Am J Transl Res 2026;18(1):524-536
www.ajtr.org /ISSN:1943-8141/AJTR0168551

Original Article

Targeting the LINC01515/miR-325-3p/ERBB4 axis suppresses gastric cancer progression

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Received September 3, 2025; Accepted December 16, 2025; Epub January 15, 2026; Published January 30, 2026

Abstract: Background: Globally, gastric cancer (GC) remains the leading cause of cancer-related death. Research on circular RNAs (circRNAs), microRNAs (miRNAs), and long non-coding RNAs (lncRNAs) is mostly focused on cancer. lncRNAs can influence gene expression by functioning as ceRNAs to sponge miRNAs. This study sought to characterize the expression patterns of miR-325-3p and LINC01515 and investigate their possible mechanisms in GC. Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to detect miR-325-3p expression. The target mRNAs and competitive lncRNAs of miR-325-3p were screened via bioinformatics analysis. Cell counting, Transwell assay, and wound-healing experiments were conducted to measure cell growth, migration, and invasion. The relationships between LINC01515, miR-325-3p, and erythroblastic oncogene B4 (ERBB4) were confirmed through the dual luciferase reporter gene assay. Results: GC cell lines and tissues showed upregulated LINC01515 and ERBB4 expression, accompanied by downregulated miR-325-3p expression. These expression patterns were associated with tumor proliferation, invasion, and migration. ERBB4 was positively correlated with LINC01515 in the context of differentially expressed miR-325-3p. These findings suggest that LINC01515 may regulate ERBB4 by functioning as a ceRNAs to sponge miR-325-3p. Conclusion: LINC01515 upregulates ERBB4 expression in GC by sponging miR-325-3p, thereby promoting tumor growth. These findings highlight the LINC01515/miR-325-3p/ERBB4 axis as a potential therapeutic target for GC.

Keywords: miR-325-3p, LINC01515, gastric cancer, ERBB4, invasion, migration

Introduction

Gastric cancer (GC) is one of the most prevalent malignancies of the digestive tract and poses a major threat to human health [1]. Global cancer data reveal over 1 million new GC cases and approximately 760,000 GC-related deaths in 2020, ranking fifth and fourth, respectively [2]. Both mortality and morbidity rank third in China. Currently, GC treatments primarily rely on surgery, supplemented with immunotherapy, molecular targeted therapy, radiotherapy, and chemotherapy, among others. Over 50% of patients are diagnosed at an advanced stage, and the chance of 5-year survival remains <20%, despite major improvements in disease awareness and treatment options [3-5]. Identifying sensitive and specific molecular markers is therefore crucial for improving treatment efficacy and patient prognosis. However, the mechanisms underlying GC development remain incompletely understood.

According to human genomics research, about 75% of the human genome is transcribed into RNAs; however, only 2% encodes proteins [6]. Since the discovery of small non-coding RNAs in the 1990s, extensive research has been conducted [7]. Currently, the research on circular RNAs, long non-coding RNAs (lncRNAs), and microRNAs (miRNAs) is mostly focused on cancer [8]. miRNAs are small RNAs that control biological processes and contribute to cancer occurrence and progression [7]. Certain miR-

NAs may function as either tumor suppressors or oncogenes in different cancer types [9]. For instance, miR-325-3p promotes epithelial-to-mesenchymal transition in colorectal cancer progression and may serve a potential treatment target [10]. In bladder cancer, reduced miR-325-3p expression regulates cell proliferation, EMT, migration, and invasion through MT3 [11]. In colorectal cancer, miR-325-3p overexpression suppresses bone resorption in bone metastasis, whereas miR-325-3p was downregulated [12]. Nevertheless, the role of miR-325-3p in GC remains unclear and warrants further investigation.

LncRNAs, typically more than 200 nucleotides and transcribed primarily by RNA polymerase II, function through signal, decoy, guide, or scaffold mechanisms [13-16]. Certain lncRNAs regulate gene expression by sequestering miRNA as competing endogenous RNAs (ceRNAs) [17]. lncRNA-based therapeutic approaches have attracted major research attention, as lncRNAs can act as suppressors or oncogenes to affect the proliferation, invasion, metastasis, and treatment resistance. For example, in GC, lncRNA PVT1 silencing enhances chemosensitivity to paclitaxel and inhibits tumor growth [18]. By binding to miR-519b-3p and elevating RCCD1 expression, LINC01419 exhibits carcinogenic tendencies in lung adenocarcinoma [19]. CNALPTC1 triggers GC development via suppressing the miR-6788-5p/PAK1 cascade [20]. LINC01116 promotes tumor proliferation and prevents apoptosis in lung adenocarcinoma by attaching to miR-9-5p [21]. In pancreatic cancer, NEAT1 sponges miR-146b-5p/traf6 to modulate pancreatic cancer progression [22]. Moreover, LINC01515, elevated in nasopharyngeal carcinoma, is closely associated with cell proliferation, migration, and invasion [23]. Erythroblastic oncogene B4 (ERBB4), a member of the epidermal growth factor receptor family, is frequently overexpressed in multiple malignancies and contributes to tumorigenesis [24-26]. However, there is limited evidence for the complex relationship between ERBB4 and LncRNAs in GC.

Given the pivotal role of miR-325-3p in tumors, this study screened and identified LINC01515-ERBB4 as a ceRNA pair interacting with miR-325-3p in GC. This research project aims to elucidate the key role and detailed mechanism of the LINC01515/miR-325-3p/ERBB4 axis in GC through bioinformatics and molecular biology methods, thereby providing more theoretical foundations and translational possibilities for more targeted GC treatment.

Materials and methods

Samples

A total of 50 paired tumor and adjacent noncancerous tissues (>5 cm from the tumor margin) were collected from GC patients who underwent radical surgery at the Third Department of General Surgery, Fourth Hospital of Hebei Medical University, between September 2023 and February 2024. Postoperative pathology confirmed gastric adenocarcinoma. No patient underwent preoperative chemotherapy, radiotherapy, immunotherapy, or targeted therapy, and none had a history of other malignancies. Histological and pathological diagnoses were independently verified by two specialists. Fresh tissue samples were immediately frozen in liquid nitrogen and maintained at -80°C for subsequent analyses. This study followed the International Ethical Guidelines for Biomedical Research Involving Human Subjects and was approved by the Ethics Committee of the Fourth Hospital of Hebei Medical University (approval number 2022ky136), with written informed consent obtained from all patients.

