

# MicroRNA-197 inhibits gastric cancer progression by directly targeting metadherin

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**Abstract.** Gastric cancer is the fifth most frequent malignancy and the fourth most common cause of cancer-associated mortality worldwide. MicroRNAs (miRNAs) are a group of small RNAs that regulate several cellular processes. In particular, a large number of miRNAs are involved in gastric cancer formation and progression. Thus, miRNAs may be considered as effective diagnostic biomarkers and therapeutic methods for gastric cancer. The aim of the current study was to detect miRNA (miR)-197 expression in gastric cancer and to investigate its biological role and associated mechanism in gastric cancer. In the present study, miR-197 expression was demonstrated to be considerably downregulated in gastric cancer tissues and cell lines. Its low expression level was associated with tumour size, invasive depth, tumour-node-metastasis staging and lymph node metastasis. High expression of miR-197 inhibited tumour cell proliferation and invasion *in vitro*. Subsequently, metadherin (MTDH) was identified as a direct target gene of miR-197 in gastric cancer, and this was confirmed by bioinformatics analysis, Dual-luciferase reporter assay, reverse transcription quantitative polymerase chain reaction and western blot analysis. MTDH expression was upregulated in gastric cancer and was inversely correlated with miR-197 expression levels. In addition, MTDH overexpression prevented the proliferation and inhibited invasion induced by miR-197 overexpression. In addition, miR-197 was demonstrated to regulate the phosphatase and tensin homolog (PTEN)/AKT signalling pathway in gastric cancer. The results of the present study suggested that miR-197 serves a tumour-suppressing role in human gastric carcinogenesis and progression by regulating the MTDH/PTEN/AKT signalling

pathway. The miR-197/MTDH axis may provide a novel effective therapeutic target for patients with gastric cancer.

## Introduction

Gastric cancer is the fifth most frequent malignancy and fourth third most common cause of cancer-associated mortality worldwide (1). Gastric cancer incidence exhibits significant regional differences, particularly in Asian regions (2). There are ~850,000 newly diagnosed cases of gastric cancer and 650,000 mortalities occur worldwide annually (3). The underlying molecular mechanism of gastric cancer is complex, and it remains poorly understood despite extensive clinical and basic research efforts. Several factors, including oncogene protein expression, *Helicobacter pylori* infection, dietary factors, tobacco use, alcohol consumption and obesity, are involved in gastric cancer occurrence and progression (4-6). Despite advancements in early detection, treatment and prevention, advanced-stage gastric cancer remains incurable, with a remarkably poor 5-year survival rate of ~4-5% (7). Therefore, the mechanisms underlying gastric cancer carcinogenesis and progression should be elucidated to identify novel biomarkers for diagnosis and to develop effective therapeutic strategies.

MicroRNAs (miRNAs) are endogenous, highly conserved, short (20-25 nucleotides) noncoding RNA molecules (8). These small molecules completely or partially bind to the 3'-untranslated regions (3'-UTRs) of their target genes to promote mRNA degradation or inhibit mRNA translation (9). miRNAs participate in the modulation of greater than one-third of human genes (10). Studies have indicated that miRNAs are dysregulated in various types of human cancers, such as gastric cancer (11), hepatocellular carcinoma (12), bladder cancer (13), ovarian cancer (14) and breast cancer (15). Abnormally expressed miRNAs have been implicated in tumour initiation and progression, and may regulate a variety of important physiological events, including cell proliferation, cell cycle, apoptosis, angiogenesis, migration and invasion and metastasis (16-18). Previous studies have also demonstrated that miRNAs may serve as oncogenes or tumour suppressors depending on the nature of their target mRNAs (19-21). Thus, miRNAs may be examined to identify a novel therapeutic treatment for gastric cancer patients.

miR-197 is aberrantly expressed in several types of human cancers, and it serves important roles in tumorigenesis and tumour development (22-24). However, studies that have

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investigated the expression levels and biological roles of miR-197 in gastric cancer are sparse. Therefore, the present study examined the expression of miR-197 in gastric cancer tissues and cell lines, and investigated its roles and the underlying mechanisms in the regulation of aggressive behaviours of gastric cancer cells.

## Materials and methods

**Human gastric cancer clinical specimens.** The present study was approved by the Ethics Committee of Renhe Hospital (Shanghai, China), and written informed consent was obtained from all patients between June 2015 and December 2016. A total of 45 paired gastric cancer tissues and adjacent non-tumoural tissues were obtained from patients who received surgery resection at Department of General Surgery, Renhe Hospital (Shanghai, China). None of the patients were treated with chemotherapy or radiotherapy prior to surgery. Tissue samples were immediately frozen in liquid nitrogen following surgical resection, and stored at  $-80^{\circ}\text{C}$  until use.

**Cell lines and culture conditions.** Human gastric cancer cell lines (SGC-7901, MGC-803, AGS and BGC-823), an immortalized gastric epithelial cell line (GES-1) and the 293T cell line were acquired from the American Type Culture Collection (Manassas, VA, USA). All cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% foetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 50 U/ml penicillin and 50  $\mu\text{g}/\text{ml}$  streptomycin in a humidified atmosphere containing 5%  $\text{CO}_2$  at  $37^{\circ}\text{C}$ .

