European Review for Medical and Pharmacological Sciences 2019; 23: 9996-10004

MiR-491 suppresses migration and invasion *via* directly targeting TPX2 in breast cancer

G.-Z. TAN[1], M. LI[2], X. TAN[3], M.-L. SHI[1], K. MOU[1]

[1]Department of Oncology, Jinan City People's Hospital, Jinan, China
[2]Department of Radiotherapy, Jinan City People's Hospital, Jinan, China
[3]Department of Invasive Technology, Jinan City People's Hospital, Jinan, China

Abstract. – OBJECTIVE: Breast cancer (BC) is one of the primary causes of tumor-related female mortalities. Although in recent years, we have made great progress in the systemic therapy and earlier diagnosis for BC patients, recurrence or distant metastasis remains leading obstacles for the successful therapy of BC. Therefore, a comprehensive understanding of the molecular mechanism underlying the progression may be crucial in developing an effective strategy against BC. The current research aimed to explore the expressions, functions and molecular mechanism of microRNA-491 (miR-491) in BC.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was performed to examine the level of miR-491 expression in 52 pairs of BC tissues and para-cancerous specimens, and the relation between miR-491 level and the clinical features of BC patient prognosis was analyzed. Transwell invasion and migration assays were conducted to determine whether miR-491 had effects on the regulation of BC metastasis. Potential target genes of miR-491 were found out using TargetScan to explore the molecular functions of miR-491 in inhibiting breast cancer cell invasion and migration. To elucidate the mechanism of TPX2 in suppressing cell invasion and migration medicated by miR-491in breast cancer, we further transfected TPX2 siRNAs into MCF-7 cells to delete endogenous TPX2, along with the transfections with miR-491 inhibitor into MCF-7 cell lines.

RESULTS: The findings demonstrated that miR-491 expressions were significantly decreased in BC tissues and cells. The miR-491 restoration suppressed the invasion and migration of BC cells. In addition, we identified the targeting protein for Xklp2 (TPX2) as a direct target of miR-491 in BC. The knockdown of TPX2 markedly reversed miR-491-medicated inhibition of cell invasion and migration in BC cell lines.

CONCLUSIONS: In short, all the results suggested that miR-491 functioned as a tumor suppressor by targeting TPX2 in BC and the miR-491 restoration may be an effective therapy for the BC treatment in the future.

Key Words:
MiR-491, Invasion, Migration, TPX2, Breast cancer.

Introduction

Breast cancer (BC), a common tumor in female, is one of the leading causes of tumor-related female mortality[1]. Early BC has a 5-year survival rate of 99%, but for patients with distant metastasis, the 5-year survival rate is only 24%[2]. Hence, early detection of patients with breast cancer is very important. In addition, even the same therapies, including chemotherapy or radiotherapy[3], are provided for breast cancer patients with the same TNM stage, some patients may benefit from the therapy but others may have tumor metastasis or recurrence[4]. Therefore, it is still hard to predict the consequence of the treatment for every single patient accurately. As a result, we know that it is urgent to find out new biomarker for the earlier diagnosis and prognosis for BC.

Recently, increasing evidence has demonstrated a significant relation between tumors and microRNAs (miRNAs). As we all know, miRNAs are small non-coding RNAs, consisting of approximately17-24 nucleotides[5]. It has been reported that miRNAs play critical regulatory roles via directly base pairing with the 3'UTR of their target mRNAs, contributing to the suppression of the mRNA expressions[6]. Notably, several studies have shown that miRNAs are related to extensive biological processes of cancer cells, such as cell differentiation and proliferation, leading to

Corresponding Author: Guozhu Tan, Ph.D; e-mail: tgz964@sina.com

increasing studies focusing on targeting miRNAs to improve the efficacy of breast cancer treatments[7, 8]. For example, Zhang et al[9] found that miR-129-5p could inhibit cell invasion and proliferation via binding to E-cadherin, vimentin, and microspherule protein 1 in lung cancer. MiR-495 was reported to suppress cell proliferation and migration by targeting FAM83D in colorectal cancer[10]. MiR-320a was found to have function in inhibiting tumor proliferation and invasion in human hepatocellular carcinoma by targeting c-Myc[11]. Additionally, miR-491 has been identified to express aberrantly in different human tumors[12,13]. However, the functions of miR-491 in modulating BC cell invasion and migration needs to be fully elucidated.

New evidence emerging in gene expression profiling suggested that differentially expressed genes in tumors can provide new targeted therapeutic strategies for patients with tumors and used as biomarkers for detecting early-stage tumor or predicting prognosis[14]. Based on this, current research has focused on the targeting protein for Xklp2 (TPX2). TPX2 has essential functions in mitotic spindles formation[15]. In recent years, several researches have shown that TPX2 is closely related to the development of various cancers such as cervical and colon cancer[16,17]. However, there are few studies on the association between TPX2 and miR-491 in BC. The insightful information about TPX2 presented in our research will provide a therapeutic target and prognostic marker for BC

Patients and Methods

Human Tissues and Cell Culture

Specimens of patients with BC (n=52) and adjacent normal tissues were collected from our hospital from 2015 to 2017. All patients involved in this study had never been subjected to any previous treatment. All tissue samples were snap-frozen in liquid nitrogen immediately after surgery for further assays. This study was approved by the Ethics Committee of Jinan City People's Hospital. Additionally, signed written informed consents were obtained from all participants before the study. Human BC cell line MCF-7 and mammary epithelial cell line MCF-10A were purchased from the American Type Culture Collection (Manassas, VA, USA). Cells involved in this study were maintained in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) which contained 10% of fetal bovine serum (FBS; Gibco, Rockville, MD, USA) in an atmosphere with 5% CO_2 at 37°C.

