SPANDIDOS PUBLICATIONS

ONCOLOGY LETTERS 18: 2232-2239, 2019

MicroRNA-589 serves as a tumor suppressor microRNA through directly targeting metastasis-associated protein 2 in breast cancer

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Received October 3, 2018; Accepted April 15, 2019

DOI: 10.3892/ol.2019.10548

Abstract. Triple-negative breast cancer (TNBC) has a poorer outcome compared with that of other subtypes of breast cancer, and the discovery of dysregulated microRNA (miRNA) and their role in tumor progression has provided a new avenue for elucidating the mechanism involved in TNBC. Previous studies have revealed that aberrant expression of miRNA-589 (miR-589) was frequently observed in various types of cancer. However, the expression and function of miR-589 in TNBC has not been well illustrated. In the present study, the expression level of miR-589 was explored in TNBC tissues and TNBC cell lines by quantitative polymerase chain reaction (qPCR). The results revealed that the expression of miR-589 was decreased in TNBC tissues and cell lines compared with that in normal tissues and breast cell lines. Furthermore, miR-589 overexpression decreased the TNBC cell proliferation, migration and invasion, whereas miR-589 silencing generated the opposite results *in vitro*. Bioinformatic algorithms predicted a direct target site for miR-589 in the 3'-untranslated region of metastasis-associated protein 2 (MTA2), which was confirmed with a dual-luciferase reporter assay and western blot analysis. Results of the qPCR and western blot analysis illustrated that miR-589 negatively regulated MTA2 expression with regard to mRNA and protein levels in the TNBC cell lines. MTA2 silencing reversed the promotion function of miR-589 inhibitor in the TNBC cell line. Finally, miR-589 could inhibit the process of epithelial-mesenchymal transition via MTA2. In summary, the present study revealed the biological function and molecular mechanism of miR-589 in the progression of TNBC. MiR-589 inhibition in the progression of TNBC may be a potential therapeutic target for TNBC.

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Key words: microRNA-589, triple negative breast cancer, migration and invasion, MTA2, epithelial-mesenchymal transition

Introduction

Breast cancer is a common type of tumor among women, and the second leading cause of cancer-associated mortality worldwide in 2017 (1,2). Triple-negative breast cancer (TNBC) refers to breast cancer lacking the expression of estrogen receptor (ER), progesterone receptor (PR) and hormone epidermal growth factor receptor-2 (HER2) (3,4). TNBC accounts for 15% of breast cancer cases, and is associated with a poorer prognosis compared with that of other subtypes (3,4). Patients with TNBC have a higher risk of distant metastasis and a poorer overall survival compared with that of patients with other types of breast cancer, partly due to the lack of effective targeted therapies (4). Although patients with TNBC are sensitive to chemotherapy, they are prone to medical treatment resistance (4,5). In addition, satisfactory targeted therapies for TNBC are unavailable due to the lack of expression of ER, PR and HER2. Therefore, the discovery of new molecular targets to treat patients with TNBC is required.

MicroRNAs (miRNAs) are endogenous non-coding small RNAs that consist of 21-22 nucleotides that are abundant in organisms (6). A number of studies have revealed that miRNAs are involved in various cell processes, including differentiation, proliferation, metastasis and apoptosis (7). Previous studies have illustrated that miRNAs are aberrantly expressed in some types of cancer, including breast cancer (8-10). Although the study of miRNAs is challenging due to the variable downstream target genes of miRNAs, the application of miRNAs for TNBC therapy is crucial. Previous studies illustrated that miRNA-589 (miR-589) serves as a tumor suppressor in non-small cell lung cancer, glioma and hepatocellular carcinoma (11-14). By contrast, miR-589 acts as an oncogene in gastric cancer (15). These results revealed that the same miRNA may serve as an oncogene or tumor suppressor depending on the tissue type. To date, the expression, biological function and molecular mechanism of miR-589 in TNBC has not been determined.

The objective of the present study was to determine the expression and biological role of miR-589 in TNBC, and to identify the direct target gene(s) that mediate the activity of miR-589 in TNBC.

Materials and methods

TNBC tissues. Paired TNBC tissues and adjacent non-cancerous tissues (>5 cm distant from the cancer tissue) were obtained

from the Central Hospital of Zibo (Shandong, China). A total of 20 female patients (age range, 34-71 years; mean age, 61 years) underwent modified radical mastectomy at Central Hospital of Zibo between January 2016 and January 2017. None of the patients had received chemotherapy or radiotherapy prior to surgery. ER and PR tumor status were determined by immunohistochemistry (IHC). In brief, paraffin-embedded sections of tumor tissue (4 μm thick) were deparaffinized in xylene, rehydrated via a graded alcohol series and blocked in methanol containing 3% hydrogen peroxide for 10 min at room temperature. The sections were incubated with ERα antibody (cat. no. sc-8002; 1:200 dilution; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) or PR antibody (cat. no. sc-166169; 1:200 dilution; Santa Cruz Biotechnology, Inc.) overnight at 4˚C. Biotinylated goat anti-mouse secondary antibodies (cat. no. ab64255; 1:1,000 dilution; Abcam, Cambridge, MA, USA) were applied and incubated for 30 min at 37˚C, and then the streptavidin-peroxidase conjugate (1:200 dilution; Fuzhou Maixin Biotech Co., Ltd.) was added and incubated at 37˚C for 30 min. Finally, the immunoreactions of sections were visualized by staining with 3'-diaminobenzidine (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) for 3 min at room temperature and counterstaining with hematoxylin for 30 sec at room temperature. Subsequently, the IHC-stained sections were observed under a light microscope (magnification, x400; Nikon Corporation, Tokyo, Japan). Cases with breast cancer that had at least 1% of cells staining positive for ER or PR were considered as ER-positive or PR-positive. HER2 tumor status was detected by fluorescence *in situ* hybridization (FISH). The PathVysion HER2/neu DNA probe kit II (Abbott Pharmaceutical Co. Ltd., Lake Bluff, IL, USA), which is designed to detect amplification of the HER2/neu gene via FISH in formalin-fixed paraffin-embedded human breast cancer tissue specimens, was used according to the manufacturer's protocol (16). A high mean copy number of HER2 (ufacturer's plificawas considered positive regardless of the HER2/chromosome enumeration probe 17 ratio. Written informed consent was obtained from all the patients and the study was approved by the Ethics Committee of the Central Hospital of Zibo (approval no. ZB 20151229005).