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR).** Total RNA was extracted from tissues (1 g) or cells ( $1.5 \times 10^6$ ) using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), following to the manufacturer's protocol. RNA concentrations were determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). To detect miR-197 expression, reverse transcription was conducted using a TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems; Thermo Fisher Scientific, Inc.), and the TaqMan miRNA assay (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used to examine miR-197 expression, with U6 as an internal control. The cycling conditions for qPCR were as follows:  $50^{\circ}\text{C}$  for 2 min,  $95^{\circ}\text{C}$  for 10 min; 40 cycles of denaturation at  $95^{\circ}\text{C}$  for 15 sec and annealing/extension at  $60^{\circ}\text{C}$  for 60 sec. To quantify MTDH mRNA expression levels, total RNA was reverse transcribed to cDNA using a PrimeScript RT Reagent kit (Takara Biotechnology Co., Ltd., Dalian, China). The relative expression levels of MTDH were determined with SYBR Premix Ex Taq (Takara Biotechnology Co., Ltd.), and normalized to GAPDH expression. The amplification was performed with cycling conditions as follows:  $95^{\circ}\text{C}$  for 5 min, followed by 40 cycles of  $95^{\circ}\text{C}$  for 30 sec and  $65^{\circ}\text{C}$  for 45 sec. The primers were designed as follows: miR-197, 5'-ATTACTTTGCCCA TATTCATTTTGA-3' (forward) and 5'-ATTCTAGAGCC GAGGCGGCCGACATGT-3' (reverse); U6, 5'-CGCTTC GGCAGCACATATAC-3' (forward), and 5'-TTCACGAAT

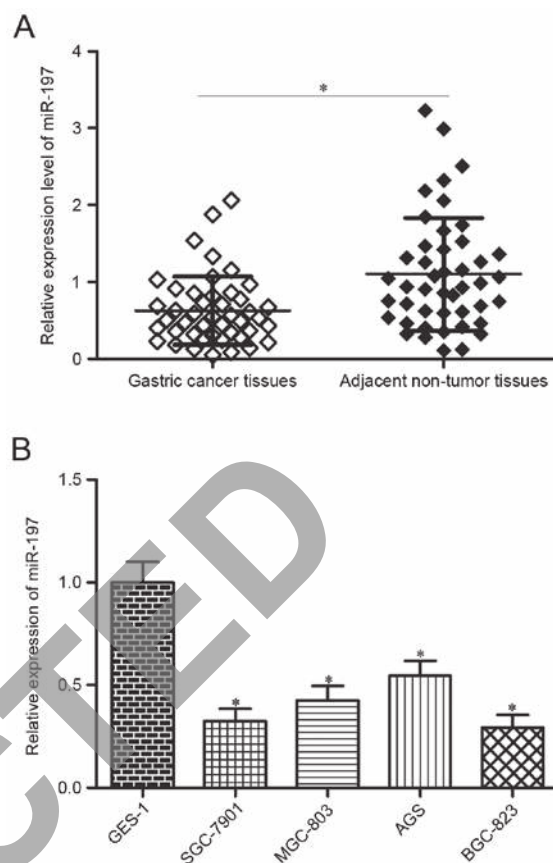


Figure 1. miR-197 expression is downregulated in gastric cancer tissues and cell lines. (A) miR-197 expression in 45 pairs of human gastric cancer tissues and adjacent non-tumoural tissues was determined using RT-qPCR;  $*P < 0.05$ . (B) RT-qPCR analysis of miR-197 expression in four gastric cancer cell lines (SGC-7901, MGC-803, AGS and BGC-823) and an immortalised normal gastric epithelial cell line (GES-1);  $*P < 0.05$  vs. GES-1. miR, microRNA; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

TTGCGTGTCAT-3' (reverse); MTDH, 5'-AAATAGCCA GCCTATCAAGACTC-3' (forward) and 5'-TTCAGACTT GGTCTGTGAAGGAG-3' (reverse); and GAPDH, 5'-GCA CCGTCAAGGCTGAGAAC-3' (forward) and 5'-TGGTGA AGACGCCAGTGGGA-3' (reverse). The relative quantification was calculated using the  $2^{-\Delta\Delta\text{Ct}}$  method (25).

**Cell transfection.** The miR-197 mimics and corresponding negative control miRNA (miR-NC) were synthesized and purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). The miR-197 mimic sequence was 5'-UUCACCACC UUCUCCACCCAGC-3' and the miR-NC sequence was 5'-UUCUCCGAACGUGUCACGUTT-3'. The MTDH over-expression plasmid (pcDNA3.1-MTDH) and empty control plasmid (pcDNA3.1) were obtained from Origene Technologies, Inc. (Rockville, MD, USA). Cells ( $7 \times 10^5$  cells/well) were seeded in 6-well plates and transfected with oligonucleotides (100 pmol) and/or plasmids (2  $\mu\text{g}$ ) using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Following a 6 h incubation at  $37^{\circ}\text{C}$ , the culture medium was replaced with fresh DMEM containing 10% FBS. Cell Counting Kit-8 assay, Matrigel invasion assay, RT-qPCR and western blot analysis were performed 24, 48, 48 and 72 h post-transfection, respectively.

Table I. Association between microRNA-197 expression and clinicopathological factors of patients with gastric cancer.

Clinicopathological factor	n	Low miR-197	High miR-197	P-value
Age (years)				0.626
<55	18	10	8	
≥55	27	13	14	
Sex				0.672
Male	28	15	13	
Female	17	8	9	
Tumour size (cm)				0.011
<5	20	6	14	
≥5	25	17	8	
Differentiation				0.668
Well and moderate	19	9	10	
Poor	26	14	12	
Invasive depth				0.005
T1+T2	21	6	15	
T3+T4	24	17	7	
TNM staging				0.011
I-II	22	7	15	
III-IV	23	16	7	
Lymph node metastasis				0.004
No	25	8	17	
Yes	20	15	5	

miR, microRNA; TNM, tumour nodes metastasis.

