MiR-548c-3p inhibits the proliferation, migration and invasion of human breast cancer cell by targeting E2F3

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Abstract MiR-548 has been reported to be involved in a variety of tumor processes, but its function in breast cancer remains unclear. In this study, we found that miR-548 was low expressed in breast cancer tissues and cells compared with normal control. We then examined whether up-regulation of miR-548 could improve the progression of breast cancer. Our results indicate that up-regulation of miR-548 significantly inhibits cell proliferation, migration and invasion, and induces apoptosis in breast cancer cells. Further studies showed that miR-548 could specifically inhibit E2F3 expression. Moreover, rescue test showed that up-regulation of E2F2 could reverse the effect of miR-548 on proliferation, migration, invasion and apoptosis of breast cancer cells. In general, miR-548 could improve the progression of breast cancer. By targeting E2F2, which may make a potential target for the treatment of breast cancer.

Keywords Breast cancer · miR-548c-3p · E2F3 · Proliferation

Introduction

Breast cancer is one of the most common malignant tumor in the world (Harbeck and Gnant 2017), it threatens the life of females and is the leading cause of death among women (Akram et al. 2017). The morbidity and mortality are highly, it is reported there are about 1.7 million patients diagnosed with breast cancer and above 0.5 million died from it, every year (Ferlay et al. 2015). The causes of breast cancer are numerous, but the mechanisms of pathogenesis remain unclear (Ren et al. 2020). Early detection and efficient systemic therapies attribute to the breast cancer without distant metastases to be potentially curable, the main treatments are surgery, radiotherapy, endocrine therapy, chemotherapy, targeted therapy. Because of the lack of standardized treatments, the disease progresses, metastatic breast cancer is considered to be incurable in some patients (Akram et al. 2017). Recently, it has been demonstrated that various miRNAs have been shown to participate in regulating the initiation, progression, metastasis or drug resistance of breast cancer (Loh 2019; Kurozumi 2017).

MicroRNAs (miRNAs) are small non-coding RNA, consist of 20–24 nucleotides. Firstly, they are transcribed by gens and then modified into mature

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microRNAs to target specific mRNAs to regulate the specific proteins by inhibiting their translation or degradation. They usually function as critical post-transcriptional gene regulators of various biological functions (Loh et al. 2019; Zendjabil et al. 2017). MiR-548 is a larger, poorly conserved primate-specific miRNA gene family, consists of 69 human miR-548 genes located in almost all human chromosomes, and the miR-548 gene family enrichment pathway analysis showed they played important roles in various human diseases (Liang et al. 2012). MiR-548c is a member of miR-548 and the mature miR-548c-3p is obtained from it. Some studies indicated miR-548c-3p functions as a regulator in many various cancers (Guo et al. 2019), including prostate Cancer (Saffari et al. 2019), esophageal cancer (Ni et al. 2018), thyroid Carcinoma (Du et al. 2019), osteosarcoma (Luo et al. 2016), glioma (Lu et al. 2017), gallbladder cancer (Kong et al. 2019), gastric cancer (Chang et al. 2015) and breast cancer (Guo et al. 2019; Tormo et al. 2015). However, it has not been clarified that the expression level of miR-548c-3p and its mechanism in breast cancer.

The aim of this study was to investigate the roles of miR-548c-3p in breast cancer. The present study demonstrated that miR-548c-3p was downregulated, and overexpression of miR-548c-3p could inhibit the proliferation, migration and invasion, and induce apoptosis in breast cancer tissues and cells. We also revealed a novel target E2F3, which mediated the inhibitory effect of miR-548c-3p on the breast cancer.

Materials and methods

Collection of tissue specimens

All breast cancer tissues and paired peritoneal tissues were obtained from patients with breast cancer diagnosed Tianjin University of Traditional Chinese Medicine. All the protocols for the usage of patient samples were approved by the Medical Ethics Committee of Tianjin University of Traditional Chinese Medicine. Informed consent was signed by all patients who participated in the study. The research was conducted in accordance with the World Medical Association Declaration of Helsinki.

Cell lines and culture conditions

Human breast cancer cell lines (MDA-MB-23, SKBr-3, MCF-7, ZR-75-30) and human normal breast cell MCF 10A were purchased from the Chinese Academy of Sciences, Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). All the cells were maintained in DMEM (Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS Hyclone, USA), and grown in a humidified 37 °C incubators supplied with 5% of CO₂. The CO₂ cell incubator purchased from Forma Scientific UK and FACS Calibri flow cytometer purchased from BD biosciences (USA). ABI7300 fluorescent quantitative PCR instrument was purchased from Applied Biosystems Inc.

