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RUSC1-AS1 promotes the malignant progression of breast cancer depending on the regulation of the miR-326/XRCC5 pathway

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Abstract

Background: Many long noncoding RNAs (lncRNAs) are the key regulators for cancer progression, including breast cancer (BC). RUSC1 antisense 1 (RUSC1-AS1) has been found to be highly expressed in BC, but its role and potential molecular mechanism in BC remain to be further elucidated.

Methods: Quantitative reverse transcription-polymerase chain reaction (RT-PCR) was utilized to measure RUSC1-AS1, microRNA (miR)-326 and X-ray repair cross-complementing group 5 (XRCC5) expression. Cell proliferation, metastasis, cell cycle, apoptosis and angiogenesis were determined by cell counting kit-8, colony formation, transwell, flow cytometry and tube formation assays. Protein expression was detected by western blot analysis. The targeted relationship between miR-326 and RUSC1-AS1 or XRCC5 was validated using dual-luciferase reporter assay and RIP assay. Xenograft models were constructed to uncover the effect of RUSC1-AS1 on BC tumorigenesis.

Results: RUSC1-AS1 was upregulated in BC, and its downregulation suppressed BC proliferation, metastasis, cell cycle, angiogenesis, and tumor growth. MiR-326 was confirmed to be sponged by RUSC1-AS1, and its inhibitor reversed the regulation of RUSC1-AS1 silencing on BC progression. XRCC5 could be targeted by miR-326. Overexpression of XRCC5 reversed the inhibitory impacts of miR-326 on BC progression.

Conclusion: RUSC1-AS1 could serve as a sponge of miR-326 to promote BC progression by targeting XRCC5, suggesting that RUSC1-AS1 might be a target for BC treatment.

KEYWORDS
breast cancer, miR-326, RUSC1-AS1, XRCC5

INTRODUCTION

As the most common malignant tumor in women, breast cancer (BC) incidence ranks first among female malignant tumors.[1,2] With the advancement of life sciences, the treatment methods of BC are constantly being improved and perfected, forming a multidisciplinary comprehensive treatment model with surgery, chemotherapy, radiotherapy, and targeted therapy.[3–5] Nevertheless, BC is still a major problem threatening women's health. Therefore, understanding the pathogenesis of BC and screening key biomarkers that regulate BC progression are of great significance to BC treatment.

Long noncoding RNA (lncRNA) are transcripts longer than 200 nts that do not code for protein, which regulates the expression level of genes in the form of RNA at multiple levels.[6,7] Studies have found that many lncRNAs can be used as the ceRNAs of microRNA (miRNA), thereby antagonizing the inhibition of miRNA on mRNAs to indirectly regulate mRNA expression.[8,9] The ceRNA mechanisms of many lncRNAs have been confirmed in BC. LncRNA DANCR has been reported to facilitate BC proliferation and metastasis

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by regulating VAPB expression via sponging miR-4319.[10] LncRNA CRRS6-AS1 has been found to be highly expressed in BC, which could promote BC proliferation and tumor growth via the miR-125a-5p/BAP1 axis.[11] Additionally, lncRNA SATB2-AS1 has been discovered to be downregulated in BC, and sponge miR-155–3p to inhibit BC cell progression and tumor growth through BRMS1L.[12]

RUSC1 antisense 1 (RUSC1-AS1) is a newly discovered lncRNA in recent years, and its abnormally high expression has been found in many cancers. RUSC1-AS1 has been found to enhance cell proliferation and metastasis in cervical cancer[13] and hepatocellular carcinoma,[14] and has been shown to be an underlying prognostic biomarker for laryngeal squamous cell carcinoma.[15] Hu et al. suggested that RUSC1-AS1 has significantly high expression in BC, and its promotion effect on BC progression suggests that RUSC1-AS1 might be an important target for BC therapy.[16] However, the molecular mechanism by which RUSC1-AS1 regulates BC progression is still unclear. Our research puts forward and confirms the hypothesis that RUSC1-AS1 regulates BC progression through the miRNA/mRNA axis. Exploring the molecular mechanism of RUSC1-AS1 is expected to provide more evidence for RUSC1-AS1 to become a potential target for BC treatment.

METHODS

Clinical tissues

A total of 30 patients with BC were recruited from the Affiliated Cancer Hospital of Xinjiang Medical University. A total of 30 paired BC tumor tissues and adjacent normal tissues were collected and stored at −80°C. The study was approved by the Ethics Committee of Affiliated Cancer Hospital of Xinjiang Medical University and was carried out according to the guidelines of Declaration of Helsinki. All patients signed written informed consent.

Immunohistochemistry staining

Tissues were prepared for paraffin-embedded sections. After that, the sections were dewaxed and rehydrated before treatment with 3% hydrogen peroxide solution. The sections were incubated with Ki67 antibody (1:50, Solarbio) or X-ray repair cross-complementing group 5 (XRCC5) antibody (1:100, Solarbio), followed by hatching with Goat Anti-Rabbit IgG antibody (1:1000, GeneTex). After the sections were stained with hematoxylin, the Ki67 and XRCC5 positive cells were observed under a microscope.

Cell culture and transfection

Human normal breast epithelial cells (MCF-10A) and BC cells (MCF-7 and MDA-MB-231) were obtained from ATCC (Manassas). All cells were cultured in RPMI-1640 medium (Gibco) containing 10% FBS (Gibco) and 1% double antibiotics (Sangon) at 37°C in a 5% CO_2 incubator.

RUSC1-AS1 siRNA and shRNA (si-RUSC1-AS1 and sh-RUSC1-AS1), miR-326 mimic and inhibitor, pcDNA XRCC5 overexpression vector (pc-XRCC5), or their negative controls, were constructed by Ribobio. When cells were cultured to 50%–60% confluence, lipofectamine 3000 reagent (Invitrogen) was used to transfect them into cells.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

TRIzol reagent (Invitrogen) was utilized to extract total RNA, and miScript reverse transcription kit was used to synthesis cDNA. QRT-PCR was carried out on PCR system using Power SYBR Green Kit (Takara). GAPDH (for lncRNA and mRNA) or U6 (for miRNA) was used as an internal reference, and data were examined using the $2^{-\Delta\Delta CT}$ method. The sequences of primers were shown as below: RUSC1-AS1, F 5′-TGTCAGTGCCAATTTTGCCG-3′, R 5′-AGGTGTATTGCAGACAGGGC-3′; miR-326, F 5′-GCCGAGCCTCTGGGCCCTTC-3′, R 5′-CTCAACTGGTGTCGTGGA-3′; XRCC5, F 5′-TGACTTCCTGGATGCACTAATCG-3′, R 5′-CCTAAGCGAAAGGGGCCAT-3′; GAPDH, F 5′-GACAGTCAGCCGCATCTTCT-3′, R 5′-GCGCCCAATACGACCAAATC-3′; U6, F 5′-CAGCACATATACTAAAATTGGAACG-3′, R 5′-ACGAATTTGCGTGTCATCC-3′.

