

miR-217-5p suppresses epithelial-mesenchymal transition and the NF- κ B signaling pathway in breast cancer via targeting of metadherin

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Abstract. MicroRNAs (miRNAs) have been associated with a number of human malignancies, including breast cancer (BC). However, the expression, biological function and fundamental underlying mechanism of miR-217-5p in BC remain unclear. Therefore, in the present study, the expression levels of miR-217-5p and metadherin (MTDH) were examined in BC tissues and BC cell lines using reverse transcription-quantitative PCR. Cell Counting Kit-8 assays, cell proliferation, wound healing assays, Transwell assays and western blotting were used to examine the effects of miR-217-5p on cell proliferation, migration, the epithelial-mesenchymal transition (EMT) and NF- κ B signaling pathway expression. The direct relationship between miR-217-5p and MTDH was assessed using a dual-luciferase reporter assay. The results demonstrated that significantly reduced expression levels of miR-217-5p but significantly increased mRNA expression levels of MTDH were observed in BC tissues from 35 patients with BC compared with non-tumor breast tissues. Furthermore, BC cell lines SK-BR3 and BT549 expressed miR-217-5p at markedly lower levels and MTDH at markedly higher levels compared with the breast epithelial MCF10A cell line. miR-217-5p overexpression significantly inhibited cell proliferation, invasion and migration and suppressed the EMT in BC cells. miR-217-5p overexpression also inhibited the NF- κ B signaling pathway by markedly decreasing p65 mRNA and protein expression levels but significantly increasing I κ B α expression levels. Furthermore, miR-217-5p knockdown markedly increased MTDH mRNA and protein expression levels. The

expression levels of miR-217-5p were negatively correlated with those of MTDH in BC tissues. These results suggested that restoration of MTDH expression levels could potentially attenuate the inhibitory effects of miR-217-5p overexpression on BC cell proliferation. Therefore, in conclusion miR-217-5p overexpression may inhibit cell migration, invasion, the EMT and NF- κ B signaling pathway in BC via targeting of MTDH. miR-217-5p may serve as an important potential target in BC therapy.

Introduction

Breast cancer (BC) is the most common malignancy in women, accounting for ~22% of all new cancer cases worldwide and >1.05 million new cases annually (1). Globally, 450,000 patients succumb to BC each year, which accounts for 3.7% of all cancer mortalities in women (1,2). BC is a heterogeneous disease that has a wide spectrum of clinical, pathological and prognostic subtypes. BC occurs when normal epithelial cells become hyperplastic and develop into carcinoma *in situ*, eventually transitioning to an invasive carcinoma to facilitate metastasis (3,4). Therefore, the molecular mechanism of BC tumorigenesis and development requires further study to identify novel therapeutic strategies for the treatment of this disease.

MicroRNAs (miRNAs/miRs) serve an important role in cancer metastasis (5). Abnormal expression profiles of miRNAs have been reported in numerous types of BC (6). Moreover, the dysregulation of miRNAs has been demonstrated to be involved in the tumorigenesis and progression of BC, including miR-142 (7), miR-205 (8) and miR-92 (9). Previous studies have identified various miRNAs that are aberrantly expressed in human malignancies (10-12). miR-217-5p is downregulated in gastric cancer (10), pancreatic ductal adenocarcinoma (11) and osteosarcoma (12). However, the biological function of miR-217-5p in BC remains to be elucidated.

The epithelial-mesenchymal transition (EMT) refers to the biological process whereby cells of the epithelial phenotype transform into those with a mesenchymal phenotype. This process serves an important role in embryonic development, chronic inflammation, tissue remodeling, cancer metastasis

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and various fibrotic disease states (13). The main characteristics of EMT are a reduction in the expression of cell adhesion molecules, including E-cadherin, and the transformation of the cyto keratin cytoskeleton (14). Vimentin-based cytoskeletons and morphological features are typical characteristics of cells with mesenchymal phenotypes (14). Through the EMT, epithelial cells lose polarity and their connection with the basement membrane, whereas other cells acquire mesenchymal phenotypes, such as increased migration and invasion, reduced apoptosis and the increased ability to degrade the extracellular matrix (15). The EMT is an important biological process that allows malignant tumor cells derived from epithelial cells to acquire metastatic capabilities (15).

Metadherin (MTDH) was first identified by Su *et al* (16) in 2002. MTDH has a functional role in several crucial aspects of tumor progression, including transformation, proliferation, cell survival, evasion of apoptosis, migration and invasion, metastasis, angiogenesis and chemoresistance. MTDH is located on the 8q22 locus and has been previously associated with the initiation and progression of numerous types of cancer. MTDH is also known as astrocyte-elevated gene-1 and is a type of oncogene (17). The upregulated expression of MTDH has been observed in BC, ovarian, prostate, lung, esophageal and colorectal cancer (17-19). Moreover, it promotes the invasiveness of human BC cells by inducing the EMT (18). The NF- κ B signaling pathway has been reported to participate in the MTDH-induced EMT of BC cells *in vitro* (18). Previous reports suggest that MTDH activates NF- κ B via the degradation of I κ B α and by directly binding to the p65 NF- κ B subunit (19,20).

miR-217 possibly functions as a tumor suppressor in pancreatic ductal adenocarcinoma by targeting KRAS (11,21). Furthermore, miR-217 has been reported to induce endothelial cell senescence via targeting of sirtuin 1 (22). Previously identified miR-217 targets include E2F transcription factor 3, enhancer of zeste homolog 2 and runt-related transcription factor 2, the suppression of which inhibits clear cell renal cell carcinoma, gastric cancer and glioma, respectively (21,23,24). However, miR-217 is also an oncogene that targets PTEN and peroxisome proliferator-activated receptor γ co-activator 1- α to enhance germinal center responses in mature B-cell lymphomagenesis (25,26). Based on these previous reports, it can be hypothesized that miR-217 can mediate context-dependent functions during carcinogenesis. miR-217-5p is the main member of the miR-217 family, and it is also the main research object of the aforementioned studies.