**Cell Counting kit-8 (CCK-8) assay.** Cell proliferation was determined by a CCK-8 assay (Dojindo Molecular Technologies, Inc., Kumamoto, Japan), according to the manufacturer's protocol. Briefly, transfected cells were collected at 24 h post-transfection, and seeded ( $3 \times 10^3$  cells/well) into 96-well plates. Proliferation was monitored everyday for 4 days. A total of 10  $\mu$ l CCK8 solution was added into each well and cultured for 2 h in a 37°C incubator with 5% CO<sub>2</sub>. The absorbance at a wavelength of 450 nm was measured using a SpectraMAX Plus microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA). Each assay was performed in triplicate and repeated three times.

**Matrigel invasion assay.** Transwell invasion assays were performed to examine the cell invasive abilities using Transwell chambers (8  $\mu$ m; BD Biosciences, San Jose, CA, USA) coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). Transfected cells were collected and resuspended in FBS-free DMEM medium. A total of  $5 \times 10^4$  transfected cells in FBS-free DMEM medium were seeded in the top chamber. DMEM supplemented with 10% FBS was added to the lower chamber and served as a chemoattractant. Following 48 h incubation at 37°C in 5% CO<sub>2</sub>, the cells remaining on the upper side were wiped away with cotton swabs and the Transwell chambers were washed with PBS, fixed in 95% methanol at room temperature for 15 min, stained with 0.1% crystal violet at room temperature for 15 min and air-dried.

The invasive cells were imaged and five independent fields were counted with an Olympus IX53 microscope (Olympus Corporation, Tokyo, Japan). The assay was repeated at least three times.

**Bioinformatics analysis.** Bioinformatics analysis was conducted to predict the candidate genes of miR-197 using TargetScan (<http://targetscan.org>) and miRanda (<http://www.microna.org/microna/home.do>).

**Dual-luciferase reporter assay.** MTDH was predicted to be a potential target of miR-197 by bioinformatics analysis. The luciferase reporter vectors, pMiR-MTDH-3'UTR wild-type (Wt) and pMiR-MTDH-3'UTR mutant-type (Mut) were synthesized and confirmed by Shanghai GenePharma, Co., Ltd. 293T cells were seeded in 24-well plates until 60-70% confluent. Subsequently, cells were transfected with either pMiR-MTDH-3'UTR Wt (0.2  $\mu$ g) or pMiR-MTDH-3'UTR Mut (0.2  $\mu$ g), and co-transfected with either miR-197 mimics (50 pmol) or miR-NC (50 pmol) were transfected into 293T cells using Lipofectamine 2000, following the manufacturer's protocol. Following 48 h transfection, luciferase activities were detected using the Dual Luciferase Reporter assay system (Promega Corporation, Madison, WI, USA). *Renilla* luciferase activities were used to normalize firefly luciferase activities. The experiment was repeated three times, and each was performed in triplicate.

