Oncology Research, Vol. 25, pp. 579–585
Printed in the USA.

0965-0407/17 $90.00 + .00
DOI: https://doi.org/10.3727/97818823455816X14760504645779
E-ISSN 1555-3906
www.cognizantcommunication.com

miR-1193 Suppresses Proliferation and Invasion of Human Breast Cancer Cells Through Directly Targeting IGF2BP2

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miRNAs are involved in breast cancer initiation and progression. In this study, we investigated the role of miR-1193, a newly found and poorly studied miRNA, in the proliferation and invasion of human breast cancer cells. Our results showed that compared with the adjacent tissues and MCF-10A human normal breast cells, miR-1193 was sharply reduced in breast cancer tissues and five breast cancer cell lines, including MDA-MB-231, MDA-MB-468, MDA-MB-435, SKBR3, and MCF-7. The oligo miR-1193 mimic or anta-miR-1193 was then transfected into MDA-MB-231 and MCF-7 breast cancer cell lines. Our results showed that the miR-1193 mimic robustly increased the miR-1193 level and decreased the proliferation and invasion in MDA-MB-231 and MCF-7 cells. In contrast, anta-miR-1193 had an opposite effect on miR-1193 expression, cell proliferation, and cell invasion. Moreover, bioinformatic and luciferase reporter gene assays confirmed that miR-1193 targeted the mRNA 3′-UTR region of insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2), an identified proto-oncogene. miR-1193 suppressed the protein level of IGF2BP2 and the activation of the ERK and PI3K/Akt signaling pathways. Moreover, suppression could be rescued by the transfection of pcDNA-IGF2BP2. In conclusion, miR-1193 suppressed proliferation and invasion of human breast cancer cells via translational suppression of IGF2BP2.

Key words: Breast cancer; miR-1193; Cell proliferation; Cell invasion; Insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2)

INTRODUCTION

Breast cancer greatly imperils women's physical and mental health[1]. With the improvement of women's health awareness, the difficulties in the discovery and early diagnosis of breast cancer have been gradually overcome[2,3]. However, it is still urgent to improve the treatment strategies and investigate novel therapeutic targets.

MEK/ERK and PI3K/Akt are well-studied signaling pathways that are frequently dysregulated in human cancer as a result of genetic alterations in their expression levels or upstream activation of cell surface receptors[3]. They are shown to be activated by a variety of cytokines and play a key role in many aspects of cancer pathogenesis, including cell proliferation/apoptosis, invasion, angiogenesis, and drug resistance[3–7]. To date, the MEK/ERK and PI3K/Akt pathways have been regarded as important diagnostic markers and therapeutic targets in multiple cancers including breast cancer[8,9]. It was proven that these two pathways were of high clinical relevance, and dual inhibition of both pathways exhibited more favorable efficacy and less toxicity compared with inhibition of either pathway[7].

miRNAs have been involved in tumor initiation and progression. There have been numerous reports that miRNAs are associated with clinical indices of breast cancer such as tumor size, clinical stage, lymph node metastasis, and prognosis[10,11]. For example, high levels of miR-21 were associated with advanced clinical stage, lymph node metastasis, and patient poor prognosis[12]. During tumor initiation and progression, miRNAs functioned through targeting and posttranscriptionally suppressing their target genes, which might be proto-oncogenes or tumor suppressor genes[13]. It was demonstrated that miRNAs targeted the marker genes in MEK/ERK and PI3K/Akt pathways, and their upstream genes could be regarded as potential therapeutic targets for breast and other cancers[14,15].

In this study, we explore the role of miR-1193, a newly found and poorly studied miRNA, in breast cancer progression. We detected the expression of miR-1193 in human primary breast cancer tissues and adjacent normal

[1]These authors provided equal contribution to this work.
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tissues, as well as in human normal breast cell lines and five breast cancer cell lines. We found that miR-1193 was sharply downregulated in the cancerous tissues and cell lines. Gain-and-loss function experiments were then used to investigate the exact role in the proliferation and invasion of human breast cancer cells. Furthermore, we predicted and confirmed the relationship between miR-1193 and insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2), a key cytokine receptor upstream of both the MEK/ERK and PI3K/Akt pathways.

MATERIALS AND METHODS

Ethical Statements

This study was approved by the ethics committee of Huaihe Hospital of Henan University (P.R. China). The subjects were informed of the experimental details, and they gave written consent.

Tissue Sampling

Breast cancer and matched normal adjacent tissues were isolated from a total of 39 primary breast cancer patients (50 ± 8.6 years) who underwent tumor resection during the last 36 months in our hospital. Patients who had ever received chemo- or radiotherapy, or suffered systemic diseases or acute splanchnic diseases, were excluded from this study.

Cell Culture and Transfection

Human normal breast epithelial cell MCF-10A; human breast cancer cell lines, including MDA-MB-231, MDA-MB-468, MDA-MB-435, SKBR3, and MCF-7; and HEK293 cells were purchased from the American Type Culture Collection Company (ATCC; Manassas, VA, USA). They were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 U/ml penicillin (Gibco), and 100 U/ml streptomycin (Gibco). The cells were incubated at a humidified atmosphere at 37°C in a 5% CO_2 incubator.

