lncRNA NEAT1 Inhibits Proliferation, Invasion and Epithelial-mesenchymal Transition of Gastric Cancer Cells by Regulating MiR-129-5p/PBX3 Axis

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Research article

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Abstract

Purpose: lncRNA NEAT1 has been reported as a tumor-promoting gene in a variety of tumors, but few studies have explored its role and mechanism in gastric cancer. In the face of increasing incidence of gastric cancer, how to improve the diagnostic accuracy and therapeutic effect of gastric cancer is a major clinical problem. Therefore, we studied the effect and mechanism of lncRNA NEAT1 on the proliferation, invasion and epithelial-mesenchymal transition of gastric cancer cells. To inquiry into the effect of lncRNA NEAT1 on the proliferation, invasion and epithelial-mesenchymal transition (EMT) of gastric cancer (GC) cells by regulating miR-129-5p/PBX3 axis.

Methods: Totally 63 GC diagnosed and treated in our hospital were selected as the study subjects, whose paired GC tissues and pericarcinomatous tissues were collected as the study specimens after obtaining their consent. QRT-PCR was employed to detect the NEAT1 expression in tissues and cells to analyze the relationship between NEAT1 and clinicopathological data of GC patients. In addition, stable and transient overexpression and inhibition vectors were established and transfected into GC cells HCG-27 and MKN-45. CCK-8, traswell, and flow cytometry were employed to evaluate the proliferation, invasion, and apoptosis of transfected cells. The correlation of miR-129-5p between PBX3 and NEAT1 was assessed using dual luciferase reporter assay, while that between NEAT1 and miR-129-5p was assessed by RNA-binding protein immunoprecipitation (RIP) . Western blot was applied for the detection of apoptosis and EMT related proteins.

Results: NEAT1 was overexpressed in GC patients and had a high diagnostic value. The expression of NEAT1 was related to the pathological stage, differentiation degree, tumor size and lymph node metastasis of patients with GC. Down-regulated NEAT1 brought decreased cell proliferation, invasion and EMT, and increased apoptosis. According to dual luciferase reporter assay, NEAT1 could target miR-129-5p, while in turn miR-129-5p could target PBX3. Functional analysis exhibited that miR-129-5p overexpression inhibited PBX3 in GC cells, affecting cell proliferation, invasion, EMT and apoptosis, and rescue experiments demonstrated that these effects were eliminated by up-regulating NEAT1 expression.

Conclusion: Inhibition of NEAT1 could mediate miR-129-5p/PBX3 axis to promote apoptosis of GC cells, and reduce cell proliferation, invasion and EMT.

Introduction

Recent years have witnessed the ongoing prevalence of malignant tumors driven by the change of social environment and people's living habits. Among them, GC, as a high-incidence malignant tumor of the digestive system, seriously hazards people's life and health due to its high incidence and mortality [1, 2]. Despite the fact that the development of medical technology has brought great benefit to the treatment of GC, its prognosis remains poor owning to its high infiltration and metastasis [3, 4]. Thus, it is particularly important to conduct in-depth research on molecular mechanism of the genesis and development of GC, which is also a research hotspot in this disease in recent years.

Previous studies showed that non-coding RNA plays a vital part in manipulating oncogenes and tumor suppressor genes in the pathogenesis of cancer [5]. As a kind of RNA with a length of over 200 nucleotides, Long non-coding RNA (LncRNA) has been well established to exert marked effects on epigenetics, transcription and other aspects [6]. And so is that with the case of GC. For example, studies [7] demonstrated that, through regulating the PIK/Akt1/mTOR pathway, down-regulating LINC01419 could inhibit the progression and metastasis of GC cells. Moreover, LINC00565 was able to inhibit the proliferation of GC cells by targeting the miR-665/Akt3 axis [8]. LncRNA NEAT1 is transcribed from a tumor syndrome multiple endocrine tumor type 1 gene located on chromosome 11q13.1, which is mainly enriched in the nucleus [9, 10]. It is well established that NEAT1 is overexpressed in a variety of tumors. For instance, studies [11] found that NEAT1 could competitively bind miR-98-5p to promote oncogene HMGA2 in prostate cancer, thereby promoting prostate cancer cells' progression and metastasis. The specific mechanism of NEAT1 in GC, however, waits for exploring. Here bioinformatics analysis had discovered the presence of binding site between miR-129-5p and NEAT1. As to the former, it is a short-chain non-coding RNA which is found to be lowly expressed in GC [12]. Whereas, the concrete relationship between the two in GC remains poorly understood.

Therefore here, we probed into the link between NEAT1 and miR-129-5p, in the hope of providing references for the treatment and diagnosis of GC.

Information And Methods

Clinical data

Sixty-three patients with GC admitted to Cangzhou Central Hospital were enrolled as the study subjects. Paired GC tissues and adjacent tissues were collected as the study specimens with the consent of the patients. Inclusion criteria: Patients diagnosed with GC by pathology, with a life expectancy of more than 3 months were included and assigned into the research group (RG). Exclusion criteria: Patients received any treatment prior to the trial, with severe liver or kidney dysfunction, other malignant tumors, infection or immune system disorders were excluded. All study subjects and their families gave their consents to take part in the experiment and signed the informed consent form. This experiment was conducted under the approval of Cangzhou Central Hospital's Medical Ethics Committee.

Culture, passage and transfection of cells

GC cell lines (BGC-823, HCG-27, MKN-45, MGC-803) and human normal gastric mucosal cell line GES were obtained from the cell bank of ATCC. The GC cell line was placed in a medium containing 10% PBS DMEM, and cultured at 37 °C, 5% CO_2. When it was observed that the cell adherent growth fusion reached 85%, 25% trypsin was added for complete digestion. After that, the medium was further cultured until passage finished. Then, miR-129-5p-inhibitor (inhibition sequence), miR-129-5p-mimics (overexpression sequence), miR negative control (miR-NC), targeted inhibition of NEAT1 (si-NEAT1), and targeted inhibition of PBX3 (si-PBX3), as well as negative control RNA (Si-NC) were respectively transfected into cells using Lipofectamine™ 2000 kit in strict accordance with the kit instructions.

