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MiRNA regulates OCT4 expression in breast cancer cells

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Abstract. – OBJECTIVE: Breast cancer cell infiltration, migration, and proliferation significantly affect its curative effect. Stemness gene octamer-binding transcription factor 4 (OCT4) upregulated in breast cancer tissue compared with normal control. MiRNA exhibits regulatory role in gene expression. This study adopted bioinformatics to predict the miRNA to regulate OCT4 gene and investigated its impact on breast cancer cell infiltration, migration, and proliferation.

MATERIALS AND METHODS: MirBase database was analyzed to explore the potential miRNA in regulating OCT4 based on human OCT4 gene sequence. MiRNA mimics and inhibitor were synthetized and transfected to BS524 cells. qRT-PCR was applied to test miRNA and OCT4 mRNA expressions in cells at 12 h, 24 h, and 48 h after transfection. Western blot was selected to detect OCT4 protein expression. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was selected to determine cell proliferation. Scratch assay was adopted to evaluate cell migration. Transwell assay was used to analyze cell infiltration.

RESULTS: MiR-145 may regulate OCT4 gene with score 82. OCT4 mRNA and protein increased at 12 h after transfection ($p > 0.05$). OCR4 gene significantly upregulated, cell proliferation, migration, and infiltration enhanced by miR-145 transfection compared with control ($p < 0.05$). OCT4 gene downregulated, while cell proliferation, infiltration, and migration markedly weakened in miR-145 inhibitor group compared with control ($p < 0.05$).

CONCLUSIONS: MiR-145 affects breast cancer BS524 cell proliferation, infiltration, and migration via positively regulating OCT4 gene expression.

Key Words:
Breast cancer cell, miR-145, OCT4 gene, Proliferation, Migration, Infiltration.

Introduction

Breast cancer is one of the most common malignant tumors in women. It accounts for about 10% of all malignant tumors, which is often associated with heredity. It mainly appears in women between 40 to 60 years old, thus seriously impacts the physical and mental health. It was shown that stemness gene was upregulated in the incidence of breast cancer[1,2]. Following the advantage and application of molecular biological technology and gene therapy in the clinic, it becomes critical to investigate the biological treatment targets[3,4].

Octamer-binding transcription factor 4 (OCT4) is a type of stemness gene located in chromosome 6. It belongs to POU transcription factor family that mainly expresses in embryonic stem cells and primordial germ cells. OCT4 gene level gradually declines following cell differentiation and the disappearance of stemness[5,6]. However, it is found that OCT4 gene expression abnormally elevated in tumor incidence and closely related to tumor development[7], such as bladder cancer, prostate cancer, liver cancer, and lung cancer. It is verified that OCT4 overexpression may promote lung cancer cell proliferation and migration[8,9]. However, the role of OCT4 in breast cancer and related mechanism has not been fully elucidated[10,11]. Thus, investigation of OCT4 gene regulation may be further used for the exploration of breast cancer biological treatment target.

MiRNA is a kind of short stranded noncoding RNA that plays an important role in regulating gene expression. MiRNA expression changes are found in multiple diseases, which may participate in regulating the pathogenesis and the severity of the disease. Under most conditions, miRNA degrades target mRNA by binding with them to inhibit gene expression. It is suggested that miRNA can regulate cell proliferation, migration, and infiltration as oncogene and tumor suppressor gene. Some miRNAs have been treated as a biomarker for tumor early detection, such as miR-122, miR-21, and miR-201, thus to play a key role in tumor diagnosis[12-14].

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Thus, this study analyzed miRNA targeting OCT4 in the tumor using bioinformatics, aiming to select miRNA regulating OCT4. Moreover, this research tested the regulatory role of miRNA on OCT4 and biological behavior in breast cancer cells.