The clinical significance of miR-217-5p expression in BC remains controversial with previous studies reporting conflicting results (27,28). The mechanism by which miR-217 targets MTDH to regulate BC cell function remains unclear. Therefore, in the present study, miR-217-5p and MTDH expression levels were examined in human BC tissue and cell lines. The possible function of miR-217-5p and MTDH in BC was also investigated.

Materials and methods

Clinical tissues. In total 35 pairs of BC tissues and matched adjacent non-tumor tissues were obtained from patients (age, 28-65 years; 35 females) who were surgically treated at the

Breast Center, The Fourth Hospital of Hebei Medical University (Shijiazhuang, China) between August 2020 and November 2021. These patients were all pathologically diagnosed with breast cancer. None of the patients received any treatment prior to surgery and written informed consent was obtained from all patients. The clinicopathological information of the patients is presented in Table I. Tissue samples were frozen in liquid nitrogen and stored in a refrigerator at -80°C. The present study was approved by the Ethics Committee of The Fourth Hospital of Hebei Medical University (approval no. 2021KY056). The present study was conducted in accordance with the principles described in The Declaration of Helsinki.

Cell culture and transfection. The human breast epithelial MCF10A cell line and BC SK-BR3, MCF7 and BT549 cell lines were purchased from Procell Life Science & Technology, Co., Ltd. Cells were cultured in RPMI-1640 media (Invitrogen; Thermo Fisher Scientific, Inc.) containing 10% FBS (Invitrogen; Thermo Fisher Scientific, Inc.) and incubated with 5% CO₂ at 37°C. miR-217-5p mimic, miR-negative control (NC) mimic, miR-217-5p inhibitor and miR-NC-inhibitor were synthesized by Shanghai GenePharma Co., Ltd., and transfected into SK-BR3 or BT549 cells at a final concentration of 100 nM using Lipofectamine® 3000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C for 6 h. Subsequent experimentation was performed 48 h after transfection. The following sequences were used: miR-217-5p mimic, 5'-UACUGCAUC AGGAACUGAUUGGA-3'; miR-NC mimic, 5'-UUGUCC GAACGUGUCACGU-3'; miR-217-5p inhibitor, 5'-UCC AAUCAGUUCUGAUGCAGUA-3'; and miR-NC inhibitor, 5'-CAGCUGGUUGAAGGGGACCAAA-3'.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) was used to extract total RNA from BC tissues and BC cell lines, including miRNA. RNA quality was assessed using the A260/A280 ratio. This value was determined using a microplate reader (Multiskan Sky; Thermo Fisher Scientific, Inc.). RNA quantity was also assessed using a microplate reader. Total RNA was reverse transcribed into complementary (c)DNA using a Transcriptor First Strand cDNA Synthesis Kit [Roche Diagnostics (Shanghai) Co., Ltd.] using the following temperature protocol according to the manufacturer's instructions: 37°C for 15 min and 85°C for 5 sec. The volume of each RT reaction system was 20 μ l, including 1 μ g of total RNA. DNase was used to remove genomic DNA interference. Subsequently, qPCR was performed to examine gene expression using a SYBR Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.). The following thermocycling conditions were used for qPCR: Initial denaturation at 95°C for 5 min; followed by 40 cycles of denaturation at 95°C for 5 sec, 60°C for 30 sec and 72°C for 30 sec, with a final extension step at 72°C for 2 min. The 2^{- $\Delta\Delta$ C_q} method was used to calculate the relative expression levels using GAPDH or U6 as the internal reference gene (29). The primer sequences used for RT-qPCR are presented in Table II.

Cell proliferation assay. Cell proliferation was analyzed using the Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.) assay. Briefly, transfected SK-BR3 or

Table I. Clinicopathological characteristics of patients with breast cancer (n=35) in the present study.

Characteristics	Patients, n (%)
Age, years	
≤50	13 (37.1)
>50	22 (62.9)
Tumor size, cm	
≤3	28 (80)
>3	7 (20)
Grade	
II	24 (68.6)
III	11 (31.4)
TNM stage	
I	14 (40)
II	15 (42.9)
III	6 (17.1)
ER status	
Positive	25 (71.4)
Negative	10 (28.6)
PR status	
Positive	20 (57.1)
Negative	15 (42.9)
HER2 status	
Positive	5 (14.3)
Negative	30 (85.7)
Ki-67, %	
≤20	13 (37.1)
>20	22 (62.9)
Lymph node status	
Positive	13 (37.1)
Negative	22 (62.9)

ER, estrogen receptor; PR, progesterone receptor.

Table II. Primer sequences used for reverse transcription-quantitative PCR.

Gene	Sequence (5'-3')
miR-217-5p	F: TACTGCATCAGGAAGTGAATTGGA R: CTCAACTGGTGTCTGCTGG
U6	F: CTGGTAGGGTGTCTGCTTCGGCAG R: CAACTGGTGTCTGCTGG AGTCGG
MTDH	F: CTGTTGAAGTGGCTGAGGG R: GGTGGCTGCTTGCTGTTA
GAPDH	F: GCACCGTCAAGGCTGAGAAC R: GGATCTCGCTCCTGGAAGATG
E-cadherin	F: CGAGAGCTACACGTTACGG R: GGGTGTCTGAGGGAATAATAGG
Vimentin	F: GACGCCATCAACACCGAGTT R: CTTTGTCTGTTGGTTAGCTGGT
slug	F: TGTGACAAGGAATATGTGAGCC R: TGAGCCCTCAGATTGACCTG
p65	F: ATGTGGAGATCATTGAGCAGC R: CCTGGTCCTGTGTAGCCATT
IκBα	F: CTCCGAGACTTTCGAGGAAATAC R: GCCATTGTAGTTGGTAGCCTTCA

MTDH, metadherin; miR, microRNA; slug, snail family transcriptional repressor 2; F, forward; R, reverse.

BT549 cells were seeded into 96-well plates at a density of 2×10^3 cells/well. Following incubation at 37°C for 24, 48 and 72 h, 10 μ l CCK-8 solution was added into each well and incubated at 37°C for 4 h. The absorbance of each well at a wavelength of 450 nm was assessed using a microplate reader (Multiskan Sky; Thermo Fisher Scientific, Inc.).

