Research Article

MiR-942 regulates the function of breast cancer cell by targeting FOXA2

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MicroRNA (MiR)-942 regulates the development of a variety of tumors, however, its function in breast cancer (BCa) has been less reported. Therefore, the present study investigated the regulatory effects of miR-942 on BCa cells. The expression of miR-942 in whole blood samples and BCa cell lines was detected by quantitative real-time (qRT)-PCR. Direct target gene for miR-942 was confirmed by dual-luciferase reporter assay. FOXA2 expression in adjacent tissues was detected by qRT-PCR. The effects of miR-942, or miR-942 with FOXA2, on the cell viability, proliferation, apoptosis, migration and invasion of BCa cells were determined by cell counting kit-8 (CCK-8), colony formation assay, flow cytometry, wound scratch and Transwell, respectively. The levels of N-Cadherin, E-Cadherin and Snail were determined by Western blot. Kaplan–Meier was used to explore the relationship among the expressions of miR-942 and FOXA2 and the prognosis of BCa patients. MiR-942 had high expressed in BCa, while its low expression significantly suppressed the cell viability, proliferation, migration and invasion of BCa, but increased cell apoptosis. Down-regulation of N-Cadherin and Snail and up-regulation of E-Cadherin were also induced by low-expression of miR-942. FOXA2, which was proved as the direct target gene for miR-942 and was low-expressed in BCa, partially reversed the effect of overexpressed miR-942 on promoting cell viability, proliferation, migration and invasion, and suppressed cell apoptosis. A lower survival rate was observed in BCa patients with a high expression of miR-942 and a low expression of FOXA2. MiR-942 promoted the progression of BCa by down-regulating the expression of FOXA2.

Introduction

As one of the most frequent malignant tumors for women, breast cancer (BCa) seriously affects women’s health [1]. BCa has a high incidence rate, with approximately one million new cases being diagnosed annually [2]. Although medical advancement has improved the 5-year survival rate of patients with BCa through surgical treatment, the long-term survival rate and prognosis of patients with BCA are still unsatisfactory [3]. The occurrence of BCa is a complex molecular biological process, in which multiple genes and factors are involved [4]. Analyses on gene expression profile of BCa showed that different gene expressions are correlated with various biological behaviors of BCa [5–7]. Therefore, conducting an in-depth study on the molecular mechanisms of the occurrence and development of BCa is significantly important in improving the cure rate and the prognosis of BCa.

MicroRNA (MiRNA) is an endogenous, non-coding RNA molecule [8], and mature expression of MiRNA is time-ordered, highly conserved and tissue-specific [9]. MiRNAs are involved in the regulation of various life activities such as cell proliferation, cell cycle, angiogenesis and metabolism [10]. In addition, studies showed that abnormal expressions of miRNAs play an important role in tumor growth, invasion and apoptosis [11]. MiR-942 is located on chromosome 1p13.1, and its role has been previously reported in esophageal squamous cell carcinoma, colorectal cancer and ovarian cancer [12–14], however,
the effect of miR-942 has been less reported on BCa. Thus, the present study explored the role of miR-942 in the biological characteristics of BCa cells by up-regulating and down-regulating the expressions of miR-942 in BCa cells.

TargetScan7.2 software predicted that there were multiple binding sites of miR-942 in the 3’ UTR region of FOXA2 gene. As FOXA2 is an important gene associated with tumor growth and is often low-expressed in multiple tumor specimens [15,16], the current study further investigated the relationship between miR-942 and FOXA2 to reveal the role of miR-942 in the development of BCa cells.

Materials and methods

Clinical specimens

Whole blood samples were obtained from 62 participants (31 BCa patients and 31 healthy subjects) who received treatment or examination from May 2017 to January 2019 in Baoding No.1 Central Hospital (HBH20170425). Anticoagulant blood specimens were stored in a cryogenic refrigerator (3695576, Shanghai Weiwu Cryogenic Vacuum Equipment Co., Ltd., https://b2b.hc360.com/supplyself/669456707.html, Shanghai, China) at −20°C. The BCa tissue and adjacent tissue samples were obtained from six BCa patients who received treatment or examination from May 2017 to January 2019 in Baoding No.1 Central Hospital. The tissue samples were kept in liquid nitrogen and maintained at −80°C. Written informed consents were signed by all subjects and the study was approved by the Ethics Committees of the hospital.

