

RESEARCH ARTICLE

miR-125a-5p promotes gastric cancer growth and invasion by regulating the Hippo pathway

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Abstract

Objective: This study was carried out to explore the potential involvement of miR-125a-5p in the oncogenic effects of EphA2, TAZ, and TEAD2 and the activity of the Hippo signaling pathway in gastric cancer progression.

Methods: In vitro transfection of miR-125a-5p mimics or inhibitors, qRT-PCR, colony formation assays, and cell invasion assays were used to assess the effect of miR-125a-5p on the growth and invasion in gastric cancer (GC). Male nude mice bearing tumors derived from human GC cells were used for evaluating the effects of miR-125a-5p on tumor growth. Luciferase reporter assay, immunofluorescence, immunohistochemistry, qRT-PCR, and immunoblotting were performed to explore the role of miR-125a-5p in the epithelial-mesenchymal transition (EMT) and association among miR-125a-5p, EphA2, TAZ, and TEAD2 in GC cells.

Results: MiR-125a-5p enhanced GC cell viability and invasion in vitro, whereas inhibition of miR-125a-5p using a specific inhibitor and antagomir suppressed cancer cell invasion and tumor growth. Moreover, inhibition of miR-125a-5p reversed EMT in vitro. miR-125a-5p upregulated the expression of EphA2, TAZ, and TEAD2, promoted TAZ nuclear translocation, and induced changes in the activity of the Hippo pathway by enhancing the expression of TAZ target genes. Finally, miR-125a-5p was overexpressed in late-stage GCs, and positive correlations were observed with its targets EphA2, TAZ, and TEAD2.

Conclusion: miR-125a-5p can promote GC growth and invasion by upregulating the expression of EphA2, TAZ, and TEAD2.

KEYWORDS

gastric cancer, Hippo pathway, miR-125a-5p, prognosis, TAZ

Ruixin Li and Zhihao Hu contributed equally to this work.

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1 | INTRODUCTION

Gastric cancer (GC) is the fifth most common malignancy and third most frequent cause of cancer-related death worldwide.¹⁻³ Surgical resection is the only curative approach for resectable GCs; however, locoregional control of advanced GCs may frequently be complicated by postsurgical recurrence and metastasis. The molecular mechanism of GC recurrence and progression is unclear, and investigation of this mechanism is critical for development of targeted therapies to inhibit local recurrence and progression.

The Hippo pathway consists of a large network of proteins and controls growth of normal and malignant (carcinoma) tissues.^{4,5} The key kinase cascade involved in the Hippo pathway is phosphorylation of upstream Yes-associated protein (YAP) and transcriptional co-activator PDZ-binding motif (TAZ). YAP and TAZ promote tissue growth and cell viability by regulating the activity of transcription factor TEA domain family members (TEADs).⁶ Multiple components of the Hippo pathway, including YAP1, TAZ, and TEAD1, are overexpressed in GCs and are closely correlated with lymphatic metastasis and tumor TNM staging.^{7,8} Thus, the Hippo pathway has been recognized as a promising and important therapeutic target.⁹

MicroRNAs (miRNAs) are small non-coding RNAs that post-transcriptionally control the translation and stability of mRNAs. miRNAs may function as oncogenes or tumor-suppressor genes to contribute to cancer development, progression, and therapeutic responses.¹⁰ *miR-125a-5p* has anticancer activities in breast cancer,¹¹ hepatocellular carcinoma,¹² lung cancer,¹³ and glioblastoma.¹⁴ However, controversial results have been reported for the function of *miR-125a-5p* in GCs in different studies. The *miR-125a-5p* expression had been reported to be significantly down-regulated in GCs, and decreased *miR-125a-5p* expression was associated with gastric carcinogenesis.^{15,16} However, *miR-125a-5p* had also been reported to be overexpressed in a GC characterized by activation of mesenchymal phenotype-related pathways, but *miR-125a-5p* expression level itself did not associate with poor prognosis.¹⁷ Moreover, *miR-125a*, the precursor of *miR-125a-5p* (which is derived from the 5' end of *pri-miR-125a*), was reported to be associated with invasion depth and clinical stage of GC.¹⁸ Thus, the specific role of *miR-125a-5p* in GCs is unclear and needs additional studies to clarify.

Here, we investigated the role of *miR-125a-5p* as an oncogene in GC by analyzing its effects on the growth, invasion, and epithelial-mesenchymal transition (EMT) in GC cells. In addition, we examined the involvement of the *miR-125a-5p* target EphA2, TAZ, and TEAD2 in these oncogenic effects and the activity of the Hippo signaling pathway in GC. Finally, we evaluated the correlations between *miR-125a-5p* expression levels and GC progression. Our results suggested that *miR-125a-5p* could be a potential therapeutic target for advanced GC.

2 | MATERIALS AND METHODS

2.1 | Patients and clinical specimens

This study was approved by the ethics committee of our hospital, and all patients with GCs had given their signed informed consent to participate. The GC adenocarcinoma tissues ($n = 65$) and corresponding noncancerous gastric tissues were obtained from samples of radical resection. No patients received neoadjuvant chemotherapy or radiotherapy before radical gastrectomy. Resected tissues were immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction. The clinical stage of cancer was based on the criteria published by the 7th American Joint Committee on Cancer TNM staging classification for carcinoma of the stomach.¹⁹

2.2 | Reagents

Antibodies specific to EphA2 (ab118882), YES1 (ab109744), TAZ (ab119253), and GAPDH (ab37168) and horseradish peroxidase-couple secondary antibodies were purchased from Abcam. Antibodies targeting TEAD2 (8870), E-cadherin (3195), N-cadherin (13116), and vimentin (5741) were from Cell Signaling Technology. *miR-125a-5p* mimics, inhibitors (antagomir), negative controls, 3'-untranslated region (UTR) reporter plasmids, small hairpin RNA (shRNA) specifically targeting TAZ, and control shRNA were synthesized by Ribobio Co. The sequences are shown in Table S1.

