luciferase reporter assay in MGC-803 and SGC-7901 cells (* *P*<0.05). (**D**) RNA pulldown assay was utilized to verify the interaction between LINC001138 and miR-1273e by using MS2-tagged LINC001138 in MGC-803 and SGC-7901 cells (* *P*<0.05). (**E**) Expression of LINC001138 was detected in NC or miR-1273e mimics-treated MGC-803 and SGC-7901 cells by qRT-PCR assay (* *P*<0.05). (**F**) Expression of miR-1273e was detected in si-NC- or si-Lnc(Mix)-treated MGC-803 and SGC-7901 cells by qRT-PCR assay (* *P*<0.05).
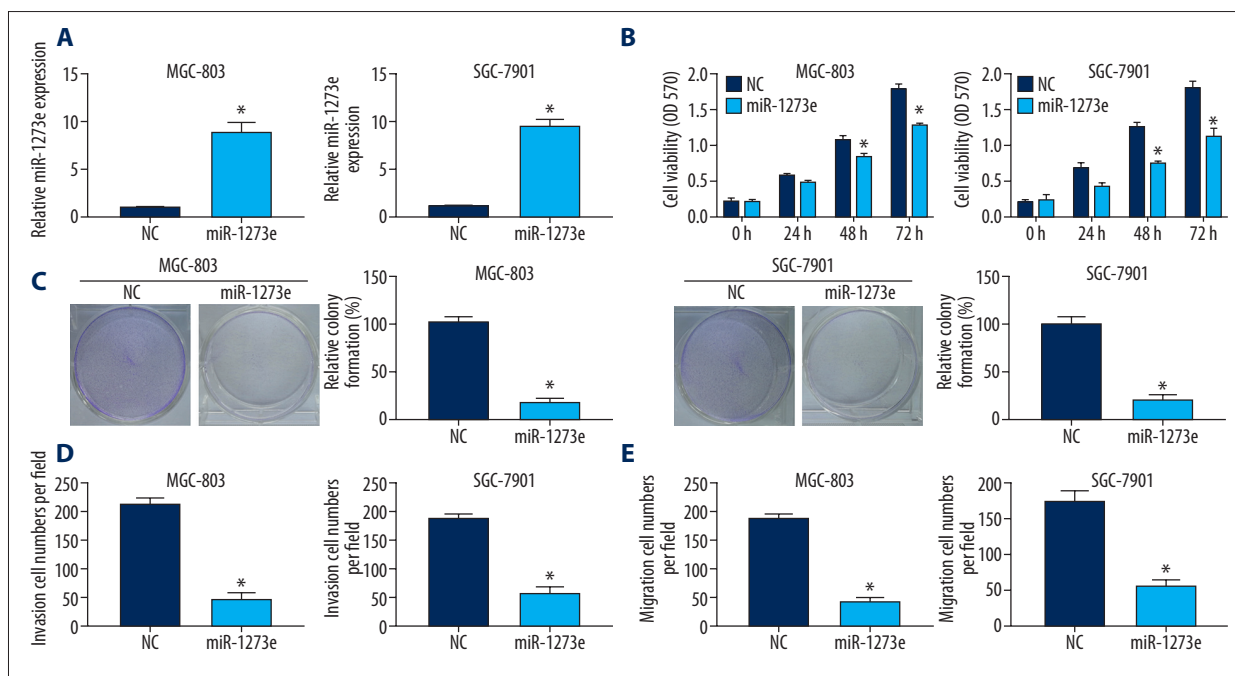

