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Circular RNA hsa_circ_0103552 Promotes Proliferation, Migration, and Invasion of Breast Cancer Cells through Upregulating Cysteine-Rich Angiogenic Inducer 61 (CYR61) Expression via Sponging MicroRNA-515-5p

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Circular RNAs (circRNAs) exert a significant regulatory function on tumor progression. This work intends to probe into the biological function and regulatory mechanism of circRNA_0103552 (circ_0103552) in breast cancer carcinogenesis. In this study, circ_0103552, microRNA-515-5p (miR-515-5p), and cysteine-rich angiogenic inducer 61 (CYR61) mRNA expressions in breast cancer cells and tissues were determined by quantitative real-time polymerase chain reaction, followed by cell counting kit 8 and Transwell experiments to examine the multiplication, migration, and invasion of breast cancer cells. Circular RNA Interactome database and StarBase database were searched, and dual-luciferase reporter gene experiments were applied to verify the targeting relationship between circ_0103552 and miR-515-5p, and between miR-515-5p and CYR61, and Western blot was adopted to the regulatory function of circ_0103552 and miR-515-5p on CYR61 protein expression. Circ_0103552 expression was found to be remarkably up-modulated in breast cancer tissues and cells, and circ_0103552 overexpression facilitated the multiplication, migration, and invasion of breast cancer cells, while knocking down circ_0103552 induced the opposite effects. Mechanistically, circ_0103552 could sponge miR-515-5p and restrained its expression in breast cancer cells. MiR-515-5p could counteract the functions of circ_0103552 in breast cancer cells. Additionally, CYR61 was revealed to be a downstream target of miR-515-5p in breast cancer cells. In summary, this study shows that circ_0103552 up-modulates CYR61 expression by targeting miR-515-5p and thus facilitates the multiplication, migration, and invasion of breast cancer cells.

Keywords: breast cancer; circular RNA; circ_0103552; cysteine-rich angiogenic inducer 61 (CYR61); microRNA-515-5p

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Introduction

Breast cancer is one of the most common cancers worldwide (Harbeck and Gnant 2017; Nagini 2017). The morbidity of breast cancer is predicted to increase to 85 per 100,000 women by 2021 (Han et al. 2013). Early diagnosis, surgery, and adjuvant therapy are crucial in improving the prognosis of breast cancer patients, but most patients are in advanced stages at the time of diagnosis, resulting in a poor prognosis (Pardo et al. 2016). Hence, it is vital to elucidate the molecular mechanism of the carcinogenesis and progression of and breast cancer, which is necessary for developing novel treatment strategies for breast cancer.

Due to the lack of free 5′ and 3′ends, circular RNAs are resistant to RNase and are more stable than linear RNAs (Patop et al. 2019). CircRNAs play different roles in regulating biological processes, including the sponge of miRNAs, transcriptional regulators, protein translation templates and so on (Ng et al. 2018). CircRNAs are differentially expressed in tumor tissues, and exert crucial regulatory effects in tumorigenesis and cancer progression (He et al. 2017; Wang et al. 2017). CircRNAs have great potential as biomarkers or therapy targets in the diagnosis and treatment of tumors (Wang et al. 2017). Reportedly, circRNA_0103552 (circ_0103552) expression is up-regulated in breast cancer tissues and cells, and circ_0103552 can promote the growth, colony formation, migration, and invasion of breast cancer cells (Yang et al. 2019b).

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Nonetheless, the role of circ_0103552 in breast cancer are far from fully elucidated.

MicroRNAs (miRNAs) are non-coding RNAs consisting of 20-22 nucleotides that are implicated in various biological processes including cell multiplication, apoptosis, and migration (Lee and Dutta 2009). MiRNAs are important modulators in cancer biology. For instance, miR-195 represses the growth and metastasis of colonic cancer cells by down-modulating WNT3A expression (Li et al. 2018). MiR-122 targets P4HA1 and modulates P4HA1 expression to impede the migration, invasion and epithelial-mesenchymal transition (EMT) of ovarian cancer cells (Duan et al. 2018). Reportedly, miRNA-515-5p (miR-515-5p) is significantly lowly-expressed in breast cancer and exerts tumor-suppressive effects (Pinho et al. 2013). Nevertheless, the role and the regulating mechanism of miR-515-5p in breast cancer warrant further investigation.

In this work, we aimed to investigate the function and underlying mechanism of circ_0103552 in the pathogenesis of breast cancer. We found that circ_0103552 could up-modulate cysteine-rich angiogenic inducer 61 (CYR61) expression via sponging miR-515-5p, thereby enhancing breast cancer cell multiplication, migration, and invasion.

Materials and Methods

Clinical tissue samples

All experiments were performed in compliance with the relevant laws and institutional guidelines. This research was approved by the Ethics Committee of Daping Hospital and was conducted in accordance with the Declaration of Helsinki. Forty-two pairs of breast cancer tissue specimens and paracancerous tissue specimens were available from Daping Hospital. All subjects were not subject to radiotherapy or chemotherapy before surgery and signed informed consent. All specimens were frozen in liquid nitrogen immediately after the excision and preserved at −80 °C.

Cell culture

Human normal mammary epithelial cell lines MCF-10A and breast cancer cell lines (MCF7, HCC1937, MDA-MB-231, ZR-75-1, and Bcap-37) were obtained from the Cell Bank of the Committee for Typical Culture Collection, Chinese Academy of Sciences (Shanghai, China). The cells were inoculated into Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% inactivated fetal bovine serum (FBS, HyClone, Logan, UT, USA), and placed in an incubator at 37°C with 5% CO_2 in saturated humidity for routine culture, and the cells were subcultured every 2-3 days. The cells in the logarithmic growth phase were harvested for subsequent experiments.