QRT-PCR detection

TRIzol kit (Invitrogen) was adopted to extract the total RNA from tissues and cells, whose concentration and purity at 260–280 nm were then detected by uv spectrophotometer, and only those with OD260/OD280 > 1.8 were selected for follow-up experiments. Next, 5 μg total RNA from tissues and cells was taken to carry out reverse transcription cDNA operation with a corresponding kit (TransGen Biotech, Beijing, China). After reverse transcription, 1 μL synthesized cDNA was amplified. PCR reaction conditions were as follows: Pre-denaturation: 94 °C/30 s, denaturation: 94 °C/ 5 s, annealing: 60 °C/30 s, totaling 40 cycles. Three replicate wells were set for each sample and the experiment was performed a total of three times. Finally, U6 was set as the internal reference for miR-129-5p, GAPDH for that of NEAT1 and PBX3, $2^{-\triangle\triangle ct}$ was applied for data analysis. Shanghai Sangon Biotechnology Co. Ltd. was responsible for the design and synthesis of all the primers. (Table 1)

Table 1
Primer sequences

Factors	Upstream primer	Downstream primer
miR-129-5p	5'-GCGGCTTTTTGCGGTCTGG-3'	5'-GTGCAGGGTCCGAGGT-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'
NEAT1	5'-TGGCTAGCTCAGGGCTTCAG-3'	5'-TCTCCTTGCCAAGCTTCCTTC-3'
PBX3	5'-GAGCTGGCCAAGAAATGCAG-3'	5'-GGGCGAATTGGTCTGGTTG-3'
GAPDH	5'-GGACCTGACCTGCCGTCTAG-3'	5'-GTAGCCCAGGATGCCCTTGA-3'

WB detection for protein expression

PBX3, Bax, Bcl-2, Caspese-3, N-cadherin, E-cadherin, vimentin, and β-Actin Antibodies were all acquired from Cell Signaling Technology Inc.

Cell protein extract (1 ml, cell lysate: protease inhibitor: phosphatase inhibitor = 98:1:1, v/v/v) was added to the culture plate with cell lines, repeatedly pipetted until the cell was completely lysed, and centrifugated at $1.2*10^4$ r/min for 15 min to collected the supernatant. Then the protein was isolated by SDS-PAGE electrophoresis, transferred to NC membrane, and then placed at room temperature for 1 h (sealed with 5% skim milk-PBS solution). Next, PBX3 (1:500), Bcl-2 (1:500), Caspese-3 (1:500), Bax (1:500), vimentin (1:500) and β-Actin (1:1000) primary antibody, N-cadherin (1:500) and E-Cadherin (1:500)) were added and sealed at 4℃ for a night. After that, the NC membrane was rinsed with PBS solution for three times, added with horseradish peroxidase-labeled goat anti-rabbit secondary antibody (1:1000), then left to stand at room temperature for 1 h. Finally, the NC membrane was rinsed with PBS solution and visualized by enhanced chemiluminescence method. With β-Actin as the internal reference protein, the relative expression level of the protein to be measured was equal to the gray value of the band to be measured/the gray value of the β-actin band.

MTT assay for cell viability

The transfected cells were collected and hydrolyzed with trypsin. Fresh medium was then added and pipetted to prepare the cell suspension. And then the cells were inoculated in the 96-well plate according to the specification of $5*10^3$/100μL per well. After incubation for 24, 48 and 72 hours, 5 μg/mL MTT solution was added at 20 μL/well and cultured at 37℃ for 4 h before the addition of 200 μL methyl sulfoxide to each well. Finally, a microplate reader was adopted to measure the OD at 570 nm. The test was carried out three times to draw the cell viability-time curve.

Transwell detection

The cells were harvested 24 hours after transfection and transfected with trypsin to prepare the cell suspension. Then the cell density was adjusted to $2*10^4$ cells/well, planted in 24-well plates, trypsinized and transferred to the upper chamber, where 200μL fetal bovine serum and 1% DMEM medium were added, while the lower chamber was filled with 500 mL DMEM medium (containing 10% FBS), and cultured at 37℃ for 48 h. The matrix and cells of the upper chamber that failed to pass through the membrane surface were wiped, triply rinsed with PBS, and fastened with polyformaldehyde for 10 min. Followed by a triple rinse with double distilled water and dyed with crystal violet (0.5%) after drying. At last, the cell invasion was observed under a microscope.

Flow cytometry for apoptosis

The sample to be tested was firstly prepared as a cell suspension of $1*10^6$ cells/mL, and then immobilized in 70% ethanol ice-cold solution for 30 minutes at 4 °C. Thereafter, the ethanol solution was removed and the cell particles were incubated in Annexin V-FITC/7-AAD mixed solution. FACScan flow cytometry (Becton Dickinson Company, USA) was applied to analyzed the apoptosis, and the targeting link between NEAT1 and miR-129-5p, miR-129-5p and PBX3 was determined using dual luciferase reporter gene assay.

PmirGLO-NEAT1-wt, pmirGLO-PBX3-wt, pmirGLO-NEAT1-mut, and pmirGLO-PBX3-mut vectors were established and co-transfected into cells with miR-129-5p-mimcs and NC, respectively. Forty-eight hours after transfection, their luciferase activity was determined by double luciferase reporter gene assay (Promega) strictly followed the instructions.

RIP experiment

Firstly, the GC cells were rinsed and crosslinked with formaldehyde (0.01%) for 15 min. Then centrifuged and lysed, and the extracted cells grew with RIP buffer containing protein A/G magnetic beads coated with anti-Ago2 or negative control anti-IgG antibodies. After culturing at 4 °C for a night, the cells and protein G-agarose were grew at 4 °C for 3 h before the RNA was isolated and analyzed with Western blot. The experiment was repeated three times.

Statistical methods

In this study, the collected data was analysed using SPSS20.0, and the picture rendering was performed by GraphPad 7. An independent t-test was employed for inter-group comparison, and one-way ANOVA was adopted for inter-group comparison (expressed as F). Post pairwise comparison was conducted by LSD-t, and repeated measurement ANOVA was applied for multi-time expression (expressed as F). Bonferroni was utilized for post-hoc test, and the correlation of miR-129-5p between NEAT1 and PBX3 was performed by Pearson test. A statistically significant difference was assumed at $P < 0.05$.

