European Review for Medical and Pharmacological Sciences 2019; 23: 9988-9995

MicroRNA-1236-3p inhibits proliferation and invasion of breast cancer cells by targeting ZEB1

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Abstract. – OBJECTIVE: This research aims to investigate the level of microRNA-1236-3p in breast cancer (BCa) tissues and to further investigate its possible mechanism in the progression of BCa.

PATIENTS AND METHODS: The level of microRNA-1236-3p in BCa tissues and adjacent tissues was detected by quantitative Real-time polymerase chain reaction (qRT-PCR). Regulatory effects of microRNA-1236-3p on cell proliferation and invasion were detected by cell counting kit-8 (CCK-8) and transwell assay. The binding relationship between microRNA-1236-3p and zinc-finger E-box binding homeobox (ZEB1) was examined by the dual-luciferase reporter gene assay. Finally, rescue experiments were conducted to verify the potential role of microRNA-1236-3p/ ZEB1 axis in BCa.

RESULTS: MicroRNA-1236-3p was downregulated in BCa tissues relative to adjacent tissues, and the similar trend was shown in BCa cell lines. Overexpression of microRNA-1236-3p in MDA-MB-231 and MCF-7 cells inhibited proliferation and attenuated invasiveness, while knockdown of microRNA-1236-3p had an opposite effect. Dual-luciferase reporter gene assay and qRT-PCR results showed that microRNA-1236-3p could target ZEB1 to degrade it. Overexpression of ZEB1 in BCa cells can partially reverse the effect of overexpressed miR-1236-3p on cell proliferative and invasive abilities.

CONCLUSIONS: MicroRNA-1236-3p could inhibit the growth and metastasis of BCa cells by inhibiting ZEB1 expression, suggesting that microRNA-1236-3p may be a potential therapeutic target for BCa.

Key Words:
MicroRNA-1236-3p, Breast cancer, Proliferation, Invasion, ZEB1.

Introduction

Breast cancer (BCa) is one of the most common cancers in females, which extremely endangers female health. The adverse effects of BCa are multifaceted[1]. The occurrence and progression of BCa is a complex process involving multiple factors and pathways. The pathogenesis of BCa involves both environmental and genetic factors, which cannot be comprehensively explained by only a few simple risk factors[2]. Therefore, early diagnosis plays a crucial role in improving the therapeutic efficacy of BCa[3]. The mechanism of BCa development needs to be further explored. More importantly, it is urgent to find sensitive and specific molecular biomarkers, which is important for the treatment of BCa. MicroRNAs (miRNAs) are a class of non-coding small RNA molecules of approximately 18-25 nucleotides in length. MiRNAs are a class of regulatory RNAs that inhibit gene expressions at the post-transcriptional level. They can bind to the target mRNA by base pairing, resulting in the mRNA degradation or translation inhibition of the target mRNA[4]. Functionally, many important life activities apoptosis and cellular behaviors are affected by miRNAs[5]. It is currently found that almost one-third of proteins are regulated by miRNAs. Abnormally expressed miRNA can lead to a variety of biological processes including tumor occurrence, development, invasion and metastasis[6]. Related studies have shown that miRNA can act as an oncogene or a tumor suppressor in different tumor tissues. As an oncogene, it can promote the progression of tumor, and its deficiency can inhibit cell growth and induce cell apoptosis[7]. As a tumor-suppressor gene, it can inhibit tumor growth, and its silence accelerates tumor progression[8]. At present, tumor-related miRNAs have been extensively explored, and these miRNAs are expected to become a new target for tumor diagnosis, treatment and prognosis. Previous studies have confirmed that microRNA-1236-3p plays a crucial role in various tumors, such as gastric cancer,

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bladder tumor, lung adenocarcinoma and ovarian tumor[9-12], but its biological function in BCa remains to be studied. In our research, we explored the effects of miRNA-1236-3p on the proliferation and invasion of BCa cells. We speculated whether microRNA-1236-3p can exert its biological functions by regulating the level of zinc-finger E-box binding homeobox (ZEB1).

Patients and Methods

Study Subjects and Sample Collection

Fresh BCa tissues and adjacent normal tissues were collected from 67 patients treated with surgery in Sichuan Luzhou TCM Hospital from July 2015 to December 2017. None of patients received any treatment before surgery, and they had no family history. BCa diagnosis was confirmed by pathological diagnosis. Patients volunteered participated in the study and signed written informed consent. This study has been approved by the Hospital Ethics Committee of The First Affiliated Hospital of Shantou University Medical College. The collected specimens were stored in a liquid nitrogen tank.

Cell Culture and Transfection

The normal cell line (HBL-100) and BCa cell lines (MDA-MB-231, MDA-MB-468, MDA-MB-435s, and MCF-7) were obtained from ATCC (Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum (FBS, Gibco, Rockville, MD, USA), 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco, Rockville, MD, USA). Cells were maintained in an incubator with a relative humidity of 95% and a 5% CO_2 at 37°C. After the cell fusion degree reached 60-70%, cells were washed once with phosphate-buffered saline (PBS). The transfection reagent mixed with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was applied. After 6 hours, the culture medium was replaced with complete medium, and the transfection effect was verified by quantitative Real-time polymerase chain reaction (qRT-PCR). The miRNA mimics, inhibitor and pcDNA-ZEB1 used in this experiment were all provided by GenePharma (Shanghai, China).