For transfection, MDA-MB-231 and MCF-7 breast cancer cells were subgrown in six-well plates. Upon reaching about 75% of confluence, 100 nM miR-1193 mimic oligo, 100 nM miR-1193 antagomir oligo, or 100 nM relative control oligo was transfected into the cells using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The miR-1193 mimic, anta-miR-1193, and their control oligos were designed and synthesized by Ribobio Co., Ltd. (Guangzhou, P.R. China). The pcDNA-IGF2BP2 was provided by Dr. Q. W. Li (College of Life Science, Northwest University).

Cell Proliferation

The cells were passed into six-well plates at a density of 10^5/per well. After transfection for 48 h, cell proliferation was evaluated using the cell counting kit-8 (CCK-8; Sigma-Aldrich, St. Louis, MO, USA) assay according to the manufacturer's instructions.

In Vitro Invasion Assay

Transwell invasion assay was used to detect cell invasion. Cells (3×10^4) with serum-free medium were plated in the top chamber of the plates (Corning, Corning, NY, USA). Medium (plus 10% bovine fetal serum) was used as a chemoattractant in the lower chamber. After incubation for 24 h, a cotton swab was used to remove the

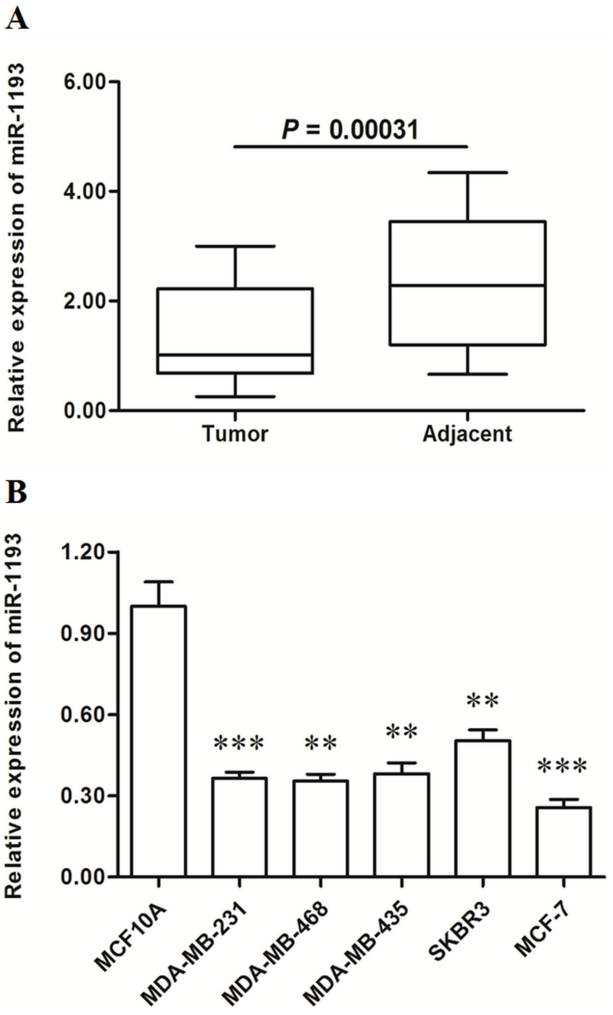

Figure 1. miR-1193 was significantly downregulated in human breast cancer tissues and cell lines. (A) miR-1193 was downregulated in breast cancer tissues. (B) miR-1193 was decreased in breast cancer cell lines. Breast cancer tissues and paired adjacent noncancerous tissues were sampled from 39 breast cancer patients. The expression levels of miR-1193 were detected in these tissues with real-time qPCR. The expression levels of miR-1193 were detected in human normal breast epithelial cell MCF-10A and human breast cancer cell lines MDA-MB-231, MDA-MB-468, MDA-MB-435, SKBR3, and MCF-7. $^{**}p < 0.01$ versus MCF-10A, $^{***}p < 0.001$ versus MCF-10A.

cells that had not migrated from the upper chamber, and the filters were stained with 2% crystal violet. The invasive cells were captured under a light microscope (100×; Olympus IX70; Olympus Corporation, Osaka, Japan) and counted.

Real-Time Quantitative PCR

Real-time qPCRs were carried out in a 25-μl system using SYBR Premix Ex Taq (Invitrogen), 0.5 mM of primers, and 200 ng of cDNA template. The primers of miR-1193 and U6 RNA (internal reference) were designed and produced by Ribobio Co., Ltd. PCR amplification cycles were performed using the SYBR Premix Ex Taq II Kit (Invitrogen). The reactions were initially denatured at 95°C for 1 min and followed by 30 cycles of 94°C for 15 s and 55°C for 1 min. The transcript abundance was calculated using the $2^{-\Delta\Delta}$Ct method.