Cell culture and transfection

GC cell lines (HGC27, MKN45, NCIN87, AGS) and the gastric epithelial cell line (GES-1) were provided by the Cell Bank of the Chinese Academy of Sciences (China). Cells were cultured in Gibco's Roswell Park Memorial Institute (RPMI)-1640 medium (Waltham, MA, USA) supplemented with 100 U/ml penicillin, 100 mg/ml streptomycin (Invitrogen, Carlsbad, CA, USA), and 10% fetal bovine serum (Gibco, FBS; MA, USA) at 37°C in a humidified incubator with 5% CO_2. For transfection, 2.5×10^5 cells were seeded into six-well plates and cultivated in 2 ml of RPMI-1640 overnight. Lipofectamine 2000 (Invitrogen) was used to transfect GC cells with siRNA ERBB4 (5'-GGGAAAGACUGAAAGAGAA-3') siLINC01515 (5'-GCAGUUAGCACACAAUUAA-3'), hsa-miR-325-3p mimic (5'-UGUGAAUGACCUGUGGAUGAUCC-3'), negative con-

Table 1. Primer sequence

Gene	Primer Sequence
LINC01515	F: 5'TGAAAGGGTGAGGCAATG3'
	R: 5'GTTAGCAGCAGGGTAAGAAAGAG3'
ERBB4	F: 5'TTGACACAACTGGCACTGC3'
	R: 5'AAGGAACAGGACAGCATCG3'
hsa-miR-325-3p	F: 5'TGTGAATGACCTGTGGATGATCC3'
	R: 5'GTCGTATCCAGTGCAGGGT3'
U6	F: 5'GAAACACCGTGCTCGCTT3'
	R: 5'TGCTAATCTTCTCTGTATCGTTCC3'
MMP2	F: 5'CCAACTACAACTTCTTCCCTCG3'
	R: 5'TCACATCGCTCCAGACTTG3'
MMP9	F: 5'AGAACCAATCTCACCGACAGG3'
	R: 5'CGACTCTCCACGCATCTCT3'
TIMP3	F: 5'TCGGTATCACCTGGGTTGT3'
	R: 5'GACACTCGTTCTTGGAAGTCAC3'
E-cadherin	F: 5'GTGGTCAAAGAGCCCTTACTG3'
	R: 5'CGTTACGAGTCACTTCAGGC3'
N-cadherin	F: 5'TCATTGCCATCCTGCTCTG3'
	R: 5'CATCCATACCACAAACATCAGC3'
Vimentin	F: 5'AAATGGCTCGTCACCTTCG3'
	R: 5'AGAAATCCTGCTCTCCTCGC3'
GAPDH	F: 5'TGAACGGGAAGCTCACTGG3'
	R: 5'GCTTCACCACCTTCTTGATGTC3'

trol (NC; 5'-CCUCUUACCUCAGUUACAAUUUAUA-3'), hsa-miR-325-3p inhibitor (5'-GGATCATCCA-CAGGTCATTCACA-3'), and corresponding controls at 70-80% confluence. Quantitative real-time polymerase chain reaction (qRT-PCR) was used to determine the transfection efficiency.

RNA extraction and qRT-PCR

Total RNA was extracted using TRNzol universal reagent (TIANGEN, China). After quantification, 3 μg of total RNA was reverse transcribed using the GoScript Reverse Transcription System (A5001, Promega, USA). qRT-PCR was performed using the A6001 GoTaq(R) qRT-PCR Master Mix (Promega, USA) on an ABI PRISM 7500 sequence detection system (Applied Biosystems). **Table 1** displays the primer sequences. Relative expression levels were determined using the 2-ΔΔCt method. U6 served as the internal miRNA control, whereas GAPDH served as endogenous control for mRNAs and lncRNAs.

Western blotting

Protein expression was analyzed using Western blotting. Total proteins from cells and clinical samples were extracted using RIPA lysis buffer (Solarbio, China), and quantified using a bicinchoninic acid (BCA) assay (Thermo, USA). Approximately 30 μg of proteins was separated on 10-12% SDS-PAGE gels and transferred to polyvinylidene fluoride membrane (Millipore, Billerica, MA). After blocking with 5% fat-free milk for 1 h at room temperature, membranes were incubated with primary antibodies overnight at 4°C, followed by incubation with secondary antibodies for 2 h. Protein bands were visualized using the Odyssey system (LI-COR Biosciences, USA). The primary antibodies, including MMP-2 (Proteintech, 10373-2-AP), β-actin (Abcam, ab8227), MMP-9 (Proteintech, 10375-2-AP), E-Cadherin (Abcam, ab231303), PCNA (Proteintech, 10205-2-AP), Vimentin (Abcam, ab92547), N-Cadherin (Abcam, ab18-203), ERBB4 (Proteintech, 19943-1-AP), and TIMP3 (Abcam, ab276134), were used.

Cell Counting Kit-8 (CCK-8) test

Cells were seeded into 96-well cell plate and cultured for 0, 24, 48, or 72 hours. Then, 10 μl of CCK-8 regent (Dojindo, Kumamoto, Japan) was added to each well and cultivated for 2 h at 37°C. Absorbance at OD450 nm was measured using a microplate reader.

Wound-healing migration assay

A total of 5×10^5 cells were seeded into six-well plates. After transfection and upon reaching >80% confluence, a linear scratch was made using a 200-μl pipette tip. The detached cells were removed by washing with PBS, and the remaining adherent cells were cultured for 24 h. Images were captured using an inverted microscope at designated time points.

Transwell invasion assay

Cell invasion was evaluated using 24-well Transwell chambers (Corning Costar, Cambridge, MA, USA). After transfection, the upper chambers were coated with a thin layer of Matrigel (BD Biosciences, USA) diluted in serum-free medium, and the cells were seeded into the upper chamber. The lower chambers were filled

with RPMI-1640 (600 μl) medium containing 10% FBS. After incubation for 24 h at 37°C in 5% CO_2, invading cells were fixed and stained with crystal violet for 10 min, and then counted under an inverted optical microscope in five randomly chosen areas.

Luciferase reporter gene assay

Predicted miR-325 binding sites in the 3'-untranslated regions (3'-UTRs) of wild-type or mutant LINC01515 or ERBB4 were cloned into a luciferase reporter vector (Promega, Madison, WI, USA). MKN45 cells were co-transfected with miR-325 mimic or control mimic and the luciferase reporter constructs. After 48 h of culture, luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega), which was then standardized to Renilla luciferase activity.