Materials and Methods

General Information

Human breast cancer cell line BS524 was purchased from Shanghai Yanjing Biological Technology co., Ltd (Shanghai, China). Dulbecco's Modified Eagle Medium (DMEM)/F12 medium and phosphate-buffered saline (PBS) were bought from Sigma-Aldrich (St. Louis, MO, USA). Cell culture related materials were produced by Corning (Corning, NY, USA). Fetal bovine serum (FBS) and trypsin were got from Hyclone (Thermo Fisher Scientific, Waltham, MA, USA). Penicillin-streptomycin was obtained from Beyotime (Suzhou, China). RNA extraction kit was supplied by Bioteke (Beijing, China). Mouse anti-human OCT4 primary monoclonal and rabbit anti-mouse secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). cDNA synthesis kit was bought from TaKaRa (Dalian, China). MiRNA mimics (5`-3`:GUCCAGUUUUCCCAGGAAUCCCU) and inhibitor (5`-3`:GGAUUCUGGAAAUACUGUUCU) were synthetized by Sangon (Shanghai, China). qRT-PCR amplification kit was supplied by Sigma-Aldrich (St. Louis, MO, USA). Western blot related reagents were got from CWBio (Beijing, China). Matrigel and transwell chamber were supplied by BD Company (Franklin Lakes, NJ, USA). Cell electronic transfection instrument was produced by Lonza (Basel, Swiss). Real-time PCR amplifier was obtained from ABI (Thermo Fisher Scientific, Waltham, MA, USA). Microplate reader was supplied by Thermo (Thermo Fisher Scientific, Waltham, MA, USA).

Methods

MiRNA Prediction

According to human OCT4 gene sequence (KT584545.1) published by NCBI, TargetScanHuman 7.1 software (http://www.targetscan.org/vert_71/) was select to predict the potential miRNA. MiRNA with a high score was used for the following investigation.

BS524 Cell Cultivation and Transfection

BS524 cells were routinely cultured in medium containing 12% FBS and penicillin-streptomycin. The cells were used for transfection after resuscitated in an incubator for 24 h. The cells were seeded in 6-well plate at 1×10^4/well for 18-24 h. After cultured in serum-free medium for 4 h, the cells were digested and transfected at 48V for 35 μs. The final concentration of miR-145 mimics and inhibitor was 3 μg/ml. The cells were centrifuged at 800 g for 2 min and seeded in 6-well plate.

qRT-PCR

Total RNA was extracted from cells after transfection for 12 h, 24 h, and 48 h, respectively. Next, the RNA was used to synthetize cDNA according to the manual. The primers used were as follows. MiR-145 F(5`-3`):CACCTTGTCCTCACGGTCCAGT, miR-145 R(5`-3`):TACTGTTCTTGAGGTCATGGTT. OCT4 F(5`-3`):TGTAAGCTGCGGCCCTTGCTG, OCT4 R(5`-3`):ACTGCAGCAGATCAGCCACAT. GAPDH F(5`-3`):CGGAGTCAACGGATTTGGTCGTA, GAPDH R(5`-3`):AGCCTTCTCCATGGTGGTGAAG. The reaction system contained 0.08 μM primer, 10 μl Master Mix, 2 μl cDNA, 0.4 μl PCR Taq enzyme, and ddH_2O. The reaction was performed at 94°C for 3 min, followed by 45 cycles of 95°C for 13 s and 63°C for 40 s. Each experiment was repeated for 3 times.

Western Blot

Total protein was collected from cells by radioimmunoprecipitation assay (RIPA) and quantified using bicinchoninic acid (BCA) kit. A total of 5 μg protein was mixed with loading buffer and boiled for 10 min. Next, the protein was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane at 25V for 25 min. Then, the membrane was incubated with primary antibody at 1:200 and secondary antibody at 1:500. After treated by developer, the membrane was detected using Bio-Rad automatic detector (Bio-Rad, Hercules, CA, USA) and analyzed by related software. β-actin was used as internal reference to calculate the relative expression of OCT4.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) Assay

The cells were seeded in 96-well plate at 1×10^4/well and incubated for 12 h, 24 h, and 48

h, respectively. Then, 20 µl MTT solution was added to each well for 4 h and 150 µl DMSO was dropped into the well for 10 min at room temperature. At last, the plate was tested to obtain the OD value to calculate the cell proliferation rate.

Scratch Assay

A total of 5 × 10^5 cells were seeded in 60 mm dish and cultured for 24 h. A pipette tip for 200 µl was used to draw a straight line on the bottom. After cultured for 12 h, 24 h, and 48 h, the cells were observed under the inverted microscope to take a picture. The width of the scratch was analyzed by Image J software to calculate cell migration.