Results

Expression and clinical significance of NEAT1 in GC

QRT-PCR indicated that, compared to pericarcinomatous normal tissues, NEAT1 was markedly up-regulated while miR-129-5p was notably dropped in GC tissues. The receiver operating characteristic (ROC) analysis exhibited that the area under the curve (AUC) of NEAT1 was 0.895, and patients were distributed into high and low expression groups on the basis of its average value. The results suggested that the expression of NEAT1 was related to pathological stage, differentiation degree, lymph node metastasis and tumor size of GC patients. While compared with GES, NEAT1 was notably elevated and miR-129-5p was markedly reduced in GC cells. Correlation analysis displayed that NEAT1 and miR-129-5p were negatively correlated in GC tissues. Moreover, NEAT1 and miR-129-5p were observed to present the most obvious changes in HCG-27 and MKN-45 cells, therefore, these two were selected as the experimental objects in this study. (Table 2, Fig. 1)

Table 2
Relationship between NEAT1 and pathological data of patients with GC

Factors		NEAT1 expression		X^2	P value
		Low expression (n = 32)	High expression (n = 31)		
Gender				0.034	0.855
	Male (n = 41)	22 (68.75)	19 (61.29)		
	Female (n = 22)	10 (31.25)	12 (38.71)		
Age				0.011	0.916
	≥ 65 (n = 37)	19 (59.38)	18 (58.06)		
	< 65 (n = 26)	13 (40.63)	13 (41.94)		
Tumor size				12.49	< 0.001
	≥ 5 cm (n = 39)	13 (40.62)	26 (83.87)		
	< 5 cm (n = 24)	19 (59.38)	5 (16.13)		
TNM staging				10.93	< 0.001
	I + II (n = 23)	18 (56.25)	5 (16.13)		
	III + IV (n = 40)	14 (43.75)	26 (83.87)		
Lymph node metastasis				10.05	< 0.001
	Transferred (n = 34)	11 (34.38)	23 (71.19)		
	Untransferred (n = 29)	21 (65.63)	8 (25.81)		
Differentiation degree				5.763	0.016
	Low + moderate differentiation	23 (71.88)	13 (41.94)		
	High differentiation	9 (28.12)	18 (58.06)		

Effects of NEAT1 on the biological function of GC cells

We hypothesized that the abnormal expression of NEAT1 might be related to GC to some extent. Thus here, we silenced NEAT1 by siRNA to explore its effect on the biological function of GC cell lines. It was observed that after silencing NEAT1 in GC cells, the NEAT1 expression was remarkably decreased in the Si-NEAT1 group in contrast with the Si-NC group, and the proliferation and invasion of HCG-27 and MKN-45 cells were notably suppressed, the apoptosis rate was greatly elevated, the anti-apoptotic proteins N-

cadherin, Bcl-2 and vimentin were remarkably declined, and the expressions of proapoptotic proteins Bax, Caspase-3 and E-Cadherin were significantly raised. (Fig. 2)

Effects of miR-129-5p on the biological function of GC cells

We transfected miR-129-5p mimcs and miR-129-5p-inhibitor into GC cell lines to observe the effect of miR-129-5p on cell biological function, so as to explore the role of miR-129-5p in GC. The results displayed that compared to the miR-NC group, the miR-129-5p mimcs group had markedly enhanced miR-129-5p expression, obviously suppressed cell invasion and proliferation, significantly increased apoptosis rate, markedly decreased anti-apoptotic proteins N-cadherin, Bcl-2 and vimentin, and remarkably increased expressions of pro-apoptotic proteins bax, Caspase-3 and E-cadherin. While the results of the miR-129-5p-inhibitor group were contrary to those of the miR-129-5p-mimcs group. (Fig. 3)

Competitive binding between NEAT1 and miR-129-5P

The targeted binding site between NEAT1 and miR-129-5p was found by online software starBase 3.0 analysis. Dual luciferase reporter assay further exhibited that the luciferase activity of miR-129-5p in NEAT1 was remarkably reduced compared to the miR-NC group. Moreover, RIP experiments presented an increase in miR-190b-WT and AFAP1-AS1 enrichment compared to miR-NC and miR-190b-MUT. (Fig. 4)

MiR-129-5p directly targeted PBX3 to regulate cell biological function

We predicted the target genes of miR-129-5p through Targetscan7.2, finding that it had targeted binding sites with PBX3. The luciferase activity of miR-129-5p-mimics in pmirGLO-PBX3-3'UTR-WT was remarkably inhibited as evaluated by dual luciferase reporter assay. To further explore the effect of PBX3 on cells, we silenced the expression of PBX3 in GC cells, and found that the proliferation and invasion of Si-PBX3 cells were greatly suppressed, the apoptosis rate was markedly increased, the anti-apoptotic proteins Bcl-2 and N-cadherin, vimentin protein were notably reduced, and the proapoptotic related proteins bax, Caspase-3 and E-Cadherin were remarkably elevated compared to the Si-NC group. (Fig. 5)

Rescue experiment

Compared to the Si-NC group, no significant difference was found in proliferation, invasion and apoptosis rate of GC cells, as well as apoptosis-related proteins and EMT-related proteins after co-transfection of Si-NEAT1 and miR-129-5p-inhibitor ($P > 0.05$). When in contrast with the Si-NEAT1 group, the proliferation, invasion and EMT of GC cells were markedly enhanced after co-transfection, the apoptosis rate was notably reduced, the anti-apoptotic proteins Bcl-2, N-cadherin and vimentin were remarkably elevated, and the proapoptotic related proteins bax, Caspase-3 and E-Cadherin was markedly decreased. (Fig. 6)

Discussion

As one of the most common cancers, GC seriously hazards human life and health due to its high morbidity and mortality [13]. Its inconspicuous early symptoms and the lack of effective means for early screening lead to the fact that many patients have developed advanced GC once diagnosed [14]. Worse still, the mainstream treatment today remains surgery combined with chemoradiotherapy, whose efficacy is limited to some extent, resulting in poor prognosis of many patients [15, 16]. Along with the in-depth development of molecular biology, more and more attention has been paid to the role of molecular markers in the diagnosis and treatment of GC, in that finding a stable and highly sensitive molecular marker is of great clinical significance for patients with GC.