Cell culture. The human TNBC cell lines (MDA-MB-231, HCC-1937 and MDA-MB-468), the immortal mammary epithelial cell line (MCF-10A) and 293T cells were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cell lines were cultured at 37˚C in a humidified incubator with 5% CO_2. The TNBC cell lines and 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA USA) containing 10% fetal bovine serum (FBS; HyClone; GE Healthcare Life Sciences, Logan, UT, USA). MCF-10A cells were cultured in DMEM/F12 medium (HyClone; GE Healthcare Life Sciences Shanghai, China) supplemented with 5% horse serum (Gibco; Thermo Fisher Scientific, Inc.).

Cell transfection. miR-589 mimic (5'-UGAGAACCACGU CUGCUCUGAGGGTATTCGCACTGGATACGACGAACT TT-3'), mimic control (miR-NC) (5'-ACUACUGAGUGACAG UAGA-3'), miR-589 inhibitor (5'-UGAGAACCACGUCUG CUCUGAG-3') and inhibitor control (anti-NC) (5'-CAGUAC UUUUGUGUAGUACAA-3') were purchased from RiboBio Co., Ltd., (Guangzhou, China). The small RNAs including si-metastasis-associated protein 2 (MTA2) and sicontrol were obtained from Santa Cruz Biotechnology, Inc. The siRNA sequences were: siMTA2, 5 MTA2ces Cruz Biotechnologyand sicontrol, 5control Cruz Biotechnology, A total of $5x10^5$ MDA-MB-468 cells were transfected with miR-NC (2.5 μg) or miR-589 mimic (2.5 μg) using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. A total of $5x10^5$ MDA-MB-231 cells were transfected with anti-NC (2.5 μg) or miR-589 inhibitor (2.5 μg) and siMTA2 (100 nM) or sicontrol (100 nM) using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Following transfection for 48 h, the efficiency of transfection was analyzed using quantitative polymerase chain reaction (qPCR) or western blot analysis.

Cell viability assay. Cell viability was assessed using the Cell Counting Kit-8 (CCK-8) assay (Beyotime Institute of Biotechnology, Haimen, China), according to the manufacturer's protocol. Briefly, cells were plated into 96-well plates ($1x10^3$/well). At set time points (0, 24, 48 and 72 h), 10 μl of CCK-8 solution was added to each well. Following incubation for 3 h, the absorbance of each well was measured using the Multiskan MK3 microplate photometer (Thermo Fisher Scientific, Inc.) at 450 nm.

Colony formation assay. The proliferation of cells was measured using the plate colony formation assay. Each well of the 6-well plates contained 300 cells/well and were cultured for 10 days in DMEM containing 10% FBS at 37˚C. Subsequently, the cells were washed with PBS and fixed with 10% methanol for 15 min at room temperature, and the colonies were stained with crystal violet (Beyotime Institute of Biotechnology, Haimen, China) for 30 min at room temperature. The colony images were obtained, and the number of colonies was counted under a microscope (Olympus Corporation, Tokyo, Japan).

Transwell and Matrigel assays. The cell migration and invasion abilities were detected using the Transwell and Matrigel assays, which were carried out in 24-well Transwell chambers (Corning Inc., Corning NY, USA). In brief, $5x10^4$ MDA-MB-468 or MDA-MB-231 cells were plated on the upper Transwell chamber either in the presence or absence of Matrigel inserts in DMEM. DMEM supplemented with 5% FBS was added to the lower chamber. The cells were maintained in a humidified incubator with 5% CO_2 for 24 h at 37˚C. Non-migrating cells in the upper chamber were scraped using a cotton swab. The migrating cells were fixed with 10% methanol for 10 min, and stained with crystal violet (Beyotime Institute of Biotechnology) for 5 min at room temperature. Finally, the stained cells from six random fields were counted, and the images were captured under a light microscope (magnification, x100).

Western blot analysis. Total proteins were extracted from tissues using the T-PER Tissue Protein Extraction Reagent (Pierce; Thermo Fisher Scientific, Inc.). Protein concentrations were determined using a bicinchoninic acid (BCA) Protein Assay kit (Pierce; Thermo Fisher Scientific, Inc.).

