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Cyclic RNA hsa_circ_0091017 inhibits proliferation, migration and invasiveness of bladder cancer cells by binding to microRNA-589-5p

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Abstract. – OBJECTIVE: To investigate the role of circular RNA hsa_circ_0091017 in the progression of bladder cancer (BCa) and its possible molecular mechanisms.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was used to detect the expression level of circular RNA hsa_circ_0091017 in 40 pairs of BCa tissues and normal adjacent tissues, as well as in BCa cell lines. MiRNAs that could bind to the circular RNA hsa_circ_0091017 were predicted by bioinformatics databases and their expression levels were verified in BCa tissues and cell lines. The circular RNA hsa_circ_0091017 overexpression plasmid and the corresponding negative control were transfected into T24 and UMUC-3 cells, and their effects on cell proliferative ability, migration, and invasiveness were determined by cell counting kit-8 (CCK-8), 5-Ethynyl-2'- deoxyuridine (EdU), and transwell assays. In addition, the Dual-Luciferase reporter gene assay was used to verify the binding relation between the circular RNA hsa_circ_0091017 and microRNA-589-5p. Furthermore, *in vitro* reversal experiments were used to confirm the biological role of microRNA-589-5p in the progression of BCa mediated by circular RNA hsa_circ_0091017.

RESULTS: QRT-PCR results revealed that the expression level of hsa_circ_0091017 was remarkably down-regulated in BCa tissues and cell lines, while the expression level of microRNA-589-5p was remarkably increased in BCa tissues and cells. Overexpression of the circular RNA hsa_circ_0091017 in BCa cells can inhibit the proliferation, migration, and invasiveness of BCa cells. At the same time, it was found that overexpression of the circular RNA hsa_circ_0091017 in BCa cells can inhibit the expression of microRNA-589-5p and there was a binding relation between the two in BCa cells. In addition, overexpression of microRNA-589-5p reversed the inhibitory effect of the circular RNA hsa_circ_0091017 on the malignant phenotype of BCa cells.

CONCLUSIONS: The circular RNA hsa_circ_0091017 can inhibit the proliferation, migration and invasiveness of BCa cells by regulating the expression of microRNA-589-5p.

Key Words:
Circular RNA hsa_circ_0091017, MicroRNA-589-5p, BCa, Circular RNA.

Introduction

In tumors, bladder cancer (BCa) ranks 4th among men and 11th among women, according to statistics, there are more than 43,000 new cases of bladder cancer every year worldwide[1]. The main pathological type of bladder cancer is transitional epithelial carcinoma, including invasive bladder cancer with a higher degree of malignancy and superficial bladder cancer with lower malignancy[2]. Bladder cancer is multifocal and recurrent and most patients are often diagnosed of non-muscle invasive bladder cancer at the time of initial diagnosis. After surgery or intravesical instillation, there is still a high probability of tumor recurrence and progression. The treatment of BCa is a great threat to human health and socio-economic development[3]. Currently, surgical treatment and chemotherapy are still the main treatment strategies for BCa. In recent years, more and more attention has been given to neoadjuvant therapy, including monoclonal antibody therapy, and immunotherapy[4]. However, for patients with advanced BCa, the 5-year survival rate is only 50% after radical treatment due to the late detection[5]. The prevention and treatment of BCa are still facing great challenges, mainly due to the lack of highly sensitive and specific molecular markers for the diagnosis of BCa. Therefore, the detection and establishment

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of key targets related to the progress of BCa have become an effective approach to solve the problem of BCa diagnosis and treatment.

At present, with the development of high-throughput sequencing technology, a new type of RNA, circRNAs, is increasingly considered to play an important role in tumor progression[6]. CircRNAs are RNAs without 5' cap structure and 3' tail structure. Although they were previously considered as the wrong splicing in the process of RNA transcription, they are now increasingly found to be a class of RNAs with rich, stable, diverse and conserved expressions, which are of great significance in the RNA regulatory network[7,8]. Circular RNA MAT2B affects the glycolysis process in liver cancer by regulating the mir-338-3p/PKM2 axis, thus leading to malignant progression of the tumor[9]. CircTADA2As can inhibit the progression and metastasis of breast cancer by regulating the expression of mir-203a-3p and its target genes[10]. CircMMP9 has been found to bind to mir-124 as a sponge RNA, promoting pleomorphic changes and malignant changes in glioma cells[11]. Previous studies[12] have also found that circRNA hsa_circ_0091017 is remarkably dysregulated in BCa and may play an important role; however, the specific mechanism of circRNA hsa_circ_0091017 in BCa progression is still unclear. In this study, *in vitro*, and *in vivo* experiments were conducted to further explore the role and specific mechanism of hsa_circ_0091017 in BCa progression.

In this research, quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was used to quantitatively detect hsa_circ_0091017 expression in BCa tissues and corresponding normal bladder tissues in clinical samples, and to verify its expression in BCa cells and normal bladder epithelial cells. Then, we overexpressed circRNA hsa_circ_0091017 in BCa cells and further determined its effect on the proliferation, migration, and invasiveness of tumor cells. Subsequently, luciferase reporter technology was used to verify the possible downstream molecular mechanism of circRNA hsa_circ_0091017 and *in vitro* reverse experiments were performed to further illustrate its key role as the downstream target of circRNA hsa_circ_0091017.

Patients and Methods

Tissue Collection

A total of 40 pairs of BCa tissues and corresponding normal bladder tissues were collected from patients who were treated with urology from April 2016 to December 2018 in Chifeng Tumor Hospital. The samples collected were confirmed by pathological examination as tumor tissue and normal epithelial tissue. All samples were stored in liquid nitrogen. This study was approved by the Chifeng Tumor Hospital Ethics Committee. All participants had been informed that their tissues would be used for scientific research and had signed a written consent. The content of the experiment was in line with the Helsinki Declaration.