Cell culture

Human normal breast epithelial cell lines (MCF-10A) and BCa cell lines (SKBR3, MCF-7, BT-549, MDA-MB-231 and MDA-MB-468) were purchased from American Type Culture Collection (Manassas, U.S.A.). The cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% fetal bovine serum (FBS, Gibco, U.S.A.) at 37°C in 5% CO2.

Transfection

As miR-942 is lowly expressed in MCF-7 cells, but highly expressed in MDA-MB-468, MCF-7 and MDA-MB-468 cells were selected to be used in subsequent experiments. The cells were digested, thoroughly mixed and seeded at 1 × 10^6/ml into the six-well plate and then evenly distributed in an orifice plate. The next day, 20 pmol miR-942 mimic, mimic control (MC), miR-942 inhibitor, inhibitor control (IC), FOXA2, siFOXA2, negative control (NC), siNC, IC+siNC, IC+siFOXA2, inhibitor+siNC, inhibitor+siFOXA2, MC+NC, MC+FOXA2, mimic+NC and mimic+FOXA2 (Shanghai GenePharma Co., Ltd., China) were respectively dissolved in 50 μl Dulbecco’s modified Eagle’s medium (DMEM, HyClone, U.S.A.) and mixed as the transfected group A. One microliter of Lipofectamine 2000 (Invitrogen, U.S.A.) was dissolved in 50 μl DMEM, set aside for 5 min at room temperature and then mixed with the transfected group A as the transfection group B. Next, the transfection group B was added into the corresponding hole of the six-well plate and maintained in a culture box at 37°C with 5% CO2 for further culture. The culture medium was changed 24 h after the transfection, and the cells were collected 72 h after the culture. MiR-942 mimic (5’-UCUUCUCUGUUUGCCAUUG-3’) and miR-942 MC (5’-UUCUCGAACGUGUCAGUTT-3’) were purchased from Shanghai GenePharma Company (Shanghai, China).

Bioinformatics analysis

The data of 1085 cancer and 104 normal samples cases with miR-942-3p expression in BRCA were downloaded and analyzed from the StarBase (http://starbase.sysu.edu.cn/).

Luciferase activity assay

For dual-luciferase reporter assay, the 3’ UTR of FOXA2 containing miR-942 binding sites were inserted into a pmirGLO dual luciferase vector (Promega, U.S.A.) to generate wild-type (WT) pmirGLO-FOXA2 3’ UTR. The mutant (MUT) 3’ UTR of FOXA2 in miR-942 binding site was synthesized using a Site-Directed Mutagenesis Kit (Thermo Fisher Scientific, U.S.A.) and inserted into a pmirGLO dual-luciferase vector to generate MUT pmirGLO-FOXA2 3’ UTR. The pmirGLO vector containing WT or MT FOXA2 3’ UTR was respectively co-transfected with miR-942 mimic into MCF-7 cells, while the pmirGLO vector containing WT or MT FOXA2 3’ UTR was co-transfected with miR-942 inhibitor into MDA-MB-468 cells by Lipofectamine2000 (Invitrogen, U.S.A.). After incubation for 48 h, the relative luciferase activities in the cells were measured by Dual-Luciferase Reporter Assay protocol (Promega, Madison, WI).
Wound scratch
The transfected cells were incubated in a six-well plate at $5 \times 10^5$ per well. After the cells adhered to the cell wall for 24 h, an uniform scratch was quickly drawn on the cells. After washing the suspended cells, the cells were cultured in 1% serum. Then, cell migrations at 0 and 48 h at the same location were recorded by taking photos and the migration distances were measured by ImageJ software version 1.8.0. Relative migration rate (%) = (0 h scratch width − 48 h scratch width)/0 h scratch width × 100%.

Transwell
After transfection for 48 h, the cells were suspended in the medium without FBS until the cell density reached $1 \times 10^6$/ml. Next, the cell suspension was added to the upper compartment of Transwell (Corning, U.S.A.), while medium containing 10% FBS serum was added to the lower compartment. After culturing the cell suspension at 37°C for 24 h, the cells at the bottom of the upper chamber were stained by 0.5% Crystal Violet, while those at the upper chamber side were removed by cotton swab. Invasion cells were counted at ×200 magnification.

