ORIGINAL RESEARCH

MIR-518a-5p Targets ZEB2 to Suppress the Migration and Invasion of Breast-cancer Cells

Li Wang, MD; Ziru Guo, MD; Shuo Zhang, MD; Xiaochong Zhang, MD; Meixiang Sang, PhD; Yunjiang Liu, PhD; Baoen Shan, PhD

ABSTRACT

Context • Although great progress has occurred in breast cancer (BC) treatment, including chemotherapy, chemoradiotherapy, and surgical resection, the rate of patients' survival is still unsatisfactory. Multiple genes and factors have proven to contribute to BC's occurrence. Thus, it's urgent to explore the molecular mechanisms in the development and progression of BC and find possible targets for therapy.

Objective • The study intended to assess the mechanisms of miR-518a-5p's effect on BC by targeting the zinc finger E-box-binding homeobox 2 (ZEB2) gene.

Design • The research team designed a laboratory study and retrospective analysis.

Setting • The study took place in the Department of Breast Surgery at the Xingtai People's Hospital in Xingtai, Hebei, China.

Outcome Measures • The study measured the miR-518a-5p expression in BC tissues and normal tissues using a real-time quantitative reverse transcription (qRT)-polymerase chain reaction (PCR) test. The research team purchased the BC cells MDA-MB-231 and MCF-7 and measured the effects of the miR-518a-5p on BC cell activity as well as epithelial–mesenchymal transition (EMT) ability, using cell scratch tests and Transwell assays. The team assessed the ZEB2 protein expression and verified the targeting relationship using a Dual-Luciferase Reporter assay.

Results • The miR-518a-5p expressed was low in the BC tissues and cell lines compared with adjacent normal tissues ($P < .05$). Overexpressing the miR-518a-5p inhibited BC-cell migration and invasion ($P < .05$). Moreover, the ZEB2 was the target gene for the miR-518a-5p. The Luciferase assay verified that the miR-518a-5p directly targeted the ZEB2 in BC cells ($P < .05$). The Transwell invasion assay, western blot analysis, and wound healing assay also showed that the miR-518a-5p inhibited BC cells by targeting ZEB2 ($P < .05$).

Conclusions • The miR-518a-5p suppressed BC migration, invasion, and process of EMT by regulating ZEB2. In the future, this method may be a new option for BC diagnosis and treatment. An in-depth understanding of the role of the miR-518a-5p in BC can help clinicians to understand the pathogenic mechanism of breast cancer more accurately. (*Altern Ther Health Med.* 2023;29(1):137-143).

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Breast cancer (BC) has the highest incidence and is the deadliest disease in women. Asia has the highest mortality from BC for both genders.[1] Moreover, the incidence rises every year in China, and BC has become the biggest threat to women's health.

Although great progress has occurred in BC treatment, including chemotherapy, chemoradiotherapy, and surgical resection, the rate of patients' survival is still unsatisfactory due to the lack of effective methods for early diagnosis.[2]

As molecular biology has developed, multiple genes and factors have proven to contribute to BC's occurrence. Thus, it's urgent to explore the molecular mechanisms in the development and progression of BC and find possible targets for therapy.[3]

MicroRNAs (miRNAs) are short, single-strand, noncoding RNA molecules with 20-30 nucleotides.[4] MiRNAs participate in posttranscriptional regulation of expression of target gene mRNA to affect cells' biological processes. Growing evidence has shown that miRNAs play a part in BC development and progression and may be vital biomarkers for diagnosis and prognosis.

For example, Yin et al found that miRNA-96-5p influences BC progression by targeting the forkhead box O3 (FOXO3) gene.[5] Also, Zhang et al identified miRNA-93 as a functional, dysregulated miRNA in triple-negative BC.[6]

Hu et al found that circular RNA hsa_circ_0078607 can suppress ovarian-cancer progression by adjusting the miR-518a-5p/Fas signaling pathway.[7] Yin et al showed that the miR-518a-5p can adjust the expression of the Chemokine receptor CCR6 in colorectal cancer cells.[5] Also investigating colorectal cancer cells, Xu et al found that the miR-518a-5p interacts with CCR6.[8] The miR-518a-5p expression decreased in BC tissues and inhibited cell proliferation.