Bioinformatics analysis

LncRNAs and target genes were screened using the Stomach Adenocarcinoma dataset from the Cancer Genome Atlas (TCGA) (http://cancergenome.nih.gov/). Differentially expressed genes (DEGs) were identified using the R packages *limma* and *ggplot2* with thresholds of $P<0.05$ and $|LogFoldChange|>1.5$. Functional enrichment analysis was performed using Metascape (https://Metascape.org/). The association between lncRNAs and DEGs was determined using the Pearson correlation test.

Statistical analysis

Data analyses were carried out using GraphPad Prism 8.0 (GraphPad, San Diego, CA, USA) and SPSS 22.0. Data were expressed as the mean ± standard deviation (SD). Comparisons between two groups were conducted using the Student's t-test (unpaired, two-tailed), while multiple-group comparisons were conducted using one-way analysis of variance followed by Tukey's post-hoc test. Categorical variables, including patients' clinicopathological characteristics, were expressed as frequencies and percentages [n (%)] and analyzed using Fisher's exact test. A *P*-value <0.05 was considered statistically significant.

Results

Expression of miR-325-3p in GC and proposal of the LINC01515/miR-325-3p/ERBB4 axis

The miR-325-3p expression was consistently downregulated in GC samples and GC cell lines compared to adjacent normal tissues and GES-1 cells (**Figure 1A**, **1B**). MKN45 cells showed lowest expression of miR-325-3p (**Figure 1B**). Based on median miR-325-3p expression, GC samples were categorized into low- and high-expression groups. Reduced miR-325-3p expression was significantly associated with higher TNM stage, deeper tumor invasion, and regional lymph node metastasis (**Table 2**).

Bioinformatics analysis was performed to identify competitive lncRNAs and target mRNAs of miR-325-3p using TCGA-GC datasets (**Figure 1C**, **1D**). DEGs were primarily enriched in pathways related to PID MYC activation, epithelial cell proliferation, and proteoglycans in cancer (**Figure 1E**). Pearson correlation analysis revealed strong positive association between ERBB4 and LINC01515, suggesting that LINC01515-ERBB4 may represent a ceRNA regulatory pair (**Figure 1F**), supporting the hypothesis of the existence of LINC01515/miR-325-3p/ERBB4 axis.

LINC01515 upregulated ERBB4 expression via sponging miR-325-3p

To validate this axis, qRT-PCR was conducted to detect the expression of ERBB4 and LINC01515 in GC cell lines and samples. Compared with adjacent non-cancerous tissues, both ERBB4 and LINC01515 were upregulated in GC samples (**Figure 2A**, **2B**). Similarly, MKN45 cells showed the highest ERBB4 and LINC01515 expression among the GC cell lines (**Figure 2C**, **2D**). The binding sites between miR-325-3p and LINC01515, as well as between miR-325-3p and ERBB4 were identified (**Figure 2E**, **2F**). Dual-luciferase assays showed co-transfection of MKN45 cells with 325-3p mimic and the LINC01515 wild-type reporter significantly reduced the luciferase activity compared with the control mimic, whereases co-transfection with the LINC01515-Mut showed unchanged activity (**Figure 2G**). Likewise, MKN45 cells co-transfected with miR-325-3p mimic and ERBB4-Wt demonstrated significantly reduced luciferase activity, while ERBB4-mutant didn't notably change luciferase activity (**Figure 2H**). These results validate the targeting relationship between miR-325-3p and LINC01515, as well as between miR-325-3p and ERBB4.

LINC01515 downregulation suppressed proliferation, migration, and invasion in GC cells

MKN45 cells, with the highest LINC01515 expression, were selected for subsequent experi-

