European Review for Medical and Pharmacological Sciences 2019; 23: 10422-10432

MicroRNA-296 functions as a tumor suppressor in breast cancer by targeting FGFR1 and regulating the Wnt/β-catenin signaling pathway

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Abstract. – OBJECTIVE: Breast cancer (BC) is a common malignancy all over the world. However, the detailed mechanism underlying BC progression remains incompletely understood. MicroRNAs (miRNAs) have been observed to play crucial roles in tumorigenesis. The present study aimed to determine the expression and function of miR-296 in BC.

PATIENTS AND METHODS: MiR-296 expressions in BC tissue samples and cell lines were examined by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). After that, we performed functional assays, including MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assays and transwell assays, to show the functions of miR-296 in BC cell proliferation, invasion and migration. Immunological histological chemistry (IHC) assays were carried out to detect the expression levels of fibroblast growth factor receptor 1 (FGFR1) in BC tissue samples. Western blot was used to explore potential mechanisms of miR-296 in regulating BC progression. A Luciferase reporter assay was carried out to confirm the target gene of miR-296.

RESULTS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) results demonstrated a significant decrease of miR-296 expressions in BC when compared to the corresponding normal controls. In addition, the decreased miR-296 was correlated with the malignant phenotypes and poorer prognosis of BC patients. The functional assays indicated that miR-296 restoration could repress the proliferation, invasion and migration abilities of BC cells. Moreover, the results of the current study revealed that miR-296 exerted the repressive functions in BC cells *via* regulating FGFR1, the Wnt/β-catenin signaling pathway and EMT. Additionally, miR-296 up-regulation could inhibit *in vivo* BC cell growth.

CONCLUSIONS: All these findings indicated that miR-296 exerted anti-BC functions, providing novel therapeutic strategies in BC treatment.

Key Words:
Breast cancer, MicroRNA-296, FGFR1, Wnt/Ð-catenin, Epithelial-mesenchymal transition.

Introduction

Breast cancer (BC), one frequent malignancy among females, remains a major threat to women's health globally[1]. Current therapies for BC mainly include surgery, radiotherapies, chemotherapies, endocrine treatments and molecular targeted therapies[2,3]. Recently, thanks to the development of effective adjuvant systemic treatments and screening tools, the mortality rate of BC patients has been declining[4]. However, metastases and recurrence following conventional treatments are leading factors for morbidities and mortalities of BC patients[5,6]. Therefore, more accurate predictions of recurrence and clinical outcome of BC patients are great challenges in BC management. Moreover, it is relevant to identify specific and sensitive tumor biomarkers in earlier diagnosis, individualized treatments, monitoring curative effects and individual prognosis of BC patients.

Scholars[7,8] have shown that miRNAs may act as potential predictive biomarkers in tumor therapies and prognosis. MiRNAs could regulate the expressions of protein-coding genes *via* binding to the 3'-untranslated regions (3'UTRs) of the target mRNAs[9,10]. MiRNAs are identified as crucial regulators of multiple biological processes, including cell metabolism, growth, apoptosis, proliferation, invasion and metastasis[11-13]. Aberrant expressions of miRNAs are implicated in many diseases, including vari-

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ous tumors. For example, Lin et al[14] proposed that miR-215 inhibited epithelial ovarian cancer growth and invasion *via* targeting NIN1/RPN12 binding protein1 homolog (NOB1); Li et al[15] reported that miR-187 promoted gastric carcinoma growth and metastasis by regulating forkhead box A2 (FOXA2); Fu et al[16] revealed that miR-19a promoted prostate cancer progression and acted as a prognostic marker *via* inhibiting vacuolar protein sorting 37A (VPS37A). Therefore, as key regulators in tumor progression, miRNAs may be potential biomarkers for clinical diagnosis, and serve as useful targets in cancer therapies.

Epithelial-mesenchymal transition (EMT)[17] is a conserved developmental process in which cells will gain mesenchymal characteristics and lose epithelial disposition. Cai et al[18] indicated that EMT played vital roles in tumor migration and invasion, being associated with tumor recurrence. Recently, accumulating data have shown[19] that miRNAs participate in the regulation of the EMT process in many malignant carcinomas. For instance, Chen et al[20] proposed that down-regulation of transient receptor potential melastatin 7 (TRPM7) inhibited prostate cancer cell migration and invasion by regulating EMT; Chen et al[21] found that murine double minute 2 (MDM2) promoted ovarian cancer cell EMT and metastasis. Moreover, in various kinds of cancers, the Wnt/β-catenin signaling pathway is frequently inappropriately activated, playing fundamental roles in tumor development and proliferation[22]. Moreover, Hu and Xie[23] also indicated that the Wnt/β-catenin pathway contributed to BC progression. Thus, the identification of novel biomarkers in regulating EMT and Wnt/β-catenin signaling to suppress BC development has pivotal roles.

Fibroblast growth factor receptor 1 (FGFR1) is a member of the FGFR family, and its expressions have been shown to vary with the types of tumors, significantly predicting poor outcome and survival[24]. Koole et al[25] indicated that FGFR1 functioned as a potential therapeutic target and prognostic biomarker for head and neck squamous cell carcinoma. Moreover, Song et al[26] showed that high amplification of FGFR1 acted as a poor prognostic factor in patients with early stage esophageal squamous cell carcinoma. However, the molecular mechanisms by which FGFR1 contributes to BC metastasis and poor prognosis are not fully known. Therefore, the aim of the present work was to assess the prognostic significance of FGFR1 in BC.

