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MiR-526b suppresses cell proliferation, cell invasion and epithelial-mesenchymal transition in breast cancer by targeting Twist1

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Abstract. – OBJECTIVE: The aberrant expression of microRNAs (miRNAs) acts as crucial regulators in the tumorigenesis of breast cancer (BC). The aim of the study is to investigate the functional effects of miR-526b expression in breast cancer progression.

PATIENTS AND METHODS: The expression level of miR-526b in breast cancer tissues and cell lines was detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Cell proliferation, migration, and invasion capacity was detected by CCK-8 cell proliferation, colony formation, and transwell invasion assays after up-regulating or down-regulating miR-526b expression in breast cancer cells. Bioinformatics analysis and Dual-Luciferase reporter gene assays were used to demonstrate that Twist1 was a target of miR-526b. Western blot analysis was also performed.

RESULTS: We showed that miR-526b expression was significantly downregulated in breast cancer tissues compared to adjacent normal tissues. Lower miR-526b expression was associated with lymph node metastasis in breast cancer patients. Function assays showed that upregulation of miR-526b expression suppressed cell proliferation, cell colony formation, and cell invasion ability in breast cancer. Furthermore, the upregulation of miR-526b suppressed EMT makers Vimentin expression but increased the E-cadherin expression. Mechanically, we showed that miR-526b inhibited cell EMT process by targeting Twist1 expression.

CONCLUSIONS: Thus, our evidence indicated that miR-526b may serve as a potential target of breast cancer treatment.

Key Words:
Breast cancer, MiR-526b, Epithelial-mesenchymal transition, Twsit1.

Introduction

Breast cancer is the most frequent malignant tumor and represents the leading cause of tumor death in females worldwide[1]. In recent years, therapeutic methods including surgical resection in combination with hormonal therapy, chemo-radiotherapy, and biological therapy have made great progress for this disease[2]. However, understanding of the molecular mechanisms involved in breast cancer still need to be explored.

MicroRNAs (miRNAs) are a class of endogenous non-coding RNAs that have been identified as key regulators in human tumors[3]. In breast cancer, some miRNAs act as oncogenes or suppressors in tumor progression[4]. As, miR-29a could affect ER-positive breast cancer cell growth and invasion and is involved in the insulin signaling pathway[5]. MicroRNA-204 could reduce the cell proliferation and metastasis of breast cancer by targeting the PI3K/AKT signaling pathway[6]. MiR-221/222 increase tumor growth and suppress cell apoptosis by targeting lncRNA GAS5 in breast cancer[7]. MicroRNA-30d regulates breast cancer cell invasion, cell migration, and EMT process by targeting KLF11 and activating STAT3 pathway[8]. However, the functional role of miR-526b in breast cancer remains unknown. Our aim is to investigate the functional effects of miR-526b expression in breast cancer.

Here, we showed that miR-526b expression was significantly upregulated in breast cancer tissues compared to adjacent normal tissues. Lower miR-526b expression was related to lymph node metastasis of patients. Function assays showed

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that upregulation of miR-526b expression suppressed cell proliferation, migration, and invasion ability of breast cancer. Furthermore, the upregulation of miR-526b suppressed EMT makers Vimentin expression but increased the E-cadherin expression. Mechanically, we showed that miR-526b inhibited cell EMT process by targeting Twist1 expression. Thus, our evidence indicated that miR-526b may serve as a potential target of breast cancer treatment.

Patients and Methods

Patient Tissue Samples

A total of 57 pairs of human breast cancer tissue samples and adjacent normal tissues was obtained between January 2012 and March 2014. After resection, the tissues were snap-frozen in liquid nitrogen and then reserved at -80°C for further RNA analysis. All the patients involved were histologically diagnosed as breast cancer. The investigation was approved from the Ethics Committee of School of Clinical Medicine, Weifang Medical University, Weifang, Shandong, China. Written informed consents were obtained from all patients.

Cell Line Culture

Breast cancer cell lines including MDA-MB-231, MCF-7, BT-474, and SKBR3 were purchased from the Chinese Academia Cell Repository (Shanghai, China). A normal human breast epithelial cell line MCF-10A was purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Carlsbad, CA, USA) and supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA). All cells lines were grown at 37°C in a humidified atmosphere containing 5% CO_2.