Cell transfection

Circ_0103552 overexpression plasmid (circ_0103552), three small interfering RNAs targeting circ_0103552 (si-circ_0103552#1, 5′-CTCAAAAGATAAACAGGCTTT-3′; si-circ_0103552#2, 5′-AACAGGCTTTTCATATTCTGG-3′ ; si-circ_0103552#3, 5′-GATAAACAGGCTTTTCATATT-3′), miR-515-5p mimics (5′-UUCUCCAAAAGAAAGCACUU UCUG-3′), miR-515-5p inhibitors (5′- CAGAAAGUGCU UUCUUUUGGAGAA-3′) and corresponding negative controls (NC for circ_0103552, si-NC (5′-GCTGTTACTATAA TTCGCCT-3′) for small interfering RNAs, miR-NC (5′-CGAUCGCAUCAGCAUCGAUUGC-3′) for miR-515-5p mimics, and inhibitors-NC (5′- CUAACGCAUGCA CAGUCGUACG-3′) for miR-515-5p inhibitors) were all provided by GenePharma (Shanghai, China). The cells were transfected using Lipofectamine™3000 (Life Technologies, San Diego, CA, USA) according to the manufacturer's instruction.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from tissues or cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA purity was determined and cDNA was obtained by reverse transcription. qRT-PCR was executed using SYBR Green (Invitrogen). The relative expression levels of the genes were quantified by $2^{-\Delta\Delta Ct}$ method, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and U6 as internal references. The primer sequences were as follows: circ_0103552 forward: 5′-GCCCTCTTTGCAAATCTCTGT-3′, reverse: 5′-TCAGGAGCTTTTTGAAGCTGT-3′; miR-515-5p forward: 5′-CGGGTTCTCCAAAAGAAAGCA-3′, reverse: 5′-CAGCCACAAAAGAGCACAAT-3′; U6 forward: 5′-CTCGCTTCGGCAGCACA-3′, reverse: 5′-AACGCTTCACGAATTTGCGT-3′; CYR61 forward: 5′-CTCCCTGTTTTTGGAATGGA-3′, reverse: 5′-TGGTCTTGCTGCATTTCTTG-3′; GADPH forward: 5′-GAAGGTCGGAGTCAACGGAT-3′, reverse: 5′-TGGAATTTGCCATGGGTGGA-3′.

Subcellular fractionation

RNA in the cytoplasm and nucleus of breast cancer cells were isolated and collected using NE-PER™ Nucleus and Cytoplasm Extraction Reagent (Thermo Fisher Scientific, Shanghai, China) and RNeasy Midi Kit (Qiagen, Dusseldorf, Germany) according to the manufacturer, and qRT-PCR was performed to detect the sub-cellular localization of circ_0103552, with GAPDH as cytoplasmic control and U6 as nucleus control.

Cell counting kit 8 (CCK-8) experiment

The breast cancer cells were transferred in 96-well plates (2×10^3 cells / well). After 12, 24, 48, 72, and 96 h of culture, 10 μL of CCK-8 solution (Beyotime Biotechnology, Shanghai, China) was supplemented to each well, with which the cells were incubated for 4 h, and the absorbance value of the cells at 450 nm was measured by the microplate reader.

Transwell experiment

Cell migration and invasion were examined with Transwell chambers (Corning Inc., Corning, NY, USA) with a pore size of 8 μM. Matrigel (BD Biosciences, San Jose, CA, USA) was employed to cover the filter in invasion assays, while it was not used in the migration assay. Briefly, the transfected cells (suspended in serum-free medium) were transferred into the upper compartment, while RPMI-1640 medium (with FBS) was added to the lower chamber. After 24 h, the migrated or invaded cells were fixed and then stained, and the number of cells which passed the filter was counted under the microscope.

Dual-luciferase reporter gene experiment

All luciferase reporter vectors (circ_0103552 wild type (WT), circ_0103552 mutant (MUT), CYR61 WT, CYR61 MUT) were constructed by Promega (Madison, WI, USA). MCF7 and ZR-75-1 cells were seeded in 48-well plates and cultured to 70% confluence. The luciferase reporter vector was then co-transfected with miR-515-5p mimics into the above cells using Lipofectamine™3000 (Life Technologies). After 48 h, the luciferase activity of the cells was measured using the dual-luciferase reporter gene assay system (Promega, Madison, WI, USA).