Huang et al found that the miR-30a-5p can adjust BC cell proliferation and migration through the miR-30a-5p/ubiquitin protein ligase E3C (UBE3C) axis.[9] Two other studies found that miRNAs can inhibit tumor growth as suppressor oncogene.[10,11] Other miRNAs can act as protooncogenes, suppress tumor-suppressor genes, and advance cell migration and metastasis.[12-14] However, the specific mechanism of miR-518a-5p is still unknown.

The zinc finger E-box-binding homeobox 2 (ZEB2) gene is a DNA-binding transcription factor, which is mainly involved in epithelial–mesenchymal transition (EMT).[15] Feng et al found that tripartite motif-containing 14 (TRIM14) can boost epithelial-mesenchymal transition in conjunction with ZEB2 in glioblastoma cells.[16] Wang et al reported that the knockdown of the small nucleolar RNA host gene 12 (SNHG12) can suppress tumor metastasis and epithelial-mesenchymal transition through the Slug/ZEB2 signaling pathway by targeting miR-218 in non-small cell lung cancer (NSCLC).[17] However, the relevant mechanism of ZEB2 in BC cells is still unclear.

The current study intended to assess the mechanisms of miR-518a-5p's effect on BC by targeting the zinc finger E-box-binding homeobox 2 (ZEB2) gene.

METHODS

The research team designed a laboratory study and retrospective analysis. The study took place in the Department of Breast Surgery at the Xingtai People's Hospital in Xingtai, Hebei, China.

Procedures

Patient information. From October 2015 to May 2017, 98 patients diagnosed with BC at the Fourth Hospital of Hebei Medical University, Tangshan people's Hospital and Qinhuangdao First Hospital were selected as the research subjects.This experiment has been approved by the ethics committee, and all research subjects signed the informed consent.

Inclusion and exclusion criteria. Inclusion criteria: diagnosed with BC by pathological biopsy, treated with radical tumor resection in our hospital after diagnosis, and aged >18 years. Exclusion criteria: patients with other tumors, combined with other cardiovascular and cerebrovascular diseases, combined with other immune deficiency diseases, combined with other mental diseases, abnormal liver and kidney function or disorders.

Sample collection. The tumor tissue and surrounding normal tissue after surgical resection of the patient were obtained for experiments. Tissues were preserved in liquid nitrogen.

Cell lines. The BC cell lines MDA-MB-231, BT-549, and MCF-7 were all purchased from ATCC (Manassas, Virginia, USA).

Cell transfection. The research team bought the miR-518a-5p mimics, inhibitor, and negative control from the RiboBio Corporation (Guangzhou, China) and obtained the ZEB2 overexpression vector from OriGene Technologies (Rockwell, Maryland, USA). The Lipofectamine 2000 was applied to cell transfection (Invitrogen, Waltham, Massachusetts, USA) in Opti-RPMI 1640 medium (Gibco, New York, California, USA). The research team used the BC cells to perform gene- or protein-expression analysis and biological experiments on cells.

Real-time quantitative reverse transcription (qRT)-polymerase chain reaction (PCR) test. The research team extracted total RNA from the primary BC tissues, normal tissue and BC cells using TRIzol (Invitrogen, Waltham, Massachusetts, USA) and reverse transcribed it to complementary DNA (cDNA) using a kit from Applied Biosystems (Waltham, Massachusetts, USA) and following the kit's instructions. The team then performed amplification reactions. For calculation of the relative expression, the reaction conditions were 94°C for 4 min; 94°C for 1 min; 60°C for 1 min; and 72°C for 1 min, using the 2-ΔΔCT [2(-Delta Delta C(T)].

Western Blot (WB) analysis. The research team: (1) lysed the cells using a radioimmunoprecipitation assay (RIPA) from Invitrogen (Waltham, Massachusetts, USA); (2) transferred them to a polyvinylidene fluoride (PVDF) membrane using sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE); (3) added a blocking buffer of 5% skim milk plus the ZEB2 primary antibody, MDA-MB-231am (company, city, state, USA); (4) cultivated them overnight at 4°C (Cell Signaling Technology, Boston, Massachusetts, USA); (5) cleaned them with Tris Buffered Saline + Tween 20 (TBST) from Invitrogen (Waltham, Massachusetts, USA) the next day; (6) added a Goat anti-rabbit secondary antibody, cultivated for 2h at 37°C; and (7) developed them using an enhanced luminol-based chemiluminescent (ECL) kit (Solarbio, Beijing, China).