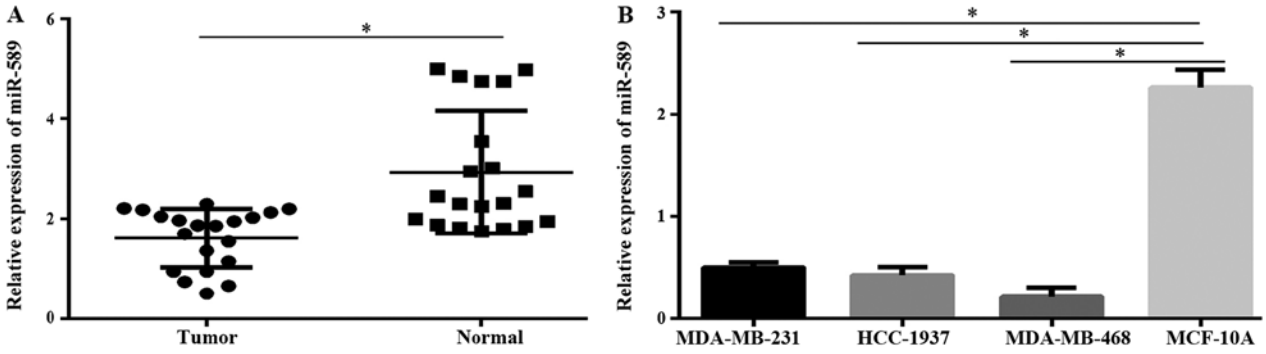

Figure 1. Expression level of miR-589 is downregulated in TNBC. (A) qPCR was used to analyze the expression levels of miR-589 in 20 paired TNBC specimens and their corresponding paired adjacent normal tissues. (B) qPCR was used to analyze the expression level of 3 TNBC cell lines (MDA-MB-231, HCC-1937 and MDA-MB-468) and the normal breast epithelial cell line, MCF-10A. U6 acted as an internal control. *P<0.05. miR, microRNA; TNBC, triple-negative breast cancer; qPCR, quantitative polymerase chain reaction.

Proteins (20 μg) were separated by SDS-PAGE (10% gels) and transferred to polyvinylidene fluoride membranes (EMD Millipore, Billerica, MD, USA). Membranes were blocked at room temperature with 5% skimmed milk for 1 h and incubated at 4˚C overnight with the following antibodies: MTA2 antibody (cat. no. sc-55566; 1:500 dilution), N-cadherin (cat. no. sc-8424; 1:500 dilution), vimentin (cat. no. sc-66002; 1:500 dilution), E-cadherin (cat. no. sc-8426; 1:500 dilution; all Santa Cruz Biotechnology, Inc.), β-actin antibody (cat. no. AF0003; 1:1,000 dilution; Beyotime Institute of Biotechnology) and subsequently with biotinylated goat anti-mouse secondary antibodies (cat. no. ab64255; 1:1,000 dilution; Abcam) at room temperature for 2 h. Specific bands of interest were visualized with enhanced chemiluminescence reagent (Nanjing KeyGen Biotech Co., Ltd., Nanjing, China) on an autoradiographic film.

Total mRNA extraction and qPCR. Total RNA was extracted from tissues and MDA-MB-231, HCC-1937, MDA-MB-468 and MCF-10A cells using the miRcute miRNA isolation kit (Tiangen Biotech Co., Ltd., Beijing, China), according to the manufacturer's protocol. Total RNA was reverse transcribed with oligodT primers using the miRcute Plus miRNA First-strand cDNA Synthesis kit (Tiangen Biotech Co., Ltd.) at 37˚C for 60 min and 85˚C for 1 min. The cDNA was amplified by qPCR using SYBR® Premix Ex Taq (Takara Biotechnology Co., Ltd., Dalian, China). The thermocycling conditions were as follows: 5 min at 95˚C, followed by 40 cycles of 30 sec at 95˚C, 60 sec at 60˚C and 30 sec at 72˚C; and 1 sec at 99˚C; 15 sec at 59˚C; 1 sec at 95˚C; followed by cooling to 40˚C. The relative expression level of miR-589 and MTA2 was calculated using the $2^{-\Delta\Delta Cq}$ method (17), and normalized to the reference gene. The primers used for qPCR were as follows: miR-589 forward, 5'-CGAGGTCAGCGTGATTTCATGG-3' and reverse 5'-TGTGTCCAAGTCCCAGCCAGAG-3'; U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse 5'-AACGCTTCACGAATTTGCGT-3'; MTA2 forward, 5'-ATCATTACCAGCCACCCA-3' and reverse 5'-CGATTATCAGATTCTCCCTC-3'; N-cadherin forward 5'-ATCAAAGACCCATCCACC-3' and reverse 5'-CCTCCTCACCACCACTA-3'; vimentin forward, 5'-CTTCCGCGCCTACGCCA-3' and reverse 5'-GCCCAGGCGACCTACTCC-3'; E-cadherin forward 5'-GTACTTGTAATGACACATCTC-3' and reverse 5'-TGCCAGTTTCTGCATCTTGC-3'; and β-actin forward 5'-GATCATTGCTCCTCCTGAGC-3' and reverse 5'-ACTCCTGCTTGCTGATCCAC-3'.

Prediction of miRNA targets. The hypothetical targets of miR-589 were predicted using TargetScan human version 7.1 (http://www.targetscan.org/vert_71/) (18). This revealed that the 3'-untranslated region (3'-UTR) of MTA2 may be complementarily paired with the seed sequences of miR-589.