Western Blotting

Total protein (25 μg) from each sample was separated by 12% SDS-PAGE gel. The protein was transferred onto a PVDF membrane (Millipore, Billerica, MA, USA). The primary antibodies ERK (1:400; Abcam, Cambridge, MA, USA), pERK (1:200; Santa Cruz Biotechnologies, Santa Cruz, CA, USA), PI3K (1:500; Abcam), pPI3K (1:400; Cell Signaling Technology, Danvers, MA, USA), Akt (1:300; Santa Cruz), pAkt (1:200; Cell Signaling Technology), IGF2BP2 (1:400; Abcam), and β-actin (1:1,000; Abcam) were used to incubate with the membrane at 4°C overnight. After incubation with the

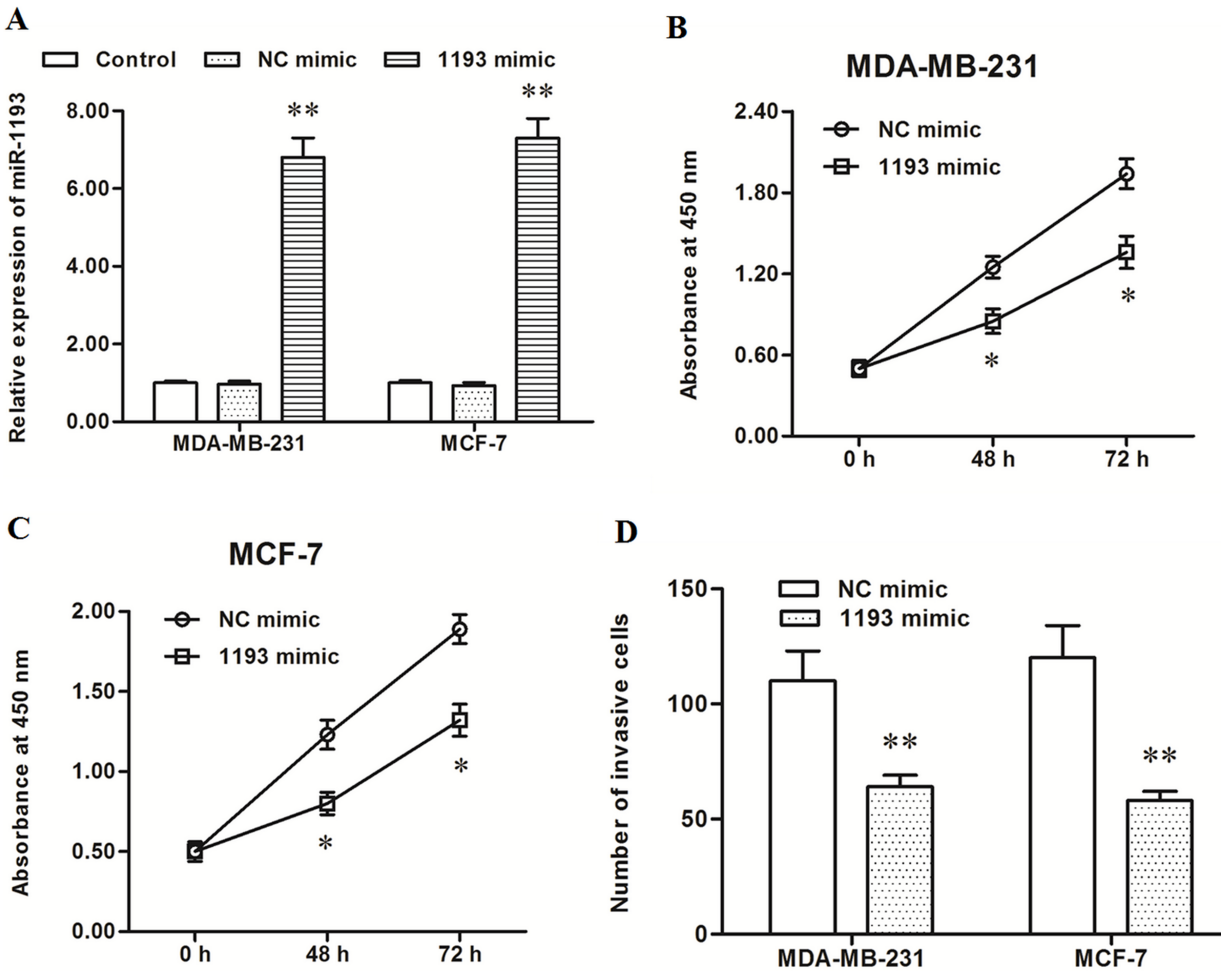

Figure 2. miR-1193 mimic transfection suppressed the proliferation and invasion of MDA-MB-231 and MCF-7 breast cancer cell lines. (A) miR-1193 expression was increased greatly by the miR-1193 mimic transfection. The miR-1193 mimic inhibited the proliferation of MDA-MB-231 (B) and MCF-7 (C) cells. (D) The miR-1193 mimic reduced the invasion of MDA-MB-231 and MCF-7 cells. The negative control (NC) mimic or miR-1193 mimic was transfected into MDA-MB-231 and MCF-7 human breast cancer cell lines. After incubation for 48 h, miR-1193 expression was detected with real-time qPCR, cell proliferation was detected with the CCK-8 approach, and cell invasion was detected with the Transwell invasion assay. *$p<0.05$ versus NC mimic, **$p<0.01$ versus NC mimic.