Patients and Methods

Human Tissues

Fifty-four pairs of BC patients who underwent surgery were recruited from The First Affiliated Hospital of Jinzhou Medical University between June 2015 and October 2017. None of the patients received prior preoperative chemotherapy or radiotherapy. Thereafter, tumor tissues and the corresponding matched para-carcinoma tissues were collected from the BC patients. All tissue samples were immediately snapped frozen in liquid nitrogen, and stored at -80°C for further use. All patients involved in the current study provided informed consent. The study was approved by the Ethics Committee of The First Affiliated Hospital of Jinzhou Medical University.

Cell Culture

Human normal mammary epithelial cell line MCF-10A and BC cell lines (MCF7, T47D, SKBR3 and MDA-MB-231) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA). Cells were incubated in a humidified incubator with 5% CO_2 at 37°C.

Cell Transfection

MiR-296 mimics, inhibitor and the negative control miRNA (NC) were obtained from GenePharma (Shanghai, China). Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) was used to transfect the corresponding miRNAs into BC cells following the manufacturer's proposals. After being cultured for 48 h at 37°C, cells were harvested for further assays.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was carried out to determine the miRNA and gene expressions. Briefly, TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was utilized to extract total RNA from BC tissues and cell lines and then Prime Script RT reagent kit (TaKaRa, Otsu, Shiga, Japan) was used to reverse transcribe the RNA into complementary deoxyribose nucleic acid (cDNA). qRT-PCR analyses for FGFR1 and miR-296 were performed with SYBR® Premix Ex Tag™ II (TaKaRa, Otsu, Shiga, Japan) and TaqMan MicroRNA Assay Kit

Table I. Primer sequences for qRT-PCR.

Primer	Sequence
miR-296 forward	5'-TGCCTAATTCAGAGGGTTGG -3'
miR-296 reverse	5'-CTCCACTCCTGGCACACAG -3'
U6 forward	5'- CTCGCTTCGGCAGCACA-3'
U6 reverse	5'- AACGCTTCACGAATTTGCGT-3'
FGFR1 forward	5'-CCTCCTCCCTTCCCAAGTAA -3'
FGFR1 reverse	5'-GGACTGATACCCCAGCTCAG-3'
GAPDH forward	5'- CCTCAAGATCATCAGCAATGCCTC -3'
GAPDH reverse	5'- GTGGTCATGAGTCCTTCCACGATA -3'

U6: small nuclear RNA, snRNA.
FGFR1: Fibroblast growth factor receptor 1.
GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

(Applied Biosystems, Foster City, CA, USA) on the ABI 7500 Real Time-PCR System (Applied Biosystems, Foster City, CA, USA). The relative expressions of miR-296 and FGFR1 were calculated with the $2^{-\Delta\Delta Ct}$ method, being normalized to U6 and GAPDH expressions, respectively. The sequences of the primers were listed in Table I.

Cell Proliferation Assay

The role of miR-296 in BC cell proliferation ability was determined by performing MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assays (Sigma-Aldrich, St. Louis, MO, USA). In brief, the transfected BC cells were seeded into 96-well plates, and incubated for 0, 24, 48 and 72 h. Thereafter, MTT solution was added into each well and incubated for another 4 h, and dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) was added into each well. Optical density $(OD)_{490}$ was detected using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Transwell Assays

Transwell assays were carried out to value the invasion and migration abilities of BC cells with different treatments using transwell chambers (8.0 μm pore size, Corning, Lowell, MA, USA). Particularly, Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) was added on the upper chamber for invasion assays. Then, the serum-free medium was added into the upper compartments and the lower compartments were supplemented with medium containing 10% FBS. Transfected BC cells were then seeded into the upper compartments and incubated under standard conditions (5% CO_2, 37°C, and saturated humidity) for 48 h. After the incubation, swabs were utilized to remove the cells which remained on the upper surface from the membrane, and on the other hand, cells that attached to the lower surface were fixed and stained with methanol and crystal violet respectively. For the migration assay, the transwell chamber was not coated with Matrigel. The numbers of invaded or migrated cells were counted in five randomly chosen fields with an inverted microscope (Olympus, Tokyo, Japan).

Western Blots

BC cells were lysed in cold radioimmunoprecipitation assay (RIPA) buffer (Beyotime, Shanghai, China) containing a protease inhibitor. The protein concentration was then quantified with the bicinchoninic acid (BCA) protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). After that, 10% of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate the protein lysates. Thereafter, the protein samples were transferred onto polyvinylidene difluoride (PVDF) membrane (Invitrogen, Carlsbad, CA, USA), which was blocked with 5%-skim milk in Tris-Buffered Saline and Tween (TBST) for 2 h at room temperature. Then, the membranes were incubated overnight at 4°C with specific primary antibodies as indicated: cyclin D1 (1:1000, Abcam, Cambridge, MA, USA), c-Myc (1:1000, Abcam, Cambridge, MA, USA), β-catenin (1:1000, Abcam, Cambridge, MA, USA), p-GSK3β (1:1000, Abcam, Cambridge, MA, USA), total GSK3β (1:1000, Abcam, Cambridge, MA, USA), E-cadherin (1:2000, Abcam, Cambridge, MA, USA), N-cadherin (1:2000, Abcam, Cambridge, MA, USA), Vimentin (1:1000, Abcam, Cambridge, MA, USA) and GAPDH (1:1000, Abcam, Cambridge, MA, USA). The membranes were then blotted for 2 h at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibody (1:3,000, Abcam, Cambridge, MA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was an internal control. Finally, the protein bands were analyzed using enhanced chemiluminescence (ECL) Western blot detection reagents (Beyotime, Shanghai, China).