Cell Transfection

MiR-491 mimics, miR-491 inhibitor, TPX2 siRNAs and corresponding controls were all obtained from RiBoBio (Guangzhou, China). Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was applied to transiently transfect them into MCF-7 cells in line with the manufacturer's instruction. 48 h after transfections, cells were collected for further study.

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

Total RNA in BC or normal tissue samples and cell lines were prepared by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocols. Then, the Prime Script RT reagent kit (TaKaRa, Dalian, China) was used to synthesize the cDNA. QRT-PCR analysis for miR-491 and TPX2 were conducted with TaqMan MicroRNA Assay Kit (Applied Biosystems, Foster City, CA, USA) and SYBR® Premix Ex Tag™ II (TaKaRa, Dalian, China) on the ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The conditions for PCR were as follows: 95°C (30 s), 40 cycles at 95°C (5 s) and 60°C (34 s). The miR-491 expression was normalized to U6 while the TPX2 was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The relative expression ratios of Genes were evaluated by the $2^{-\Delta\Delta CT}$ method. The primers involved in this assay were shown in Table I.

Western Blots

MCF-7 cells were collected 72 h after the transfections and the total protein was prepared by a radioimmunoprecipitation assay (RIPA) buffer (Beyotime Biotechnology, Shanghai, China). Then, the protein concentrations were measured by bicinchoninic acid (BCA) Protein Assay Kit (Beyotime Biotechnology, Shanghai, China). Subsequently, the protein sample was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) which was blocked in Tris-Buffered Saline and Tween (TBST) with 5% non-fat dry milk for 2 h. Next, we incubated the membrane with primary antibodies at 4°C overnight. After that, wash it

Table I. Primer sequences for qRT-PCR.

Primer	Sequence
miR-491 forward	5'-ACACTCCAGCTGGGAGTGGGGAACCCTTCC-3'
miR-491 reverse	5'-TGGTGTCGTGGAGTCG-3'
U6 forward	5'-CTCGCTTCGGCAGCACA-3'
U6 reverse	5'-AACGCTTCACGAATTTGCGT-3'
TPX2 forward	5'-ACCTTGCCCTACTAAGATT-3'
TPX2 reverse	5'-AATGTGGCACAGGTTGAGC-3'
GAPDH forward	5'-ACCTGACCTGCCGTCTAGAA-3'
GAPDH reverse	5'-TCCACCACCCTGTTGCTGTA-3'

U6: small nuclear RNA, snRNA; TPX2: targeting protein for Xklp2; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

with TBST, followed by secondary antibody incubation for 2 h at room temperature. Primary antibodies involved in this assay were as follows: anti-TPX2 (1:1000; ab71816; Abcam, Cambridge, MA, USA); anti-GAPDH (1:1000; ab9485; Abcam, Cambridge, MA, USA). The secondary antibody was Anti-Rabbit IgG (1:4000; ab150077; Abcam, Cambridge, MA, USA). Protein levels were measured by a chemiluminescent detection system (Beyotime, Shanghai, China). GAPDH was an internal reference.

Transwell Assays

After treated with miR-491 mimics, inhibitor or TPX2 siRNA, MCF-7 cell lines were seeded in the top chamber. For invasion and migration assays, transwell chambers were pretreated with or without matrigel (BD Biosciences, Franklin Lakes, NJ, USA), respectively. The top chamber contained a serum-free medium when the bottom chamber contained DMEM medium with FBS. After incubated for 48h at 37°C, the cells remained on the top surface were eliminated by a cotton swab. At the same time, those adhered to the bottom surface were fixed using 4% formaldehyde and stained with 0.1% of crystal violet for detecting the images using a microscope (Olympus Corporation, Tokyo, Japan).

Luciferase Reporter Assay

The amplified TPX2 wild- or mutant-type 3'UTR containing the miR-491 target sites was inserted into the pMIR-GLO luciferase reporter vectors (Ambion, Foster City, CA, USA). Then, according to the manufacturer's protocols, Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA) was used to transfect miR-491 mimics and luciferase reporter vectors of the wide type or mutant type 3'UTR of TPX2 gene into MCF-7 cells. Dual-Luciferase Reporter assay system (Promega, Madison, WI, USA) was used to measure the luciferase activities of the MCF-7 48 h after the transfection.

Statistical Analysis

All the above experiments were performed 3 times. The statistical analysis was evaluated by the GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA) together with Statistical Product and Service Solutions (SPSS) 18.0 version (SPSS Inc. Chicago, IL, USA). Student's *t*-test was applied to evaluate the differences between the two groups. The data was indicated as means ± SD (standard deviation). The differences were identified as statistically significant when $p<0.05$.