2.3 | Cell culture and transfection

The gastric epithelial cell line GES-1, the GC cell lines AGS, SGC-7901, BGC-823, MGC-803, MKN-45, N87, SNU-5, and KATO-III, and HEK293T cells were purchased from the Cell Resource Center of Xiangya Medical School, Central South University. GC cells were cultured in RPMI 1640 medium, whereas GES-1 and HEK293T cells were cultured in Dulbecco's modified Eagle medium (Gibco) with 10% fetal bovine serum (Hyclone) and incubated in 5% CO_2 at 37°C . Cells were seeded into six-well plates, incubated overnight, and then transfected with miRNA mimics, inhibitors, or shRNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

2.4 | RNA quantification

Total RNA was extracted from tissues and cells with TRIzol reagent (Invitrogen). Reverse transcription reactions were conducted using a RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas). Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was conducted using a qSYBR-Green-containing PCR kit (Qiagen) on an

ABI 7500 System (Applied Biosystems). RNU6 and β -actin were used as internal controls for miRNA and mRNA, respectively. Relative expression levels were determined using the $2^{-\Delta\Delta C_t}$ method. The primers for qRT-PCR detection were synthesized by GenePharma Co., and the sequences are presented in Table S2.

2.5 | Colony formation assay

Gastric cancer cells were seeded in 3.5-cm dishes at 1×10^3 cells per dish and incubated at 37°C for 2 weeks. Colonies were fixed with methanol and stained with 0.1% crystal violet for 10 min. The number of colonies was counted using an inverted microscope (Olympus). The colony formation rate was calculated according to the following formula: colony formation rate = number of colonies/number of seeded cells.

2.6 | Cell invasion assays

Briefly, cells transfected with *miR-125a-5p* mimics or inhibitors and their negative normal controls were plated onto the basement membrane matrix in the inserts of 24-well culture plates (8 μ m; BD Biosciences) at a density of 1×10^5 cells per insert. The cells were incubated at 37°C for 48 h, and noninvasive cells and matrix were then gently removed with a cotton swab. The invaded cells on the lower membrane surface were fixed with methanol for 10 min, stained with crystal violet, counted, and imaged.

2.7 | Luciferase reporter assay

The 3'-UTRs of *EphA2*, *YES1*, *TAZ*, and *TEAD2* mRNA containing the intact *miR-125a-5p* recognition sequence or mutant sequences were amplified by PCR and subcloned into the pmir-RB-REPORT vector (Ribobio Co.). The *miR-125a-5p* mimics were cotransfected with luciferase reporter plasmids into cultured HEK293T cells. At 48 h after transfection, the cells were assayed for luciferase activity using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. The relative luciferase activity was normalized to the renilla luciferase activity.

2.8 | Immunoblotting

After transfection and culture, cells were harvested and lysed in RIPA buffer (Promega). Total proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto polyvinylidene fluoride membranes (Millipore). After blocking of nonspecific binding, the transferred membranes were subsequently incubated overnight at 4°C with primary and

secondary antibodies for 1 h. Protein bands were visualized using enhanced chemiluminescence detection (Millipore).

2.9 | Immunohistochemistry

Immunohistochemistry was performed as previously described.²⁰ Briefly, peroxidase activity within the sections was blocked with 2.5% hydrogen peroxide in methanol for 30 min at room temperature, and the slides were then incubated with primary antibodies overnight at 4°C. After washing with phosphate-buffered saline, the sections were incubated with peroxidase-labeled polymer and substrate chromogen. Finally, the slides were visualized in diaminobenzidine solution at room temperature.

2.10 | Immunofluorescence

Cells grown on glass coverslips were fixed in 4% paraformaldehyde and permeabilized using 0.5% Triton X-100. The primary antibodies were applied for 1 h at 37°C and visualized using Alexa-Fluor 488-conjugated secondary antibodies (Invitrogen). Cells were counterstained with 4',6-diamidino-2-phenylindole and then examined by confocal microscopy (LSM5 PASCAL, Carl Zeiss).

2.11 | In vivo assay

Male nude mice bearing tumors derived from human GC cells were used for evaluating the effects of *miR-125a-5p* on tumor growth. For preparation of the subcutaneous xenograft model, 1×10^6 N87 cells were subcutaneously injected into the flanks of mice. Two weeks after subcutaneous injection, 10 nmol antagomir in 0.1 ml saline buffer was locally injected into the tumor mass once every 3 days for 2 weeks.²¹ The length (a) and width (b) of the tumor were measured every 3 days. The tumor volume (V) was calculated according to the formula $V = (ab)^2/2$.²² All mouse experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, with the approval of the Animal Ethics Committee of Central South University.

2.12 | Statistical analyses

Continuous variables were presented as means \pm standard deviations (SDs). Comparisons between groups were analyzed using the t test and chi-squared test. Correlations between the expression levels of two mRNAs were evaluated by Pearson's coefficient correlation analyses. Differences with *p* values of <0.05 were considered significant. Statistical analyses were conducted using GraphPad Prism5 software (GraphPad Software, Inc.).

3 | RESULTS

3.1 | *miR-125a-5p* promoted GC growth and invasion

To select suitable GC cells for use, the expression levels of *miR-125a-5p* were tested in a series of GC cell lines and a normal gastric epithelial cell line (GES-1 cells). The highest expression of *miR-125a-5p* was observed in N87 cells, whereas the lowest level was demonstrated in the KATO-III cells (Figure 1A). Therefore, the N87 and KATO-III cells were selected for functional deficient and acquired studies, respectively.