RNA Extraction

1 mL of TRIzol (Invitrogen, Carlsbad, CA, USA) was added in tissue or cells. The mixture was thoroughly homogenized, incubated with chloroform, and then centrifuged at 12,000 rpm, 4°C for 10 minutes. The RNA was precipitated with isopropanol and washed with 75% ethanol. The RNA precipitate was dried at room temperature and dissolved in diethyl pyrocarbonate (DEPC) water (Beyotime, Shanghai, China) according to the amount of RNA precipitation. The RNA concentration and purity were measured with a NanoDrop 2000 spectrophotometer (Thermo-Fisher, Waltham, MA, USA).

QRT-PCR

The reverse transcription reaction system was prepared on ice using the PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan), and the reaction was terminated to obtain complementary deoxyribose nucleic acid (cDNA). The reverse transcript template was added to the RNase-free water to a final concentration of 10 ng/µL. Quantitative PCR operations were performed according to the SYBR Green PCR Kit instructions (TaKaRa, Otsu, Shiga, Japan). The total reaction system was 10 µL. The reaction procedure was as follows: 95°C for 10 min, 94°C for 30 s, 60°C for 30 s, 72°C for 30 s and a final extension at 72°C for 10 min, for a total of 40 cycles. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 were used as internal references. Relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method. Primer sequences used in this study were as follows: ZEB1, F: 5'-CAACAGGCCTCCCCACTTTC-3', R: 5'-CGGTAGAGTGTTCCAGGGAA-3'; microRNA-1236-3p, F: 5'-GTGGGAACGTAATCGACCTCTG-3', R: 5'-ATGTCGTCGTGGTGACGGT-3'; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Cell Counting Kit-8 (CCK-8) Assay

After 24 h of transfection, cells were collected and seeded into 96-well plates with 5000 cells/well. At the appointed time points, 10 µL of CCK-8 solution (Dojindo Molecular Technologies, Kumamoto, Japan) was added to each well, and incubated at 37°C for 1 h. The absorbance of each well at 450 nm was measured by a microplate reader.

Cell Invasion Assay

MDA-MB-231 and MCF-7 cells were prepared for suspension with 3×10^5 cells/mL, and then applied in the Matrigel pre-coated transwell chamber that was inserted into the 24-well plate. 100 µL of Dulbecco's Modified Eagle's Medium (DMEM)