Results

MiR-491 Expressions Was Reduced and TPX2 Expressions Was Up-Regulated in BC

To confirm the effects of miR-491 in BC, we first measured the miR-491 expressions in BC tissues and cell lines respectively. The results of qRT-PCR indicated that the miR-491 expressions in BC tissues declined significantly in contrast to that in normal breast tissue samples (Figure 1A). Additionally, to confirm miR-491 expressions were downregulated in BC, we further detected the miR-491 expression in breast cancer MCF-7 cells using qRT-PCR, the results indicated that miR-491 expression was also decreased significantly in contrast with the normal MCF10A cells (Figure 1B). Furthermore, the TPX2 expression level was measured in BC cell lines. The qRT-PCR analysis showed significantly higher mRNA levels of TPX2 in MCF-7 cells in contrast with that in normal MCF10A cells (Figure 1C). Spear-

man's correlation analysis disclosed a negative correlation between miR-491 and TPX2 expressions (Figure 1D).

MiR-491 Inhibited BC Cell Invasion and Migration

Transwell invasion and migration assays were conducted to determine whether miR-491 had effects on the regulation of BC metastasis. First, miR-491 mimics or inhibitor was transfected into MCF-7 cell lines and the efficiency was confirmed by RT-qPCR. Results revealed that the miR-491 expressions in MCF-7 cell lines treated with miR-491 mimics were up-regulated while that in MCF-7 cell lines with miR-491 inhibitor transfections was down-regulated (Figure 2A). We found that, compared to control group, treatment with the miR-491 mimics could significantly reduce the invasion ability of MCF-7 cells (Figure 2B and 2D). Moreover, by comparison with the control group, miR-491 overexpression also could repress the migration capacity of MCF-7 cells significantly (Figure 2C and 2E). In brief, the above findings suggested that miR-491 played a suppressive role in BC cell lines.

MiR-491 Targeted TPX2 and Reduced TPX2 Expression in BC Cell Lines

Potential target genes of miR-491 were found out using TargetScan to explore the molecular functions of miR-491 in inhibiting breast cancer cell invasion and migration. As shown in Figure 3A, the TPX2 3'UTR included a putative miR-491 binding site. Subsequently, luciferase reporter assays were carried out to determine whether TPX2 was a target gene of miR-491. MCF-7 cell lines were cotransfected with luciferase reporter vectors containing TPX2-3'UTR-Wt or TPX2-3'UTR-Mut, along with miR-491 mimics. Results indicated that, compared with the control group,