Next, the effects of *miR-125a-5p* were examined on GC growth and invasion. *miR-125a-5p* mimics significantly increased *miR-125a-5p* expression in KATO-III cells, whereas antisense oligonucleotides significantly decreased *miR-125a-5p* expression in N87 cells (Figure 1B). Increased expression of *miR-125a-5p* enhanced the viability of KATO-III cells, whereas loss of *miR-125a-5p* significantly attenuated N87 cell viability (Figure 1C). To identify the effects of *miR-125a-5p* on cell growth, subcutaneous xenografts were established from N87 cells in nude mice. Two weeks after implantation, intratumoral injection of *miR-125a-5p* antagomir was performed. The *miR-125a-5p* antagomir significantly inhibited the growth of subcutaneous tumors after 10 days of antagomir treatment (Figure 1D). Moreover, ectopic expression of *miR-125a-5p* in KATO-III cells significantly increased cell invasion, whereas inhibition of *miR-125a-5p* by antisense oligonucleotides inhibited the invasion of N87 cells (Figure 1E). Thus, these results demonstrated that *miR-125a-5p* promoted GC growth and invasion. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

3.2 | Inhibition of *miR-125a-5p* suppressed the EMT

Because overexpression of *miR-125a-5p* has been shown to activate mesenchymal phenotype-related pathways, including the EMT, and regulate mesenchymal cell proliferation in GC tissues,¹⁷ the effects of *miR-125a-5p* on the EMT were subsequently tested. Loss of *miR-125a-5p* resulted in a decrease in the number of spindle-like N87 cells (Figure 2A). Moreover, in cells treated with a *miR-125a-5p* inhibitor, E-cadherin protein levels were increased significantly compared with that in the control. Inversely, the expression levels of N-cadherin and vimentin were significantly decreased in cells treated with *miR-125a-5p* inhibitors (Figure 2B). The same phenomenon was observed using immunofluorescence (Figure 2C).

To further investigate the role of *miR-125a-5p* in EMT, the epithelial marker E-cadherin and the mesenchymal markers N-cadherin and vimentin were detected in subcutaneous tumors using immunohistochemistry. As shown in Figure 2D, the expression of E-cadherin was increased, whereas those of N-cadherin and vimentin were decreased in tumors treated with the *miR-125a-5p* antagomir. Thus, inhibition of *miR-125a-5p* reversed the EMT.

3.3 | *miR-125a-5p* regulated EphA2 and multiple components of the Hippo pathway

Different miRNA target-predicting algorithms such as TargetScan, Pictar, and miRDB were used to identify potential effectors of *miR-125a-5p*, and conserved *miR-125a-5p* sites were found at the 3'-UTR of three genes related to the Hippo pathway (YES1, TAZ, and TEAD2) and of EphA2 (Figure S1A), an Eph family member that is overexpressed in GCs and has been shown to promote the proliferation, invasion, and EMT in GC cells through the Wnt/ β -catenin pathway.²⁰ To further validate targets of *miR-125a-5p*, the 3'-UTRs of EphA2, YES1, TAZ, and TEAD2 (containing the putative *miR-125a-5p* target sites) were cloned into luciferase reporter constructs. The luciferase activity of plasmids containing the wild-type 3'-UTRs of EphA2, YES1, TAZ, and TEAD2 was significantly reduced in the presence of *miR-125a-5p* but not in the mutant 3'-UTR (Figure S1B). However, the expression of EphA2, TAZ, and TEAD2 mRNA and protein was significantly increased by transfection with *miR-125a-5p* mimics and decreased by transfection with *miR-125a-5p* inhibitors, and no significant changes were present in the YES1 expression (Figure 3A and Figure S2). Moreover, transfection with the *miR-125a-5p* antagomir significantly downregulated the expression of EphA2, TAZ, and TEAD2 in subcutaneous tumors (Figure 3B).

Because TAZ is a key co-activator of the Hippo pathway, the subcellular distribution of TAZ was tested by immunofluorescence, and the expression of TAZ target genes, including connective tissue growth factor, plasminogen activator inhibitor 1, and surviving, was detected by qPCR.²³ It was found that *miR-125a-5p* promoted the nuclear translocation of TAZ and activated the transcription of TAZ target genes (Figure 3C,D). Taken together, these findings suggested that *miR-125a-5p* contributed to activation of EphA2, TAZ, and TEAD2. (* $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

3.4 | Silencing of TAZ reversed the effects of *miR-125a-5p* on GC cells

The above findings indicated that TAZ was a candidate effector of *miR-125a-5p*. TAZ is a nuclear effector of Hippo-related pathways that regulate EMT.²⁴ Moreover, inhibition of TAZ by miRNA has been shown to inhibit tumor growth and metastasis in GCs.²⁵ Therefore, it was hypothesized that *miR-125a-5p* promoted EMT in GC cells through modulation of TAZ. To test this hypothesis, TAZ was silenced using specific shRNA in GC cells overexpressing *miR-125a-5p* (Figure 4A). It was demonstrated that knockdown of TAZ inhibited the effects of *miR-125a-5p* on promotion of EMT, cell viability, and invasion in GC cells (Figure 4B,C). These data implied that *miR-125a-5p* contributed to cell growth, invasion, and EMT in GCs through the key Hippo pathway component TAZ.

3.5 | Clinical correlation between *miR-125a-5p* and its targets in GCs

To further elucidate the potential biological significance of *miR-125a-5p* expression in GC progression, the expression levels of