Cell proliferation assay. The research team seeded the BC cells, at 1 × 103 cells/well, into a 96-hole plate with 100 uL

of Dulbecco's Modified Eagle Medium (DMEM) from Invitrogen (Waltham, Massachusetts, USA and 10% fetal bovine serum (FBS) from Invitrogen (Waltham, Massachusetts, USA. At the appointed time, the team put 10 uL of cell counting kit-8 (CCK-8) buffer from abcam (Cambridge, Cambridgeshire, UK into each hole, and the cultivated the cells for 2 h. Then, the team measured the OD450 absorbance value using a microplate reader (Tecan Infinite F50, Männedorf, Zürich, Switzerland).

Wound healing assay. The research team implanted 2×105 cells/well into a six-hole plate with DMEM serum-free medium and scraped them with 10-uL pipette tips. The team graphed the cell-migration image using five random fields at 0 h and 24 h after wound injury and measured the migration distance of the cells.

Transwell invasion assay. The research team put 2 × 105 cells/well into a Transwell chamber from Corning (Shanghai, China with DMEM serum-free medium and filled the lower chamber with DMEM complete medium. Twenty-four hours later, the team immobilized the cells with 4% paraformaldehyde and then dyed them with crystal violet.

Dual-Luciferase Reporter assay. The research team cloned the ZEB2 wild-type 3'UTR or mutant 3'UTRs into the downstream of the plasmid-mediated beta-lactamase (pMIR) with ZEB2-MT and co-transfected the ZEB2-MUT with the miR-518a-5p using Lipofectamine 2000 transfection reagent Invitrogen (Waltham, Massachusetts, USA. Forty-eight hours later, the team measured the Luciferase activity using a Dual-Luciferase Reporter Assay kit (Promega, Madison, Wisconsin, USA), following the instructions.

Outcome Measures

Mir-518a-5p levels. The study compared the miR-518a-5p levels in the BC and normal tissues (NC group) adjacent to the cancer as well as the levels in the three BC cell lines—MDA-MB-231, BT-549, and MCF-7 using a qRT-PCR test. Subsequently, to further confirm the relationship between the miR-518a-5p and BC, the study also analyzed the relationship between the miR-518a-5p and the pathological features of BC.

Cell proliferation. To assess the miR-518a-5p's cell scratch test and migration rate in BC, the research team transfected an miR-518a-5p mimics and inhibitor into the MDA-MB-231 and MCF-7 cell lines. Using a cell scratch assay, the study evaluated migration ability of cells and determined the migration rate. The higher the migration rate, the stronger the ability of the cells to move and the greater the possibility of tumor metastasis.

Effects on EMT. By detecting EMT marker proteins to understand miR-518a-5p's effect on the EMT ability of the MDA-MB-231 and MCF-7 cells lines transfected with the miR-518a-5p mimics and inhibitor cells, the research team measured the EMT markers N-cadherin, E-cadherin, and Vimentin. First, the research team performed a qRT-PCR assay to obtain the data and then verified the results using WB analysis.

Binding sites between miR-518a-5p and ZEB2. The research team used RNA sequencing (RNA-seq) to find potential targets of miR-518a-5p to assess its specific mechanisms in BC cells. The team used the targets ZEB2, which had great potential for being targeted by the miR-518a-5p, and the empty vector (EV). To identify what role the miR-518a-5p plays in targeting ZEB2, the research team conducted a Dual-Luciferase Reporter assay.