RNA Extraction and Quantitative Real Time-PCR (qRT-PCR)

Total RNA was extracted using TRIzol reagent (TaKaRa, Dalian, China) following the protocol of the manufacturers. Reverse-transcribed complementary DNAs (cDNAs) were prepared from 100 ng of the total RNA using the TaqMan Universal PCR kit (TaKaRa, Dalian, China). SYBR Prime Script RT-PCR Kit (TaKaRa, Dalian, China) was used to detect mRNA expression on an ABI Prism 7500 HT sequence detection system using (Applied Biosystems; Foster City, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or U6 mRNA fold were identified as the endogenous control. The primers used in the study for miR-526b-forward: 5'-GCGCTCTTGAGGGAAGCACT-3', miR-526b-reverse: 5'-TACGTTCCATAGTCT ACCA-3'. U6-forward: 5'-GCGCGTCGTGAAGCGTTC-3' U6-revese: 5'-GTGCAGGGTCCGAGGT-3', Twist1-forward: 5'-ACGAGCTGGACTCCAAGATG-3' and Twist1-reverse: 5'-CACGCCCTGTTTCTTTGAAT-3' and GAPDH-forward: 5'-GGTCTCCTCTGACTTCAACA-3', GAPDH-reverse: 5'-GCCAAATTCGTTGTCATAC-3' were synthesized and purchased from Sangon Biotech (Shanghai, China). The mRNA fold change was analyzed using the relative quantification ($2^{-\Delta\Delta Ct}$) methods.

Cell Counting Kit-8 (CCK-8) Assay

MDA-MB-231 and SKBR3 cells were seeded in 96-well plates (2000 cells /per well). After cell transfection at 24, 48, 72, and 96 h, 10 μL CCK-8 (Beyotime Institute of Biotechnology, Shanghai, China) solution was added into each well, and cells were incubated for 2 h in a humidified incubator. Cell proliferation was detected by a microplate reader (BioTek Instruments, Bio-Tek, Winooski, VT, USA) and the optical density was measured at 450 nm.

Colony Formation Assays

For cell colony formation assays, transfected MDA-MB-231 and SKBR3 cells were seeded in 6-well plates (Nunc, Roskilde, Denmark) at 37°C with 5% CO_2 at a density of 500 cells per well. The cells were continued to be maintained at 37°C with 5% CO_2 for approximately 2 weeks. At the indicated time, the cell colonies were fixed with ethanol, treated with 0.1% crystal violet solution (Sangong, Songjiang, Shanghai, China) for 30 min, washed twice by deionized water, and photographed by an inverted microscope (IX71, Olympus, Tokyo, Japan).

Transwell Assays

Transwell assays were used to evaluate the migration and invasion capabilities of MDA-MB-231 and SKBR3 cells. The transwell invasion assays were performed using an 8-μm pore polycarbonate membrane chamber insert in a 24-well plate (BD Biosciences, Franklin Lakes, NJ, USA). The chamber inserts were pre-coated with 20 μL of Matrigel (200 mg/mL; BD Biosciences, Franklin Lakes, NJ, USA). Then, cells were re-suspended in serum-free medium and

were added into the upper chamber. The medium containing 10% FBS was added into the lower chamber. After 48 h, the invasive cells in the lower chamber were fixed with 100% methanol and then stained with 0.1% crystal violet solution (Beyotime Institute of Biotechnology, Shanghai, China). The cells were counted and photographed by using an inverted microscope (IX71, Olympus, Tokyo, Japan).

Western Blot Analysis

Western blot analysis was carried out as previously described. Briefly, equal amount of protein was separated on 10%-12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and then was transferred to nitrocellulose membranes (EMD Millipore, Billerica, MA, USA). Next, the membranes were blocked with 5% nonfat milk for 1 h and incubated with primary antibodies with E-cadherin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), Vimentin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and Twist 1 (Abcam, Cambridge, MA, USA) and GAPDH (Abcam, Cambridge, MA, USA). The signals were detected using an enhanced chemiluminescence (ECL) detection system (Beyotime Institute of Biotechnology, Shanghai, China). GAPDH expression level was used as internal control.

Luciferase Reporter Assay

The Wild-type (WT) and mutated putative miR-526b sequences binding sites in Twist1 3' untranslated regions (UTR) were constructed and inserted into the pmiRNA-Report vectors (Genechem, Shanghai, China). MDA-MB-231 cells (5000/well) were seeded in a 96-well plate. Cells were co-transfected with Twist1 3'-UTR wild-type (WT) or Twist1 3'-UTR mutated (MUT) reporter plasmid and miR-526b mimic or miR-NC and the internal control *Renilla* plasmids. Luciferase activities were detected at 48 h after transfection using the Promega Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA). Relative Luciferase activity was analyzed as the ratio of firefly Luciferase activity to *Renilla* Luciferase activity.

Statistical Analysis

Data were analyzed using Statistical Product and Service Solutions 18.0 Statistical Software (SPSS 18.0; IBM, Armonk, NY, USA) and Prism GraphPad version 5.0 (GraphPad Software Inc., La Jolla, CA, USA) software. Results were presented as mean ± SD. A Student's *t*-test was used to evaluate the statistical significance. $p<0.05$ was considered as statistically significant.