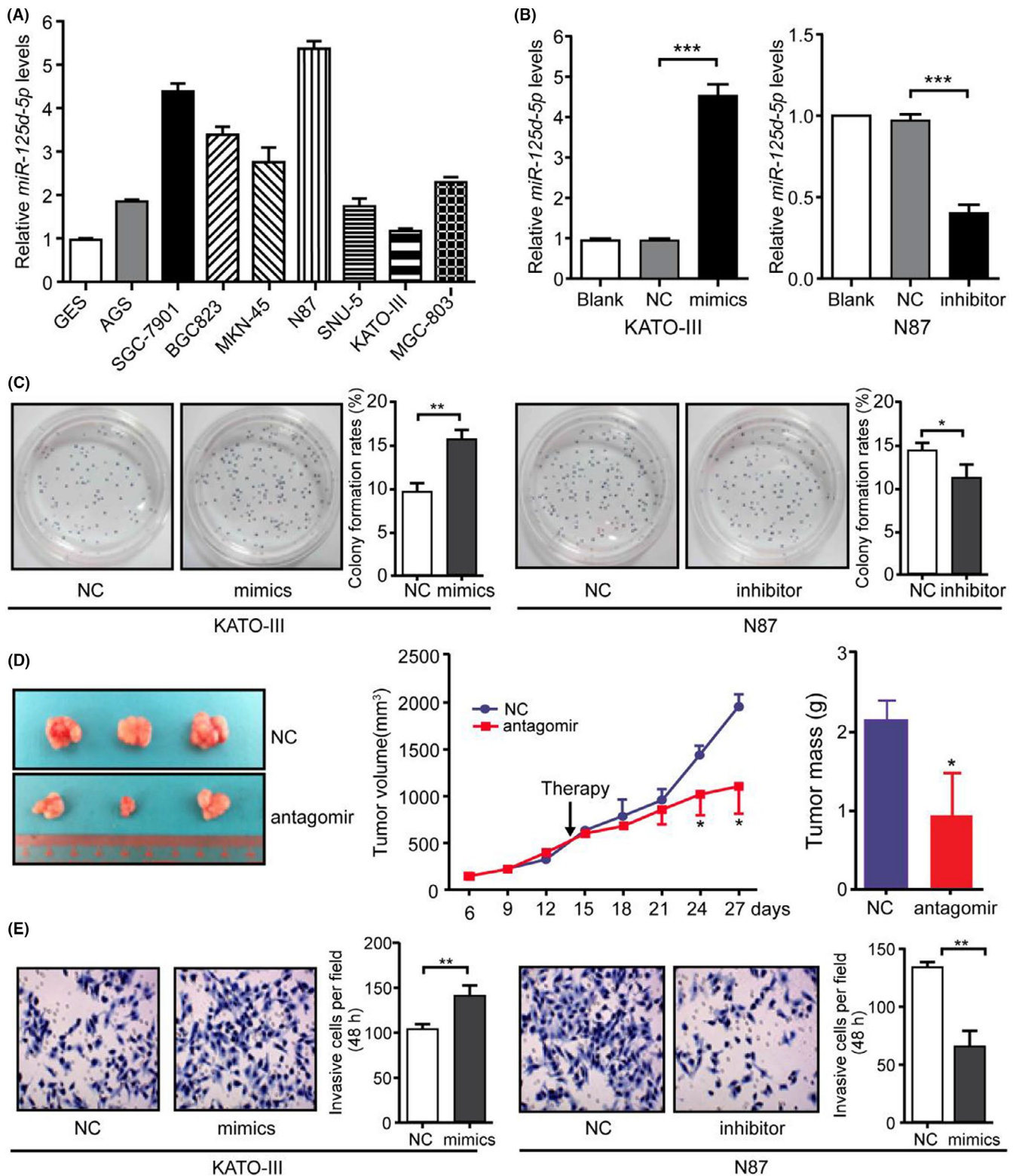


FIGURE 1 *miR-125a-5p* promoted gastric cancer (GC) growth and invasion. (A) *miR-125a-5p* expression in the gastric epithelial cell line GES-1 and the GC cell lines AGS, SGC-7901, BGC-823, MGC-803, MKN-45, N87, SNU-5, and KATO-III was detected using real-time qRT-PCR. (B) KATO-III and N87 cells were transfected with *miR-125a-5p* mimics and inhibitors at a final concentration of 50 nM. *miR-125a-5p* expression was detected using real-time qRT-PCR at 48 h after transfection. (C) Cell viability was evaluated using colony formation assays at the indicated times after transfection. (D) The effects of *miR-125a-5p* antagonist on N87 tumor growth were evaluated using the growth curve and tumor weights. (E) Invasive capacity was evaluated in cells treated with *miR-125a-5p* mimics and inhibitors as described in B. Blank, blank control; NC, negative control; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