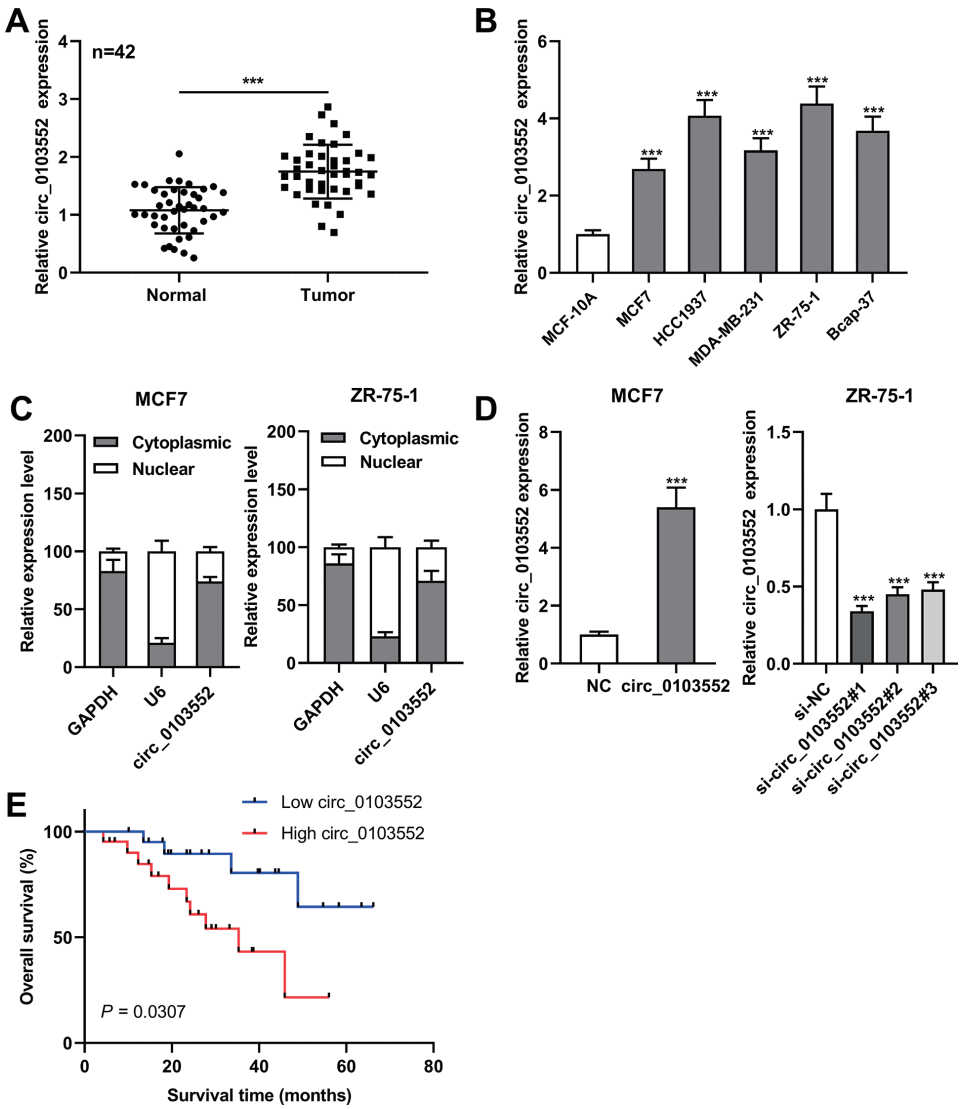

Fig. 1. Circ_0103552 expression was up-modulated in breast cancer tissues and cells.
(A) qRT-PCR was performed to detect the relative expression of circ_0103552 in breast cancer tissues (tumor group) and paracancerous tissues (normal group) (n = 42). (B) qRT-PCR was performed to detect circ_0103552 expression in breast cancer cells (MCF7, HCC1937, MDA-MB-231, ZR-75-1, and Bcap-37) and MCF-10A cells. (C) The expression levels of circ_0103552, U6, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the nucleus and cytoplasm of MCF7 and ZR-75-1 cells were detected by qRT-PCR. (D) qRT-PCR was performed to detect the relative expression of circ_0103552 in MCF7 cells with circ_0103552 overexpression and ZR-75-1 cells with circ_0103552 knockdown. (E) Kaplan-Meier survival curve was used to evaluate the correlation between circ_0103552 expression and the overall survival of breast cancer patients. Error bars represented the mean ± SD of at least three independent experiments. ***$P < 0.001$; compared with the normal group, MCF-10A cells, negative control (NC), or siRNA negative control (si-NC) group.

Table 1. Correlation between circ_0103552 expression and clinicopathological characteristics in breast cancer.

Characteristics	Number (n = 42)	Circ_0103552 expression		χ^2	*P* value
		Low	High		
Age (years)					
< 50	17	10	7	0.889	0.346
≥ 50	25	11	14		
Tumor size					
< 2 cm	18	14	4	9.722	0.002**
≥ 2 cm	24	7	17		
Lymphatic metastasis					
Negative	20	14	6	6.109	0.013*
Positive	22	7	15		
ER status					
Positive	14	6	8	0.429	0.513
Negative	28	15	13		
PR status					
Positive	17	10	7	0.889	0.346
Negative	25	11	14		
HER-2 status					
Positive	10	4	6	0.525	0.469
Negative	32	17	15		
Molecular subtype					
Luminal like	22	12	10	0.515	0.773
HER-2 positive	12	5	7		
Triple negative	8	4	4		

ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor-2.
*$P < 0.05$; **$P < 0.01$.

Western blot

The cells were lysed with RIPA lysis buffer (Beyotime Biotechnology) on the ice for 30 min and then the mixtures were centrifuged at 8,000 × *g* at 4°C for 10 min, and then the supernatants were collected as the protein samples. Protein concentration was determined using a bicinchoninic acid (BCA) kit (Beyotime Biotechnology), and then the protein samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), respectively, and then transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After being blocked with 5% skimmed milk for 1 h, the membranes were incubated with the primary antibodies anti-CYR61 (Abcam, Shanghai, China, ab24448, 1:1,000) and anti-GAPDH (Abcam, ab181602, 1:2,000) at 4°C for 12 h, followed by the incubation with goat anti-rabbit IgG secondary antibody (Abcam, ab6721, 1:5,000) for 1 h at 37°C. A ECL kit (Amersham Pharmacia Biotech, Little Chalfont, UK) was applied for developing the protein bands, and the images were captured with ImageQuant LAS4000 micro-biomolecular imager. GAPDH was used as the internal reference, and the quantification of protein bands was executed using ImageJ software (NIH, Bethesda, MD, USA).

Statistical analysis

Each experiment was conducted three times. SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was employed for statistical analysis, and the experimental data were presented as mean ± standard deviation. Paired *t*-test was used to measure the differences between two groups of paired data that conformed to a normal distribution with equal variances, and unpaired data were tested using unpaired *t*-tests. One-way ANOVA was applied to compare the data among multiple groups. Kaplan-Meier survival curve was used to compare the overall survival time of the patients. The correlation of expression levels between the two genes was analyzed by Pearson's correlation analysis. $P < 0.05$ signified statistical significance.