Cell migration with ZEB2 regulation. In the prior analysis, the research team found that ZEB2 is the miR-518a-5p's target gene in BC and that ZEB2 is targeted in its 3'-untranslated region (3'UTR) position. To explore the relationship between the miR-518a-5p and ZEB2, the research team also conducted wound healing, qRT-PCR, and WB analyses at 0 and 24 hours to determine miR-518a-5p's ability to inhibit cell migration through regulating ZEB2. The test compared the NC+EV group, the mir-518a-5p+EV group, the NC+ZEB2 group, and the mir-518a-5p+ZEB2 group.

Table 1. Relationship Between miR-518a-5p Expression and Clinicopathological Features in Breast-cancer Tissues (N = 98)

Parameters	n (%)	Mir-518a-5p Expression		χ^2	*P* value
		Low	High		
Age, y				0.304	.581
≤50	40 (40.82)	27	13		
>50	58 (59.18)	36	22		
Tumor Size, cm^3				0.048	.826
≤5	63 (64.29)	40	23		
>5	35 (37.51)	23	12		
Histological Grade				3.61	.057
Low	73 (74.49)	43	30		
High	25 (25.51)	20	5		
Clinical Stage				19.006	<.001[a]
Low	44 (44.90)	18	26		
High	54 (55.10)	45	9		
Lymph Node Metastasis				0.236	.627
no	67 (68.37)	42	25		
yes	31 (31.63)	21	10		

[a]$P < .001$, indicating that a significant difference existed among participants at different clinical stages

Statistical Analysis

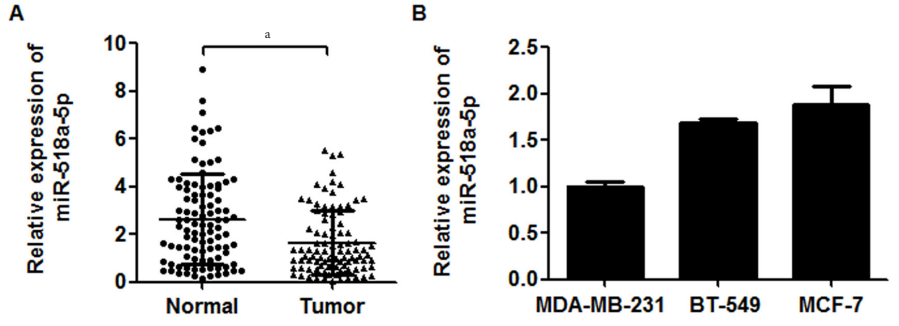
The study statistically analyzed the data using SPSS 21.0 software (IBM Corporation, Armonk, New York, USA). The research team assessed the quantitative data using student's t-test and expressed it as means ± standard derivations (SDs). The qualitative data were assessed using the Chi-square test. $P < .05$ was considered to be a statistically significant difference.

RESULTS

Participants

As Table 1 shows, no significant differences existed in the miR-518a-5p expression among BC patients of different ages, tumor sizes, histological grades, and time of lymph-node metastasis ($P > .05$), but a significant difference existed

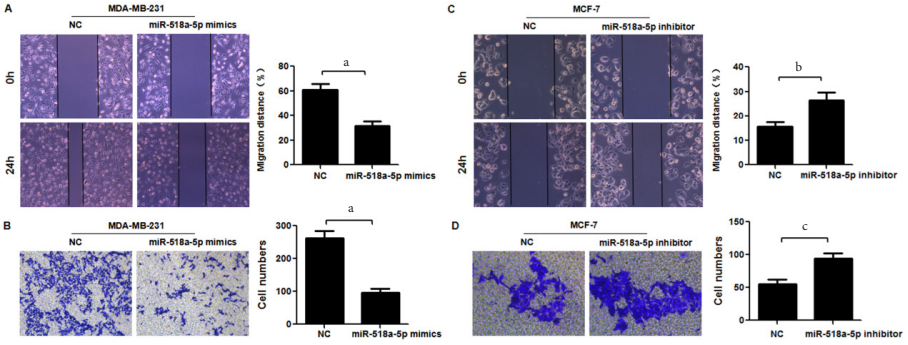
Figure 1. MiR-518a-5p Level in with Tissues and Cells. Figure 1A shows the miR-518a-5p level in BC and normal control tissues; Figure 1B shows the expression of miR-518a-5p in the BC cell—MDA-MB-231, BT-549, and MCF-7.