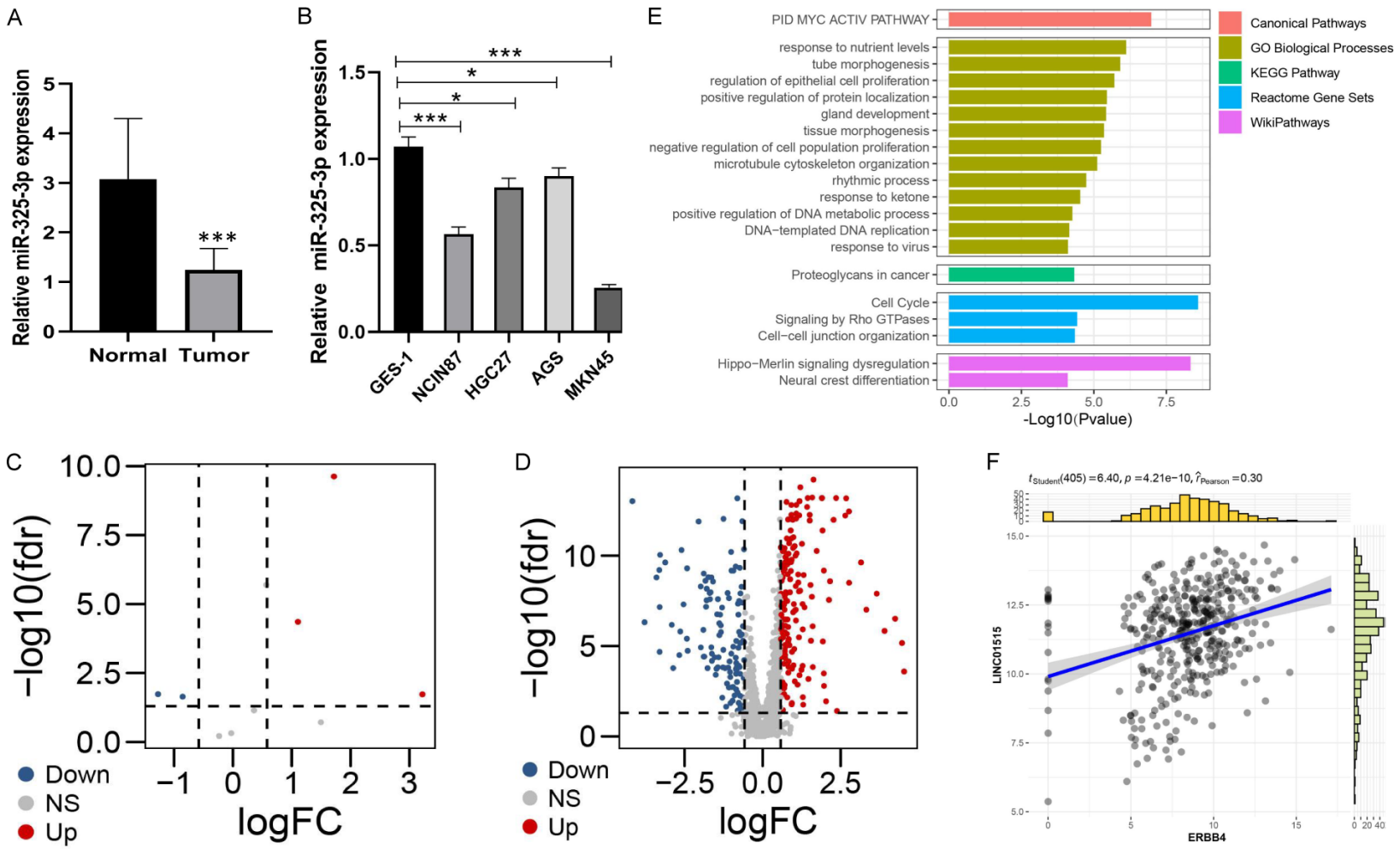

Figure 1. Expression of miR-325-3p in GC samples and cells and proposal of the LINC01515/miR-325-3p/ERBB4 axis. A. miR-325-3p expression in GC and the adjacent healthy tissues assessed by qRT-PCR; B. miR-325-3p expression in GC cell lines (MKN45, HGC27, AGS and NCIN87) and GES-1 assessed by qRT-PCR; C, D. Bioinformatic prediction of target mRNAs and competitive lncRNAs of miR-325-3p; E. Enrichment analysis of differentially expressed genes; F. Pearson correlation analysis showing a positive correlation between ERBB4 and LINC01515. *P<0.05, **P<0.01, ***P<0.001.

Table 2. Association between miR-325-3p expression and clinicopathological features in 50 patients with gastric cancer

Clinicopathological parameters	n	Expression of miR-325-3p		χ^2	*P*-value
		Higher expression	Lower expression		
Gender				0.192	0.775
Male	28	11	17		
Female	22	10	12		
Age (years)				0.321	0.571
≤55	26	12	14		
>55	24	13	11		
Tumor size (cm)				0.009	1.00
≤5	18	7	11		
>5	32	12	20		
Infiltrating depth				7.892	0.033*
T1	5	2	3		
T2	8	4	4		
T3	11	5	6		
T4	26	3	23		
Nodal status				7.969	0.035*
N0	5	1	4		
N1	7	4	3		
N2	13	7	6		
N3	25	4	21		
Distant metastases				0	1.00
M0	48	22	26		
M1	2	1	1		
TNM stage				7.038	0.047*
I	7	3	4		
II	16	7	9		
III	25	3	22		
IV	2	1	1		

*$P<0.05$.

ments. siRNA knockdown efficiency was first confirmed (**Figure 3A**). CCK-8 results showed that LINC01515 knockdown significantly reduced the proliferation of MKN45 cells (**Figure 3B**). Transwell invasion and wound healing assays showed that LINC01515 knockdown substantially reduced MKN45 cell invasion and migration (**Figure 3C**, **3D**). Additionally, the expression of genes related to cell invasion and migration was assessed: TIMP3 and E-cadherin levels were increased, while MMP9, Vimentin, MMP2, and N-cadherin levels were decreased (**Figure 3E**). Collectively, LINC01515 knockdown inhibited GC cell growth, migration, and invasion.

miR-325-3p upregulation impaired GC cell growth, migration, and invasion

miR-325-3p overexpression was achieved in MKN45 cells using a miR-325-3p mimic (**Figure 4A**). CCK-8 assays indicated that miR-325-3p overexpression significantly suppressed MKN45 cell proliferation (**Figure 4B**). Transwell invasion and wound-healing assays showed that miR-325-3p overexpression notably reduced the migratory and invasion of MKN45 cells (**Figure 4C**, **4D**). Molecular analyses demonstrated that the expression levels of MMP9, Vimentin, MMP2, and N-cadherin were decreased, whereas the levels of E-cadherin and TIMP3 were increased (**Figure 4E**).

ERBB4 downregulation suppressed GC cell proliferation, migration, and invasion

qRT-PCR confirmed that ERBB4 mRNA levels were significantly reduced after ERBB4-siRNA transfection (**Figure 5A**). As shown by CCK-8 assays, ERBB4 knockdown significantly inhibited the proliferation of MKN45 cells (**Figure 5B**). Transwell and wound-healing assays showed that the invasion and migration abilities of MKN45 cells were notably decreased after ERBB4 knockdown (**Figure 5C**, **5D**). Moreover, the expression of E-cadherin and TIMP3 was increased, while the expression of N-cadherin, MMP9, Vimentin, and MMP2 was suppressed, which align with the phenotypic changes (**Figure 5E**).

LINC01515 promoted GC progression by sponging miR-325-3p

LINC01515-siRNA significantly decreased the protein and mRNA expression of ERBB4 in MKN45 cells; whereas co-transfection with miR-325-3p inhibitors restored ERBB4 expression (**Figure 6A**, **6B**). The co-transfection also restored the impaired proliferative capaci-