Figure 5. miR-1273e inhibited cell proliferation, migration, and invasion. (**A**) Expression of miR-1273e was assessed by qRT-PCR assay in NC or miR-1273e mimics-treated MGC-803 and SGC-7901 cells (* P<0.05). (**B**) Cell viability was examined in NC or miR-1273e-mimics treated MGC-803 and SGC-7901 cells via MTT assay (* P<0.05). (**C**) Cell proliferation was measured by colony formation assay in NC or miR-1273e mimics-treated MGC-803 and SGC-7901 cells (* P<0.05). (**D, E**) Cell migration and invasion of MGC-803 and SGC-7901 cells treated with NC or miR-1273e mimics were evaluated by Transwell assay (* P<0.05).
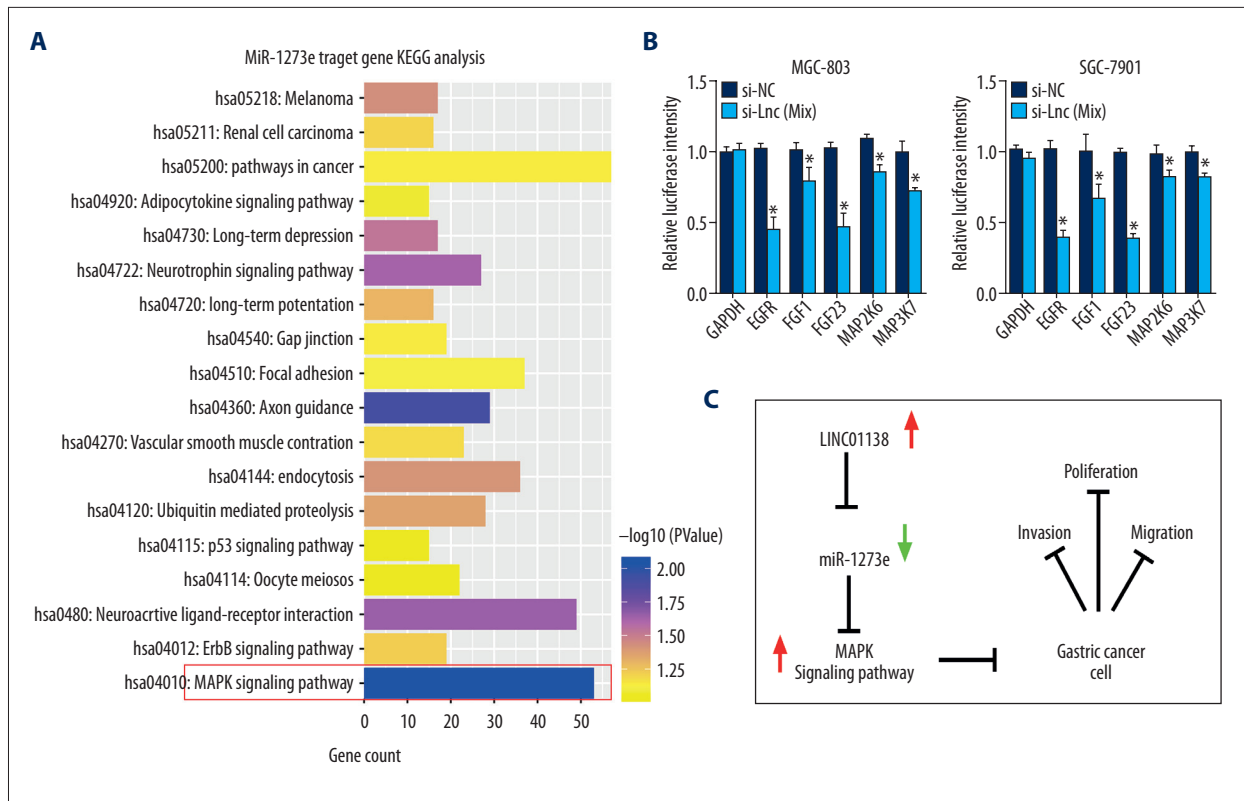
Figure 6. LINC01138 knockdown inhibited MAPK signaling pathway. (**A**) KEGG pathway enrichments of predicted target genes of miR-1273e. (**B**) Expressions of EGFR, FGF1, FGF23, MAP2K6, and MAP3K7 were measured by qRT-PCR assay in LINC01138-blocked MGC-803 and SGC-7901 cells (* *P*<0.05). (**C**) Diagram of the mechanisms underlying the LIN01138-miR-1273e-MAPK axis in GC.