LncRNA, as a kind of long non-coding RNA, has enjoyed more and more attention for its role in tumor development [17]. Among them, NEAT1 is a classic LncRNA that has also been reported to act on a variety of tumors. For example, studies [18] supported that NEAT1 could influence lung cancer cells' proliferation and apoptosis by modulating miR-1223/KLF3 axis. Other studies [19] revealed that NEAT1 could promote autophagy of HCC cells by regulating miR-204/ATG3. In our study, NEAT1 was observed to be overexpressed in both GC tissues and cells, and the inhibited NEAT1 expression in GC cells brought about greatly inhibited proliferation, invasion and EMT, and remarkably dropped apoptosis rate of GC cells, indicating that NEAT1 might act as an oncogene in GC. Studies in the past [20] demonstrated that NEAT1 was up-regulated in GC, and the overexpressed NEAT1 could synergize with EZH2 to promote the invasion and metastasis of GC cells, which validated our results and explains the possible mechanism of NEAT1 in GC from another aspect.

It is well known that LncRNA can act as a ceRNA to competitively bind miRNAs and thus play a sponge role, and the sponge effect of LncRNA in GC has also been widely concerned [21, 22]. For example, some studies [23] indicated that lncRNA GAS5 could act as a molecular sponge to bind to miR-23a, thereby enhancing the inhibitory effect of MT2A on the proliferation of GC cells. Other studies [24] demonstrated that LINC01303 could promote the metastasis of GC cells through sponge action on miR-101-3p. In present study, we found that there were binding sites between NEAT1 and miR-129-5p through online tool analysis. Then we further verified it by dual luciferase reporter assay, and observed the enrichment phenomenon between the two through RIP experiment, which further validated the ceRNA link between them. Subsequently, we detected the underexpression of miR-129-5p in GC tissues and cells, and the up-regulated miR-129-5p brought about enormously inhibited proliferation, invasion and EMT of GC cells, markedly elevated apoptosis rate, and conversely, the overexpression of miR-129-5p resulted in opposite results. Additionally, the rescue experiment revealed that proliferation, invasion and apoptosis were offset after co-transfection with Si-NEAT1 + miR-129-5p-inhibitor, and there was no difference compared to the si-NC group. As was reported [25], miR-129-5p could hinder the proliferation and migration of GC cells, which was in line with our results. What's more, dual luciferase reporter validated the existence of binding sites between miR-129-5p and PBX3. PBX3 is a well-known regulatory and highly conserved homologous domain[26, 27], which has been shown to function as an oncogene in a variety of tumors like colorectal cancer [28] and prostate cancer [29]. After the inhibition of PBX3 expression in GC cells in our study, the proliferation, invasion and EMT of GC cells were greatly hindered, and meanwhile the apoptosis rate was

markedly ascended. Previous studies [30] clearly indicated that PBX3 could promote EMT in GC cells by enhancing the phosphorylation of AKT, which accorded with our conclusion.

In short, by regulating miR-129-5p/PBX3 axis, NEAT1 is able to promote the proliferation, invasion and EMT of GC cells and inhibit apoptosis, which may be a new target direction in the diagnosing and treating of GC. However, there are some deficiencies in this study. For example, we have not conduct tumor formation experiment in nude mice to validate the effect of NEAT1 on in vivo tumor growth, nor have we explored the downstream mechanism of PBX3, which are waiting for us to carry out more in-depth basic experiments.

Abbreviations

epithelial-mesenchymal transition (EMT)

gastric cancer (GC)

protein immunoprecipitation (RIP)

Long non-coding RNA (LncRNA)

research group (RG)

miR negative control (miR-NC)

receiver operating characteristic (ROC)

area under the curve (AUC)

Declarations

- Ethics approval and consent to participate

Not applicable

- Consent for publication

Not applicable

- Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article.

- Competing interests

The authors declare that they have no competing interests.

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- Authors' contributions

Hanshu Ji conducted the experiments; Xiaoyu Zhang designed the experiments and wrote the paper.

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NEAT1	5'-TGGCTAGCTCAGGGCTTCAG-3′	5'-TCTCCTTGCCAAGCTTCCTTC-3'
PBX3	5'-GAGCTGGCCAAGAAATGCAG-3'	5'-GGGCGAATTGGTCTGGTTG-3'
GAPDH	5'-GGACCTGACCTGCCGTCTAG-3'	5'-GTAGCCCAGGATGCCCTTGA-3'

Table 2. Relationship between NEAT1 and pathological data of patients with GC

Factors		NEAT1 expression		X^2	P value
		Low expression (n=32)	High expression (n=31)		
Gender				0.034	0.855
	Male (n=41)	22 (68.75)	19 (61.29)		
	Female (n=22)	10 (31.25)	12 (38.71)		
Age				0.011	0.916
	≥65 (n=37)	19 (59.38)	18 (58.06)		
	<65 (n=26)	13 (40.63)	13 (41.94)		
Tumor size				12.49	<0.001
	≥5cm (n=39)	13 (40.62)	26 (83.87)		
	<5cm (n=24)	19 (59.38)	5 (16.13)		
TNM staging				10.93	<0.001
	I+II (n=23)	18 (56.25)	5 (16.13)		
	III+IV (n=40)	14 (43.75)	26 (83.87)		
Lymph node metastasis				10.05	<0.001
	Transferred (n=34)	11 (34.38)	23 (71.19)		
	Untransferred (n=29)	21 (65.63)	8 (25.81)		
Differentiation degree				5.763	0.016
	Low+moderate differentiation	23 (71.88)	13 (41.94)		
	High differentiation	9 (28.12)	18 (58.06)		