Colony formation assay. Colony formation assays were performed to detect cell proliferation. Transfected SK-BR3 and BT549 cells were seeded at 1×10^3 cells/well in six-well plates and incubated with 5% CO₂ at 37°C. After 15 days, colonies were visible to the naked eye. These colonies were fixed with 4% paraformaldehyde for 15 min at ambient temperature and stained with 1% crystal violet (Beyotime Institute of Biotechnology) for 15 min at ambient temperature. The number of colonies (containing >50 cells) was counted microscopically.

Wound healing assay. After 48 h of transfection, 2×10^5 SK-BR3 or BT549 cells were transferred into a 35-mm²

petri dish, cultured at 37°C and grown to 90% confluence. A wound was then created using a 200- μ l pipette tip to scratch the monolayer before any detached cells were washed away with PBS. The remaining cells were cultured in serum-free culture media, and wound closure was then observed. Images of the wounds were taken at 0 and 24 h after the scratch was made using a light microscope (magnification, x100; Olympus Corporation). Migratory ability was calculated as the mobility ratio using the following formula: Mobility ratio=(scratch width at 24 h-scratch width at 0 h)/scratch width at 0 h.

Western blotting. Total protein was extracted using RIPA lysis buffer (Beyotime Institute of Biotechnology) from transfected SK-BR3 and BT549 cell lines. Protein concentration was determined by BCA assay (Thermo Fisher Scientific, Inc.). Next, the protein lysates (30 μ g) were separated using SDS-PAGE on a 10% gel and separated proteins were then transferred onto PVDF membranes (MilliporeSigma). Subsequently, the membranes were incubated with 5% skimmed milk at room temperature for 2 h, before they were incubated with the following primary antibodies against: MTDH (1:2,000; cat. no. ab227981; Abcam), GAPDH (1:10,000; cat. no. 10494-1-Ig; ProteinTech Group, Inc.), p65 (1:2,000; cat. no. 10745-1-Ig; ProteinTech Group, Inc.), p-p65 (1:2,000; cat. no. 3033; Cell Signaling Technology), IκBα (1:2,000; cat. no. 10268-1-Ig; ProteinTech Group, Inc.), E-cadherin (1:2,000; cat. no. 20874-1-Ig; ProteinTech Group, Inc.), Vimentin (1:20,000; cat. no. 10366-1-Ig; ProteinTech Group, Inc.) and snail family transcriptional repressor 2 (slug; 1:2,000; cat. no. 12129-1-AP; ProteinTech Group, Inc.),

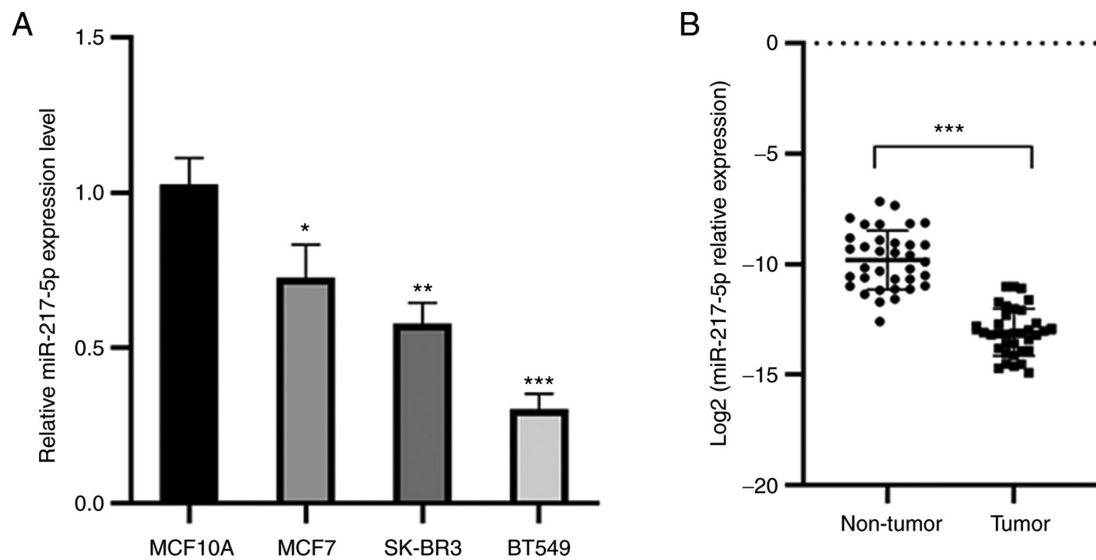


Figure 1. miR-217-5p is downregulated in BC lines and tissues. Reverse transcription-quantitative-PCR analysis of miR-217-5p expression levels in (A) BC cell lines and (B) BC tissues. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.005$. miR, microRNA; BC, breast cancer.

overnight at 4°C. After washing with TBST (0.05% Tween 20), the membranes were incubated with the HRP-conjugated goat polyclonal anti-rabbit IgG secondary antibody (1:10,000; cat. no. SA00001-2; ProteinTech Group, Inc.) for 1 h. Protein expression was determined by enhanced chemiluminescence (MilliporeSigma).

Invasion assay. After transfection with miR-217-5p mimic, miR-NC mimic, miR-217-5p inhibitor and miR-NC inhibitor for 48 h at 37°C, a total of 5×10^5 SK-BR3 and BT549 cells were resuspended in 200 μ l serum-free medium and subsequently seeded into the upper chamber of a Transwell insert (Corning, Inc.) precoated with 1 μ g/ μ l Matrigel (BD Biosciences) overnight at 37°C. The lower chamber was filled with 500 μ l basal medium with 10% FBS to stimulate cell invasion. Following 48 h of cell culture at 37°C, the cells that did not cross the membrane were wiped with a cotton swab, whereas the cells adhering to the lower surface of the membrane were stained with 0.1% crystal violet solution at room temperature for 10 min. The number of invading cells was counted in five randomly selected fields using a light microscope (magnification, x200; Olympus Corporation).