[a]$P < .001$, showing that the miR-518a-5p level in tumor tissue was significantly lower than that in normal tissue

Abbreviations: BC, breast cancer.

Figure 2. MiR-518a-5p Inhibits Cell Proliferation. Figure 2A compares the cell scratch test showing the migration rate for MDA-MB-231 cells transfected with miR-518a-5p mimic cells and for normal control cells; Figure 2B shows the number of invasive cells of MDA-MB-231 transfected with miR-518a-5p mimic cells and for normal control cells; Figure 2C compares the cell scratch test showing the migration rate of MCF-7 cells transfected with miR-518a-5p inhibitor cells and for normal control cells; and Figure 2D shows the number of invasive cells of MCF-7 transfected with miR-518a-5p inhibitor cells and for normal control cells.



[a]$P < .001$, indicating that cell migration rate and number of invasive cells of MDA-MB-231 after transfection with miR-518a-5p mimics were significantly lower than those of the normal control cells

[b]$P < .05$, indicating that the cell migration rate of MCF-7 after transfection with the miR-518a-5p-inhibitor was significantly higher than that of the normal control cells

[c]$P < .01$, indicating that the number of invasive cells of MCF-7 after transfection with the miR-518a-5p-inhibitor was significantly higher than that of the normal control cells

Abbreviations: MDA-MB-231 and MCF-7, breast cancer cell lines; NC, normal control tissue.

among participants at different clinical stages ($P < .001$), indicating that a close relationship exists between the miR-518a-5p and the clinical stage of BC.

Mir-518a-5p Levels

Figure 1A shows that the miR-518a-5p level in the BC tissues was significantly lower than that in the NC group ($P < .01$), indicating that the miR-518a-5p level in BC is low. In the assay of the BC cell lines (Figure 1B), the miR-518a-5p level in the MDA-MB-231 cell line was significantly lower than that in the BT-549 and MCF-7 cell lines ($P < .05$), while the level in the MCF-7 cell line was significantly higher than that of the other two lines ($P < .05$). Therefore, the research team selected MDA-MB-231 and MCF-7 to evaluate cell proliferation.

Mir-518a-5p and Cell Proliferation

Figure 2A shows that the cell migration rate of MDA-MB-231, after transfection with the miR-518a-5p mimics, was significantly lower than that of the NC group ($P < .05$). Figure 2B shows that the number of invasive cells of MDA-MB-231 was significantly lower after transfection with the miR-518a-5p mimics than that of the NC group ($P < .05$). These findings indicated that the miR-518a-5p mimics could inhibit the migration and invasion of MDA-MB-231. Figure 2C shows that the cell migration rate of MCF-7 after the miR-518a-5p-inhibitor transfection was significantly higher than that of the NC group ($P < .05$). Figure 2D shows that the number of invasive cells of MCF-7 after the miR-518a-5p-inhibitor transfection was also significantly higher than that of the NC group ($P < .05$), indicating that the miR-518a-5p inhibitor can promote the migration and invasion of MCF-7.