Cell viability detection
The cells were seeded into 96-well plate, and 10 μl cell counting kit-8 (CCK-8) (CK04, Japanese Tongren Chemical Company, https://china.guidechem.com/trade/pdetail8419763.html, Japan) was added to each well and incubated for 4 h. The absorbance at 450 nm was determined by an enzyme microscopy (Multiskan GO, Shanghai Bajiu Industrial Co., Ltd., http://www.89-china.com/, Shanghai, China). The experiment was conducted in triplicate, and the average value was calculated.

Colony formation assay
The cells at 100 cells/well were transfected and digested, counted and cultured in 12-well plates at 37°C for 3 weeks, and the culture medium was changed every 3 days to observe the formation of clones. The culture was terminated when the colonies filled 50–150 fields, and the medium was discarded. Next, the cells were rinsed twice in Dulbecco's Phosphate Buffered Saline (DPBS, D8662, Sigma–Aldrich, U.S.A.), and 1 ml methanol (34860, Sigma–Aldrich, U.S.A.) was added into each well to fix the cells for 15 min. One milliliter Giemsa (999D715, Thermo Fisher Scientific, U.S.A.) was added into each well and held for 30 min. Colony formation rate was calculated by colony formation rate = (number of colonies/number of seeded cells) × 100%. Each treatment was carried out in triplicate.

Cell apoptosis
After transfection for 48 h, the cells ($1 \times 10^6$/ml) were resuspended in a 1 × Annexin binding buffer (A21009-100 ml, Alpha Applied Bioscience, U.S.A.), 5 μl fluorescein isothiocyanate (FITC) Annexin V (KGA108-1, KeyGEN BioTECH, http://www.keygentec.com.cn/index.html, China), 1 μl 100 μg/ml Propidium Iodide (PI, C0080, Beijing Suolai Bao Technology Co., Ltd., http://www.solarbio.com/search.php, China) solution and 300 μl of 1 × Annexin Binding Buffer were added to the cell suspension for 15 min to stain the cells. Finally, the stained cells were analyzed by flow cytometry (version 10.0, FlowJo, FACS Calibur™, BD, Franklin Lakes, NJ, U.S.A.).

Western blotting
The cells were washed twice by cold PBS, and total proteins were lysed in RIPA buffer (Tianjin Yitailong Technology Co., Ltd., Tianjin, China). Next, the proteins were boiled for 5 min at 100°C for denaturation, isolated on 15% SDS/PAGE, and then moved to polyvinylidene fluoride (PVDF) membranes. The membranes were sealed with 5% milk at room temperature for 1 h and then incubated with specific antibodies (anti-N-cadherin (anti-N-cad, mouse, CST, 14215, 1:1000), anti-Snail (goat, abcam, ab53519, 1:1000), anti-glyceraldehyde-3-phosphate dehydrogenase (anti-GAPDH, mouse, CST, 14215, 1:1000), anti-E-cadherin (anti-E-cad, mouse, CST, 14472, 1:1000), anti-FOXA2 (mouse, abcam, ab60721, 1:1000), anti-N-cadherin (anti-E-cad, mouse, CST, 14472, 1:1000), anti-glyceraldehyde-3-phosphate dehydrogenase (anti-GAPDH, mouse, 1:2000, ab8245, Abcam, U.S.A.)) at 4°C overnight. The membranes were then respectively incubated with goat anti-mouse, goat anti-rabbit and goat anti-goat IgG (H+L) HRP–conjugated secondary antibody (Proteintech, U.S.A.) for 2 h and then washed with PBS for three times. The protein bands were detected by ECL Western blotting kit (93–K820-500, MULTI SCIENCES, Hangzhou, China) and scanned by super sensitive multifunctional imager (Image), version 4.7, National Institutes of Health, U.S.A.).

