Original paper

Downregulated lncRNA LINC00908 correlates with a poor prognosis and increasing malignancy of gastric cancer

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Long non-coding RNA LINC00908 is a functional biomarker in regulating tumour progression. Its dysregulation in gastric cancer implies its potential functional role. Few studies have noted the functional role of LINC00908 in gastric cancer. The potential of LINC00908 to serve as a biomarker in gastric cancer was evaluated.

A total of 113 paired gastric cancer tissues and normal tissues were collected from patients with gastric cancer. LINC00908 levels were evaluated by polymerase chain reaction, and its significance in disease progression and patients' prognosis was assessed. *In vitro*, the function of LINC00908 in tumour-related cellular processes was evaluated with CCK8 and Transwell assay. Significant downregulation of LINC00908 was observed in gastric cancer and was negatively associated with disease development and overall survival of patients. LINC00908 showed significant inhibitory effects on the proliferation, migration, and invasion of gastric cancer cells.

Additionally, miR-627-3p was sponged by LINC00908 and therefore mediated the function of LINC00908 in gastric cancer cells. LINC00908 functioned as a prognostic biomarker and tumour suppressor of gastric cancer, providing a therapeutic target for gastric cancer.

Key words: gastric cancer, lncRNA LINC00908, prognosis, progression, cell processes.

Introduction

Gastric cancer, a type of malignant tumour originating from the gastric mucosa, is one of the most frequently diagnosed cancers. It is also one of the most common leading causes of cancer death [1]. The poor prognosis is mainly due to late diagnosis and uncontrolled tumour progression. Importantly, with the increasing incidence and mortality, monitoring disease onset and development is of great necessity. Moreover, multi-drug resistance, recurrence, and distant metastasis are also significant hurdles in the clinical management of gastric cancer, resulting in poor outcomes for patients [2–4]. Identifying novel biomarkers and exploring the mechanisms associated with disease progression and severity could benefit the therapeutic strategy.

Long noncoding RNAs (lncRNAs), a series of RNAs without open reading frames and over 200 nucleotides in length, are widely expressed in humans. Interestingly, lncRNAs appear to play crucial roles in various human diseases. In addition, due to their high sensitivity and specificity in serum, tissues, urine, and other types of body fluids, lncRNAs could assist in increasing diagnostic sensitivity [5–7]. Numerous lncRNAs are associated with tumour progression and mediate cell processes, such as cell growth and cell differentiation [8–11]. LncRNA

LINC00908 (LINC00908) was reported to be involved in the development of malignant tumours with abnormal expression levels. LINC00908 also acted as a tumour suppressor in prostate cancer by negatively regulating miR-483-5p [12]. Previous identification of abnormally expressed lncRNAs in gastric cancer has revealed the dysregulation of LINC00908, but the function of LINC00908 remains unclear [13].

Due to the heterogeneity of lncRNA expression in different cancers, the expression of LINC00908 in gastric cancer needs to be validated. The present study could also disclose the clinical significance and biological effect of LINC00908 on gastric cancer progression. In addition, according to the prevalent ceRNA theory, LINC00908 was speculated to sponge function miRNAs to display its regulatory effect. Among predicted downstream miRNAs of LINC00908 using the online database, miR-627-3p was reported to regulate malignant tumour progression and mediate the function of various lncRNAs [14–16]. However, whether miR-627-3p is involved in gastric cancer development and whether it mediates the function of LINC00908 remains unclear, which was confirmed in the present study.

Material and methods

Study subjects

From 2013 to 2015, a total of 113 patients diagnosed with gastric cancer, who received surgical therapy at Hengshui People's Hospital, were included in this study. The clinical data of the enrolled patients are summarised in Table 1. Briefly, the enrolled gastric cancer patients were composed of 68 males and 45 females with an average age of 61.45 ±9.36 years. Patients were staged at tumour-node-metastasis (TNM) I–III. Specifically, there were 33 patients at stage I, 42 patients at stage II, and 36 patients at stage III. Tumour tissues and adjacent normal tis-

Table 1. Association between LINC00908 expression and the clinicopathological features of patients