Results

Circ_0103552 was markedly overexpressed in breast cancer tissues and cells

To probe into the expression characteristics of circ_0103552 in breast cancer, qRT-PCR was employed to examine circ_0103552 expression in breast cancer tissues and cells. The data revealed that circ_0103552 expression was up-modulated in breast cancer tissues relative to paracancerous tissues (Fig. 1A). Moreover, circ_0103552

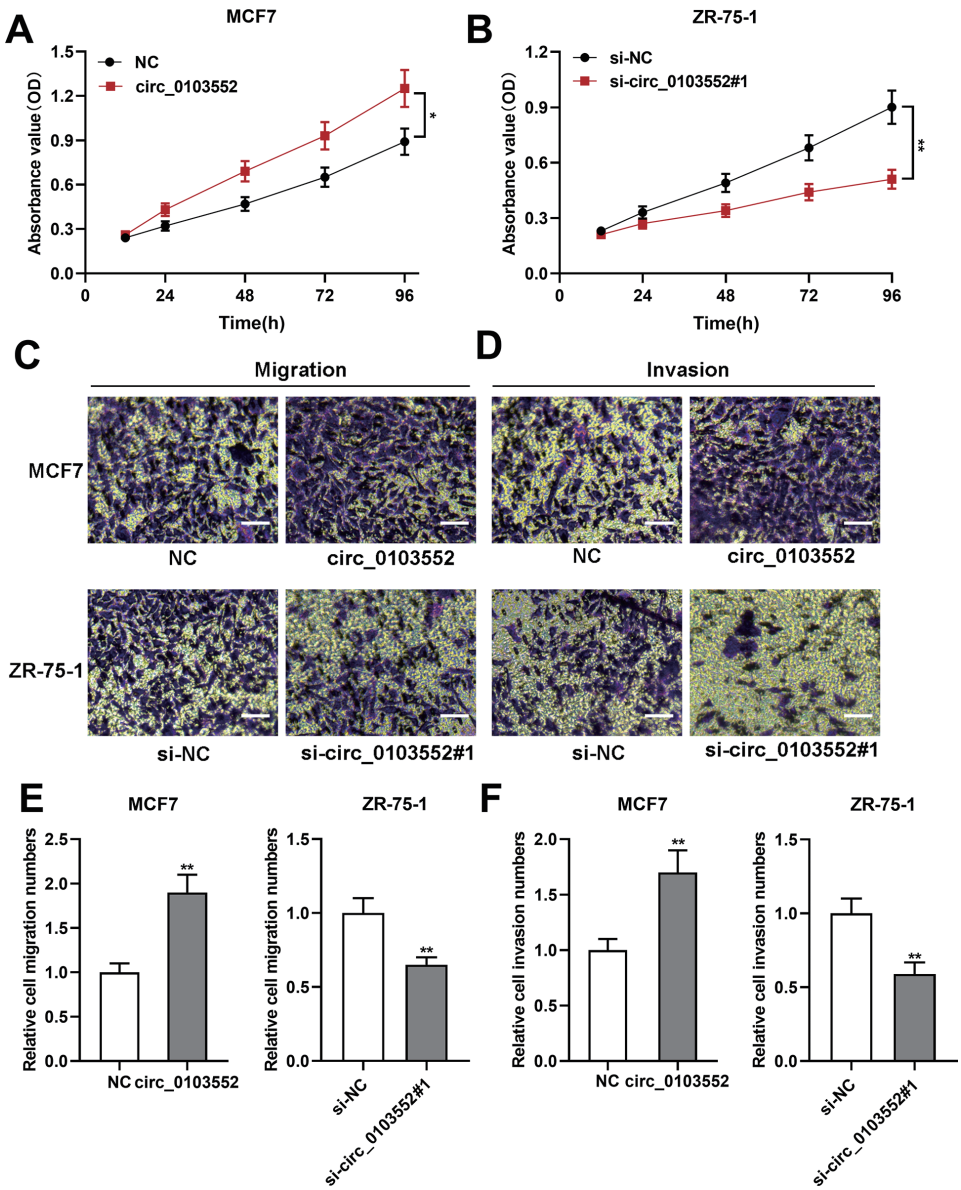

Fig. 2. Circ_0103552 promoted the multiplication, migration, and invasion of breast cancer cells. (A-B) CCK-8 assay was used to detect the effects of circ_0103552 overexpression or knockdown on the multiplication of MCF7 and ZR-75-1 cells. (C-F) Transwell assay was used to detect the effects of circ_0103552 overexpression or knockdown on the migration and invasion of MCF7 and ZR-75-1 cells. Scale bars: 250 μm. Error bars represented the mean ± SD of at least three independent experiments. *$P < 0.05$; **$P < 0.01$; compared with the negative control (NC) or siRNA negative control (si-NC) group.

expression level was markedly elevated in breast cancer cell lines (MCF7, HCC1937, MDA-MB-231, ZR-75-1, and Bcap-37 cells) compared with that in MCF-10A cells (Fig. 1B). Additionally, the subcellular distribution of circ_0103552 was analyzed by qRT-PCR, and it was demonstrated that circ_0103552 was mainly located in the cytoplasm of breast cancer cells (Fig. 1C). To pinpoint the function of circ_0103552 in breast cancer, circ_0103552 overexpression plasmid was transfected into MCF7 cells, while si-circ_0103552 was transfected into ZR-75-1 cells. The transfection efficiency was verified by qRT-PCR (Fig. 1D). Kaplan-Meier survival curve analysis showed that the overall survival time of breast cancer patients with high expression of circ_0103552 was significantly shorter than that of those with low expression of circ_0103552 (Fig. 1E). In addition, Chi-square test manifested that high expression of circ_0103552 was associated with larger tumor size and positive lymphatic metastasis of breast cancer patients (Table 1).