Dual-Luciferase Reporter Assays

The mutant (MUT) or wild-type (WT) FGFR13’-UTR which contained miR-296 binding sites were inserted into pGL3 Luciferase vectors (Promega, Madison, WI, USA). Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) was used to co-transfect miR-296 mimics and FGFR1-3’UTR-WT or FGFR1-3’UTR-MUT into

BC cells following the manufacturer's protocols. A Dual--Luciferase Reporter Assay kit (Promega, Madison, WI, USA) was utilized to quantify the Luciferase activity 48 h after the transfection.

Xenograft Tumor Formation Assay

Four to six weeks old female nude mice were used for *in vivo* assays and randomly divided into two groups. All animal experiments were carried out in strict accordance with the protocol approved by the Jinzhou Medical University. The over-expressed miR-296 or control miRNA was transfected into MDA-MB-231 cells by lentivirus infection. Stably transfected cells were subcutaneously injected into the left flank of nude mice. The volumes of tumors were monitored every 3 days with the formula: volume = length × width^2/2. The mice were sacrificed after 29 days and the tumors were isolated.

Statistical Analysis

All experiments in the current study were repeated at least three times. The statistical analysis was performed by Statistical Product and Service Solutions (SPSS) software version 17.0 (SPSS Inc., Chicago, IL, USA). Student's *t*-test, analysis of variance (ANOVA) and Scheffe's post-hoc analysis were applied, where appropriated. Kaplan-Meier method and log-rank test were applied to estimate the survival rates and compare the survival curves respectively. $p<0.05$ was regarded as a statistically significant difference.

Results

MiR-296 Was Dramatically Downregulated in BC Tissues

To verify the functions of miR-296 in BC development, we first measured the expressions of miR-296 in BC tissue samples. The results of qRT-PCR indicated that miR-296 expressions in BC tissues were notably decreased compared to that in adjacent normal tissues (Figure 1A). Moreover, we performed the Kaplan-Meier analysis to determine the effects of miR-296 on the prognosis of BC patients. Findings demonstrated that BC patients with low miR-296 expressions presented shorter overall survival in comparison with the patients who had high miR-296 expressions (Figure 1B). Additionally, we investigated the association between miR-296 expressions and the clinicopathologic features of BC patients. Briefly, BC patients involved in the current study were assigned into two groups with the mean miR-296 expression level as the cutoff. As shown in Table II, we found that decreased miR-296 expression in BC patients was associated with the malignant characteristics of BC patients.

MiR-296 Overexpression Inhibited BC Cell Proliferation

As we indicated that miR-296 expression in BC tissues was decreased, we further explored the functions of miR-296 in BC cells. First, the expressions of miR-296 in BC cells were detected using qRT-PCR. As expected, there was a prominent decrease of miR-296 expressions in BC cells compared to MCF-

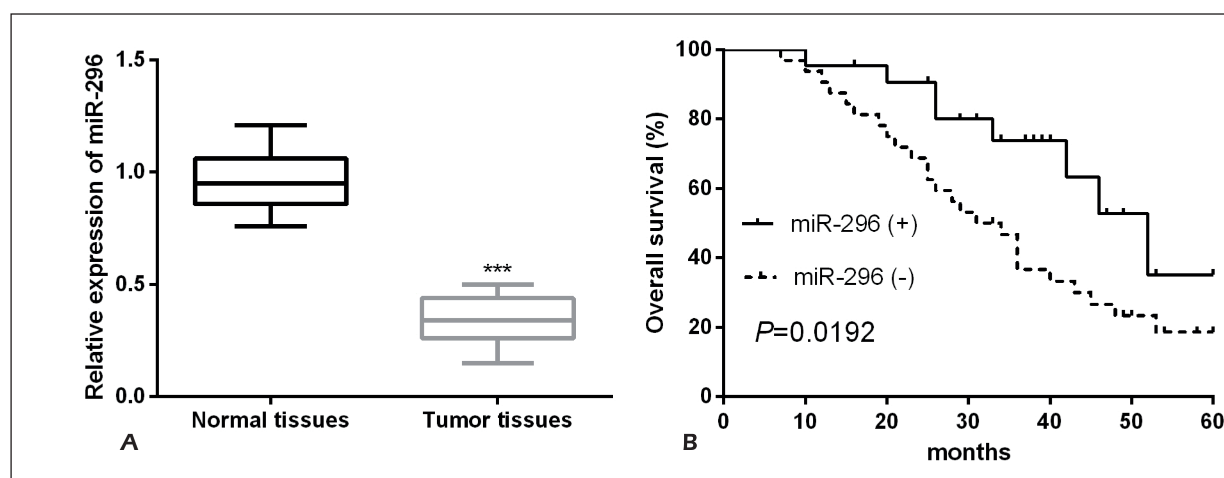

Figure 1. Decreased miR-296 expressions in BC tissues demonstrated a poor prognosis of BC patients. **A**, MiR-296 expressions in BC tissues were detected by qRT-PCR. **B**, The Kaplan-Meier analysis was performed to determine the association between miR-296 expressions and overall survival rate of BC patients. ***$p<0.001$.