Parameters	Total (N = 113)	LINC00908 expression		*p*-value
		Low (n = 58)	High (n = 55)	
Age (years)				0.656
< 60	51	25	26	
≥ 60	62	33	29	
Sex				0.673
Male	68	36	32	
Female	45	22	23	
Differentiation				0.232
Well + moderate	74	41	33	
Poor	39	17	22	
Regional lymph node metastasis				0.026
Absent	64	27	37	
Present	49	31	18	
TNM stage				0.008
I–II	77	33	44	
III	36	25	11	
Location				0.368
Body + cardia	65	31	34	
Antrum	48	27	21	
Tumour size (mm)				0.216
< 50	59	27	32	
≥ 50	54	31	23	
Macroscopic type				0.206
Localised	50	29	21	
Diffuse	63	29	34	

TNM – tumour-node metastasis.

sues were resected during surgery and confirmed by at least 2 pathologists. Collected tissues were immediately frozen in liquid nitrogen and stored at –80°C for the following analyses.

A 5-year follow-up survey was conducted to obtain the survival information of all study subjects. Patients were followed up for 3–60 months. The survival data were analysed by the Kaplan-Meier curve followed by a log-rank test. All human samples were obtained with written informed consent from patients. The Ethics Committee of Hengshui People's Hospital approved the research use of these tissues. All studies on human subjects should be conducted in accordance with the Declaration of Helsinki.

LINC00908 expression evaluation

The expression of LINC00908 was evaluated by real-time quantitative polymerase chain reaction. At first, total RNA was extracted and reverse transcribed to cDNA with the help of TRIzol (Invitrogen, USA) and Human lncRNA Profiler cDNA synthesis buffer (Funeng Biological Technology, China), respectively. The expression of LINC00908 was evaluated using SYBR Green kit on the ABI PRISM 7300 system. The $2^{-\Delta\Delta Ct}$ method was used to calculate the expression level of LINC00908 with normalisation to β-actin.

Cell culture and cell transfection

Human gastric cancer cells, SNU-484, HGC-27, MKN-45, and AGS cells, and a normal cell, GES-1 cell, were obtained from ATCC. All cells were cultured in the DMEM medium with 10% FBS (Thermo, USA) at 37°C with 5% CO_2.

Cells were available when they reached the logarithmic period. The overexpression-LINC00908 vectors (oe-LINC00908, pcDNA3.1 plasmid of LINC00908) were constructed and transfected into gastric cancer cells to overexpress LINC00908. The small interference RNA of LINC00908 (si-LINC00908) was also transfected into gastric cancer cells for the knockdown of LINC00908. The empty vectors were used as the negative control. Cells were seeded into a 6-well plate and cell transfection was conducted with a Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions. Transfected cells were available for the following experiments after 24 hours of transfection.

Cell viability assessment

Cells were seeded into the 96-well plates and cultured at 37°C with 5% CO_2. After 24, 48, and 72 hours of the cell culture, CCK8 (Dojindo, China) was added to each well and incubated for another 2 hours. Absorbance at 450 nm was measured from 5 random fields *per* well detected with a microplate reader. Both the experiment and the determination were repeated 3 times.

Cell migration and invasion evaluation

For cell migration experiments, cells (1×10^5 cells/well) were seeded into the upper chamber of a Transwell chamber (24-well, Corning, USA). Cells were supplied with an FBS-free culture medium, while the completed culture medium was placed in the lower chamber. For the invasion experiments, the upper chamber was pre-coated with Matrigel. The chambers were incubated for 24 hours. Then, the cells that remained on the top surface were removed, and the cells on the subsurface were fixed with paraformaldehyde and stained with 0.1% crystal violet. After washing 3 times, the cells were counted under a light microscope (Nikon, Japan).

Interaction between LINC00908 and miR-627-3p

The downstream targets of LINC00908 were predicted from the online database. AGS cells were seeded into the 12-well plates for the dual-luciferase reporter assay. Cells were co-transfected with the LINC00908 wild-type (containing the binding sequences) or mutant-type (containing the mutated sequences) vector and miR-627-3p mimic or inhibitor using Lipofectamine 2000 transfection reagent (Invitrogen, USA). The luciferase activity of LINC00908 was evaluated using the Dual-Luciferase Reporter Assay System (Promega, USA) relative to Renilla.

Data analysis

All data are presented as mean value ±SD obtained from 3 independent experiments. The differences between groups were estimated using Student's *t*-test and one-way ANOVA using SPSS 20.0 software. The role of LINC00908 in gastric cancer development was evaluated by its association with patients' clinicopathological features using the χ^2 test. The receiving operating characteristic (ROC) curve analysis was performed to find the cut-off of LINC00908 grouping gastric cancer patients. $P < 0.05$ was considered statistically significant.

