European Review for Medical and Pharmacological Sciences 2020; 24: 6185-6194

MiR-566 protects the malignant progression of breast cancer by negatively regulating WNT6

M.-C. ZHAO[1], M.-M. ZHANG[2], T. LI[2], Z.-H. TAO[1], Y.-Q. DU[1], L.-P. WANG[1], J. ZHANG[1], B.-Y. WANG[1], X.-C. HU[1]

[1]Department of Medical Oncology, Fudan University Shanghai Cancer Center, Fudan University School of Medicine, Shanghai, China.
[2]National Center for Drug Screening, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China.

Mingchuan Zhao and Mengmeng Zhang contributed equally to this work

Abstract. – OBJECTIVE: To elucidate the relationship between microRNA-566 (miR-566) and prognosis in breast cancer (BC) and to clarify the influences of miR-566 and WNT6 in its locus region on BC progression.

PATIENTS AND METHODS: MiR-566 and WNT6 levels in 44 pairs of BC samples were detected by quantitative real-time polymerase chain reaction (qRT-PCR). The influences of miR-566 on clinical features and prognosis in BC patients were analyzed. According to the differential expressions of miR-566 in the tested BC cell lines, MDA-MB-231 and MCF-7 cells were selected for generating miR-566 knockdown and overexpression models, respectively. Cell counting kit-8 (CCK-8), 5-Ethynyl-2'-deoxyuridine (EdU) and transwell assays were conducted to explore the role of miR-566 in BC cell functions. Besides, the regulatory interaction between miR-566 and its downstream gene WNT6 was assessed by performing Dual-Luciferase reporter assay. Finally, the co-regulation of miR-566 and WNT6 in BC cell functions was examined.

RESULTS: MiR-566 was downregulated in BC tissues. BC patients with a low expression level of miR-566 were prone to suffering a large tumor size, advanced tumor grade, high incidence of lymphatic metastasis and poor prognosis. Overexpression of miR-566 weakened proliferative and migratory abilities in MCF-7 cells, whereas knockdown of miR-566 produced the opposite results in MDA-MB-231 cells. WNT6 was the target gene binding to miR-566, and they displayed a negative expression correlation in BC tissues. Regulatory effects of miR-566 on BC progression could be reversed by WNT6.

CONCLUSIONS: MiR-566 is closely related to tumor size, tumor grade, lymphatic metastasis and prognosis in BC. It protects the malignant progression of BC by negatively regulating WNT6.

Key Words:
MiR-566, WNT6, Breast cancer.

Introduction

Breast cancer (BC) is a globally prevalent tumor that mainly affects women aged 40-60 years old[1,2]. It is reported that there are 1.38 million (23%) new cases of BC and 450,000 (14%) deaths of BC each year in the world[3,4]. The incidence and mortality of BC are both in the first place in either developing or developed countries[5,6]. The incidence of BC shows an increasing trend in our country, and its onset appears to be manifesting at a younger age, especially in urban women[6]. Therapeutic strategies for BC have been largely improved[7]. However, gradually developed drug resistance severely restricts the clinical outcomes of BC patients[7,8]. It is believed that intracellular drug consumption through transporters and enzymes, DNA repair pathways, therapeutic target mutations, resistance to apoptosis, and recently discovered miRNAs are involved in BC resistance[9,10].

MicroRNAs (miRNAs) are endogenous, noncoding RNAs containing 21-25 nucleotides[11,12]. The genome is transcribed into a pri-miRNA in the nucleus, where a hairpin-structure pre-miRNA containing 60-70 nucleotides is produced under the guidance of RNase III Drosha and its cofactor DGCR8[13,14]. With the help of Exportin 5, a pre-miRNA is transported to the cytoplasm, where a double-stranded RNA is produced by Dicer[14,15]. Later, two strands of the short dsRNA

Corresponding Authors: Xichun Hu, MD; e-mail: huxichun2017@163.com

are separated by RNA helicase, one of which is integrated into the RNA-induced silencing complex (RISC) and the other is discarded from the complex[16,17]. Finally, the complete RISC-miRNA complex induces mRNA degradation or translation inhibition through miRNA-mRNA base pairing in the mRNA 3′-untranslated region (3′-UTR), therefore silencing the target proteins[8,19]. MiR-566 is abnormally expressed in colorectal cancer and renal cancer samples, and its expression is closely related to tumor stage, clinical grade and prognosis[20,21].

Through targeting the downstream genes, miRNAs are widely involved in tumor progression[18,19]. In this paper, WNT6 was predicted as a target binding to miR-566. The abnormality of the WNT signaling may influence the transcription of multiple downstream genes, thereafter changing biological characteristics[22,23]. As a vital member of the WNT family, WNT6 is reported to participate in tumor progression[24,25]. In the present study, the regulation of miR-566-WNT6 interaction on the malignant progression of BC was mainly explored.