HRP-conjugated secondary antibody (1:2,000) for 1 h at room temperature, proteins were analyzed with an ECL kit (Millipore) and analyzed with a UV transilluminator (Bio-Rad, Hercules, CA, USA).

3′-UTR Luciferase Gene Reporter Assay

The 3′-UTR sequences of human IGF2BP2 mRNA were amplified by PCR. The sequences were, respectively, purified and inserted into a psiCHECK™-2 Vector (Promega, Madison, WI, USA). The negative control (NC) mimic and miR-1193 mimic were, respectively, cotransfected with psiCHECK-IGF2BP2 3′-UTR into the HEK293 cells using Lipofectamine 3000 (Invitrogen). The cells were then incubated for 72 h, and luciferase activity was measured using a microplate reader (PT-3502; Potenov, Beijing, P.R. China).

Statistical Analysis

All measurements were obtained from more than three independent experiments. The data were expressed as means ± SEM. Statistics were calculated with SPSS v22.0 (Chicago, IL, USA). Multiple comparisons were assessed by one-way ANOVA followed by Dunnett's tests. A value of $p < 0.05$ was considered statistically significant.

RESULTS

miR-1193 Was Markedly Downregulated in Human Breast Cancer Tissues and Cell Lines

Expression of miR-1193 was detected in breast cancer tissues and matched adjacent normal tissues with real-time qPCR. The results showed that miR-1193 was reduced by more than 50% in the cancerous tissues ($p = 0.00031$) (Fig. 1A). Then the miR-1193 level was