Dual-luciferase assay. To validate the direct target gene of miR-589, an online bioinformatics search using TargetScan was performed. MTA2-3'-UTR wild-type and MTA2-3'-UTR mutant were synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China) and cloned into the psiCHECK2 vector (Promega Corporation, Madison, WI, USA). The psiCHECK2-3'-UTR-wild-type or psiCHECK2-3'-UTR-mutant luciferase plasmids were transfected into 293T cells using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). At 24 h following transfection, luciferase activity was measured using the dual-luciferase reporter assay kit (Promega Corporation), according to the manufacturer's protocol. The activity of firefly luciferase was normalized to the corresponding *Renilla* luciferase activity.

Statistical analysis. All experiments were performed three times, and all values are presented as the mean ± standard deviation (SD). Statistical analysis was performed using SPSS (11.0; SPSS, Inc., Chicago, IL, USA). The differences between 2 groups were assessed using a two-tailed unpaired Student's t-test. Data of >2 groups were analyzed using one-way analysis of variance with the Tukey's post hoc test. Pearson's correlation analysis was used to analyze the correlation between the expression of miR-589 and MTA2. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of miR-589 decreases in TNBC tissues and cell lines. To investigate the role of miR-589 in TNBC, the expression of miR-589 in TNBC patient samples and adjacent non-tumor tissues were evaluated. Results from the qPCR demonstrated that miR-589 was downregulated in TNBC tissues compared with that in the adjacent normal

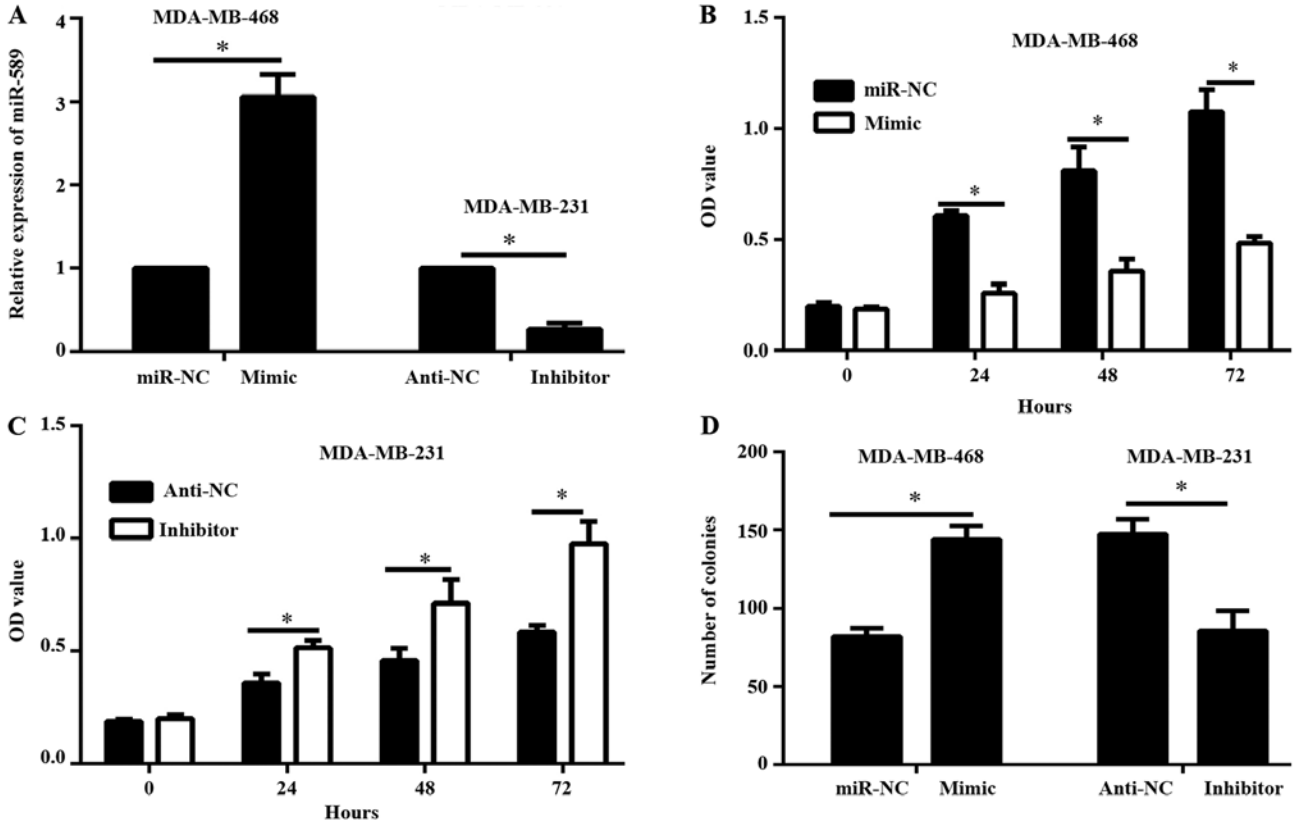

Figure 2. miR-589 inhibits TNBC cell viability and proliferation. (A) The expression level of miR-589 in 2 TNBC cell lines transfected with miR-589 mimic or inhibitor were analyzed by quantitative polymerase chain reaction. Cell Counting Kit-8 was used to evaluate the viability of the (B) MDA-MB-468 and (C) MDA-MB-231 cells. (D) The proliferation of MDA-MB-468 and MDA-MB-231 was detected using colony formation assay. *P<0.05. miR, microRNA; TNBC, triple-negative breast cancer; NC, negative control.