Table II. Correlation of miR-296 expression with the clinicopathological characteristics of the breast cancer patients.

Clinicopathological features	Cases (No. =54)	miRNA-296a expression		p-value
		High (No. =20)	Low (No. =34)	
Age (years)				0.2518
> 50	29	9	20	
≤ 50	25	11	14	
Tumor size (cm)				0.0691
≥ 5.0	24	8	16	
< 5.0	30	12	18	
Histological grade				0.0767
I–II	27	9	18	
III	27	11	16	
TNM stage				0.0031*
I-II	26	16	10	
III	28	4	24	
Lymph-node metastasis				0.0026*
Yes	16	5	11	
No	38	15	23	
ER status				0.3518
Positive	24	10	14	
Negative	30	10	20	
PR status				0.2941
Positive	28	13	15	
Negative	26	7	19	
HER2 status				0.2196
Positive	29	8	21	
Negative	25	12	13	

TNM: tumor-node-metastasis; ER: estrogen receptor; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2.
[a]The mean expression level of miR-296 was used as the cutoff.
*Statistically significant.

10A (Figure 2A). Then, we analyzed the effects of gain or loss of miR-296 on BC cell development. In brief, we obtained miR-296-over-expressed or -suppressed BC cells by transfecting miR-296 mimics or inhibitor into MDA-MB-231 or MCF7 cells which presented relatively low or high endogenous miR-296 expressions. The transfection efficiencies were confirmed by qRT-PCR (Figure 2B and 2C). Moreover, MTT assay was carried out to determine the roles of miR-296 in BC cell proliferation. Data demonstrated that miR-296 over-expression significantly inhibited BC cell proliferation ability (Figure 2D). On the other hand, miR-296 knockdown dramatically promoted the proliferation capacity of BC cells (Figure 2E). Overall, miR-296 exerted tumor suppressive functions in BC cell proliferation.

MiR-296 Restoration Significantly Repressed BC Cell Invasion and Migration

To further demonstrate the functional roles of miR-296 in BC progression, we determined the impact of miR-296 on BC cell invasion and migration by performing transwell assays. As expected, the results indicated that miR-296 over-expression resulted in significantly decreased invasion and migration abilities of MDA-MB-231 cells (Figure 3A and 3B). In contrast, miR-296 suppression in MCF7 cells markedly facilitated the invasion and migration capacities (Figure 3C and 3D). All these results revealed that miR-296 repressed BC cell invasion and migration.

FGFR1 Was a Direct Target of MiR-296 in BC Cells

As we all know, miRNAs exert their function by down-regulating expressions of their downstream target genes. Thus, we predicted the potential downstream target genes of miR-296 using Targetscan database. The results demonstrated that FGFR1 was a candidate target of miR-296 (Figure 4A). To verify the association between FGFR1 and miR-296, we performed Luciferase reporter assays by co-transfecting FGFR1-3'UTR-WT or FGFR1-3'UTR-MUT and miR-296 mimics into BC cells. As illustrated in Figure