Results

MiR-526b Expression Is Downregulated In Human Breast Cancer Tissues and Cells

MiR-526b expression level in breast cancer tissues and adjacent normal tissues was determined using qRT-PCR analysis. The analysis results demonstrated that miR-526b expression was significantly lower in breast cancer tissues compared with adjacent normal tissues (Figure 1A). Furthermore, the association between miR-526b expression and clinicopathological feathers was analyzed using Chi-square test. Results demonstrated that low miR-526b expression was significantly associated with lymph node metastasis in patients (Table I, Figure 1B). Moreover, we detected the expression of miR-526b in four human breast cell lines MDA-MB-231, MCF-7, BT-474, and SKBR3 and a normal human breast epithelial cell line MCF-10A. Our results indicated that miR-526b expression was also downregulated in breast cancer cells compared to that in the MCF-10A (Figure 1C).

MiR-526b Inhibits Cell Proliferation and Cell Colony Formation Ability In Breast Cancer

To detect the effects of miR-526b expression on breast cancer cells proliferation capacity, we performed gain and loss function assays. The miR-526b mimic and miR-526b inhibitor were used to upregulate or downregulate the expression of miR-526b in breast cancer (Figure 1D). The results of CCK-8 cell proliferation assays showed that miR-526b mimic significantly inhibited cell proliferation ability; however, miR-526b inhibitor promoted cell proliferation, compared to corresponding control groups in MDA-MB-231 and SKBR3 cells (Figure 2A-2B). Furthermore, cell colony formed assays showed that miR-526b mimic significantly reduced cell colony number; however, miR-526b inhibitor increased cell colony number, compared to corresponding control groups in MDA-MB-231 and SKBR3 cells (Figure 2C-2D). These results indicated that miR-526b could inhibit cell proliferation ability of breast cancer.

Table I. The association between clinicopathological factors and miR-526b expression in breast cancer patients.

Factors	No. of Patients	MiR-526b expression		*p*-value
		Lower (n=29)	Higher (n=28)	
Age (years)				0.503
≤45	30	14	16	
>45	27	15	12	
Tumor size				0.903
≤2 cm	26	13	13	
>2 cm	31	16	15	
Histological grade				0.516
G1/2	33	18	15	
G3	24	11	13	
Lymph node metastasis				0.002*
Negative	33	11	22	
Positive	24	18	6	
TNM				0.105
I/II	39	17	22	
III	18	12	6	

*p <0.05.