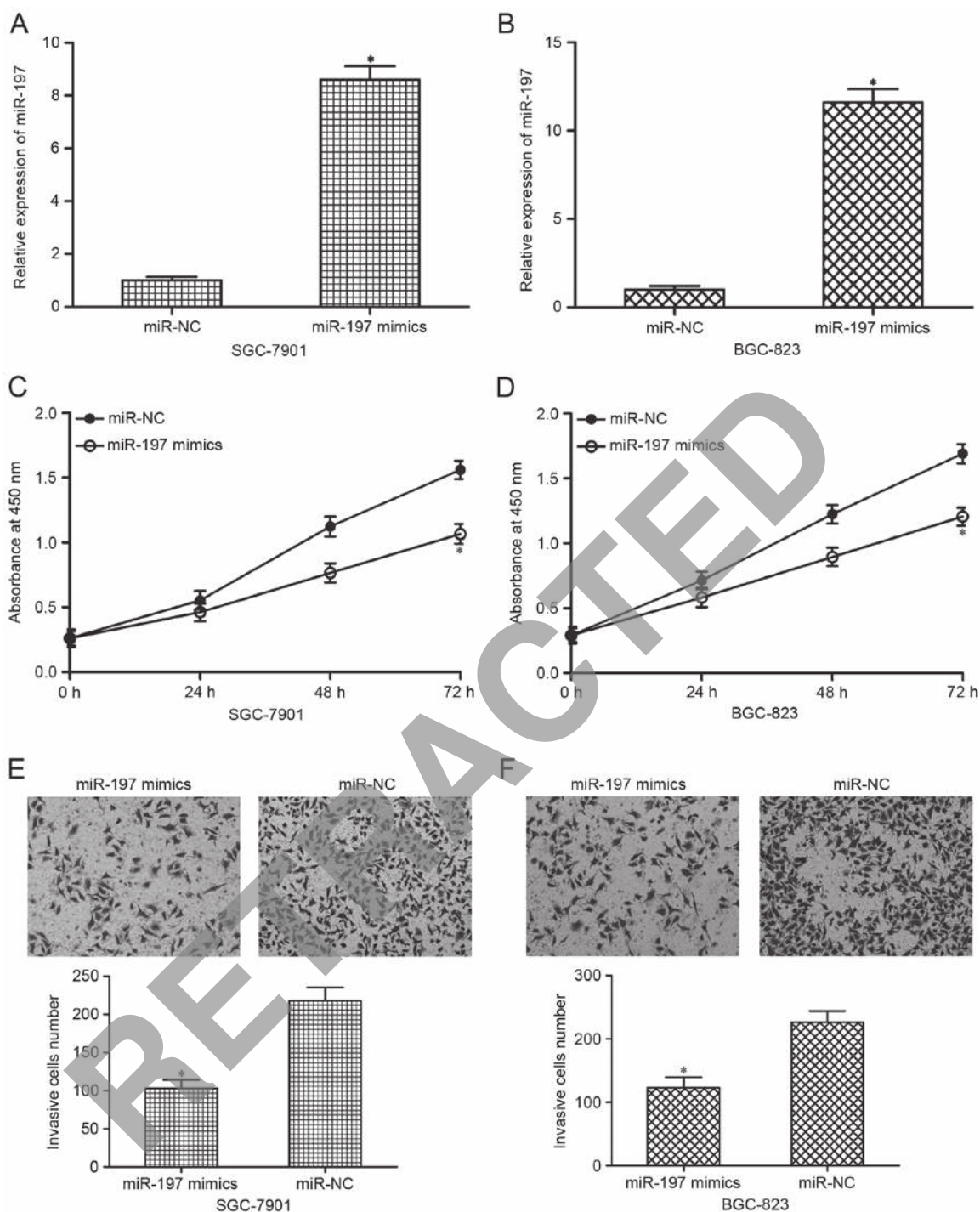


Figure 2. miR-197 inhibits SGC-7901 and BGC-823 cell proliferation and invasion. (A and B) miR-197 mimics transfection significantly increased the expression levels of miR-197 in (A) SGC-7901 and (B) BGC-823 cells; \* $P < 0.05$  vs. miR-NC. (C and D) Cell Counting kit-8 assay was performed to evaluate cell proliferation of (C) SGC-7901 and (D) BGC-823 cells following transfection with miR-197 mimics or miR-NC; \* $P < 0.05$  vs. miR-NC. (E and F) Transwell invasion assays were performed to examine the invasive abilities of (E) SGC-7901 and (F) BGC-823 cells transfected with miR-197 mimics or miR-NC; \* $P < 0.05$  vs. miR-NC. miR, microRNA; NC, negative control.

**Western blot analysis.** Cells ( $1.5 \times 10^6$ ) or tissue specimens (1 g) were lysed in radioimmunoprecipitation lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) containing protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, USA). Protein quantification was performed using a Bicinchoninic Acid Protein assay (Pierce; Thermo Fisher Scientific, Inc.). Equal amounts of protein (30  $\mu$ g) were separated by 10% SDS-PAGE and electrotransferred

to polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). Following blocking with 5% non-fat milk in Tris-buffered saline containing 0.05% Tween-20 (TBST) at room temperature for 1 h, the membranes were incubated overnight at 4°C with primary antibodies against: MTDH (1:1,000 dilution; cat. no. sc-517220; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), PTEN (sc-133197; 1:1,000 dilution), p-AKT (sc-271966; 1:1,000 dilution), AKT (sc-81434; 1:1,000 dilution)

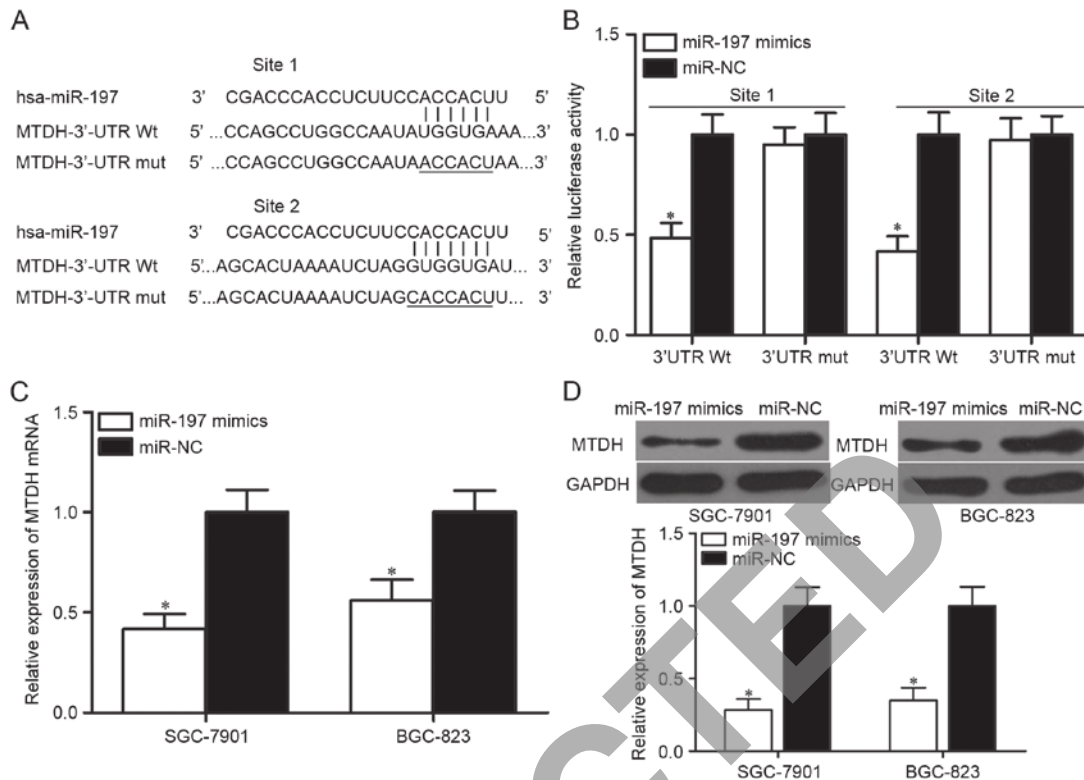


Figure 3. MTDH is a direct target of miR-197 in gastric cancer cells. (A) Two putative binding sites of miR-197 in the 3'-UTR of Wt MTDH were predicted through bioinformatics analysis; the Wt and Mut sites within the MTDH-3'-UTR are indicated. (B) Relative luciferase activity was detected in 293T cells co-transfected with MTDH-3'-UTR Wt or MTDH-3'-UTR Mut reporter plasmids and miR-197 mimics or miR-NC. (C) MTDH mRNA levels were detected by reverse transcription-quantitative polymerase chain reaction in SGC-7901 and BGC-823 cells transfected with miR-197 mimics or miR-NC. (D) MTDH protein expression levels were examined by western blot analysis in SGC-7901 and BGC-823 cells transfected with miR-197 mimics or miR-NC. \* $P < 0.05$  vs. miR-NC. 3'-UTR, 3' untranslated region; miR, microRNA; MTDH, metadherin; mut, mutant; NC, negative control; wt, wild-type.