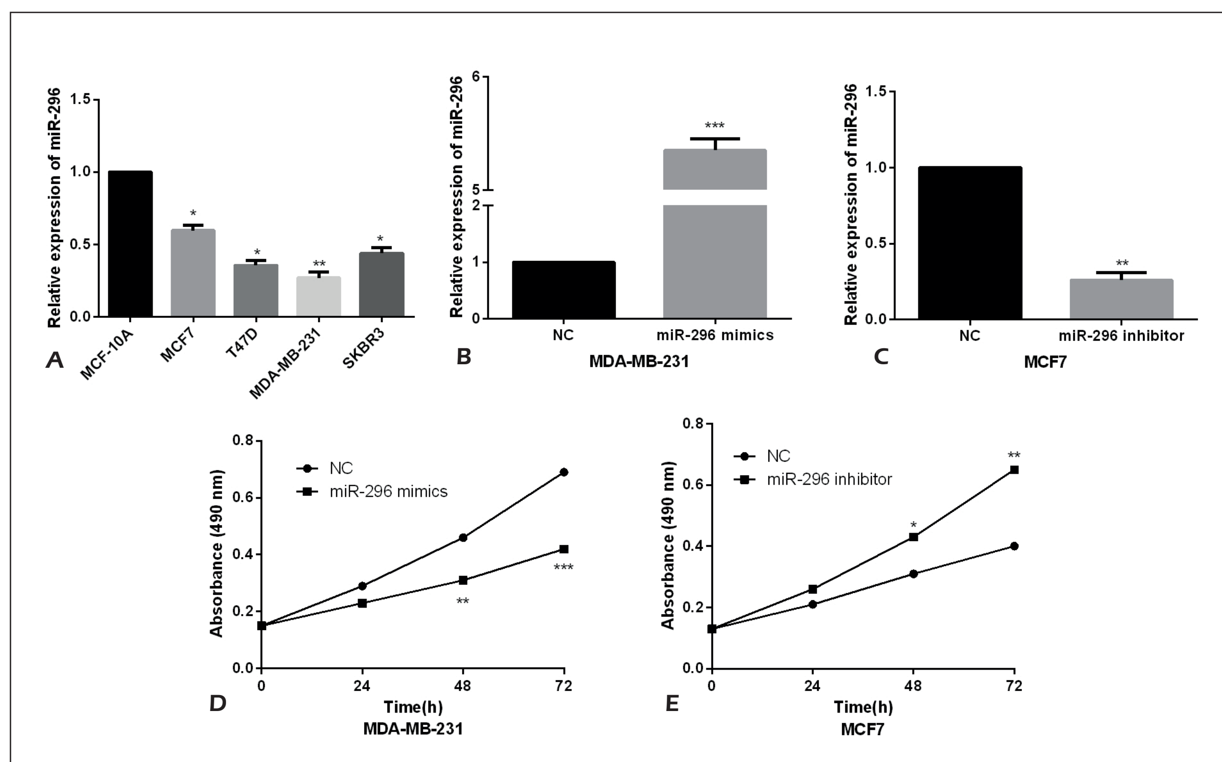

Figure 2. MiR-296 up-regulation prominently inhibited BC cell proliferation. **A**, MiR-296 expressions in BC cell lines were measured by qRT-PCR. **B**, MiR-296 expressions in MDA-MB-231 cells, which were transfected with miR-296 mimics, were examined. **C**, MiR-296 expressions in MCF cells, which were transfected with miR-296 inhibitors, were detected. **D**-**E**, Proliferation abilities of miR-296-over-expressed MDA-MB-231 cells or miR-296-inhibited MCF cells were determined by MTT assays. *p<0.05, **p<0.01, ***p<0.001.

4B, we found that miR-296 mimics could prominently decrease the Luciferase activities of FGFR1-3'UTR-WT, and, on the other hand, miR-296 mimics had no significant impact on the Luciferase activities of FGFR1-3'UTR-MUT compared to the controls. Furthermore, we investigated the regulatory functions of miR-296 in FGFR1 expressions. The findings showed that FGFR1 expressions were dramatically inhibited by miR-296 over-expression in MDA-MB-231 cells (Figure 4C). Moreover, miR-296 inhibition in MCF7 cells notably promoted FGFR1 expressions (Figure 4D). All the above results showed that FGFR1 was a direct target of miR-296 in BC cells.

MiR-296 Upregulation Modulated EMT and Wnt/β-Catenin Pathway in BC Cells

As we had detected that FGFR1 was a direct target of miR-296 in BC cells, we next measured FGFR1 expression levels in BC tissues and cells. Data demonstrated that FGFR1 expressions were prominently increased both in BC tissues and cells in comparison with normal controls (Figure 5A and 5B). Moreover, the prognostic values of FGFR1 in BC patients were determined by performing the Kaplan-Meier analysis. It was found that BC patients with high FGFR1 expressions presented worse OS in comparison with the patients who had low FGFR1 expressions (Figure 5C). We next investigated the underlying mechanisms of miR-296 in BC cell progression by performing Western blot analysis. As shown in Figure 5D, in miR-296 over-expressed-MDA-MB-231 cells, the expressions of E-cadherin was markedly up-regulated while the vimentin and N-cadherin expressions were remarkably down-regulated; in the meantime, miR-296 restoration significantly decreased the protein expressions of cyclin D1, c-Myc, activated β-catenin and p-GSK3β. On the other hand, we also found that miR-296 repression in MCF7 cells could remarkably reduce the E-cadherin expressions whereas enhancing the N-cadherin and vimentin expressions. Additionally, the cyclin D1, c-Myc, activated β-catenin and p-GSK3β expressions in miR-296-suppressed MCF7 cells were