Migration assay. A total of 5×10^5 transfected SK-BR3 and BT549 cells were resuspended in 200 μ l serum-free medium and were subsequently seeded into the upper chamber of a Transwell insert without Matrigel. All other procedures were performed as described for the invasion assay.

Bioinformatics analysis. The TargetScan (http://www.targetscan.org/vert_70/) database was used to predict the target genes of miR-217-5p.

Dual-luciferase reporter assay. The 3'-untranslated regions (UTR) of wild type or mutant MTDH were inserted into the pmirGLO luciferase vector (Shanghai GeneChem Co., Ltd.). These vectors and miR-217-5p mimic or miR-NC mimic were co-transfected into BT549 cells using Lipofectamine® 3000

(Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h at 37°C. The lysate was cleared and the luciferase reporter activity was assayed at 48 h post-transfection using the Dual-Luciferase Reporter Assay System (Promega Corporation). The firefly luciferase activity was normalized using *Renilla* luciferase activity.

Statistical analysis. Data were analyzed using SPSS 23.0 (IBM Corp.) and GraphPad Prism 8 (GraphPad Software, Inc.). Data are presented as the mean \pm SD. All comparisons between two groups of cells were performed using unpaired Student's t-tests. Paired Student's t-tests were performed for comparisons between paired tumor and adjacent non-tumor tissues. Two-way ANOVA followed by Bonferroni's post hoc test were used to compare data from the dual-luciferase reporter assay. The gene expression correlation between miR-217-5p and MTDH was assessed using the Pearson's correlation coefficient test. All experiments were performed at least three times. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

miR-217-5p expression levels are reduced in BC tissues and cell lines. miR-217-5p expression levels were demonstrated to be markedly reduced in MCF7, BT549 and SK-BR3 cell lines compared with MCF10A cells (Fig. 1A). Furthermore, the expression levels of miR-217-5p were significantly lower in BC tissues compared with those in the adjacent non-tumor tissues (Fig. 1B). This indicated that miR-217-5p expression levels were lower in both BC cell lines and tissues.

Overexpression of miR-217-5p inhibits cell proliferation, colony formation, migration and invasion of BC cells. To investigate the specific function of miR-217-5p in BC, miR-217-5p mimic was transfected into SK-BR3 cells, whereas miR-217-5p inhibitor was transfected into BT549 cells (two cell lines with highest transfection efficiency). Compared with