Patients and Methods

BC Sample Collection

A total of 44 pairs of BC and paracancerous tissues were collected and stored at -80°C. This investigation was approved by the Ethics Committee of Fudan University Shanghai Cancer Center. In this study, none of the enrolled subjects were preoperatively treated with anti-tumor therapy. In addition, tumor stage was assessed based on the guideline proposed by the Union for International Cancer Control (UICC). Exclusion criteria: patients complicated with other malignancies, those with mental disease, or those complicated with heart failure or other chronic diseases. In addition, this study was in line with the declaration of Helsinki and good clinical practice guidelines. Informed consent was obtained from each subject before sample collection.

Cell Lines and Reagents

BC cell lines (BT474, SKBR-3, ZR-75-30, MCF-7, MDA-MB-453, MDA-MB-231) and the normal breast epithelial cell line (MCF-10A) were provided by American Type Culture Collection (ATCC; Manassas, VA, USA). Except for BT474 and SKBR-3 cells that were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA), the remaining cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 100 U/mL penicillin and 100 μg/mL streptomycin.

Transfection

Transfection plasmids were purchased from GenePharma (Shanghai, China). Cells were inoculated in the 6-well plate and cultured to a density of 30-50%. Later, transfection was conducted using Lipofectamine™2000 (Invitrogen, Carlsbad, CA, USA). After 48 h, transfected cells were collected for functional experiments.

Cell Proliferation Assay

2×10^3 cells were implanted in each well of a 6-well plate and cultured for indicated time points, where 10 μL of CCK-8 solution was added (TaKaRa, Dalian, China). After 1-h culturing in the dark, the optical density at 450 nm was measured using a microplate reader. Blank group was set by adding medium and experimental solution without cells.

5-Ethynyl-2'-Deoxyuridine (EdU) Assay

10 μL of EdU (50 μmol/L; Sigma-Aldrich, St. Louis, MO, USA) was applied in each well for cell labeling. After 12 hours, cells were incubated with 4% paraformaldehyde, followed by phosphate-buffered saline (PBS) washing and incubation with 0.5% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA) for another 20 min. Subsequently, 1 mL of 4',6-diamidino-2-phenylindole (DAPI) was applied for nuclei staining in the dark. Finally, cells were washed in PBS and captured.

Transwell Migration Assay

200 μL of suspension (5×10^5 cells/mL) and 600 μL of serum-free medium were added in the upper and bottom transwell chambers, respectively, for balancing the pressure (Corning, Corning, NY, USA). After 48-h incubation, transwell chambers were taken out. Cells in the bottom were subjected to methanol fixation for 15 min, followed by staining with crystal violet (0.2%) for 20 min. Finally, migratory cells were counted in 5 randomly selected fields per sample.

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

RNAs extracted from pancreatic cancer cells and tissues using TRIzol (Invitrogen, Carlsbad, CA, USA) were reversely transcribed by

means of Primescript RT Reagent (TaKaRa, Dalian, China). QRT-PCR was conducted using SYBR®Premix Ex Taq™ (TaKaRa, Dalian, China) on the StepOne Plus Real-time PCR system (Applied Biosystems, Foster City, CA, USA). Relative level was calculated by $2^{-\Delta\Delta Ct}$ method. miR-566: forward: 5'-GGGCGCCUGUGAUCCCAAC-3', reverse: 5'-UGGGAUCACAGGCGCCCUU-3'; U6: forward: 5'-GCGCGTCGTGAAGCGTTC-3', reverse: 5'-GTGCAGGGTCCGAGGT-3'; WNT6: forward: 5'-CACCCTGCCGCCCTTACCCTCC-3', reverse: 5'-GATCCGGGTCACAGGCAGAGGC-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH): forward: 5'-GGAGCGAGATCCCTCCAAAAT-3', reverse: 5'-GGCTGTTGTCATACTTCTCATGG-3'.

Western Blot

Cells were lysed on ice for 30 min and centrifuged at 4°C, 14000×g for 15 min to isolate proteins, followed by electrophoresis. Protein samples were loaded on polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Subsequently, membranes were soaked in 5% skim milk for 2 hours. Primary and secondary antibodies were applied for indicated time. Finally, protein bands were exposed and analyzed.

Dual-Luciferase Reporter Assay

Shared binding sites to miR-566 seed sequence in WNT6 3′-UTR were predicted online, which were inserted into pmirGLO vectors for generating wild-type pmirGLO-WNT6. The mutant-type pmirGLO-WNT6 was generated by inserting mutated sequences. They were transfected into HEK293T cells with either miR-566 mimic or negative control for 48 h, followed by luciferase activity measurement (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

GraphPad Prism 5 V5.01 (La Jolla, CA, USA) was responsible for statistical analysis. Differences between two groups were analyzed by using the Student's *t*-test. Comparison among multiple groups was done using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). Each experiment was repeated in triplicate and data were expressed as mean ± standard deviation. Kaplan-Meier curves were depicted for survival analysis. $p<0.05$ was considered statistically significant.

Results

MiR-566 Was Lowly Expressed In BC Samples

We collected 44 pairs of BC and paracancerous tissues. QRT-PCR data revealed a lower level of miR-566 in BC tissues than that in paracancerous ones (Figure 1A). Meanwhile, miR-566 was downregulated in BC cell lines as well (Figure 1B). It is suggested that miR-566 may be a tumor-suppressive miRNA involved in BC progression.