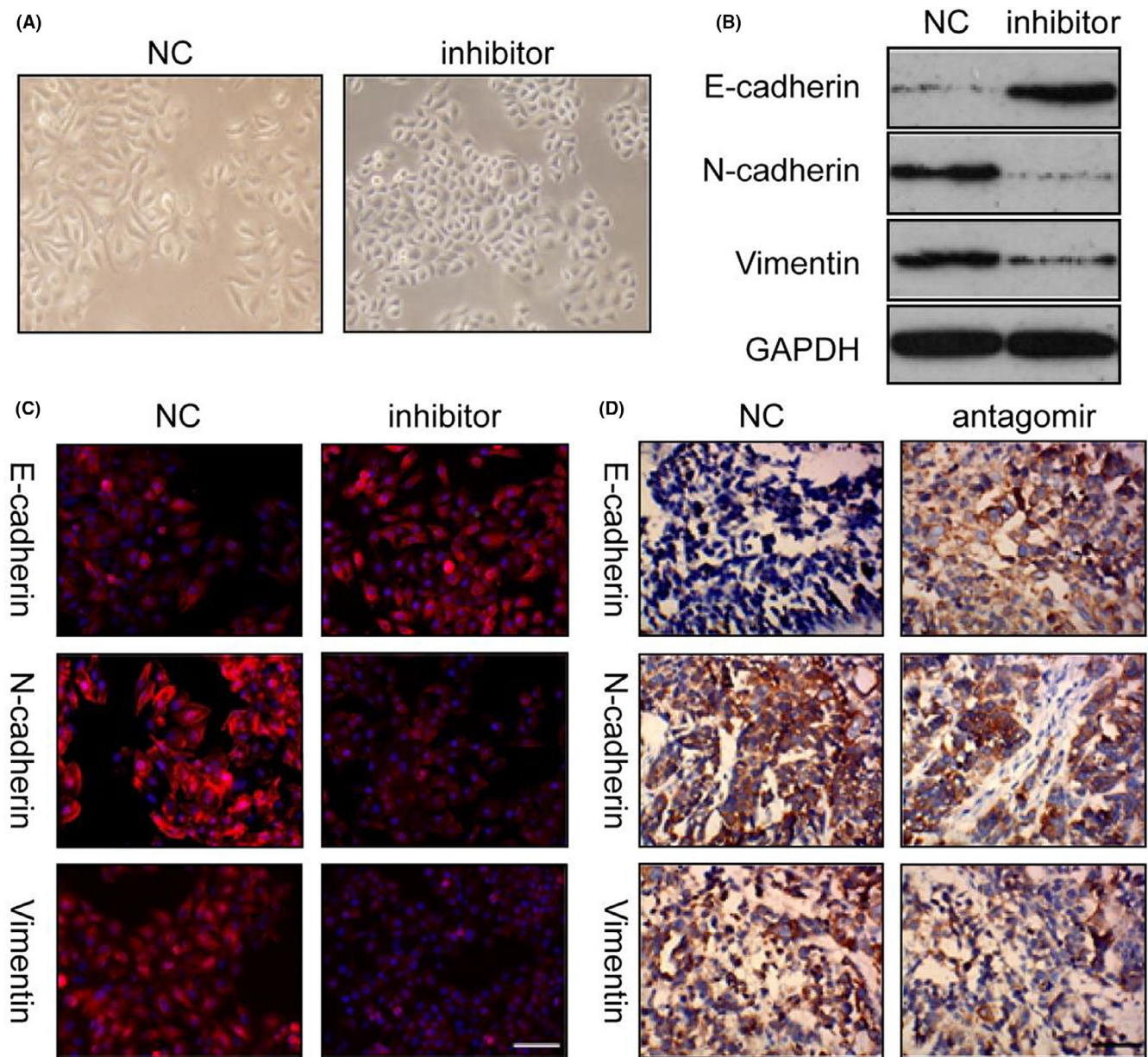


FIGURE 2 *miR-125a-5p* antagonist inhibited the epithelial-mesenchymal transition (EMT). (A) N87 cells treated with *miR-125a-5p* inhibitor lost mesenchymal characteristics (200 \times). (B) Expression of epithelial and mesenchymal markers in N87 cells transfected with *miR-125a-5p* inhibitors. (C) EMT markers were detected using immunofluorescence. (D) E-cadherin, N-cadherin, and vimentin were detected in subcutaneous tumors using immunohistochemistry. NC, negative control. Bar in C, 100 μ m; bar in D, 50 μ m

miR-125a-5p were detected in tumor specimens and corresponding noncancerous gastric tissues. The results showed that the expression levels of *miR-125a-5p* in GC tissues were significantly higher than those in noncancerous gastric tissues (Figure 5A). Next, the expression of *miR-125a-5p* was analyzed in patients with GCs of different grades. *miR-125a-5p* expression was significantly higher in stage III and IV GCs than in stage I and II GCs. No significant differences were detected among noncancerous gastric tissues, stage I GCs, and stage II GCs (Figure 5B).

The expression of *EphA2*, *YES1*, *TAZ*, and *TEAD2* was tested in 65 pairs of gastric adenocarcinoma tissues by qRT-PCR. Pearson's correlation coefficient analysis revealed significant positive correlations

in *miR-125a-5p* expression with *EphA2*, *TAZ*, and *TEAD2* mRNA levels. However, no correlation was detected between *miR-125a-5p* and *YES1* mRNA expression (Figure 5C). These results indicated that *miR-125a-5p* upregulation was correlated with tumor staging and may play a significant role in the progression of advanced GCs.

4 | DISCUSSION

In this study investigating the role of *miR-125a-5p* in the progression of GCs, it was found that *miR-125a-5p* played a substantial role in the growth, invasion, and EMT in GC cells in vitro and in an animal