and GAPDH (sc-32233; 1:1,000 dilution) (all from Santa Cruz Biotechnology, Inc.). Membranes were washed with TBST and probed with horseradish peroxidase-conjugated goat-anti mouse secondary antibody (1:5,000 dilution; cat. no. sc-2005; Santa Cruz Biotechnology, Inc.) at room temperature for 2 h. Immunoreactive bands were visualized using an Enhanced Chemiluminescence Detection kit (Thermo Fisher Scientific, Inc.). Quantification of band intensity was performed with Quantity One software (version 4.62; Bio-Rad Laboratories, Inc., Hercules, CA, USA). GAPDH served as a loading control.

**Statistical analysis.** Data are expressed as the mean  $\pm$  standard deviation, and all statistical analyses were performed with a two-tailed Student's *t* test or one-way analysis of variance followed by a Student-Newman-Keuls test, using SPSS version 16.0 software (SPSS, Inc., Chicago, IL, USA). Correlation of miR-197 expression with that of MTDH mRNA expression was conducted with the Spearman's correlation analysis.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**miR-197 is downregulated in gastric cancer tissues and cell lines.** miR-197 expression was measured in 45 pairs of gastric cancer tissues and adjacent non-tumoural tissues by RT-qPCR to evaluate the significance of miR-197 in gastric cancer. Expression of miR-197 was significantly lower in gastric cancer

tissues compared with expression in adjacent non-tumoural tissues ( $P < 0.05$ ; Fig. 1A). In addition, associations between miR-197 expression and clinicopathological factors of patients with gastric cancer were evaluated. Patients were divided into the following groups according to their median miR-197 values (Table I): Low-miR-197 group ( $n = 23$ ) and high-miR-197 group ( $n = 22$ ). Low miR-197 expression was significantly associated with tumour size ( $P = 0.011$ ), invasive depth ( $P = 0.005$ ), tumour nodes metastasis (TNM) staging ( $P = 0.011$ ) and lymph node metastasis ( $P = 0.004$ ). However, no significant association was observed between miR-197 expression and age ( $P = 0.626$ ), sex ( $P = 0.672$ ) or differentiation ( $P = 0.668$ ).

miR-197 expression levels were also examined in a panel of gastric cancer cell lines (SGC-7901, MGC-803, AGS and BGC-823) and an immortalised normal gastric epithelial cell line (GES-1). miR-197 expression levels in all of the gastric cancer cell lines were significantly decreased compared with that of GES-1 ( $P < 0.05$ ; Fig. 1B). These results suggested that miR-197 may serve important roles in gastric cancer progression. Notably, SGC-7901 and BGC-823 cells expressed the lowest levels of miR-197; thus, these two cell lines were selected as models for subsequent experiments.

**miR-197 overexpression inhibits proliferation and invasion of gastric cancer cells.** miR-197 mimics were transfected into SGC-7901 and BGC-823 cells to overexpress the total miR-197 levels and to investigate the biological roles of miR-197 in gastric cancer progression. RT-qPCR analysis demonstrated

that miR-197 was significantly upregulated in SGC-7901 and BGC-823 cells following transfection with miR-197 mimics compared with cells transfected with miR-NC ( $P < 0.05$ ; Fig. 2A and B, respectively). Subsequently, the effects of miR-197 overexpression on the proliferation and invasion of SGC-7901 and BGC-823 cells were investigated. CCK-8 assays demonstrated that miR-197 overexpression suppressed SGC-7901 and BGC-823 cell proliferation compared with the miR-NC ( $P < 0.05$ ; Fig. 2C and D, respectively). Results from the Matrigel invasion assays revealed that overexpression of miR-197 decreased the invasive capacities of the SGC-7901 and BGC-823 cells compared with the miR-NC ( $P < 0.05$ ; Fig. 2E and F). These results suggested that miR-197 may act as a tumour suppressor in gastric cancer.

*miR-197 directly targets MTDH by binding to its 3'-UTR to inhibit MTDH expression.* Bioinformatics analysis was performed to predict candidate target genes and to determine the molecular mechanism underlying miR-197-mediated suppression of gastric cancer cell proliferation and invasion. Among the predicted targets, MTDH was selected for further analysis, as it has been reported to be highly expressed in gastric cancer and may serve oncogenic roles in gastric cancer initiation and progression (26,27). Two putative target sites of miR-197 in the 3'-UTR of MTDH were identified (Fig. 3A).

Luciferase reporter assays were performed to determine whether MTDH is a direct target of miR-197. 293T cells were transfected with MTDH-3'-UTR Wt or MTDH-3'-UTR Mut and co-transfected with miR-197 mimics or miR-NC. Overexpression of miR-197 significantly decreased the luciferase activities of MTDH-3'-UTR Wt site 1 and MTDH-3'-UTR Wt site 2 in 293T cells compared with cells transfected with the miR-NC ( $P < 0.05$ ; Fig. 3B). By contrast, no significant suppression of luciferase activity was observed on the MTDH-3'-UTR Mut reporter plasmid following miR-197 mimics transfection. In addition, the influence of miR-197 overexpression on the levels of MTDH mRNA and protein expression were determined in SGC-7901 and BGC-823 cells transfected with miR-197 mimics or miR-NC. RT-qPCR and western blot analysis results indicated that miR-197 overexpression resulted in decreased MTDH expression in the SGC-7901 and BGC-823 cells at both the mRNA ( $P < 0.05$ ; Fig. 3C) and the protein ( $P < 0.05$ ; Fig. 3D) level. These results suggested that MTDH may be a direct target gene of miR-197 in gastric cancer.