Circ_0103552 enhanced the multiplication, migration, and invasion of breast cancer cells

After the gain-of-function and loss-of-function models were established, the multiplication, migration, and inva-

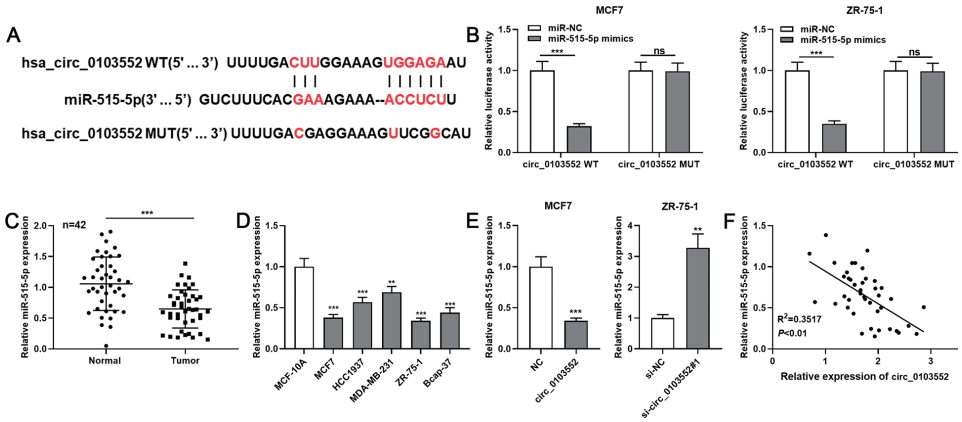

Fig. 3. Circ_0103552 targeted miR-515-5p in breast cancer cells.
(A) Bioinformatics (https://circinteractome.nia.nih.gov/) predicted the binding sites between circ_0103552 and miR-515-5p. (B) Dual-luciferase reporter gene experiments were used to verify the interaction of circ_0103552 and miR-515-5p. (C-D) qRT-PCR was used to detect miR-515-5p expression in breast cancer tissues (tumor group), paracancerous tissues (normal group), breast cancer cells (MCF7, HCC1937, MDA-MB-231, ZR-75-1, and Bcap-37), and MCF-10A cells. (E) qRT-PCR was used to quantify the relative expression of miR-515-5p in MCF7 and ZR-75-1 cells after overexpression and knockdown of circ_0103552. (F) Pearson's analysis of the correlation between circ_0103552 expression and miR-515-5p expression in 42 cases of breast cancer tissues.
Error bars represented the mean ± SD of at least three independent experiments. $^{**}P < 0.01$; $^{***}P < 0.001$; ns, $P > 0.05$; compared with the miR-515-5p mimic negative control (miR-NC), normal group, MCF-10A cells, negative control (NC), or siRNA negative control (si-NC) group. WT, wild type; MUT, mutant.

sion of MCF7 and ZR-75-1 cells were further examined by the CCK-8 and Transwell experiments, respectively. The data revealed that circ_0103552 overexpression enhanced multiplication, migration, and invasion of breast cancer cells, while knocking down circ_0103552 worked oppositely (Fig. 2A-F).

Circ_0103552 specifically targeted miR-515-5p

We then continued to examine the potential mechanism of circ_0103552 in breast cancer progression. Circular RNA Interactome database suggested that miR-515-5p might be one of the functional target miRNAs of circ_0103552 (Fig. 3A). Subsequently, the targeting relationship between circ_0103552 and miR-515-5p was verified by dual-luciferase reporter gene experiments, which revealed that miR-515-5p overexpression markedly repressed the luciferase activity of circ_0103552 WT reporter, whereas it had little effect on the luciferase activity of circ_0103552 MUT reporter (Fig. 3B). Furthermore, qRT-PCR showed that miR-515-5p expression level was remarkably reduced in breast cancer tissues and cell lines (Fig. 3C, D). Additionally, miR-515-5p expression was down-modulated in MCF7 cells with circ_0103552 overexpression, and up-modulated in ZR-75-1 cells with circ_0103552 knockdown (Fig. 3E). Besides, Pearson's correlation analysis unveiled a negative correlation between circ_0103552 and miR-515-5p expressions in breast cancer tissues (Fig. 3F). The above data indicated that circ_0103552 sponged miR-515-5p and negatively modulated its expression in breast cancer cells.

MiR-515-5p impeded the multiplication, migration, and invasion of breast cancer cells

To elaborate on the biological function of miR-515-5p in breast cancer, miR-515-5p inhibitors and mimics were transfected into MCF7 and ZR-75-1 cells, respectively. qRT-PCR verified that the transfection was successful (Fig. 4A). Following that, the role of miR-515-5p on multiplication, migration, and invasion of breast cancer cells was investigated by CCK-8 and Transwell experiments. The data implied that miR-515-5p overexpression suppressed the multiplication, migration, and invasion of breast cancer cells, and inhibiting miR-515-5p promoted these malignant phenotypes (Fig. 4B-G).

Circ_0103552 modulated breast cancer cell multiplication, migration, and invasion by sponging miR-515-5p

To investigate the function of the circ_0103552/miR-515-5p axis in breast cancer progression, miR-515-5p mimics and circ_0103552 were co-transfected into MCF7 cells; miR-515-5p inhibitors and si-circ_0103552#1 were co-transfected into ZR-75-1 cells (Fig. 5A). Moreover, CCK-8 and Transwell experiments were employed to detect cell multiplication, migration, and invasion. The miR-515-5p mimic was revealed to attenuate the promoting effects of circ_0103552 overexpression on the multiplication, migration, and invasion of breast cancer cells; miR-515-5p inhibitor partially counteracted the inhibitory effects of knockdown of circ_0103552 on the malignant biological behaviors of breast cancer cells (Fig. 5B-E).