Figures

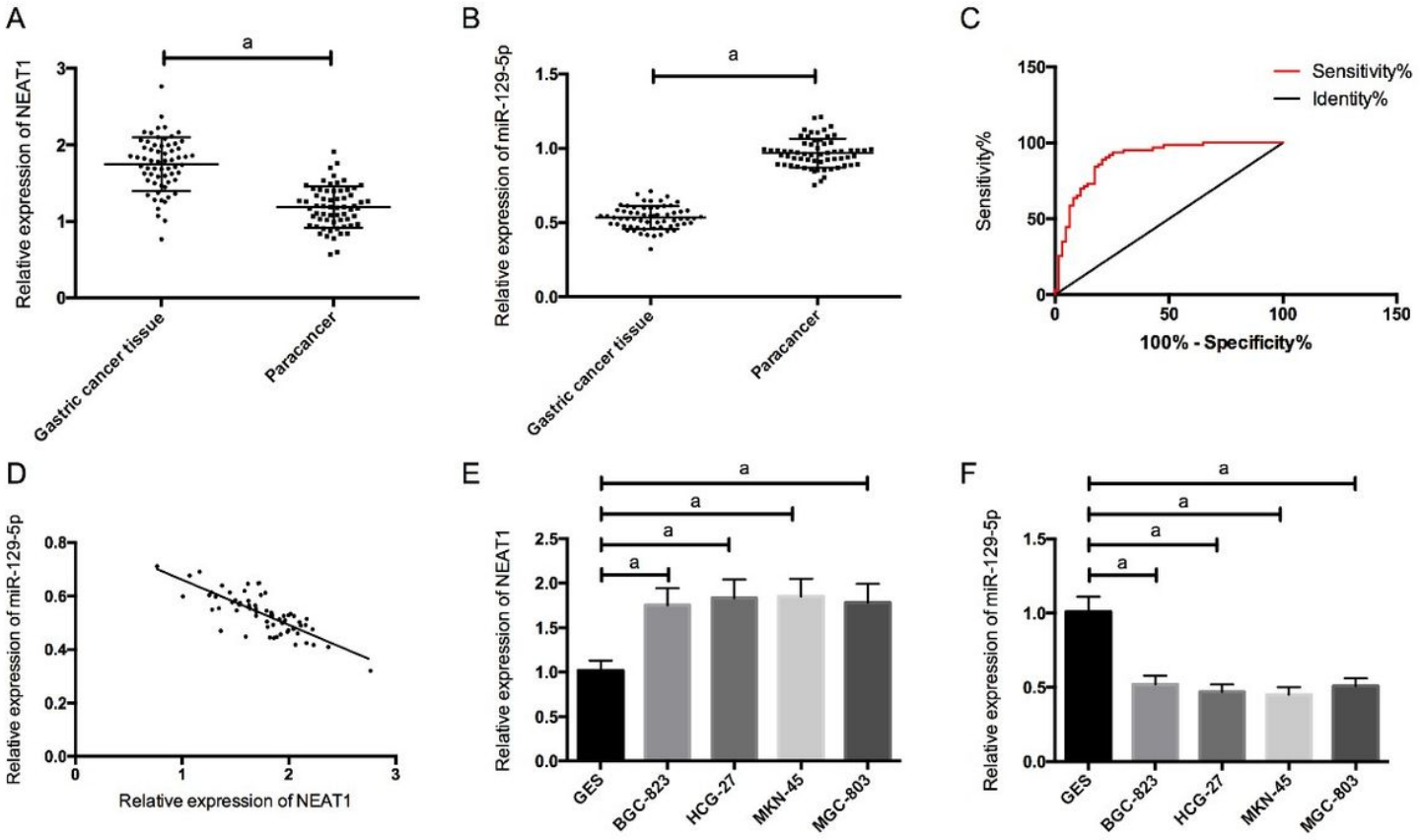

Figure 1

Expression and clinical significance of NEAT1 in GC tissues. A: Expression of NEAT1 in GC tissues; B: Expression of miR-129-5p in GC tissues; C: ROC analysis of NEAT1 in the diagnosis of GC; D: Correlation analysis between NEAT1 and miR-129-5p in GC tissues; E: Expression of NEAT1 in GC cells; F: Expression of miR-129-5p in GC cells. a indicates $P<0.05$.