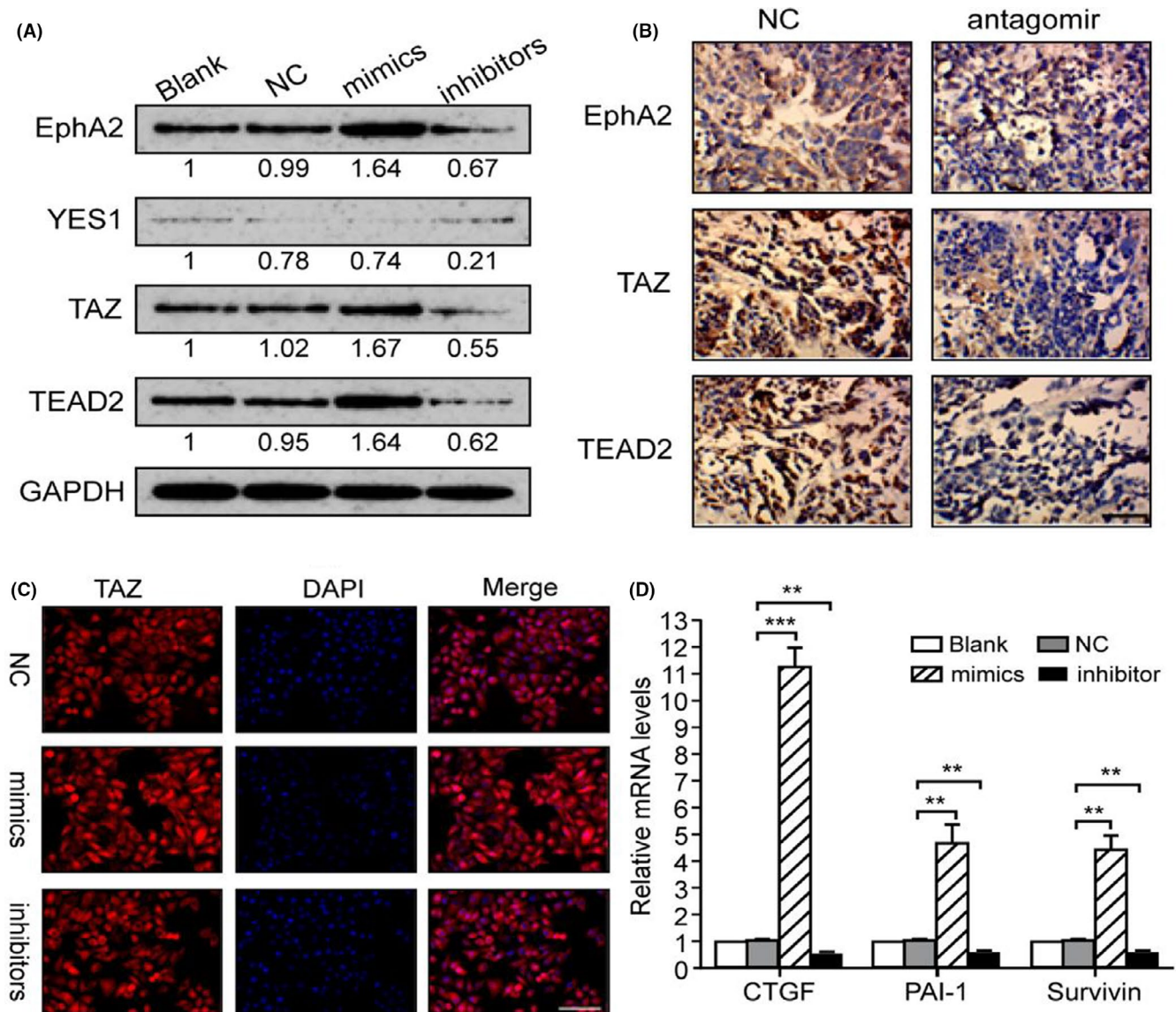


FIGURE 3 Targeting of EphA2 and the Hippo pathway by *miR-125a-5p*. (A) Proteins levels of EphA2, YES1, TAZ, and TEAD2 were detected after transfection with *miR-125a-5p* mimics or inhibitors. (B) EphA2, TAZ, and TEAD2 were detected in subcutaneous tumors using immunohistochemistry. (C) Effects of *miR-125a-5p* on TAZ nuclear translocation were detected with immunofluorescence. (D) Expression levels of TAZ target genes were tested using real-time qRT-PCR. Blank, blank control; NC, negative control; miR, *miR-125a-5p* mimics; WT, wild-type 3'-UTR plasmid; Mut, mutant 3'-UTR plasmid; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Bar in B, 50 μm ; Bar in C, 100 μm

model through the Hippo pathway. Additionally, *miR-125a-5p* expression was significantly associated with clinical stages in patients with GCs. This comprehensive study provided in vitro, in vivo, and clinical evidence supporting the significant role of *miR-125a-5p* in the progression of GCs.

MiR-125a-5p expression was significantly higher in stage III and IV GCs than in stage I and II GCs, but no significant differences were detected in *miR-125a-5p* levels between noncancerous tissues, stage I and II GCs. These data indicated that *miR-125a-5p* played a significant role in the progression of GCs of advanced rather than early stages. It has been found that *miR-125a-5p* expression did not associate with poor prognosis in patients with the mesenchymal subtype GC.¹⁷ However, controversies also existed

regarding the expression and role of *miR-125a-5p* in GCs. Nishida et al.²⁶ reported that *miR-125a-5p* was downregulated in GC tissues and that *miR-125a-5p* potentially suppressed the proliferation of GC cells by directly targeting *ERBB2*. The exact reason for this is unknown, but may be related to intratumor heterogeneity²⁷ and racial variations. However, other reasons may also account for this difference. The use of different GC cell lines may contribute to the difference. In our study, the GC cell lines used were the N87 cells with the highest *miR-125a-5p* expression and the KATO-III cells with the lowest *miR-125a-5p* expression. Other studies used different cell lines including MGC-803 and SGC-7901 in the study by Song et al.,¹⁷ NUGC4 by Nishida et al.,²⁶ OE19 and OE33 by Fassan et al.,²⁸ and AGS and MGC-803 by Xu et al.¹⁶ In one study