MiR-566 Expression Was Correlated With Prognosis In BC

Recruited BC patients were divided into high and low miR-566 expression groups, respectively. The differences in clinical features of BC patients between groups were analyzed. It was shown that miR-566 level was markedly related to tumor size, tumor grade and lymphatic metastasis in BC patients, whereas it was unrelated to age and distant metastasis (Table I). To explore the relationship between miR-566 and prognosis in BC, we collected follow-up data of recruited BC patients. Kaplan-Meier curves showed a low survival rate in low miR-566 expression group, suggesting that low level of miR-566 predicts poor prognosis in BC (Figure 1C). MiR-566 could be a biomarker for predicting the prognosis in BC.

MiR-566 Inhibited Proliferation and Metastasis In BC

To elucidate the biological functions of miR-566 in BC cell behaviors, we generated miR-566 overexpression and knockdown models in MCF-7 and MDA-MB-231 cells, respectively. Transfection efficacy of miR-566 mimic and inhibitor was tested by qRT-PCR (Figure 1D). In MCF-7 cells overexpressing miR-566, the cell viability and the percentage of EdU-positive cells markedly decreased (Figure 2A and 2B, left). Knockdown of miR-566 in MDA-MB-231 cells yielded the opposite results (Figure 2A and 2B, right). In addition, transwell assay uncovered that the migratory ability was inhibited after overexpression of miR-566 in MCF-7 cells, which was markedly stimulated in MDA-MB-231 cells with miR-566 knockdown (Figure 2C). It is concluded that miR-566 inhibits the proliferative and migratory abilities in BC.

MiR-566-WNT6 Interaction

By detecting the differential level of WNT6 in BC tissues, it was upregulated in tumor tissues compared with that in paracancerous ones (Fig-

Table I. Association of miR-566 expression with clinicopathologic characteristics of breast cancer.

Parameters	No. of cases	miR-566 expression		*p*-value
		High (%)	Low (%)	
Age (years)				0.907
<60	16	10	6	
≥60	28	17	11	
Tumor size (years)				0.029
<4	27	20	7	
≥4	17	7	10	
T stage				0.029
T1-T2	27	20	7	
T3-T4	17	7	10	
Lymph node metastasis				0.014
No	28	21	7	
Yes	16	6	10	
Distance metastasis				0.431
No	29	19	10	
Yes	15	8	7	

ure 3A) and negatively correlated with miR-566 level (Figure 3F). As expected, WNT6 was highly expressed in BC cell lines (Figure 3B). In addition, protein levels of WNT6 and β-catenin were downregulated in MCF-7 cells overexpressing miR-566 (Figure 3C). Moreover, shared binding sites to miR-566 seed sequence in WNT6 3′-UTR were predicted using the bioinformatics tool (Figure 3D). Overexpression of miR-566 was able to decrease luciferase activity in pmirGLO-WNT6-WT, whereas it failed to mediate the luciferase activity in the mutant-type one. It was also indicated that WNT6 was the target gene binding to miR-566 (Figure 3E). Knockdown of miR-566 in MDA-MB-231 cells upregulated vital genes in the WNT signaling.

WNT6 Was Involved In MiR-566-Modulated BC Cell Functions

We next explored the role of WNT6 in BC progression. Transfection efficacy of pcDNA3.1-WNT6 and si-WNT6 was examined in MCF-7 and MDA-MB-231 cells by Western blot, respectively (Figure 4A). Compared with those in cells overexpressing miR-566, the viability and percentage of EdU-positive cells were increased in MCF-7 cells co-overexpressing miR-566 and WNT6 (Figure 4B and 4C, left). In addition, lower proliferative ability was observed in MDA-MB-231 cells with co-silence of miR-566 and WNT6 than those with miR-566 knockdown (Figure 4B and 4C, right). Similarly, migratory changes induced by miR-566 intervention were abolished by WNT6 (Figure 4D).

Discussion

Malignant tumor has become the number one killer severely endangering human lives. BC is a major reason for cancer deaths in females. It is responsible for 25% of female cancer deaths and 15% of total cancer deaths. During the past decade, the prevalence of BC in our country has sharply increased owing to the rapid development of the living habits[1-5]. Although great efforts have been made in timely diagnosing BC, controlling BC aggravation and improving life quality of BC patients, the increasing trends of incidence and mortality of BC have not been well controlled[5-8]. Therefore, it is urgently necessary to clarify the molecular mechanisms of BC progression and seek effective methods to reverse the growth and metastasis of BC[9,10].

A growing number of studies have reported the abnormally expressed miRNAs in tumor cells. They are vital regulators during the growth, metastasis and progression of tumors *via* targeting the downstream genes[11-16]. Aiming to provide a novel idea in clinical treatment of BC, the objective of this study was first to elucidate the oncogenic role of miR-566 in the progression of BC, as well as its specific mechanism. Therefore, we collected BC samples and generated *in vitro* miR-566 knockdown and overexpression models in BC cells. MiR-566 was found to be downregulated in BC tissues compared with that in paracancerous ones and its level was linked to tumor size, tumor grade, lymphatic metastasis and prognosis in BC patients, suggesting that miR-566 serves as a

tumor suppressor in BC. Subsequently, cell function experiments uncovered the role of miR-566 in inhibiting proliferative and migratory abilities in BC cells.

