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Research Article

Downregulation of miR-142-3p Contributes to Breast Cancer Oncogenesis

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ABSTRACT

Breast cancer is one of the commonly-encountered malignant tumors, and its morbidity is on the rise year by year. A better understanding of the molecular mechanisms of breast cancer is important. This can improve the prevention, diagnosis, and treatment. The miR-142-3p has been shown to inhibit carcinogenesis by regulating various cellular processes, including cell cycle progression, cell migration, apoptosis, and invasion. We surgically recruited 96 breast cancer patients and the breast cancer samples, each with matched adjacent normal breast tissue were obtained, MDA-MB-231 and MCF-7 cells were cultured for experiments in vitro and measured the expression of miR-142-3p via quantitative real-time polymerase chain reaction (qRT-PCR). Cell viability, apoptosis, migration, and invasion were determined by MTT, flow cytometry, and trans-well assays. The expression of miR-142-3p was down-regulated in both cancer cell lines and cancer specimens. The reduced expression of miR-142-3p was associated with tumor size, lymph node metastasis, and tumor-node-metastasis (TNM) stage of patients ($p < 0.05$). However, there was no significant correlation between miR-142-3p expression in tumor tissues with age at diagnosis ($p > 0.05$). Overexpression of miR-142-3p repressed cell viability, migration, and invasion, while it induced apoptosis, whereas its depletion promoted it. The results suggest miR-142-3p acts as a tumor suppressor and can act as a novel target for the treatment of breast cancer. Thus, restoration of miR-142-3p expression, for example, via miRNA replacement therapy, may represent an effective strategy for the treatment of breast cancer patients.

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Introduction

Breast cancer is one of the commonly-encountered malignant tumors, and its morbidity is on the rise year by year [1, 2]. Based on the statistics, breast cancer mortality in China has increased over the last thirty years [3, 4]. Furthermore, breast cancer has become one of the most common malignant tumors in women and is therefore one of the most significant

threats to a woman's health [3-5]. Although mastectomy plus radiotherapy and lymphadenectomy significantly reduced the non-cancer mortality of primary and secondary breast cancer, the prognosis and survival rate of advanced metastatic breast cancer are still worthy of attention. Early detection, diagnosis, and treatment plays an important role in the prognosis of breast cancer [6]. As a result, numerous studies worldwide have sought to determine the most effective ways to early

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diagnosis, treat breast cancer, assess the therapeutic effects, correctly evaluate prognosis, and identify postoperative recurrence in patients. There is a great development in exploring therapeutic targets for breast cancer [7]. However, effective strategies remain limited in breast cancer treatment. Therefore, novel targets are needed to be elucidated for understanding the mechanism of breast cancer development and improving the therapeutic effect.

Noncoding RNAs, including long noncoding RNAs (lncRNAs) and microRNAs (miRNAs), could serve as important targets for therapeutic and diagnosis of diseases [8]. miRNA is evolutionarily conserved with 20-24 nucleotide non-coding RNAs and no protein-coding potential play vital roles in cancer development. The essential roles miRNA in controlling gene expression are consistent with the estimates showing that approximately more than one-third of the human genome is conserved as miRNA targets [9]. miRNAs can act as a dual role in oncogenes or tumor suppressors in different types of cancer including breast, gastrointestinal, lung hepatic, and pancreatic cancers [10-12]. Hsa-miR-142-3p, as a member of miRNAs, is involved in the regulation of various physiological activities such as tumorigenesis, cell differentiation, and hematopoiesis. miR-142-3p overexpression decrease cell proliferation and induced apoptosis in non-small-cell lung carcinoma [13].