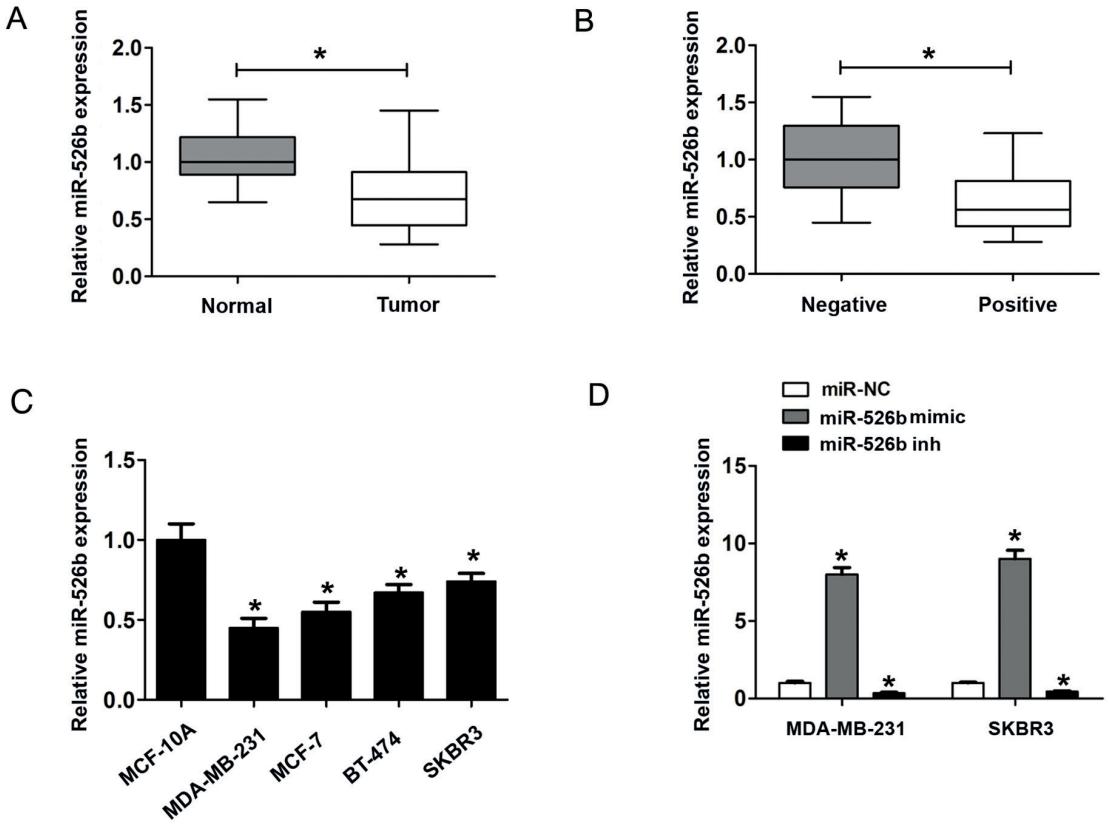

Figure 1. MiR-526b expression is lower in human breast cancer tissues and cells. **A,** Expression of miR-526b was examined using qRT-PCR in breast cancer tissues and adjacent normal tissues. **B,** Expression of miR-526b was associated with lymph node metastasis in patients using qRT-PCR. **C,** Expression of miR-526b in four human breast cancer cell lines MDA-MB-231, MCF-7, BT-47, SKBR3, and A normal human breast epithelial cell line MCF-10A was detected using qRT-PCR. **D,** Expression of miR-526b in MDA-MB-231 and SKBR3 cells was detected using qRT-PCR after cells were introduced with miR-526b mimic, miR-526b inhibitor or miR-NC. *p<0.05.

MiR-526b Inhibits Cell Invasion and EMT Process In Breast Cancer

To define the character of miR-526b in breast cancer cell invasion and EMT process, miR-526b mimic or miR-NC was transfected into MDA-MB-231 and SKBR3 cells to explore the effects of miR-526b on the invasion and EMT process of breast cancer cell lines, respectively. The transwell invasion assays results shown that miR-526b mimic significantly reduced cell migration and invasion number compared to corresponding control groups in MDA-MB-231 and SKBR3 cells (Figure 3A-3D). Furthermore, we showed that miR-526b mimic significantly suppressed the Vimentin expression and upregulated the E-cadherin expression in MDA-MB-231 and SKBR3 cells, suggesting miR-526b significantly suppressed EMT process in breast cancer (Figure 4A-4B).