Quantitative real-time polymerase chain reaction
Total RNA was extracted using TRizol reagent (BY11909, Hefei Bomei Biotechnology Bo., Ltd., China), and Nanodrop (Thermo Scientific™, San Diego, CA, U.S.A.) was used to measure the concentration of RNA, which was then diluted...
Table 1 Primes used in real-time PCR analysis

| Gene   | Primer sequence | Species |
|--------|-----------------|---------|
| miR-942| Forward: 5′-GCATGGATCCGCTTTAACAATGGTTCCTCCG-3′  
Reverse: 5′-GCCGGTCTAGAAGCACCTTTTGTTTCTATTATCACG-3′. | Human |
| FOXA2  | Forward: 5′-CTGAGGCCCACCTGAAGCC-3′  
Reverse: 5′-GTAGCCGGGGTAGTGCATCA-3′. | Human |
| U6     | Forward: 5′-TGACTTCCAAGTACCATCGCCA-3′  
Reverse: 5′-TTGTAGAGGTAGGTGTGCAGCAT-3′. | Human |
| GAPDH  | Forward: 5′-GGTGAAAGGTGAGTGCTCAAGC-3′  
Reverse: 5′-CAGAAATCTGAGAATTATCT-3′ | Human |

to 500 ng/μl. Superscript II first-strand cDNA synthesis System (Invitrogen, U.S.A.) was used to determine reverse transcription. The mRNA expression levels were determined by quantitative real-time polymerase chain reaction (qRT-PCR) using SYBR Green Real Time PCR kit (204057, QIAGEN, https://www.qiagen.com/cn/products/, China). Next, 4 μl cDNA was mixed with 5 μl SYBR (code DRR041A, Takara, Japan) and 1 μl Primer. PCR cycle was conducted as follows: pretreatment at 94°C for 2 min, at 94°C for 30 s, at 63°C for 30 s, at 72°C for 1 min (35 cycles), finally, chain extension at 72°C for 7 min and kept at 4°C. Sequences of primers used were listed in Table 1. The expression levels of RT-PCR products were determined by the 2^−ΔΔC_T method [17].

Statistical analysis
Prism 6 (version 6.01, GraphPad Software, Inc., San Diego, CA, U.S.A.) was used for data analysis. Results were shown as mean ± standard deviation (SD) of at least three independent experiments, and t test was used to compare the differences in the mean between the continuous variables. Differences between multiple groups were analyzed by one-way analysis of variance (ANOVA), followed by Bonferroni post hoc. Kaplan–Meier was used to analyze the relationship between miR-942 and overall survival rates of BCa patients and the relationship between FOXA2 and survival in BCa patients without distant metastasis. The correlation between the miR-942 and FOXA2 mRNA expression was analyzed by Pearson. P < 0.05 was considered as statistically significant.

Results
The expression of miR-942 in BCa cell lines and its effect on the cell viability of BCa cells
The result demonstrated that the expression level of miR-942 in BCa patients was significantly higher than that in normal people (P < 0.001, Figure 1A). Moreover, Kaplan–Meier analysis showed that the survival rate of BCa patients with a high expression of miR-942 was lower than that of patients with a low expression of miR-942 (P < 0.05, Figure 1B). According to the results from database, miR-942-3p had high expression in cancer than that in normal tissues (P < 0.001, Figure 1C). The expression levels of miR-942 in BCa cell lines (SKBR3, MCF-7, BT-549, MDA-MB-231 and MDA-MB-468) were significantly higher than that in normal BCa cells (MCF-10A) (P < 0.001, Figure 1D). Our result also showed that the expression level of miR-942 was greatly up-regulated in MCF-7 cells transfected mimic, while the expression level of miR-942 in MDA-MB-468 cells transfected with the miR-942 inhibitor was significantly inhibited (P < 0.001, Figure 1E,F). The cell viability could be significantly promoted by overexpressing miR-942 and greatly inhibited by inhibiting the expression of miR-942 (P < 0.001, Figure 1G,H). Colony formation assay showed that the overexpression of miR-942 promoted the cell proliferation (P < 0.001, Figure 1I,J), which, however, could be inhibited by the low expression of miR-942 (P < 0.001, Figure 1K,L).