tissues (P<0.05; Fig. 1A). The results also revealed that the expression level of miR-589 was significantly downregulated in the MDA-MB-231, MDA-MB-468 and HCC-1937 TNBC cell lines compared with that in the normal breast epithelial cell line, MCF-10A (P<0.05; Fig. 1B). These data revealed that miR-589 may serve a critical role in the progression of TNBC. The expression level of miR-589 was highest in the MDA-MB-231 cells, while it was lowest in the MDA-MB-468 cells. Subsequently, these 2 cancer cell lines were used in further investigations.

miR-589 decreases TNBC cell viability and proliferation. Subsequently, the role of miR-589 in TNBC cell viability and proliferation was determined. miR-589 mimic and miR-NC were transfected into the MDA-MB-468 cells, while the miR-589 inhibitor and anti-NC were transfected into the MDA-MB-231 cells. Results of the qPCR demonstrated that the expression level of miR-589 was enhanced in MDA-MB-468 cells transfected with miR-589 mimic compared with MDA-MB-468 cells containing miR-NC; while the expression level of miR-589 was reduced in the MDA-MB-231 cells transfected with miR-589 inhibitor compared with that in the anti-NC group (P<0.05; Fig. 2A). Results of the CCK-8 and colony formation assays demonstrated that miR-589 overexpression decreased the cell viability and proliferation of MDA-MB-468 cells, while miR-589 silencing remarkably enhanced the cell viability and proliferation of MDA-MB-231 cells (P<0.05; Fig. 2B-D).

miR-589 inhibits TNBC cell migration and invasion in vitro. Furthermore, the ability of miR-589 to regulate TNBC cell migration and invasion was determined. The results of the migration and invasion assays revealed that exogenous miR-589 expression significantly decreased the capability of migration and invasion of MDA-MB-468 cells, whereas miR-589 silencing produced the opposite results in MDA-MB-231 cells (P>0.05; Fig. 3A-D). In addition, the results of the Transwell assay revealed that MTA2 silencing decreased the migration and invasion capability of MDA-MB-231 cells, which was initially enhanced by miR-589 inhibitor (P<0.05; Fig. 3B and D).

Identification of MTA2 as a target of miR-589. The potential targets of miR-589 were predicted via bioinformatics analysis to investigate the underlying mechanism by which miR-589 decreases TNBC cell progression. MTA2 serves as an oncogene in TNBC and was thus selected for further confirmation (Fig. 4A). The 293T cells were co-transfected with miR-589 mimic or miR-589 inhibitor and either the wild-type or mutated MTA2 3'-UTR reporter and subsequently subjected to dual-luciferase assay. The results illustrated that miR-589 mimic decreased the luciferase intensity of MTA2 3'-UTR, whereas a mutation within the binding site abolished the inhibitory role of miR-589 in MTA2 3'-UTR (P<0.05; Fig. 4B). The miR-589 inhibitor increased the luciferase intensity of MTA2 3'-UTR (P<0.05; Fig. 4B). Furthermore, qPCR (P<0.05; Fig. 4C) and western blot analysis (Fig. 4D) revealed