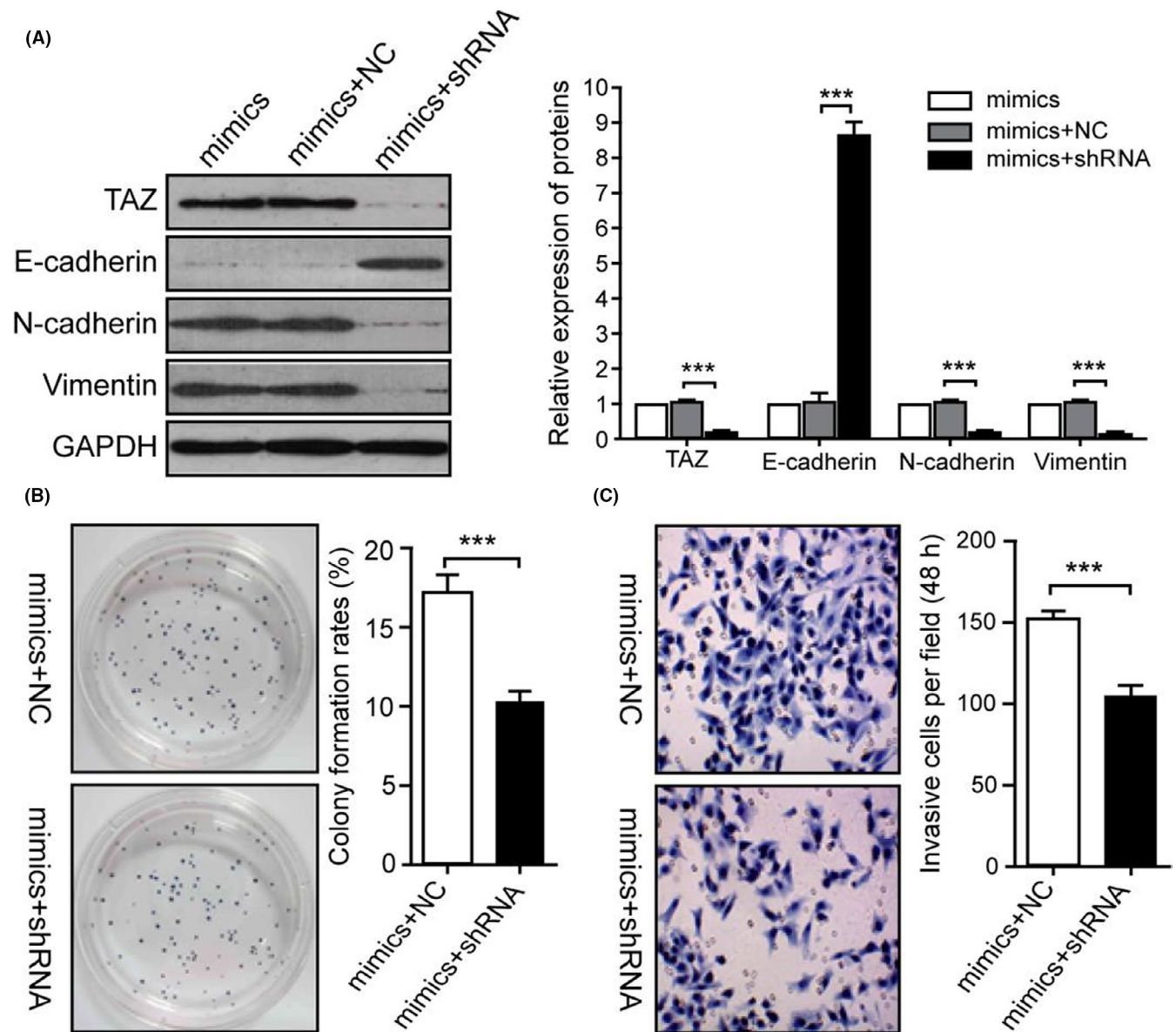


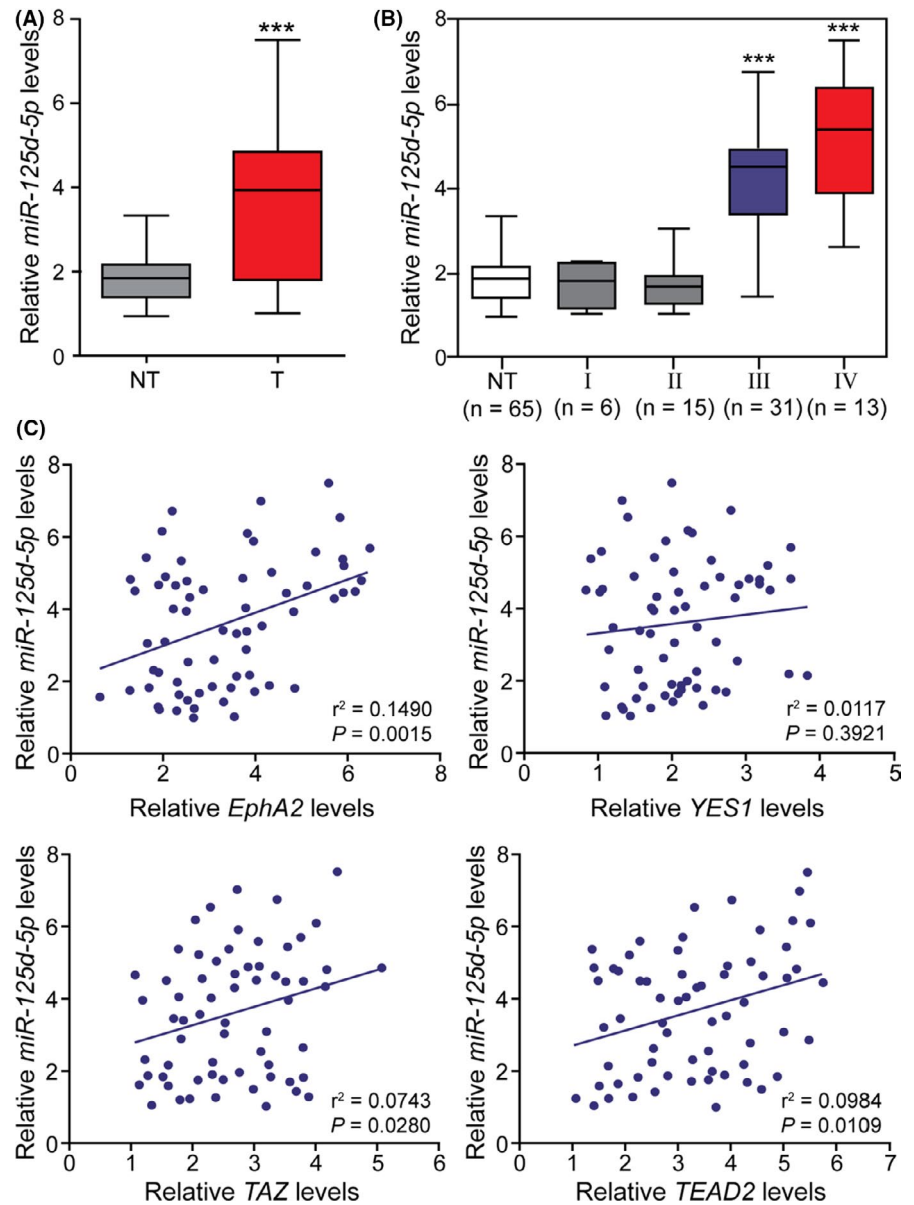
FIGURE 4 Silencing of TAZ inhibited the effects of *miR-125a-5p* in GC cells. (A) Proteins levels of E-cadherin, N-cadherin, and vimentin were detected in KATO-III cells transfected with *miR-125a-5p* mimics in the presence of TAZ shRNA. (B) Cell viability was evaluated using colony formation assays. (C) The invasive capacity of cells was determined using transwell assays. NC, negative control; shRNA, TAZ-shRNA; *** $p < 0.001$

investigating somatic mutations in human cancers,²⁹ the MKN45 GC cell lines which encode a p53 heterozygous mutation did not have either *miR-125a-5p* overexpression or Dies1 promoter methylation in regulating GC pathogenesis. However, in other cell lines of MKN28, MKN45, SNU1, GP202, and AGS, the Dies1 expression existed to adjust the GC pathogenesis.³⁰ Moreover, combinatory mechanisms may exist to play a role in the initiation and development of GCs. For example, p53 mutations were present in the MKN 28 GC cell lines and might play a combinatory effect with Dies1 in regulating GC pathogenesis.^{29,30} The initiation, development, aggravation, or regression of GCs are caused by the total effect of all factors. In our study, the Hippo pathway and its regulator *miR-125a-5p* were investigated, whereas other studies explored varied

pathways like the HER2-*miR125a-5p*/*miR125b* loop in the study by Fassan et al.,²⁸ the *miR-125a-5p* expression and the targeting E2F3 by Xu et al.,¹⁶ and *miR125a-5p* and GC prognosis by Nishida et al.²⁶ Every study focused only on one point or one series of issues related to the pathogenesis of GCs. Different pathways and targeting points exist to play combining effects in the same GC cells and may result in different overall outcomes in the initiation, development, aggravation, regression, or relief.

miR-125a-5p is a tumor-suppressive miRNA in breast cancer,¹¹ hepatocellular carcinoma¹² lung cancer,¹³ and glioblastoma.¹⁴ However, the study by Song et al. did not find the *miR-125a-5p* expression to be significantly associated with poor prognosis in patients with the mesenchymal subtype GC,¹⁷ and our results indicated that *miR-125a-5p*