The ceRNA hypothesis proposed that there is a complicated regulatory interaction between different types of RNAs, including protein-encoding mRNAs and noncoding RNAs. They share common miRNA binding sites. By abolishing or alleviating the inhibitory effects on target genes, these ceRNAs are widely involved in either promoting or inhibiting tumor progression[18,19]. The balance between mRNAs and miRNAs can control mRNA abundance to ensure normal expressions and functions of proteins[19]. Once the balance is broken, abnormally expressed miRNAs can cause either carcinogenic or tumor-suppressive effect[15-17,21]. In WNT6 3′-UTR, shared binding sites to miR-566 seed sequence were predicted by bioinformatics analysis. MiR-566 failed to enrich WNT6 after mutation of the binding sites, further supporting our finding that WNT6 was the tar-

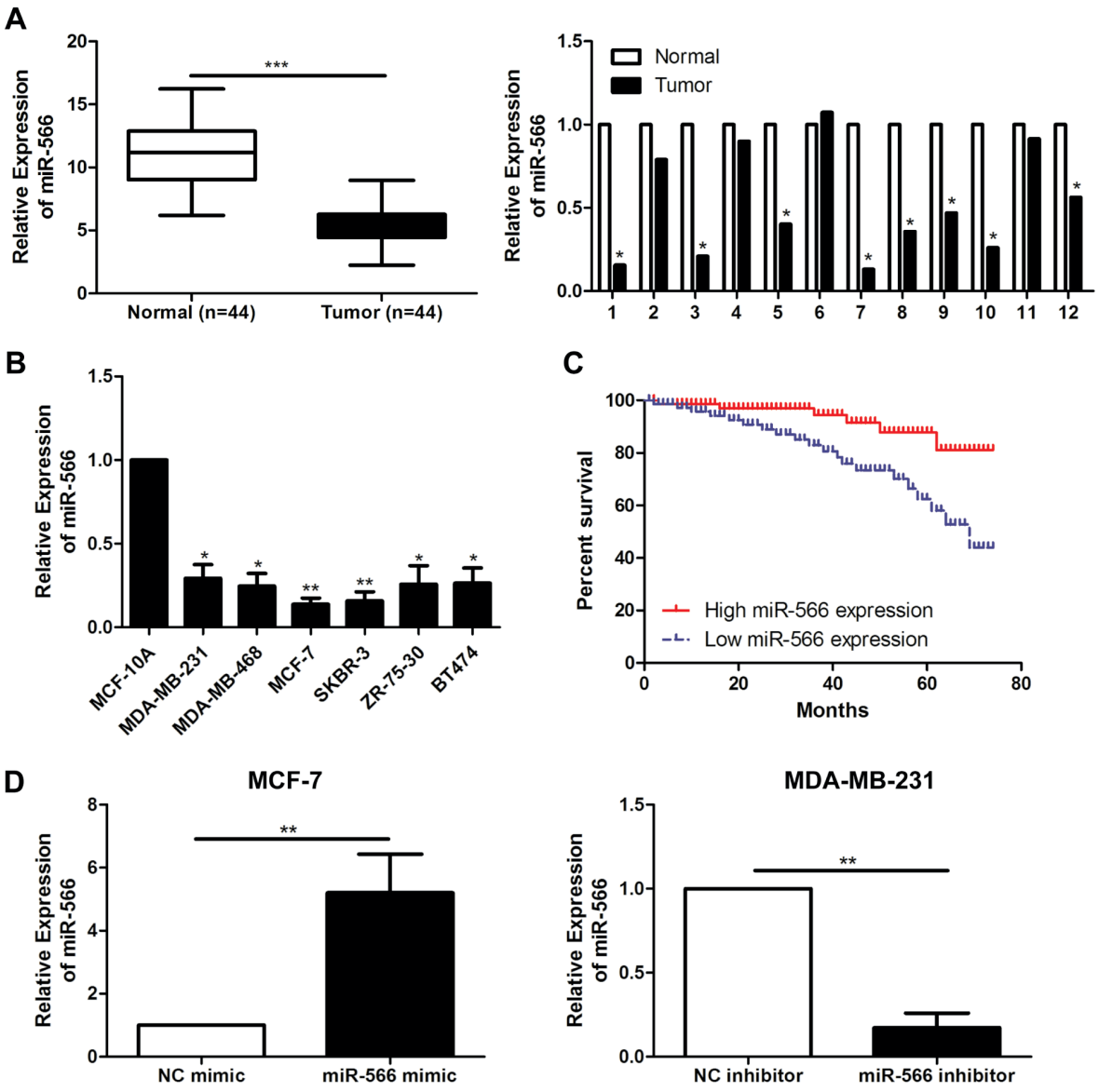

Figure 1. MiR-566 was lowly expressed in BC samples. **A,** Differential levels of miR-566 in BC tissues and paracancerous ones; **B,** MiR-566 levels in BC cell lines; **C,** Overall survival of BC patients depending on the expression of miR-566; **D,** Transfection efficacy of miR-566 mimic and inhibitor in MCF-7 and MDA-MB-231 cells. Data were expressed as mean ± SD. *p<0.05, **p<0.01, ***p<0.001.