Cell counting kit-8 assay

Breast cancer cells were plated into 96-well plates. After 48 h, the cells were incubated with cell counting kit-8 (CCK-8) solution (Dojindo). Absorbance value was determined at 450 nm using a microplate reader.

Colony formation assay

Breast cancer cells were seeded in a six-well plate (200 cells/well) and cultured for 2 weeks. The colonies were fixed with paraformaldehyde, stained with crystal violet solution, and then counted under a microscope.

Transwell assay

We used 24-well transwell chambers (8 μm, Corning Inc.) for cell migration and invasion. Additionally, the upper chamber was precoated with Matrigel (Corning Inc.) for the invasion assay. BC cells in serum-free medium were added to the upper chamber, and serum medium was added to the lower chamber. Then, 24 h later, the cells were photographed and counted using a microscope (100×) after staining with crystal violet.

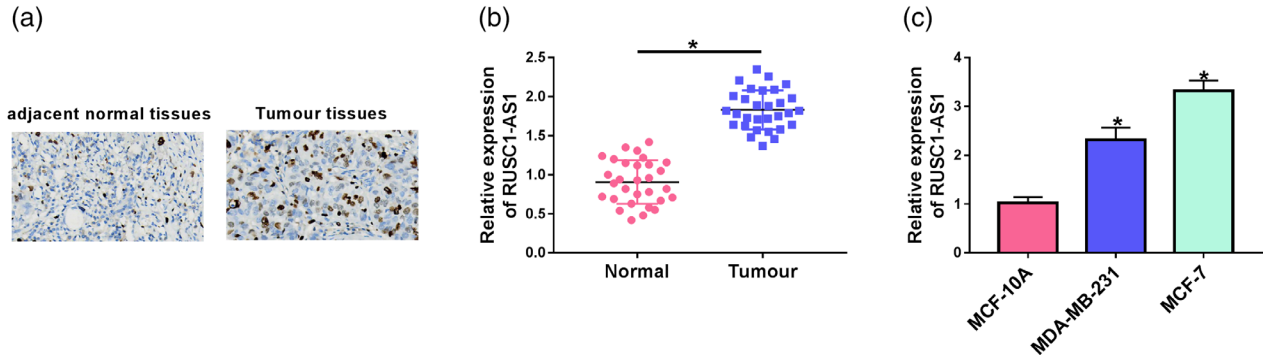

FIGURE 1 Upregulation of RUSC1-AS1 in breast cancer (BC). (a) Immunohistochemistry staining was used to assess the Ki67 positive cells in BC tumor tissue and adjacent normal tissues. (b) RUSC1-AS1 expression in BC tissues and adjacent normal tissues was detected by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). (c) RUSC1-AS1 expression in MCF-10A cells and both BC cells was measured by qRT-PCR. $^*p < 0.05$.

Flow cytometry

BC cells were harvested and washed with cold phosphate-buffered saline (PBS). For the cell cycle, the cells were fixed in 70% cold ethanol solution (diluted with PBS), and then incubated with RNase A and propidine iodide (PI) staining buffer according to the instructions of the cell cycle detection kit (Haigene). For cell apoptosis, cells were suspended in binding buffer, and then stained with annexin V-FITC and PI (BestBio). The cell cycle and apoptosis rate were examined using a flow cytometer.

Tube formation assay

The BC cell media were collected and the supernatants obtained to prepare the conditioned medium. Human umbilical vein endothelial cells were suspended with conditioned medium and seeded into 24-well plates coated with 100 μL Matrigel (Corning Inc.). The images were collected under a microscope, and the number of branches were counted after 24 h using Image J angiogenesis analyzer.

Western blot (WB) analysis

Proteins were isolated using radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime). Protein samples (30 μg) were segregated by SDS-PAGE gel and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore). The membranes were blocked in skim milk, and then incubated with primary antibodies: anti-E-cadherin (1:5000, GeneTex), anti-N-cadherin (1:3000, GeneTex), anti-fibronectin (1:3000, GeneTex), anti-XRCC5 (1:2000, Solarbio) or anti-GAPDH (1:10000, GeneTex). Then, the membranes were reacted with secondary antibody (Goat Anti-Rabbit IgG, 1:50000, GeneTex). Finally, protein bands were examined using a western blotting detection kit (Solarbio).

Dual-luciferase reporter assay

The miR-326 binding sequences and matching mutant sequences in RUSC1-AS1 or the 3′UTR of XRCC5 were inserted into the pGL3 reporter vector, which were termed as the WT/MUT-RUSC1-AS1 vector or WT/MUT-XRCC5-3′UTR vector. BC cells were transfected with miR-326 mimic and the above vectors for 48 h. Luciferase activity was determined using a dual-luciferase reporter assay kit (Beyotime).

Radioimmunoprecipitation assay

According to the instruments of Magna RIP Kit (Millipore), BC cells were lysed with RIP buffer, and then the cell lysate was incubated with magnetic beads precoated with anti-Ago2 or anti-IgG. QRT-PCR was applied to measure RNA enrichment in the immunoprecipitated complexes. The RNA extracted from cell lysates nonincubated with magnetic beads was used as Input.