Cell Culture

Human BCa cell lines (5637, EJ, T24, UMUC-3, RT4) and human normal bladder epithelial cells (SV-HUC-1) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (HyClone, South Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 100 UI/mL of penicillin and 100 μg/mL of streptomycin. The condition of the cell culture incubator was set to a constant temperature of 37°C and the CO_2 concentration was maintained at 5%.

QRT-PCR

RNA was extracted from tissues and cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. 1 μg of RNA was reverse transcribed into complementary deoxyribose nucleic acid (cDNA) using the Reverse Transcription Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. The qRT-PCR experiment was performed using SYBR® Premix Ex Taq II (TaKaRa, Otsu, Shiga, Japan). The quantification of microRNA-589-5p was measured using a TaqMan MicroRNA Assay Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. All experimental results were based on the $2^{-\Delta\Delta Ct}$ method calculation. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 were used for internal reference to quantify the relative quantification of circular RNAs hsa_circ_0091017 and microRNA-589-5p, respectively. The primer sequences of all qRT-PCR were as follows: hsa_circ_0091017: Forward 5'-ATCATTCGGACAAGACAGG-3', Reverse :5'-TCCATAT ACCAGATCCTCATTG-3'; microRNA-589-5p: Forward: 5'-CGAGGTCAGCGTGATTTCATGG-3', Reverse: 5'-TGTGTCCAAGTCCCAGCCAGAG-3'; GAPDH: For-

ward: 5-GGAGCGAGATCCCTCCAAAAT-3', Reverse: 5'-GGCTGTTGTCATACTTCTCATGG-3'; U6: Forward: 5'-CTCGCTTCGGCAGCACATATA-3' Reverse: 5'-AAATATGGAACGCTTCACGA-3';

Cell Transfection

The overexpression plasmids of the circular RNAs hsa_circ_0091017 and microRNA-589-5p and the corresponding control plasmids were designed and synthesized by GenePharma (Shanghai, China), Shanghai Gemma Biotech Co., Ltd. (Shanghai, China). Transfection was performed using the Lipofectamine 3000 kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and the transfection efficiency was verified by qRT-PCR.

Cell Counting Kit-8 (CCK-8) Experiment

The CCK-8 assay was used to determine the proliferative capacity of T24 and UMUC-3 BCa cells. 24 h after transfection, cells were seeded in 24-well plates at a density of 2000 cells/well and incubation was continued under standard conditions by adding an appropriate amount of complete medium. After the cells were attached, 10 μL of CCK-8 reagent (Dojindo Laboratories, Kumamoto, Japan) was added at 0, 24, 48, and 72 h, respectively, and incubation was continued for 2 h, and then a microplate reader was used to detect the absorbance of each well at a wavelength of 450 nm.

5-Ethynyl-2'-Deoxyuridine (EdU) Experiment

The EDU assay kit (Beyotime Inst Biotech, Shanghai, China) was used to determine the proliferative capacity of T24 cells and UMUC-3 cells. About 1*10^5 cells per well were uniformly seeded in 24-well plates. After the cells were attached, 100 μL of 50 μM EdU was added to each well for 2 h, and the cells were fixed with 50 μL of 4% paraformaldehyde for 30 min. Subsequently, 2 mg/mL glycine and a permeabilizing agent were sequentially added according to the product instructions, and the cells were washed twice with phosphate-buffered saline (PBS) solution. Cells were fluorescently stained with 1X Apollo solution in the dark. After aspirating the solvent, cells were further stained with 100 μL of 1X 4',6-diamidino-2-phenylindole (DAPI) solution at room temperature for 30 min, and the cells were washed several times with PBS, and then photographed and analyzed by a fluorescence microscope.

Transwell Experiment
Cell Migration Ability Experiment

The migration capacity of T24 and UMUC-3 cells was determined using an 8 um transwell chamber (Corning, Corning, NY, USA). The experimental procedure was carried out in strict accordance with the instructions. The cell suspension prepared in serum-free RPMI-1640 medium was added to the upper layer of the chamber, and RPMI-1640 medium containing 20% fetal calf serum (FCS) was added to the lower layer of the chamber to induce cell migration downward. After the cells were cultured for 48 h under standard conditions, the lower layer migrated cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet dye. Cells that were not migrated in the upper part of the chamber were gently scraped off to avoid affecting staining observation. The migrated cells were counted and analyzed under a microscope.

Cell Invasion Ability Experiment

Cell invasion ability experiments were similar to cell migration experiments. 100 μl of 1 μg/μl Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) was pre-filled in the chamber and stored at 4°C overnight. The cells were then added to the chamber in the same manner as the migration experiment, and the experimental procedure was carried out in strict accordance with the product specifications. After 48 h of cell culture, the lower layer migrated cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet dye. Cells that were not migrated in the upper part of the chamber were gently scraped off to avoid affecting staining observation. The migrated cells were counted and analyzed under a microscope.

Luciferase Reporter Gene Assay

Luciferase reporter assays were used to further verify the ability of the circular RNA hsa_circ_0091017 to bind to the downstream target gene microRNA-589-5p. The circular RNA hsa_circ_0091017 sequence was introduced into the reporter plasmid by cytomegalovirus and the predicted binding site mutation sequence of microRNA-589-5p was introduced into the vector plasmid *via* the Mut Express II Fast Mutagenesis kit (Vazyme, Piscataway, NJ, USA). T24 cells and UMUC-3 cells were seeded at a density of 1000 cells/well in 96-well plates, and after 24 h of culture, a co-transfection operation was carried

out using a Lipofectamine 3000 kit (Invitrogen, Carlsbad, CA, USA). The luciferase reporter gene was added, and after 48 h of further culture, the luciferase activity was measured using a dual luciferase reporter gene system (Promega, Madison, WI, USA).