The studies have been exposed that miR-142-3p is downregulated in diverse types of cancer and suggesting that it acts as a tumor suppressor [14-15]. Emerging evidence suggested that multiple miRNAs are dysregulated in breast cancer [16-19]. Several reports described that miRNAs could mediate breast cancer progression by regulating proliferation, apoptosis, migration, and invasion. But the exact role and mechanism of miRNAs in breast cancer remain dismal. Aberrantly expressed miRNAs have been reported to play essential roles in angiogenesis and progression of breast cancer [20]. Moreover, increasing efforts suggested that miRNAs are implicated in the prognosis and progression of breast cancer [21, 22]. In this research, we focused on the function of miR-142-3p on breast cancer development both clinical tissue and *in vitro*, aimed to explore the role of miR-142-3p in development, progress and prognosis. Our research revealed an important tumor-suppressive role of miR-142-3p in breast cancer growth and downregulation of miR-142-3p contributes to breast cancer oncogenesis.

Material and Methods

I Human Tissue

This investigation was approved by the Ethics Committee of the Rizhao People's Hospital. The methods used were performed in accordance with relevant guidelines and regulations. Ninety-six patients' pairs of primary breast cancers tissues and adjacent normal breast tissues were obtained from patients in Affiliated Rizhao People's Hospital of Jining Medical University from January 2016 to December 2018. The age of the 96 patients diagnosed breast carcinomas ranged from 28 to 74 years, mean 46.8 years. All the patients received excision surgery at our hospital. The clinical features of the patients were displayed in Table 1. All tissues obtained by surgery were stored at -80°C.

II Cell Culture

Human breast cancer cell lines (MDA-MB-231 and MCF-7) and normal human breast epithelial cell line MCF-10A were obtained from BeNa Culture Collection (Beijing, China). The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA) containing 10% fetal bovine serum (FBS) in a 95% humidified atmosphere containing with 5% CO₂ at 37°C.

III Cell Transfection

The miR-142-3p mimic (miR-142-3p), mimic negative control (miR-NC), miR-142-3p inhibitor (anti-miR-142-3p), and inhibitor negative control (anti-NC) were generated by GenePharm (Shanghai, China). These oligonucleotides with a final concentration of 30 nM were transfected into MDA-MB-231 and MCF-7 cells with Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) for 24 h.

IV MTT

After indicated transfection, MDA-MB-231 and MCF-7 cells (5 x 10³/well) were seeded into 96-well plates in triplicates. After culture for 0, 24, 48 or 72 h, 10 µl MTT solution (Beyotime, Shanghai, China) was added to each well and the plates were maintained at 37°C for 4 h. Then, 100 µl dimethyl sulfoxide (DMSO) was added to each well to solubilize the formed formazan. Cell viability was determined by detecting the absorbance at 490 nm with a microplate reader (Bio-Rad, Hercules, CA, USA).

V Transwell Assay

The transwell chambers with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) were used for invasion assay, and those without Matrigel were used for migration assay. Transfected MDA-MB-231 and MCF-7 cells (1 x 10⁵/ml) were resuspended in serum-free Dulbecco's Modified Eagle's Medium (DMEM), and 200 µl cell suspension was added in the upper chambers. The lower chambers were filled with 500 µl Dulbecco's Modified Eagle's Medium (DMEM) medium with 10% fetal bovine serum (FBS). After the incubation for 12 h, the non-traversed cells were wiped away, and traversed cells were stained with 0.1% crystal violet. The number of migrated or invasive cells was counted with three random fields using a 200x magnification microscope.

VI Flow Cytometry

Flow cytometry was used to determine cell apoptosis with an Annexin V-FITC Apoptosis Detection Kit (Beyotime, Shanghai, China). Transfected MDA-MB-231 and MCF-7 cells were cultured for 72 h and then were resuspended in a binding buffer. Subsequently, cells were stained with Annexin V-FITC and PI for 10 min in the dark, and the cell apoptosis was analyzed with a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA).

VII qRT-PCR

TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) was used for RNA isolation, and the concentration of total RNAs was

measured by Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). 500 ng RNA was reversely transcribed to cDNA using All-in-One miRNA or mRNA First-Strand cDNA Synthesis Kit (Fulengen, Guangzhou, China) or Prime-Script RT reagent kit (TaKaRa, Dalian, China) following the manufacturer's instructions. The qRT-PCR was performed using SYBR mix (Fulengen, Guangzhou, China) on CFX96 Real-time PCR Systems (Bio-Rad, Hercules, CA, USA). The relative expression levels of miR-142-3p was normalized to GAPDH or U6, respectively, and calculated by the $2^{-\Delta\Delta Ct}$ method.