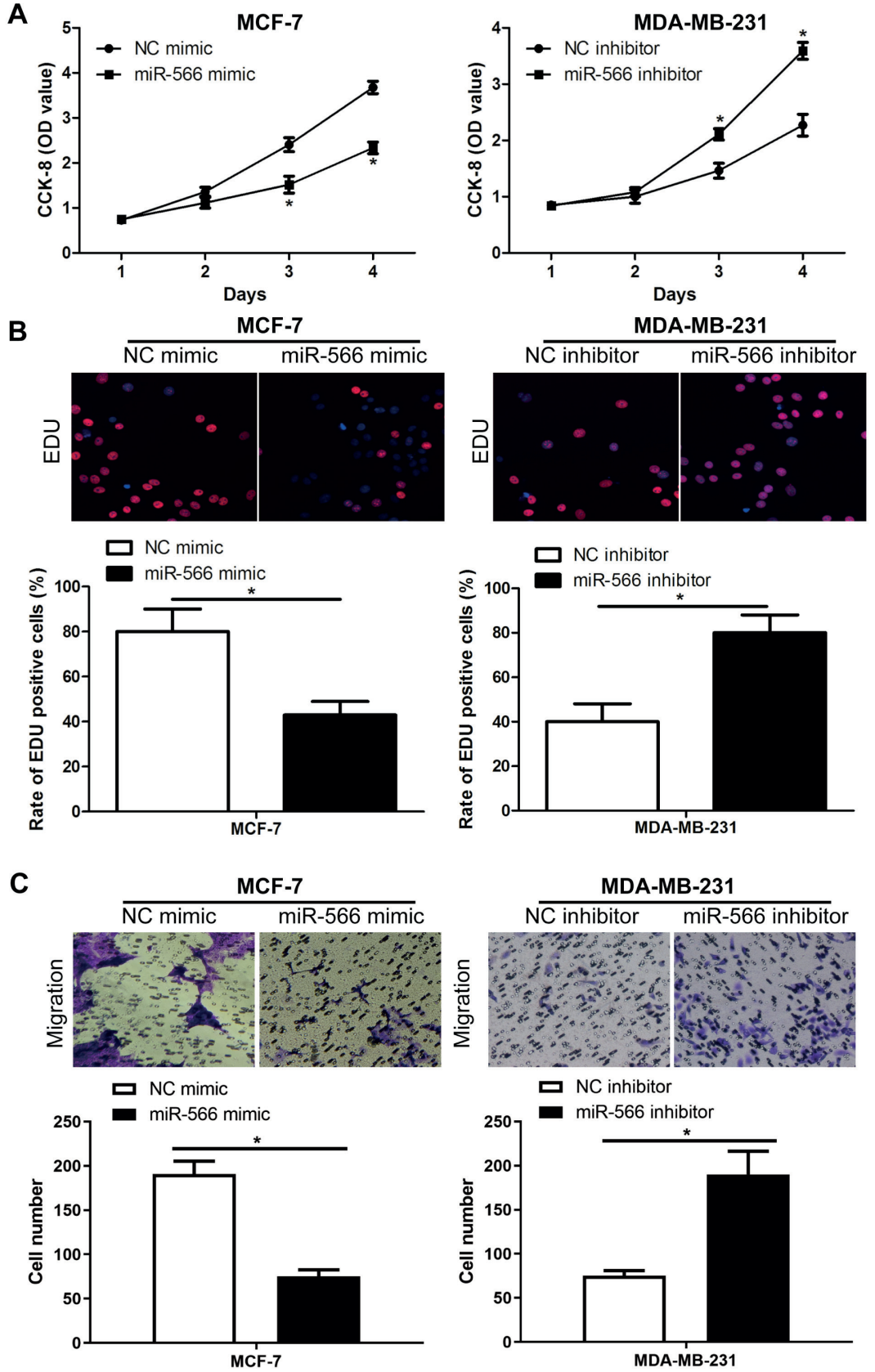

Figure 2. MiR-566 inhibited proliferation and metastasis in BC. **A,** Viability in MCF-7 and MDA-MB-231 cells regulated by miR-566; **B,** The percentage of EdU-positive MCF-7 and MDA-MB-231 cells regulated by miR-566 (magnification 40×); **C,** Migration in MCF-7 and MDA-MB-231 cells regulated by miR-566 (magnification 40×). Data were expressed as mean ± SD. *p<0.05.