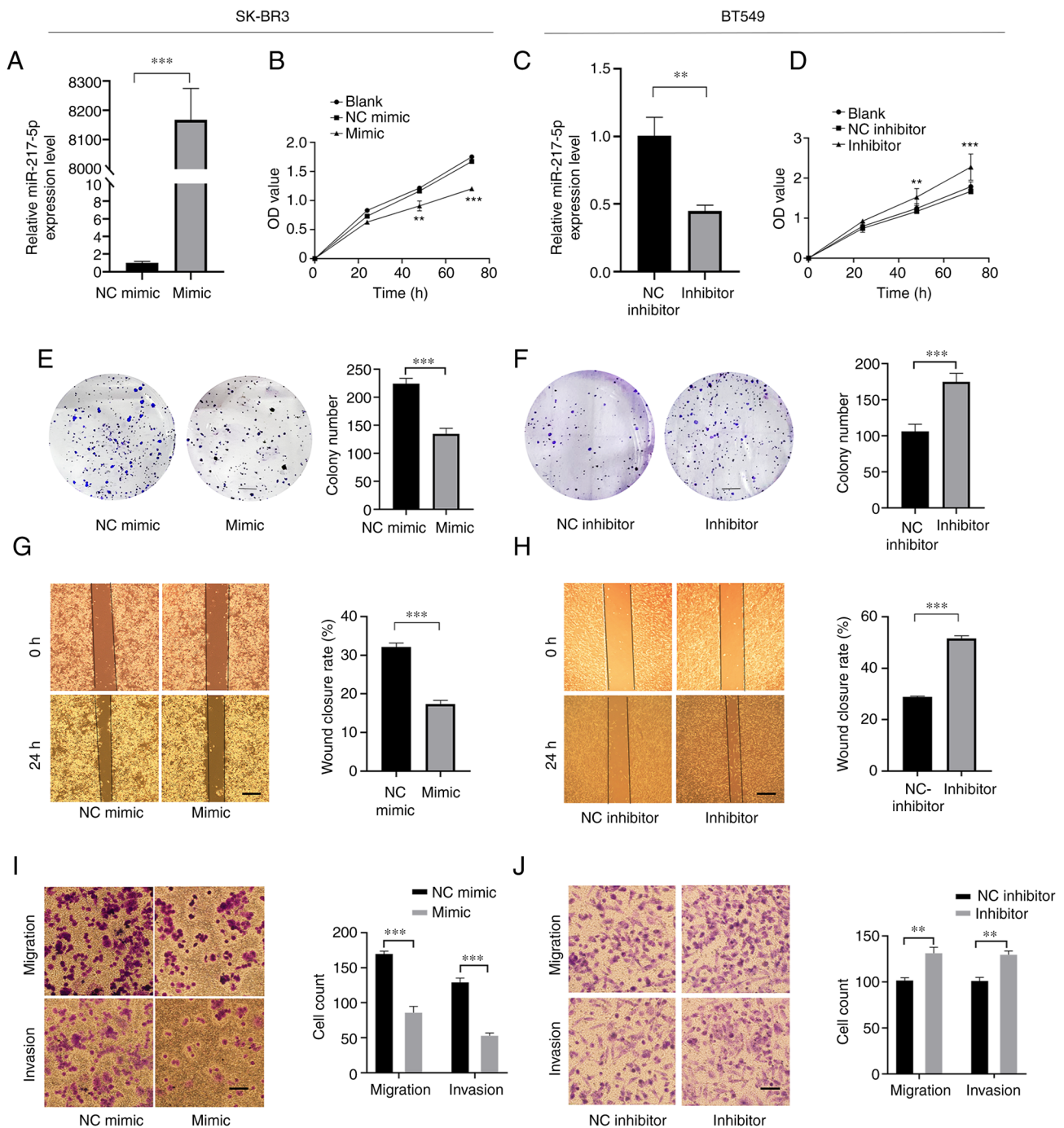


Figure 2. Overexpression of miR-217-5p inhibits cell proliferation, colony formation, migration and invasion of breast cancer cells. (A) RT-qPCR analysis of miR-217-5p expression levels in SK-BR3 cells transfected with miR-217-5p or miR-NC mimic. (B) Cell proliferation in SK-BR3 cells transfected with miR-217-5p mimic or miR-NC mimic. (C) RT-qPCR analysis of miR-217-5p expression levels in BT549 cells. (D) Cell proliferation in SK-BR3 cells transfected with miR-217-5p inhibitor or the miR-NC inhibitor. Colony formation by (E) SK-BR3 and (F) BT549 cells transfected with miR-217-5p mimic or inhibitor, respectively, or the corresponding NCs. Scale bar, 5 mm. Wound healing assays for (G) SK-BR3 and (H) BT549 cells transfected with miR-217-5p mimic or inhibitor, respectively, or the corresponding NCs. Scale bar, 500 μ m. Magnification, x100. Transwell assay of (I) SK-BR3 and (J) BT549 cells transfected with miR-217-5p mimic or inhibitor, respectively, or the corresponding NCs. Scale bar, 100 μ m. Magnification, x200. **P<0.01 and ***P<0.005. miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; OD, optical density.

the negative control (NC), the results demonstrated that transfection with the miR-217-5p mimic significantly increased miR-217-5p expression levels, whereas miR-217-5p inhibitor transfection significantly decreased miR-217-5p expression levels (Fig. 2A and C). The CCK-8 assay demonstrated that compared with the NC, transfection with the miR-217-5p mimic in SK-BR3 cells significantly inhibited cell proliferation at 48

and 72 h, whereas transfection with the miR-217-5p inhibitor into BT549 cells significantly promoted cell proliferation at 48 and 72 h (Fig. 2B and D). Furthermore, colony formation assays demonstrated that miR-217-5p overexpression resulted in the significant inhibition of BC cell proliferation, whereas miR-217-5p expression knockdown significantly promoted colony formation compared with the NC group (Fig. 2E and F).

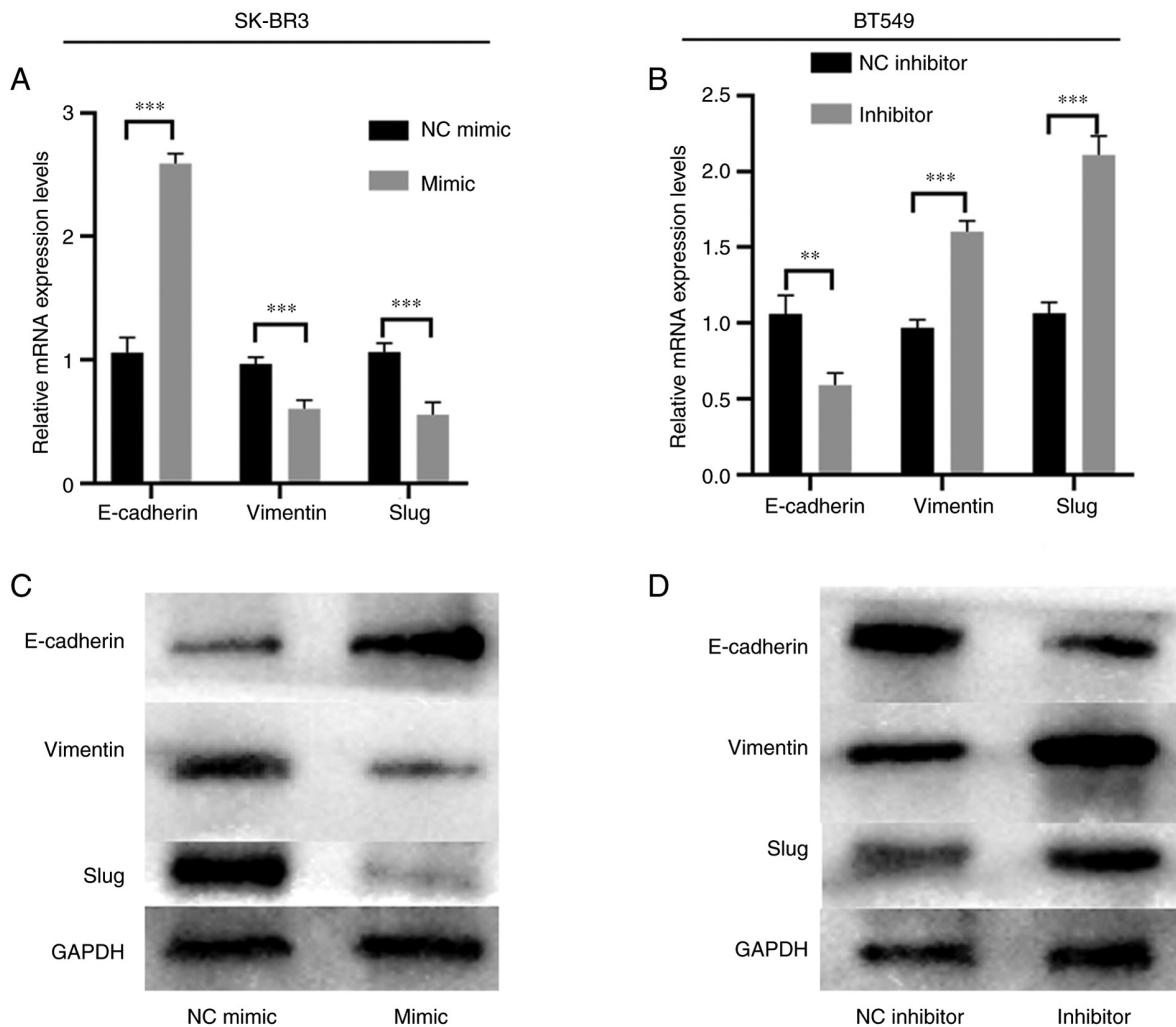


Figure 3. Overexpression of miR-217-5p prevents the epithelial-mesenchymal transition in breast cancer cells. (A) RT-qPCR and (C) Western blotting analysis of the expression level of E-cadherin, N-cadherin and Vimentin in SK-BR3 cells transfected with miR-217-5p mimic and miR-NC mimic. (B) RT-qPCR and (D) Western blotting analysis of the expression level of E-cadherin, N-cadherin and Vimentin in BT549 transfected with miR-217-5p inhibitor and miR-NC inhibitor. ** $P < 0.01$ and *** $P < 0.005$. miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; slug, snail family transcriptional repressor 2.