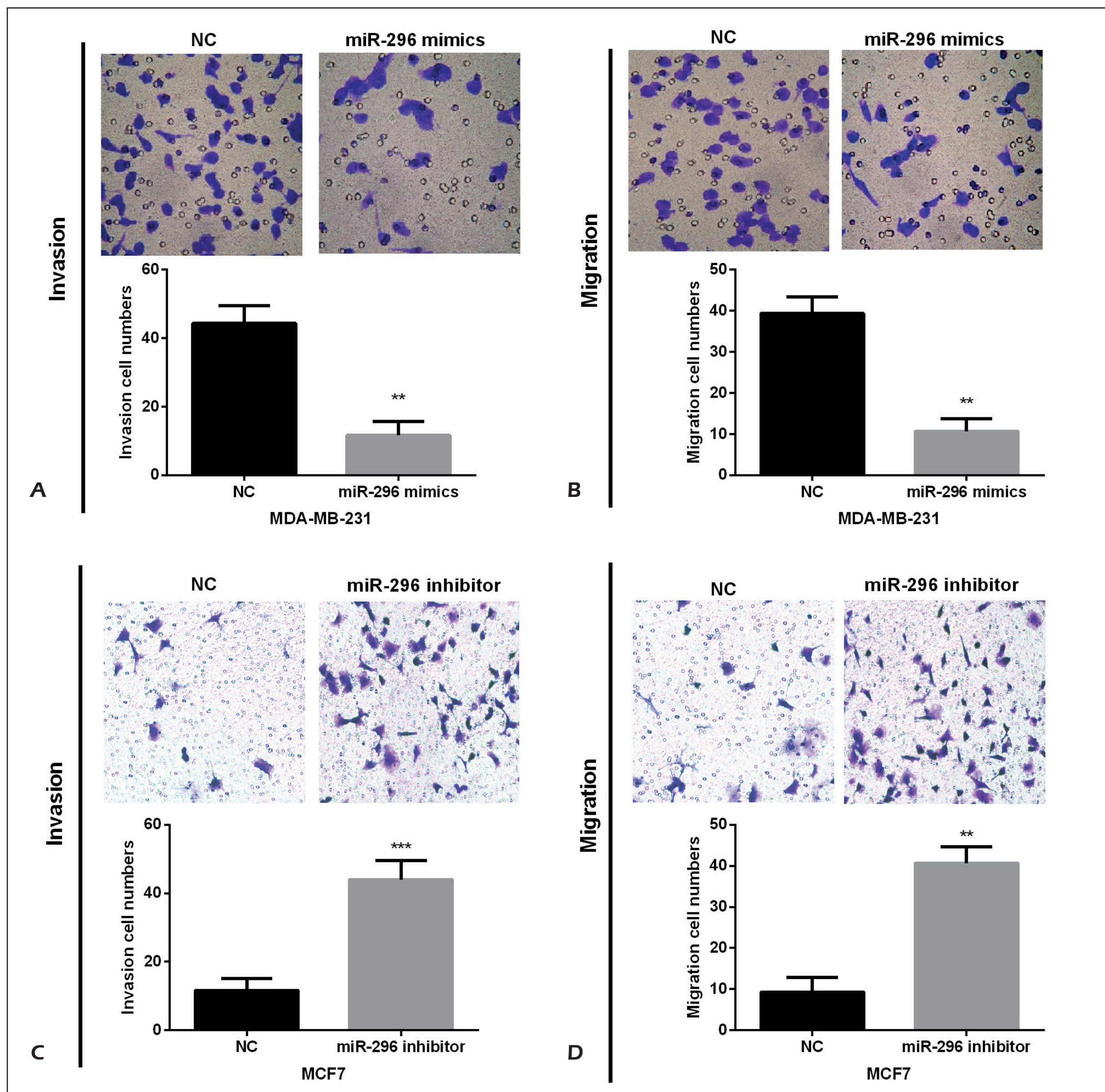

Figure 3. MiR-296 over-expression repressed BC cell invasion and migration. **A-B**, Transwell assays were conducted to analyze the invasion and migration capacities of miR-296-over-expressed MDA-MB-231 cells (Magnification × 40). **C-D**, The invasion and migration capacities of miR-296-inhibited MCF cells were detected by transwell assays (Magnification × 40). $^{**}p<0.01$, $^{***}p<0.001$.

prominently promoted (Figure 5D). All these results suggested that miR-296 exerted tumor suppressive functions in BC cells *via* the regulation of EMT and Wnt/β-catenin pathway.

In Vivo Effects of MiR-296on BC Tumorigenesis

We next determined the impacts of miR-296 on tumor growth *in vivo* and findings showed that miR-296 over-expression resulted in decreased tumor growth and tumor volumes of BC xenograft models (Figure 6A and 6B). Collectively, our data demonstrated that miR-296 repressed BC tumorigenesis *in vivo*.

Discussion

BC is one common malignant carcinoma among women worldwide, and the morbidity of BC has been increasing in recent years[27]. However, current therapeutic strategies for BC demon-

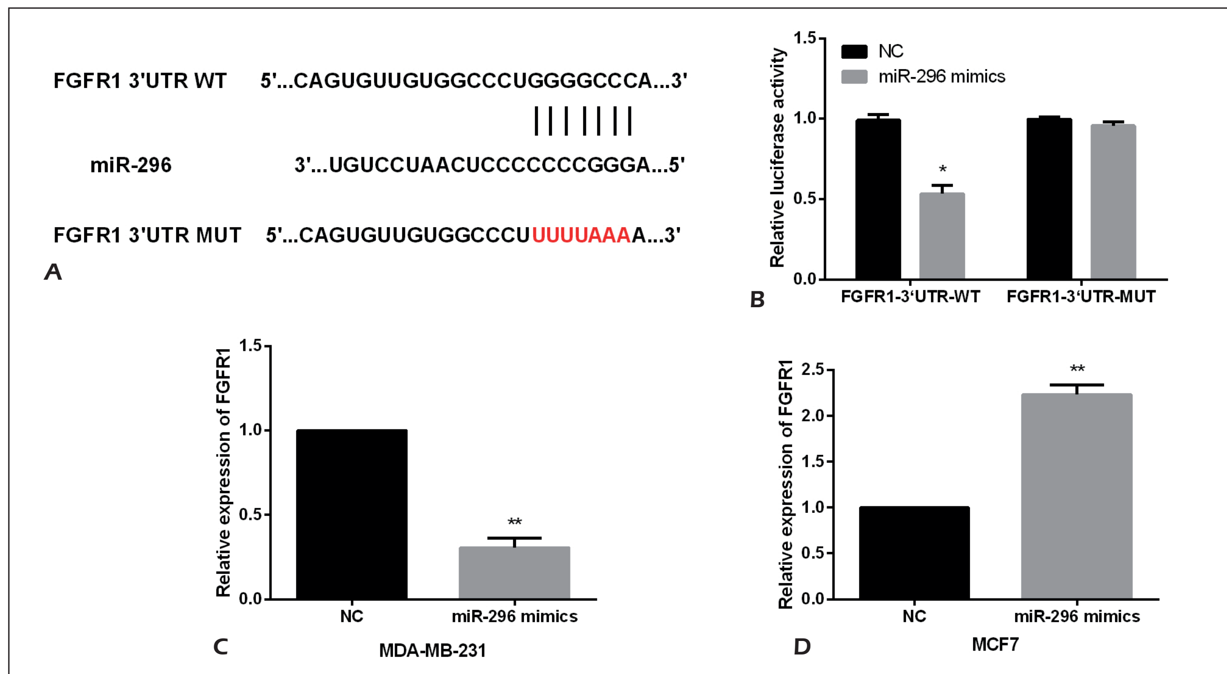

Figure 4. FGFR1 was a direct target of miR-296 in BC cells. **A**, Putative binding sites of miR-296 in the FGFR1 3'UTRs were shown. **B**, Analysis of the Luciferase activities of the Luciferase reporter plasmids containing either FGFR1-3'UTR-WT or FGFR1-3'UTR-MUT was performed. **C-D**, FGFR1 expressions in miR-296-over-expressed MDA-MB-231 cells or miR-296-inhibited MCF cells were measured. *$p<0.05$, **$p<0.01$.