Results

Expression of LINC00908 in gastric cancer

In collected tissues, LINC00908 was observed to be downregulated in tumour tissues in comparison with adjacent normal tissues ($p < 0.001$) (Fig. 1A). Consistently, the relative expression of LINC00908 in gastric cancer cells (SNU-484, HGC-27, MKN-45, and AGS cells) was also found to be significantly lower than that in normal cells ($p < 0.001$) (Fig. 1B).

Clinical significance of LINC00908 in gastric cancer

Based on the survival status of the patients, the ROC analysis of the enrolled patients was performed (Fig. 2A).

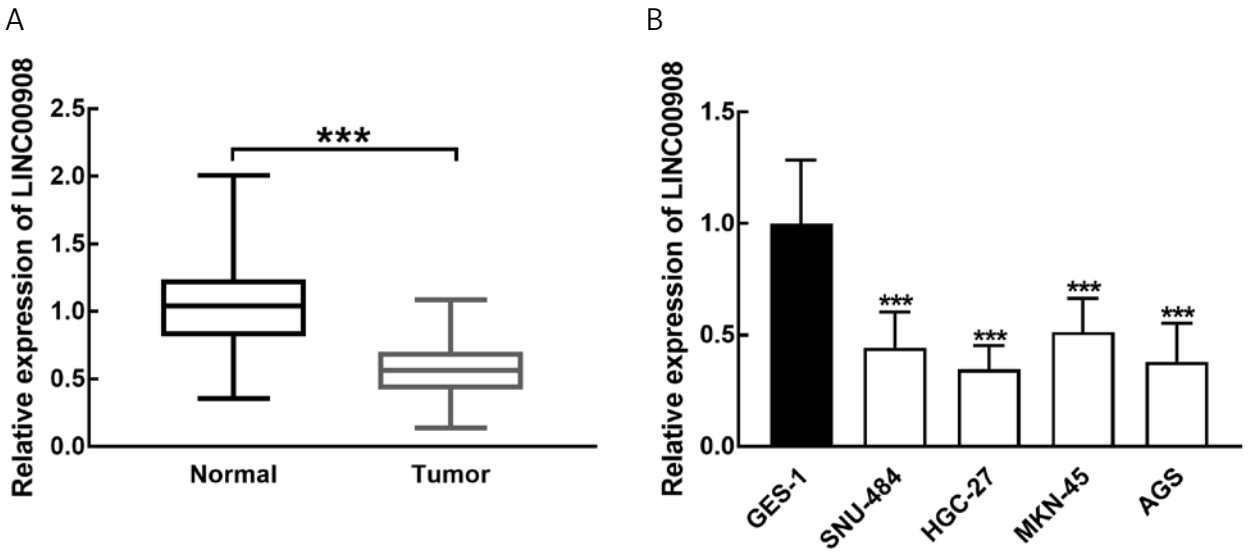

Fig. 1. A) Expression of LINC00908 in gastric cancer tissues. B) Expression of LINC00908 in cells