Xenograft models

MDA-MB-231 cells transfected with sh-RUSC1-AS1 or sh-NC were subcutaneously injected into the back of BALB/c nude mice ($n = 5$/group) (female, 5-week-old; Vital River, Beijing, China). The tumor length and width were measured once a week when the tumor grew to 100 mm^3 to calculate tumor volume. The mice were euthanized after 4 weeks and the tumors were obtained for further analysis. Animal experiments were ratified by the Ethics Committee of Affiliated Cancer Hospital of Xinjiang Medical University and performed in accordance

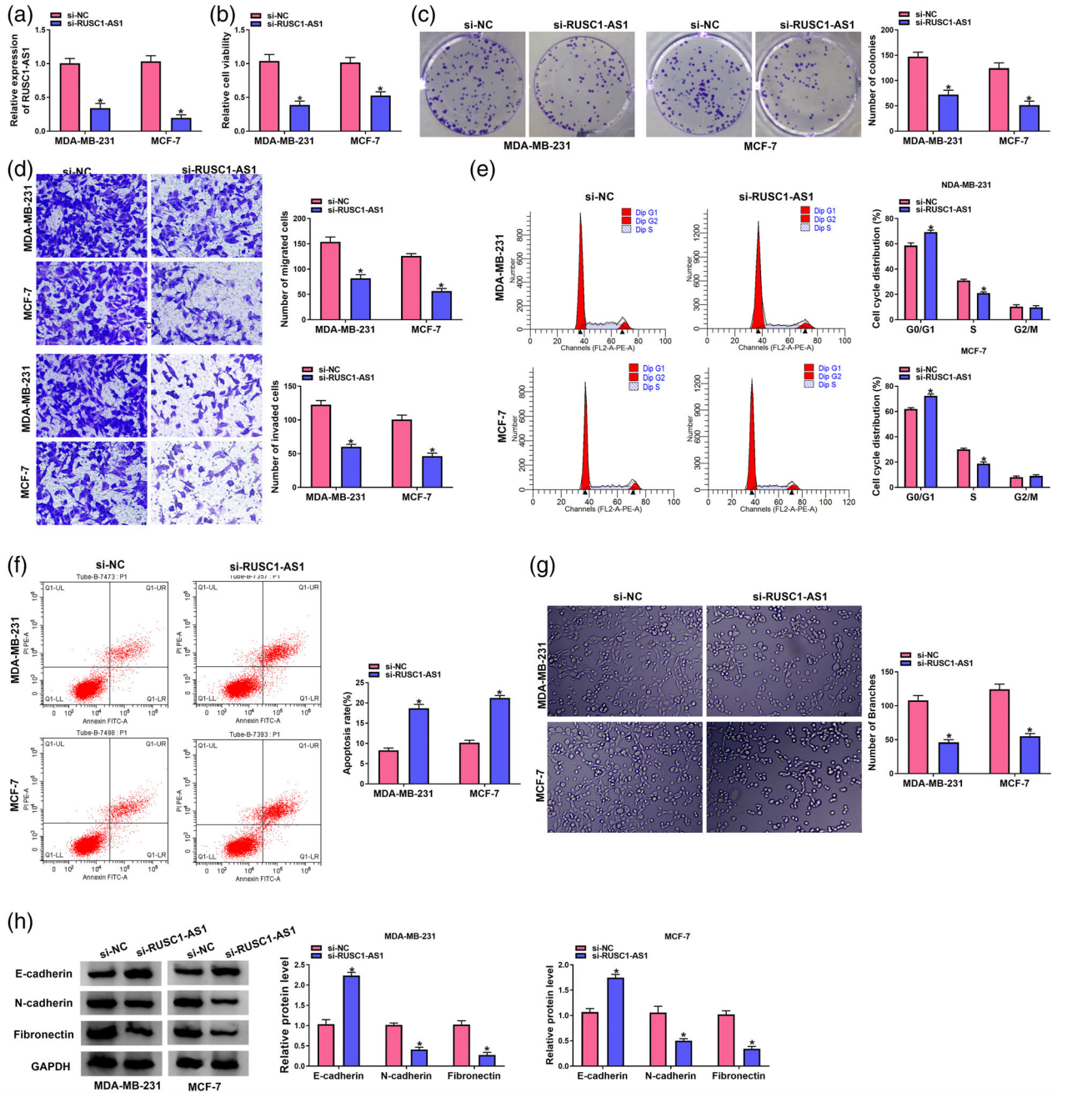

FIGURE 2 Silencing of RUSC1-AS1 inhibited breast cancer (BC) cell progression. BC cells were transfected with si-NC or si-RUSC1-AS1. (a) RUSC1-AS1 expression was measured by qRT-PCR. (b) Cell counting kit-8 (CCK-8) assay, (c) colony formation assay, (d) transwell assay, (e, f) flow cytometry and (g) tube formation assay were used to determine cell viability, number of colonies, number of migrated and invaded cells, cell cycle, apoptosis, and number of branches. (h) E-cadherin, N-cadherin and fibronectin protein levels were detected by WB analysis. *$p < 0.05$.

with the guidelines of the National Animal Care and Ethics Institution.

Statistical analysis

Data are expressed as mean ± SD from three independent experiments. A student's *t*-test and one-way ANOVA followed by Tukey's test were used to analyze the differences. GraphPad Prism 7.0 was used to analyze the data. Statistically significant differences were defined as $p < 0.05$.

RESULTS

RUSC1-AS1 was highly expressed in BC

Immunohistochemistry (IHC) staining results showed that the Ki67 positive cells in BC tissue were significantly higher