Wound healing assays determined that transfection with the miR-217-5p inhibitor into BT549 cells significantly promoted cell migration, whereas miR-217-5p overexpression in SK-BR3 cells significantly suppressed cell migration, compared with the NC group (Fig. 2G and H). Transwell assays demonstrated that miR-217-5p knockdown significantly promoted cell migration and invasion in BT549 cells, but miR-217-5p overexpression significantly inhibited SK-BR3 cell migration and invasion compared with the NC group (Fig. 2I and J). The aforementioned results indicated that miR-217-5p exerted a tumor suppressive role in BC cells.

Overexpression of miR-217-5p prevents the EMT in BC cells. E-cadherin, Vimentin and slug mRNA and protein expression levels were analyzed using RT-qPCR and western blotting to explore the effects of miR-217-5p on SK-BR3 and BT549 cells further. Overexpression of miR-217-5p markedly enhanced E-cadherin mRNA and protein expression levels, whereas Vimentin and slug expression levels were markedly inhibited compared with the NC group (Fig. 3A and C). However, compared with the NC, miR-217-5p knockdown markedly

decreased E-cadherin mRNA and protein expression levels but markedly promoted Vimentin and slug mRNA and protein expression levels (Fig. 3B and D). These results suggested that miR-217-5p overexpression may suppress the EMT in BC.

Overexpression of miR-217-5p inhibits the NF- κ B signaling pathway. The mRNA and protein expression levels of NF- κ B markers were subsequently assessed to determine the effects of miR-217-5p on SK-BR3 and BT549 cells. RT-qPCR and western blotting demonstrated that miR-217-5p overexpression markedly inhibited p65 phosphorylation but markedly promoted I κ B α expression levels compared with the NC (Fig. 4A and C). However, miR-217-5p knockdown markedly suppressed I κ B α mRNA and protein expression levels but markedly promoted p65 phosphorylation compared with the NC (Fig. 4B and D). These results indicated that the down-regulation of miR-217-5p may activate the NF- κ B signaling pathway.

miR-217-5p directly targets MTDH in BC cells. TargetScan (http://www.targetscan.org/vert_71/) was used to predict