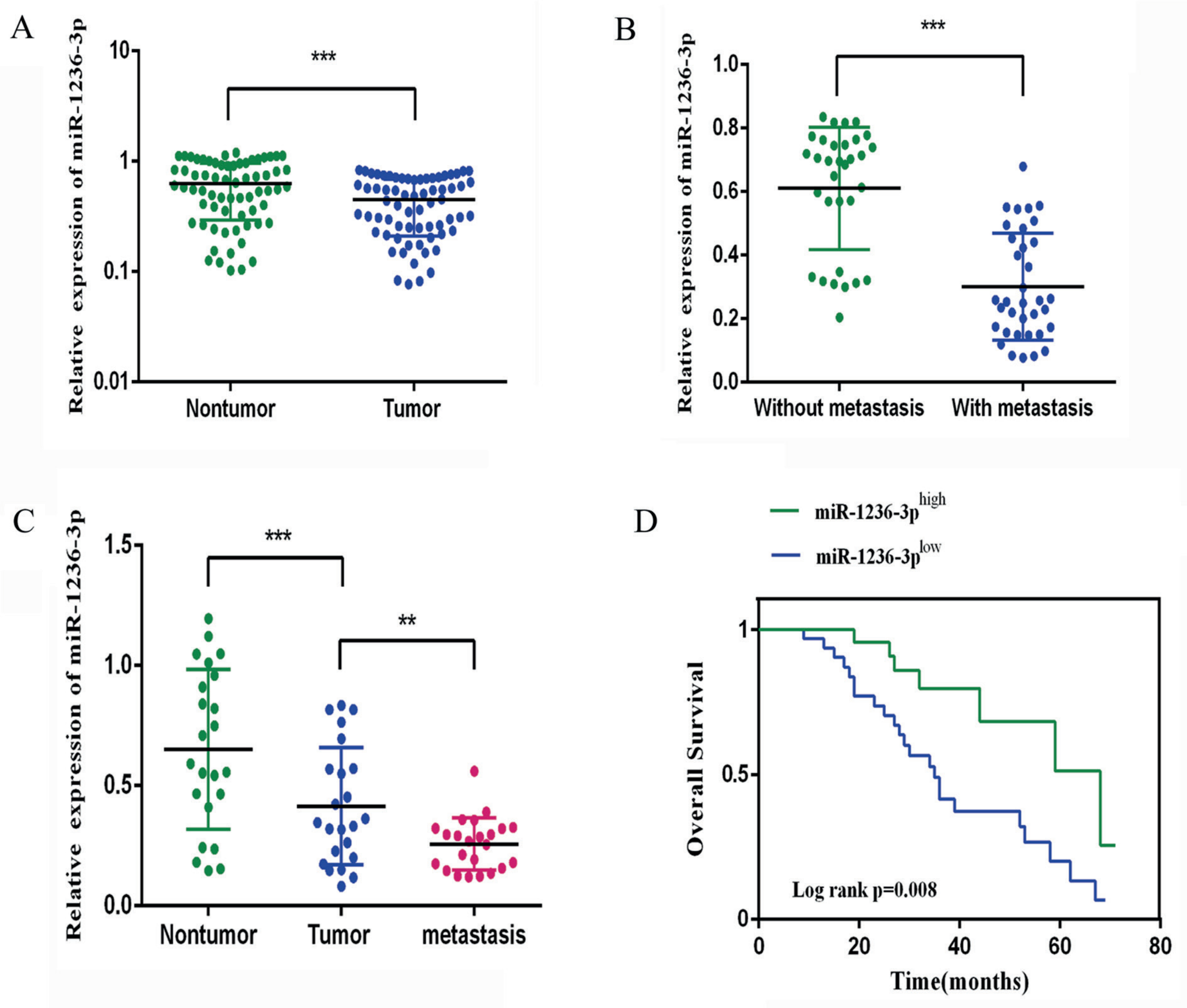

Figure 1. MicroRNA-1236-3p is downregulated in BCa tissues and associated with poor prognosis. **A,** MicroRNA-1236-3p level in 67 BCa tissues and corresponding adjacent tissues. **B,** MicroRNA-1236-3p level in 32 BCa patients with no tumor metastasis and 35 patients with tumor metastasis. **C,** MicroRNA-1236-3p level in 23 non-tumor tissues, matched tumor tissues, and metastatic tissues. **D,** Analysis of the relationship between microRNA-1236-3p level and prognosis of BCa patients by Kaplan-Meier. Data are presented as mean±SD; $^{**}p<0.01$, $^{***}p<0.001$.

was added to the upper chamber, while 600 μL of DMEM containing 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) was added to the bottom chamber. After incubating for 48 h, the chamber was removed. Remaining cells were fixed with ethanol for 15 min, and stained with crystal violet for 20 min. Penetrating cells were counted in 5 randomly selected fields per sample. The experiment was repeated three times.

Dual-Luciferase Reporter Gene Assay

The 3'UTR sequences of ZEB1 were downloaded from the NCBI website to construct the wild-type plasmid (ZEB1 WT 3'-UTR) and mutant-type plasmid (ZEB1 MUT 3'-UTR). Cells were then seeded in 96-well plates. Subsequently, 50 pmol/L microRNA-1236-3p mimics or negative control was co-transfected in cells with 80 ng ZEB1 WT 3'-UTR or ZEB1 MUT 3'-UTR. After 48 h of transfection, the fluorescence intensity was measured using the dual-luciferase reporter gene detection system (Promega, Madison, WI, USA).

Statistical Analysis

Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) 22.0 statistical software (IBM, Armonk, NY, USA). GraphPad Prism 7 software (La Jolla, CA, USA) was used for depicting figures. Measurement data were expressed as mean ± standard devia-