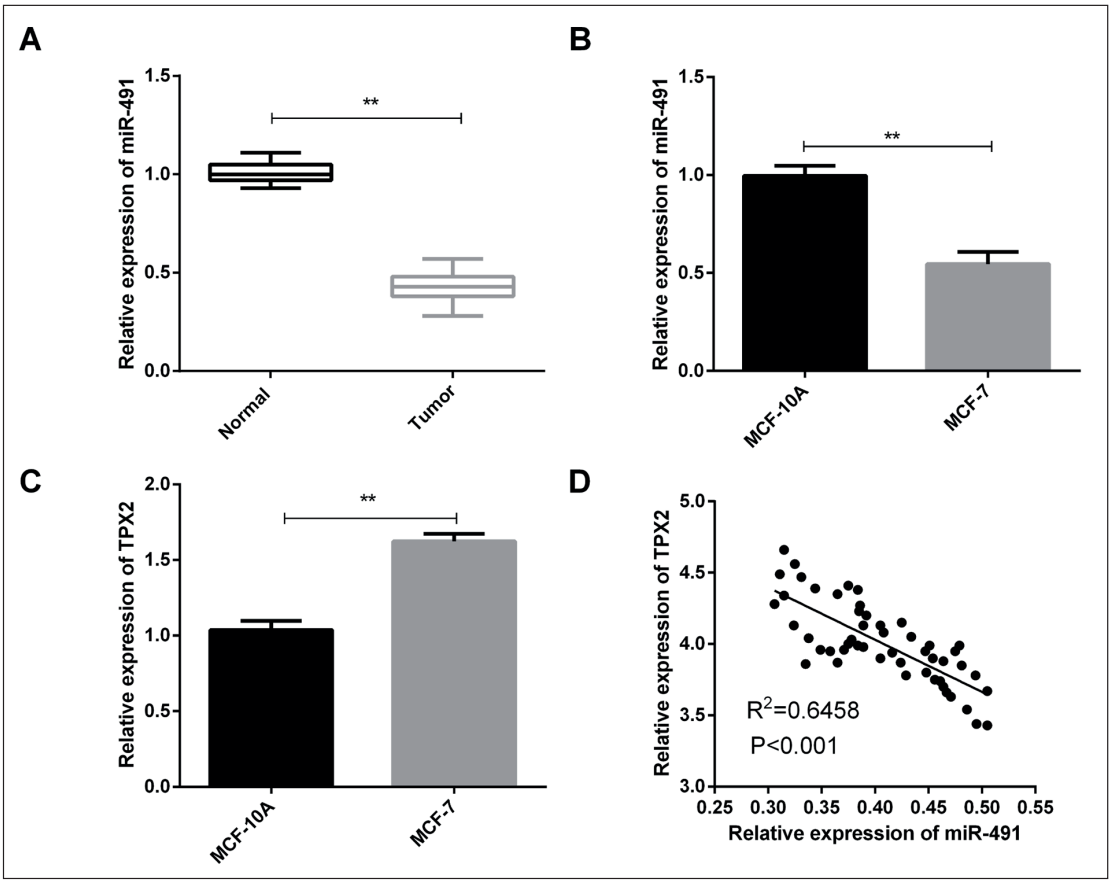

Figure 1. MiR-491 expression reduced and TPX2 expression elevated in BC. **A,** QRT-PCR was applied to measure the miR-491 expressions in BC tissues (n=52) (***p*<0.01). **B,** MiR-491 expressions in BC cells were decreased by qRT-PCR (***p*<0.01). **C,** TPX2 expression was measured using qRT-PCR in breast cancer cells (***p*<0.01). **D,** Regression analysis of correlation between miR-491 and TPX2 expressions.

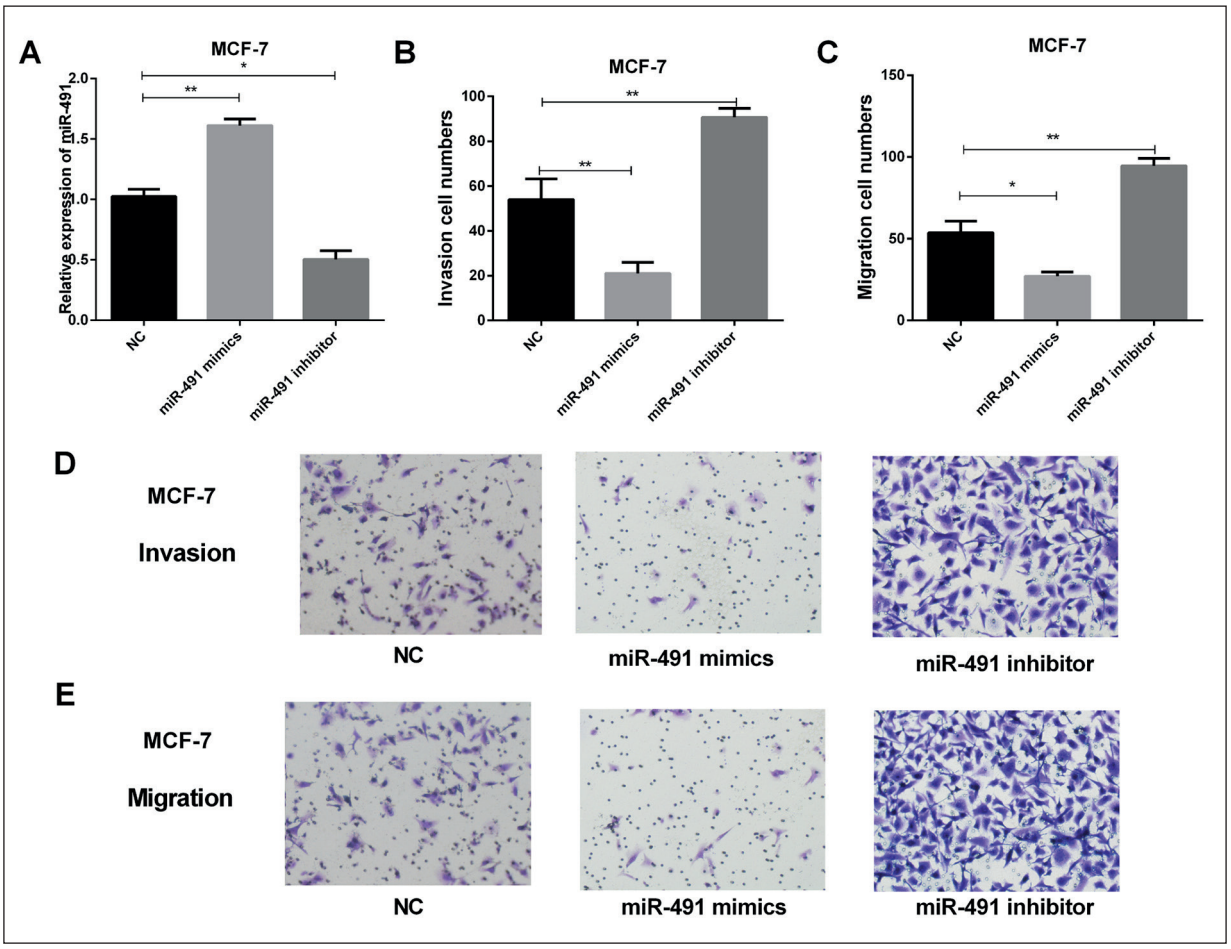

Figure 2. MiR-491 suppressed BC cell invasion and migration. **A,** MiR-491 expressions in transfected breast cancer cells were measured using qRT-PCR (*p<0.05, **p<0.01). **B,** Invasion cell numbers of BC cells were counted (**p<0.01). **C,** Migration cell numbers of BC cells were counted (*p<0.05, **p<0.01). **D,** Cell invasion was observed by the transwell assay in transfected BC cells. **E,** Transwell assay was conducted to detect cell migration in transfected BC cells (magnification: 40×).