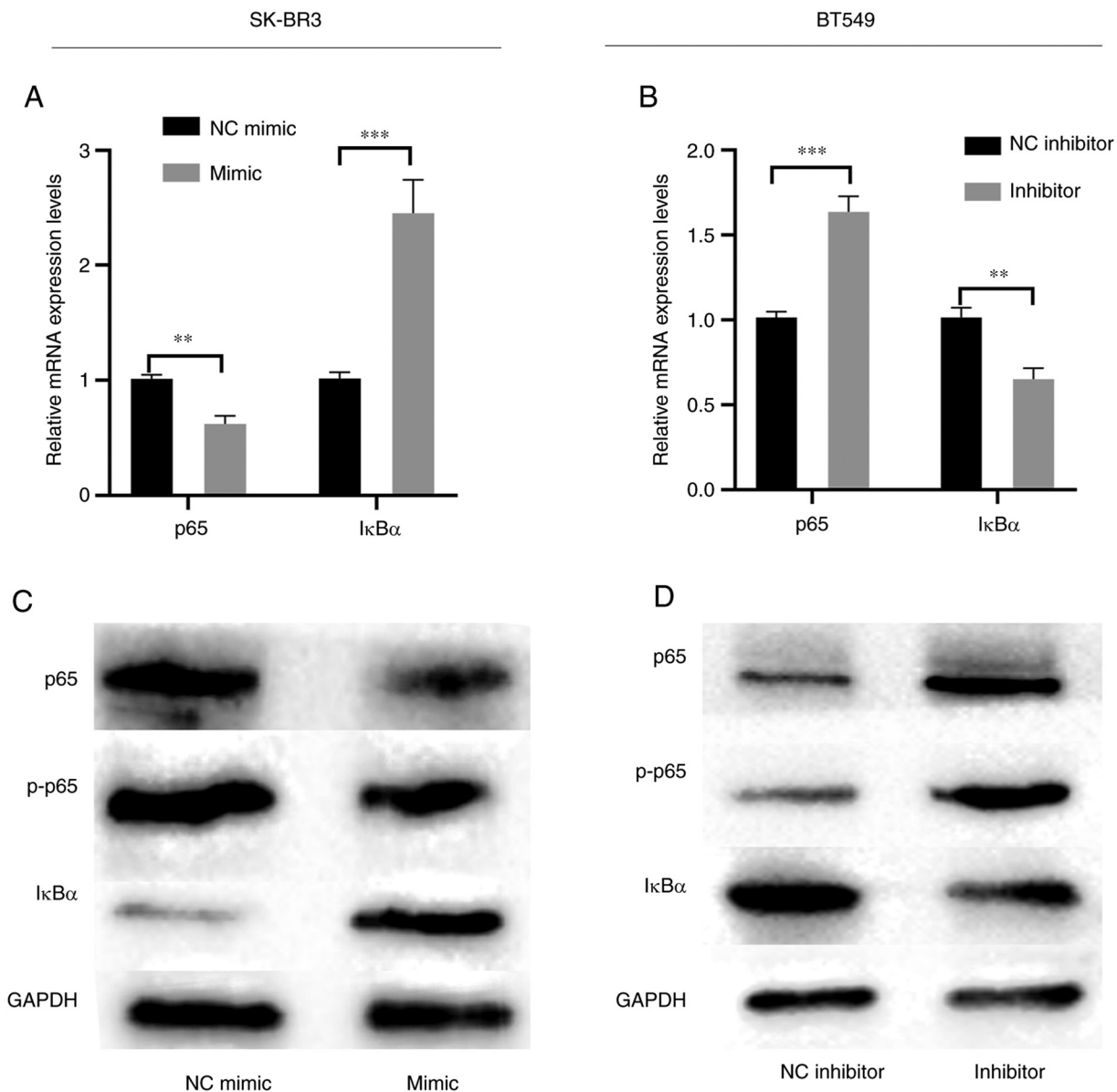


Figure 4. Overexpression of miR-217-5p inhibits the NF-κB signaling pathway. (A) RT-qPCR analysis of the mRNA expression levels of p65 and IκBα in SK-BR3 cells transfected with miR-217-5p mimic or miR-NC mimic. (B) RT-qPCR analysis of the mRNA expression levels of p65 and IκBα in BT549 cells transfected with miR-217-5p inhibitor or miR-NC inhibitor. (C) Western blotting analysis of the protein expression levels of p65, p-p65 and IκBα in SK-BR3 cells transfected with miR-217-5p mimic or miR-NC mimic. (D) Western blotting analysis of the protein expression levels of p65, p-p65 and IκBα in BT549 cells transfected with miR-217-5p inhibitor or miR-NC inhibitor. ** $P < 0.01$ and *** $P < 0.005$. miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; p, phosphorylated.

miR-217-5p target genes. MTDH was determined to be a candidate target for miR-217-5p. Therefore, dual-luciferase reporter assays were performed to assess the potential binding between MTDH mRNA and miR-217-5p (Fig. 5A). MTDH mRNA expression levels were demonstrated to be markedly increased in MCF7, BT549 and SK-BR3 cell lines compared with MCF10A cells (Fig. 5B). Moreover, significantly higher MTDH mRNA expression levels were demonstrated in BC tissues compared with adjacent non-tumor tissues (Fig. 5C). Transfection with miR-217-5p mimic significantly reduced the luciferase activity of the wild-type MTDH 3'UTR compared with the miR-NC mimic. However, there was no change in luciferase activity in the mutant-MTDH 3'UTR group (Fig. 5D). Furthermore, MTDH mRNA expression levels were demonstrated to be significantly inversely correlated

with those of miR-217-5p in BC tissues (Fig. 5E). The results also demonstrated that compared with the NC, miR-217-5p overexpression markedly inhibited MTDH mRNA and protein expression levels (Fig. 5F), whereas miR-217-5p knockdown markedly promoted MTDH mRNA and protein expression levels (Fig. 5G). These data therefore suggested that miR-217-5p may directly target MTDH and that the expression of miR-217-5p is inversely correlated with MTDH in BC cells.

Discussion

Previous studies have demonstrated that miRNAs serve important roles in the growth and metastasis of malignant types of cancer, including BC (7,30). miR-217 has been reported to suppress tumors associated with gastric cancer (24),