Cell transfection

NC mimic (5'-UUCUCCGAACGUGUCACGUTT-3') and Hsa-miR-548c-3p mimic (5'-CAAAAUCUCAAUACUUUUGC-3') were provided by Gene Pharma (Shanghai, China). Transfection of ZR-75-30 and MCF-7 cells were conducted using Lipofectamine 2000 transfection reagent (Thermo Fisher Scientific, USA). Cells were cultured in six-well plates for 48 h and collected for subsequent analyses.

RNA extraction and RT-qPCR

Total RNAs were extracted from ZR-75-30 and MCF-7 with TRIZOL reagent (Thermo Fisher, USA), and were reverse-transcribed to cDNA by Revert Aid First Strand cDNA Synthesis kit (Thermo Fisher Scientific). RT-qPCR was conducted by SYBR-Green PCR Master Mix kit (Takara, Tokyo, Japan) and ABI 7500 Real-Time PCR System (Thermo Fisher Scientific). The reverse transcription reactions were: 37 °C for 15 min, 85 °C for 5 s. Real time quantitative PCR was performed using a Taqman PCR kit on an Applied Biosystems 7300 sequence detection systems (ThermoFisher, USA). These actions were performed in a 96 well plate at 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 1 min. GAPDH were used as the internal control.

GAPDH upstream primer: 5'-AATCCCATCAC-CATCTTC 3', downstream primer: 5'-AGGCTGTTGTCATACTTC 3'; miR-548c-3p upstream primer: 5'-TGTCCTTAG

CACCTGGTACGGACCGCT-3', downstream primer:5'-TGCA ATCTGGACTACCGATGG-3'.

Cell counting kit-8 (CCK-8) assay

After 48-h transfection, cells were collected and resuspended in the culture medium. In total, 100 μ l of cell suspension containing 2×10^3 cells were inoculated into each well of a 96 well plate. Cells were incubated in the humidified incubator at 37 °C and 5% CO₂ to determine the cellular proliferation at specific time points: 0, 24, 48, and 72 h after inoculation. At each time point, 10 μ l of the CCK-8 solution (Syngene, Nanjing, China) was added into each well, followed by incubation at 37 °C for another 2 h. The absorbance of each well was detected at a 450 nm wavelength on a microplate reader (ThermoFisher, USA).

Colony formation assay

After transfection with miR-548c-3p, the cells were trypsinized, counted, seeded for colony formation assay in 6-well plates at the density of 100 cells per well. During colony growth, the culture medium was replaced every 3 days. The colony was counted only if contained more than 50 cells, and the number of colonies was counted the 6th day after seeding. Colony formation rate was calculated with equation; colony formation rate = (number of colonies/number of seeded cells) $\times$ 100%. Each treatment was carried out in triplicate.

EdU assay

ZR-75-30 and MCF-7 cell lines were in 96-well plates and were transfected with NC mimics and miR-548c-3p mimics for 48 h. Cell lines were cultured using EdU (Syngene, Nanjing, China) and fixed with 1 ml 4% paraformaldehyde (Syngene, Nanjing, China) at room temperature for 30 min, then cell lines were washed with 3% BSA for two times, 5 min each time, and permeated with 1 ml osmotic agent (0.5% TritonX-100) to permeate the cytoplasm and nuclear membrane. After 20 min of permeation, each cell line was washed with 3% BSA twice for 5 min each time to remove the osmotic agent. 0.5 ml of the KeyFluor488 (Syngene, Nanjing, China) click-it reaction mixture was added to each culture dish and incubated at room

temperature for 30 min in darkness. Click-it reaction mixture was discarded, and the reaction mixture was rinsed with 3% BSA twice, 5 min each time. The reaction liquid was removed, and then antifluorescence quenching agent was added to observe the fluorescence by using a fluorescence microscope (ThermoFisher, MA, USA).

Flow cytometry analysis

ZR-75-30 and MCF-7 cell lines were transfected with NC mimics and miR-548c-3p mimics for 48 h and the cells were washed, digested and transferred into centrifuge tubes and centrifuged at 1000g for 5 min, then the cells were collected. 100ul buffer solution were added to resuspend the cells to make sure the cell concentration was 1×10^6 . 5 μ l Annexin V-FITC and 10 μ l propidium iodide (PI) dye were further added into each tube for 10 min. Finally, the cell apoptosis was detected within 1 h by Flow cytometer. The green fluorescence of FITC was detected in FL1 channel, while red fluorescence of PI was in FL2 or FL3 channel and then draw the two color dot plot, the abscissa is FITC and the ordinate is PI.