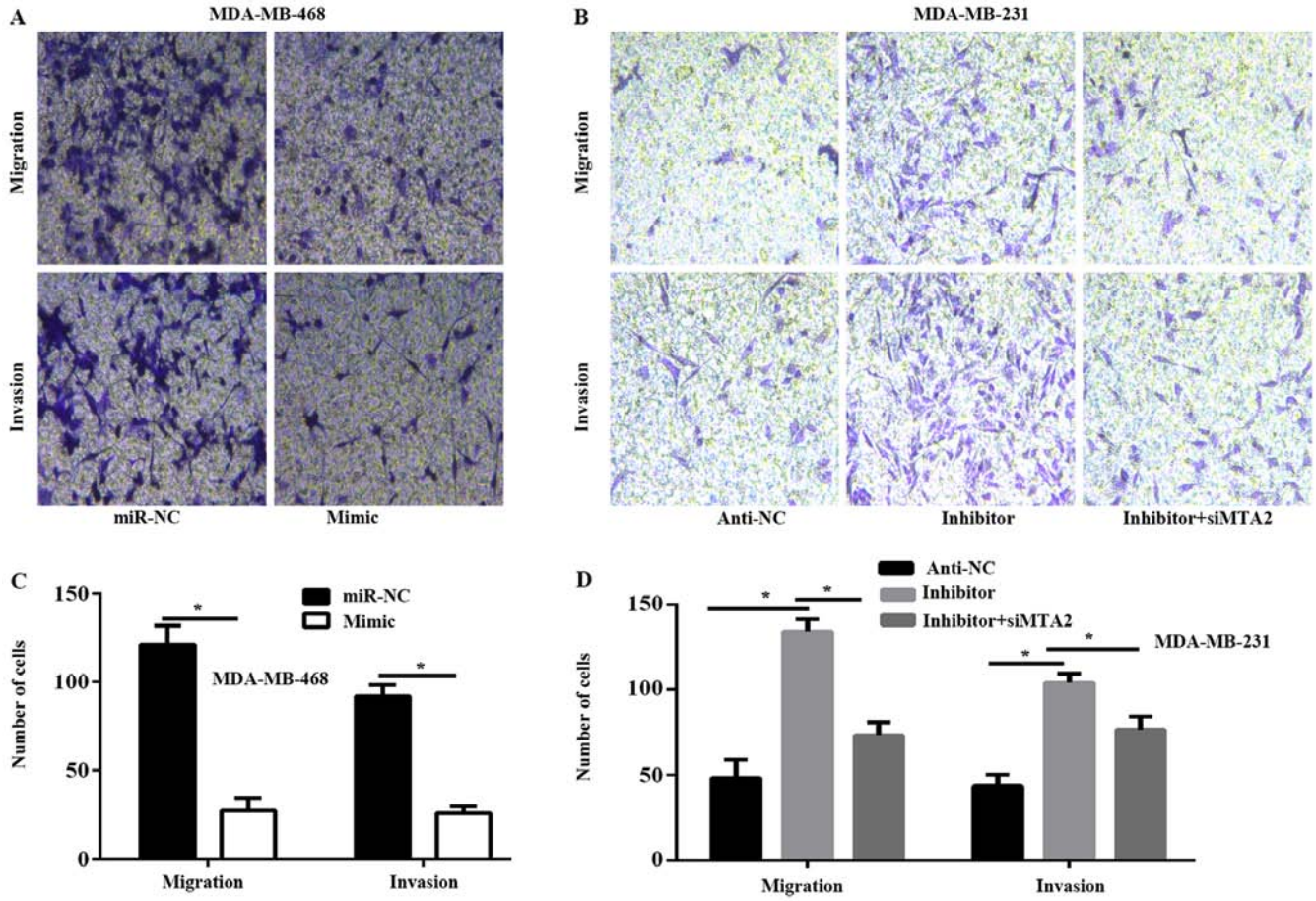

Figure 3. miR-589 reduces the cell migration and invasion of TNBC cells. Transwell assay was used to detect the migration and invasion ability of MDA-MB-231 and MDA-MB-468 cells. Cell migration and invasion images of (A) MDA-MB-468 and (B) MDA-MB-231 cells. Quantification of cell migration and invasion of (C) MDA-MB-468 and (D) MDA-MB-231 cells. *P<0.05. miR, microRNA; TNBC, triple-negative breast cancer; NC, negative control; si, small interfering RNA; MTA2, metastasis-associated protein 2.

that the expression levels of MTA2 mRNA and protein were inhibited in the MDA-MB-468 cells transfected with miR-589 mimic, while miR-589 silencing produced higher expression levels in the MDA-MB-231 cells. In addition, the mRNA expression level of MTA2 was upregulated in the TNBC tissues compared with that in the adjacent normal tissues (P<0.05; Fig. 4E), and was negatively correlated with miR-589 expression in TNBC tissues (P<0.05; Fig. 4F).

MTA2 is a functional target of miR-589. The MDA-MB-231 cells were transfected with miR-589 inhibitor or miR-589 inhibitor and siMTA2 to determine if MTA2 is a functional target of miR-589 in TNBC. The protein expression of MTA2 was detected using western blot analysis. The results showed that the expression of MTA2 was decreased in response to siMTA2 compared to control. In addition, the expression of MTA2 was markedly reduced when cells were treated with siMTA2 and miR-589 inhibitor compared with miR-589 inhibitor only (Fig. 4D).

miR-589 inhibits the epithelial-mesenchymal transition (EMT) progression via MTA2. A previous study has revealed that MTA2 promoted EMT progression (19). To further discover the mechanism of miR-589, whether miR-589 regulates EMT in breast cancer cells was investigated. As revealed in Fig. 5A and B, miR-589 mimic reduced the expression of the mesenchymal cell markers N-cadherin and vimentin, but enhanced the expression levels of the epithelial cell marker E-cadherin. However, miR-589 silencing resulted in increased expression of N-cadherin and vimentin, and decreased the expression of E-cadherin in the MDA-MB-231 cells (Fig. 5A and C). These data revealed that miR-589 silencing triggered more aggressive EMT, and promoted cell migration and invasion. Furthermore, the present study demonstrated that the expression of N-cadherin and vimentin in the siMTA2 and miR-589 inhibitor group was significantly decreased compared with the miR-589 inhibitor group, while the expression of E-cadherin was markedly enhanced in the siMTA2 and miR-589 inhibitor group compared with the miR-589 inhibitor group (P<0.05; Fig. 5A and C). These results suggested that miR-589 inhibited the process of EMT via MTA2.

Discussion

miRNA is a non-coding RNA consisting of 21-22 nucleotides in length in eukaryotes, and its primary function is to regulate the expression of target genes (6,7). miRNAs serve in a number of crucial roles in the development and progression of many types of cancer (6,7). As a novel cancer-associated miRNA, miR-589 serves as an oncogene in gastric cancer (15).