Statistical Analysis

Statistical analysis of the data was performed using GraphPad Prism 7.0 software (GraphPad Software, Inc., La Jolla, CA, USA). All experimental nodes were derived from 3 independent repetitive experiments and the mean ± standard error of the test results (mean ± SEM) was included in the final data analysis. A two-tailed *t*-test was used to analyze the two-tailed Student's *t*-tests. The expression level of the circular RNA hsa_circ_0091017 and the microRNA-589-5p correlation were analyzed by Pearson correlation. $p<0.05$ was considered statistically significant.

Results

The Expression Level of Circular RNA hsa_circ_0091017 in BCa Tissues and Cells was Remarkably Down-Regulated

QRT-PCR was applied to measure the expression level of hRNA_h_0091017 in BCa tissues. Results showed that the expression level of hRNA_h_0091017 was remarkably down-regulated in 40 pairs of BCa samples than in adjacent normal tissues (Figure 1A). Subsequently, we measured the expression level of the circular RNA hsa_circ_0091017 in BCa cells (5637, EJ,

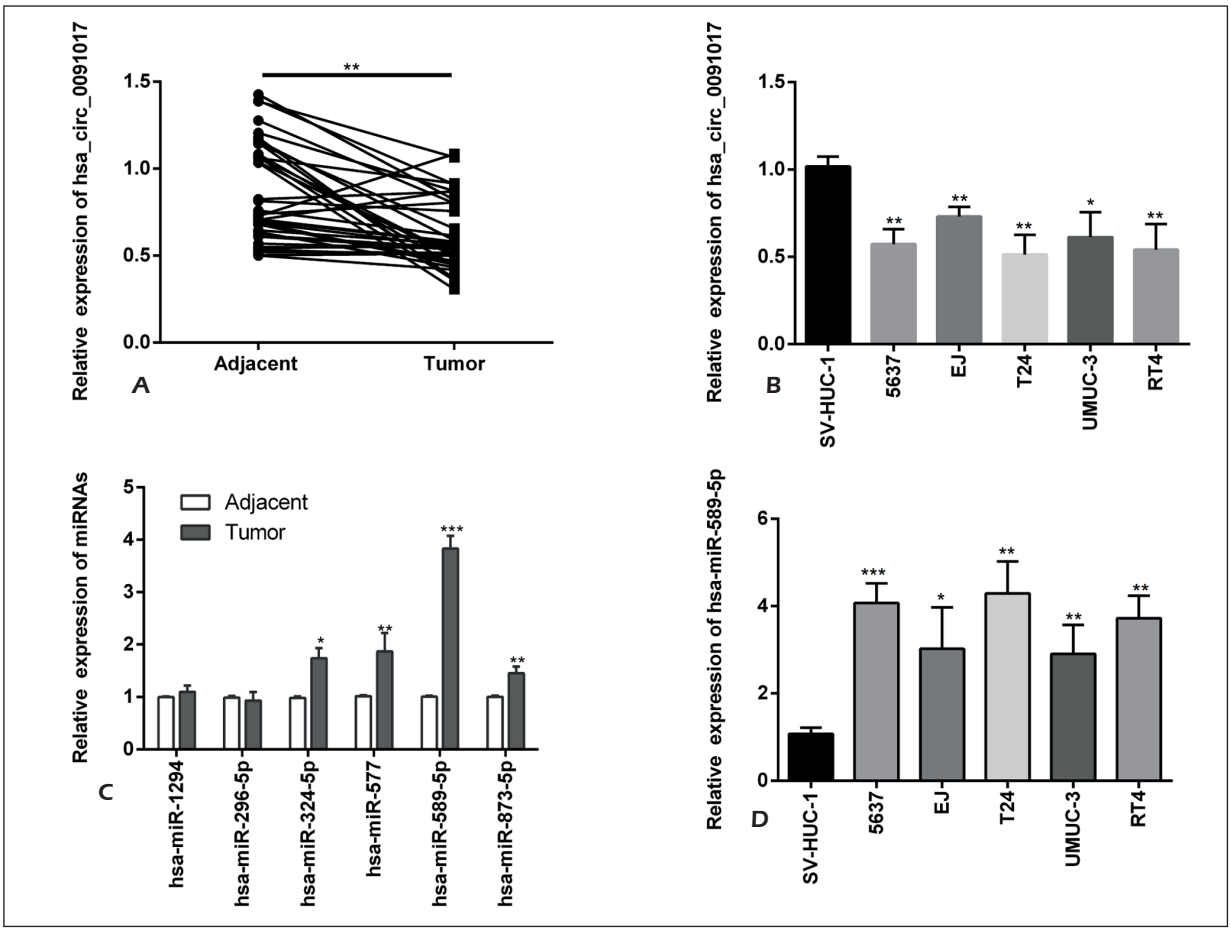

Figure 1. **A**, Expression of circular RNA hsa_circ_0091017 in bladder cancer tissues was lower than that of matched adjacent normal bladder tissues; **B**, Expression of circular RNA hsa_circ_0091017 in bladder cancer cell lines was significantly lower than that in normal bladder epithelial cell lines; **C**, Prediction of the expression of miRNAs that bind to the circular RNA hsa_circ_0091017 in bladder cancer tissues and matched paracancerous tissues; **D**, Expression level of miR-589-5p in bladder cancer cell lines was significantly higher than that in normal bladder epithelial cell lines. (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

T24, UMUC-3, RT4), as well as human normal bladder epithelial cells (SV-HUC-1), and found that the expression level of circular RNA hsa_circ_0091017 in BCa cells was lower than that in normal bladder epithelial cells (Figure 1B), suggesting that hsa_circ_0091017 might play a pivotal role in the progression of BCa.