Western blot analysis

ZR-75-30 and MCF-7 cell lines were seeded in 24-well plates and were transfected with NC mimics and miR-548c-3p mimics for 48 h. Cells were then cracked in RIPA lysis buffer plus PMSF in low temperature. BCA assay kit (Santa Cruz, California, USA) were used to detect total protein concentration. Prepared protein samples were separated in SDS-PAGE, and transferred into 0.22 μ m PVDF membranes and incubated with prepared antibodies, the primary antibody incubated overnight, and the secondary antibody for 2 h. Finally, enhanced chemiluminescence (ThermoFisher, MA, USA) visualized this membrane. The antibody β -actin was purchased from CST (Beverly, Massachusetts, USA). Antibodies against the Bax, Bcl-2, MMP-2 and MMP-9 were purchased from Abcam (Shanghai, China).

Scratch assay

After transfected with NC mimics and miR-548c-3p mimics for 48 h, ZR-75-30 and MCF-7 cell lines were cultured in 24-well plates. When cells were grown to

90% confluence, a single wound was created and phase contrast images were digitally photographed immediately and 24 h after incubation. The original opening distances of the wound were set as 100%. The opening distances after 24 h were measured from three areas randomly selected per well, and the distances in three wells of each group were quantified and normalized by the original opening distance. The experiment was performed three times in triplicate, and the percentage of the migration rate was calculated by measuring the length of cell migration and expressed as a percentage compared to the control group. Migration rates = (treatment group cell migration distance/control group migration distance) $\times$ 100%.

Transwell chamber assay

The capacity for cell migration was assessed using Transwell chambers (8 μ m diameters; Shanghai, China). After transfected with NC mimics and miR-548c-3p mimics for 48 h, ZR-75-30 and MCF-7 cells were harvested, washed with PBS, and resuspended in DMEM without FBS. In total, 100 μ l of suspension containing 5×10^4 cells was added into the upper chambers. The transwell chambers were placed into a 24-well plate that had already been covered with 500 μ l of DMEM containing 10% of FBS. 24 h later, non-migratory cells were gently removed, and the migratory cells were fixed with 100% methanol, stained with 0.5% crystal violet, washed with PBS, and imaged using an inverted microscope (Olympus, Tokyo, Japan). The migratory and invasive abilities were measured by counting respectively the migratory and invading cells.

Luciferase reporter assay

ZR-75-30 and MCF-7 cells were seeded in 24-well plates until reaching 60% confluence. 3'UTR region of the E2F3 WT or E2F3 Mut were synthesised by Shanghai GenePharma Co., Ltd. (Shanghai, China), and cloned into the pGL3 promoter vector (Promega Corporation, Madison, WI, USA) for luciferase reporter experiments. Sequences that could be binding to miR-548c-3p were partly mutated and inserted into the reporters in order to identify the binding specificity. Each well was co-transfected with luciferase reporter plasmids (0.5 μ g) and NC mimics and miR-

548c-3p mimics (100 nM) using Lipofectamine 2000 (ThermoFisher, USA) according to the manufacturer's protocol. The luciferase activity was measured after 48-h transfection by using the Dual Luciferase Reporter Assay (Promega Shanghai, China).

Statistical analyses

All experiments were repeated three times and the data are presented as the mean $\pm$ standard deviation (SD) using SPSS 18.0 (SPSS, inc.). An unpaired *t*-test was performed to compare the difference of the two groups. At the meanwhile, a one-way ANOVA followed by Bonferroni multiple comparison test was used to detect differences between the two or more groups. *P* < 0.05 was considered as statistically significant.

Results

The expression level of miR-548c-3p is downregulated both in breast cancer tissues and its cell lines

To detect the potential function of miR-548c-3p in breast cancer, we first measured its expression in breast cancer tissues and its cell lines. RT-qPCR analysis indicated that the expression level of miR-548c-3p was decreased in cancer tissues compared with the normal tissue (Fig. 1a). Consistently, miR-548c-3p expression was downregulated in breast cancer cell (MDA-MB-231, SKBr-3, MCF7, and ZR-75-30) compared with normal breast cell (MCF-10A). In all breast cancer cells, the low expression of miR-548c-3p in MCF-7 and ZR-75-30 is more significant (Fig. 1b). After transfection with NC mimic and miR-548c-3p mimic in MCF-7 and ZR-75-30 cells, we carried out further investigation to evaluate the transfection efficiency. Under the fluorescence microscope, we observed green fluorescent protein expressed in MCF-7, ZR-75-30 cells to confirm whether NC mimic or miR-548c-3p mimic were transfected (Fig. 1c). And the expression levels of miR-548c-3p were increased after transfected with miR-548c-3p mimic (Fig. 1d).