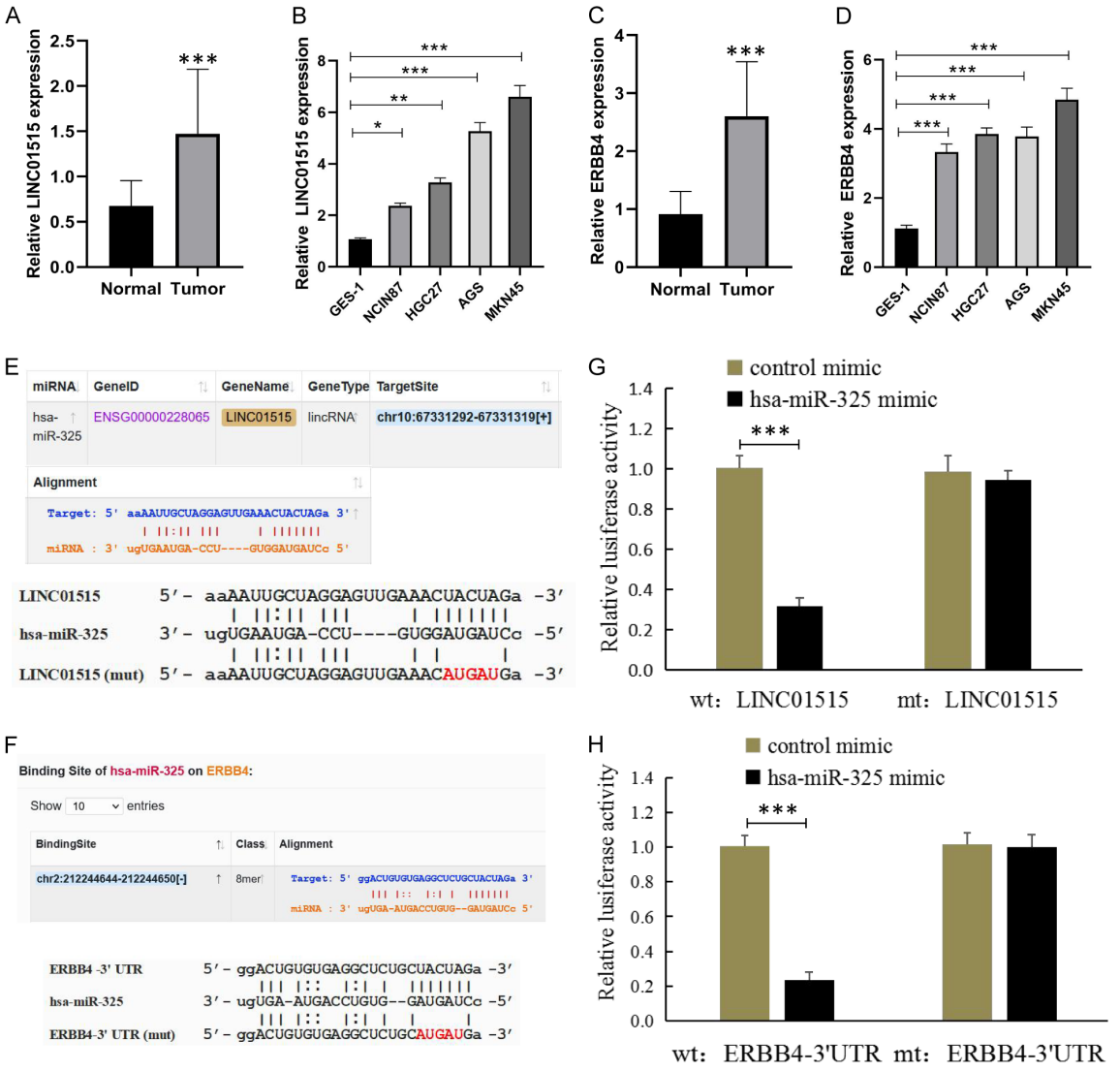

Figure 2. LINC01515 upregulated ERBB4 expression via sponging miR-325-3p. A, B. The mRNA expression levels of LINC01515 and ERBB4 in GC and adjacent healthy tissues assessed by qRT-PCR; C, D. The expression levels of LINC01515 and ERBB4 in GC cell lines (MKN45, HGC27, AGS and NCIN87) and GES-1 assessed by qRT-PCR; E, F. The binding sites between LINC01515 and miR-325-3p, as well as between miR-325-3p and ERBB4; G. Dual luciferase reporter assays showing that mutation of LINC01515 abolished the impact of miR-325-3p up-regulation on reporter gene expression; H. Dual luciferase reporter assays showing that mutation of ERBB4 abolished the impact of miR-325-3p up-regulation on reporter gene expression. *P<0.05, **P<0.01, ***P<0.001.

ty caused by LINC01515-siRNA (**Figure 6C**). Transwell and wound healing assays consistently demonstrated that the reduced invasion and migration abilities caused by LINC01515-siRNA were reversed by co-transfection with miR-325-3p inhibitors (**Figure 6D**, **6E**). Similarly, co-transfection notably restored the expression of migration- and invasion-related genes (**Figure 6F**, **6G**).

Discussion

miRNAs play major roles in regulating tumor-associated genes. MiR-325 is a critical modulator in several malignancies, including colorectal, breast, and bladder cancers. Yu et al. reported that miR-325 inhibits non-small cell lung cancer cell proliferation and migration via targeting DNA ligase 1 [27]. In hepatocellular