FIGURE 5 Clinical correlations between *miR-125a-5p* and its targets in gastric cancers (GCs). (A) The expression of *miR-125a-5p* was detected in 65 GC tissues and noncancerous gastric tissues. (B) Expression levels of *miR-125a-5p* in GCs of different stages. (C) Correlations between *miR-125a-5p* levels and the expression levels of putative targets in GCs were demonstrated. NC, noncancerous gastric tissues; *** $p < 0.001$ compared with NC, stage I GCs, and stage II GCs



may serve as an oncogenic miRNA in GCs. These findings strongly suggest that the function of *miR-125a-5p* as a tumor suppressor or oncogenic promoter is tissue specific, consistent with findings of *miR-146* and *miR-29*, which have opposing roles in tumorigenesis depending on the cellular context.³¹ Interestingly, the same miRNA may have opposing roles in the same type of cancer. For example, *miR-125a-5p* expression is lower in non-small cell lung cancer tissues than in adjacent normal lung tissues, and an inverse relationship between *miR-125a-5p* expression and pathological stage or lymph node metastasis is observed. In contrast, *miR-125a-5p* has been shown to enhance lung cancer cell migration and invasion.³² Therefore, more refined analyses of *miR-125a-5p* function in GCs are needed.

One unique characteristic of the relationship between miRNAs and their targets is that a single miRNA sequence may be able to target numerous genes that are simultaneously involved in the same functional event.³³ Our experimental findings showed that *miR-125a-5p* targeted *EphA2*, *TAZ*, and *TEAD2* mRNAs at the same time.

Although dual-luciferase reporter assays indicated that the 3'-UTR of *YES1* was a target of *miR-125a-5p*, cellular and clinical validation studies did not support this result. Even though *miR-125a-5p* mimics reduced the fluorescent signal, the mRNA and protein levels of *EphA2*, *TAZ*, and *TEAD2* were elevated in the presence of *miR-125a-5p* mimics. This result demonstrated that *miR-125a-5p* activated rather than inhibited the translation of these targets.

miRNAs primarily recognize complementary sequences in the 3'-UTRs of their target mRNAs, resulting in inhibition of the expression of target genes by translational repression or mRNA degradation.³⁴ However, recent studies have reported that miRNAs can also bind to the 5'-UTR of promoter and upregulate the translation of target genes.³⁵ Moreover, complementarity of seven or more bases with the 5' end of miRNA is sufficient to confer regulation, even if the target 3'-UTR contains only a single site.³⁶ Based on bioinformatics analysis from GenBank, in addition to the 3'-UTR, sequences that are complementary with the *miR-125a-5p* sequence were also found

in the promoter regions of *EphA2*, *TAZ*, and *TEAD2*. Although the effects of *miR-125a-5p* were not investigated on the promotion of luciferase reporter activity, it was speculated that *miR-125a-5p* might directly target the promoters of *EphA2*, *TAZ*, and *TEAD2*, thereby activating gene expression.

Epithelial-mesenchymal transition is a biological process that allows polarized epithelial cancer cells to undergo multiple biochemical changes, enabling the cells to assume a mesenchymal cell phenotype.³⁷ Various miRNAs can regulate EMT through distinct mechanisms.³⁸ GCs exhibiting *miR-125a-5p* overexpression also show activation of EMT-related pathways.¹⁷ Thus, *miR-125a-5p* plays a significant role in EMT. Our findings showed that inhibition of *miR-125a-5p* suppressed EMT by upregulating E-cadherin but downregulating N-cadherin and vimentin. Induction of *TAZ* expression has been shown to trigger EMT by stimulating E-cadherin transcriptional inhibitors Snail and Slug.^{24,39} Moreover, as a critical component in the Hippo pathway, *TAZ* is a target of *miR-125a-5p*. Our study showed that silencing of *TAZ* blocked the function of *miR-125a-5p* in promoting GC growth, invasion, and EMT. Collectively, these data supported the idea that *miR-125a-5p* may promote cell growth, invasion, and EMT in GCs by activation of the Hippo pathway through *TAZ*.

Recently, studies have also explored the role of miRNAs in GCs including *miR-148a-3p*⁴⁰ and *miR-212*.⁴¹ Bao et al. investigated the diagnostic potential and biological function of *miR-148a-3p* in GC progression in the GC tissues and plasma of patients and found that *miR-148a-3p* was significantly downregulated in both the gastric tissue and plasma of GC patients, suggesting that *miR-148a-3p* may inhibit cancer progression and is a novel diagnostic biomarker for GC.⁴⁰ Shao et al.⁴¹ analyzed the relationship of serum level of *miR-212* with GC through detecting the serum level of *miR-212* in 100 healthy people and 110 GC patients, and these authors found that *miR-212* was epigenetically downregulated in GC and could directly regulate the 3'UTR of *SOX4* mRNA to suppress p53 and Bax, suggesting that low level of *miR-212* can be a potential circulation biomarker and poor prognosis predictor of GC. Novel findings in our study and in other studies^{40,41} will ultimately accumulate to clarify the mechanism of GC and clear the way for better treatment of this cancer.

In conclusion, *miR-125a-5p* can promote GC growth and invasion by upregulating the expression of *EphA2*, *TAZ*, and *TEAD2*. *miR-125a-5p* may thus serve as a potential therapeutic target in the treatment of advanced GCs.

CONFLICT OF INTEREST

None.

AUTHORS' CONTRIBUTIONS

Study design: Ruixin Li, Guojun Wang. Data collection: Ruixin Li, Zhihao Hu, Zhuoyin Wang, Tianyu Zhu, Bulang Gao, Jingtao Wang, Xiumei Deng. Data analysis: Ruixin Li, Zhihao Hu. Supervision: Ruixin Li. Writing of the original version: Ruixin Li. Revision: Bulang Gao.

CODE AVAILABILITY

None.

CONSENT TO PARTICIPATE

All subjects agreed to participate in the study.

CONSENT FOR PUBLICATION

All subjects agreed to the publication of the data in the study.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author on reasonable account.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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