Fig. 1 The expression of miR-548c-3p in breast cancer tissues and cell lines and transfection efficiency of miR-548c-3p. **a** The expression level of miR-548c-3p in normal and cancer tissues. **b** The expression level of miR-548c-3p in normal breast cells and breast cancer cell lines. **c** The transfection efficiency of ZR-75-30 and MCF-7 cells after transfected with NC mimic and

miR-548c-3p mimic. **d** The expression level of miR-548c-3p in ZR-75-30 and MCF-7 cells transfected with NC mimic and miR-548c-3p mimic. Asterisks indicated significant differences from the control (* $P < 0.05$, ** $P < 0.01$, compared to Normal tissues or MCF10A group, # $P < 0.05$ and ### $P < 0.01$, compared to NC mimic group). All the data were presented as mean $\pm$ SD

Overexpression of miR-548c-3p inhibits proliferation and promotes apoptosis in breast cancer cells

After transfection with NC mimic and miR-548c-3p mimic, the proliferation and apoptosis of breast cancer cells were validated. CCK-8, colony formation assay and EdU incorporation assays indicated that the overexpressed miR-548c-3p significantly inhibited proliferation of MCF-7 and ZR-75-30 cell lines (Fig. 2a–c). In addition, flow cytometry analysis revealed the overexpression of miR-548c-3p promoted the apoptosis in MCF-7 and ZR-75-30 cell lines (Fig. 3a). Of note, Bcl-2, Bax, caspase-3 and caspase-9 play important roles in the cell apoptosis progression. Bcl-2 could inhibit the apoptosis, whereas the Bax, caspase-3 and caspase-9 promote apoptosis (Hassan et al. 2014; Katayama et al. 2015; Li et al. 2017a). Western blot assay revealed the

overexpression of miR-548c-3p significantly down-regulated the expression level of Bcl-2 and upregulated the expression levels of Bax, Cleaved-caspase3, Cleaved-caspase-9 (Fig. 3b).

Overexpression of miR-548c-3p inhibits migration and invasion in breast cancer cells

We next performed scratch and transwell chamber assays to examine the effects of miR-548c-3p on migration and invasion in breast cancer cell lines. As shown in Fig. 4a, b, miR-548c-3p significantly inhibited the migration and invasion both in MCF-7 and ZR-75-30 cell lines. At the molecular level, cyclooxygenase2 (COX-2) is the subtype of COX, which is the key enzymes of arachidonic acid metabolism, could catalyze arachidonic acid into prostaglandins (Li et al. 2017b; Serra et al. 2016). MMP-2 and MMP-9, as dominant matrix metalloproteinases (MMPs), which

Fig. 2 The effects of overexpressed miR-548c-3p on proliferation in breast cancer cells. MCF-7 and ZR-75-30 cells were transfected with NC mimic and miR-548c-3p mimic. **a** CCK-8 assays for proliferation. **b** Colony formation assays for

proliferation. **c** EdU assays for proliferation. (* $P < 0.05$, ** $P < 0.01$, compared to NC mimic group). All the data were presented as mean $\pm$ SD

Fig. 3 The effects of overexpressed miR-548c-3p on apoptosis in breast cancer cells. MCF-7 and ZR-75-30 cells were transfected with NC mimic and miR-548c-3p mimic. **a** Flow cytometry assays for apoptosis. **b** Western blot assays for

apoptosis related proteins. Asterisks indicated significant differences from the control (* $P < 0.05$, ** $P < 0.01$, compared to NC mimic group). All the data were presented as mean $\pm$ SD

are correlated with migration and invasion (Huang 2018; Kalhori and Törnquist 2015). Western blot assays indicated the overexpression of miR-548c-3p downregulated the COX-2, MMP-2 and MMP-9 (Fig. 4c). These results indicated that miR-548c-3p functions as a tumor suppressor in breast cancer progression.

miR-548c-3p targets on the E2F3

MiRNAs exert multiple biological functions through interaction with 3'UTR region of various target gene. In our study, bioinformatics analysis indicated that 3'UTR region of E2F3 possessed the potential binding site of miR-548c-3p (Fig. 5a). We next investigated whether miR-548c-3p could directly regulate E2F3 expression by using luciferase reporter assays, we found that miR-548c-3p mimic suppressed the luciferase activity of E2F3 WT, whereas exhibited modest