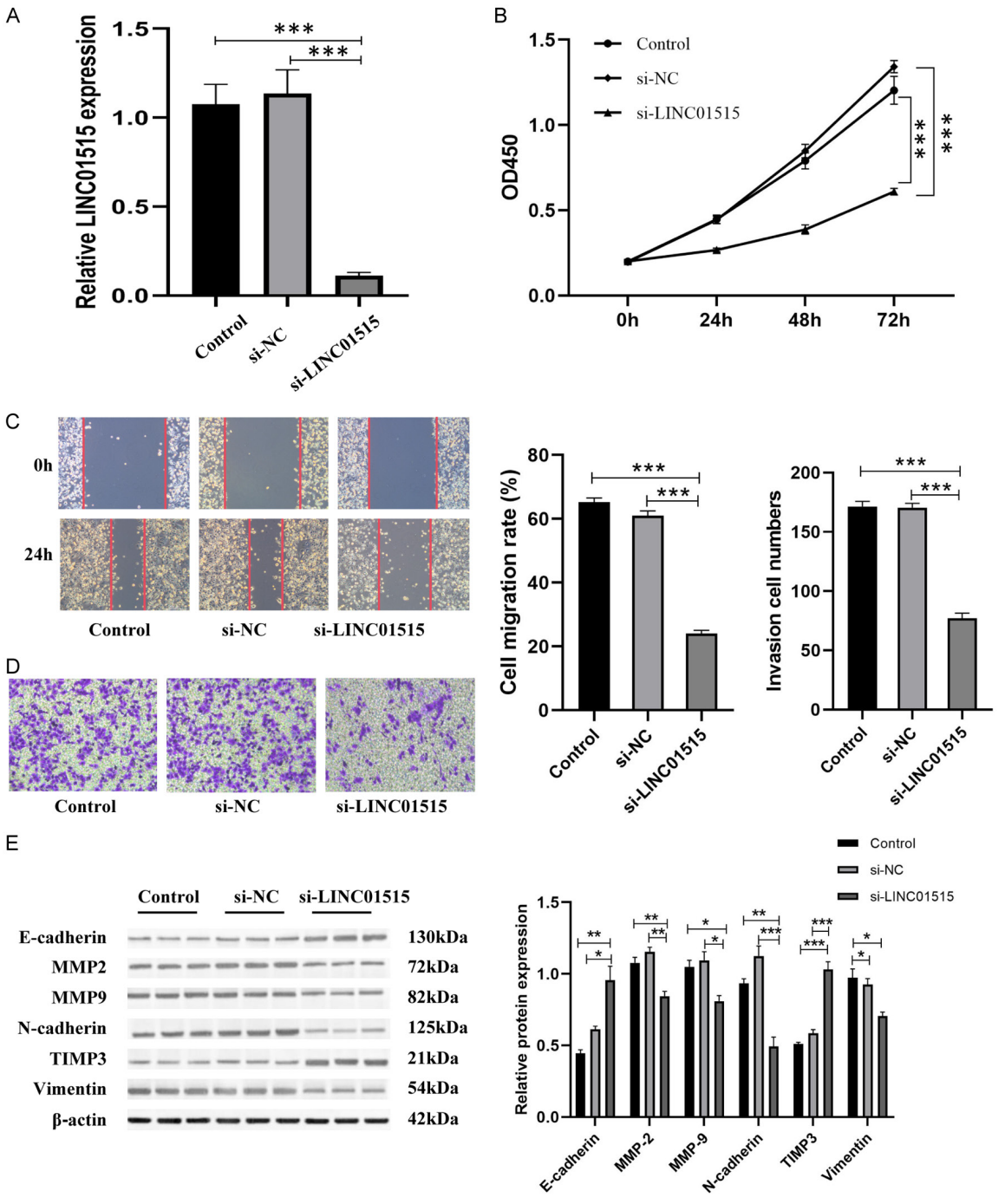

Figure 3. Downregulation of LINC01515 suppressed GC cells proliferation, migration, and invasion. A. Verification of si-LINC01515 knockdown efficiency; B. Effects of LINC01515 knockdown on MKN45 cell proliferation assessed by CCK-8 assay; C, D. Effects of LINC01515 knockdown on MKN45 cell migration and invasion detected by wound-healing and Transwell assays; scale bar, 200 μm; E. Effects of LINC01515 knockdown on the expression of invasion- and metastasis-related genes in MKN45 cells detected by qRT-PCR and Western blot. *P<0.05, **P<0.01, ***P<0.001.

carcinoma, MiR-325-3p may serve as a therapeutic biomarker as it regulates angiogenesis through the CXCL17/CXCR8 axis [28]. In breast cancer, miR-325 inhibit tumor growth and metastasis by targeting lipocalin 15 [29]. In this study, miR-325-3p expression was downregu-

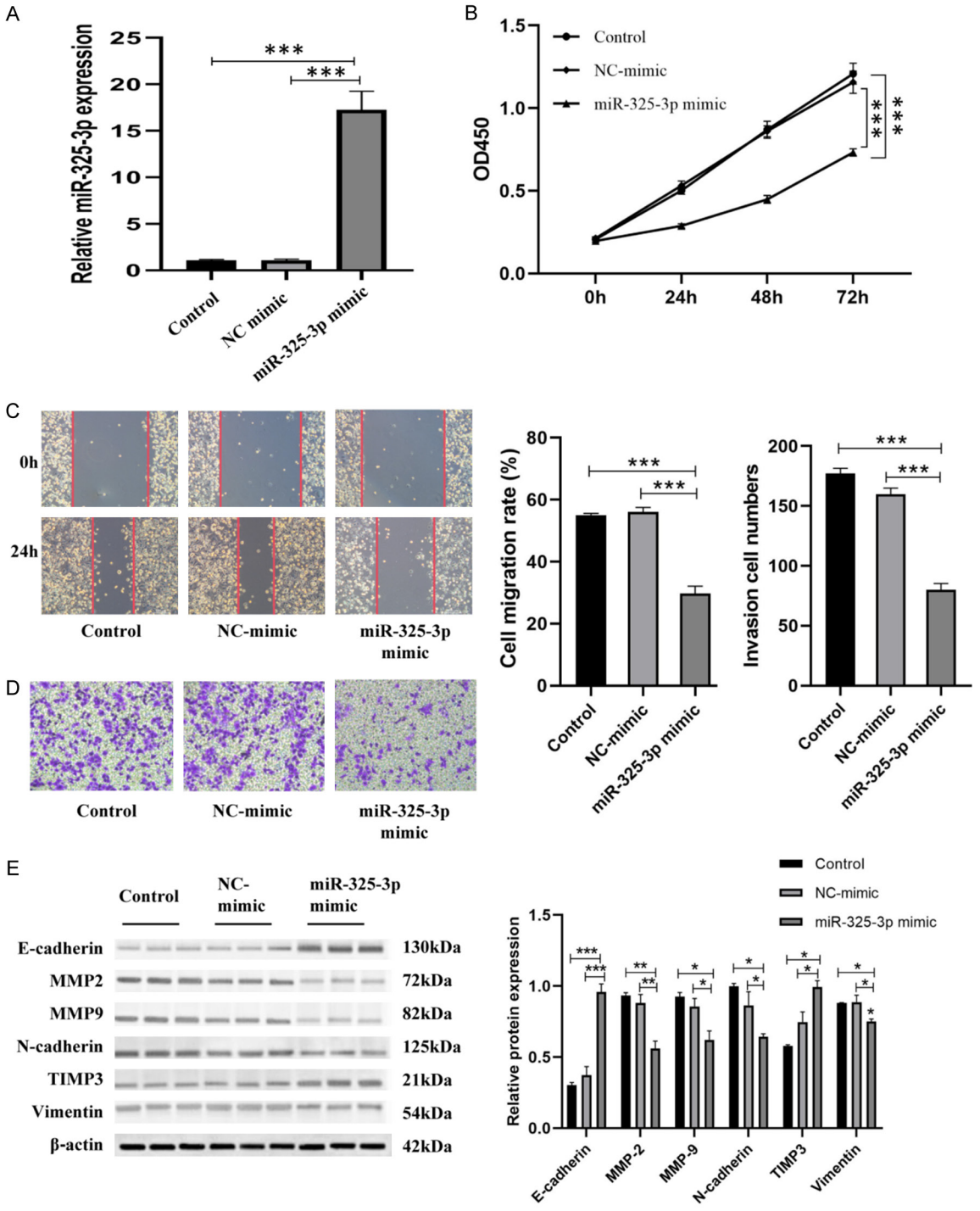

Figure 4. Upregulation of miR-325-3p inhibited GC cell proliferation, migration, and invasion. A. Verification of miR-325-3p overexpression in MKN45 cells by qRT-PCR; B. Effects of miR-325-3p overexpression on MKN45 cell proliferation detected by CCK-8 assay; C, D. Effects of miR-325-3p overexpression on MKN45 cell migration and invasion tested by the wound-healing and Transwell assays; scale bar, 200 μm; E. Effects of miR-325-3p overexpression on the expression of invasion- and metastasis-related genes in MKN45 cells detected by qRT-PCR and Western blot. *P<0.05, **P<0.01, ***P<0.001.