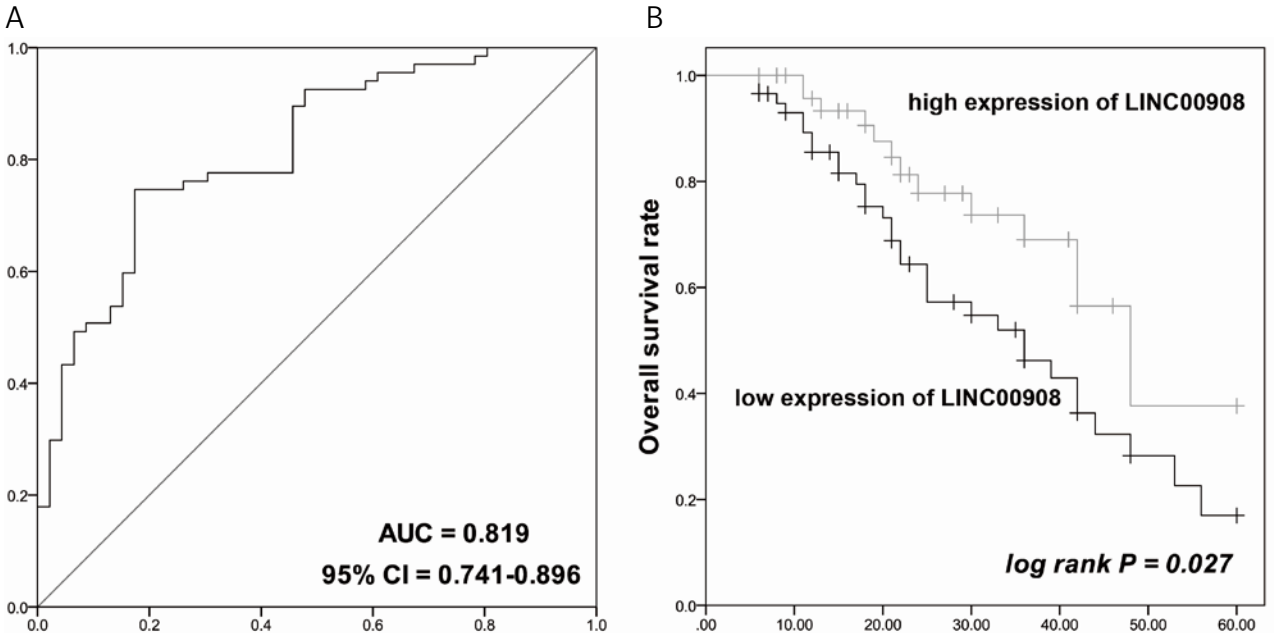

Fig. 2. Significance of LINC00908 in distinguishing patients' overall survival. A) Receiver operating characteristic analysis evaluated the predictive accuracy of the prognostic value of LINC00908 in assessing patients' overall survival. B) Kaplan-Meier analysis based on the expression level of LINC00908 assessed the prognostic value of LINC00908 in gastric cancer

****$p < 0.001$ relative to normal tissues or cell lines*

According to the cut-off (0.5797) obtained from the ROC analysis, patients ($n = 113$) were divided into a low-LINC00908 group ($n = 58$) and a high-LINC00908 group ($n = 55$). In the low-LINC00908 group, most patients had regional lymph node metastasis and were in an advanced TNM stage. The significant correlation of LINC00908 expression with regional lymph node metastasis status ($p = 0.026$) and TNM stage ($p = 0.008$) of patients was observed (Table 1).

According to the ROC analysis, LINC00908 was able to distinguish the gastric cancer patients with an AUC of 0.819 (95% CI: 0.741–0.896) (Fig. 2A). Also, the Kaplan-Meier showed that patients in the low-LINC00908 group had a poorer overall survival rate than patients in the high-LINC00908 group (log-rank $p = 0.027$) (Fig. 2B). Additionally, Cox regression analysis highlighted the dramatical significance of LINC00908 expression (HR = 2.357, 95% CI: 1.185–4.690, $p = 0.015$) and TNM stage (HR = 2.187, 95% CI: 1.058–4.524, $p = 0.035$) in predicting the prognosis of gastric cancer patients (Table 2).

The biological effect of LINC00908 in gastric cancer

The AGS and HGC-27 cells showed a relatively low expression of LINC00908, indicating their high

Table 2. Association between clinicopathological features and overall survival of patients

Parameters	HR value	95% CI	*p*-value
LINC00908	2.357	1.185–4.690	0.015
Age	1.353	0.689–2.658	0.379
Sex	1.463	0.775–2.760	0.240
Differentiation	1.496	0.761–2.940	0.243
Regional lymph node metastasis	1.567	0.850–2.890	0.150
TNM stage	2.187	1.058–4.524	0.035
Location	1.508	0.759–2.994	0.241
Tumour size	1.447	0.785–2.667	0.236
Macroscopic type	1.430	0.757–2.701	0.271

TNM – tumour-node metastasis.

sensitivity to LINC00908 dysregulation. Hence, these cells were selected for the following *in vitro* experiments. LINC00908 was significantly elevated by pcDNA 3.1-LINC00908 transfection and reduced by si-LINC00908 in AGS and HGC-27 cells ($p < 0.001$) (Fig. 3A). The overexpression of LINC00908 dramatically inhibited AGS and HGC-27 cell proliferation, and its knockdown showed opposite effects ($p < 0.05$) (Fig. 3B). Similarly, the migration (Fig. 3C) and invasion (Fig. 3D) of AGS and HGC-27 cells were suppressed by LINC00908 overexpression and enhanced by its silencing ($p < 0.01, p < 0.001$).