*MTDH is upregulated in gastric cancer tissues and is inversely correlated with miR-197 expression levels.* MTDH mRNA and protein expression levels were measured in gastric cancer tissues and adjacent non-tumoural tissues. MTDH mRNA expression levels were significantly higher in gastric cancer tissues compared with adjacent non-tumoural tissues ( $P < 0.05$ ; Fig. 4A). Similarly, a notable increase in the protein expression levels of MTDH were observed in gastric cancer tissues compared with normal tissue ( $P < 0.05$ ; Fig. 4B and C). In addition, an inverse correlation between MTDH mRNA and miR-197 expression levels was observed by Spearman's correlation analysis in gastric cancer tissues ( $P < 0.0001$ ;  $r = -0.6351$ ; Fig. 4D).

*MTDH overexpression reverses the suppressive roles of miR-197 on the proliferation and invasion of gastric cancer*

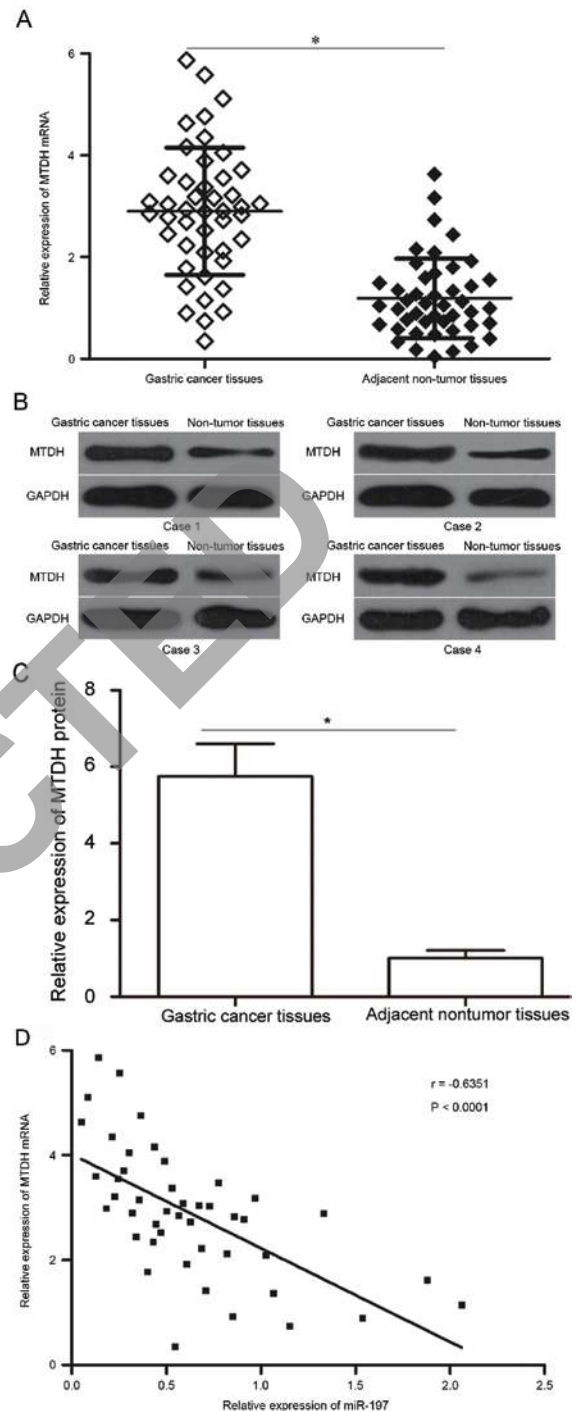


Figure 4. MTDH is upregulated and negatively correlated with miR-197 expression in gastric cancer tissues. (A) Reverse transcription-quantitative polymerase chain reaction revealed that MTDH mRNA expression levels, respectively, were increased in gastric cancer tissues.  $*P < 0.05$  vs. adjacent non-tumoural tissues. (B) Western blot analysis of MTDH expression levels in four paired gastric cancer tissues and adjacent non-tumoural tissues. (C) Densitometric analysis of MTDH expression from all patient samples.  $*P < 0.05$  vs. adjacent non-tumoural tissues. (D) Spearman correlation analysis results indicated an inverse correlation between MTDH mRNA and miR-197 expression levels in gastric cancer specimens;  $r = -0.6351$ ;  $P < 0.0001$ . miR, microRNA; MTDH, metadherin.

*cells.* Following the validation of MTDH as a direct target of miR-197, the present study examined whether MTDH upregulation may prevent the suppressive effects of miR-197 on gastric cancer cell proliferation and invasion. miR-197