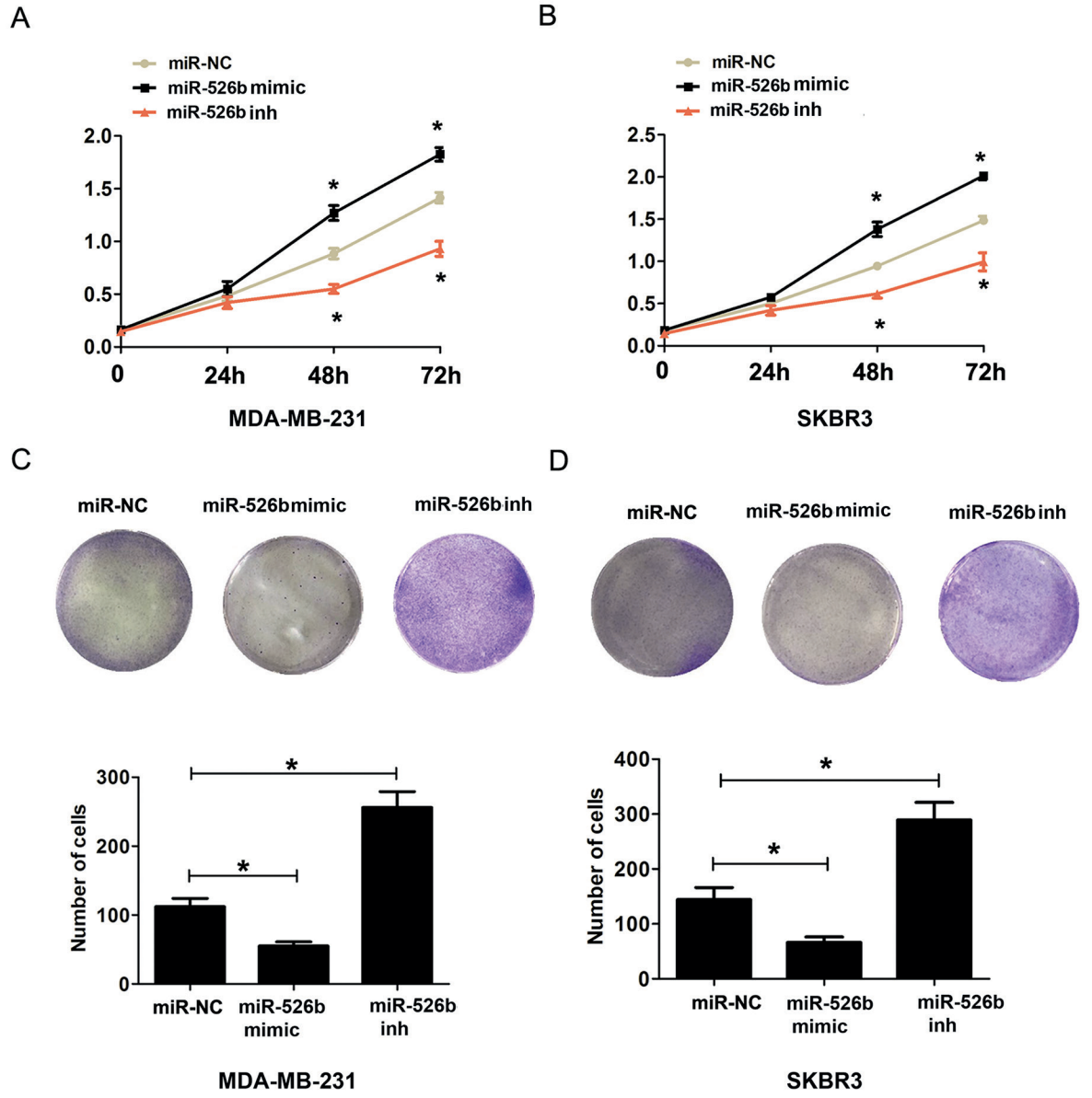

Figure 2. MiR-526b overexpression suppressed breast cancer cell proliferation ability. **A-B**, Cell proliferation ability in MDA-MB-231 and SKBR3 cells was detected using CCK-8 cell proliferation after MDA-MB-231 and SKBR3 cells were introduced with miR-526b mimic, miR-526b inhibitor or miR-NC. **C-D**, Cell colony formation ability and cell forming number in MDA-MB-231 and SKBR3 cells was detected after MDA-MB-231 and SKBR3 cells were introduced with miR-526b mimic, miR-526b inhibitor or miR-NC. (Magnification 200×), *p<0.05.

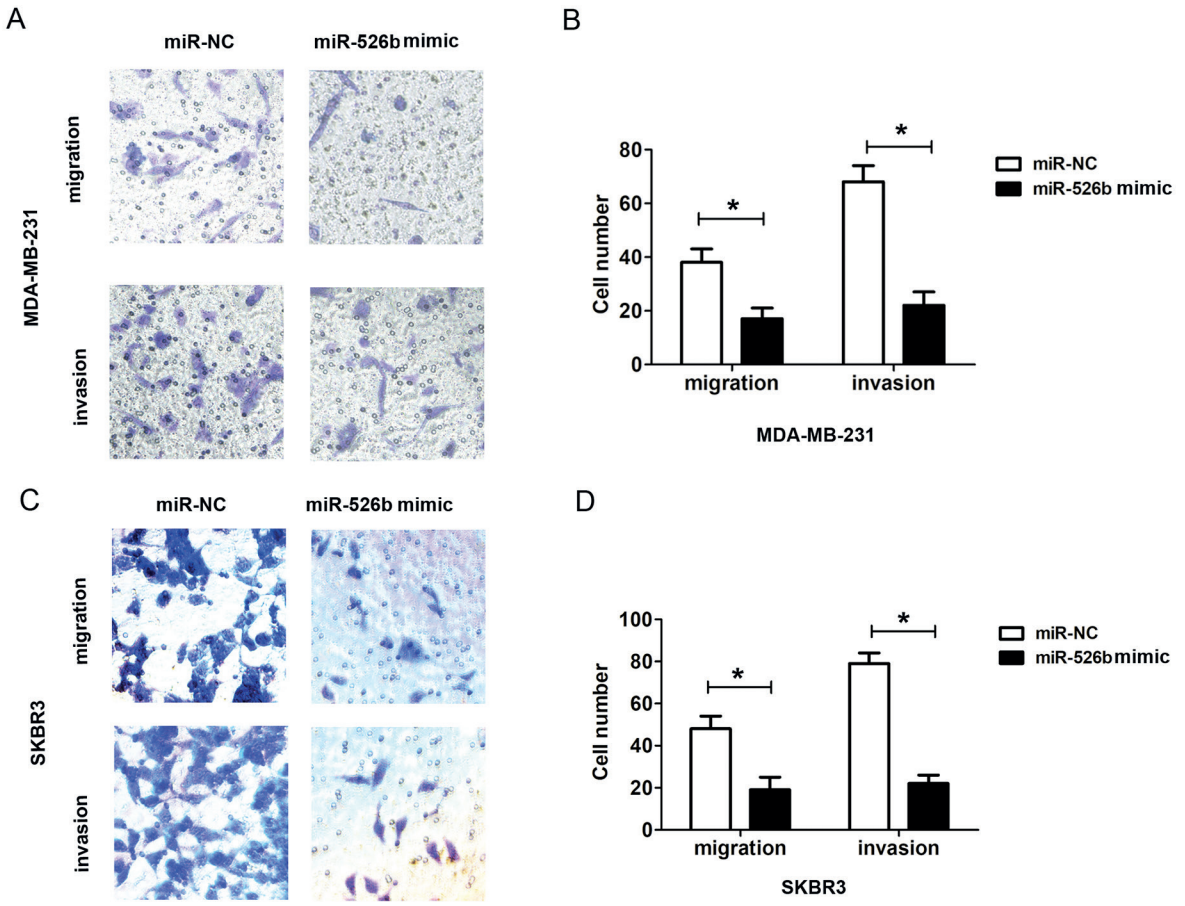

Figure 3. MiR-526b overexpression suppressed breast cancer cell migration and invasion ability. **A-B**, Cell migration and invasion ability and cell forming number in MDA-MB-231 cells was detected after MDA-MB-231 cells were introduced with miR-526b mimic or miR-NC (magnification 200X). **C-D**, Cell migration and invasion ability and cell forming number in SKBR3 cells was detected after SKBR3 cells were introduced with miR-526b mimic or miR-NC. Magnification 200X, *p<0.05.