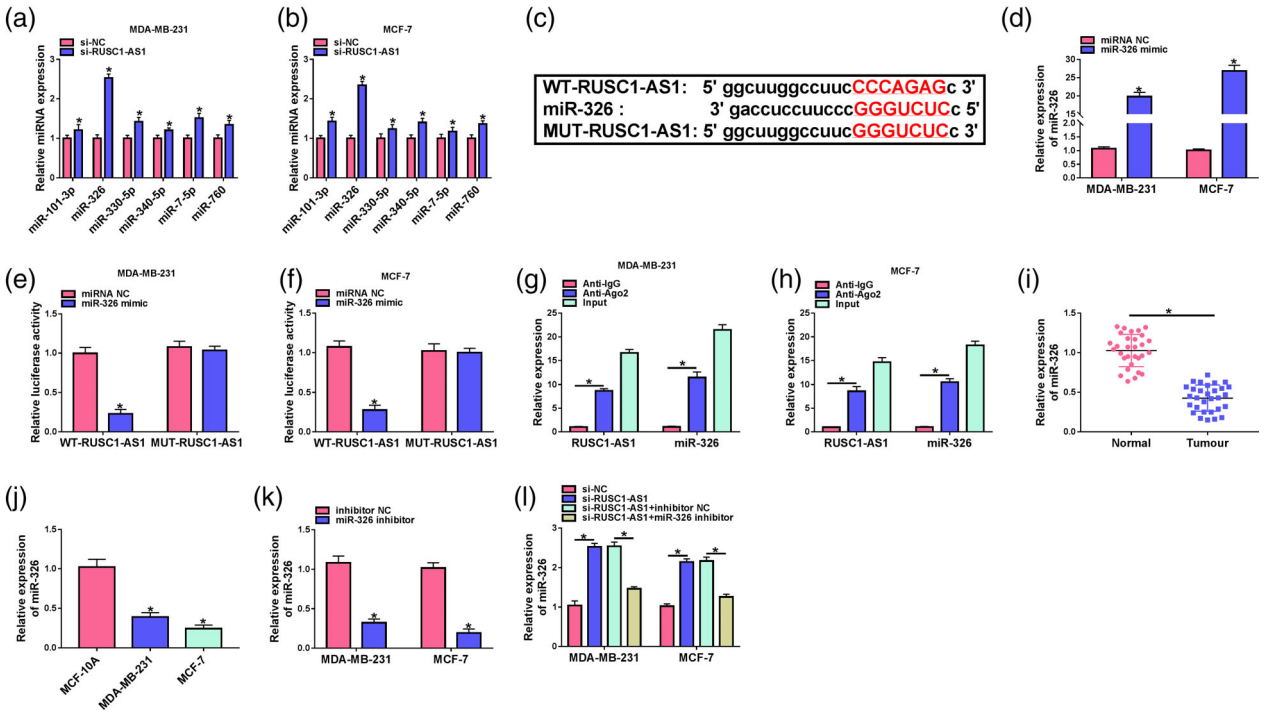

FIGURE 3 MiR-326 was sponged by RUSC1-AS1. (a, b) The expression of candidate miRNAs was measured by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) in breast cancer (BC) cells transfected with si-NC or si-RUSC1-AS1. (c) The RUSC1-AS1 and miR-326 binding sites predicted by starBase online software are shown. (d) The transfection efficiency of miR-326 mimic was assessed by detecting miR-326 expression using qRT-PCR. (e, f) Dual-luciferase reporter assay and (g, h) radioimmunoprecipitation (RIP) assay were performed to verify the interaction between RUSC1-AS1 and miR-326. (i) QRT-PCR was used to measure miR-326 expression in BC tissues and adjacent normal tissues. (j) The expression of miR-326 in MCF-10A cells and both BC cells was detected by qRT-PCR. (k) The transfection efficiency of miR-326 inhibitor was evaluated by measuring miR-326 expression using qRT-PCR. (l) BC cells were transfected with si-NC, si-RUSC1-AS1, si-RUSC1-AS1 + inhibitor NC or si-RUSC1-AS1 + miR-326 inhibitor. The miR-326 expression was determined using qRT-PCR. $^*p < 0.05$.

than that in the adjacent normal tissues, indicating that the proliferation ability of tumor cells was strong (Figure 1a). RUSC1-AS1 was notably upregulated in BC tissues compared to adjacent normal tissues (Figure 1b). Similarly, RUSC1-AS1 expression also was higher in both BC cells than in MCF-10A cells (Figure 1c). Therefore, we confirmed that RUSC1-AS1 might have an important role in BC.

Silencing RUSC1-AS1 inhibited the proliferation, metastasis, cell cycle, angiogenesis, and promoted the apoptosis of BC cells

In view of the high expression of RUSC1-AS1 in BC, we explored the role of RUSC1-AS1 in BC by decreasing its expression. After transfecting with si-RUSC1-AS1 into MDA-MB-231 and MCF-7 cells, RUSC1-AS1 expression was markedly reduced (Figure 2a). RUSC1-AS1 knockdown repressed cell viability and the number of colonies (Figure 2b,c), indicating that RUSC1-AS1 promoted BC cell proliferation. Furthermore, the numbers of migrated and invaded cells were also decreased by RUSC1-AS1 knockdown (Figure 2d). Moreover, we found that silencing RUSC1-AS1 induced cell cycle arrest in G0/G1 phase (Figure 2e). In addition, the cell apoptosis rate was markedly enhanced in the presence of si-RUSC1-AS1 (Figure 2f). Tube formation assay showed that the number of branches was significantly inhibited after RUSC1-AS1 knockdown, suggesting that RUSC1-AS1 might enhance the angiogenesis of BC cells (Figure 2g). To further evaluate the role of RUSC1-AS1 on BC metastasis, we determined the protein levels of epithelial-mesenchymal transition (EMT) markers and discovered that RUSC1-AS1 silencing increased E-cadherin level and decreased N-cadherin and fibronectin levels (Figure 2h). These data confirmed that RUSC1-AS1 played an active role in BC progression.

MiR-326 was a targeted by RUSC1-AS1

To explore the mechanism of RUSC1-AS1 in BC, the starBase online software was used to predict the targeted miRNAs of RUSC1-AS1. Through preliminary screening, we selected 6 miRNAs reported to play a role in BC as candidate miRNAs (miR-101-3p, miR-326, miR-330-5p, miR-340-5p, miR-7-5p and miR-760). After RUSC1-AS1 knockdown, we detected the expression of each miRNA and found that miR-326 expression was increased most significantly, so miR-326 was selected as the target of RUSC1-AS1