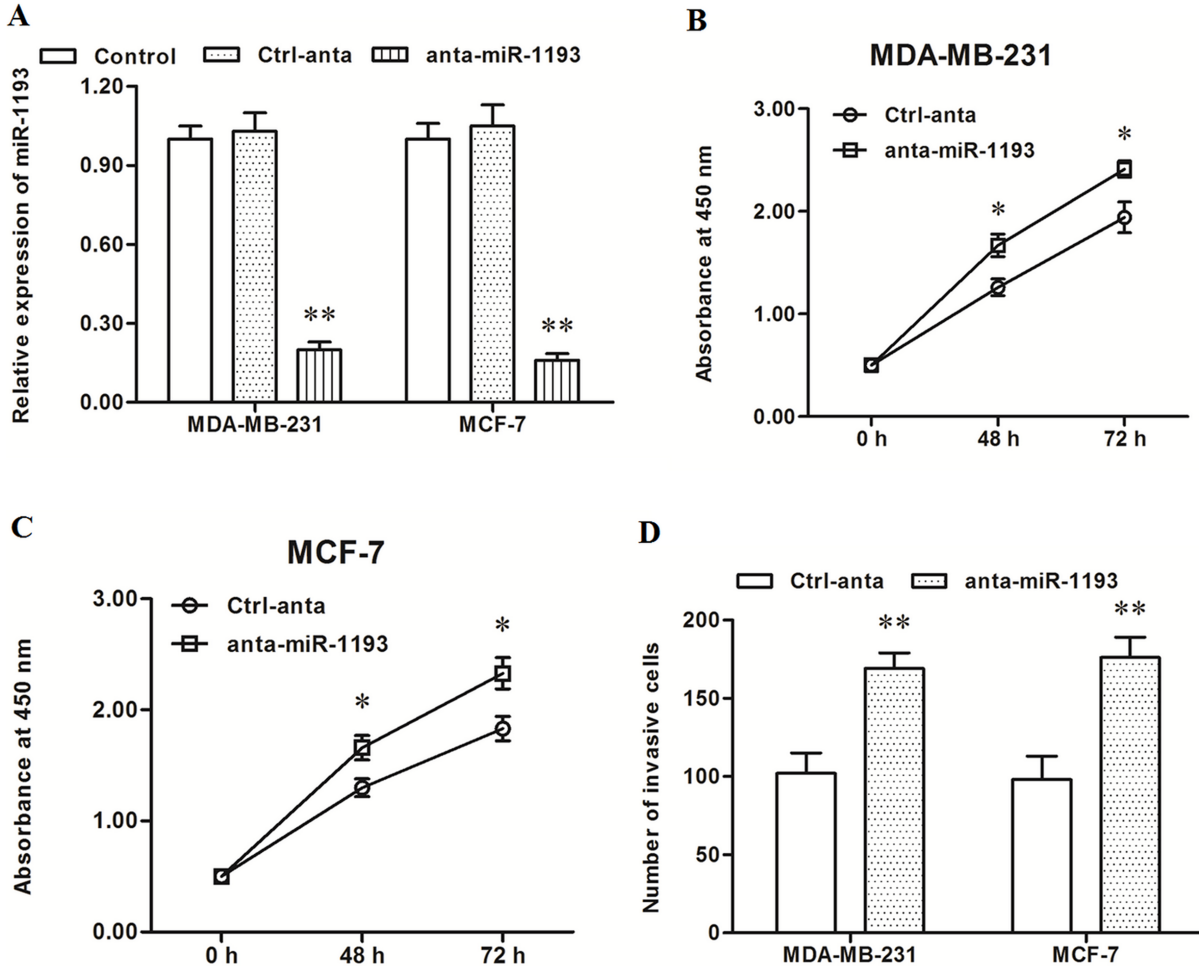

Figure 3. miR-1193 antagomir increased proliferation and invasion of MDA-MB-231 and MCF-7 cells. (A) miR-1193 level was sharply decreased by the anta-miR-1193 transfection. (B, C) The anta-miR-1193 promoted the proliferation of MDA-MB-231 (B) and MCF-7 (C) cells. (D) The anta-miR-1193 enhanced the invasion of MDA-MB-231 and MCF-7 cells. The oligo control or miR-1193 antagomir was transfected into MDA-MB-231 and MCF-7 cells. After incubation for 48 h, miR-1193 expression, cell proliferation, and cell invasion were detected. *$p < 0.05$ versus Ctrl-anta, **$p < 0.01$ versus Ctrl-anta.

detected in human normal breast epithelial cell line and five breast cancer cell lines. miR-1193 was decreased by >50% in all the cancer cell lines ($p<0.01$) (Fig. 1B).

miR-1193 Mimic Transfection Suppressed Proliferation and Invasion of Human Breast Cancer Cells

To explore the role of miR-1193 in the progression of human breast cancer, a NC mimic and miR-1193 mimic were transfected into MDA-MB-231 and MCF-7 breast cancer cells, respectively. The data showed that miR-1193 expression was increased about sevenfold by the miR-1193 mimic transfection in both MDA-MB-231 and MCF-7 cells ($p<0.01$) (Fig. 2A). CCK-8 and Transwell invasion assays showed that the miR-1193 mimic suppressed the proliferation and invasion of MDA-MB-231 and MCF-7 cells ($p<0.05$) (Fig. 2B–D).

miR-1193 Silencing Promoted Proliferation and Invasion of MDA-MB-231 and MCF-7 Cells

Subsequently, the control oligo antagomir and miR-1193 antagomir were transfected into MDA-MB-231 and MCF-7 cells, respectively. In contrast to the results from the miR-1193 mimic transfection, miR-1193 expression was decreased by about 80% in both cell lines ($p<0.01$) (Fig. 3A). The miR-1193 antagomir sharply reduced proliferation and invasion of the two cell lines ($p<0.05$) (Fig. 3B–D). These results, combined with those from the mimic transfection, demonstrated that miR-1193 negatively regulated the proliferation and invasion of breast cancer cells.

miR-1193 Directly Targeted the Proto-oncogene IGF2BP2 to Suppress its Expression and Activation of the ERK and PI3K/Akt Pathways

To explore the potential mechanism from which miR-1193 suppressed the proliferation and invasion of breast cancer cells, bioinformatics analysis was applied to search for potential targets. The output of miRDB displayed that the seed of miR-1193 completely matched with seven consecutive bases in the 3′-UTR region of the IGF2BP2 mRNA sequence (Fig. 4A). The 3′-UTR luciferase reporter gene assay showed that miR-1193