miR-627-3p mediates the biological effect of LINC00908

A significant upregulation of miR-627-3p was observed in gastric cancer cells, compared with normal cells ($p < 0.001$) (Fig. 4A). *In vitro* overexpression of miR-627-3p dramatically inhibited the luciferase activity of LINC00908, and its knockdown significantly enhanced LINC00908 luciferase activity ($p < 0.001$) (Fig. 4B). Overexpressing LINC00908 significantly suppressed the expression of miR-627-3p in AGS cells, which was attenuated by its mimic transfection ($p < 0.01, p < 0.001$) (Fig. 4C). Interestingly, the inhibitory effects of LINC00908 overexpression on the proliferation (Fig. 4D), migration (Fig. 4E), and invasion (Fig. 4F) of AGS cells were reversed by the elevated miR-627-3p level ($p < 0.01, p < 0.001$).

Discussion

Surgical resection is the primary first-line approach to gastric cancer therapy [17, 18]. Although the therapeutic strategies for gastric cancer have improved and several clinical biomarkers have been found in the past decades, various drawbacks still result in inefficient diagnosis and unsatisfying prognosis [18, 19]. Recently, lncRNAs have drawn attention in tumour-related research. It is widely acknowledged that lncRNAs, especially dysregulated lncRNAs, participate in tumour progression and play vital roles in regulating biological processes [20, 21]. For example, the upregulation of lncRNA BCRT1 significantly promoted breast cancer development by regulating the miR-1303/PTBP3 axis [22]. lncRNA MNX1-AS1 was revealed to contribute to the development of gastric cancer and predict the prognosis of patients [23]. Here, the expression of LINC00908 was analysed to evaluate its potential function in tumour progression of gastric cancer.

In previous studies, LINC00908 was identified as an effective biomarker in various diseases and was correlated with disease development. In hepatocellular carcinoma, LINC00908was upregulated and was associated with advanced disease development, promoting cellular processes *via* modulating Sox-4 [24]. Among the identified dysregulated lncRNAs in lung adenocarcinoma, downregulated LINC00908 was associated with patients' poor prognosis [25]. However, the expression of LINC00908 is controversial in different tumours. Aberration of LINC00908 in gastric cancer was observed primarily in a lncRNA expression profile, which implies its potential function in tumour progression [13]. Here, decreased expression of LINC00908 in gastric cancer and its involvement in the disease development was observed, showing a significant relationship with the regional lymph node metastasis status and TNM stage of patients. Besides the function in tumour progression, the prognostic value of lncRNAs has also been widely reported. For instance, upregulated LINC00659 was associated with the poorer overall survival of gastric cancer patients [26]. LINC00908 was reported to correlate with the advanced neoplasm grade and poorer prognosis of glioma [27]. In the present study, LIN00908 also showed a significantly prognostic value in gastric cancer because it was negatively correlated with the overall survival rate of patients.

In previous studies, LINC00908 promoted proliferation and showed an anti-apoptotic effect on