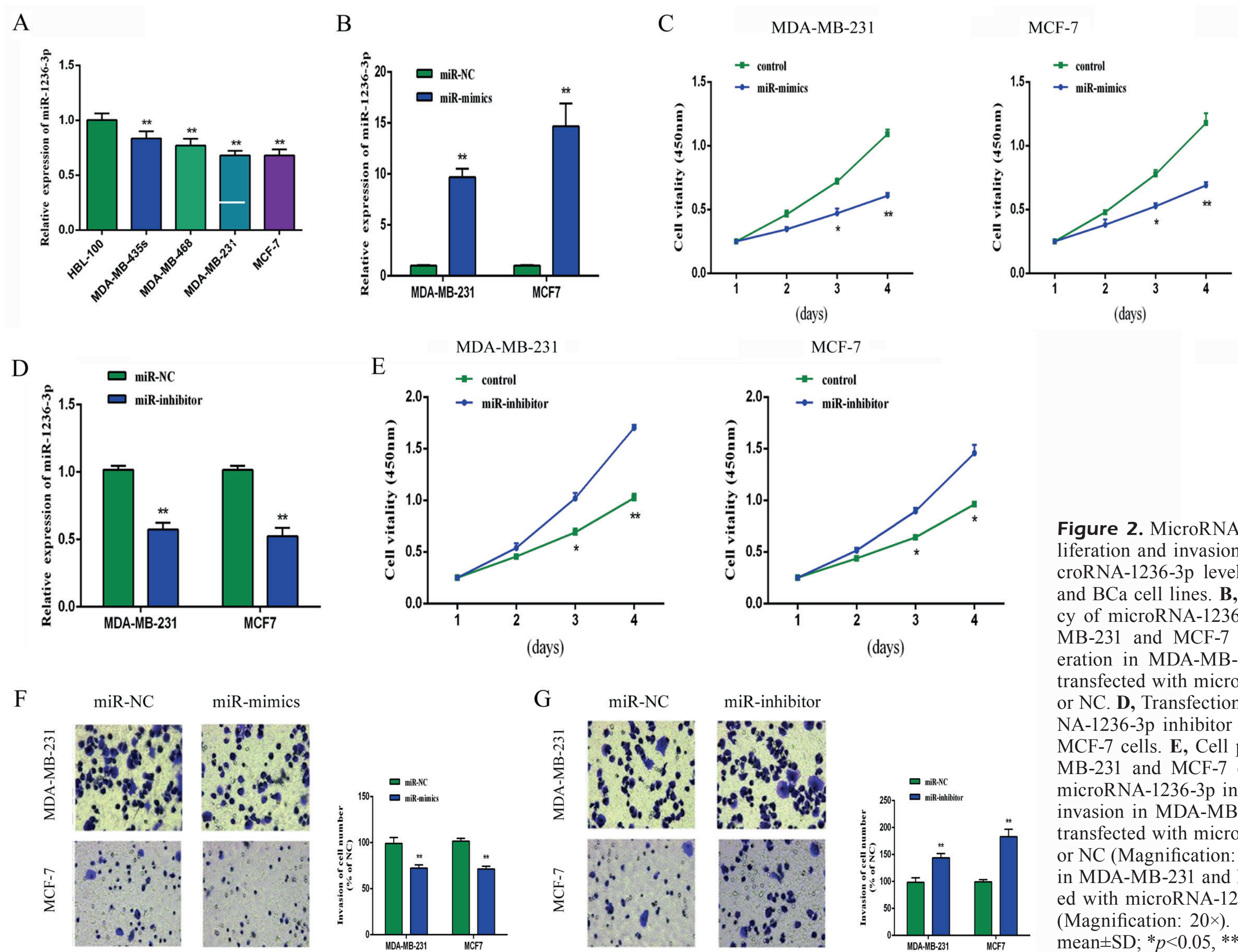

Figure 2. MicroRNA-1236-3p inhibits proliferation and invasion of BCa cells. **A,** MicroRNA-1236-3p level in normal cell lines and BCa cell lines. **B,** Transfection efficiency of microRNA-1236-3p mimics in MDA-MB-231 and MCF-7 cells. **C,** Cell proliferation in MDA-MB-231 and MCF-7 cells transfected with microRNA-1236-3p mimics or NC. **D,** Transfection efficiency of microRNA-1236-3p inhibitor in MDA-MB-231 and MCF-7 cells. **E,** Cell proliferation in MDA-MB-231 and MCF-7 cells transfected with microRNA-1236-3p inhibitor or NC. **F,** Cell invasion in MDA-MB-231 and MCF-7 cells transfected with microRNA-1236-3p mimics or NC (Magnification: 20×). **G,** Cell invasion in MDA-MB-231 and MCF-7 cells transfected with microRNA-1236-3p inhibitor or NC (Magnification: 20×). Data are presented as mean±SD; *p<0.05, **p<0.01.

tion. The correlation coefficient (R) was analyzed using Kaplan-Meier, and intergroup differences were compared by *t*-test. $p<0.05$ was considered statistically significant.

Results

MicroRNA-1236-3p Was Lowly Expressed in BCa Tissues

First, we detected microRNA-1236-3p expression in 67 BCa tissues and corresponding adjacent tissues by qRT-PCR. As shown in Figure 1A, microRNA-1236-3p was downregulated in BCa tissues, suggesting that microRNA-1236-3p might play a vital role in the development of BCa. Clinical information revealed that the level of microRNA-1236-3p in metastatic BCa patients was lower than that in the non-metastatic group (Figure 1B). In 23 matched non-tumor, tumor and metastatic tissues, the level of microRNA-1236-3p gradually decreased from non-tumor tissue to metastatic one (Figure 1C). We further explored the prognostic potential of microRNA-1236-3p in BCa. Kaplan-Meier analysis revealed that the survival rate of BCa patients with low level of microRNA-1236-3p was worse than those with high level. The experimental results indicated that microRNA-1236-3p could be used as a biomarker for predicting patient's prognosis.

MicroRNA-1236-3p Enhanced Proliferative and Invasive Capacities of BCa Cells

We subsequently examined microRNA-1236-3p expression in BCa cell lines using qRT-PCR. The results showed that microRNA-1236-3p was down-regulated in BCa cell lines compared to HBL-100 (Figure 2A). MDA-MB-231 and MCF-7 cell lines were selected for subsequent experiments. Transfection of microRNA-1236-3p mimics into BCa cell lines markedly upregulated microRNA-1236-3p level (Figure 2B). Further, CCK-8 result showed that overexpression of microRNA-1236-3p in BCa cells inhibited cell growth (Figure 2C). Similarly, we found that transfection of microRNA-1236-3p inhibitor could inhibit microRNA-1236-3p expression (Figure 2D). Knockdown of microRNA-1236-3p enhanced cell proliferation (Figure 2E). Subsequently, transwell assay revealed that overexpression of microRNA-1236-3p notably inhibited invasion of BCa cells, while knockdown of microRNA-1236-3p enhanced it (Figure 2F-2G).