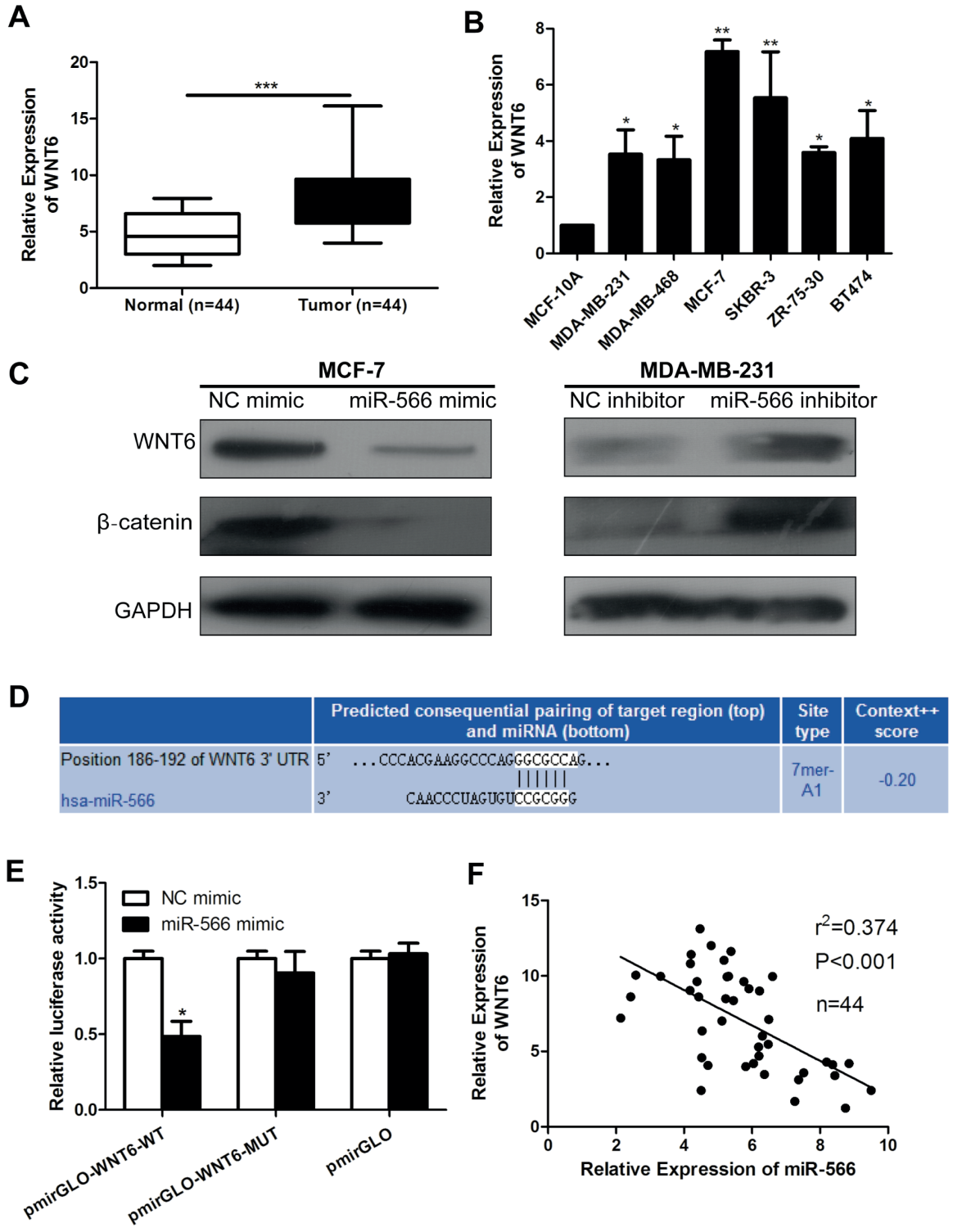

Figure 3. MiR-566-WNT6 interaction. **A,** Differential levels of WNT6 in BC tissues and paracancerous ones; **B,** WNT6 levels in BC cell lines; **C,** Protein levels of WNT6 and β-catenin in MCF-7 and MDA-MB-231 cells regulated by miR-566; **D,** Binding sites to miR-566 seed sequence in WNT6 3′-UTR; **E,** Luciferase activity in HEK293 cells; F, A negative correlation between WNT6 and miR-566 levels in BC tissues. Data were expressed as mean ± SD. *p<0.05, **p<0.01, ***p<0.001.