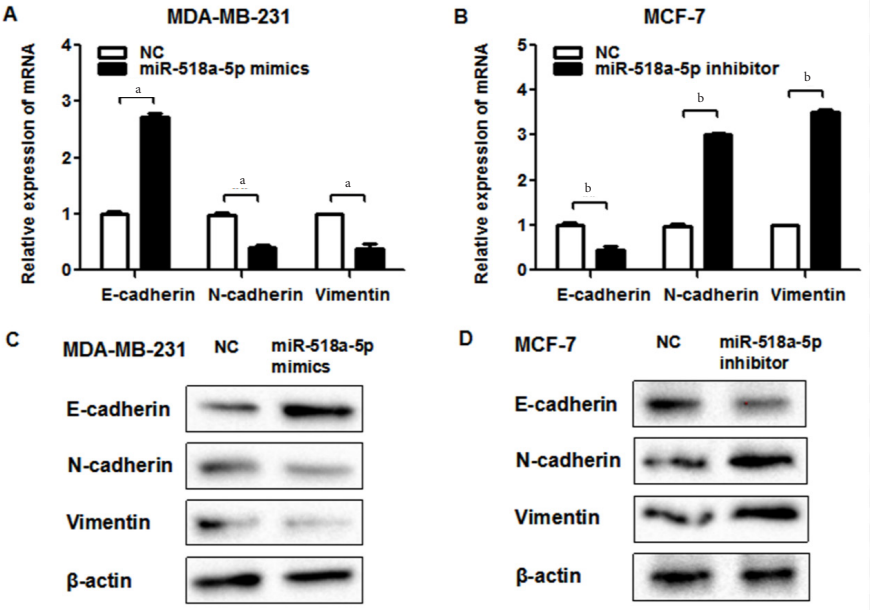
Mir-518a-5p and EMT Capability

Figure 3A shows that the E-cadherin mNRA levels were significantly higher for the MDA-MB-231 transfected with the miR-518a-5p mimics compared to those of the NC group ($P < .05$), while the N-cadherin and Vimentin mRNA were significantly lower ($P < .05$), indicating that the miR-518a-5p mimics can inhibit the EMT process of MDA-MB-231. In contrast, Figure 3B shows that the E-cadherin mNRA, in the MCF-7 transfected with the miR-

518a-5p inhibitor, were significantly lower than those of the NC group ($P < .05$), and the N-cadherin and Vimentin mRNA were significantly higher ($P < .05$), suggesting that the miR-518a-5p inhibitor can promote EMT in MCF-7.

Figure 3C shows that the E-cadherin protein level in MDA-MB-231 after transfection with the miR-518a-5p mimics was significantly higher than that of the NC group and the N-cadherin and Vimentin protein levels were significantly lower. Figure 3D shows that the E-cadherin protein level in the MCF-7 after transfection with the miR-518a-5p inhibitor was significantly lower than that of the NC group and the N-cadherin and Vimentin were significantly higher in the MCF-7 after transfection with the miR-518a-5p inhibitor, indicating that the miR-518a-5p is also relevant to the EMT of BC cells.

Figure 3. Influence of Mir-518a-5p on EMT Capability. Figure 3A compares the expression of EMT protein in the MDA-MB-231 cells transfected with miR-518a-5p mimic cells and in normal control cells, as tested using qRT-PCR; Figure 3B compares the expression of EMT protein in MCF-7 cells transfected with miR-518a-5p mimic cells and in normal control cells, as tested using qRT-PCR; Figure 3C compares the expression of EMT protein in MDA-MB-231 cells transfected with miR-518a-5p inhibitor cells and in normal control cells, as tested using WB; and Figure 3D compares the expression of EMT protein in MCF-7 cells transfected with miR-518a-5p inhibitor cells and in normal control cells, as tested using WB.



[a]$P < .01$, indicating that the E-cadherin mNRA levels were significantly higher for the MDA-MB-231 transfected with miR-518a-5p mimics compared to those of the normal control cells, while the N-cadherin and Vimentin mRNA were significantly lower

[b]$P < .01$, indicating that the E-cadherin mNRA levels were significantly lower for the MCF-7 transfected with miR-518a-5p inhibitor compared to those of the normal control cells, while the N-cadherin and Vimentin mRNA were significantly higher

Abbreviations: EMT, epithelial–mesenchymal transition; MDA-MB-231 and MCF-7, breast cancer cell lines; NC, normal control tissue; qRT-PCR, real-time quantitative reverse transcription-polymerase chain reaction test; WB, Western blot analysis.

ZEB2 and Mir-518a-5p

Figure 4A shows the binding sites between the miR-518a-5p and ZEB2. No significant difference existed after transfection of ZEB2-EV with the miR-518a-5p mimics compared to the NC group (Figure 4B). The Luciferase activity of the miR-518a-5p mimics group was significantly lower after co-transfection with ZEB2 3'UTR plasmid as compared with the NC group.

Figures 4C and 4D show that the ZEB2 level was significantly lower in the MDA-MB-231 cells transfected with the miR-518a-5p mimics but was significantly higher in the MCF-7 cells transfected with the miR-518a-5p inhibitor as compared with the corresponding NC groups.