The effects of miR-942 on the apoptosis and expressions of EMT-related proteins of BCa cells
Overexpressed miR-942 inhibited the apoptosis of MCF-7 cells, while inhibiting the expression of miR-942 promoted the apoptosis of MDA-MB-468 cells (P < 0.001, Figure 2A–D). Moreover, the overexpression of miR-942 was also found to promote the expressions of N-Cad and Snail and inhibit the expression of E-Cad, while the inhibition of miR-942 expression of the above proteins produced the opposite results (P < 0.001, Figure 2E–H).
Figure 1. The expression of miR-942 in BCa cell lines and its effect on the cell viability of BCa cells

(A) QRT-PCR was performed to detect the expression of miR-942 in the whole blood of 31 BCa patients and 31 normal controls (n=3, **P<0.001, vs. normal). (B) Kaplan–Meier method was used to analyze the relationship between miR-942 and the overall survival rate of 60 BCa patients. (C) The expression of miR-942-3p with 1085 cancer and 104 normal samples in BRCA from database (P=0.001). (D) QRT-PCR was performed to detect the expressions of miR-942 in MCF-10A and BCa cell lines of normal breast epithelial cells (n=3, **P<0.001, vs. MCF-10A). (E,F) The transfection of miR-942 was detected by qRT-PCR (n=3, **P<0.001, vs Blank; ##P<0.001, vs MC or IC). (G,H) CCK-8 was used to detect cell viability (n=3, **P<0.001, vs Blank; ##P<0.001, vs MC or IC). (I-L) Colony formation assay was performed to detect the effect of miR-942 expression on cell proliferation (n=3, **P<0.001, vs Blank; ##P<0.001, vs MC or IC). U6 served as intrinsic parameters of experiment.
Figure 2. The effects of miR-942 on the apoptosis and expressions of EMT-related proteins of BCa cells

(A–D) Cell apoptosis was detected by flow cytometry (n=3, **P<0.001, vs Blank; #P<0.001, vs MC or IC).

(E–H) Western blotting was used to detect the expressions of N-Cad, E-Cad and Snail (n=3, **P<0.001, vs Blank; #P<0.001, vs MC or IC). Abbreviations: E-Cad, E-cadherin; N-Cad, N-cadherin.

The effects of miR-942 on the invasion and migration of BCa cells

Scratch experiments showed that the overexpression of miR-942 promoted cell migration, which, however, was found to be inhibited by the inhibition of miR-942 expression (P<0.001, Figure 3A–D). Transwell assay showed that overexpression of miR-942 promoted cell invasion, while inhibition of miR-942 expression inhibited cell invasion (P<0.001, Figure 3E–H).

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Figure 3. The effects of miR-942 on the invasion and migration of BCa cells
(A–D) The wound scratch assay was used to measure the ability of cells to migrate (n=3, **P<0.001, vs. Blank; #P<0.001, vs. MC or IC). (E–H) Transwell was used to detect the ability of BCa cells to invade (n=3, **P<0.001, vs. Blank; #P<0.001, vs. MC or IC).
FOXA2 directly targets miR-942

TargetScan 7.2 predicted that miR-942 binds to the 3′ UTR of FOXA2 (P < 0.001, Figure 4A). Moreover, we constructed two pmirGLO dual-luciferase reporter vectors, namely, FOXA2-3′-UTR and FOXA2-3′-UTR mut, and respectively transfected the two vectors with miR-942 mimic and inhibitor into the cells, the results showed that the FOXA2 was the target gene for miR-942, and that miR-942 inhibited the expression of FOXA2 (P < 0.001, Figure 4B,C).

The expression of FOXA2 in BCa tissue and the correlation between miR-942 and FOXA2

The expression of FOXA2 in BCa tissues was significantly lower than that in adjacent tissues (P < 0.001, Figure 4D,F–I). As shown in Figure 4E, miR-942 was in negative correlation with FOXA2 (P = 0.016, Figure 4E). In addition, the survival rate for patients who have a low FOXA2 expression and without distant metastasis was significantly lower than that those with a high FOXA2 expression (P < 0.001, Figure 4I).

MiR-942 affects the viability, proliferation and apoptosis of BCa cells by targeting FOXA2

After FOXA2 and mimic were co-transfected into MCF-7 cells, the results showed that the expression of FOXA2 was decreased significantly in mimic and FOXA2 co-transfection group than that in MC and FOXA2 co-transfection group. However, the expression of FOXA2 was increased greatly in MDA-MB-468 cells co-transfected with inhibitor and siFOXA2 (P < 0.001, Figure 5A,B). Moreover, the cells viability was significantly inhibited in mimic and FOXA2 co-transfection group in comparison with the overexpressed miR-942 group, but was greatly increased in inhibitor and siFOXA2 co-transfection groups (P < 0.001, Figure 5C,D). Colony formation assay demonstrated that FOXA2 partially reversed the effect of overexpressed miR-942 on promoting cell proliferation and the inhibitory effect of low-expressed miR-942 on cell proliferation (P < 0.001, Figure 6A–D). We also found that FOXA2 could reverse the inhibitory effect of overexpressed miR-942 on cell apoptosis and the promotive effect of low-expressed miR-942 on cell apoptosis (P < 0.001, Figure 6E–H).