strate poor efficacy for certain BC patients[28]. Moreover, patients with BC usually die from tumor recurrence even after radical surgeries and adjuvant therapies[29]. There have been emerging studies revealing that miRNAs play key roles in BC development and progression. For example, Ahmad et al[30] reported that miR-135a exerted tumor-suppressive functions in BC cell proliferation *via* targeting oncogene ETS domain-containing protein Elk-1 (ELK1). and ETS domain-containing protein Elk-3 (ELK3). Cheng et al[31] found that miR-183-5p facilitated BC cell proliferation and inhibited apoptosis by targeting programmed cell death protein 4 (PDCD4). Li et al[32] claimed that miR-101 inhibited BC metastasis and growth by targeting CX chemokine receptor 7. In the current work, we investigated the biological roles of miR-296 in human BC progression.

MiR-296 has been found to serve crucial roles in various malignancies. For example, recently He et al[33] indicated that miR-296 inhibited colorectal cancer metastasis and EMT by targeting S100 calcium binding protein A4 (S100A4); Wang et al[34] found that miR-296 inhibited hepatocellular carcinoma proliferation and induced apoptosis by targeting fibroblast growth factor receptor 1(FGFR1); Lv et al[35] demonstrated that miR-296 targeted specificity protein 1 to suppress cervical cancer cell invasion and proliferation. In the present work, we investigated the functional roles of miR-296 in BC development. Findings indicated that miR-296 down-regulation in BC was associated with the adverse clinicopathologic features of BC patients. We also found that miR-296 up-regulation could significantly inhibit BC cell proliferation, invasion and migration *via* the regulation of the Wnt/β-catenin signaling pathway and EMT. Additionally, we observed that miR-296 restoration could suppress BC tumorigenesis *in vivo*. Hence, all results demonstrated the tumor suppressive functions and potential therapeutic potential of miR-296 in patients with BC.

Recent studies have revealed that the FGFR family, especially FGFR1, is frequently abnormally highly expressed in a variety of tumor tissues like gastric cancer[36], lung cancer[37] and oral squamous cell carcinoma[38]. Moreover, in the current work, to further investigate the mechanism underlying the suppressive functions of miR-296 in BC, we identified the potential target of miR-296. Data demonstrated that FGFR1 served as a functional target of miR-296 in BC cells.