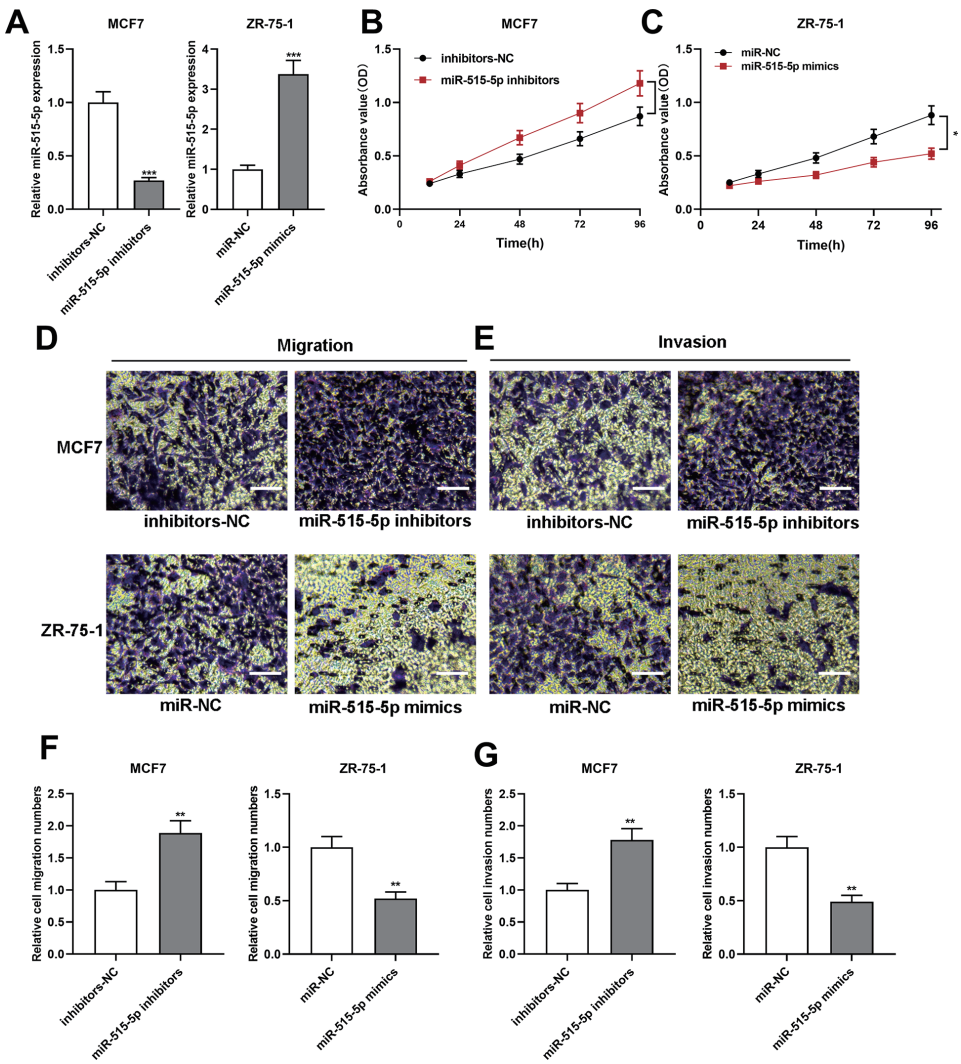

Fig. 4. MiR-515-5p exerted tumor-suppressive effects in breast cancer.
(A) The transfection efficiencies of miR-515-5p inhibitors and miR-515-5p mimics in MCF7 and ZR-75-1 cells were verified by qRT-PCR. (B-C) CCK-8 assay was employed to detect the effects of miR-515-5p mimics and inhibitors on the multiplication of MCF7 and ZR-75-1 cells. (D-G) Transwell experiments detected the migration and invasion of MCF7 and ZR-75-1 cells after the transfection with miR-515-5p inhibitors or mimics. Scale bars: 250 μm. Error bars represented the mean ± SD of at least three independent experiments. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; compared with the miR-515-5p inhibitors negative control (inhibitors-NC) or miR-515-5p mimic negative control (miR-NC) group.

Circ_0103552/miR-515-5p axis was involved in modulating CYR61 expression

CYR61 is reported to facilitate the growth and aggressiveness of breast cancer cells (Hellinger et al. 2019). StarBase database predicted that CYR61 was a possible downstream target of miR-515-5p (Fig. 6A). The targeting relationship between miR-515-5p and CYR61 was further confirmed by dual-luciferase reporter gene experiments (Fig. 6B). The data of qRT-PCR and Western blot showed that circ_0103552 overexpression markedly increased CYR61 mRNA and protein expressions, and this effect was partly attenuated by the transfection of miR-515-5p mimics; besides, knockdown of circ_0103552 significantly repressed CYR61 expression and co-transfected miR-515-5p inhibitors reversed this effect (Fig. 6C-E). Furthermore, CYR61 mRNA was observed to be remarkably overexpressed in breast cancer tissues and cells, and it was also negatively correlated with miR-515-5p expression and positively correlated with circ_0103552 expression in breast cancer tissues (Fig. 6F-I). The above results implied that CYR61 was a downstream target of miR-515-5p in breast cancer cells, and its expression was modulated by circ_0103552/miR-515-5p axis.