VIII Statistical Analyses

The researches were repeated three times and data were presented as mean \pm standard deviation (S.D). The statistical analysis was performed using GraphPad Prism 7 software (GraphPad Inc., La Jolla, CA, USA) and Student's *t* test or one-way ANOVA followed by Tukey's test was used to compare the difference between the different groups. The association between clinicopathologic features of breast cancer patients and level of miR-142-3p was assessed by chi-squared (χ^2) test. Statistical significance was defined as $p < 0.05$.

Results

I miR-142-3p Expression Down-Regulated in Breast Cancer Tissues

To determine the expression of miR-142-3p in breast cancer, we surgically collected 96 breast cancer samples, each with matched adjacent normal breast tissue, and measured the expression of miR-142-3p via quantitative real-time polymerase chain reaction (qRT-PCR). Our results showed that miR-142-3p expression was aberrantly decreased in breast cancer tissues. Compared with the control group, the difference of miR-142-3p expression was statistically significant (Figure 1A).

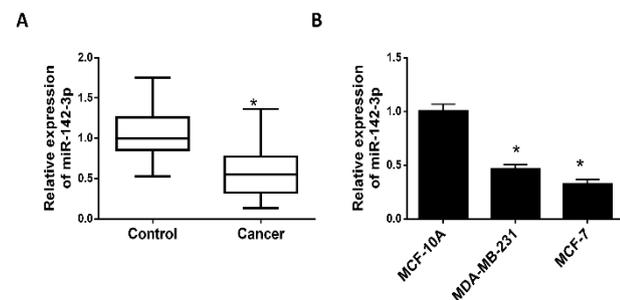


Figure 1: The expression of miR-142-3p is decreased in breast cancer tissue and cancer cell. qRT-PCR was performed to detect the expression of miR-142-3p in cancer tissues and normal samples(A). * $p < 0.05$ compared with control. Level of miR-142-3p was measured in cancer cells (MDA-MB-231 and MCF-7) cells and control cells (MCF-10A) by qRT-PCR(B). * $p < 0.05$ compared with MCF-10A group.

II Relationship between miR-142-3p Expression and Clinicopathological Factors

The patients with breast cancer were divided into high ($n=38$) or low ($n=58$) miR-142-3p expression group. Table I summarized that reduced

expression of miR-142-3p was associated with tumor size, lymph node metastasis, and tumor-node-metastasis (TNM) stage of patients ($p < 0.05$). However, there was no significant correlation between miR-142-3p expression in tumor tissues with age at diagnosis ($p > 0.05$) (Table 1). These findings suggested that miR-142-3p downregulated expression indicated the poor outcome of breast cancer patients.

Table 1: Correlations between miR-142-3p expression and clinical characteristics in breast cancer patients.

Clinical parameters	High	Low	<i>p</i> -value
Age			>0.05
< 50 years	15	24	
≥ 50 years	23	34	
Tumor size			<0.05
≤ 2	21	10	
> 2	17	48	
Lymph node metastasis			<0.05
Yes	21	40	
No	17	18	
TNM stage			<0.05
I-II	17	8	
III-IV	21	50	

III Expression of miR-142-3p in Breast Cancer Cell

The level of miR-142-3p was measured in breast cancer cells, and results displayed similar to our above observations in patient samples, we achieved that the the expression of miR-142-3p was remarkably down-regulated in the MDA-MB-231 and MCF-7 cells line compared with that in normal breast cell line MCF-10A (Figure 1B).