Fig. 4 The effects of overexpressed miR-548c-3p on migration and invasion in breast cancer cells. MCF-7 and ZR-75-30 cells were transfected with NC mimic and miR-548c-3p mimic. **a** Scratch assays for migration. **b** Transwell chamber assays for

migration and invasion. **c** Western blot assays for migration and invasion related proteins. Asterisks indicated significant differences from the control (* $P < 0.05$, ** $P < 0.01$, compared to NC mimic group). All the data were presented as mean $\pm$ SD

impact on the E2F3 MUT both in MCF7 and ZR-75-30 cell lines (Fig. 5b). Additionally, RT-qPCR and western blot analysis indicated that overexpression of miR-548c-3p significantly decreased the expression of E2F3 at both mRNA and protein levels (Fig. 5c, d).

E2F3 mediates the effect of miR-548c-3p on breast cancer progression

After transfection with the E2F3 expressing plasmids, the expression levels of E2F3 were increased (Fig. 6a). CCK-8 and EdU assays indicated that

miR-548c-3p mimic inhibited MCF-7 and ZR-75-30 proliferation, while the E2F3 expressing plasmid partially blocked this inhibition (Fig. 6b, c). In addition, flow cytometry analysis indicated that miR-548c-3p mimic promoted MCF-7 and ZR-75-30 apoptosis, while the promotion was partially antagonized by E2F3 expressing plasmid (Fig. 7d). Besides, the cell migration and invasion analyses in the miR-538-3p overexpression breast cancer cells revealed the similar changes proliferation analysis in response to E2F3 overexpression (Fig. 7e). All the results suggested that the E2F3 mediated the miR-548c-3p-

Fig. 5 E2F3 is a target gene of miR-548c-3p. MCF-7 and ZR-75-30 cells were transfected with NC mimic and miR-548c-3p mimic. **a** The sequencing assay for E2F3 and miR-548c-3p. **b** Relative values of luciferase signal. **c** The expression level of E2F3 in MCF-7 and ZR-75-30 cells. **d** The protein expression

level of E2F3 in MCF-7 and ZR-75-30 cells. Asterisks indicated significant differences from the control (* $P < 0.05$, ** $P < 0.01$, compared to NC mimic group). All the data were presented as mean $\pm$ SD

induced inhibition of proliferation and migration/invasion, as well as miR-548c-3p-induced cell apoptosis.

Discussion

Breast cancer accounts for about 23% of female malignancies and it is considered a serious for the high morbidity and mortality among women (Wu et al.

Fig. 6 E2F3 mediates the effect of miR-548c-3p on breast cancer cell proliferation. **a** RT-qPCR assays for the expression levels of E2F3 after in MCF-7 and ZR-75-30 cells transfected with E2F3 expressing plasmid (** $P < 0.01$, compared to vector group). **b** CCK-8 assays for proliferation of MCF-7 and ZR-75-30 cells co-transfected with miR-548c-3p and E2F3 **c** EdU

assays for proliferation of MCF-7 and ZR-75-30 cells co-transfected with miR-548c-3p and E2F3. (* $P < 0.05$, ** $P < 0.01$, compared to NC mimic + vector group, # $P < 0.05$ and ## $P < 0.01$, compared to miR-548c-3p mimic + vector group). All the data were presented as mean $\pm$ SD

2020). The incidence of breast cancer is related to many factors. Some studies suggested miRNA is a potential element in carcinomas, miRNA-224-5p inhibits autophagy in breast cancer cells via targeting Smad4 (Cheng et al. 2018). MiRNA-223 can maintain cell proliferation of breast cancer cell through targeting FOXO1 (Wei et al. 2017). MiR-548c-3p inhibited the proliferation of breast cancer cells by regulating the expression of ECHS1 (Shi et al. 2015). In breast cancer, miRNA has been proven to be selectively secreted from malignant cells in the mammary gland, and expressed differently in the blood of healthy individuals and patients affected with patients so, it could function as a future screening marker (Zubor et al. 2018). The present study focused on the effects of miR-548c-3p on breast cancer. The results suggested that miR-548c-3p played a crucial role in breast cancer cell proliferation, migration, invasion, and apoptosis. The relative expression levels of miR-548c-3p were lower in primary breast cancer tissues and its cell lines compared with in normal tissues and the MCF10A cells. MiR-548c-3p overexpression could inhibit MCF-7 and ZR-75-30 cells proliferation, migration, invasion and promotes apoptosis.