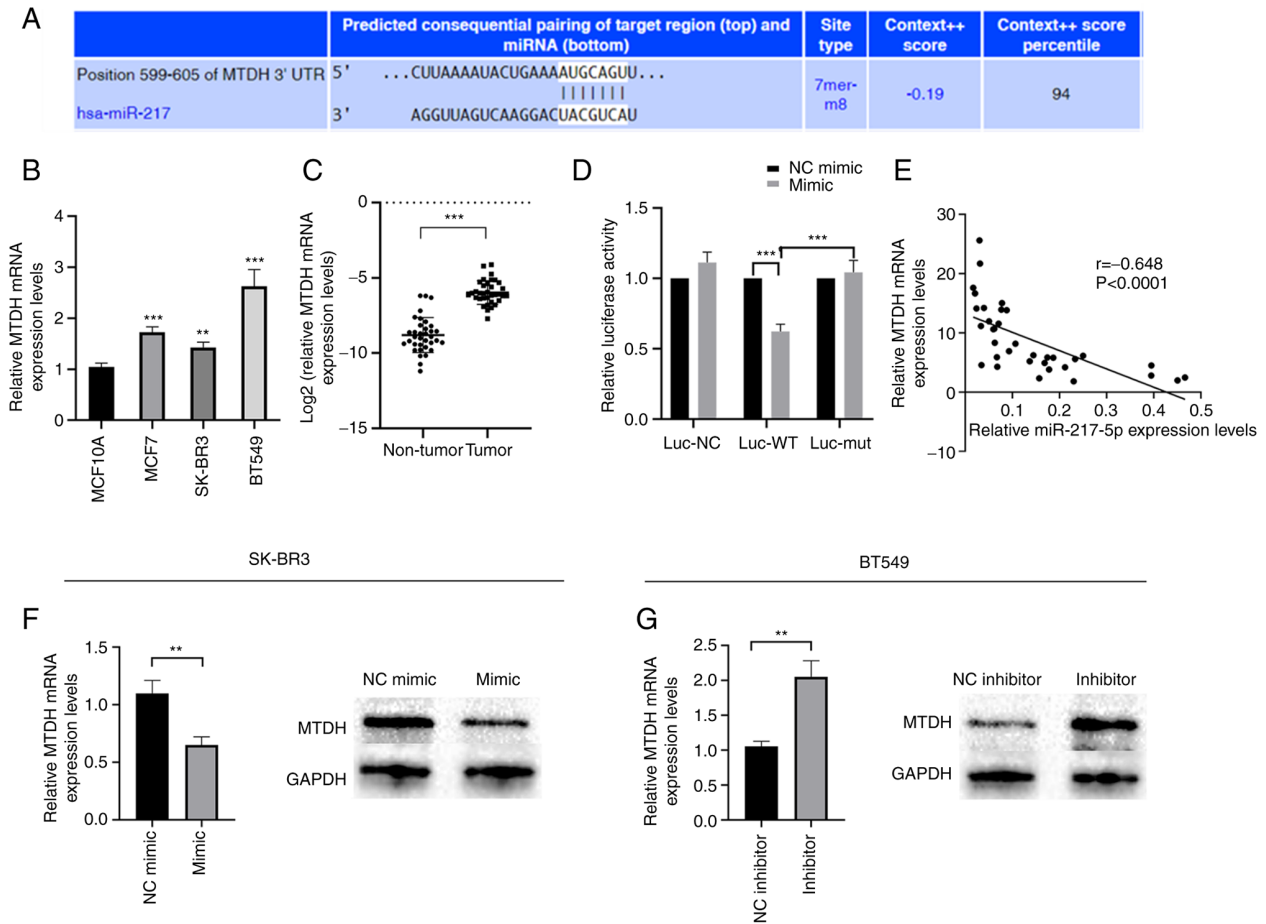


Figure 5. MTDH is a direct target of miR-217-5p. (A) Schematic representation of MTDH 3'-UTR demonstrating the putative miR-217-5p target site. RT-qPCR analysis of MTDH mRNA expression levels in (B) BC cell lines and (C) BC tissues. (D) Analysis of the relative luciferase activities of MTDH-WT and MTDH-mut. (E) Pearson's correlation coefficient analysis of miR-217-5p and MTDH expression levels in BC tissues. RT-qPCR and western blotting analysis of MTDH expression levels in (F) SK-BR3 and (G) BT549 cells transfected with miR-217-5p mimic or inhibitor, respectively, or the corresponding negative control. ** $P<0.01$ and *** $P<0.005$. MTDH, metadherin; miR, microRNA; UTR, untranslated region; RT-qPCR, reverse transcription-quantitative PCR; BC, breast cancer; WT, wild-type; mut, mutant; NC, negative control; luc, luciferase.

pancreatic ductal adenocarcinoma (23), hepatocellular carcinoma (31), osteosarcoma (12) and chronic myelogenous leukemia (32). However, results from previous studies on BC are controversial. Zhang *et al* (27) reported that miR-217 is frequently overexpressed in BC, which enhances tumor growth by targeting Dachshund homolog 1 expression and promoting cell cycle progression. However, Zhou *et al* (28) demonstrated that miR-217 suppresses triple-negative BC cell proliferation, migration and invasion, at least in part by down-regulating Kruppel-like factor 5 expression. miR-217-5p is an important member of the miR-217 family. In the present study, miR-217-5p downregulation was detected in BC tissues and BT549 and SK-BR3 cells. Therefore, miR-217-5p may serve distinct roles in different BC cell lines.

The EMT is a process that describes the molecular reprogramming and phenotypic changes during the transition of polarized, immotile epithelial cells into motile mesenchymal cells, which exhibit increased migratory and invasive capacities (13). The activation of the EMT is characterized by the reduced expression of epithelial markers, such as E-cadherin, coupled with the increased expression of mesenchymal markers, such as Vimentin and slug. It has also been reported that miRNAs can serve as regulators of malignant

transformation and metastasis (14). In the present study, miR-217-5p was found to regulate the mRNA and protein expression levels of E-cadherin, Vimentin and slug. Therefore, these results indicated that miR-217-5p expression potentially inhibits the EMT of BC cells.

Previous studies have demonstrated that the activation of the NF- κ B signaling pathway is associated with BC development (33,34). I κ B is a specific intracellular inhibitor of NF- κ B. In the majority of cell types, NF- κ B exists in an inactive form by forming a complex with I κ B in the cytoplasm (34). A previous study reported that NF- κ B is constitutively activated in hepatocellular carcinoma cell lines (31). Results from the present study demonstrated that the overexpression of miR-217-5p significantly inhibited NF- κ B signaling. These results therefore indicated that miR-217-5p may prevent BC progression by potentially regulating the NF- κ B signaling pathway.

Given the potential importance of miR-217-5p in BC, further experiments were performed to assess the consequences of miR-217-5p activation. Using bioinformatics analysis combined with the dual-luciferase reporter assay, MTDH was demonstrated to be a direct target of miR-217-5p. A previous study reported that miR-217 can target MTDH to promote the

progression of meningioma (35). MTDH has also been reported to be an important oncogene in the carcinogenesis, progression and metastasis of numerous malignancies, via regulating and PI3K/AKT, ERK/MAPK, Wnt/ β -catenin and aurora-A kinase signaling pathways (36,37). Furthermore, the positive effects of MTDH on cell migration, invasion and EMT have also been previously determined in BC (36). Furthermore, MTDH has been reported to regulate tumorigenesis by interacting with several miRNAs, including miR-145 (38) and miR-630 (39). In the present study, miR-217-5p was demonstrated to significantly negatively regulate MTDH expression in BC. These results suggested that miR-217-5p may be involved in BC progression via the inhibition of MTDH.

A previous study by Wang *et al* (40) reported that decreased miR-217 expression is associated with poor prognosis in patients with colorectal cancer. In the present study, based on the available data, none of the enrolled patients with BC experienced disease progression or death. As the prognosis of breast cancer is relatively good, it is usually difficult to observe differences in survival after a short period of follow-up, such as several months. The small sample size may also cause some bias. Therefore, in future research, the sample size will be expanded and the follow-up time extended, and related research on the impact of miR-217-5p on the prognosis of breast cancer will continue to be conducted.

In conclusion, the data from the present study suggested that miR-217-5p overexpression may inhibit cell migration, invasion and the EMT via targeting MTDH expression in BC. It can therefore be hypothesized that the miR-217-5p/MTDH axis is likely to be involved in BC via the regulation of the NF- κ B signaling pathway. These findings indicated that miR-217-5p may serve as an important potential target in BC therapy.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZS designed and conceptualized the present study. LXY, BX and SL were responsible for data acquisition. LY, XK and MW analyzed and interpreted the data. LXY drafted the manuscript. ZS and XK revised the manuscript for critically important intellectual content. LXY and ZS confirm the authenticity of all the raw data. All authors contributed to the article and read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of The Fourth Hospital of Hebei Medical University (approval no. 2021KY056). The present study was conducted in accordance with the principles described in The Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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