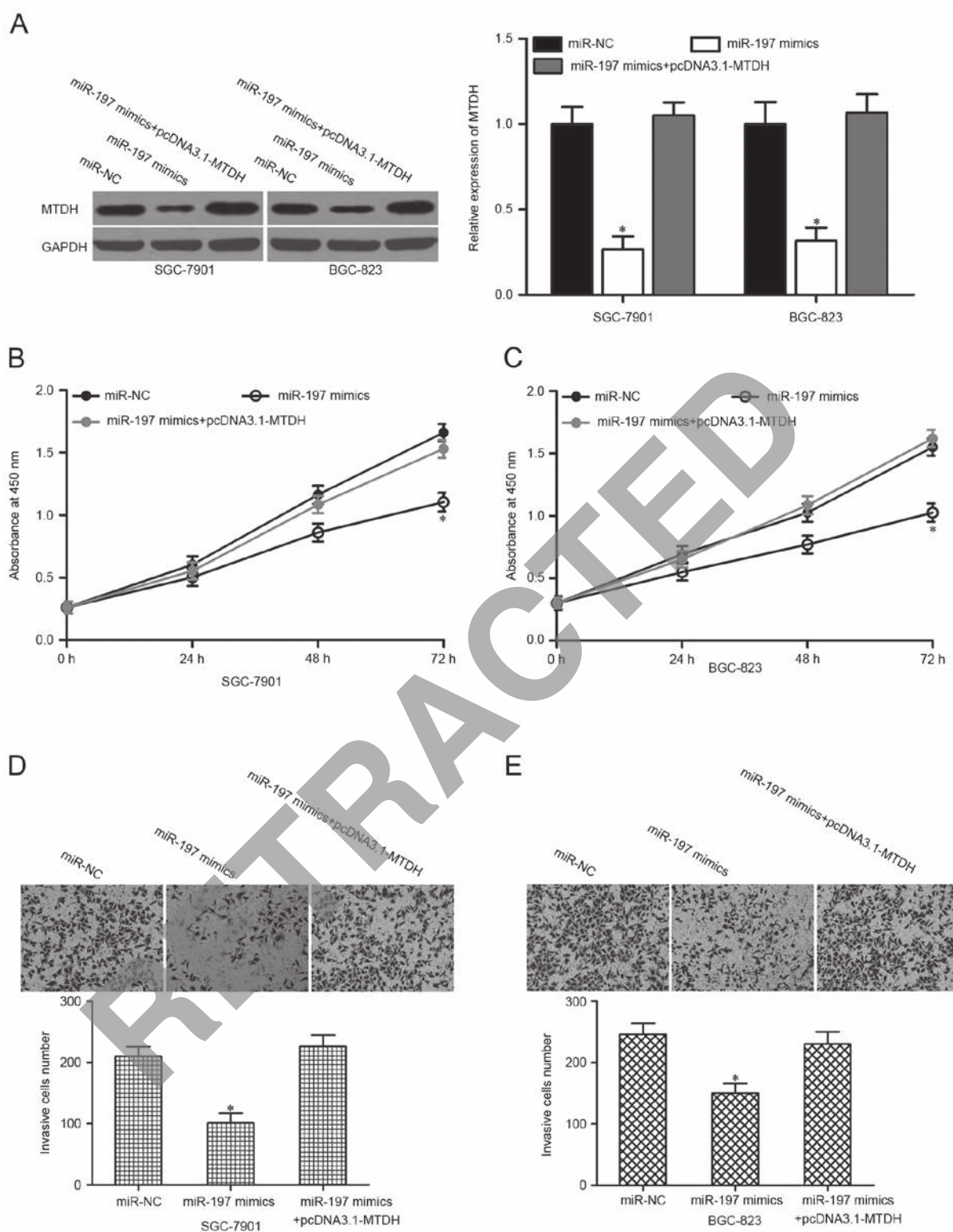


Figure 5. MTDH overexpression partially prevents the tumour-suppressing effects of miR-197 in gastric cancer cells. (A) Western blot analysis of MTDH expression levels in the SGC-7901 and BGC-823 cells transfected with miR-197 mimics with or without pcDNA3.1-MTDH. (B-D) Co-transfection with the miR-197 mimics and pcDNA3.1-MTDH reversed the suppressive effects of miR-197 overexpression on the proliferation of (B) SGC-7901 and (C) BGC-823 and invasion of (D) SGC-7901 and (E) BGC-823 cells. \* $P < 0.05$  vs. miR-NC and miR-197 mimics + pcDNA3.1-MTDH. MiR, microRNA; MTDH, metadherin; NC, negative control.

mimics were transfected into SGC-7901 and BGC-823 cells with or without pcDNA3.1-MTDH. Co-transfection of pcDNA3.1-MTDH and miR-197 mimics enabled the recovery of MTDH expression ( $P < 0.05$ ; Fig. 5A). Furthermore, MTDH restoration rescued the proliferation (Fig. 5B and C;  $P < 0.05$ )

and invasion (Fig. 5D and E;  $P < 0.05$ ) inhibition induced by miR-197 overexpression in SGC-7901 and BGC-823 cells. These results suggested that MTDH inhibition by miR-197 may partially contribute to tumour-suppression in human gastric cancer.

*miR-197 regulates the phosphatase and tensin homolog (PTEN)/AKT pathway in gastric cancer.* MTDH negatively regulates PTEN expression by blocking PTEN transcription (28,29). Western blot analysis was conducted to detect PTEN, AKT and phosphorylated (p)-AKT protein expression levels in SGC-7901 and BGC-823 cells following transfection with miR-197 mimics or miR-NC. The results revealed that PTEN protein levels were notably increased in cells transfected with miR-197 mimics, compared with miR-NC-transfected cells (Fig. 6). p-AKT expression was reduced by overexpression of miR-197, and AKT expression levels were unaltered by miR-197 overexpression in SGC-7901 and BGC-823 cells (Fig. 6;  $P < 0.05$ ). These results suggested that miR-197 may indirectly regulate the PTEN/AKT signalling pathway in gastric cancer by regulating MTDH expression.

## Discussion

miRNAs are a group of small RNAs that regulate several cellular processes (30,31). A large number of miRNAs are involved in gastric cancer formation and progression and thus may be used as effective diagnostic biomarkers for gastric cancer therapy (32). In the present study, miR-197 expression was demonstrated to be downregulated in the gastric cancer tissues and cell lines. Low miR-197 expression was significantly associated with tumour size, invasive depth, TNM staging and lymph node metastasis in gastric cancer. The results also demonstrated that miR-197 overexpression significantly inhibited gastric cancer cell proliferation and invasion *in vitro*. Notably, MTDH was confirmed as a direct target of miR-197. These findings may contribute to the establishment of effective therapeutic targets for gastric cancer treatment.