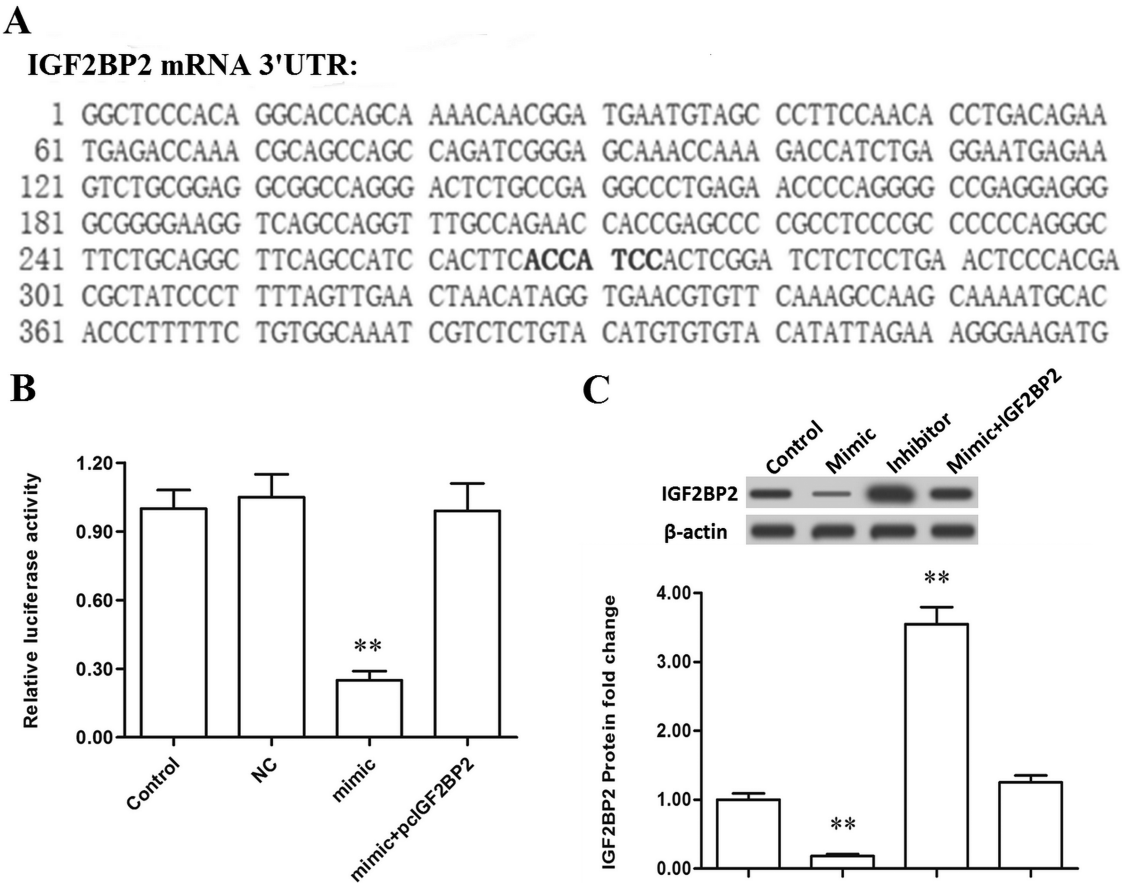

Figure 4. miR-1193 directly targeted IGF2BP2 mRNA at the 3′-UTR region. (A) The output of the miRDB online server on the binding of miR-1193 with the 3′-UTR region of IGF2BP2 mRNA. (B) Validation of the binding of miR-1193 with IGF2BP2 mRNA by the 3′-UTR luciferase reporter gene assay. (C) miR-1193 suppressed the expression of the IGF2BP2 protein, and the suppression could be rescued by pcDNA-IGF2BP2 transfection. **$p<0.01$ versus control.

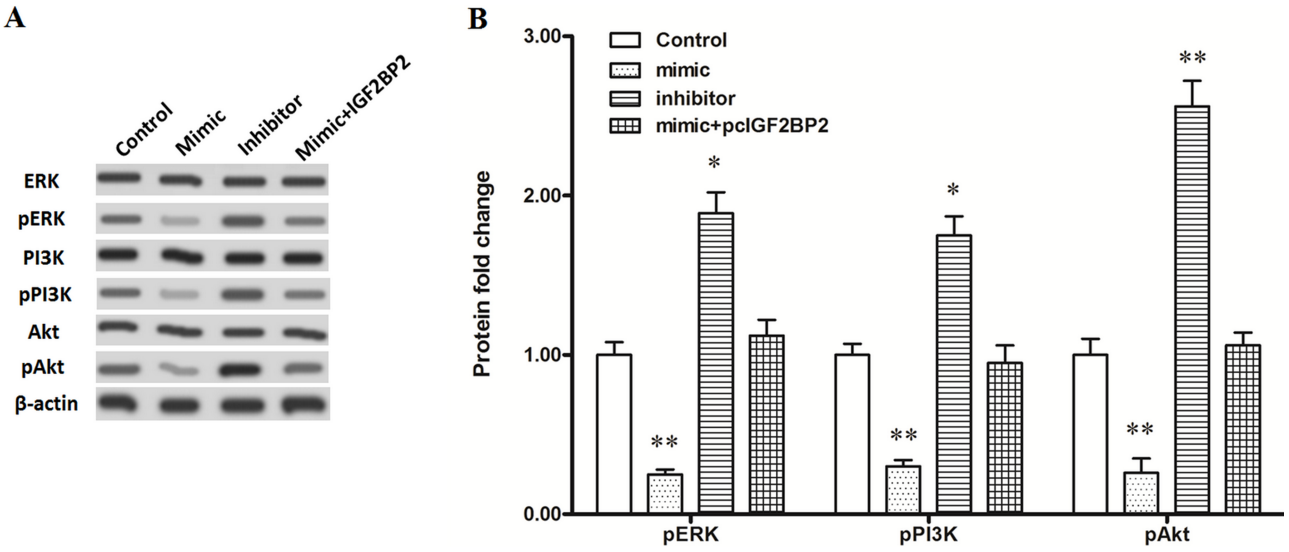

Figure 5. miR-1193 suppressed activation of the ERK and PI3K/Akt pathways. (A) The blots of the key proteins in the ERK and PI3K/Akt pathways. (B) Statistical analysis for (A). The MDA-MB-231 cells were transfected with 100 nM NC mimic, 100 nM miR-1193 antagomir, 100 nM miR-1193 mimic plus 4 μg of pcDNA-IGF2BP2. After incubation for 48 h, Western blotting was used to detect the protein levels of ERK, pERK, PI3K, pPI3K, Akt, and pAkt. *$p<0.05$, **$p<0.01$ versus Control.