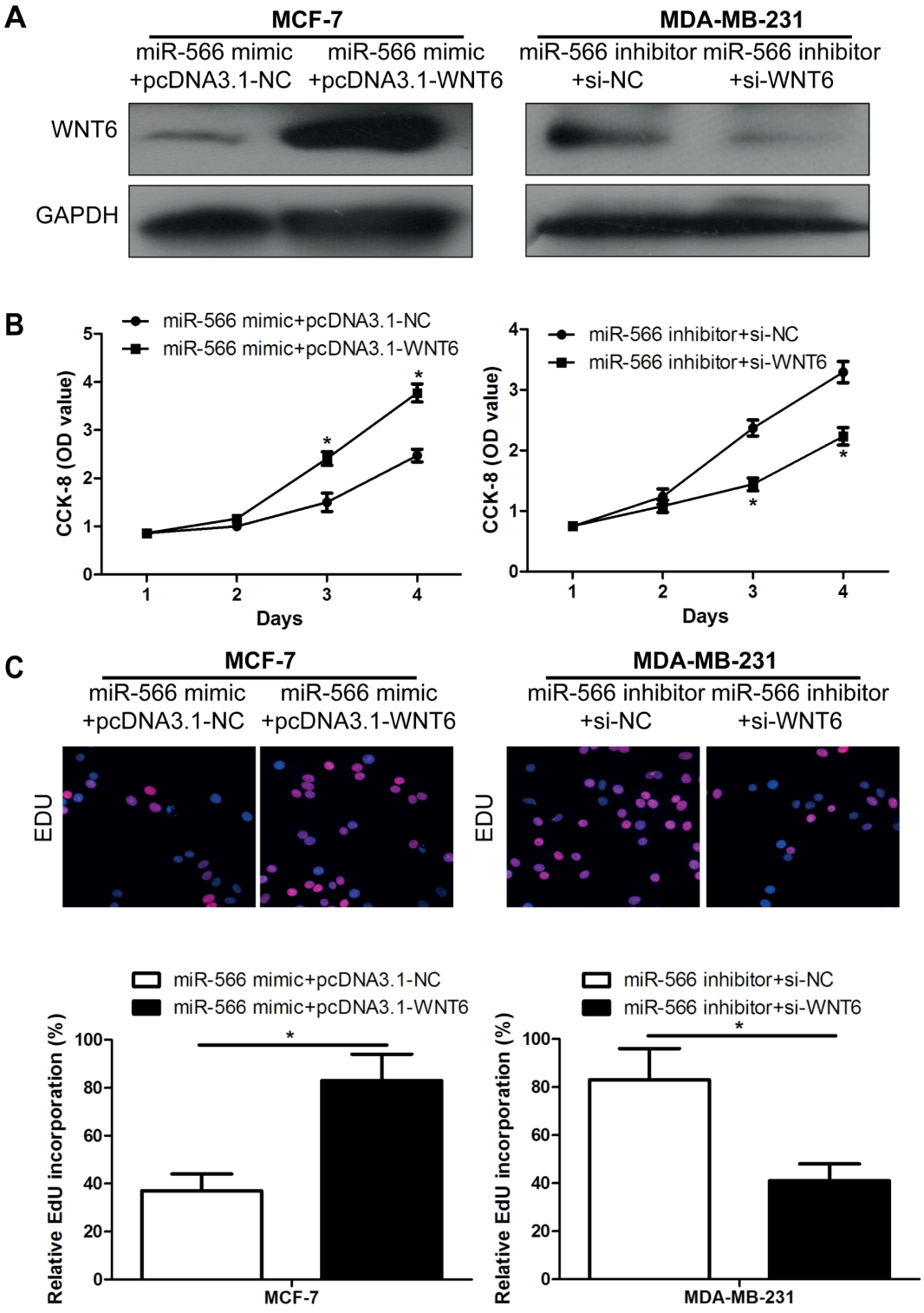

Figure 4. WNT6 was involved in miR-566-modulated BC cell functions. **A,** Transfection efficacy of pcDNA3.1-WNT6 and si-WNT6 in MCF-7 and MDA-MB-231 cells; **B,** Viability in MCF-7 and MDA-MB-231 cells co-regulated by miR-566 and WNT6; **C,** The percentage of EdU-positive MCF-7 and MDA-MB-231 cells co-regulated by miR-566 and WNT6 (magnification 40×); **D,** Migration in MCF-7 and MDA-MB-231 cells co-regulated by miR-566 and WNT6 (magnification 40×). Data were expressed as mean ± SD. *p<0.05.

get gene binding to miR-566. The WNT pathway plays a critical role in controlling the enormous tissue expansion and remodeling during the development of BC, and its deregulation has been implicated in BC initiation and progression[26]. Western blot analyses uncovered that the vital genes in the WNT signaling, including WNT6 and β-catenin, were downregulated by overexpression of miR-566 in MCF-7 cells. Besides, WNT6 level was negatively related to miR-566 level in BC samples. We thereafter speculated that WNT6 was involved in miR-566-regulated BC progression. By performing rescue experiments, it is found that WNT6 was able to abolish the regulatory effects of miR-566 on proliferative and migratory abilities in BC. To sum up, the protective role of miR-566 in alleviating the malignant progression of BC relied on the involvement of WNT6. These findings provided new insights and ideas for the diagnosis and treatment of BC, and might become a key to making breakthrough progress in the subsequent years.