The effects of miR-942 on the invasion and migration of BCa cells by targeting FOXA2

FOXA2 could reverse the effect of overexpressed miR-942 on promoting cell migration and the inhibitory effect of low-expressed miR-942 on cell migration (P < 0.001, Figure 7A–D). Moreover, FOXA2 was also observed to be able to reverse the promotive effect of overexpressed miR-942 on cell invasion and the inhibitory effect of low-expressed miR-942 on cell invasion (P < 0.001, Figure 7E–H).

Discussion

BCa patients are likely to develop tumor recurrence and metastasis after surgical treatment [18]. In recent years, miRNAs in BCa research have attracted increasing research attention [7,19], for example studies on the interaction between miRNA with related target genes and the regulative role of signaling pathways revealed the critical effects of miRNAs on tumors [20,21]. Thus, discovering clinical targets indicating the prognosis of BCa may contribute to the treatment of BCa.

MiR-942 has been found to regulate the development of esophageal squamous cell carcinoma, colorectal cancer and ovarian cancer [12–14]. The overexpression of miR-942 promotes the growth of esophageal squamous cell carcinoma tumors, with higher miR-942 indicating a higher incidence of esophageal squamous cell carcinoma [12]. Currently, the mechanism of miR-942 on BCa is still unclear, therefore, the present study aimed to investigate the role of miR-942 in BCa. We found that the expression of miR-942 in the whole blood samples derived from BCa patients was significantly higher than that from the normal patients, and that the miR-942 expression was significantly higher in BCa cell lines than that in the normal breast epithelial cells, which was consistent with the high expression observed in the esophageal squamous cell carcinoma [12]. In addition, it was also found that BCa patients with a low expression of miR-210 had significantly better overall survival rate than those with a high expression of miR-210 [22]. Therefore, we further investigated the effect of miR-942 expression on the survival rate of BCa patients. Kaplan–Meier analysis showed that the survival rate of BCa patients with a high expression of miR-942 was lower than that of patients with a low expression of miR-942.
Figure 4. FOXA2 directly targets miR-942

(A) TargetScan7.2 was used to predict the possible target genes for miR-942. (B) Dual-luciferase reporter assay was used to analyze the fluorescence activity of FOXA2 in overexpressed miR-942 \( (n=3, **P<0.001, \text{ vs. Blank}) \). (C) Dual-luciferase reporter assay was used to analyze the fluorescence activity of FOXA2 in low-expressed miR-942 \( (n=3, **P<0.001, \text{ vs. Blank}) \). (D) QRT-PCR was used to detect FOXA2 expression in 31 BCa patients and 31 adjacent tissues \( (n=3, **P<0.001, \text{ vs. normal}) \). (E) miR-942 was in negative correlation with FOXA2 in 31 BCa patient samples \( (r=-0.429, P=0.016) \). (F, G) Western blotting was used to detect the FOXA2 expression in BCa patients and adjacent tissues \( (n=3, **P<0.001, \text{ vs. normal 1}; ##P<0.001, \text{ vs. normal 2}) \). (H, I) Western blotting was used to detect the FOXA2 expression in BCa patients and adjacent tissues \( (n=3, **P<0.001, \text{ vs. normal 4}; ##P<0.001, \text{ vs. normal 5}; ^{\wedge\wedge}P<0.001, \text{ vs. normal 6}) \). (J) Kaplan–Meier method was used to analyze the relationship between FOXA2 and survival without distant metastasis in BCa patients.
The effects of miR-942 on the biological characteristics of BCa cells were explored, and the results showed that overexpressed miR-942 significantly promoted cell viability and proliferation, and inhibited cell apoptosis rate. However, inhibiting the expression of miR-942 in cells significantly inhibited cell viability and proliferation, and greatly promoted cell apoptosis. The invasion, metastasis and drug resistance of cancer cells are closely related to EMT [23], a cellular development process, in which epithelial cells undergo structural changes, loss of polarity and cell adhesion and acquisition of mesenchymal properties, and such a process allows cancer cells to become more aggressive and metastatic [24,25]. E-cadherin is indispensable in maintaining stable adhesion, the loss of which in epithelial cells during EMT will lead to decreased cell adhesion, increased expressions of N-cadherin and Snail, therefore the cells will be transformed into mesenchymal cells [26–28]. The regulation of EMT by miRNA in BCa cells has previously been reported, for example miR-138 can regulate EMT in BCa cells by targeting vimentin [29], and miR-520c-3p can regulate EMT in BCa cells by targeting IL-8 negative [30]. In this study, the overexpression of miR-942 inhibited the expression of E-cadherin and promoted the expressions of N-cadherin and Snail, suggesting that miR-942 can
Figure 6. MiR-942 affects the viability, proliferation and apoptosis of BCa cells by targeting FOXA2