IV Overexpression of miR-142-3p Suppresses Progression of Breast Cancer Cells

The role of miR-142-3p was investigated in breast cancer cells by overexpressing its abundance, which was confirmed by in (Figure 2A). The overexpression of miR-142-3p led to significant reduction of cell viability in MDA-MB-231 and MCF-7 cells line at 72 h (Figure 2B). In addition, the apoptosis of MDA-MB-231 and MCF-7 cells line was notably induced by the addition of miR-142-3p (Figure 2C). Moreover, as shown in Figure 2D and 2E, the up-regulation of miR-142-3p in MDA-MB-231 and MCF-7 cells significantly repressed cell migration and invasion. The abundance of miR-142-3p in the MDA-MB-231 and MCF-7 cells was remarkably decreased by the transfection of anti-miR-142-3p, rescue experiments demonstrated that the deficiency of miR-142-3p promoted the breast cancer progression, revealed by the increase of viability, migration, and invasion, as well as reduction of apoptosis.

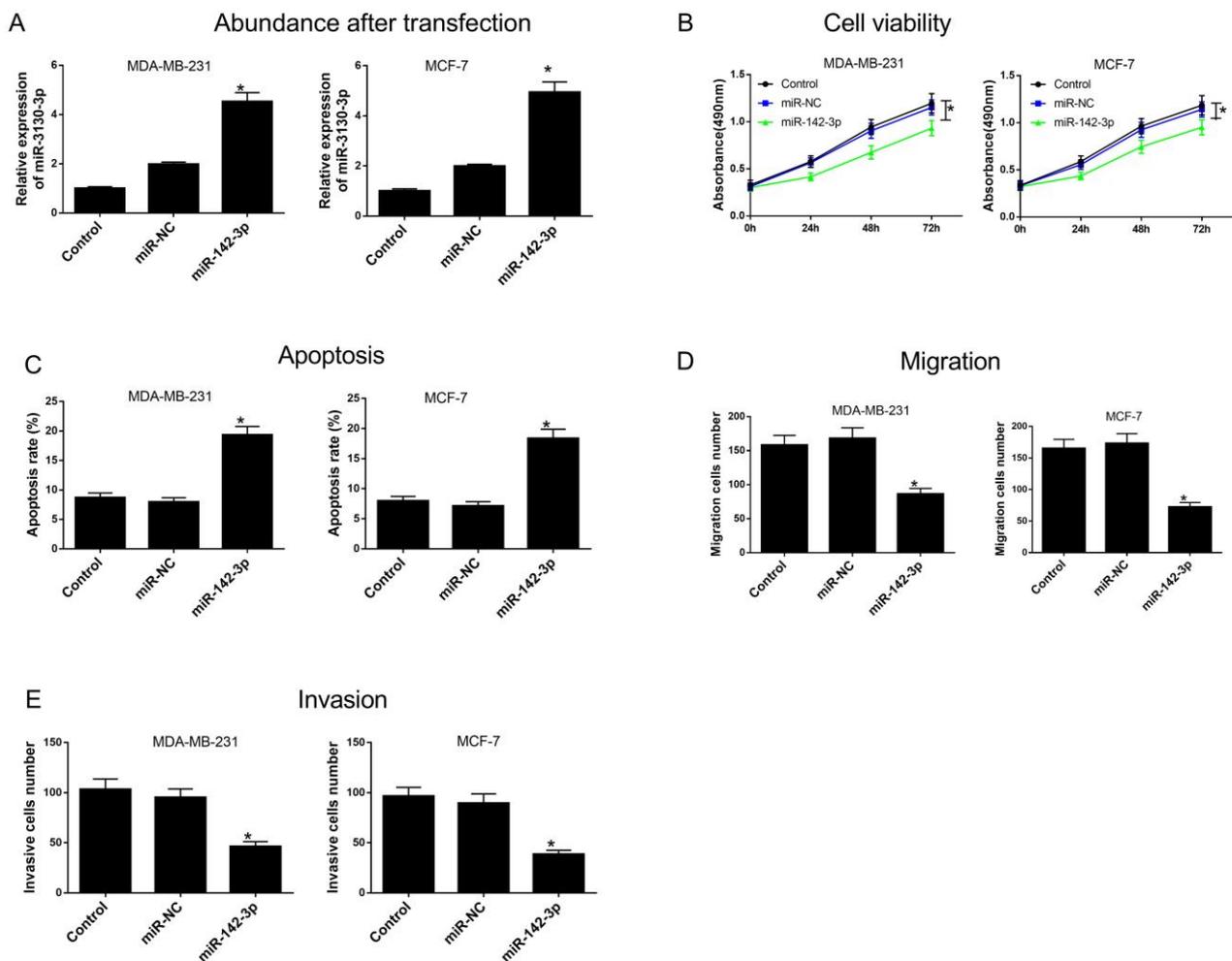


Figure 2: Overexpression of miR-142-3p suppresses progression of breast cells. (A), Abundance of miR-142-3p in MDA-MB-231 and MCF-7 cells was detected after transfection of miR-142-3p or miR-NC. Cell viability (B), apoptosis (C), migration (D) and invasion (E) of MDA-MB-231 and MCF-7 cells transfected with miR-142-3p or miR-NC were analyzed by MTT, flow cytometry and trans-well assays, respectively. Control is the non-transfected group. * $p < 0.05$ compared with miR-NC group.

Discussion

Breast cancer is the most common form of cancer in women and the second leading cause of death due to malignant disease in females after lung cancer [23]. In recent years, there has been an upward trend in the incidence of breast cancer in china, and it tends to occur at a younger age [4]. Furthermore, breast cancer has become one of the most significant threats to a woman's health. Early detection, diagnosis, and treatment plays an important role in the prognosis of breast cancer. As a result, numerous studies worldwide have sought to determine the most effective ways to early diagnosis, treat breast cancer, assess the therapeutic effects, correctly evaluate prognosis, and identify postoperative recurrence in patients. MicroRNAs (miRNAs) contribute to control of cell cycle progression and are frequently deregulated in cancer. miRNA is evolutionarily conserved with 20-24 nucleotide non-coding RNAs and no protein-coding potential play vital roles in cancer development. They act on their specific gene targets by translational repression or mRNA cleavage after assembling themselves into an RNA-induced silencing complex (RISC) [24, 25]. They exert their functions by binding to