MiRNAs exert their biological function by regulating their target genes. In this study, we proved that E2F3 is the target of miR-548c-3p. E2F3 is a member of the E2F transcription factor family and plays important roles in cell proliferation, apoptosis, and differentiation, overexpresses in lung, ovarian,

bladder, gastric, and prostate cancers, the dysregulation of E2F3 could contribute to oncogenesis (Wang et al. 2019). In breast cancer miR-34a could suppress cell growth, migration, and invasion, miR-125b associates with chemotherapeutic resistance (Gao et al. 2017). Given that both E2F3 and miR-548c-3p could affect breast cancer progression, but the relationship between E2F3 and miR-548c-3p in breast cancer remains unclear. Then we investigated whether miR-548c-3p could regulate E2F3, our results showed that miR-548c-3p mimic suppressed the luciferase activity of E2F3 WT, whereas exhibited modest impact on the E2F3 MUT both in MCF7 and ZR-75-30 cell lines. The RT-qPCR and western blot assays also indicated the miR-548c-3p suppressed the expression level of E2F3. Moreover, we demonstrated that after transfection with the E2F3 expressing plasmid, the expression levels of E2F3 were increased, E2F3 enhanced the effect on proliferation, migration and invasion of miR-548c-3p in MCF-7 and ZR-75-30 cells while weaken the effect of apoptosis. So our study indicated miR-548c-3p inhibits the proliferation, migration and invasion of human breast cancer cell by targeting E2F3.

In breast cancer, miRNA has been shown to act on targets through several signaling pathways to regulate cell progression, apoptosis, angiogenesis, epithelial-mesenchymal transition, tumor microenvironment, migration, invasion, metastasis and drug resistance (Liu et al. 2017). For example, miR-145 affects breast

Fig. 7 E2F3 mediates the effect of miR-548c-3p on breast cancer cell apoptosis, migration and invasion. **a** Flow cytometry for the apoptosis of MCF-7 and ZR-75-30 cells co-transfected with miR-548c-3p and E2F3. **b** Transwell chamber assays for migration and invasion of MCF-7 and ZR-75-30 cells co-transfected with miR-548c-3p and E2F3. Asterisks indicated

significant differences from the control. (* $P < 0.05$, ** $P < 0.01$, compared to NC mimic + vector group, # $P < 0.05$ and ## $P < 0.01$, compared to miR-548c-3p mimic + vector group). All the data were presented as mean $\pm$ SD

cancer BS524 cell proliferation, infiltration, and migration via positively regulating OCT4 gene expression (Kuang et al. 2018), miRNA-140-5p inhibits invasion and angiogenesis through targeting VEGF-A in breast cancer (Lu et al. 2017), the overexpression of miR-449a suppresses CRIP2, which then affects the tumor vasculature in breast cancer, likely via NF- κ B/p65 complex-mediated transcription of VEGF (Shi et al. 2016), sh-HIF1A-AS2 inhibited the proliferation, invasion, and pathological damage of breast cancer via targeting miR-548c-3p through regulating HIF-1 α /VEGF pathway in vitro and vivo (Guo et al. 2019). Hence, miRNAs were defined as pivotal factors in defining the specificity and sensitivity of post-transcriptional gene silencing, their target genes inferring the functions and regulatory mechanisms are critical in understanding biological processes of organisms and may shed light on deciphering their roles in the pathophysiology of diseases (Liu et al. 2014). In our study, we found that miR-548c-3p as a target played an important role in regulating the occurrence and progression of breast cancer (Guo et al. 2019; Tormo et al. 2015), and we hoped to find a new target of miR-548c-3p in breast cancer. Our investigation further corroborated that E2F3 is a target of miR-548c-3p and it played important roles in cell proliferation, apoptosis, and provided a novel mechanism through which miR-548c-3p could regulate the breast cancer progression. Also, it improved the network between miRNAs and target genes.

In conclusion, the present study demonstrated that miR-548c-3p is a key regulator in the proliferation, migration, invasion and apoptosis of breast cancer cells by targeting E2F3. Thus, miR-548c-3p/E2F3 axis might be used as a diagnosed biomarker or therapeutic target for the breast cancer and its associated diseases.

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Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflicts of interest The authors declare that there are no conflicts of interest.