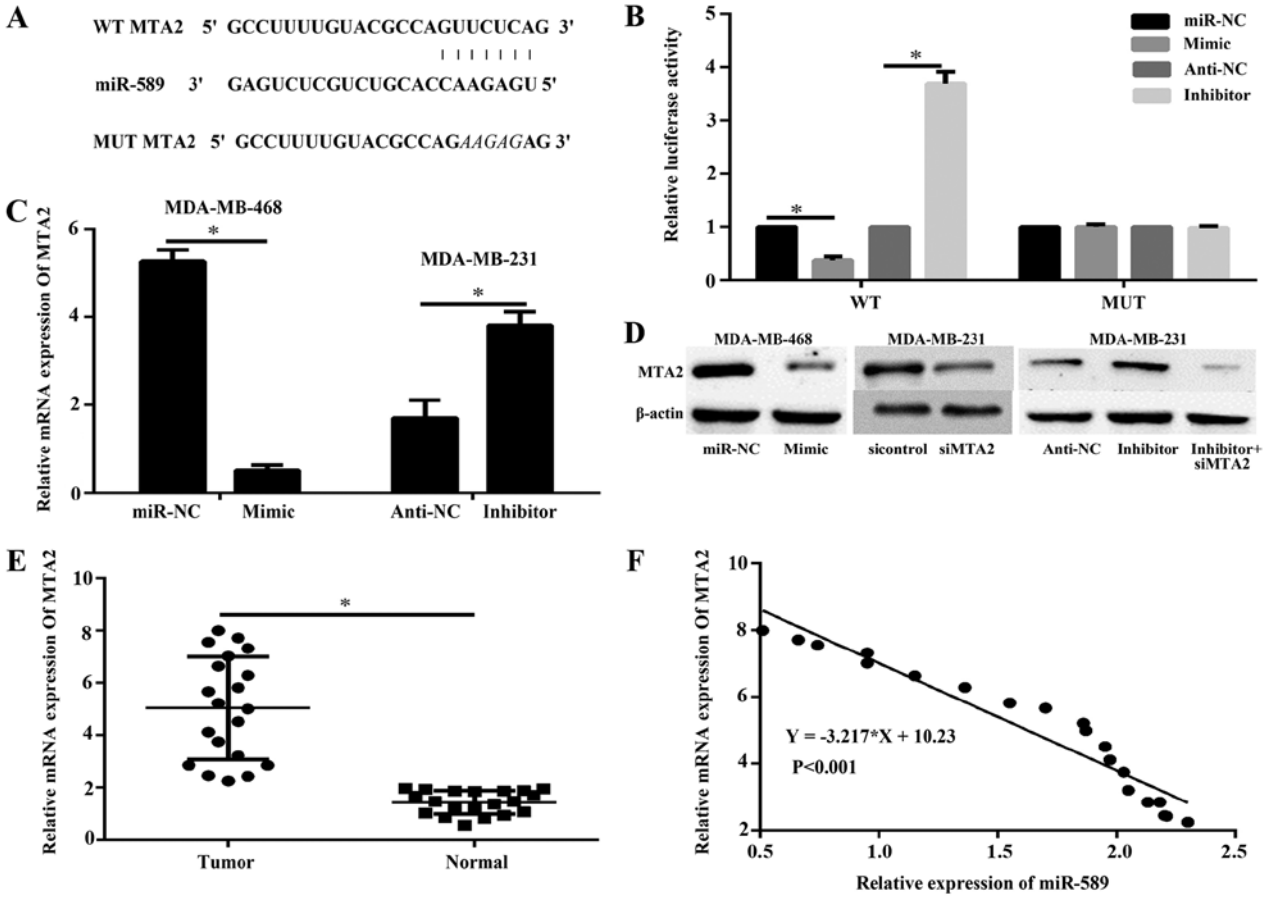

Figure 4. MTA2 is a direct target gene of miR-589. (A) Sequence alignment of miR-589 with 3etastasis-associated asusing TargetScan. (B) Dual-luciferase reporter assays were performed in 293T cells. (C) The mRNA expression level of MTA2 in MDA-MB-231 and MDA-MB-468 cells was analyzed using quantitative polymerase chain reaction. β-actin acted as an internal control. (D) The protein expression level of MTA2 in MDA-MB-231 and MDA-MB-468 cells was examined usng western blot analysis. β-actin acted as an internal control. (E) The mRNA expression level of MTA2 was analyzed in TNBC tissues and adjacent normal breast tissues. β-actin served as the loading control. (F) Pearson's correlation analysis examined the correlations between the mRNA expression levels of miR-589 and MTA2 in TNBC tissues. *P<0.05. MTA2, metastasis-associated protein 2; miR, microRNA; TNBC, triple-negative breast cancer; NC, negative control; si, small interfering RNA.