(A–D) The effects of miR-942 and FOXA2 on cell proliferation was detected by colony formation assay (n=3, **P<0.001, vs. MC + NC or NC + siNC; ^P<0.001, vs. MC + FOXA2 or IC + si FOXA2; ^ ^ P<0.001, vs. mimic + NC or inhibitor + siNC). (E–H) Cell apoptosis was detected by flow cytometry (n=3, **P<0.001, vs. MC + NC or IC + siNC; ^ ^ P<0.001, vs. MC + FOXA2 or IC + si FOXA2; ^ ^ ^ P<0.001, vs. mimic + NC or inhibitor + siNC).

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Figure 7. The effects of miR-942 on the invasion and migration of BCa cells by targeting FOXA2

(A–D) The wound scratch assay was used to measure the ability of cells to migrate (n=3, **P<0.001, vs. MC + NC or IC + siNC; ##P<0.001, vs. MC + FOXA2 or IC + si FOXA2; ^&^P<0.001, vs. mimic + NC or inhibitor + siNC).

(E–H) Transwell was used to detect the ability of BCa cells to invade (n=3, **P<0.001, vs. MC + NC or IC + siNC; ##P<0.001, vs. MC + FOXA2 or IC + si FOXA2; ^&^P<0.001, vs. mimic + NC or inhibitor + siNC).
regulate the EMT in BCa cells, moreover, we found that overexpressed miR-942 can promote the ability of BCa cell migration and invasion.

It is currently unknown whether miR-942 inhibits the EMT of OSCC cells by regulating certain genes, however, in the present study, TargetScan7.1 predicted that FOXA2 is a potential target gene for miR-942, and confirmed by the fluorescence experiment report. FOXA2 was found to effectively inhibit the progression and development of lung cancer, liver cancer and prostate cancer [31–33], and tumor metastasis by inhibiting the EMT process. The present study showed that FOXA2 expression in BCa was significantly lower than that in the adjacent tissues, moreover, Kaplan–Meier analysis showed that the survival rate of patients with a low expression of FOXA2 was significantly lower than that with a high expression of FOXA2 but without distant metastasis. Furthermore, we found that FOXA2 partially reversed the effects of overexpressed miR-942 on enhancing the activity, proliferation of BCa cells and on inhibiting apoptosis and promoting cell migration and invasion, and such results were further confirmed by rescue experiment.

In conclusion, miR-942 promote BCa progression by down-regulating FOXA2 expression, indicating that miR-942 could be used as a potential therapeutic target for the treatment of BCa patients.

Competing Interests
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Author Contribution
Substantial contributions to conception and design: J.Z. and Z.Z. Data acquisition, data analysis and interpretation: J.S., Q.M. and W.Z. Drafting the article or critically revising it for important intellectual content: X.C. and H.Q. Final approval of the version to be published: all authors. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved: J.Z.

Abbreviations
BCa, breast cancer; BRCA, breast cancer; EMT, epithelial-mesenchymal transition; FBS, fetal bovine serum; FOXA2, forkhead box transcript factor A2; HRP, horseradish peroxidase; IC, inhibitor control; MC, mimic control; miR/miRNA, microRNA; MUT, mutant; NC, negative control; RIPA, radio-immunoprecipitation assay; WT, wild-type.

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