Increased Expression of MicroRNA-589-5p in BCa Tissues and BCa Cells

We used the circular RNA downstream target prediction database starbase (http://starbase.sysu.edu.cn) and the circular RNA interactome database (https://circinteractome.nia.nih.gov/miRNA_Target_Sites) to predict the potential targets of circular RNA hsa_circ_0091017. The results showed that hsa-miR-1294, hsa-miR-296-5p, hsa-miR-324-5p, hsa-miR-577, hsa-miR-589-5p, hsa-miR-873-5p were both predicted to be target genes in two databases. Subsequently, we used qRT-PCR to detect the expression levels of these miRNAs in clinical samples and found that the expression level of hsa-microRNA-589-5p in BCa tissues was remarkably increased (Figure 1C). *In vitro* experiments also showed that hsa-microRNA-589-5p was also remarkably up-regulated in BCa cells (Figure 1D). According to the bioinformatics localization suggestion, hsa-microRNA-589-5p was located in the region of mRNA TAF1 (TATA-box binding protein associated factor 1) and could form a circular RNA (Figure 2A) through a special cyclic structure.

Overexpression of Circular RNA hsa_circ_0091017 Inhibits Proliferation of BCa Cells

We subsequently used CCK-8 and EDU experiments to further validate the effect of circular RNA hsa_circ_0091017 over-expression on the proliferation of BCa cells. We transfected the circ_0091017 overexpression plasmid in T24 and UMUC-3 cells and verified the transfection efficiency by qRT-PCR (Figure 2B), and subsequently, the change in cell proliferation ability was determined. The results of CCK-8 and EDU experiments consistently suggested that overexpression of circular RNA hsa_circ_0091017 can inhibit the proliferation of BCa cells (Figure 2C-2E).

Overexpression of Circular RNA hsa_circ_0091017 Inhibits Migration and Invasion of BCa Cells

We used transwell migration and invasion assays to further validate the effect of overexpression of circular RNA hsa_circ_0091017 on BCa metastasis. We specifically overexpressed circular RNA hsa_circ_0091017 in T24 and UMUC-3 cells, and then inoculated the cells in a transwell chamber, using high-concentration serum-containing medium to induce cells to pass through the basement membrane of the chamber. Cell counting was used to determine changes in BCa cell migration and invasion. Transwell experiments and Invasion experiments showed that overexpression of circular RNA hsa_circ_0091017 can inhibit the migration and invasion of BCa cells (Figure 3A-3B).

Overexpression of Circular RNA hsa_circ_0091017 in BCa Cells Inhibits the Expression of MicroRNA-589-5p

To further validate the regulatory relation between the circular RNA hsa_circ_0091017 and microRNA-589-5p in BCa, we used a luciferase reporter gene assay to verify the binding ability of the two. First, we performed a Pearson correlation analysis of the expression levels of hsa_circ_0091017 and microRNA-589-5p in BCa tissues. The results showed that there was a significant negative correlation between their expression levels of BCa tissues (R^2=0.3948) (p<0.001) (Figure 4A). By detecting the expression of microRNA-589-5p in T24 and UMUC-3 cells expressing the circular RNA hsa_circ_0091017, we found that high expression of the circular RNA hsa_circ_0091017 in BCa cells can inhibit the expression of microRNA-589-5p (Figure 4C-4D). Subsequently, we constructed a mutant sequence of hsa_circ_0091017 binding site by predicting the possible binding sites of the two and further showed that the two had a binding relationship (Figure 4E-4F) using the dual luciferase reporter gene assay.

Overexpression of MicroRNA-589-5p Reverses the Inhibition of Circulating Cancer hsa_circ_0091017 on BCa Cells

We further clarified the key role of microRNA-589-5p in hsa_circ_0091017-modulated BCa process by *in vitro* reversal experiments. We co-transfected hsa_circ_0091017 and microRNA-589-5p overexpressing plasmids in T24 and UMUC-3 cells and assayed the proliferative ability, migration, and invasiveness of BCa cells by CCK-8, EDU, transwell, and invasion assays. It was found that overexpression of microRNA-589-5p partially reversed the inhibitory ef-