Discussion

CircRNAs are first discovered within RNA viruses and are characterized by structural stability, sequence conservatism, and expression specificity (Conn et al. 2015). Accumulating evidence implies that circRNAs are crucial modulators of physiological and pathological processes and

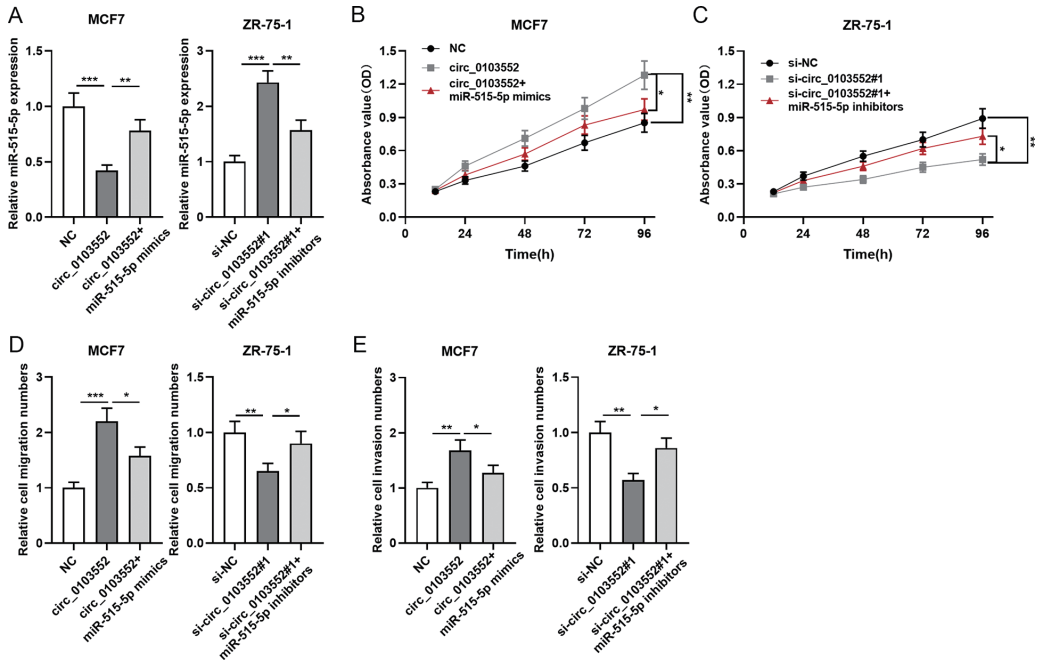

Fig. 5. MiR-515-5p partially reversed the oncogenic effects of circ_0103552 on breast cancer cells.
MCF7 cells were transfected with circ_0103552 overexpression plasmid or co-transfected with circ_0103552 overexpression plasmid and miR-515-5p mimics, and ZR-75-1 cells were transfected with si-circ_0103552#1 or co-transfected with si-circ_0103552#1 and miR-515-5p inhibitors.
(A) The expression of miR-515-5p in MCF7 and ZR-75-1 cells was detected by qRT-PCR. (B-C) CCK-8 assay was used to detect the multiplication of MCF7 and ZR-75-1 cells after the transfection. (D-E) Transwell was applied to detect the migration and invasion of MCF7 and ZR-75-1 cells after the transfection. Error bars represented the mean ± SD of at least three independent experiments. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; compared with the negative control (NC), siRNA negative control (si-NC), circ_0103552 overexpression plasmid (circ_0103552) or targeting circ_0103552 small interfering RNA (si-circ_0103552#1) group.

they enhance or repress the malignancy of tumor cells. For instance, Circ-HuR represses HuR expression and gastric cancer progression by suppressing CNBP activation (Yang et al. 2019a). Circ_0058124 promotes the aggressiveness of papillary thyroid cancer cells through regulating NOTCH3/GATAD2A axis (Yao et al. 2019). A lot of studies report that circRNAs participate in regulating the malignant biological behaviors of breast cancer cells, including viability, apoptosis, cell cycle progression, metastasis and so on (Ojha et al. 2018). For instance, circ_0067934 enhances the multiplication and cell cycle progression of breast cancer cells by up-modulating Mcl-1 expression (Wang et al. 2020). Circ_001569 enhances breast cancer cell multiplication and metastasis through regulating PI3K-AKT pathway (Xu et al. 2019), and Circ-ABCB10 modulates the paclitaxel resistance of breast cancer cells through the let-7a-5p/DUSP7 axis (Yang et al. 2020). In this work, circ_0103552 was observed to be markedly overexpressed in breast cancer tissues and cells, which was consistent with previous findings (Yang et al. 2019b). We also demonstrated that high expression of circ_0103552 was associated with unfavorable pathological characteristics of breast cancer patients and related to the poor prognosis. Additionally, the function of circ_0103552 in enhancing breast cancer cell multiplication, migration, and invasion was further verified with MCF7 and ZR-75-1 cells. These findings indicated the carcinogenic effect of circ_0103552 in breast cancer.

Reportedly, miRNAs feature prominently in cancer progression, including breast cancer, and are implicated in the modulation of cell multiplication, apoptosis, metastasis, and chemoresistance (Loh et al. 2019). Previously, miR-515-5p is found to be lowly expressed in multiple tumors, such as prostate cancer, lung cancer, and bladder cancer, and participates in modulating the malignant biological behaviors of tumor cells (Zhang et al. 2019; Ye et al. 2020; Gong et al. 2020). In breast cancer, miR-515-5p restrains tumorigenesis by modulating MARK4 expression (Pardo et al. 2016). In addition, inhibiting miR-515-5p expression enhances the multiplication and metastasis of breast cancer cells (Pinho et al. 2013; Qiao et al. 2020). Accumulating studies reveal that circRNAs are crucial in tumor progression by acting as ceRNAs to target miRNAs. For instance, circ_0103552 promotes the migration and invasion of thyroid cancer cells via sponge miR-127 (Zheng et al. 2020); circ_0103552 promotes breast cancer cell proliferation and invasion by sponging miR-1236 (Yang et al. 2019b). The present research substantiated that miR-515-5p played a tumor-suppressive role in breast cancer, which was consistent with previous studies (Pinho et al. 2013; Qiao et al.