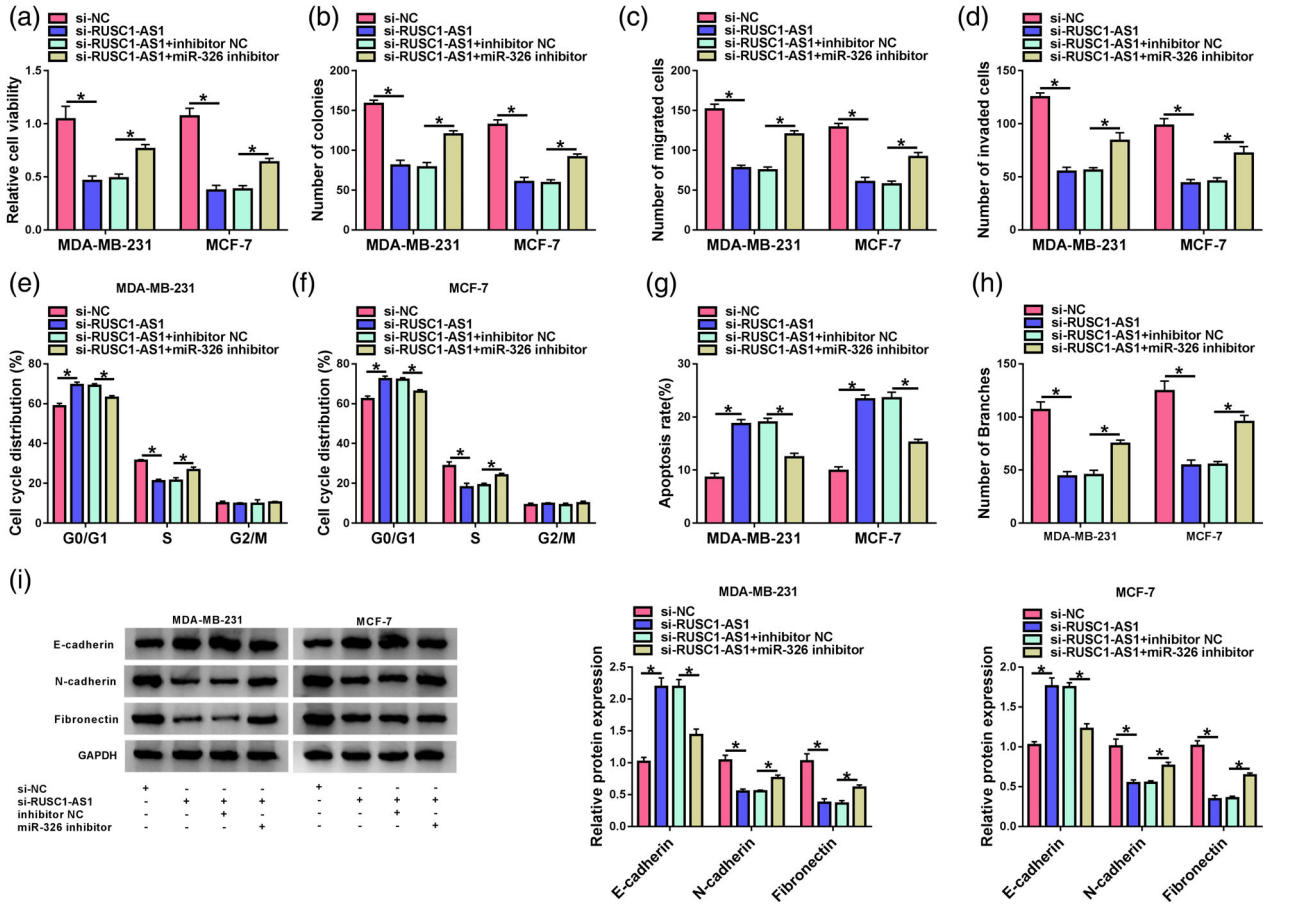

FIGURE 4 RUSC1-AS1 regulated breast cancer (BC) progression by sponging miR-326. BC cells were transfected with si-NC, si-RUSC1-AS1, si-RUSC1-AS1 + inhibitor NC or si-RUSC1-AS1 + miR-326 inhibitor. (a) Cell counting kit-8 (CCK8) assay, (b) colony formation assay, (c, d) transwell assay, (e–g) flow cytometry and (h) tube formation assay (h) were performed to measure the cell viability, the number of colonies, the numbers of migrated and invaded cells, cell cycle, apoptosis, and the number of branches. (i) Western blot analysis was used to detect E-cadherin, N-cadherin and fibronectin protein levels. $^*p < 0.05$.

for study (Figure 3a,b). The binding site between miR-326 and RUSC1-AS1 is shown in Figure 3c. To further confirm the interaction between miR-326 and RUSC1-AS1, miR-326 mimic was constructed and its transfection efficiency was confirmed by detecting miR-326 expression in BC cells transfected with miR-326 mimic (Figure 3d). Dual-luciferase reporter assay revealed that miR-326 overexpression inhibited the luciferase activity of WT-RUSC1-AS1 vector, while it had no effect on the MUT-RUSC1-AS1 vector (Figure 3e,f). RIP assay also showed that RUSC1-AS1 and miR-326 were markedly enriched in anti-Ago2 (Figure 3g,h). In addition, we discovered that miR-326 was remarkably downregulated in BC tissues and cells (Figure 3i,j). In order to further confirm that RUSC1-AS1 regulated BC progression by sponging miR-326, we also constructed miR-326 inhibitor for rescue experiments. The results of Figure 3k show that miR-326 inhibitor could effectively inhibit the expression of miR-326. After the cotransfection of si-RUSC1-AS1 and miR-326 inhibitor into MDA-MB-231 and MCF-7 cells, we found that the promotion effect of RUSC1-AS1 on miR-326 expression was reversed by the miR-326 inhibitor (Figure 3l). All data suggested that RUSC1-AS1 could sponge miR-326.

MiR-326 inhibitor reversed the negatively regulation of RUSC1-AS1 silencing on BC progression

Function experiments revealed that miR-326 inhibitor partially reversed the inhibitory effects of RUSC1-AS1 knockdown on cell viability (Figure 4a), the number of colonies (Figure 4b and Figure S1a), and the number of migrated and invaded cells (Figure 4c,d and Figure S1b,c) in BC cells. Furthermore, the blocking effect of RUSC1-AS1 silencing on cell cycle process was also reversed by miR-326 inhibitor (Figure 4e,f and Figure S1d). The promotion of RUSC1-AS1 knockdown on cell apoptosis rate also was abolished by miR-326 inhibitor (Figure 4g and Figure S1e). Also, the number of branches suppressed by RUSC1-AS1 silencing was also recovered by the miR-326