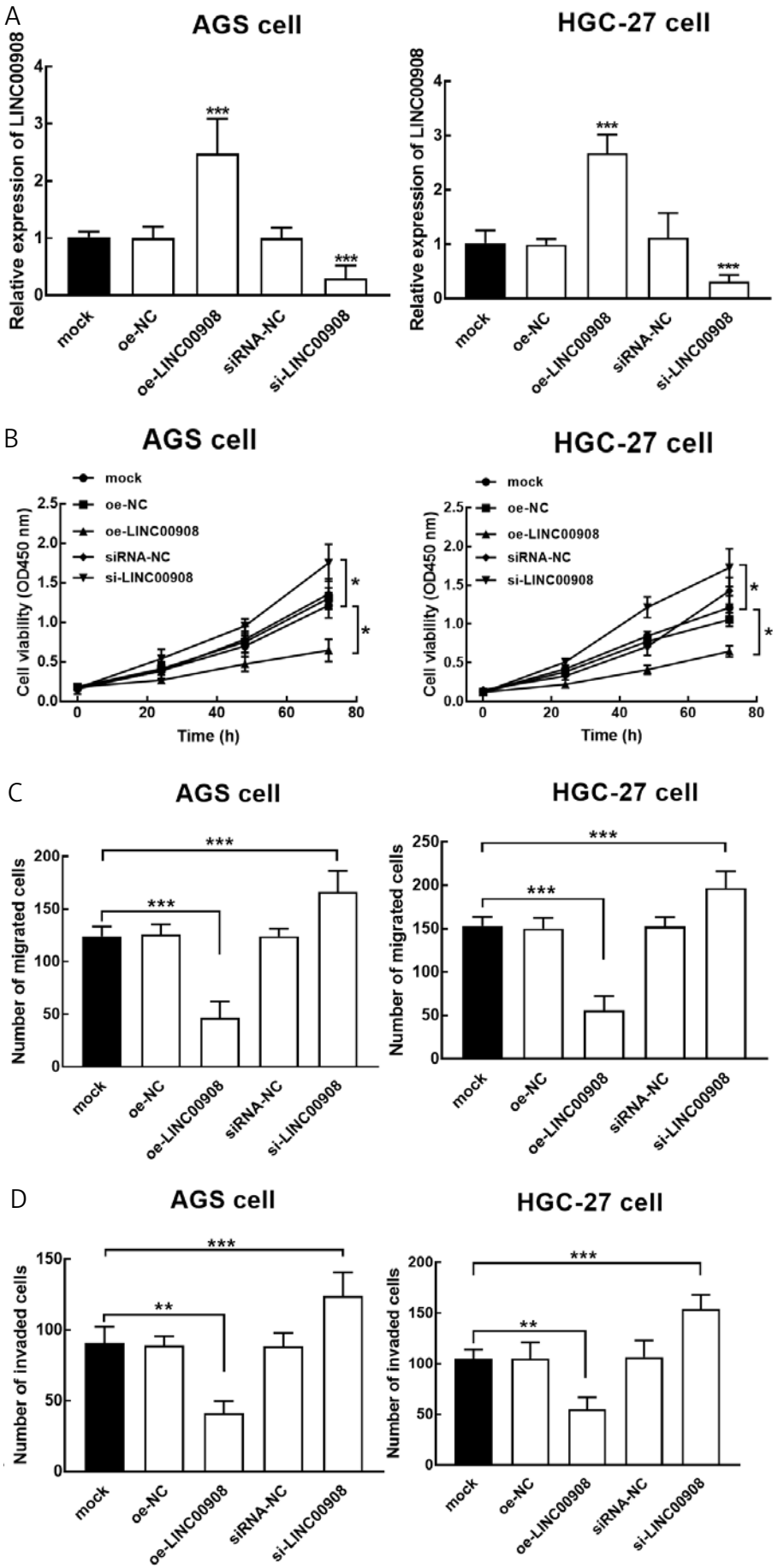

Fig. 3. Significance of LINC00908 in mediating cellular processes of gastric cancer. A) Evaluation of cell transfection B–D) Effects of LINC00908 on the proliferation (B), migration (C), and invasion (D) of AGS and HGC-27 cells