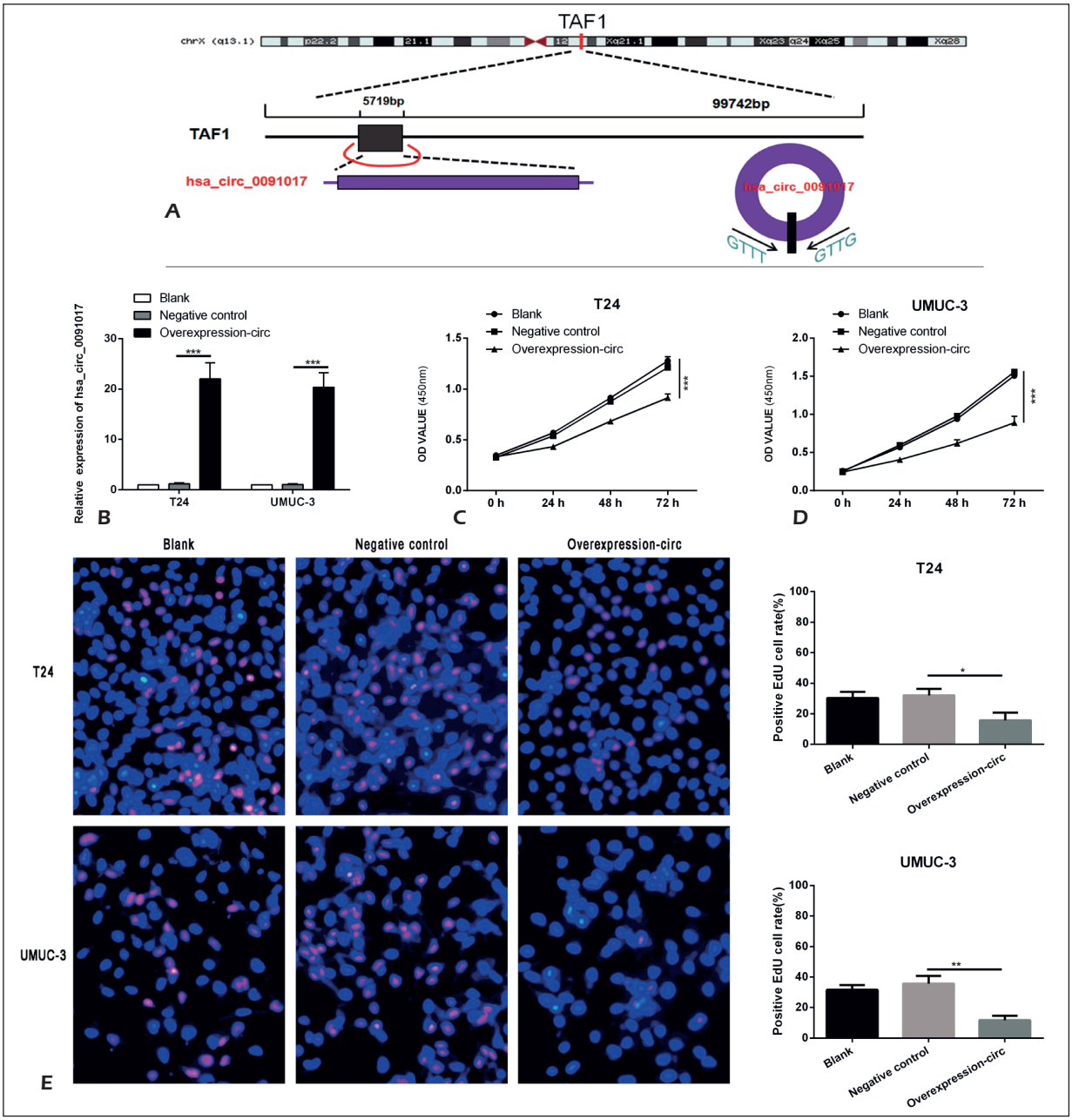

Figure 2. A, Circular locus of hRNA_circ_0091017 in the UCSC website; **B**, After transfection of the circular RNA hsa_circ_0091017 overexpression vector, real-time quantitative PCR was used to determine the expression of hRNA_h_0091017 in the bladder cancer cell lines T24 and UMUC-3; **C**, After overexpression of the circular RNA hsa_circ_0091017, the results of CCK-8 showed that the proliferation of bladder cancer cell line T24 was decreased; **D**, After overexpression of the circular RNA hsa_circ_0091017, the results of CCK-8 showed that the proliferation of bladder cancer cell line UMUC-3 was decreased; **E**, After over-expression of the circular RNA hsa_circ_0091017, EDU results showed that the proliferation of bladder cancer cell lines T24 and UMUC-3 decreased (*p<0.05, **p<0.01, ***p<0.001) (magnification: 40×).

fect of hsa_circ_0091017 overexpression on the malignant ability of BCa cells (Figure 5A-5F). These results indicated that microRNA-589-5p might play a critical role in the malignant progression of BCa mediated by circular RNA hsa_circ_0091017.

Discussion

As a newly discovered RNA species, the number of newly named circular RNAs has increased year by year, and the mechanisms by which they work are becoming more complex[13]. Among them, the