Twist1 Is a Direct Target of MiR-526b In Breast Cancer Cells

Twist1 was identified as oncogene in EMT process in breast cancer, as Xu et al[9] showed that Twist1 promotes breast cancer invasion and metastasis by silencing Foxa1 expression. Our results showed that Twist1 mRNA expression was increased in breast cancer tissues compared to adjacent normal tissues (Figure 5A). Moreover, higher Twist1 expression showed a negative correlation with lower miR-526b expression using Pearson's analysis (Figure 5B). Subsequently, the Wild-type (WT) and mutated putative miR-526b sequences binding sites in Twist1 3' untranslated regions (UTR) were constructed and inserted into the pmiRNA-Report vectors (Figure 5C). MDA-MB-231 cells were transfected with wild-type (WT) and mutated type Twist1 3' untranslated regions (UTR) Luciferase reporter vectors and miR-526b mimic or miR-NC. The results demonstrated that miR-526b mimic significantly inhibited the Luciferase activity of wild-type (WT) Twist1 3' untranslated regions (UTR) reporter vector, but not mutated type Twist1 3' untranslated regions (UTR) (Figure 5D). Thus, the results showed that Twist1 was a potential target of miR-526b in breast cancer.

The qRT-PCR results showed that miR-526b mimic inhibited the mRNA expression of Twist1; however, miR-526b inhibitor suppressed the mRNA expression of Twist1 in MDA-MB-231 and SKBR3 cells (Figure 5E). Furthermore, Western blot analysis results showed that miR-526b mimic inhibited the protein expression of Twist1 (Figure 5F). Thus, these results indicated that miR-526b may function as tumor suppressor in breast cancer by regulating Twist1 expression.

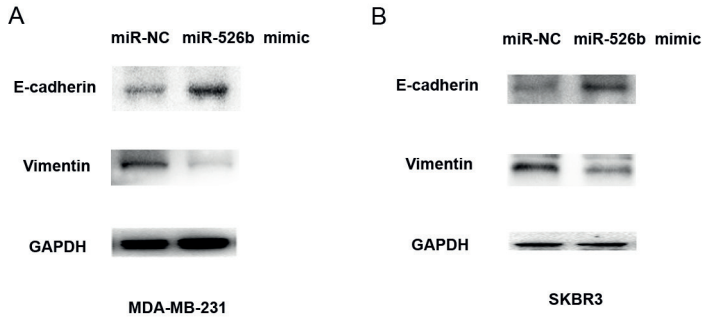

Figure 4. MiR-526b overexpression inhibited EMT relative protein expression. **A,** Protein expression of E-cadherin and Vimentin was detected after cells were introduced with miR-526b mimic or miR-NC in MDA-MB-231 cells. **B,** Protein expression of E-cadherin and Vimentin was detected after cells were introduced with miR-526b mimic or miR-NC in SKBR3 cells.

Discussion

Aberrant expression of miRNAs has been reported to be involved in breast cancer proliferation, migration, invasion, and metastasis[10]. Our results observed that miR-526b expression was lower in breast cancer compared to adjacent normal tissues. Lower miR-526b expression significantly associated with lymph node metastasis. Thus, these results suggested that miR-526b acted as a tumor suppressor in breast cancer. In the previous study, miR-526b has been identified as tumor suppressor in several tumors. MiR-526b-3p expression was found downregulated and acted as a tumor suppressor by down-regulation of HIF-1α expression in colon cancer[11] and could be a new diagnosis or therapeutic target. MiR-526b-3p serves as a prognostic factor and regulates the proliferation, invasion, and migration of glioma by targeting WEE1[12]. MicroRNA-526b acts as a prognostic factor and shows tumor suppressive property by targeting Sirtuin 7 in hepatocellular carcinoma[13]. By reducing PBX3, miR-526b expression suppresses the epithelial-mesenchymal transition process in cervical cancer cells[14]. However, the role of miR-526b in breast cancer progression still needs to be explored.

We showed that miR-526b expression is upregulated in breast cancer cells. MiR-526b overexpression inhibited cell proliferation ability and cell colony formation ability, but downregulation of miR-526b expression enhanced cell proliferation ability and cell colony formation ability in breast cancer. Besides, we found that miR-526b overexpression inhibited cell migration and invasion ability in breast cancer.