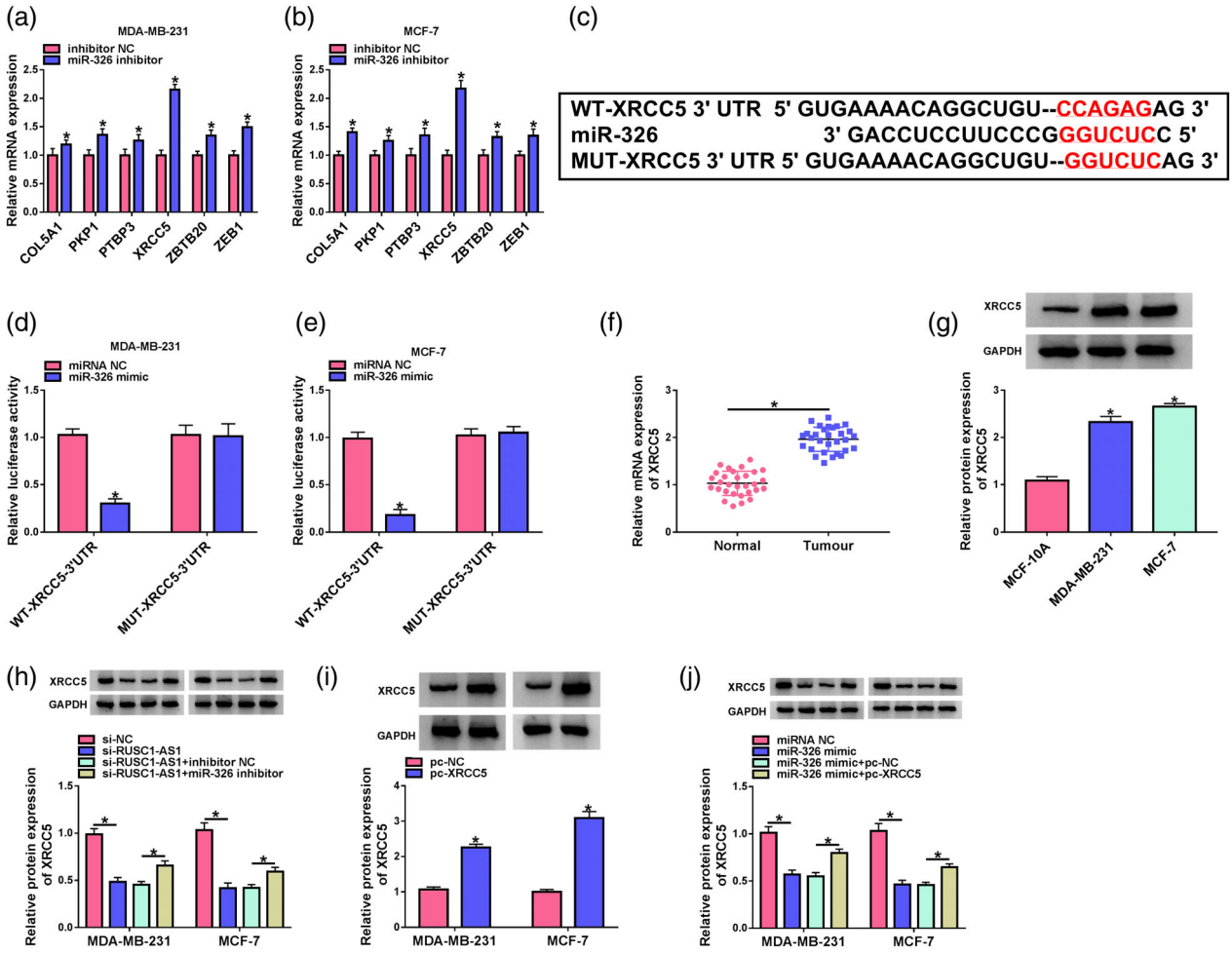

FIGURE 5 XRCC5 was a target of miR-326. (a, b) The expression of candidate targets was tested by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) in breast cancer (BC) cells transfected with inhibitor NC or miR-326 inhibitor. (c) The binding sites between miR-326 and XRCC5 3′UTR predicted by TargetScan online software are shown. (d, e) Dual-luciferase reporter assay was used to confirm the interaction between miR-326 and XRCC5 3′UTR. (f) The mRNA expression of XRCC5 in BC tissues and adjacent normal tissues was examined by qRT-PCR. (g) The protein expression of XRCC5 in MCF-10A cells and both BC cells was determined using western blot (WB) analysis. (h) XRCC5 protein expression was tested by WB analysis in BC cells transfected with si-NC, si-RUSC1-AS1, si-RUSC1-AS1 + inhibitor NC or si-RUSC1-AS1 + miR-326 inhibitor. (i) The transfection efficiency of pc-XRCC5 was assessed by measuring XRCC5 protein expression using WB analysis. (j) BC cells were transfected with miRNA NC, miR-326 mimic, miR-326 mimic + pc-NC or miR-326 mimic + pc-XRCC5. WB analysis was used to detect XRCC5 protein expression. $^*p < 0.05$.

inhibitor (Figure 4h). Additionally, WB analysis results showed that the increasing effect of RUSC1-AS1 silencing on E-cadherin protein level and the suppressive on N-cadherin and fibronectin protein levels could also be reversed by miR-326 inhibitor (Figure 4i). These results showed that RUSC1-AS1 sponged miR-326 to regulate BC progression.

XRCC5 was a target of miR-326

MiRNA has been found to affect cell biological functions by binding to the 3′UTR of mRNA. Using the TargetScan online software, we screened out six genes reported to play a vital role in BC as candidate targets (COL5A1, PKP1, PTBP3, XRCC5, ZBTB20 and ZEB1). After transfection with miR-326 inhibitor into BC cells, we discovered that XRCC5 expression was most significantly increased (Figure 5a,b). Therefore, we selected XRCC5 as a target of miR-326 for the study. The binding and mutant sites between miR-326 and XRCC5 3′UTR are presented in Figure 5c. In addition, dual-luciferase reporter assay results showed that the luciferase activity in WT-XRCC5-3′UTR vector was decreased by miR-326 overexpression, but that in the MUT-XRCC5-3′UTR vector did not change (Figure 5d,e). In BC tissues, the expression of XRCC5 was notably higher than that in adjacent normal tissues (Figure 5f). By detecting XRCC5 protein expression in BC cells, we found that XRCC5 was also upregulated in both BC cells compared to MCF-10A cells (Figure 5g). To evaluate the regulation of RUSC1-AS1 on XRCC5, we determined XRCC5 protein expression in BC cells cotransfected with si-RUSC1-AS1 and miR-326 inhibitor. The results indicated that silencing RUSC1-AS1 could markedly inhibit XRCC5 expression, while this effect could be reversed by miR-326