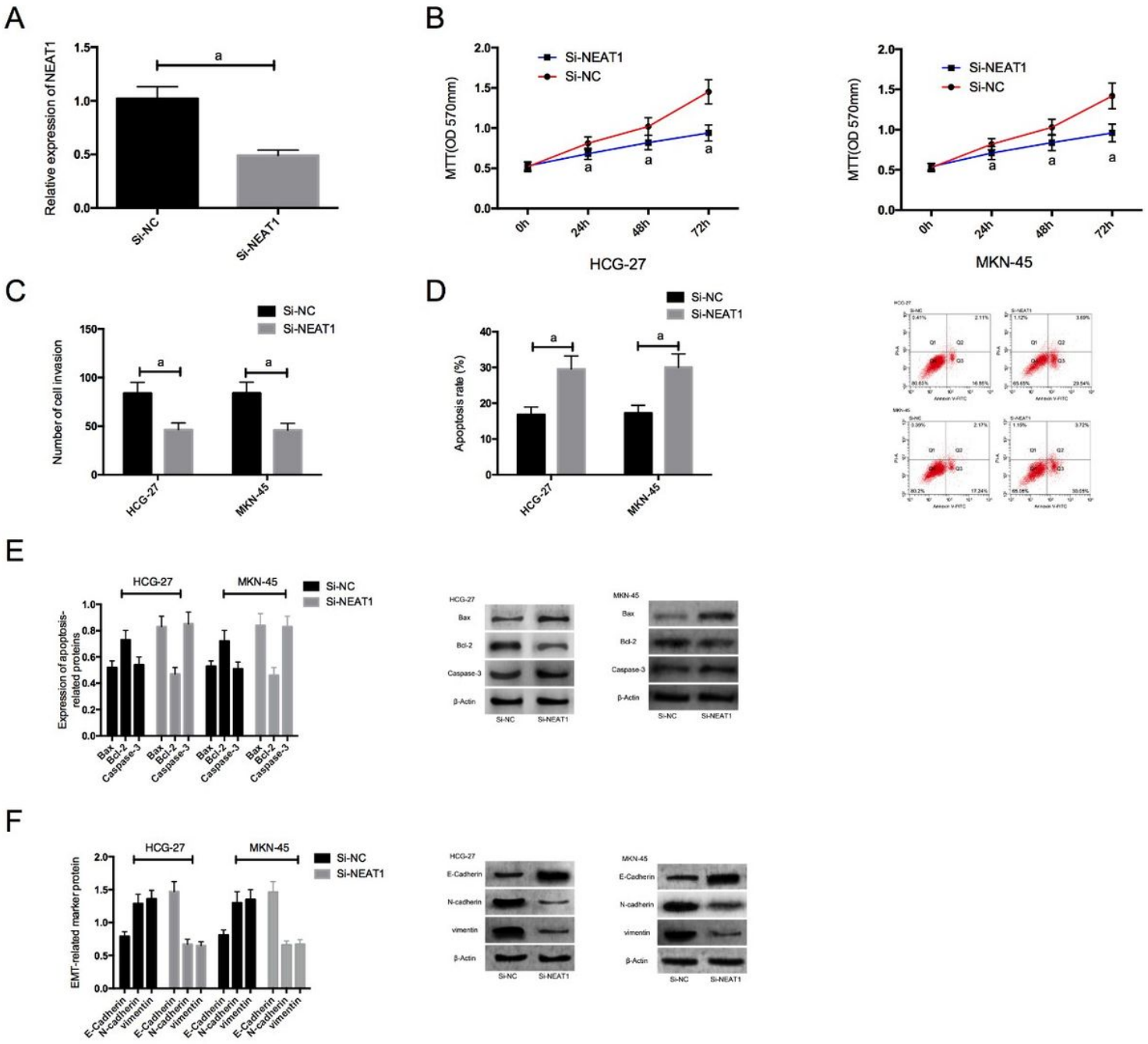

Figure 2

Effects of NEAT1 on the biological function of GC cells. A: Expression of NEAT1 in GC cells after transfection; B: Effects of silencing NEAT1 expression on the proliferation of GC cells; C: Effects of silencing NEAT1 expression on the invasion of GC cells; D: Effects of silencing NEAT1 expression on the apoptosis rate of GC cells; E: Effects of silencing NEAT1 expression on apoptosis-related proteins in GC cells; F: Effects of silencing NEAT1 expression on EMT-related proteins in GC cells. a indicates $P<0.05$.

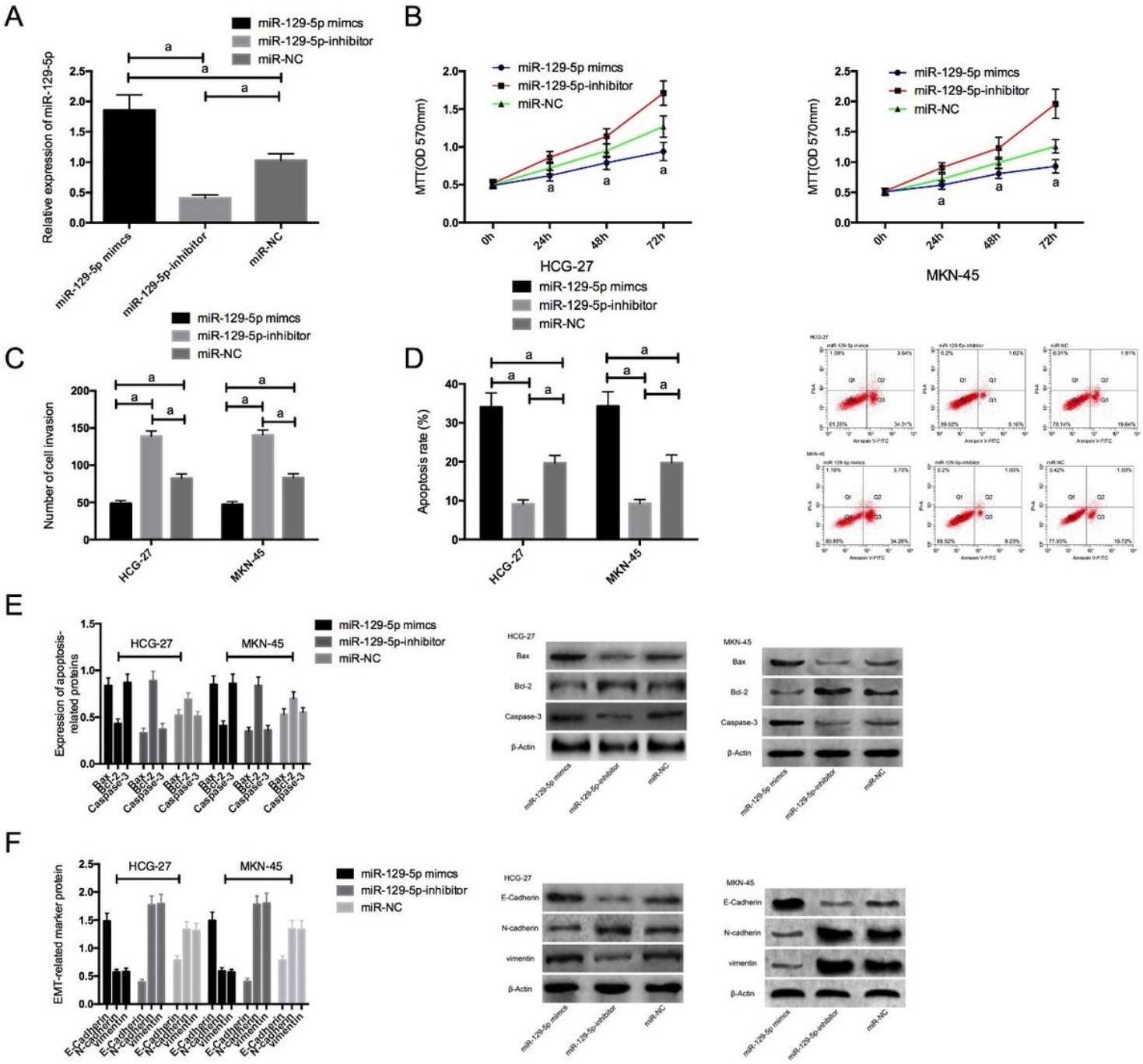

Figure 3

Effects of miR-129-5p on the biological function of GC cells. A: Expression of miR-129-5p in GC cells after transfection; B: Effects of miR-129-5p on the proliferation of GC cells; C: Effects of miR-129-5p on the invasion of GC cells; D: Effects of miR-129-5p on the apoptosis rate of GC cells; E: Effects of miR-129-5p on apoptosis-related proteins in GC cells; F: Effects of miR-129-5p on EMT-related proteins in GC cells. a indicates P<0.05.