Conclusions

In brief, miR-566 is closely related to tumor size, tumor grade, lymphatic metastasis and prognosis in BC. It protects the malignant progression of BC by negatively regulating WNT6.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Makhoul I, Atiq M, Alwbari A, Kieber-Emmons T. Breast cancer immunotherapy: an update. B Breast Cancer (Auckl) 2018; 12: 1178223418774802.
2) Bahcecioglu G, Basara G, Ellis BW, Ren X, Zorlutuna P. Breast cancer models: engineering the tumor microenvironment. Acta Biomater 2020; 106: 1-21.
3) Ito H, Matsuo K. Molecular epidemiology, and possible real-world applications in breast cancer. Breast Cancer-Tokyo 2016; 23: 33-38.
4) Albeshan SM, Mackey MG, Hossain SZ, Alfuraih AA, Brennan PC. Breast cancer epidemiology in Gulf Cooperation Council Countries: a regional and international comparison. Clin Breast Cancer 2018; 18: e381-e392.
5) Fan L, Strasser-Weippl K, Li JJ, St LJ, Finkelstein DM, Yu KD, Chen WQ, Shao ZM, Goss PE. Breast cancer in China. Lancet Oncol 2014; 15: e279-e289.
6) Li T, Mello-Thoms C, Brennan PC. Descriptive epidemiology of breast cancer in China: incidence, mortality, survival and prevalence. Breast Cancer Res Treat 2016; 159: 395-406.
7) Tang Y, Wang Y, Kiani MF, Wang B. Classification, treatment strategy, and associated drug resistance in breast cancer. Clin Breast Cancer 2016; 16: 335-343.
8) Rivera-Franco MM, Leon-Rodriguez E. Delays in breast cancer detection and treatment in developing countries. Breast Cancer (Auckl) 2018; 12: 2075657115.
9) He Y, Deng F, Yang S, Wang D, Chen X, Zhong S, Zhao J, Tang J. Exosomal microRNA: a novel biomarker for breast cancer. Biomark Med 2018; 12: 177-188.
10) Hesari A, Golrokh MS, Siasi A, Rahmani M, Behboodi N, Rastgar-Moghadam A, Ferns GA, Ghasemi F, Avan A. Tumor-derived exosomes: potential biomarker or therapeutic target in breast cancer? J Cell Biochem 2018; 119: 4236-4240.
11) Xu J, Shao T, Ding N, Li Y, Li X. MiRNA-miRNA crosstalk: from genomics to phenomics. Brief Bioinform 2017; 18: 1002-1011.
12) Hsieh PL, Liao YW, Pichler M, Yu CC. MicroRNAs as theranostics targets in oral carcinoma stem cells. Cancers (Basel) 2020; 12: 340.
13) Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 2010; 11: 597-610.
14) Hembrom AA, Srivastava S, Garg I, Kumar B. MicroRNAs in venous thrombo-embolism. Clin Chim Acta 2020; 504: 66-72.
15) Radbakhsh S, Sathyapalan T, Banach M, Sahebkar A. Incretins and microRNAs: Interactions and physiological relevance. Pharmacol Res 2020; 153: 104662.
16) Masliah G, Maris C, Konig SL, Yulikov M, Aeschimann F, Malinowska AL, Mabille J, Weiler J, Holla A, Hunziker J, Meisner-Kober N, Schuler B, Jeschke G, Allain FH. Structural basis of siRNA recognition by TRBP double-stranded RNA binding domains. EMBO J 2018; 37: e97089.
17) Schraivogel D, Meister G. Import routes and nuclear functions of Argonaute and other small RNA-silencing proteins. Trends Biochem Sci 2014; 39: 420-431.
18) Huang Y. The novel regulatory role of lncRNA-miRNA-mRNA axis in cardiovascular diseases. J Cell Mol Med 2018; 22: 5768-5775.
19) Li M, Duan L, Li Y, Liu B. Long noncoding RNA/circular noncoding RNA-miRNA-mRNA axes in cardiovascular diseases. Life Sci 2019; 233: 116440.
20) Zhang Y, Zhang S, Yin J, Xu R. MiR-566 mediates cell migration and invasion in colon cancer cells by direct targeting of PSKH1. Cancer Cell Int 2019; 19: 333.
21) Pan X, Quan J, Li Z, Zhao L, Zhou L, Jinling X, Weijie X, Guan X, Li H, Yang S, Gui Y, Lai Y. miR-566 functions as an oncogene and a potential biomarker for prognosis in renal cell carcinoma. Biomed Pharmacother 2018; 102: 718-727.

22) Jung YS, Park JI. Wnt signaling in cancer: therapeutic targeting of Wnt signaling beyond beta-catenin and the destruction complex. Exp Mol Med 2020; 52: 183-191.

23) Takahashi T. Roles of nAChR and Wnt signaling in intestinal stem cell function and inflammation. Int Immunopharmacol 2020; 81: 106260.

24) Jiang K, Li S, Li L, Wang X, Gu Y, Jin Z. WNT6 is an effective marker for osteosarcoma diagnosis and prognosis. Medicine (Baltimore) 2018; 97: e13011.

25) Zheng XL, Yu HG. Wnt6 contributes tumorigenesis and development of colon cancer via its effects on cell proliferation, apoptosis, cell-cycle and migration. Oncol Lett 2018; 16: 1163-1172.

26) Tocci JM, Felcher CM, Garcia SM, Kordon EC. R-spondin-mediated WNT signaling potentiation in mammary and breast cancer development. IUBMB Life 2020 Mar 31. doi: 10.1002/iub.2278. [Epub ahead of print].