miR-491 mimics could significantly decrease the luciferase activities of PmirGLO-TPX2-3' UTR Wt (Figure 3B), nevertheless, the luciferase activities of PmirGLO-TPX2-3'UTR Mut were not affected by miR-491 mimics, suggesting that miR-491 bound to the TPX2 3'UTR directly. Moreover, qRT-PCR and Western blotting were next conducted to measure the TPX2 expressions in MCF-7 cell lines which were transfected with miR-491 mimic or inhibitor. Results of qRT-PCR and Western blotting both indicated that, compared with that in control group, miR-491 restoration in MCF-7 cell lines suppressed the TPX2 expression; on the contrary, the down expression of miR-491 in MCF-7 cell lines resulted in a significant increase in TPX2 expressions (Figure 3C and 3D). Taken together, TPX2 was identified to be a new target of miR-491 in breast cancer.

Knockdown of TPX2 Markedly Reversed MiR-491-Medicated Inhibition of Cell Invasion and Migration in BC Cell Lines

To elucidate the mechanism of TPX2 in suppressing cell invasion and migration medicated by miR-491in breast cancer, we further transfected TPX2 siRNAs into MCF-7 cells to delete endogenous TPX2, along with the transfections with miR-491 inhibitor into MCF-7 cell lines. We next examined the mRNA and protein expressions in miR-491 down-regulated MCF-7 cells or miR-491 down-regulated and TPX2-silenced MCF-7 cells. Results showed that, compared to the control group, TPX2 expressions in MCF-7 cells cotransfected with TPX2 siRNAs and miR-491inhibitor were significantly decreased (Figure 4A and 4B). Then, we next performed transwell assays to detect the effects of TPX2

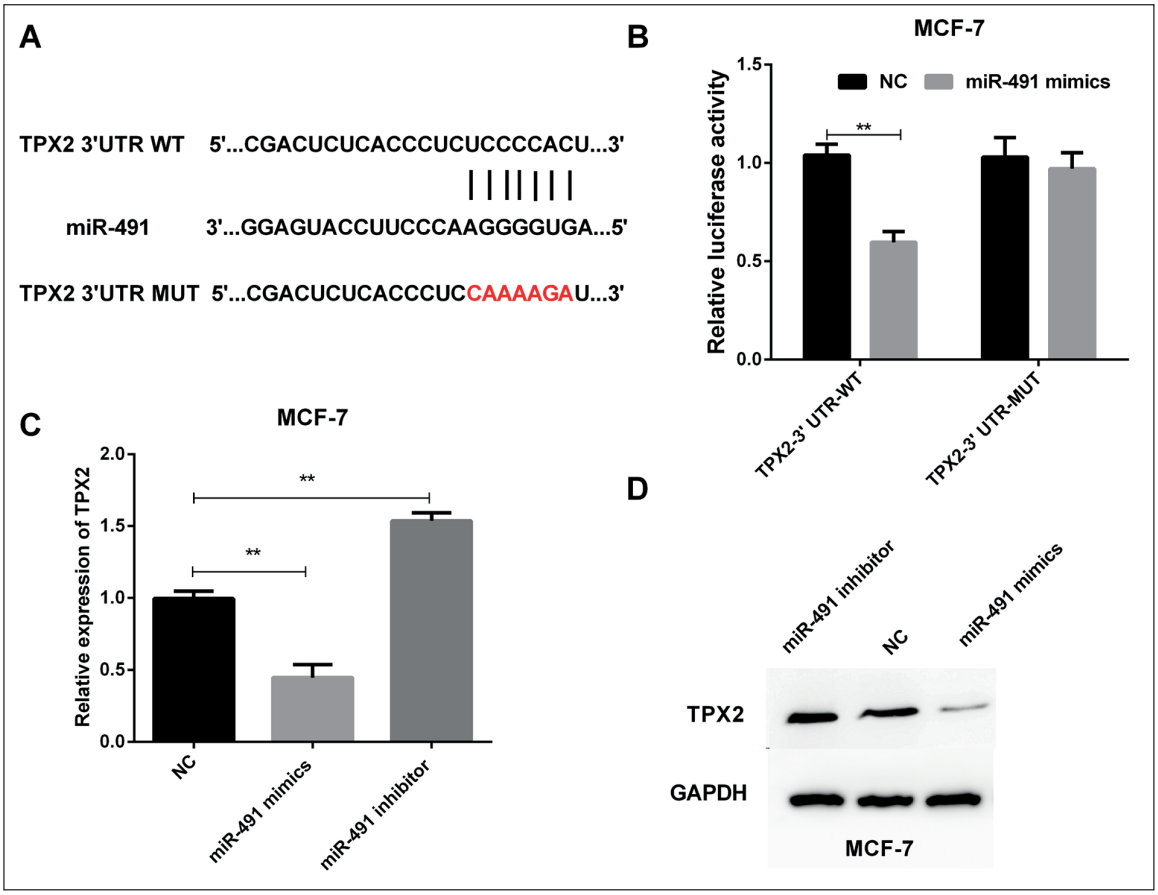

Figure 3. MiR-491 de-regulated TPX2 expression through binding its 3'UTR directly. **A,** Target site of miR-491 in the TPX2 3'UTR. **B,** Luciferase reporter gene assay was carried out to detect the fluorescence activities of the TPX2 3'UTR in BC cells which were co-transfected with wild-type TPX2 3'UTR or mutational type TPX2 3'UTR and miR-491 mimics, respectively (**p<0.01). **C,** TPX2 mRNA expressions in transfected BC cells was determined by qRT-PCR (**p<0.01). **D,** TPX2 protein expressions in transfected BC cells were evaluated by Western blotting.