MiR-197 is downregulated in multiple malignant tumours. For example, expression levels of miR-197 is decreased in patients with oesophageal cancer with poor prognosis, and Kaplan-Meier analysis indicated that miR-197 expression is associated with survival rate of oesophageal cancer patients (33). Furthermore, patients with oesophageal cancer with low miR-197 levels commonly had short survival outcomes (33). Low miR-197 expression was also reported in colorectal cancer (22), glioblastoma (23) and multiple myeloma (24). However, in hepatocellular carcinoma, miR-197 expression levels are increased in tumour tissues and cell lines (34). In lung cancer, miR-197 is upregulated and significantly associated with large tumours and squamous cell carcinoma histotype (35). In addition, high expression of miR-197 is a poor prognosis marker for patients with non-small cell lung cancer (35). Notably, miR-197 is highly expressed in breast cancer (36), Wilms tumour (37) and pancreatic cancer (38). These conflicting results suggested that miR-197 expression is varied and tissue-dependent in human cancers.

miR-197 serves as a tumour suppressor in tumourigenicity and tumour progression. It has been reported that miR-197 inhibits cell growth and metastasis of glioblastoma (23,39). A previous study revealed that miR-197 upregulation increased the sensitivity of colorectal cancer cells to 5-fluorouracil (40), and another demonstrated that restoration of

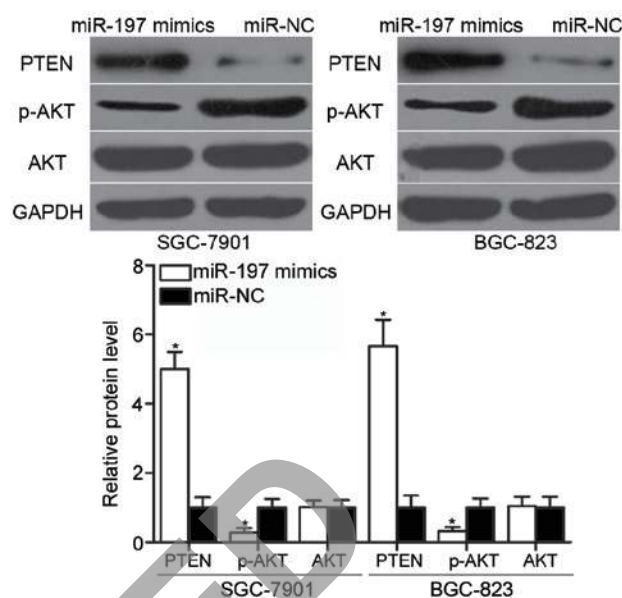


Figure 6. miR-197 regulates the PTEN/AKT signalling pathway in SGC-7901 and BGC-823 cells. After transfection with miR-197 mimics or miR-NC, western blot analysis was performed to detect PTEN, AKT and p-AKT expression levels. \* $P < 0.05$  vs. respective miR-NC. miR-197, microRNA-197; miR-NC, microRNA negative control; PTEN, phosphatase and tensin homolog; AKT, protein kinase B; p-AKT, phosphorylated-protein kinase B.

miR-197 expression represses cell viability, colony formation and cell migration, and induces apoptosis in multiple myeloma (24). However, Dai (34) *et al* and Wang (41) *et al* demonstrated that miR-197 serves oncogenic roles in hepatocellular carcinoma by promoting cell proliferation, migration and invasion *in vitro* and *in vivo*. miR-197 knockdown was reported to attenuate Wilms tumour cell proliferation and promote apoptosis (37); whereas miR-197 overexpression was demonstrated to promote epithelial-mesenchymal transition in pancreatic cancer (38). These conflicting findings indicated that the roles of miR-197 are tissue specific in tumourigenesis and tumour development. These studies have also suggested that miR-197 contributes to these types of human cancers and may serve as a potential therapeutic target for their treatment.

Several miR-197 target genes have previously been identified, including fusion 1 (23), GRB2-associated-binding protein in glioblastoma (39), kangai 1/CD82 in hepatocellular carcinoma (34), thymidylate synthase in colorectal cancer (40), induced myeloid leukaemia cell differentiation protein MCL-1 in multiple myeloma (24), insulin-like growth factor-binding protein 3 in Wilms tumour (37) and p120 catenin in pancreatic cancer (38). In the present study, MTDH was identified as a novel, direct and functional target of miR-197 in gastric cancer. MTDH is located on chromosome 8q22 (42). MTDH is upregulated in numerous human cancers, such as colorectal cancer (43), breast cancer (44), cervical cancer (45) and bladder cancer (46). MTDH serves important roles in various biological processes in cancer occurrence and progression, including cellular growth, apoptosis, metastasis and angiogenesis (47,48).

In gastric cancer, MTDH expression levels are increased, and it was significantly associated with differentiation status,



TNM staging, invasive depth and lymph node metastasis (26). MTDH overexpression is associated with poor survival in patients with gastric cancer. Multivariate analyses have demonstrated that MTDH is an independent prognostic factor for gastric cancer (27). The results of functional assays revealed that MTDH is involved in gastric cancer proliferation, cell cycle arrest, angiogenesis and metastasis (49-52). The results of the current study demonstrated that miR-197 inhibited the proliferation and invasion of gastric cancer cells by regulating the MTDH/PTEN/AKT signalling pathway. Therefore, the miR-197/MTDH axis may provide a novel effective therapeutic target for the disease.

In conclusion, miR-197 expression was downregulated in gastric cancer and aberrantly expressed miR-197 may partially influence gastric cancer cell proliferation and invasion by directly targeting MTDH.

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