Mir-518a-5p, Cell Migration and ZEB2

The cell migration rate in the miR-518a-5p+EV group was the lowest among the four groups ($P < .05$), while that in the NC+ZEB2 group was the highest, with the results being statistically significant ($P < .05$). Figure 5A shows that the cell migration rate in the miR-518a-5p+ZEB2 group was significantly higher that in the NC+EV group ($P > .001$), indicating that ZEB2 can reverse the effects of the miR-518a-5p on BC cell migration.

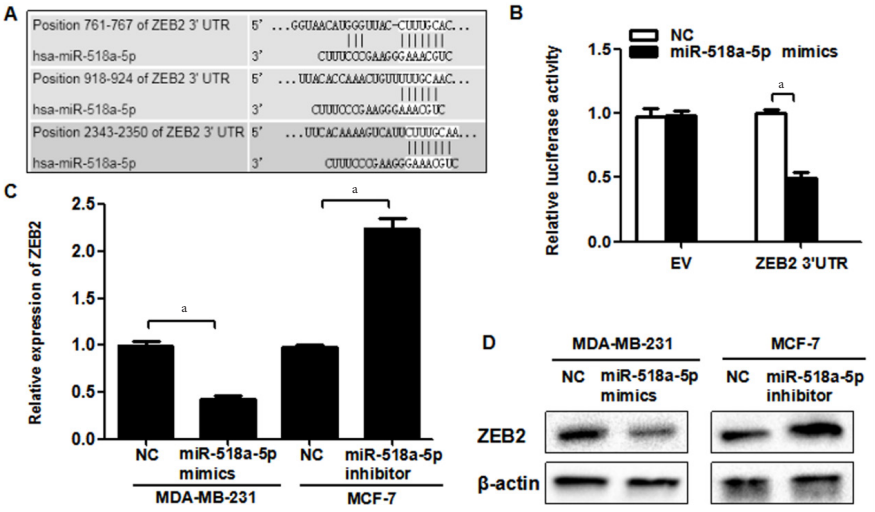
Figure 5B shows that the number of invasive cells counted using the Transwell invasion assay after co-transfection of ZEB2 and the miR-518a-5p in the miR-518a-5p+ ZEB2 group, was significantly higher that of the NC+EV group ($P > .001$,), which confirms that the miR-518a-5p can play a biological role by regulating ZEB2.

Finally, Figure 5C from the WB assay shows that the E-cadherin protein level was significantly lower and the N-cadherin and Vimentin proteins were significantly higher in the MDA-MB-231 cells transfected with ZEB2 ($P < .05$) as compared with the EV, indicating that the increase in ZEB2 can also promote the EMT process of MDA-MB-231.

DISCUSSION

The current study found that the miR-518a-5p-suppressed BC-cell migration and invasion by targeting ZEB2 and found it might be possible targets for therapy. The

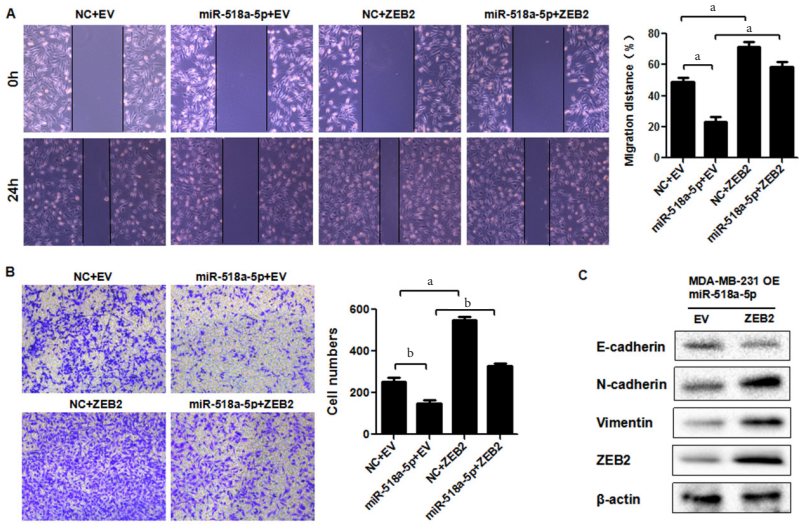
Figure 4. Targeting Relationship Between Mir-518a-5p and ZEB2. Figure 4A shows the binding sites between miR-518a-5p and ZEB2 using RNA sequencing; Figure 4B shows the results from the double fluorescein-reporter enzyme on the targets ZEB2 and EV; Figure 4C shows the effects of miR-518a-5p on ZEB2 in MDA-MB-231 and MCF-7 cells trasfected with miR-518a-5p mimic and inhibitor cells; and Figure 4D shows the effects of miR-518a-5p on ZEB2 and B-actin in MDA-MB-231 and MCF-7 cells transfected with miR-518a-5p mimic and inhibitor cells.