on BC invasion and migration. Concretely, the invasion and migration capacities in MCF-7 cell lines co-transfected with TPX2 siRNAs and miR-491inhibitor were measured and the results demonstrated that the deletion of TPX2 markedly reversed miR-491 inhibitor-medicated promotion of cell invasion and migration in MCF-7 cell lines (Figure 4D and 4E). Collectively, these data suggested that TPX2 may reverse the partial function of miR-491 in MCF-7 cell lines.

Discussion

As we all know, BC is one of the primary causes of tumor deaths in females worldwide[18]. Preventing tumor growth and metastasis is the central problem in breast cancer treatments. However, sometimes the examination is too late on account of metastasis may have already occurred at that moment[19]. Hence, exploring a new biomarker for earlier diagnosis of BC with metastasis or recurrence is an emergency. miRNAs are new approaches to develop the novel tumor therapies, suppressing tumor relapse, treatment resistance, and metastasis[20]. MiRNAs have been considered to have crucial functions in cancer development because expressions of miRNAs in tumors are different from normal tissues, and miRNAs may have a unique role in tumor regulation[21].

Li et al[22] indicated that the function of miRNA varies with tumor type, even in the same tumor type. MiR-491 has relevance to tumorigenesis in a wide range of tumors, such as colorectal cancer[23], gastric cancer[24], and hepatocellular cancer[25]. The current study aimed to explore the functions of miR-491 in BC and the findings