markedly suppressed luciferase activity, and pcDNA-IGF2BP2 could rescue the suppression (Fig. 4B). To further verify that IGF2BP2 was the target of miR-1193, the miR-1193 mimic was transfected alone or cotransfected with pcDNA-IGF2BP2 into MDA-MB-231 cells. The IGF2BP2 protein was sharply reduced by the miR-1193 mimic transfection, and the reduction could be retrieved by the pcDNA-IGF2BP2 transfection (Fig. 4C). The ERK and PI3K/Akt pathways were downstream of IGF2BP2 in the carcinogenesis regulatory networks. We then detected the levels of ERK, PI3K, and Akt proteins and their phosphorylated forms after miR-1193 mimic or antagomir transfection. The results showed that pERK, pPI3K, and pAkt were sharply reduced by the miR-1193 mimic transfection, and the reduction was rescued by the pcDNA-IGF2BP2 transfection, while their activation was markedly enhanced by the miR-1193 antagomir transfection (Fig. 5A and B).

DISCUSSION

miR-1193 was found in the melanoma miRNAome by miRNA deep sequencing[16]. To date, there has been no report indicating the exact role of miR-1193 in breast cancer progression. In this study, we report that miR-1193 expression was distinctly downregulated in human breast cancer tissues and cell lines, compared with normal adjacent tissues and epithelial cell lines. Our data on miR-1193 mimic and antagomir transfection showed that miR-1193 had a suppressive effect on the proliferation and invasion of breast cancer cells.

Cytokines interact with cell surface receptors and initiate signaling cascades that promote cell growth and division while inhibiting the pathways of apoptotic cell death. IGFs, including IGF-1 and IGF2, are protein cytokines with high sequence similarity to insulin[17]. They form a complex system that cells use to communicate with their physiologic environment. The complex system consists of IGFs, two cell-surface receptors (IGF1R and IGF2R), and a family of six high-affinity IGF-binding proteins (IGFBP-1 to IGFBP-6) and other enzymes[18,19]. IGF2BP2 was originally isolated from a hepatocellular carcinoma patient, in which overexpression could induce a steatotic phenotype in mouse liver[20]. Recently, it was reported that the interaction of IGF2BP2 and IGF2 was propitious to the tumor-promoting nature of IGF2[21,22]. IGF2BP2 has been regarded as a novel potential target for carcinogenesis regulation[21]. miR-1275 was shown to target IGF2BP1, IGF2BP2, and IGF2BP3, and hinder tumor growth in hepatocellular carcinoma[23]. Another example is that miR-126 negatively regulates endothelial recruitment and metastasis in breast cancer cells by targeting IGF2BP2[24]. In this study, we characterized miR-1193 as a targeting regulator of the IGF2BP2 gene by bioinformatic and luciferase reporter assays. Our findings suggest that miR-1193 may be regarded as a potential target for breast cancer treatment.

The MEK/ERK and PI3K/Akt signaling pathways could be activated by a variety of cytokines that function to potentiate carcinogenesis. These cytokines include interleukin, colony-stimulating factor, tumor-transforming

factors, and IGFs[4,5]. Our data on miR-1193 mimic and antagomir transfection indicated that miR-1193 negatively regulated the expression of IGF2BP2 and the downstream pathways ERK and PI3K/Akt. Moreover, the negative effect could be rescued by the pcDNA-IGF2BP2.

In conclusion, miR-1193 was found to be downregulated in breast cancer tissues and cell lines and played a negative role in the proliferation and invasion of breast cancer cells by directly targeting IGF2BP2.

ACKNOWLEDGMENT: This research was funded by the Scientific and Technological Projects in Henan Province (Grant Nos. 102102310419, 112102310090, and 162102310080).