References

- Akram M, Iqbal M, Daniyal M, Khan AU (2017) Awareness and current knowledge of breast cancer. *Biol Res* 50(1):33. <https://doi.org/10.1186/s40659-017-0140-9>
- Chang H, Kim N, Park JH et al (2015) Different microRNA expression levels in gastric cancer depending on *Helicobacter pylori* infection. *Gut Liver*. 9(2):188–196. <https://doi.org/10.5009/gnl13371>
- Cheng Y, Li Z, Xie J et al (2018) MiRNA-224-5p inhibits autophagy in breast cancer cells via targeting Smad4. *Biochem Biophys Res Commun* 506(4):793–798. <https://doi.org/10.1016/j.bbrc.2018.10.150>
- Du Y, Zhu J, Chu BF, Yang YP, Zhang SL (2019) MiR-548c-3p suppressed the progression of papillary thyroid carcinoma via inhibition of the HIF1 α -mediated VEGF signaling pathway. *Eur Rev Med Pharmacol Sci*. 23(15):6570–6578. https://doi.org/10.26355/eurrev_201908_18543
- Ferlay J, Soerjomataram I, Dikshit R et al (2015) Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 136(5):E359–E386. <https://doi.org/10.1002/ijc.29210>
- Gao Y, Feng B, Lu L et al (2017) MiRNAs and E2F3: a complex network of reciprocal regulations in human cancers. *Oncotarget*. 8(36):60624–60639. <https://doi.org/10.18632/oncotarget.17364>
- Guo X, Lee S, Cao P (2019) The inhibitive effect of sh-HIF1A-AS2 on the proliferation, invasion, and pathological damage of breast cancer via targeting miR-548c-3p through regulating HIF-1 α /VEGF pathway in vitro and vivo. *Onco Targets Ther*. 12:825–834. <https://doi.org/10.2147/ott.s192377>
- Harbeck N, Gnant M (2017) Breast cancer. *Lancet* 389(10074):1134–1150. [https://doi.org/10.1016/S0140-6736\(16\)31891-8](https://doi.org/10.1016/S0140-6736(16)31891-8)
- Hassan M, Watari H, AbuAlmaaty A, Ohba Y, Sakuragi N (2014) Apoptosis and molecular targeting therapy in cancer. *Biomed Res Int* 2014:150845. <https://doi.org/10.1155/2014/150845>
- Huang H (2018) Matrix Metalloproteinase-9 (MMP-9) as a cancer biomarker and MMP-9 biosensors: recent advances. *Sensors (Basel)*. 18(10):3249. <https://doi.org/10.3390/s18103249>
- Kalhor V, Törnquist K (2015) MMP2 and MMP9 participate in S1P-induced invasion of follicular ML-1 thyroid cancer cells. *Mol Cell Endocrinol* 404:113–122. <https://doi.org/10.1016/j.mce.2015.01.037>
- Katayama S, Shimoda K, Takenaga Y (2015) Loss of ADAR1 in human iPS cells promotes caspase3-mediated apoptotic cell death. *Genes Cells* 20(8):675–680. <https://doi.org/10.1111/gtc.12261>
- Kong L, Wu Q, Zhao L, Ye J, Li N, Yang H (2019) Identification of messenger and long noncoding RNAs associated with gallbladder cancer via gene expression profile analysis. *J Cell Biochem* 120(12):19377–19387. <https://doi.org/10.1002/jcb.28953>
- Kuang WB, Deng Q, Deng CT et al (2018) MiRNA regulates OCT4 expression in breast cancer cells. *Eur Rev Med Pharmacol Sci*. 22(5):1351–1357. https://doi.org/10.26355/eurrev_201803_14478