MicroRNA-1236 -3p Could Target ZEB1

We predicted the target genes of microRNA-1236-3p by bioinformatics and ZEB1 was selected (Figure 3A). Subsequently, we examined the binding relationship between the two genes by dual-luciferase reporter gene assay. The results showed that luciferase activity decreased in the ZEB1-WT 3'UTR group, but there was no significant difference in ZEB1-MUT 3'UTR group (Figure 3B), indicating that microRNA-1236-3p could bind to ZEB1. In addition, we found that ZEB1 was upregulated in BCa tissues (Figure 3C). We then identified a negative correlation between microRNA-1236-3p and ZEB1 (Figure 3D). The mRNA level of ZEB1 was detected in MDA-MB-231 and MCF-7 cells transfected with miR-NC, or microRNA-1236-3p inhibitor. It was found that microRNA-1236-3p mimics significantly reduced ZEB1 level, while transfection of microRNA-1236-3p inhibitor enhanced it (Figure 3E-3F). The above results indicated that microRNA-1236-3p could target ZEB1 to inhibit its expression.

Overexpression of ZEB1 Could Reverse the Inhibitory Effect of microRNA-1236-3p on Cell Proliferative and Invasive Abilities

ZEB1 level was notably increased after transfection of pcDNA-ZEB1 in BCa cells (Figure 4A). CCK8 results indicated that up-regulated microRNA-1236-3p weakened cell proliferative ability, which was reversed by overexpression of ZEB1 (Figure 4B). Meanwhile, a similar change was observed in cell invasiveness (Figure 4C). The above results demonstrated that microRNA-1236-3p might inhibit the growth and invasiveness of BCa cells by inhibiting ZEB1 expression.

Discussion

BCa is one of the most malignant tumors with the highest morbidity and mortality, and the non-specific invasive lesion may be an important reason. Normal mammary epithelial cells occur atypical change, followed by ductal epithelial hyperplasia (ADH) and ductal carcinoma *in situ* (ductal carcinoma is sire, DCIS), and eventually develop into invasive ductal carcinoma (IDC). It is still a challenge to distinguish between ADH, DCIS and IDC by X-ray and core needle biopsy (CNB)[13]. Surgical resection cannot ultimately confirm the diagnosis, and the prognosis of BCa is extremely poor. So far, the most