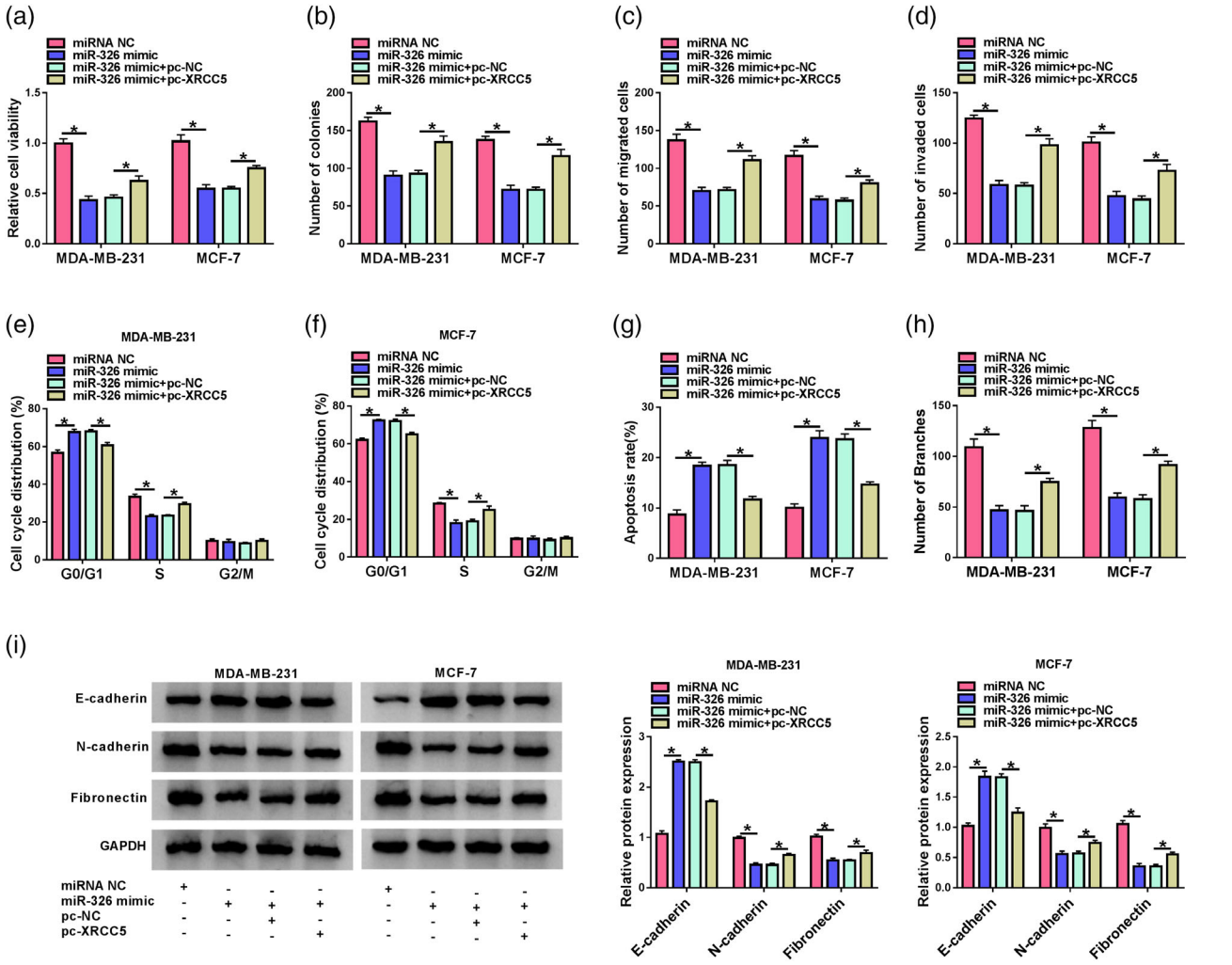

FIGURE 6 MiR-326 regulated breast cancer (BC) progression by targeting XRCC5. Breast cancer (BC) cells were transfected with miRNA NC, miR-326 mimic, miR-326 mimic + pc-NC or miR-326 mimic + pc-XRCC5. The cell viability, number of colonies, number of migrated and invaded cells, cell cycle, apoptosis, and number of branches were determined using cell counting kit-8 (CCK-8) assay (a), colony formation assay (b), transwell assay (c, d), flow cytometry (e–g) and tube formation assay (h). (i) Western blot analysis was performed to examine E-cadherin, N-cadherin and fibronectin protein levels. $^*p < 0.05$.

inhibitor (Figure 5h). In further experiments, we constructed the pc-XRCC5 and confirmed that pc-XRCC5 could enhance XRCC5 expression in BC cells (Figure 5i). Then, miR-326 mimic and pc-XRCC5 were cotransfected into BC cells. Through measuring XRCC5 expression, we uncovered that miR-326 overexpression obviously decreased XRCC5 protein expression, and the addition of pc-XRCC5 partially reversed this effect (Figure 5j). Hence, we confirmed that RUSC1-AS1 could regulate XRCC5 by sponging miR-326.

Overexpression of XRCC5 reversed the regulation of miR-326 on BC progression

MiR-326 overexpression suppressed cell viability and the number of colonies in BC cells, while this effect could be abolished by XRCC5 overexpression (Figure 6a,b and Figure S2a). Moreover, overexpressed XRCC5 also reversed the inhibitory effect of miR-326 on the number of migrated and invaded BC cells (Figure 6c,d and Figure S2b,c). Upregulated XRCC5 also inverted the negative regulation of miR-326 on cell cycle and the promotion effect on the apoptosis rate of BC cells (Figure 6e–g and Figure S2d,e). In addition, the decreasing effect of miR-326 overexpression on the number of branches could also be abolished by XRCC5 overexpression (Figure 6h). Furthermore, miR-326 enhanced E-cadherin protein expression and hindered N-cadherin and fibronectin protein expression in BC cells. However, the regulation of miR-326 on the protein expression of E-cadherin, N-cadherin and fibronectin could also be reversed by overexpressing XRCC5 (Figure 6i). These results illuminated that miR-326 indeed targeted XRCC5 to participate in BC progression.