- Kurozumi S, Yamaguchi Y, Kurosumi M, Ohira M, Matsumoto H, Horiguchi J (2017) Recent trends in microRNA research into breast cancer with particular focus on the associations between microRNAs and intrinsic subtypes. *J Hum Genet* 62(1):15–24. <https://doi.org/10.1038/jhg.2016.89>
- Li P, Zhou L, Zhao T et al (2017a) Caspase-9: structure, mechanisms and clinical application. *Oncotarget* 8(14):23996–24008. <https://doi.org/10.18632/oncotarget.15098>
- Li B, Li X, Xiong H et al (2017b) Inhibition of COX2 enhances the chemosensitivity of dichloroacetate in cervical cancer cells. *Oncotarget* 8(31):51748–51757. <https://doi.org/10.18632/oncotarget.18518>
- Liang T, Guo L, Liu C (2012) Genome-wide analysis of mir-548 gene family reveals evolutionary and functional implications. *J Biomed Biotechnol* 2012:679563. <https://doi.org/10.1155/2012/679563>
- Liu B, Li J, Cairns MJ (2014) Identifying miRNAs, targets and functions. *Brief Bioinform* 15(1):1–19. <https://doi.org/10.1093/bib/bbs075>
- Liu DZ, Chang B, Li XD, Zhang QH, Zou YH (2017) MicroRNA-9 promotes the proliferation, migration, and invasion of breast cancer cells via down-regulating FOXO1. *Clin Transl Oncol* 19(9):1133–1140. <https://doi.org/10.1007/s12094-017-1650-1>
- Loh HY, Norman BP, Lai KS, Rahman NM, Alitheen NBM, Osman MA (2019) The regulatory role of MicroRNAs in breast cancer. *Int J Mol Sci* 20(19):4940. <https://doi.org/10.3390/ijms20194940>
- Lu J, Zhang M, Yang X, Cui T, Dai J (2017a) MicroRNA-548c-3p inhibits T98G glioma cell proliferation and migration by downregulating c-Myb. *Oncol Lett* 13(5):3866–3872. <https://doi.org/10.3892/ol.2017.5870>
- Lu Y, Qin T, Li J et al (2017b) MicroRNA-140-5p inhibits invasion and angiogenesis through targeting VEGF-A in breast cancer. *Cancer Gene Ther* 24(9):386–392. <https://doi.org/10.1038/cgt.2017.30>
- Luo Z, Li D, Luo X et al (2016) Decreased expression of miR-548c-3p in osteosarcoma contributes to cell proliferation via targeting ITGAV. *Cancer Biother Radiopharm* 31(5):153–158. <https://doi.org/10.1089/cbr.2016.1995>
- Ni XF, Zhao LH, Li G et al (2018) MicroRNA-548-3p and MicroRNA-576-5p enhance the migration and invasion of esophageal squamous cell carcinoma cells via NRIP1 down-regulation. *Neoplasma* 65(6):881–887. https://doi.org/10.4149/neo_2018_171206N803
- Ren L, Li Y, Zhao Q et al (2020) miR-519 regulates the proliferation of breast cancer cells via targeting human antigen R. *Oncol Lett* 19(2):1567–1576. <https://doi.org/10.3892/ol.2019.11230>
- Saffari M, Ghaderian SMH, Omrani MD, Afsharpad M, Shankaie K, Samadaian N (2019) The association of miR-let 7b and miR-548 with PTEN in prostate cancer. *Urol J* 16(3):267–273. <https://doi.org/10.22037/uj.v0i0.4564>
- Serra KP, Peres RM, Sarian LO et al (2016) Cyclooxygenase-2 (COX2) and p53 protein expression are interdependent in breast cancer but not associated with clinico-pathological surrogate subtypes, tumor aggressiveness and patient survival. *Acta Histochem* 118(2):176–182. <https://doi.org/10.1016/j.acthis.2015.12.009>
- Shi Y, Qiu M, Wu Y, Hai L (2015) MiR-548c-3p functions as an anti-oncogenic regulator in breast cancer. *Biomed Pharmacother* 75:111–116. <https://doi.org/10.1016/j.biopha.2015.07.027>
- Shi W, Bruce J, Lee M et al (2016) MiR-449a promotes breast cancer progression by targeting CRIP2. *Oncotarget* 7(14):18906–18918. <https://doi.org/10.18632/oncotarget.7753>
- Tormo E, Pineda B, Serna E et al (2015) MicroRNA profile in response to doxorubicin treatment in breast cancer. *J Cell Biochem* 116(9):2061–2073. <https://doi.org/10.1002/jcb.25162>
- Wang T, Du M, Zhang W et al (2019) MicroRNA-432 suppresses invasion and migration via E2F3 in nasopharyngeal carcinoma. *Onco Targets Ther* 12:11271–11280. <https://doi.org/10.2147/ott.s233435>
- Wei YT, Guo DW, Hou XZ, Jiang DQ (2017) miRNA-223 suppresses FOXO1 and functions as a potential tumor marker in breast cancer. *Cell Mol Biol* 63(5):113–118. <https://doi.org/10.14715/cmb/2017.63.5.21>
- Wu X, Chen S, Lu C (2020) Amyloid precursor protein promotes the migration and invasion of breast cancer cells by regulating the MAPK signaling pathway. *Int J Mol Med* 45(1):162–174. <https://doi.org/10.3892/ijmm.2019.4404>
- Zendjabil M, Favard S, Tse C, Abbou O, Hainque B (2017) Les microRNA comme biomarqueurs: quelles perspectives. *C R Biol* 340(2):114–131. <https://doi.org/10.1016/j.crvi.2016.12.001>
- Zubor P, Kubatka P, Dankova Z et al (2018) miRNA in a multiomic context for diagnosis, treatment monitoring and personalized management of metastatic breast cancer. *Future Oncol* 14(18):1847–1867. <https://doi.org/10.2217/fon-2018-0061>

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