3'UTR of target mRNAs, and thus modulate their cellular abundance or expression [26]. shsa-miR-142-3p, as a member of miRNAs, is involved in the regulation of various physiological activities such as tumorigenesis, cell differentiation, and hematopoiesis. The focus of this study was to determine effects of miR-142-3p on the cell cycle progression and cancer cell proliferation. Here we found that miR-142-3p was down-regulated in both cancer cell lines and cancer specimens. In addition, we confirmed its overexpression suppressed proliferation, whereas its depletion promoted it. The essential roles miRNA in controlling gene expression are consistent with the estimates showing that approximately more than one-third of the human genome is conserved as miRNA targets. Specific miRNAs can act as a double-edged sword in controlling human cancers by acting either as oncogenes or tumor suppressors in different types of cancer including breast, gastrointestinal, lung hepatic, and pancreatic cancers [10-12]. Our previous studies have revealed the important roles of miR-3130-3p in controlling breast cancer, and that miR-142-3p was down-regulated in breast cancer tissues and cells. By inhibiting cell viability, migration, and invasion and promoting apoptosis, miR-3130-3p might be a therapeutic

target for breast cancer treatment [27]. Furthermore, rescue experiments uncovered that LINC01096 suppressed breast cancer progression by sponging miR-3130-3p [27]. This realization helps explain why much effort is being committed to characterizing miRNA expression profiles because such insight has pinpointed changes reflective of differences in cancer stages, prognosis, and assessment of treatment outcome. Our study shown association between changes in miR-142-3p expression level and tumor size and TNM stage has made it possible to use it as a marker of tumorigenesis. Both in vitro and in vivo experiments have revealed that miR-142-3p upregulation can effectively inhibit the proliferation or migration of breast, cervical, and hepatocellular carcinoma cells [28]. In conclusion, we demonstrate that miR-142-3p decreased in breast cancer and show that miR-142-3p can as a tumor suppressor in breast cancer cells. miRNA replacement therapy is used increasingly more often in clinical trials. Thus, our research here suggests that, via miRNA replacement therapy, replacement of miR-142-3p could be an effective and novel strategy for the treatment breast cancer patients in the future.

Conflicts of Interest

The authors declare no conflict of interest.

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