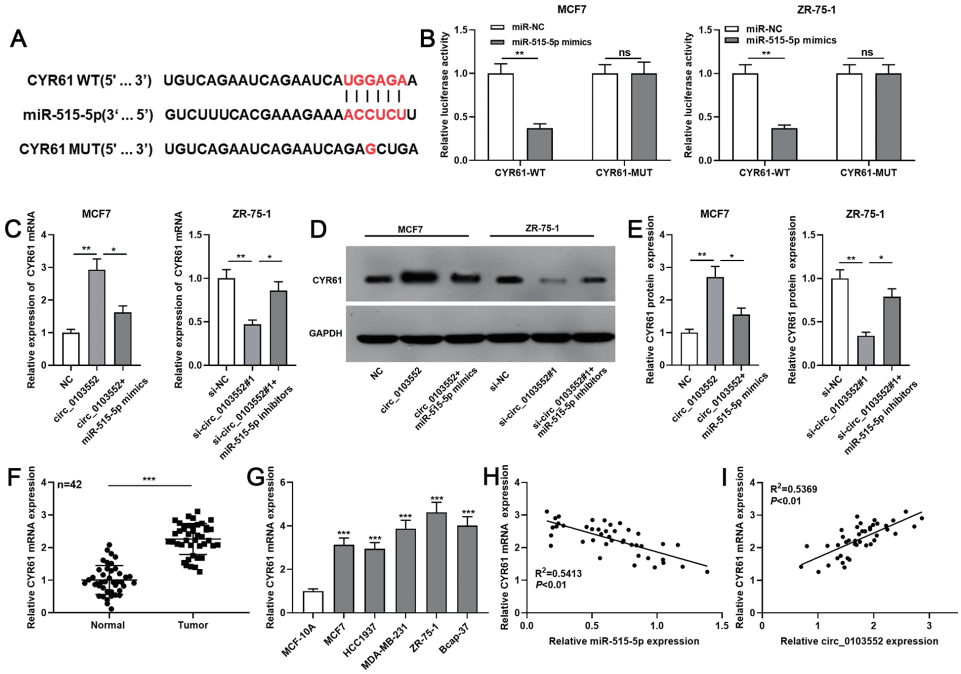

Fig. 6. Circ_0103552 modulated CYR61 expression by targeting miR-515-5p.
(A) StarBase database predicted that CYR61 3'-UTR was a downstream target of miR-515-5p. (B) Dual-luciferase reporter gene experiment validated the binding sequences between miR-515-5p and CYR61 3'-UTR. (C-E) The expressions of CYR61 mRNA and protein in MCF7 and ZR-75-1 cells after transfection were detected by qRT-PCR and Western blot, respectively. (F-G) The expression of CYR61 mRNA in breast cancer tissues (tumor group), paracancerous tissues (normal group), breast cancer cells (MCF7, HCC1937, MDA-MB-231, ZR-75-1, and Bcap-37), and MCF-10A cells was detected by qRT-PCR. (H-I) Pearson's analysis was used to evaluate the correlation between CYR61 mRNA and miR-515-5p expressions, and CYR61 mRNA and circ_0103552 expressions in 42 cases of breast cancer tissues. Error bars represented the mean ± SD of at least three independent experiments. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; ns, $P > 0.05$; compared with the miR-515-5p mimic negative control (miR-NC), negative control (NC), siRNA negative control (si-NC), circ_0103552 overexpression plasmid (circ_0103552), targeting circ_0103552 small interfering RNA (si-circ_0103552#1), normal group, or MCF-10A cells. WT, wild type; MUT, mutant.

2020). Importantly, it was found that circ_0103552 could serve as a ceRNA for miR-515-5p to repress its expression in breast cancer cells. Furthermore, it was revealed that miR-515-5p counteracted the biological function of circ_0103552 in breast cancer cells. These findings indicated that circ_0103552 facilitated the multiplication, migration, and invasion of breast cancer cells by targeting miR-515-5p.

CYR61, also known as cellular communication network factor 1 (CCN1), is a 40 kDa sized secreted protein. Its expression could be induced by growth factor, and it interacts with integrins and heparan sulfate proteoglycan, modulating and remodeling of extracellular matrix, inflammatory response, tissue repair, fibrosis, and the phenotypes of cancer cells (Lau 2016; Kim et al. 2018). Reportedly, CYR61 is markedly overexpressed in colorectal cancer cells and is associated with poor prognosis (Jeong et al. 2014). Additionally, CYR61 enhances the viability of glioblastoma cells by activating the PI3K/Akt/mTor signaling pathway (Cheng et al. 2015). Furthermore, CYR61 is a key regulator of breast cancer progression, and functionally, CYR61 facilitates breast cancer cell migration by up-modulating matrix metalloproteinase-1 expression and activating its receptor (Tsai et al. 2002; Nguyen et al. 2006; O'Kelly et al. 2008). Another study reports that CYR61 mediates the Notch signaling pathway to promote breast cancer cell metastasis (Ilhan et al. 2020). In this work, we reconfirmed that CYR61 was overexpressed in breast cancer tissues and cells, and validated that CYR61 was a downstream target of miR-515-5p. CYR61 mRNA expression in breast cancer tissues was positively correlated with circ_0103552 expression and negatively correlated with miR-515-5p expression. Besides, CYR61 expression in breast cancer cells was modulated by the circ_0103552/miR-515-5p axis. These data support that there is a ceRNA network consisting of circ_0103552, miR-515-5p and CYR61 in the progression of breast cancer.

Taken together, this work reveals that the circ_0103552/miR-515-5p/CYR61 axis facilitates the development of breast cancer. Circ_0103552, as an oncogenic factor, has great potential in breast cancer diagnosis and treatment, and deserves further in-depth study.

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Author Contributions

Xiaohua Zhang and Qi Huang designed the experiments and the structure of article; Qi Huang and Yujun He collected clinical samples and conducted the experiments; Qi Huang, Yujun He, and Lingji Guo analyzed the results; Qi Huang and Xiaohua Zhang wrote the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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