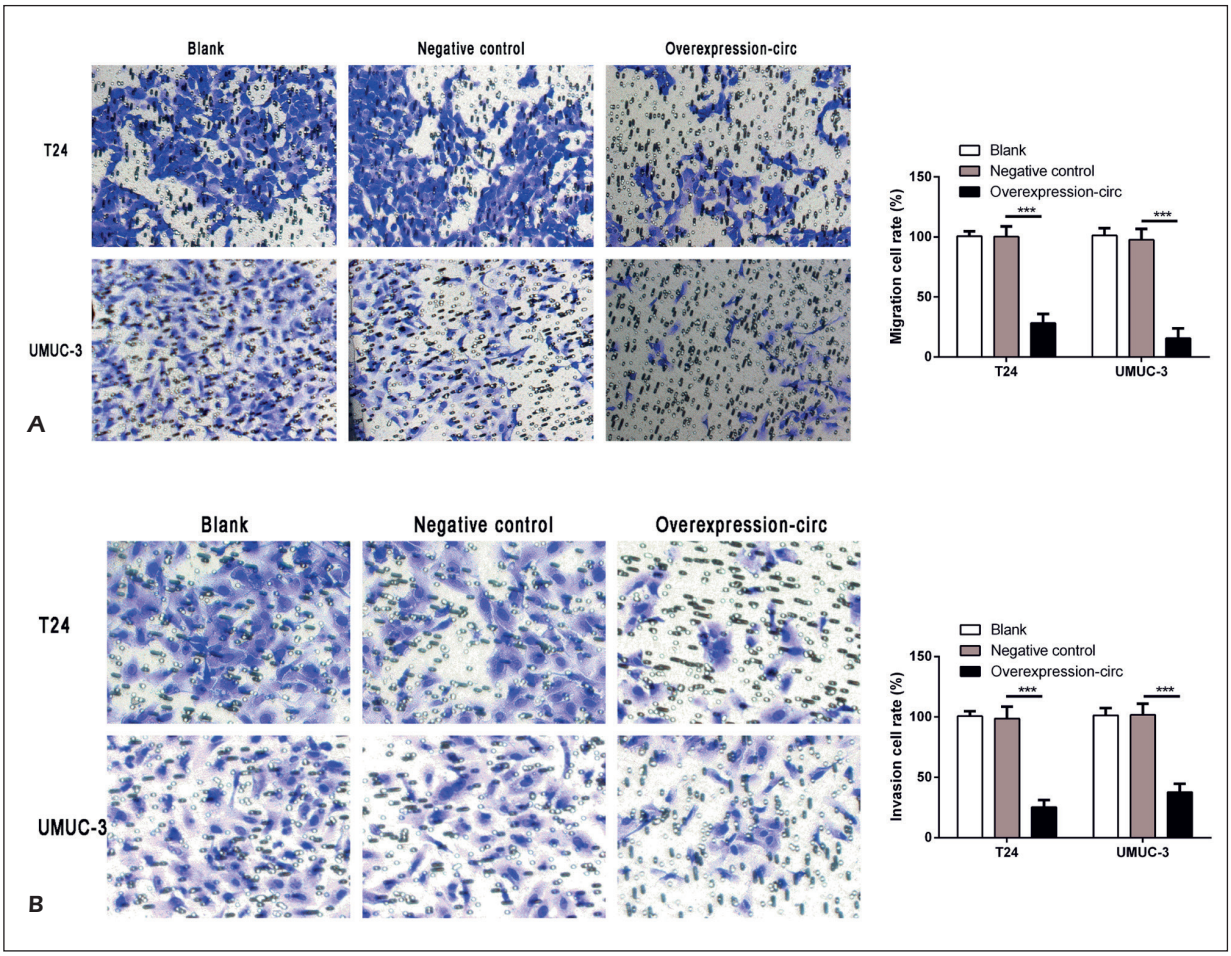

Figure 3. A, After overexpression of the circular RNA hsa_circ_0091017, transwell cell migration assay showed that the migration ability of bladder cancer cell lines T24 and UMUC-3 was inhibited (magnification: 40×); **B**, After overexpression of the circular RNA hsa_circ_0091017, the invasion of bladder cancer cell lines T24 and UMUC-3 was inhibited (*p<0.05, **p<0.01, ***p<0.001) (magnification: 40×).

mechanism by which circular RNA plays a regulatory role as a competitive endogenous RNA has received more and more attention[14]. Due to its special structure, circular RNA has more stable characteristics than ordinary linear lncRNA, so it has unique advantages as a molecular marker. In previous studies, the role of circular RNA in BCa has been confirmed experimentally. hsa_circ_0068871 was found to regulate the expression of miR-181a-5p, thereby promoting the progression of BCa[15]; while hsa_circ_0002024 was found to inhibit the proliferation, migration and invasiveness of BCa cells by adsorption of miR-197-3p[16]. In addition, studies[17] have found that circular RNA circELP3 can participate in the process of chemotherapy resistance of BCa, while inhibition of its expression can reverse the chemotherapy resistance of BCa. These studies suggest that circular RNA also plays a huge role in the progression of BCa.

As a newly discovered circular RNA, hsa_circ_0091017 was found to have abnormally low expression in BCa patients in previous studies[18] and may be associated with poor prognosis of tumors. However, the specific role and mechanism of hsa_circ_0091017 in BCa is still unclear. We foundd through a series of *in vitro* and *in vivo* experiments that the circular RNA hsa_circ_0091017 had significant low expression in BCa tissues and cells, and can affect the ability of cancer cells to proliferate, migrate, and invade. The reference gene of hsa_circ_0091017 is mRNA TAF1 (TATA-box binding protein associated factor 1, Position: chrX: 71366305-71464046, NCBI: 6872), which can act as a coactivator to participate in basal transcription, promoter recognition or modification. Previous studies have showed that the TAF1 gene may be a key gene for some tu-