lated in GC cell lines and samples. Its aberrant expression was related to regional lymph node metastasis, tumor invasion depth, and TNM stage, suggesting its potential role in GC pro-

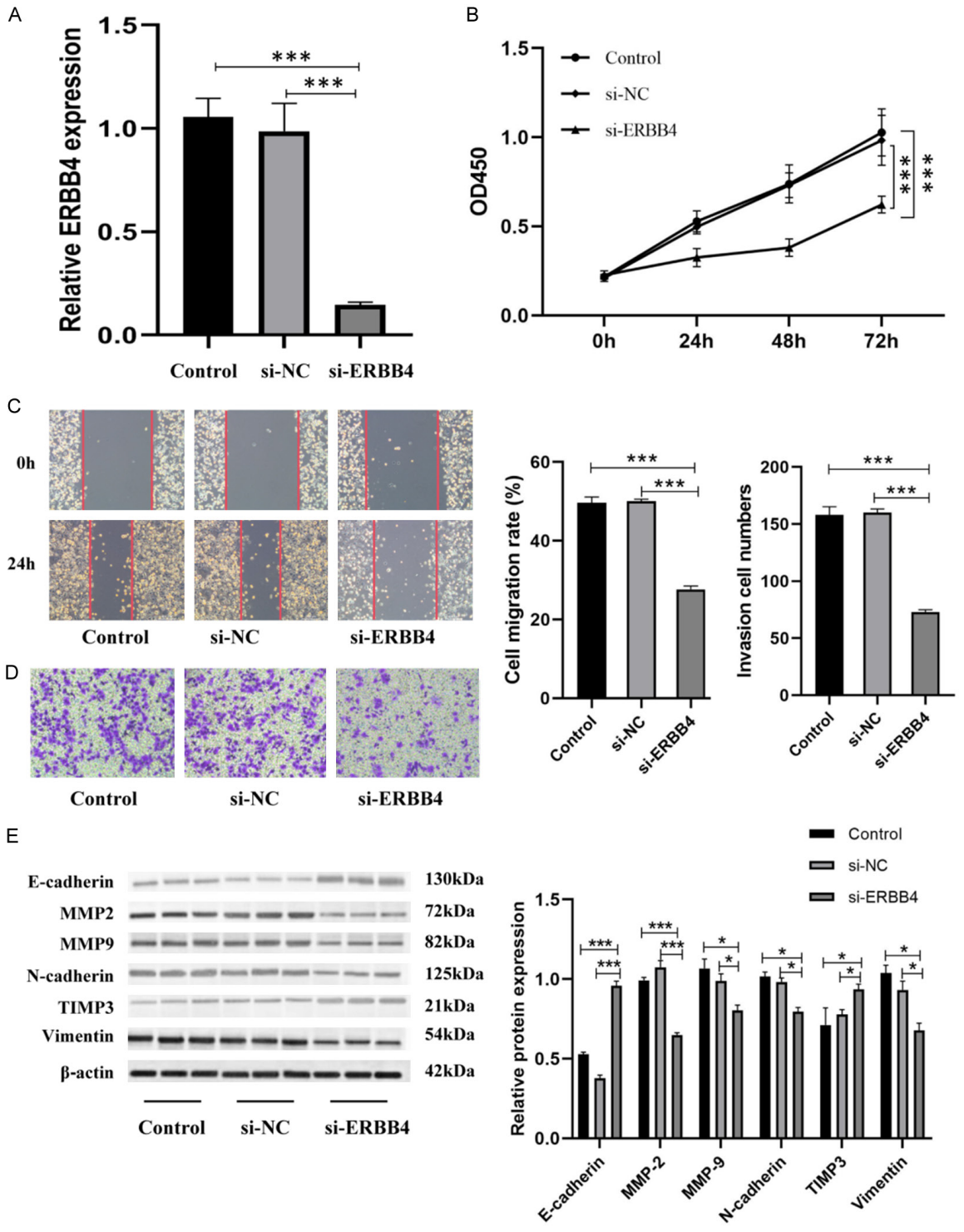

Figure 5. Downregulation of ERBB4 suppressed GC cell proliferation, migration, and invasion. A. Verification of si-ERBB4 knockdown efficiency detected by qRT-PCR. B. Effects of ERBB4 knockdown on MKN45 cell proliferation detected by CCK-8 assay. C, D. Effects of ERBB4 knockdown on MKN45 cell migration and invasion tested by the wound-healing and Transwell assays; scale bar, 200 μm. E. Effects of ERBB4 knockdown on the expression of invasion- and metastasis-related genes in MKN45 cells detected by qRT-PCR and Western blot. *P<0.05, **P<0.01, ***P<0.001.