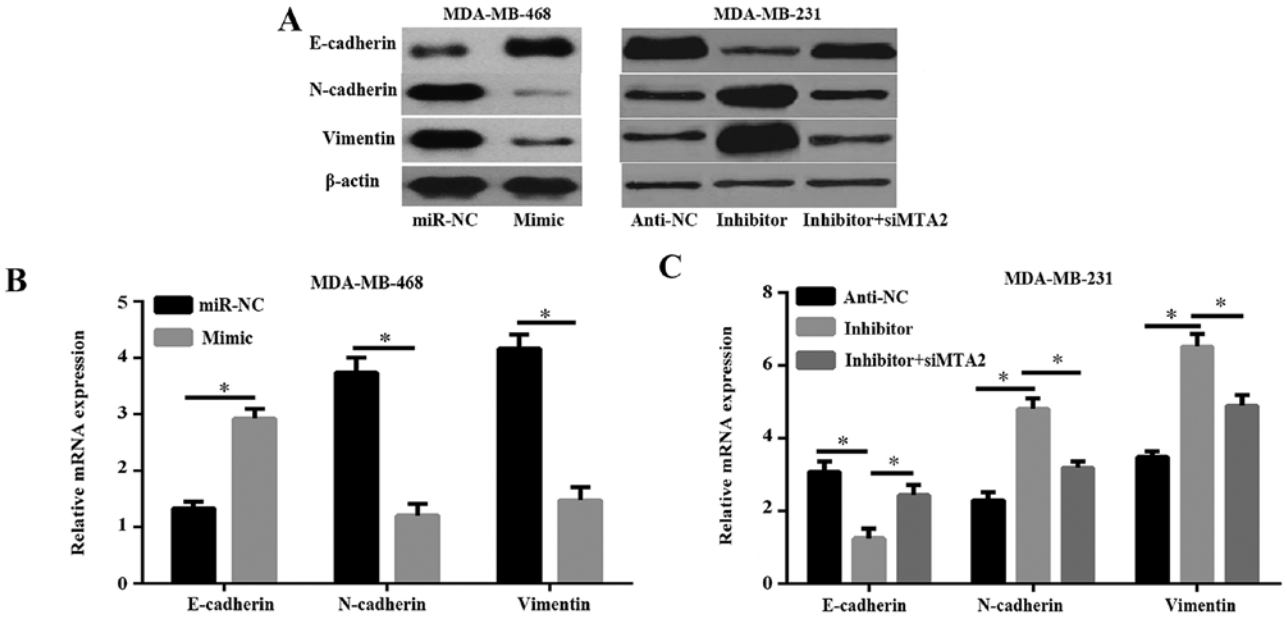

Figure 5. miR-589 inhibits the EMT progression via MTA2. (A) The protein expression of N-cadherin, vimentin and E-cadherin was analyzed using western blot analysis in MDA-MB-468 and MDA-MB-231 cells. The mRNA expression of N-cadherin, vimentin and E-cadherin was detected using quantitative polymerase chain reaction in (B) MDA-MB-468 and (C) MDA-MB-231 cells. *P<0.05. EMT, epithelial-mesenchymal transition; MTA2, metastasis-associated protein 2; miR, microRNA; NC, negative control; si, small interfering RNA.

However, miR-589 may act as a tumor suppressor in hepatocellular carcinoma, glioma and lung cancer (11-14). The role of miR-589 in TNBC has yet to be established. In the present study, the expression level of miR-589 in tumor and adjacent normal tissues from TNBC patients was measured using qPCR and identified that miR-589 is significantly downregulated in TNBC tissues compared with that in normal tissue. In addition, miR-589 was downregulated in 3 TNBC cell lines. Furthermore, miR-589 inhibited the proliferation and metastasis of breast cancer cells. MTA2 was identified as a direct target gene of miR-589, and MTA2 silencing inhibited the cell proliferation and metastasis induced by miR-589 silencing.

miR-589 serves as an oncogene or tumor suppressor via a number of molecular mechanisms in different types of cancer. A recent study revealed that miR-589 is overexpressed and promotes the progression of gastric cancer via a leukemia inhibitory factor receptor (LIFR)-phosphoinositide 3-kinase/protein kinase B-c-Jun regulatory feedback loop (15). However, in non-small lung cancer, miR-589 serves as a tumor suppressor and negatively regulates histone deacetylases (HDAC) (13). These studies revealed that the same miRNA may serve as different roles in the progression of cancer via different mechanisms depending on the tissue type. In addition, the same miRNA may serve as different roles in different biological functions in the same cancer tissue. In hepatocellular carcinoma, miR-589 serves a contrary role in the progression of cancer cells, including inhibiting the proliferation of cancer cell proliferation and maintaining the stemness and enhancing the chemoresistance to doxorubicin (11,14,20). The same miRNA may regulate different genes in cancer, and the gene may be regulated by different miRNAs in different tissues. Previous studies revealed that miR-589 could regulate many genes, including LIFR, zinc finger MYND-type containing 19, signal transducer and activator of transcription 3, HDAC5 and mitogen-activated protein kinase 8 (11-15,20). To explore the molecular mechanism of miR-589 in inhibiting TNBC progression, publicly available bioinformatic algorithms were used to analyze the target gene of miR-589. MTA2 was identified as a direct and functional target of miR-589 using bioinformatics, dual-luciferase reporter assays, qPCR and western blot analysis. MTA2 is a member of a small protein family (including MTA1, MTA2 and MTA3) (21). A previous study revealed that MTA2 promotes tumor progression (22). A study in 2013 revealed that MTA2 enhances the metastasis of TNBC cells by inhibiting Rho pathway signaling (23). In the present study, a significant negative correlation was observed between MTA2 and miR-589 expression in patients with TNBC. More importantly, the silencing of MTA2 could abolish the inducing effect of miR-589 silencing on the progression of TNBC cells. These data suggest that miR-589 inhibits TNBC, at least in part, by the downregulation of MTA2.

However, the present study has limitations. The number of patients is small and does not include Luminal A, Luminal B and HER-2 positive subtypes of breast cancer. Thus, the association of miR-589 and the other types of breast cancer was not determined. Future studies, would aim to recruit more patients and investigate additional subtypes of breast cancer. In addition, other target genes or signaling pathways via which miR-589 inhibits breast cancer require further investigation.

In summary, the present study is the first to report that miR-589 inhibits TNBC progression by directly targeting MTA2. These findings indicate that miR-589 is a potential tumor suppressor in TNBC through enhancing cellular proliferation and metastasis. The present study suggests that miR-589 is a novel biomarker in improving the therapeutic care of patients with TNBC.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

JC conceived, designed and conducted all experiments, and wrote and revised the manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all the patients and the study was approved by the Ethics Committee of the Central Hospital of Zibo (approval no: ZB 20151229005).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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