Next, we demonstrated that Twist1 was a direct target of miR-526b. MiR-526b overexpression inhibited the mRNA and protein expression of Twist1 in breast cancer cells. In the previous study, Twsit1 functioned as an oncogene to affect cell invasion and EMT process in breast cancer. Twist1 and Slug mediate H2AX-regulated epithelial-mesenchymal transition in breast cells[15]. Twist promotes reprogramming of glucose metabolism in breast cancer cells through PI3K/AKT and p53 signaling pathways[16]. MiR-720 inhibits tumor invasion and migration in breast cancer by targeting TWIST1[17]. Our results showed that miR-526b could inhibit Vimentin expression and upregulate E-cadherin expression. Twist1 is a critical oncogene that is overexpressed and plays critical roles in EMT process in various tumors by affecting E-cadherin expression[18]. In the study, we found that miR-526b could inhibit Twsit1 expression. Thus, these results may imply that miR-526b regulated cell invasion and EMT process by targeting Twist1 in breast cancer.

Conclusions

We found that miR-526b expression was lower in breast cancer tissues and cells. *In vitro* assays, we demonstrated that miR-526b inhibited tumor growth and invasion ability. Moreover, we identified that miR-526b targeted Twist1 and regulated its expression and promotes cell invasion and EMT process in breast cancer cells. Thus, these results indicated that miR-526b acts as a tumor suppressor in breast cancer, which provided potential target for breast cancer treatment.