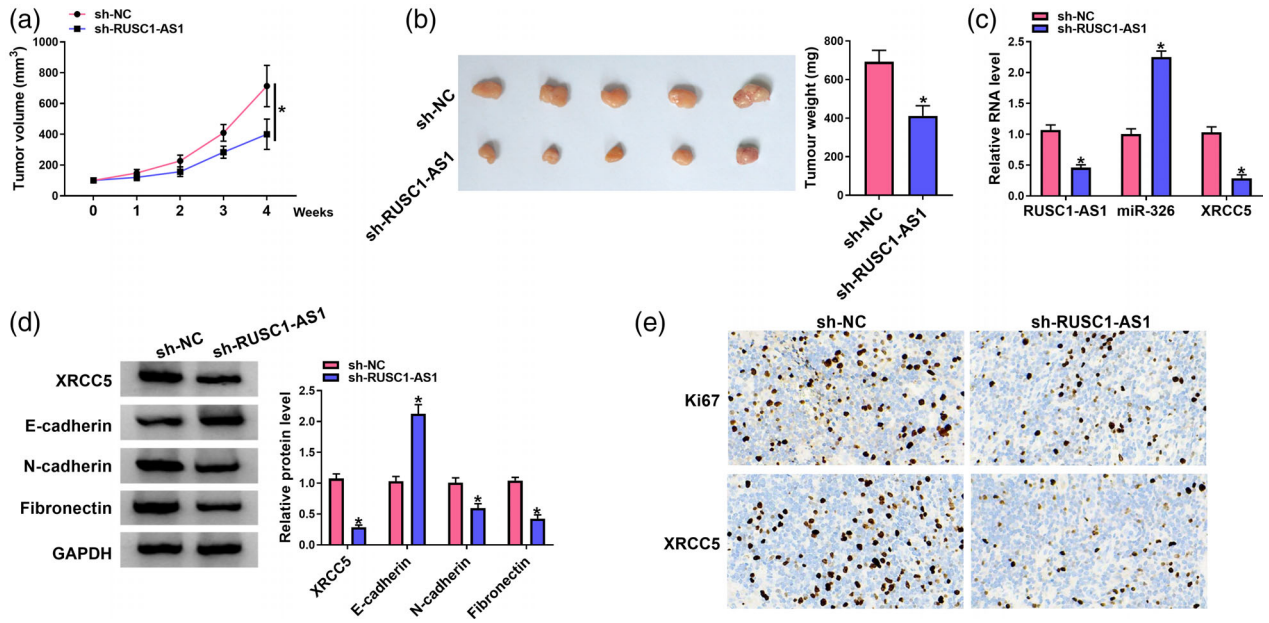

FIGURE 7 Knockdown of RUSC1-AS1 inhibited the tumorigenesis of breast cancer (BC) in vivo. (a) The tumor volume was measured every week. (b) After 4 weeks, the tumor was photographed and weighed. (c) RUSC1-AS1, miR-326 and XRCC5 expression levels were detected by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). (d) XRCC5, E-cadherin, N-cadherin and fibronectin protein levels were determined by western blot analysis. (e) Immunohistochemical (IHC) staining was used to detect the Ki67 and XRCC5 positive cells in each group. $^*p < 0.05$.

Knockdown of RUSC1-AS1 inhibited the tumorigenesis of BC in vivo

To further evaluate the role of RUSC1-AS1 in BC, we constructed a subcutaneous xenograft tumor for BC. Our data showed that silencing RUSC1-AS1 could markedly reduce the tumor volume and tumor weight (Figure 7a,b). In the tumor tissues of the sh-RUSC1-AS1 group, we found that RUSC1-AS1 and XRCC5 expression was obviously decreased, while miR-326 expression was remarkably increased (Figure 7c). In addition, the protein levels of XRCC5, N-cadherin and fibronectin were significantly reduced, while the protein level of E-cadherin was notably improved in the sh-RUSC1-AS1 group (Figure 7d). Furthermore, IHC staining revealed that the Ki67 and XRCC5 positive cells were markedly decreased in the tumor tissues of the sh-RUSC1-AS1 group (Figure 7e). Therefore, our results revealed that RUSC1-AS1 could promote the tumorigenesis of BC via regulating the miR-326/XRCC5 axis.

DISCUSSION

BC is a medical problem with a high incidence worldwide, which seriously threatens womens' physical and mental health.[1,2] It is therefore imperative to clarify the pathogenesis of BC and explore innovative and effective treatment strategies. LncRNAs are important regulators of cancer development and have been reported to participate in BC progression.[17,18] In a previous study, the novel lncRNA RUSC1-AS1 was elucidated to hinder proliferation and enhance apoptosis in BC cells by targeting KLF2 and CDKN1A.[16] Here, we further confirmed that RUSC1-AS1 had elevated expression in BC tissues and cells. In addition to regulating proliferation and apoptosis, we also found that RUSC1-AS1 had positive regulatory effects on cell migration, invasion, cell cycle and angiogenesis. Importantly, our data revealed that RUSC1-AS1 silencing also reduced BC tumorigenesis in vivo. In our study, we confirmed that RUSC1-AS1 has a cancer-promoting effect in BC, which is consistent with its role in other cancers.[13–15]

In previous studies, RUSC1-AS1 was found to interact with miR-7-5p, miR-744 and miR-10, thereby indirectly regulating downstream gene expression to participate in cancer progression.[13–15] In this study, we discovered that miR-326 could be sponged by RUSC1-AS1. MiR-326 has been shown to be a low-expressed miRNA in cancer and can be used as a tumor suppressor to regulate cancer progression. For example, miR-326 suppressed hepatocellular carcinoma proliferation, metastasis and glycolysis by targeting IGF1R,[19] and miR-326 could target WNT2B to inhibit non-small cell lung cancer invasion and EMT.[20] MiR-326 has been identified as a potential biomarker for cancer, and its anticancer effect provides new insights for targeted cancer therapy.[21] Similarly, many studies have confirmed the negative regulatory effect of miR-326 on BC progression.[22,23] In our study, we uncovered that RUSC1-AS1 negatively regulated miR-326 expression in vitro and in vivo, and confirmed that it sponged miR-326 to accelerate the progression of BC. Combined with our results, we determined that RUSC1-AS1 promoted BC progression mainly by inhibiting the expression of tumor suppressor miR-326.

MiR-326 has been shown to target multiple genes that regulate BC progression, including E2S1,[24] ELK1,[25] ITGA5[22] and TFF1.[23] Here, we found that miR-326 could target a new gene, XRCC5. The protein encoded by the XRCC5 gene (also known as Ku86) plays a vital role in the process of DNA damage repair.[26,27] Numerous studies have shown that the XRCC5 gene and its expression products may also be related to tumor progression and metastasis-related gene mutation repair ability.[28–30] The results of Ma et al. suggested that Ku86 was overexpressed in serous ovarian cancer, and that knockdown could inhibit cell proliferation, cell cycle, and promote radiotherapy sensitivity.[31] In BC, XRCC5 was determined to facilitate cell proliferation and metastasis to promote BC progression.[32] In this study, we found that XRCC5 was upregulated in BC, and its expression was negatively regulated by miR-326. Further experiments revealed that miR-326 targeted XRCC5 to inhibit BC proliferation, metastasis, cell cycle, angiogenesis, and promote apoptosis. The positive regulation of RUSC1-AS1 on the expression of XRCC5 confirmed that RUSC1-AS1 promoted BC progression via sponging miR-326 to increase XRCC5 expression.

In conclusion, our research proposes a new ceRNA pathway of RUSC1-AS1 in BC. The results showed that RUSC1-AS1 promoted BC progression through the miR-326/XRCC5 axis which might therefore provide a new reference for BC treatment in the future.

AUTHOR CONTRIBUTION

Aisikeer Ayoufu conceived and designed the study, and drafted the first draft of the manuscript. All experiments were completed by all authors. Puerkaiti Paierhati, Lei Qiao, Nan Zhang, Muzhapaer Abudukeremu analyzed and collated the results. All authors reviewed and critiqued the manuscript, and agreed to the final submission of the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no competing interests.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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