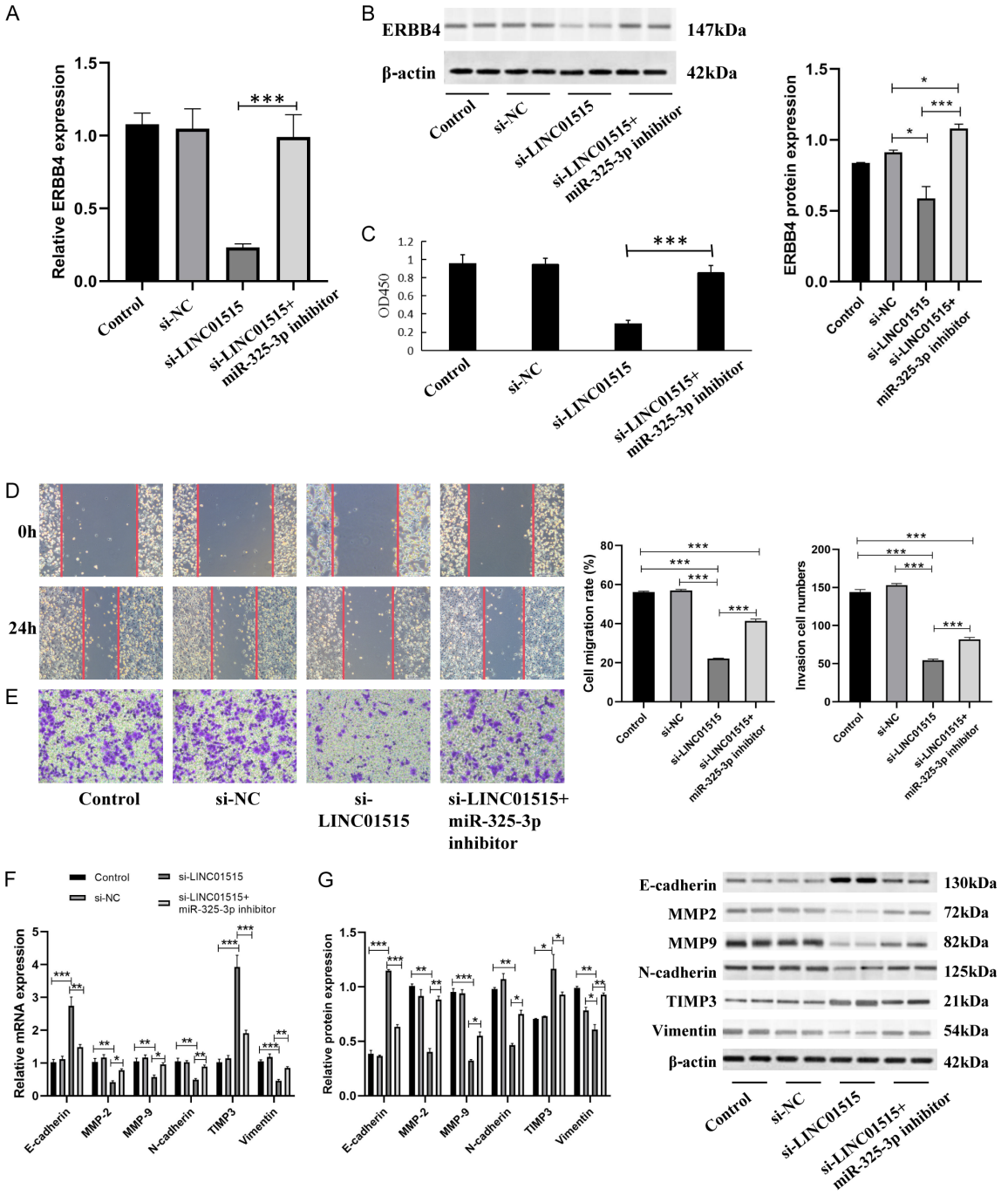

Figure 6. LINC01515 promoted GC progression by sponging miR-325-3p. A, B. The mRNA and protein levels of ERBB4 detected by qRT-PCR and WB; C. The proliferation of MNK45 cells measured by CCK-8 assay; D, E. Effects of co-transfection of si-LINC01515 and miR-325-3P inhibitor on MNK45 cell migration and invasion detected by wound-healing and Transwell assays; scale bar, 200 μm; F, G. Expression levels of invasion- and metastasis-related genes detected by qRT-PCR and Western blot. *P<0.05, **P<0.01, ***P<0.001.

gression. Functional assays demonstrated that miR-325-3p overexpression significantly inhibited GC cell proliferation, invasion, and migration, evidenced by CCK-8, Transwell, and wound healing assays.

LncRNAs can regulate target genes by acting as ceRNAs to sponge miRNAs [30]. LncRNAs with aberrant expression are increasingly recognized as critical contributors to cancer biology, by regulating invasion, proliferation, metas-

tasis, and treatment resistance, as well as key gene regulatory networks [31, 32]. To further clarify the regulatory mechanisms of miR-325-3p in GC, bioinformatics was used to screen differentially expressed competitive lncRNAs and target mRNAs. The results showed a positive relationship between ERBB4 and LINC01515, leading us to hypothesize that LINC01515 regulates ERBB4 by sponging miR-325-3p. Then, The predicted binding sites among LINC01515, miR-325-3p, and ERBB4 were identified, which were further verified using Luciferase reporter gene assays. LINC01515 was reported to be upregulated in nasopharyngeal cancer, where it promotes cell proliferation by acting as a miR-325 sponge [23]. However, its role in GC remained unclear. In this study, LINC01515 was significantly upregulated in GC cell lines and tissues. LINC01515 silencing significantly inhibited GC cell proliferation, invasion, and migration, whereas this suppressive effect was partially reversed by co-transfection with miR-325-3p inhibitors. Mechanistically, LINC01515 acts as a ceRNA to elevate ERBB4 expression by sequestering miR-325-3p. High ERBB4 expression has been linked to poor prognosis in multiple cancer types. In our study, ERBB4 was upregulated in GC samples compared with healthy tissues, and its expression was downregulated after LINC01515 knockdown; and this reduction was rescued by miR-325-3p inhibitors, further supporting the regulatory relationship within LINC01515/miR-325-3p/ERBB4 axis. Subsequently, ERBB4 knockdown suppressed cell proliferation, invasion, and migration, suggesting that ERBB4 may contribute to tumor development.

Conclusion

Collectively, GC cell lines and samples showed downregulated miR-325-3p. LINC01515 promotes GC progression by sponging miR-325-3p and consequently upregulating ERBB4. These findings indicate that the LINC01515/miR-325-3p/ERBB4 axis mechanism represents a promising target for GC treatment.

Acknowledgements

We acknowledge TopEdit LLC for the linguistic editing and proofreading during the preparation of this manuscript. This study was supported by the S&T Program of Hebei (22377701D) and Medical Science Research Project of Hebei (20210886).

Disclosure of conflict of interest

None.

Abbreviations

GC, Gastric cancer; miRNAs, microRNAs; lncRNAs, Long non-coding RNAs; ceRNAs, Competing endogenous RNAs; siRNAs, Interfering RNAs; qRT-PCR, Quantitative real-time polymerase chain reaction; CCK8, Cell Counting Kit-8; ERBB4, Erythroblastic oncogene B4.

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