REFERENCES

1. Donepudi MS, Kondapalli K, Amos SJ, Venkanteshan P. Breast cancer statistics and markers. J Cancer Res Ther. 2014;10:506–11.
2. Dubey AK, Gupta U, Jain S. Breast cancer statistics and prediction methodology: A systematic review and analysis. Asian Pac J Cancer P. 2015;16:4237–45.
3. Ward EM, Desantis CE, Lin CC, Kramer JL, Jemal A, Kohler B, Brawley OW, Gansler T. Cancer statistics: Breast cancer in situ. CA Cancer J Clin. 2015;65:481–95.
4. Mccubrey JA, Steelman LS, Abrams SL, Lee JT, Chang F, Bertrand FE, Navolanic PM, Terrian DM, Franklin RA, DAssoro AB. Roles of the RAF/MEK/ERK and PI3K/PTEN/AKT pathways in malignant transformation and drug resistance. Adv Enzyme Regul. 2006;46:249–79.
5. Steelman LS, Pohnert SC, Shelton JG, Franklin RA, Bertrand FE, Mccubrey JA. JAK/STAT, Raf/MEK/ERK, PI3K/Akt and BCR-ABL in cell cycle progression and leukemogenesis. Leukemia 2004;18:189–218.
6. Saxena NK, Sharma D, Ding X, Lin S, Marra F, Merlin D, Anania FA. Concomitant activation of the JAK/STAT, PI3K/AKT, and ERK signaling is involved in leptin-mediated promotion of invasion and migration of hepatocellular carcinoma cells. Cancer Res. 2007;67:2497–507.
7. Steelman LS, Chappell WH, Abrams SL, Kempf CR, Long J, Laidler P, Mijatovic S, Maksimovicivanic D, Stivala F, Mazzarino MC. Roles of the Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways in controlling growth and sensitivity to therapy-implications for cancer and aging. Aging (Albany NY) 2011;3:192–22.
8. Saini KS, Loi S, Azambuja ED, Metzger-Filho O, Saini ML, Ignatiadis M, Dancey JE, Piccart-Gebhart MJ. Targeting the PI3K/AKT/mTOR and Raf/MEK/ERK pathways in the treatment of breast cancer. Cancer Treat Rev. 2013;39:935–46.
9. Shimizu T, Tolcher AW, Papadopoulos KP, Beeram M, Rasco DW, Smith LS, Gunn S, Smetzer L, Mays TA, Kaiser B. The clinical effect of the dual-targeting strategy involving PI3K/AKT/mTOR and RAS/MEK/ERK pathways in patients with advanced cancer. Clin Cancer Res. 2012;18:2316–25.
10. Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M. MicroRNA gene expression deregulation in human breast cancer. Cancer Res. 2005;65:7065–70.
11. Mattie MD, Benz CC, Bowers J, Sensinger K, Wong L, Scott GK, Fedele V, Ginzinger D, Getts R, Haqq C. Optimized high-throughput microRNA expression profiling provides novel biomarker assessment of clinical prostate and breast cancer biopsies. Mol Cancer 2006;5:24.
12. Yan LX, Huang XF, Shao Q, Huang MY, Deng L, Wu QL, Zeng YX, Shao JY. MicroRNA miR-21 overexpression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. RNA 2008;14:2348–60.
13. Banerjee N, Talcott S, Safe S, Mertens-Talcott SU. Cytotoxicity of pomegranate polyphenolics in breast cancer cells in vitro and vivo: Potential role of miRNA-27a and miRNA-155 in cell survival and inflammation. Breast Cancer Res Treat. 2012;136:21–34.
14. Liu LZ, Li C, Chen Q, Jing Y, Carpenter R, Jiang Y, Kung HF, Lai L, Jiang BH. miR-21 induced angiogenesis through AKT and ERK activation and HIF-1α expression. PLoS One 2011;6:e19139.
15. Wang W, Ren F, Wu Q, Jiang D, Li H, Shi H. MicroRNA-497 suppresses angiogenesis by targeting vascular endothelial growth factor A through the PI3K/AKT and MAPK/ERK pathways in ovarian cancer. Oncol Rep. 2014;32:2127–33.
16. Stark MS, Tyagi S, Nancarrow DJ, Boyle GM, Cook AL, Whiteman DC, Parsons PG, Schmidt C, Sturm RA, Hayward NK. Characterization of the melanoma miRNAome by deep sequencing. PLoS One 2010;5:9685.
17. Djiogue S, Kamdje AHN, Vecchio L, Kipanyula MJ, Farahna M, Aldebasi Y, Etet PFS. Insulin resistance and cancer: The role of insulin and IGFs. Endocr Relat Cancer 2013;20:R1–17.
18. Matsumoto T, Gargosky SE, Iwasaki K, Rosenfeld RG. Identification and characterization of insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs), and IGFBP proteases in human synovial fluid. J Clin Endocr Metab. 1996;81:150–55.
19. Gargosky SE, Wilson KF, Fielder PJ, Vaccarello MA, Guevara-Aguirre J, Diamond FB, Baxter RC, Rosenbloom AL, Rosenfeld RG. The composition and distribution of insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBPs) in the serum of growth hormone receptor-deficient patients: Effects of IGF-I therapy on IGFBP-3. J Clin Endocr Metab. 1993;77:1683–89.
20. Zhang JY, EK LC, Peng XX, Eng M, Tan EM. A novel cytoplasmic protein with RNA-binding motifs is an autoantigen in human hepatocellular carcinoma. J Exp Med. 1999;189:1101–10.
21. Ye S, Song W, Xu X, Zhao X, Yang L. IGF2BP2 promotes colorectal cancer cell proliferation and survival through interfering with RAF-1 degradation by miR-195. FEBS Lett. 2016;590:1641–50.
22. Brouwer-Visser J, Huang GS. IGF2 signaling and regulation in cancer. Cytokine Growth Factor Rev. 2015;26:371–7.
23. Fawzy IO, Hamza MT, Hosny KA, Esmat G, El Tayebi HM, Abdelaziz AI. miR-1275: A single microRNA that targets the three IGF2-mRNA-binding proteins hindering tumor growth in hepatocellular carcinoma. FEBS Lett. 2015;589:2257–65.
24. Png KJ, Halberg N, Yoshida M, Tavazoie SF. A microRNA regulon that mediates endothelial recruitment and metastasis by cancer cells. Nature 2012;481:190–4.