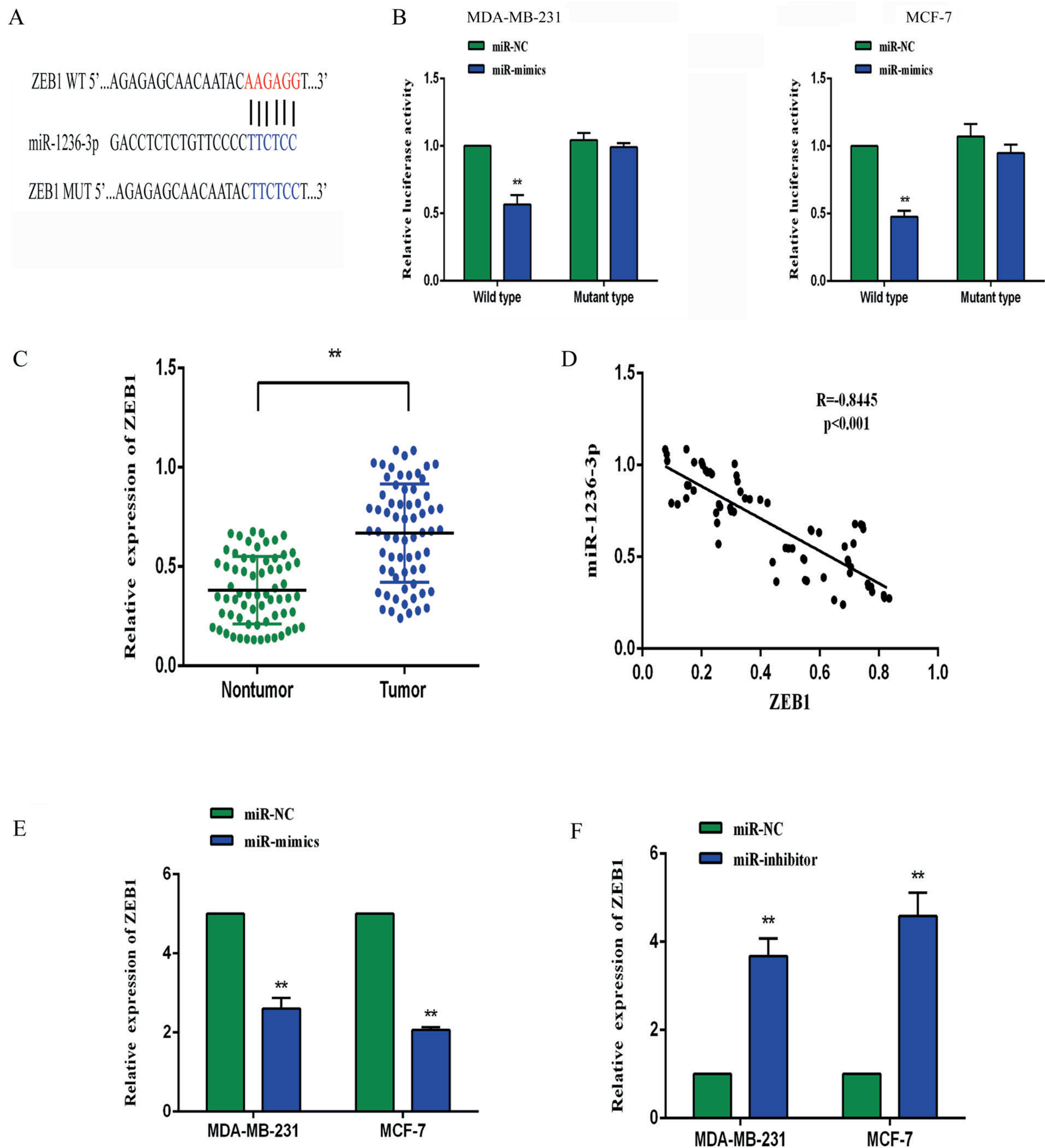

Figure 3. MicroRNA-1236-3p can target ZEB1. **A,** Prediction of the binding sites of microRNA-1236-3p to ZEB1 by bioinformatics. **B,** Relative luciferase activity in ZEB1-WT 3'UTR group and ZEB1-MUT 3'UTR group. **C,** ZEB1 level in BCa tissues and adjacent tissues. **D,** A negative correlation between microRNA-1236-3p and ZEB1 level in BCa tissues. **E,** ZEB1 level in MDA-MB-231 and MCF-7 cells transfected with microRNA-1236-3p mimics or NC. **F,** ZEB1 level in MDA-MB-231 and MCF-7 cells transfected with microRNA-1236-3p inhibitor or NC. Data are presented as mean±SD; $^{**}p<0.01$.

common method for the treatment of early-stage BCa is surgical resection. For advanced BCa, surgical resection combined with chemotherapy is preferred. Chemotherapy is frequently applied for BCa patients with metastasis[14]. Prevention and diagnosis of BCa as early as possible contribute to the well prognosis[15,16]. Therefore, improving our understanding of early-stage BCa and tumor progression are critical for the treatment of BCa patients. Researches have demonstrated that miRNAs can exert a vital

influence on the progression of BCa[17]. For example, miR-449a inhibits tamoxifen-resistance in human BCa cells by targeting ADAM22[18]. MiR-22 inhibits its tumorigenesis by targeting Sirt1 and improves radiosensitivity of BCa cells[19]. MiR-539 acts as a tumor suppressor by targeting epidermal growth factor receptors in BCa[20]. This experiment focused on the function of microRNA-1236-3p in BCa, and explored its possible mechanisms affecting the cellular behaviors of BCa cells. We found that microRNA-1236-3p was downregulated in tumor tissues of BCa patients. Similar results were further verified in BCa cell lines. These results suggested that microRNA-1236-3p was closely related to certain biological functions of tumors. Based on the above studies, we selected the MDA-MB-231 and MCF-7 cell lines for functional studies of microRNA-1236-3p. The results showed that down-regulation of microRNA-1236-3p promoted the proliferation and metastasis of BCa cells. BCa is the result of multiple genes and factors. In basic research, it is found that the common transcription factors regulating EMT are Twist, Snail, zinc-finger E-box binding homeobox (ZEB1) family[21]. EMT is a critical step to promote the metastasis of epithelial cancer cells. During the progression of EMT, epithelial cells acquire the biological characteristics of mesenchymal cells through various biochemical changes[22]. Upregulated E-cadherin and downregulated N-cadherin are features in EMT. The ZEB family, the E-box-binding zinc finger protein family, also includes ZEB1 and ZEB2 proteins. ZEB1 mainly promotes the development of T cells and cartilage in normal physiological processes. It is mainly related to the development of cancer in pathological processes. ZEB1 is upregulated in various cancer tissues including BCa, and it can downregulate E-cadherin level and induce EMT[23]. We first predicted through bioinformatics that ZEB1 might be a downstream target gene of microRNA-1236-3p. ZEB1 was found to be highly ex-