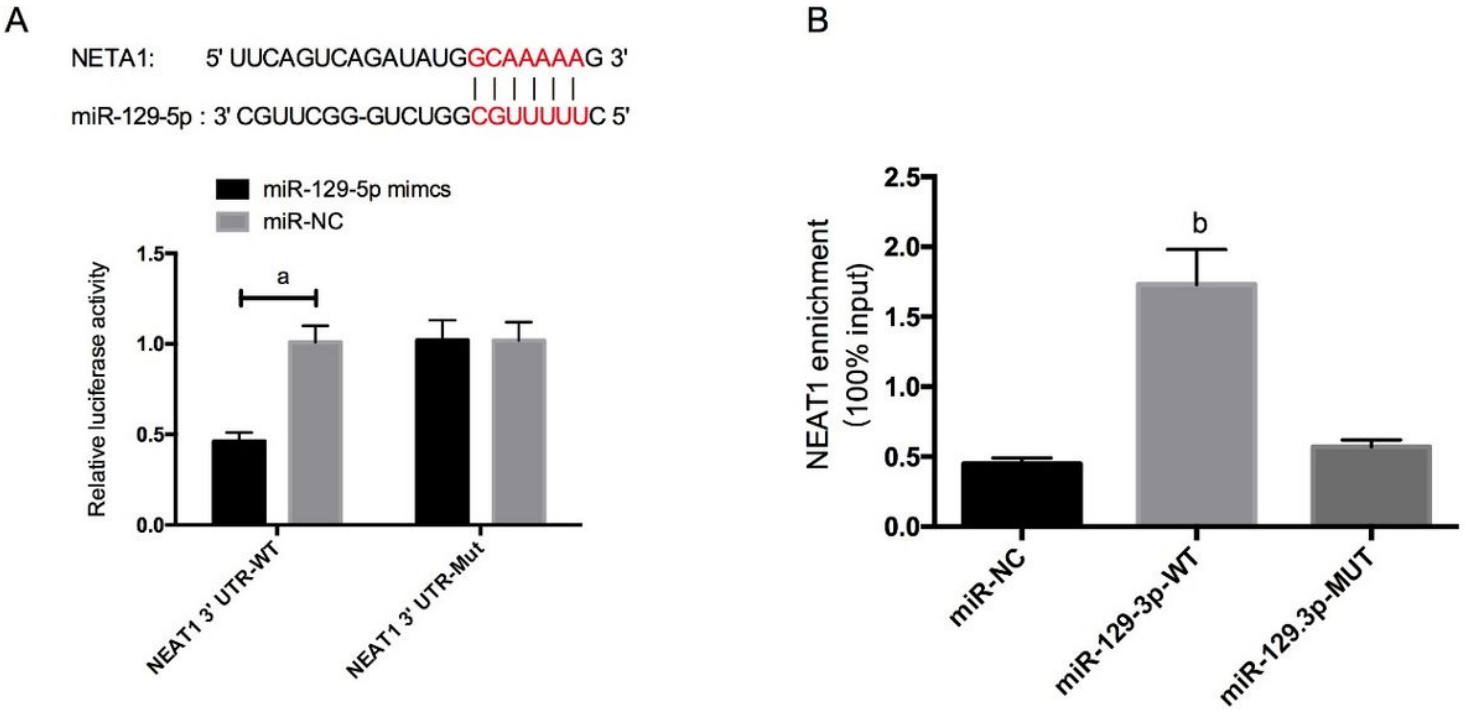

Figure 4

Competitive binding between NEAT1 and miR-129-5P. A: Dual luciferase reporter assay; B: RIP experiment. a indicates P<0.05, and b indicates P<0.05 compared with miR-NC.