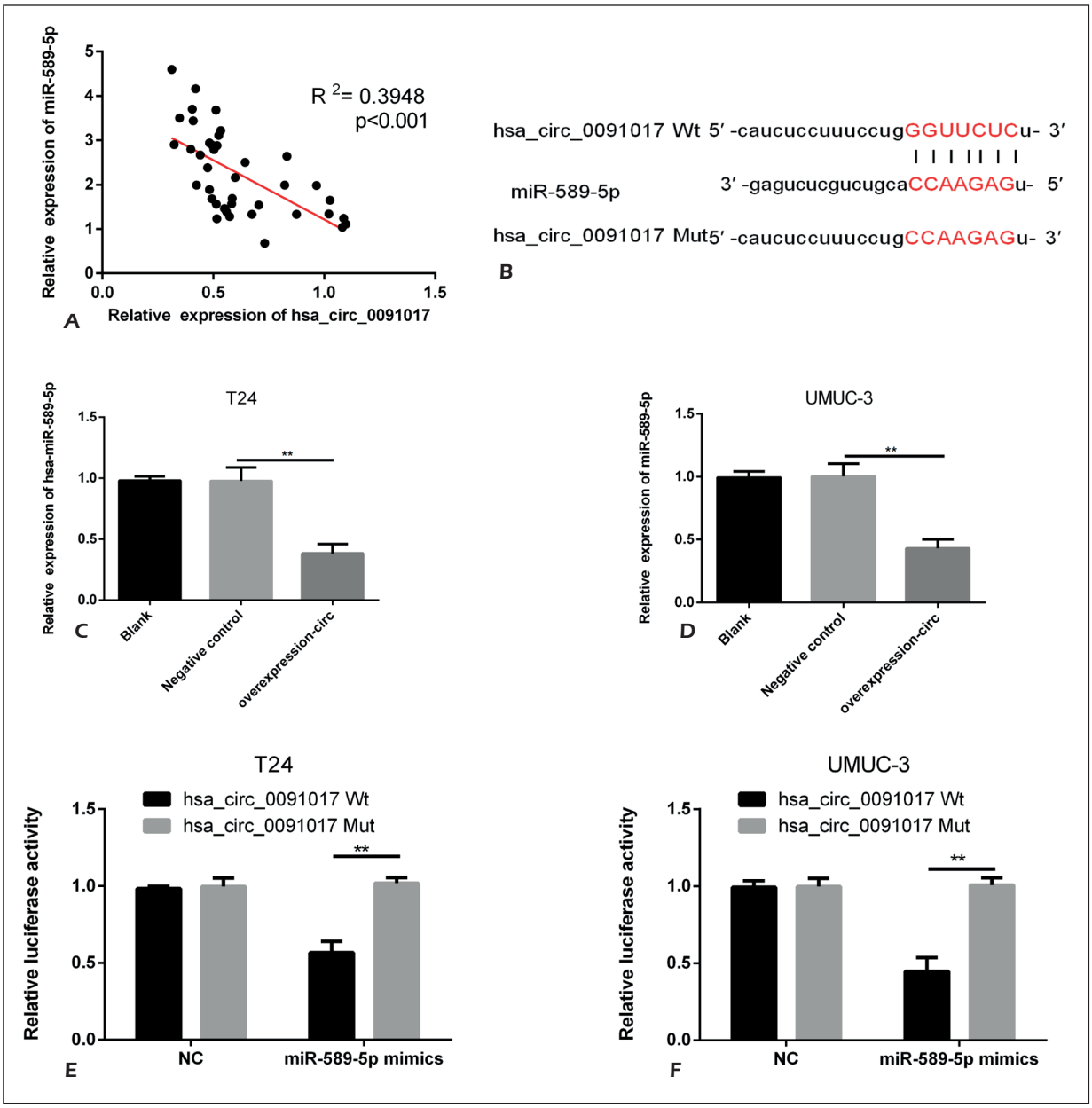

Figure 4. A, Using Pearson correlation analysis, the expression levels of hRNA_h_0091017 and miR-589-5p in bladder cancer tissues were correlated; **B**, Bioinformatics predicts that the circular RNA hsa_circ_0091017 and miR-589-5p have binding sites; **C**, After overexpression of the circular RNA hsa_circ_0091017, the expression level of miR-589-5p in T24 cells was determined by real-time quantitative PCR, and the expression level was down-regulated. **D**, After overexpression of the circular RNA hsa_circ_0091017, the expression level of miR-589-5p in UMUC-3 cells was determined by real-time quantitative PCR, and the expression level was down-regulated. **E**, Luciferase reporter gene assay showed that the transfection of the circular RNA hsa_circ_0091017 binding site mutation sequence vector can inhibit the binding ability of the circular RNA hsa_circ_0091017 and miR-589-5p in the T24 cell line; **F**, Luciferase reporter gene assay showed that the transfection of the circular RNA hsa_circ_0091017 binding site mutation sequence vector can inhibit the binding ability of the circular RNA hsa_circ_0091017 and miR-589-5p in the UMUC-3 cell line; (*p<0.05, **p<0.01, ***p<0.001).

mor progression, including esophageal cancer and breast cancer. TAF1 was found to be mutated in chemotherapy-resistant esophageal cancer tissues, and these mutations were found in patients who had not received chemotherapy. Thus, it is believed that monitoring the level of the mutant gene can predict the therapeutic effect, and the same TAF1 is also considered to be one of the important mol-