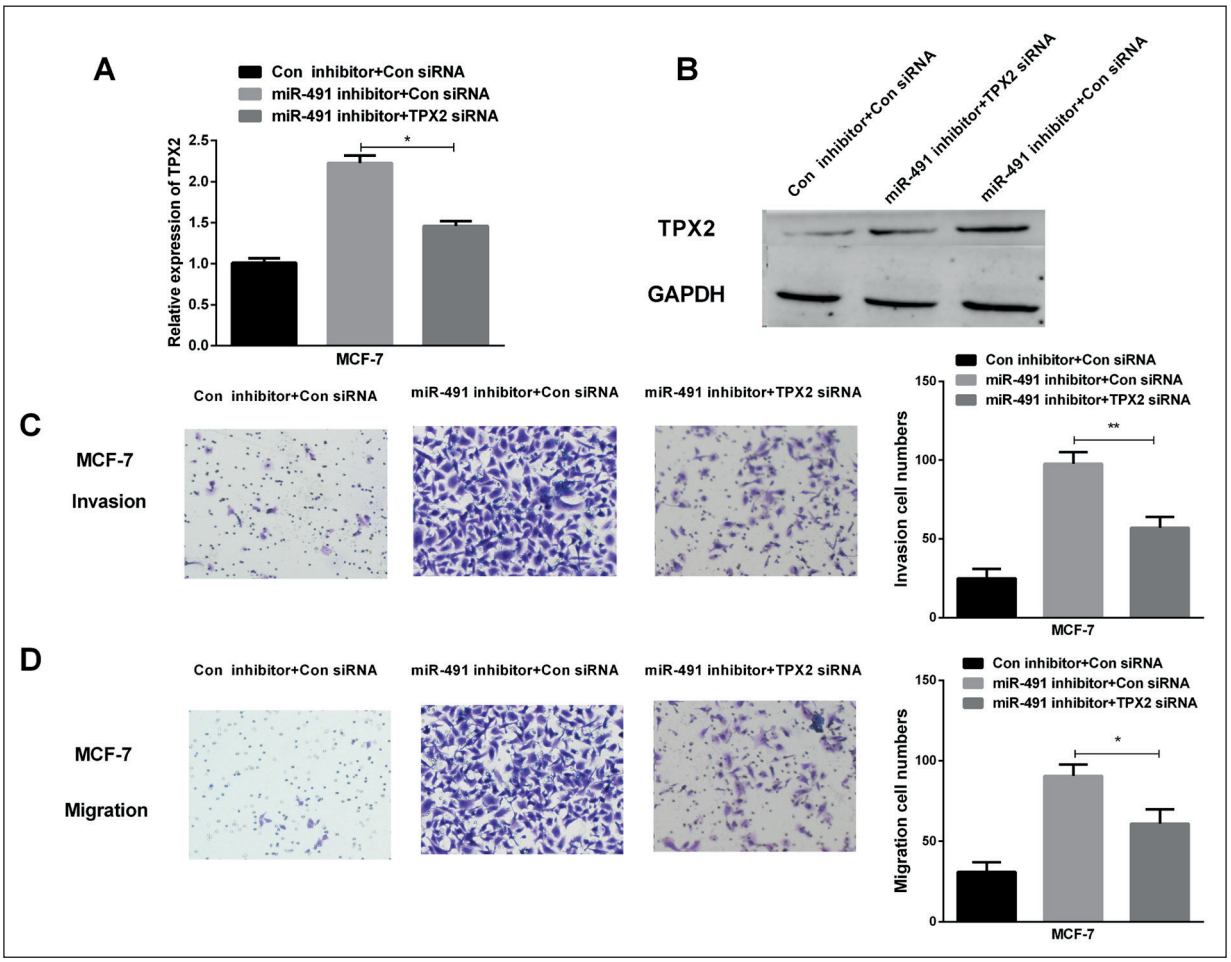

Figure 4. Depletion of TPX2 could reverse partial function of miR-491 in BC cells. **A, B,** TPX2 mRNA or protein expression level was detected by western blot or qRT-PCR in BC cells co-transfected with TPX2 siRNA and miR-491 inhibitor (*p<0.05). **C, D,** Transwell assays were carried out to detect invasion and migration abilities of BC cells co-transfected with TPX2 siRNA and miR-491 inhibitor (magnification: 40×) (*p<0.05, **p<0.01).

revealed that miR-491 was down-regulated in BC cells; moreover, we further determined that over-expression of miR-491 could inhibit the activities of BC cells by targeting TPX2. In short, the findings of this research demonstrated that miR-491 plays important roles in tumor development, including breast cancer. Qi et al[26] support our findings. For example, miR-491 was reported to have a function in regulating glioma cells proliferation by targeting TRIM28; Wang et al[27] found that miR-491 inhibited lung metastasis and chemoresistance in osteosarcoma via regulating alphaB-crystallin directly.

TPX2 is a microtubule-associated protein, and recently, increasing studies have shown that TPX2 is associated with the tumorigenesis of many tumors including breast cancer[28]. Being reported to be over-expressed in many cancers, TPX2 is considered to be a new candidate for malignant tumors diagnosis as well as prognosis[29]. Therefore, seeking a TPX2 gene-targeted therapy for breast cancer may be a curative method which would alleviate associated side effects. The current study demonstrated that TPX2 was a direct target of miR-491, in addition, the findings of this study also found that knockdown of TPX2 significantly inhibited human BC cell invasion and migration.

Conclusions

We found that the down-regulation of miR-491 in our 52 BC tissue samples and in one human BC cell line. In addition, we identified that TPX2 was a functional and direct target