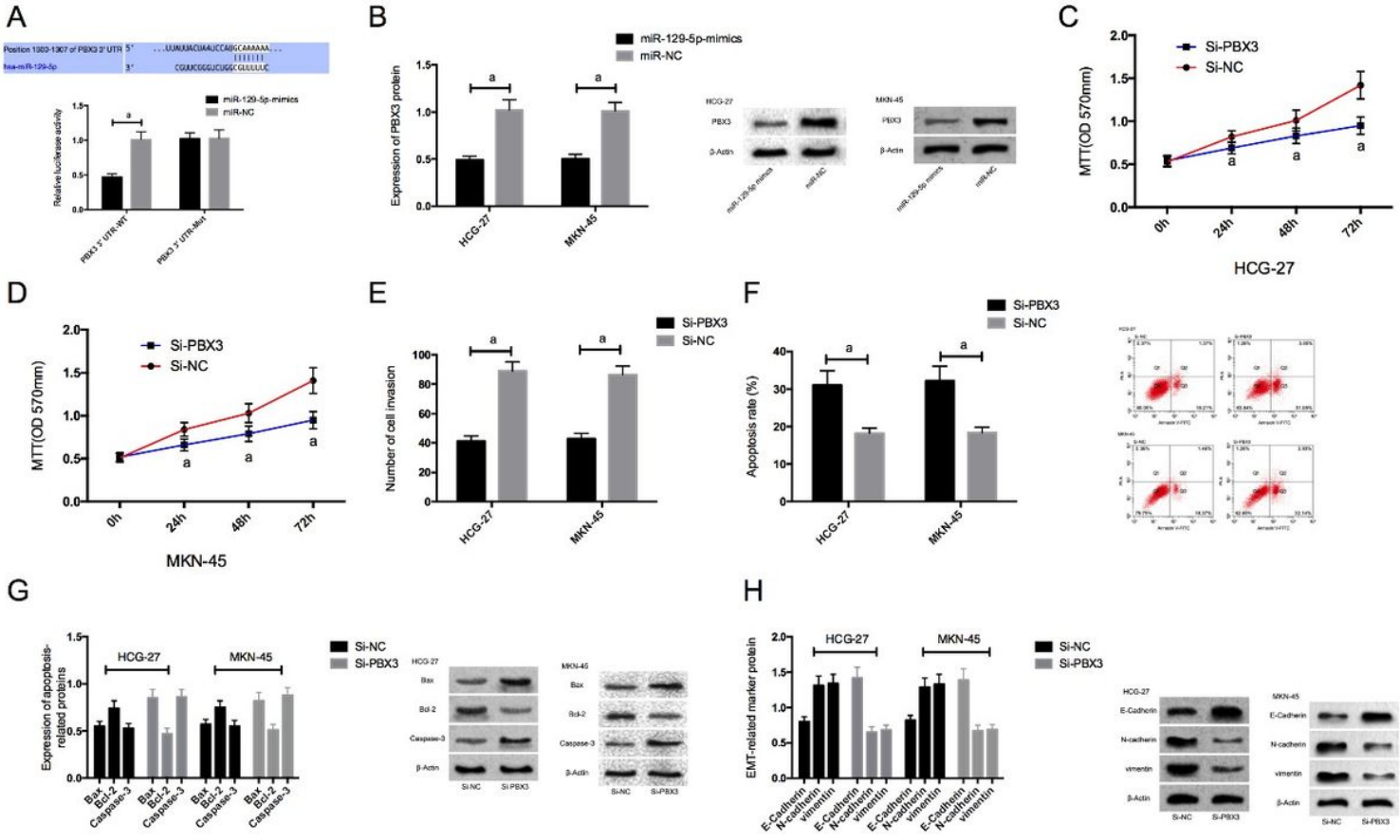

Figure 5

MiR-129-5p directly targeted PBX3 to regulate cell biological function. A: Dual luciferase reporter assay; B: Effects of miR-129-5p on PBX3 protein expression; C, D: Effects of PBX3 on the proliferation of GC cells; E: Effects of PBX3 on the invasion of GC cells; F: Effects of PBX3 on the apoptosis rate of GC cells; G: Effects of PBX3 on apoptosis-related proteins in GC cells; H: Effects of PBX3 on EMT-related proteins in GC cells.

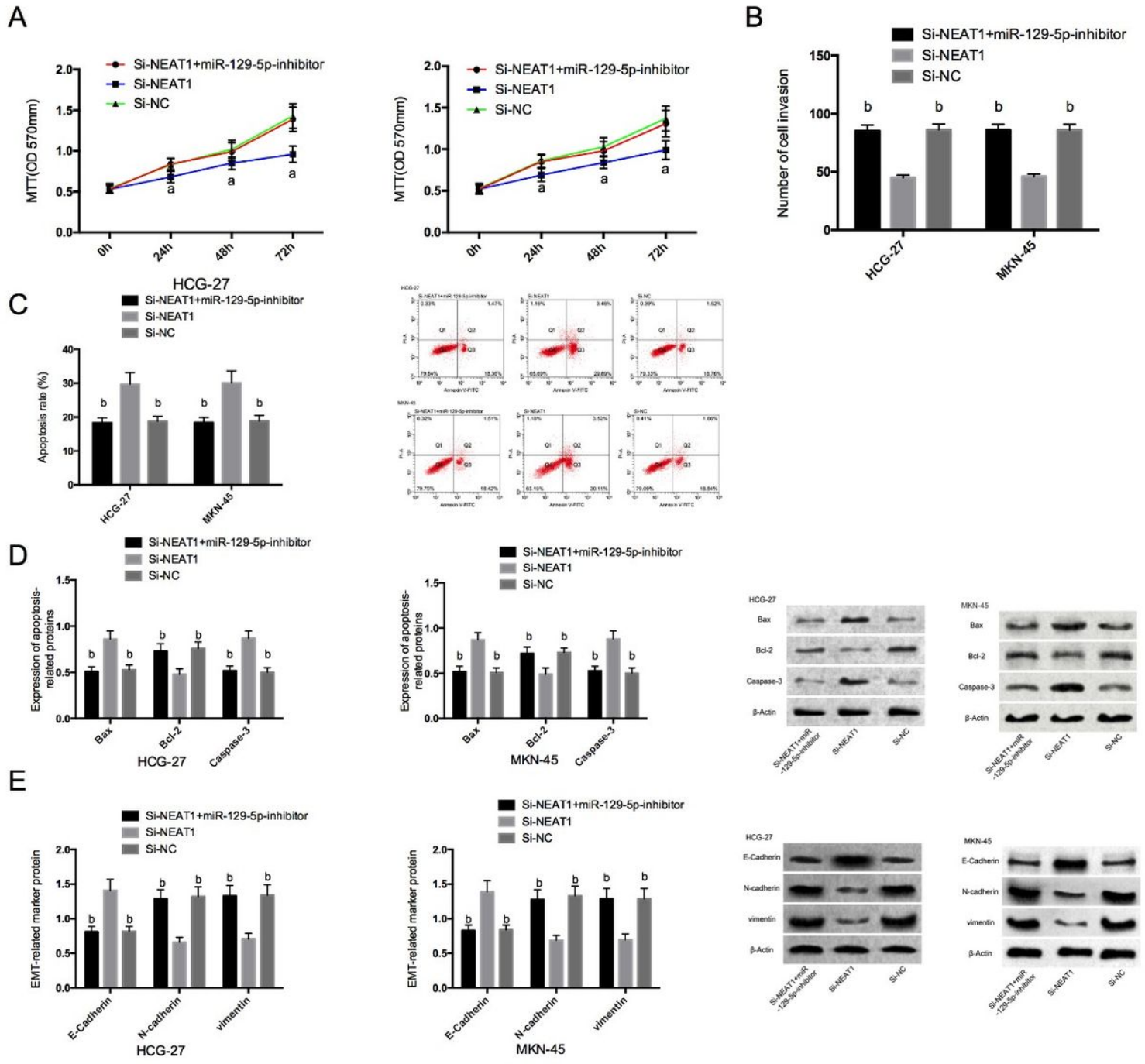

Figure 6

Rescue experiment. A: Effects of co-transfection of Si-NEAT1+miR-129-5p-inhibitor on the proliferation of GC cells; B: Effects of co-transfection of Si-NEAT1+miR-129-5p-inhibitor on the invasion of GC cells; C: Effects of co-transfection of Si-NEAT1+miR-129-5p-inhibitor on the apoptosis rate of GC cells; D: Effects of co-transfection of Si-NEAT1+miR-129-5p-inhibitor on apoptosis-related proteins in GC cells; E: Effects of co-transfection of Si-NEAT1+miR-129-5p-inhibitor on EMT-related proteins in GC cells.