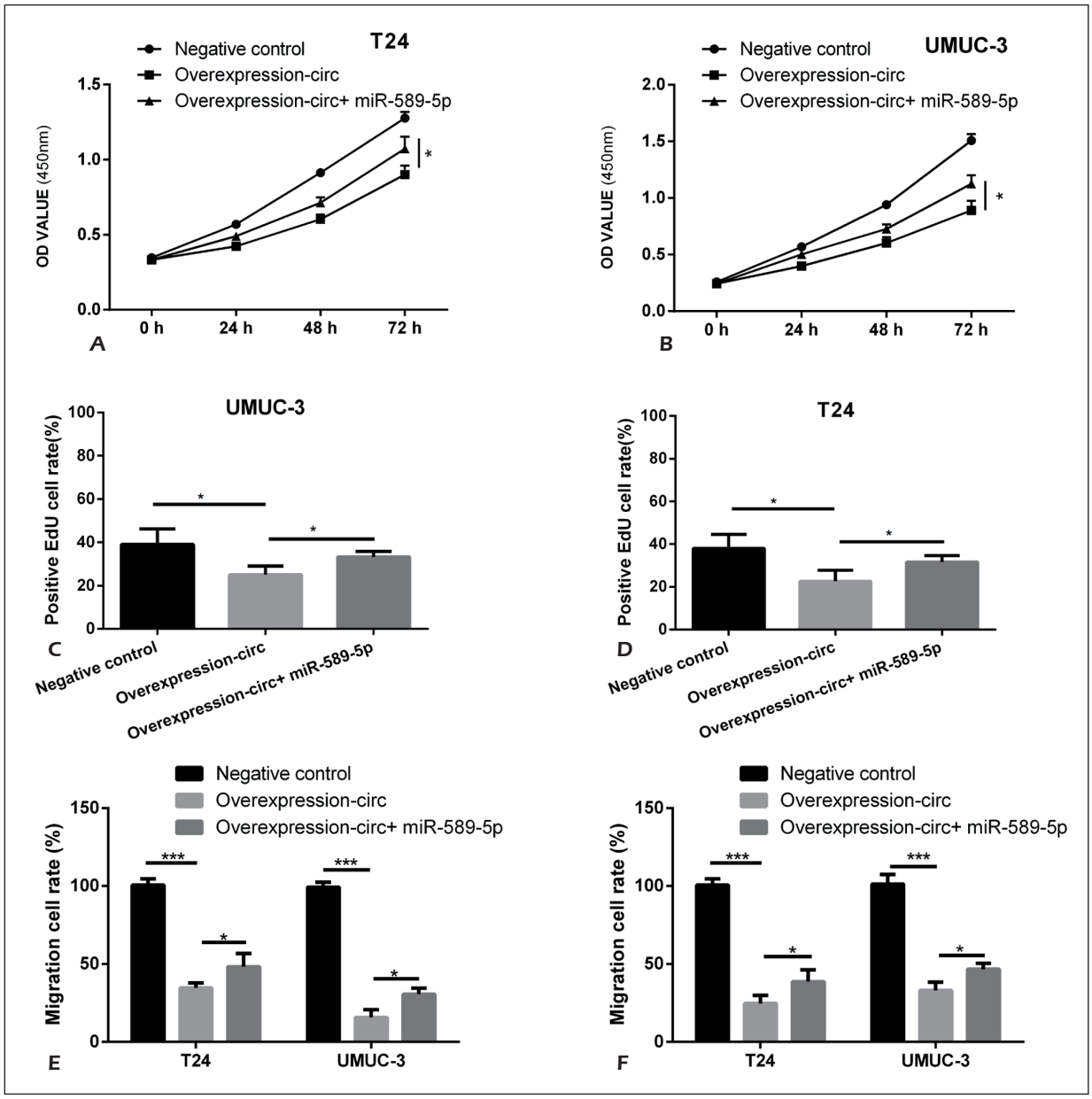

Figure 5. A, After co-transfection of hsa_circ_0091017 and miR-589-5p plasmids, CCK-8 assay found that re-recovery of miR-589-5p can repair the weakening of T24 cell proliferation by hRNA_circ_0091017; **B**, After co-transfection of hsa_circ_0091017 and miR-589-5p plasmids, CCK-8 assay revealed that re-recovery of miR-589-5p could repair the weakening of UMUC-3 cell proliferation by hRNA_circ_0091017; **C**, After co-transfection of hsa_circ_0091017 and miR-589-5p plasmids, EDU experiments showed that the re-recovery of miR-589-5p partially restored the proliferation of UMUC-3 cells; **D**, After co-transfection of hsa_circ_0091017 and miR-589-5p plasmids, EDU experiments showed that the re-recovery of miR-589-5p partially restored the proliferation of T24 cells; **E**, Transwell results showed that co-transfection of hsa_circ_0091017 and miR-589-5p plasmids in T24 and UMUC-3 cells reversed the inhibition of cell migration by hsa_circ_0091017; **F**, Transwell results showed that co-transfection of hsa_circ_0091017 and miR-589-5p plasmids in T24 and UMUC-3 cells reversed the inhibitory effect of hsa_circ_0091017 on cell invasion. (*p<0.05, ***p<0.001).

ecules driving the progression of triple-negative breast cancer[19,20]. However, the role of TAF1 is not fully understood in the progression of BCa. Our experiments revealed the important role of hsa_circ_0091017 in the progression of BCa, and indicated by dual luciferase and *in vitro* rescue experiments that microRNA-589-5p may be an important downstream molecular. MicroRNA-589-5p has been found to play a role in inhibiting the dry transition of CD90+ tumor cells in liver cancer and

is closely related to the overall prognosis of patients with liver cancer[21]. Similarly, the role of microRNA-589-5p in BCa has not been studied before, and our researches demonstrated its role in the malignant progression of BCa for the first time. In this experiment, we co-transfected the vector overexpressing microRNA-589-5p and hsa_circ_0091017, and then using *in vitro* experimental techniques to determine the proliferative ability, migration, and invasiveness of BCa cells, and found that the resumption of microRNA-589-5p can reverse the inhibition effect of hsa_circ_0091017 on the malignant phenotype of BCa cells.

In summary, we demonstrated by quantitative PCR that hsa_circ_0091017 was abnormally downregulated in BCa tissues and cells, and it was confirmed by CCK-8, EDU, transwell, and invasion experiments that hsa_circ_0091017 inhibited the proliferation and migration of BCa cells. Furthermore, we detected by dual luciferase reporter gene assay and *in vitro* rescue experiments that hsa_circ_0091017 can regulate microRNA-589-5p expression while overexpression of microRNA-589-5p can partially reverse the inhibition effect of circular RNA hsa_circ_0091017 on the malignant phenotype of BCa cells.

Conclusions

These results indicated that the circular RNA hsa_circ_0091017 could inhibit the proliferative ability, migration and invasiveness of BCa cells by regulating the expression level of microRNA-589-5p.

Conflict of Interests

The Authors declared that they have no conflict of interests.

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