receptor (EGFR), fibroblast growth factor 1 (FGF 1), fibroblast growth factor 23 (FGF 23), MAP kinase kinase 6 (MAP2K 6), and MAP kinase kinase 7 (MAP3K 7) were significantly downregulated in LINC01138-silenced MGC-803 and SGC-7901 cells (*P*<0.05 *vs.* si-NC, Figure 6B). Taken together, our results show that increased LINC01138 expression can activate the MAPK signaling pathway by inhibiting miR-1273e to promote GC cell proliferation, invasion, and migration (Figure 6C).

Discussion

Recently, abnormal lncRNAs expression profiles were found in various of human cancers, such as thyroid cancer, cervical cancer, and hepatocellular carcinoma (HCC), suggesting that lncRNAs participate in the progression of cancer [14–16]. LINC01138, located at 1q21.2, is a novel lnRNA that was found to be associated with poor outcome of patients with HCC [17]. LINC01138 was demonstrated to promote HCC growth and metastasis by interacting with arginine methyltransferase 5 (PRMT5) and increasing its protein stability by blocking ubiquitin/proteasome-dependent degradation [17]. Moreover, LINC01138 was identified to be upregulated in prostate cancer samples by performing analysis of global lncRNA expression

pattern and microarray, indicating that LINC01138 can act as a novel molecular biomarker for the diagnosis of prostate cancer [18]. In the present study, we found LINC01138 was significantly increased in GC samples, and it served as an oncogenic driver that promoted GC cell proliferation, invasion, and migration and inhibited GC cell apoptosis. Blockage of LINC01138 significantly inhibited the growth, invasion, and migration of GC cells. These findings suggest that LINC01138 could be a target in GC treatment. However, in the further studies, we may focus on how to deliver the LINC01138 siRNAs to the tumor within the GC patient.

miRNAs, characterized by short sequence and non-protein-coding ability, were recently reported to mediate the effects of lncRNAs on tumor progression of GC by regulating the transcription of target genes [19,20]. For instance, overexpression of lncRNA MEG3 was demonstrated to inhibit the EMT and migration of GC cells by reducing the expression of MMP-3, MMP-9 and VEGF via miR-21 [21]. Moreover, the GC-related lncRNA1 (GCRL1) was reported to promote GC progression by positively regulating cyclin-dependent kinase 4 (CDK4) expression via reducing miR-885-3p expression [22]. In the present study, LINC01138 was identified as a miRNA sponge for miR-1273e, and the promotive effects of LINC01138 on GC progression

were mediated by miR-1273. As miRNAs were found to connect multiple proteins involved in a variety of signaling pathways, we subsequently performed miR-1273e target gene KEGG analysis. The MAPK/ERK signaling pathway, which controls multiple cellular physiological processes [23], was identified as a miR-1273e-related pathway in the present study. The MAPK/ERK signaling pathway includes a variety of proteins such as EGFR, FGF, MAP2K, and MAP3K [23,24]. Once mutation occurred in any proteins of the MAPK pathway, it could be stuck in the "on" or "off" phase, and hence affected the initiation and progression of many tumors [23,25]. In this study, we found the expressions of EGFR, FGF1, FGF23, MAP2K6, and MAP3K7 were reduced in the LINC01138-silenced GC cells, indicating that the MAPK pathway was suppressed. However, the relationship between the LINC01138/miR-1273e axis and the MAPK family members mutation is not clear. In future research, we intend to focus on this mechanism.

Conclusions

In summary, we found increased LINC01138 expression in malignant tissues from GC patients and human GC cell lines. Moreover, the *in vitro* experiments demonstrated that LINC01138 promotes GC cell growth, invasion, and migration through activation of the MAPK pathway by negatively regulating miR-1273e expression. These findings contribute to better understanding of GC pathogenesis, suggesting that the LINC01138/miR-1273e/MAPK axis might provide novel therapeutic targets for GC patients.

Conflict of interests

None.

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