of miR-491. Moreover, the findings also revealed that TPX2 inhibition may reverse the partial function of down-regulated miR-491-induced cell migration and invasion in BC. All the above findings demonstrated a regulatory function of miR-491 on TPX2 by base-pairing with its 3'UTR, suggesting that miR-491 might be an effective biomarker and therapeutic strategy for breast cancer treatment in the future.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Kalager M. Breast cancer screening. BMJ 2017; 359: j5625.
2) Zhuang X, Wang J. Correlations of MRP1 gene with serum TGF-beta1 and IL-8 in breast cancer patients during chemotherapy. J BUON 2018; 23: 1302-1308.
3) Jafari SH, Saadatpour Z, Salmaninejad A, Momeni F, Mokhtari M, Nahand JS, Rahmati M, Mirzaei H, Kianmehr M. Breast cancer diagnosis: imaging techniques and biochemical markers. J Cell Physiol 2018; 233: 5200-5213.
4) Ponti A, Frigerio A, Marra V, Armaroli P, Segnan N. Breast cancer screening frequency and overdiagnosis. J Am Coll Radiol 2017; 14: 1520.
5) Soheilyfar S, Velashjerdi Z, Sayed Hajizadeh Y, Fathi Maroufi N, Amini Z, Khorrami A, Haj Azimian S, Isazadeh A, Taefehshokr S, Taefehshokr N. In vivo and in vitro impact of miR-31 and miR-143 on the suppression of metastasis and invasion in breast cancer. J BUON 2018; 23: 1290-1296.
6) Lajos R, Braicu C, Jurj A, Chira S, Cojocneanu-Petric R, Pileczki V, Berindan-Neagoe I. A miRNAs profile evolution of triple negative breast cancer cells in the presence of a possible adjuvant therapy and senescence inducer. J BUON 2018; 23: 692-705.
7) Toffanin S, Sia D, Villanueva A. MicroRNAs: new ways to block tumor angiogenesis? J Hepatol 2012; 57: 490-491.
8) Plummer PN, Freeman R, Taft RJ, Vider J, Sax M, Umer BA, Gao D, Johns C, Mattick JS, Wilton SD, Ferro V, McMillan NA, Swarbrick A, Mittal V, Mellick AS. MicroRNAs regulate tumor angiogenesis modulated by endothelial progenitor cells. Cancer Res 2013; 73: 341-352.
9) Zhang Y, An J, Lv W, Lou T, Liu Y, Kang W. MiRNA-129-5p suppresses cell proliferation and invasion in lung cancer by targeting microspherule protein 1, E-cadherin and vimentin. Oncol Lett 2016; 12: 5163-5169.
10) Yan L, Yao J, Qiu J. MiRNA-495 suppresses proliferation and migration of colorectal cancer cells by targeting FAM83D. Biomed Pharmacother 2017; 96: 974-981.
11) Xie F, Yuan Y, Xie L, Ran P, Xiang X, Huang Q, Qi G, Guo X, Xiao C, Zheng S. MiRNA-320a inhibits tumor proliferation and invasion by targeting c-Myc in human hepatocellular carcinoma. Onco Targets Ther 2017; 10: 885-894.
12) Cheng Q, Xu X, Jiang H, Xu L, Li Q. Knockdown of long non-coding RNA XIST suppresses nasopharyngeal carcinoma progression by activating miR-491-5p. J Cell Biochem 2018; 119: 3936-3944.
13) Denoyelle C, Lambert B, Meryet-Figuiere M, Vigneron N, Brotin E, Lecerf C, Abeilard E, Giffard F, Louis MH, Gauduchon P, Juin P, Poulain L. MiR-491-5p-induced apoptosis in ovarian carcinoma depends on the direct inhibition of both BCL-XL and EGFR leading to BIM activation. Cell Death Dis 2014; 5: e1445.
14) Thiel A, Ristimaki A. Targeted therapy in gastric cancer. APMIS 2015; 123: 365-372.
15) Neumayer G, Belzil C, Gruss OJ, Nguyen MD. TPX2: of spindle assembly, DNA damage response, and cancer. Cell Mol Life Sci 2014; 71: 3027-3047.
16) Chang H, Wang J, Tian Y, Xu J, Gou X, Cheng J. The TPX2 gene is a promising diagnostic and therapeutic target for cervical cancer. Oncol Rep 2012; 27: 1353-1359.
17) Wei P, Zhang N, Xu Y, Li X, Shi D, Wang Y, Li D, Cai S. TPX2 is a novel prognostic marker for the growth and metastasis of colon cancer. J Transl Med 2013; 11: 313.
18) Zhao J, Jiang GQ. MiR-4282 inhibits proliferation, invasion and metastasis of human breast cancer by targeting Myc. Eur Rev Med Pharmacol Sci 2018; 22: 8763-8771.
19) Fang C, Cao Y, Liu X, Zeng XT, Li Y. Serum CA125 is a predictive marker for breast cancer outcomes and correlates with molecular subtypes. Oncotarget 2017; 8: 63963-63970.
20) Chakraborty C, Sharma AR, Patra BC, Bhattacharya M, Sharma G, Lee SS. MicroRNAs mediated regulation of MAPK signaling pathways in chronic myeloid leukemia. Oncotarget 2016; 7: 42683-42697.
21) Osaki M, Okada F, Ochiya T. MiRNA therapy targeting cancer stem cells: a new paradigm for cancer treatment and prevention of tumor recurrence. Ther Deliv 2015; 6: 323-337.
22) Li J, Lai Y, Ma J, Liu Y, Bi J, Zhang L, Chen L, Yao C, Lv W, Chang G, Wang S, Ouyang M, Wang W. MiR-17-5p suppresses cell proliferation and invasion by targeting ETV1 in triple-negative breast cancer. BMC Cancer 2017; 17: 745.
23) Nakano H, Miyazawa T, Kinoshita K, Yamada Y, Yoshida T. Functional screening identifies a microRNA, miR-491 that induces apoptosis by targeting Bcl-X(L) in colorectal cancer cells. Int J Cancer 2010; 127: 1072-1080.

24) Zhang Y, Xu W, Ni P, Li A, Zhou J, Xu S. MiR-99a and miR-491 regulate cisplatin resistance in human gastric cancer cells by targeting CAPNS1. Int J Biol Sci 2016; 12: 1437-1447.

25) Zhao Y, Qi X, Chen J, Wei W, Yu C, Yan H, Pu M, Li Y, Miao L, Li C, Ren J. The miR-491-3p/Sp3/ABCB1 axis attenuates multidrug resistance of hepatocellular carcinoma. Cancer Lett 2017; 408: 102-111.

26) Qi Z, Cai S, Cai J, Chen L, Yao Y, Chen L, Mao Y. MiR-491 regulates glioma cells proliferation by targeting TRIM28 in vitro. BMC Neurol 2016; 16: 248.

27) Wang SN, Luo S, Liu C, Piao Z, Gou W, Wang Y, Guan W, Li Q, Zou H, Yang ZZ, Wang D, Wang Y, Xu M, Jin H, Xu CX. MiR-491 inhibits osteosarcoma lung metastasis and chemoresistance by targeting αB-crystallin. Mol Ther 2017; 25: 2140-2149.

28) Newlaczyl AU, Yu LG. Galectin-3--a jack-of-all-trades in cancer. Cancer Lett 2011; 313: 123-128.

29) Liu Q, Tu K, Zhang H, Zheng X, Yao Y, Liu Q. TPX2 as a novel prognostic biomarker for hepatocellular carcinoma. Hepatol Res 2015; 45: 906-918.