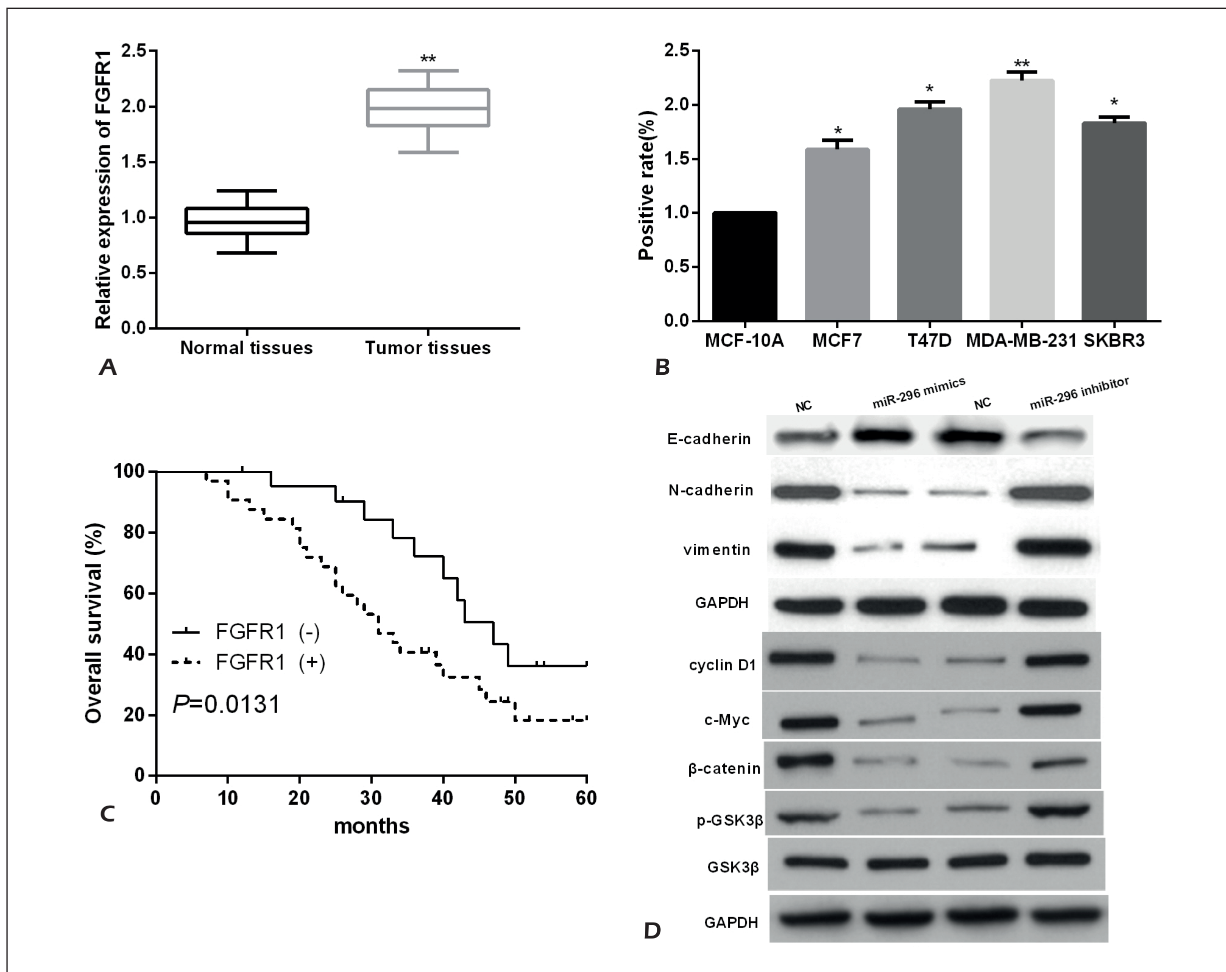

Figure 5. MiR-296 regulated Wnt/β-catenin signaling pathway and EMT in BC cells. **A**-**B**, qRT-PCR assays were performed to detect the expressions of FGFR1 in BC tissue samples and cell lines. **C**, The Kaplan-Meier analysis was performed to determine the overall survival rate of BC patients with high or low FGFR1 expressions, respectively. **D**, Western blot was carried out to investigate the roles of miR-296 in BC cell Wnt/β-catenin signaling pathway and EMT. *$p<0.05$, **$p<0.01$.

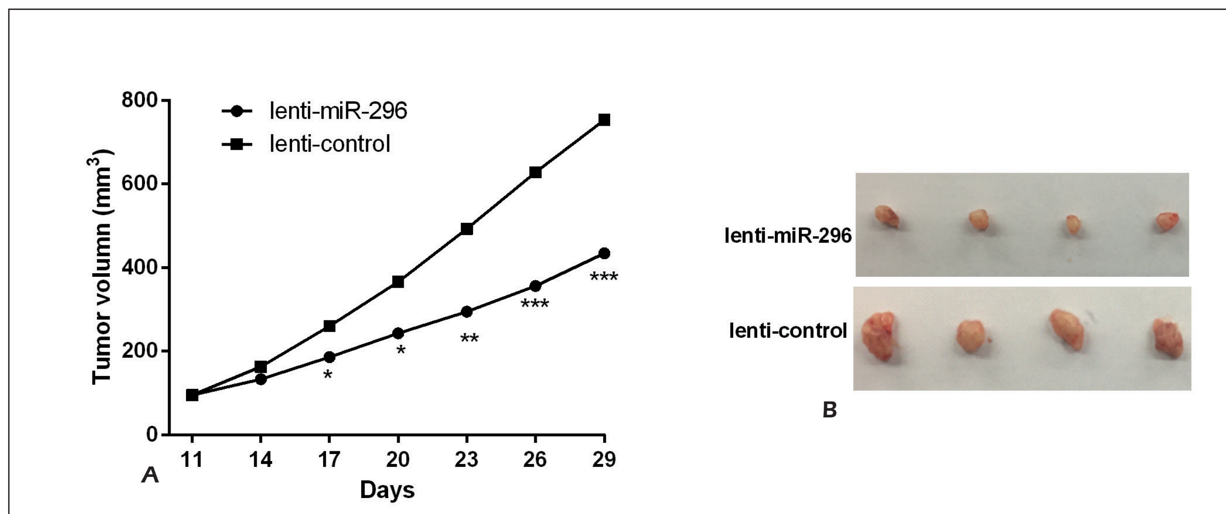

Figure 6. MiR-296 restoration markedly inhibited BC cell tumorigenesis in vivo. **A**, Tumor volumes of xenograft mice were monitored. **B**, Representative tumor photographs of xenograft mice were shown. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Consistent with the findings of previous studies, we also found that FGFR1 was up-regulated in BC tissues, which was associated with the poor prognosis of BC patients. All these results indicated that miR-296 exerted anti-tumor functions in BC at least *via* the regulation of FGFR1 in part.

Conclusions

We demonstrated that decreased miR-296 was frequently found in BC. In addition, the ectopic down-regulation of miR-296 in BC patients was associated with the worse clinicopathologic characteristics and poor prognosis. Moreover, the results of functional assays indicated that the over-expression of miR-296 markedly suppressed BC cell proliferation, invasion and migration capacities by regulating the Wnt/β-catenin signaling pathway and EMT. In the meantime, we discovered that miR-296 restoration could dramatically suppress *in vivo* tumor growth. Furthermore, FGFR1 was identified as a potential target of miR-296, indicating that miR-296 may serve as a BC suppressor partially by targeting FGFR1. The findings suggested that miR-296 acted as a novel therapeutic target in BC treatment.

Conflict of Interests

The authors declared no conflict of interest.

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