[a]$P < .01$, indicating that the ZEB2 level was significantly lower in the MDA-MB-231 cells transfected with the miR-518a-5p mimics but was significantly higher in the MCF-7 cells transfected with the miR-518a-5p inhibitor as compared with the corresponding normal control cells

Abbreviations: EV, empty vector; MDA-MB-231 and MCF-7, breast cancer cell lines; NC, normal control tissue; ZEB2, zinc finger E-box-binding homeobox 2 gene; ZEB2 3'UTR, ZEB2 wild-type 3'-untranslated region.

Figure 5. Mir-518a-5p Inhibits Cell Migration Through Regulating ZEB2 at 0 and 24 hours. The figures compare four groups: the normal control tissues + the empty vector (EV), the mir-518a-5p cells + the EV, the normal control tissues + the ZEB2, and the mir-518a-5p cells + the ZEB2. Figure 5A shows the migration distance as tested using the wound healing assay; Figure 5B shows the number of invasive cells as photographed as well as counted under a microscope using the Transwell invasion assay; and Figure 5C shows the EMT protein and ZEB2 levels as tested through WB analysis.



[a]$P < .001$, indicating that the cell migration rate in the miR-518a-5p+EV group was significantly lower than that of the NC+EV group, that the cell migration rate and number of invasive cells in the NC+ZEB2 group were significantly higher than that of the NC+EV group, and that the cell migration rate and number of invasive cells in the miR-518a-5p+EV group were significantly lower than that of the miR-518a-5p+ ZEB2 group

[b]$P < .01$, indicating that the number of invasive cells in the miR-518a-5p+EV group was significantly lower than that of the NC+EV group

Abbreviations: EMT, epithelial–mesenchymal transition; EV, empty vector; MDA-MB-231 and MCF-7, breast cancer cell lines; NC, normal control tissue; ZEB2, zinc finger E-box-binding homeobox 2 gene; ZEB2 3'UTR, ZEB2 wild-type 3'-untranslated region

ZEB2 was up-regulated in BC cells. The qRT-PCR and WB analysis showed that ZEB2 was the target gene of the miR-518A-5p. The Luciferase reporter assay verified the findings. The Transwell invasion assay and wound healing assay identified that miR-518a-5p inhibits cell migration and invasion via targeting ZEB2. Overall, the current demonstrated that the miR-518a-5p can facilitate cell migration and invasion targeting ZEB2. It showed that the miR-518a-5p might be a potential target and molecular diagnostic marker for BC treatment. The specific mechanism of the miR-518a-5p in BC cells is still vague, and further research is needed.

In follow-up experiments, we also need to carry out randomized controlled trials as soon as possible to confirm the expression of miR-518A-5p and ZEB2 in BC. At the same time, we need to add more experiments to further confirm the effect of miR-518A-5p and ZEB2 on BC. For example, we can analyze the effect of miR-518A-5p and ZEB2 on the actual growth of BC through the tumorigenesis experiment in nude mice. Finally, we also need to confirm the prognostic significance of miR-518A-5p and ZEB2 in BC through clinical follow-up investigations.

CONCLUSIONS

The miR-518a-5p suppressed BC cell migration, invasion, and process of EMT by regulating ZEB2. In the future, this method may be a new option for BC diagnosis and treatment. An in-depth understanding of the role of the miR-518a-5p in BC can help clinicians to understand the pathogenic mechanism of breast cancer more accurately.

AUTHORS' DISCLOSURE STATEMENT

The authors have no conflicts of interest to declare.

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