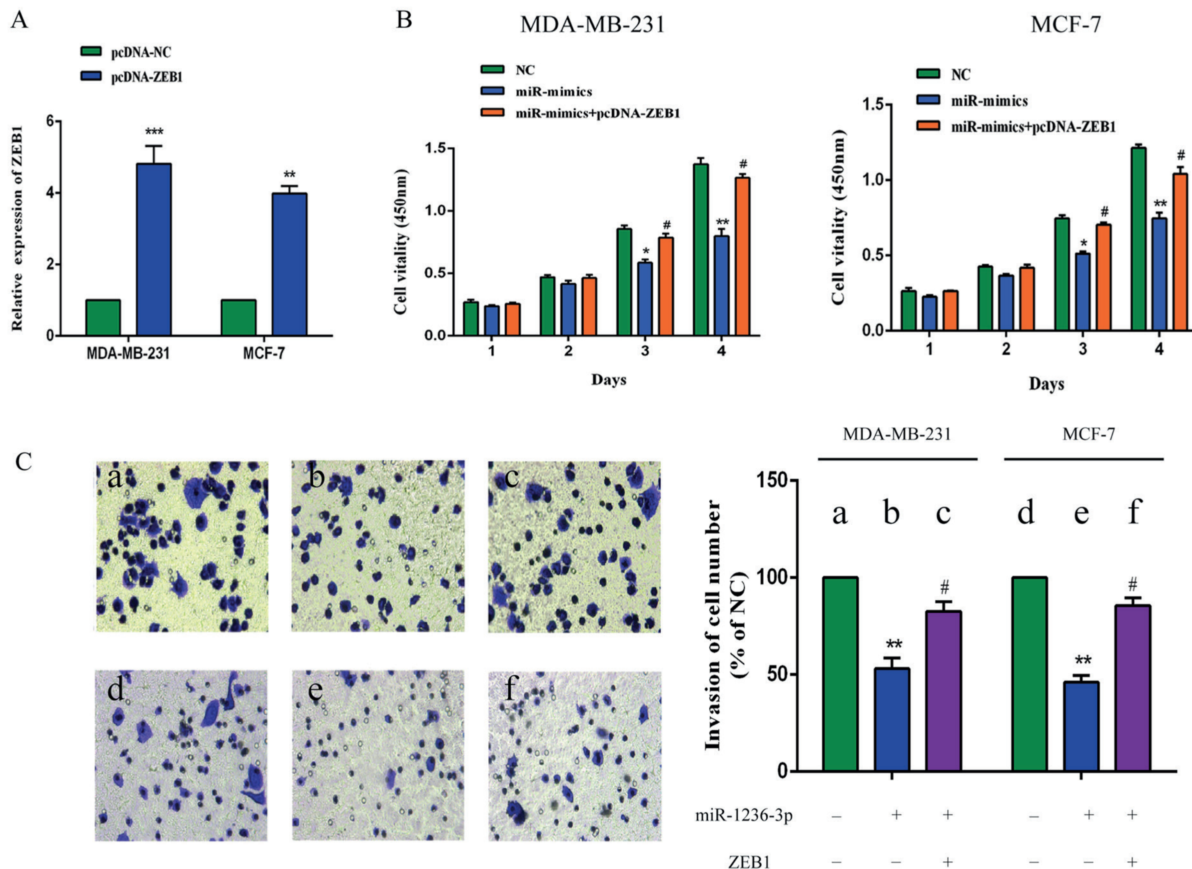

Figure 4. Overexpression of ZEB1 reverses the effect of microRNA-1236-3p on cell proliferation and invasion. **A,** The mRNA level of ZEB1 in MDA-MB-231 and MCF-7 cells transfected with pcDNA-ZEB1 or pcDNA-NC. MDA-MB-231 and MCF-7 cells were transfected with NC, microRNA-1236-3p mimics or microRNA-1236-3p mimics+pcDNA-ZEB1. **B,** Cell proliferation in each group. **C,** Cell invasion in each group (Magnification: 20×). Data are presented as mean±SD; *p<0.05, **p<0.01, ***p<0.001, #p<0.05

pressed in BCa tissues and played a vital role in tumor development. Further, dual-luciferase reporter gene assay revealed that microRNA-1236-3p could directly bind to ZEB1. In BCa cells, ZEB1 level was negatively regulated by microRNA-1236-3p. Notably, overexpression of ZEB1 reversed the inhibitory effect of microRNA-1236-3p on proliferative rate or invasiveness of BCa cells. We indicated that microRNA-1236-3p might exert its influence by reducing ZEB1 expression.

Conclusions

In short, microRNA-1236-3p could inhibit the growth and metastasis of BCa cells by downregulating ZEB1 level, suggesting that microRNA-1236-3p may be a potential therapeutic target for BCa.

Conflict of Interests

The Authors declare that they have no conflict of interests.