** $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ relative to control*

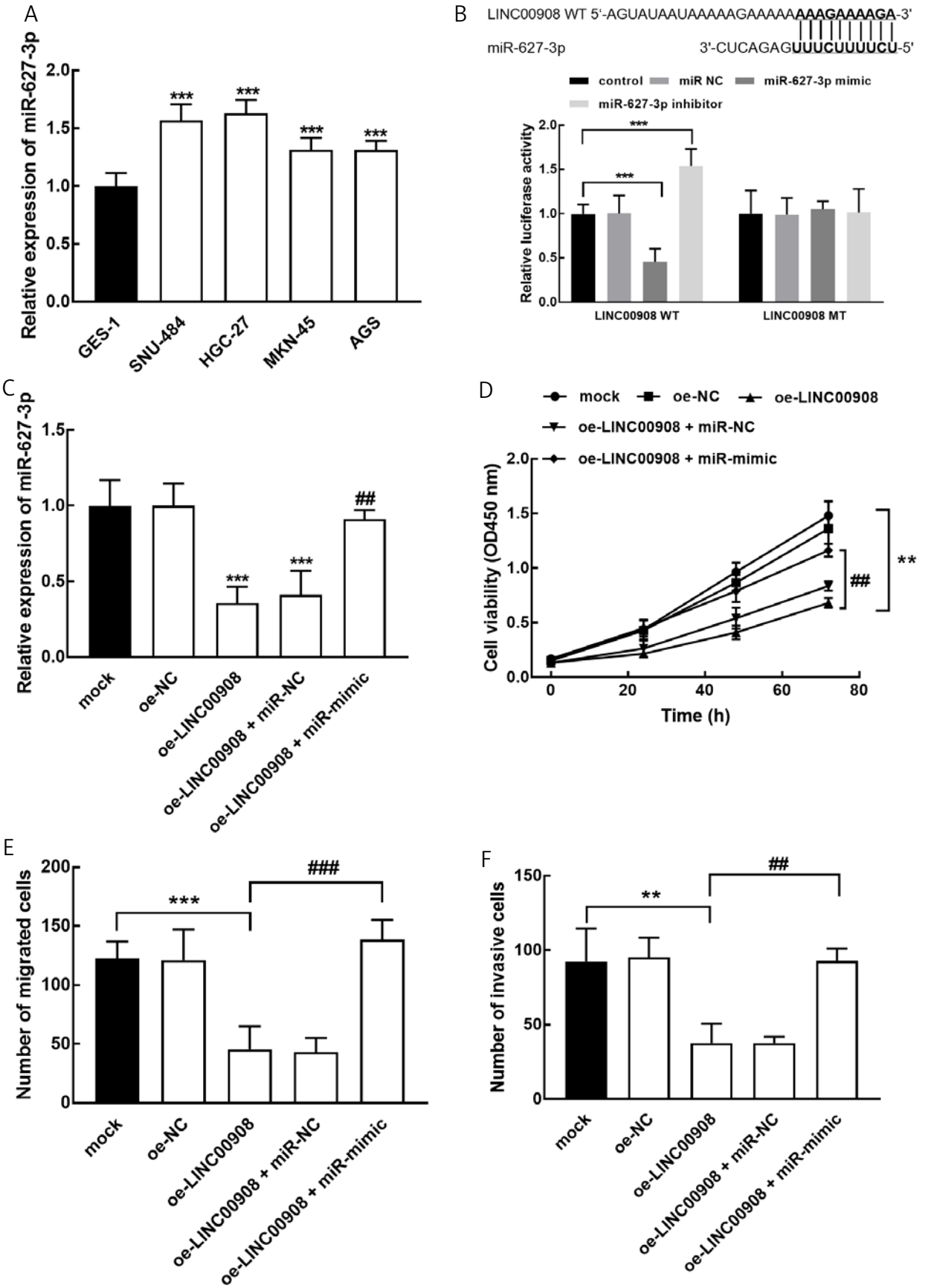

Fig. 4. A–C) Expression of miR-627-3p in gastric cancer cells (A) and its interaction with LINC00908 by luciferase (B) and polymerase chain reaction (C). D–F) The regulatory effects of LINC00908 and miR-627-3p on the proliferation (D), migration (E), and invasion (F) of AGS cells

*** $p < 0.01$, *** $p < 0.001$ compared with the mock group; ## $p < 0.01$, ### $p < 0.001$ compared with the oe-LINC00908 group*

colon cancer cells *via* regulating KLF5, suggesting its tumour promoter role [28]. The suppressor role of LINC00908 was observed in prostate cancer with a significant inhibitory effect on disease development [12]. In the main cellular processes that correlated with gastric cancer development, it was found that silencing of LINC00908 promoted the proliferation, migration, and invasion of gastric cancer cells, which was facilitated by its overexpression. These results demonstrated the inhibitor and the biomarker role of LINC00908 in gastric cancer.

The underlying mechanism is also an essential part of disclosing the function of LINC00908 in gastric cancer. Previously, the inhibitory effect of LINC00908 in prostate cancer was illustrated by the regulation of the miR-483-5p/TSPYL5 axis [12]. In mechanism, miR-627-3p was found to regulate the luciferase activity of LINC00908. Previously, miR-627 was identified as a non-invasive diagnostic biomarker of gastric cancer, which discriminated gastric cancer patients from healthy individuals with high sensitivity and specificity [29]. Additionally, the rs2620381 polymorphism of miR-627 was closely associated with gastric cancer pathogenesis and development [30]. Herein, LINC00908 was found to regulate the expression of miR-627-3p in gastric cancer cells, and its tumour-suppressed effect was mediated by miR-627-3p.

Conclusions

Downregulated LINC00908 in gastric cancer was associated with patients' advanced development and poor prognosis. LINC00908 might suppress gastric cancer *via* sponging miR-627-3p, which is a potential therapeutic target for gastric cancer.

Disclosures

1. Institutional review board statement: Not applicable.
2. Assistance with the article: None.
3. Financial support and sponsorship: None.
4. Conflicts of interest: None.

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