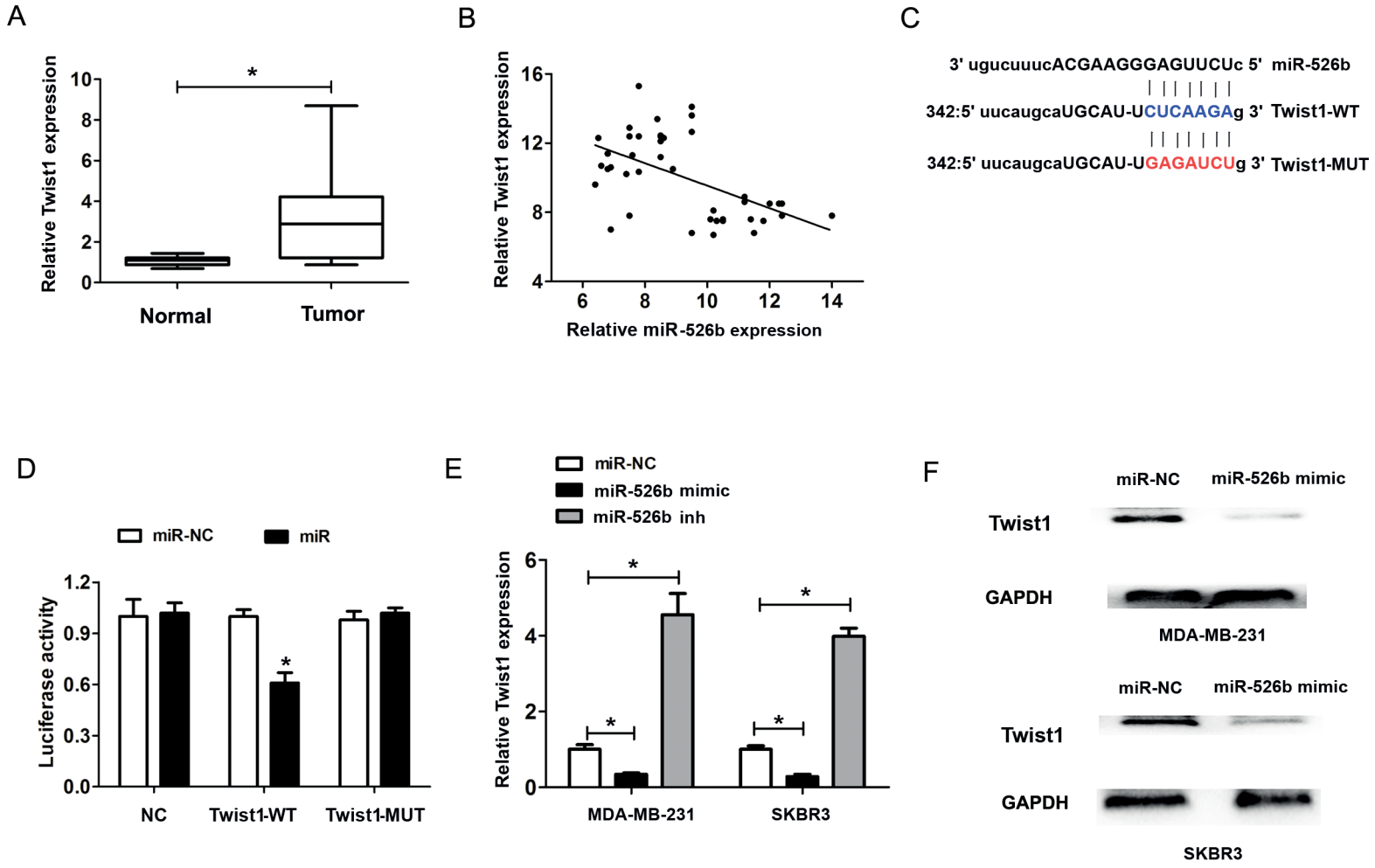

Figure 5. Twist1 is a direct target of miR-526b. **A,** mRNA expression of Twist1 was examined using qRT-PCR in breast cancer tissues and adjacent normal tissues. **B,** Higher Twist1 expression showed a negative correlation with lower miR-526b expression by using Pearson's analysis. **C,** Wild-type (WT) and mutated type Twist1 3' untranslated regions (UTR) was constructed. **D,** MDA-MB-231 cells were transfected with wild-type (WT) and mutated type Twist1 3' untranslated regions (UTR) Luciferase reporter vectors and miR-526b mimic or miR-NC. Luciferase activities were detected at 48 h after transfection. **E,** mRNA expression of Twist1 was detected after MDA-MB-231 and SKBR3 cells were introduced with miR-526b mimic, miR-526b inhibitor or miR-NC. *$p<0.05$. **F,** Protein expression of Twist1 was detected after cells were introduced with miR-526b-3p mimic, or miR-NC in MDA-MB-231 cells and SKBR3 cells.

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Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.
2) Cleator S, Heller W, Coombes RC. Triple-negative breast cancer: therapeutic options. Lancet Oncol 2007; 8: 235-244.
3) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
4) Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350-355.
5) Li ZH, Xiong QY, Xu L, Duan P, Yang QO, Zhou P, Tu JH. MiR-29a regulated ER-positive breast cancer cell growth and invasion and is involved in the insulin signaling pathway. Oncotarget 2017; 8: 32566-32575.
6) Fan X, Fang X, Liu G, Xiong Q, Li Z, Zhou W. MicroRNA-204 inhibits the proliferation and metastasis of breast cancer cells by targeting PI3K/AKT pathway. J BUON 2019; 24: 1054-1059.
7) Zong Y, Zhang Y, Sun X, Xu T, Cheng X, Qin Y. MiR-221/222 promote tumor growth and suppress apoptosis by targeting lncRNA GAS5 in breast cancer. Biosci Rep 2019; pii: BSR20181859.
8) Han M, Wang Y, Guo G, Li L, Dou D, Ge X, Lv P, Wang F, Gu Y. MicroRNA-30d mediated breast cancer invasion, migration, and EMT by targeting KLF11 and activating STAT3 pathway. J Cell Biochem 2018; 119: 8138-8145.
9) Xu Y, Qin L, Sun T, Wu H, He T, Yang Z, Mo Q, Liao L, Xu J. Twist1 promotes breast cancer invasion and metastasis by silencing Foxa1 expression. Oncogene 2017; 36: 1157-1166.
10) Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. Annu Rev Pathol 2014; 9: 287-314.
11) Zhang R, Zhao J, Xu J, Wang J, Jia J. MiR-526b-3p functions as a tumor suppressor in colon cancer by regulating HIF-1alpha. Am J Transl Res 2016; 8: 2783-2789.
12) Wu M, Li X, Liu Q, Xie Y, Yuan J, Wanggou S. MiR-526b-3p serves as a prognostic factor and regulates the proliferation, invasion, and migration of glioma through targeting WEE1. Cancer Manag Res 2019; 11: 3099-3110.
13) Liu X, Yang L, Tu J, Cai W, Zhang M, Shou Z, Yao Y, Xu Q. MicroRNA-526b servers as a prognostic factor and exhibits tumor suppressive property by targeting Sirtuin 7 in hepatocellular carcinoma. Oncotarget 2017; 8: 87737-87749.
14) Li H, Wang J, Xu F, Wang L, Sun G, Wang J , Yang Y. By downregulating PBX3, miR-526b suppresses the epithelial-mesenchymal transition process in cervical cancer cells. Future Oncol 2019; 15: 1577-1591.
15) Weyemi U, Redon CE, Sethi TK, Burrell AS, Jailwala P, Kasoji M, Abrams N, Merchant A, Bonner WM. Twist1 and Slug mediate H2AX-regulated epithelial-mesenchymal transition in breast cells. Cell Cycle 2016; 15: 2398-2404.
16) Yang L, Hou Y, Yuan J, Tang S, Zhang H, Zhu Q, Du YE, Zhou M, Wen S, Xu L, Tang X, Cui X, Liu M. Twist promotes reprogramming of glucose metabolism in breast cancer cells through PI3K/AKT and p53 signaling pathways. Oncotarget 2015; 6: 25755-25769.
17) Li LZ, Zhang CZ, Liu LL, Yi C, Lu SX, Zhou X, Zhang ZJ, Peng YH, Yang YZ, Yun JP. MiR-720 inhibits tumor invasion and migration in breast cancer by targeting TWIST1. Carcinogenesis 2014; 35: 469-478.
18) Jin C, Feng Y, Ni Y1, Shan Z. MicroRNA-610 suppresses osteosarcoma oncogenicity via targeting TWIST1 expression. Oncotarget 2017; 8: 56174-56184.