References

1) Wang Y, Chen L, Wu Z, Wang M, Jin F, Wang N, Hu X, Liu Z, Zhang CY, Zen K, Chen J, Liang H, Zhang Y, Chen X. miR-124-3p functions as a tumor suppressor in breast cancer by targeting CBL. BMC Cancer 2016; 16: 826.
2) Skol AD, Sasaki MM, Onel K. The genetics of breast cancer risk in the post-genome era: thoughts on study design to move past BRCA and towards clinical relevance. Breast Cancer Res 2016; 18: 99.
3) Ruffini E, Asioli S, Filosso PL, Lyberis P, Bruna MC, Macri L, Daniele L, Oliaro A. Clinical significance of tumor-infiltrating lymphocytes in lung neoplasms. Ann Thorac Surg 2009; 87: 365-371, 371-372.
4) Hammond SM. An overview of microRNAs. Adv Drug Deliv Rev 2015; 87: 3-14.
5) Zhao Y, Liu X, Lu YX. MicroRNA-143 regulates the proliferation and apoptosis of cervical cancer cells by targeting HIF-1alpha. Eur Rev Med Pharmacol Sci 2017; 21: 5580-5586.
6) Sekar TV, Mohanram RK, Foygel K, Paulmurugan R. Therapeutic evaluation of microRNAs by molecular imaging. Theranostics 2013; 3: 964-985.
7) Czochor JR, Glazer PM. microRNAs in cancer cell response to ionizing radiation. Antioxid Redox Signal 2014; 21: 293-312.
8) Ganju A, Khan S, Hafeez BB, Behrman SW, Yallapu MM, Chauhan SC, Jaggi M. miRNA nanotherapeutics for cancer. Drug Discov Today 2017; 22: 424-432.
9) Zhang Q, Miao S, Li C, Cui K, Ge Q, Chen Z. S-phase kinase-associated protein 2 impairs the inhibitory effects of miR-1236-3p on bladder tumors. Am J Transl Res 2018; 10: 731-743.
10) Bian T, Jiang D, Liu J, Yuan X, Feng J, Li Q, Zhang Q, Li X, Liu Y, Zhang J. miR-1236-3p suppresses the migration and invasion by targeting KLF8 in lung adenocarcinoma A549 cells. Biochem Biophys Res Commun 2017; 492: 461-467.
11) Wang Y, Yan S, Liu X, Zhang W, Li Y, Dong R, Zhang Q, Yang Q, Yuan C, Shen K, Kong B. miR-1236-3p represses the cell migration and invasion abilities by targeting ZEB1 in high-grade serous ovarian carcinoma. Oncol Rep 2014; 31: 1905-1910.
12) An JX, Ma MH, Zhang CD, Shao S, Zhou NM, Dai DQ. miR-1236-3p inhibits invasion and metastasis in gastric cancer by targeting MTA2. Cancer Cell Int 2018; 18: 66.
13) Liu M, Yu X, Chen Z, Yang T, Yang D, Liu Q, Du K, Li B, Wang Z, Li S, Deng Y, He N. Aptamer selection and applications for breast cancer diagnostics and therapy. J Nanobiotechnology 2017; 15: 81.
14) Martin M, Lopez-Tarruella S, Gilarranz YJ. Endocrine therapy for hormone treatment-naive advanced breast cancer. Breast 2016; 28: 161-166.
15) Goncalves R, Warner WA, Luo J, Ellis MJ. New concepts in breast cancer genomics and genetics. Breast Cancer Res 2014; 16: 460.
16) Thorat MA, Cuzick J. Preventing invasive breast cancer using endocrine therapy. Breast 2017; 34 Suppl 1: S47-S54.
17) Campos-Parra AD, Mitznahuatl GC, Pedroza-Torres A, Romo RV, Reyes F, Lopez-Urrutia E, Perez-Plasencia C. Micro-RNAs as potential predictors of response to breast cancer systemic therapy: future clinical implications. Int J Mol Sci 2017; 18. pii: E1182.
18) Li J, Lu M, Jin J, Lu X, Xu T, Jin S. miR-449a suppresses tamoxifen resistance in human breast cancer cells by targeting ADAM22. Cell Physiol Biochem 2018; 50: 136-149.
19) Zhang X, Li Y, Wang D, Wei X. miR-22 suppresses tumorigenesis and improves radiosensitivity of breast cancer cells by targeting Sirt1. Biol Res 2017; 50: 27.
20) Guo J, Gong G, Zhang B. miR-539 acts as a tumor suppressor by targeting epidermal growth factor receptor in breast cancer. Sci Rep 2018; 8: 2073.
21) Diaz-Lopez A, Diaz-Martin J, Moreno-Bueno G, Cuevas EP, Santos V, Olmeda D, Portillo F, Palacios J, Cano A. Zeb1 and Snail1 engage miR-200f transcriptional and epigenetic regulation during EMT. Int J Cancer 2015; 136: E62-E73.
22) Mock K, Preca BT, Brummer T, Brabletz S, Stemmler MP, Brabletz T. The EMT-activator ZEB1 induces bone metastasis associated genes including BMP-inhibitors. Oncotarget 2015; 6: 14399-14412.
23) Preca BT, Bajdak K, Mock K, Lehmann W, Sundararajan V, Bronsert P, Matzge-Ogi A, Orian-Rousseau V, Brabletz S, Brabletz T, Maurer J, Stemmler MP. A novel ZEB1/HAS2 positive feedback loop promotes EMT in breast cancer. Oncotarget 2017; 8: 11530-11543.