Transwell Assay

The cells were seeded in 24-well plate at $1×10^4$/well and stained by crystal violet according to the manual. The penetrated cells after transfection for 12 h, 24 h, and 48 h were observed under the microscope to calculate the cell number.

Statistical Analysis

All data analyses were performed on SPSS11.3 software (Chicago, IL, USA). The data were compared by ANOVA. LSD was performed as post-hoc test. $p < 0.05$ was considered as statistically significant.

Results

MiR-145 and OCT4 mRNA Expressions in Breast Cancer Cells

qRT-PCR was adopted to test miR-145 expression in breast cancer BS524 cells (Figure 1A). MiR-145 exhibited no statistical changes after transfection for 12 h ($p > 0.05$). After 24 h, miR-145 level significantly increased in BS524 cells transfected with miR-145 mimic ($p < 0.05$). MiR-145 inhibitor suppressed miR-145 expression in BS524 cells after 24 h ($p < 0.05$). The difference became more significant after 48 h ($p < 0.01$). It indicated that miR-145 mimic and inhibitor can effectively regulate miR-145 expression in BS524 cells. OCT4 mRNA expression demonstrated markedly negative correlation with miR-145 (Figure 1B, $p < 0.05$).

OCT4 Protein Expression in Breast Cancer Cells Transfected with miR-145

Western blot was adopted to determine OCT4 protein expression in BS524 cells after transfection for 12 h, 24 h, and 48 h. As shown in Figure 2, OCT4 expression gradually declined in miR-145 mimic transfection group compared with control, while it gradually elevated in miR-145 inhibitor transfection group following time extension ($p < 0.05$).

The Impact of OCT4 on BS524 Cell Proliferation

MTT assay was selected to evaluate the impact of OCT4 on cell proliferation. As shown in Figure 3, OCT4 overexpression enhanced BS524 cell proliferation with time extension.

The Impact of OCT4 on BS524 Cell Migration

As shown in Figure 4 and Table I, BS524 cell migration was determined by scratch assay. OCT4 upregulation markedly promoted BS524 cell migration, while its reduction significantly attenuated cell migration. The difference was significant after transfection for 24 h ($p < 0.05$).

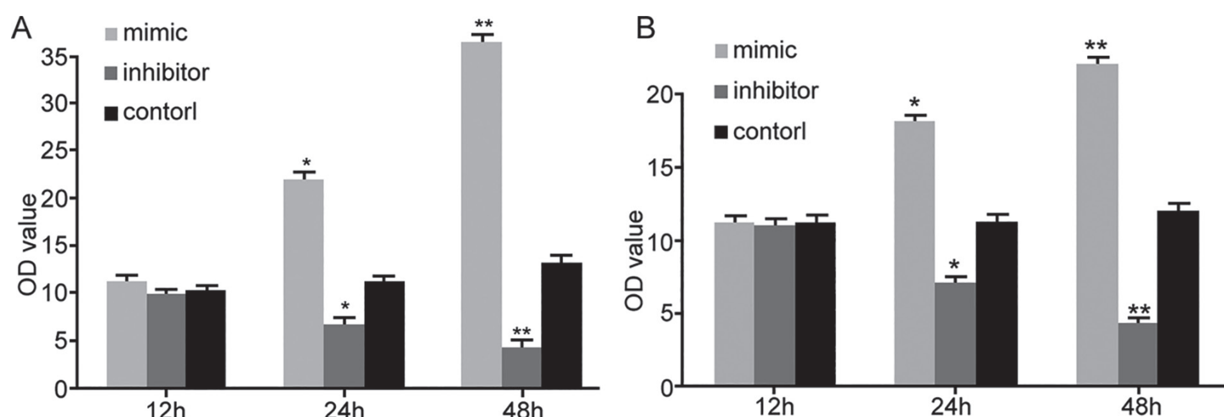

Figure 1. MiR-145 and OCT4 mRNA expressions in breast cancer cells after transfection. *$p < 0.05$, compared with control; **$p < 0.01$, compared with control.

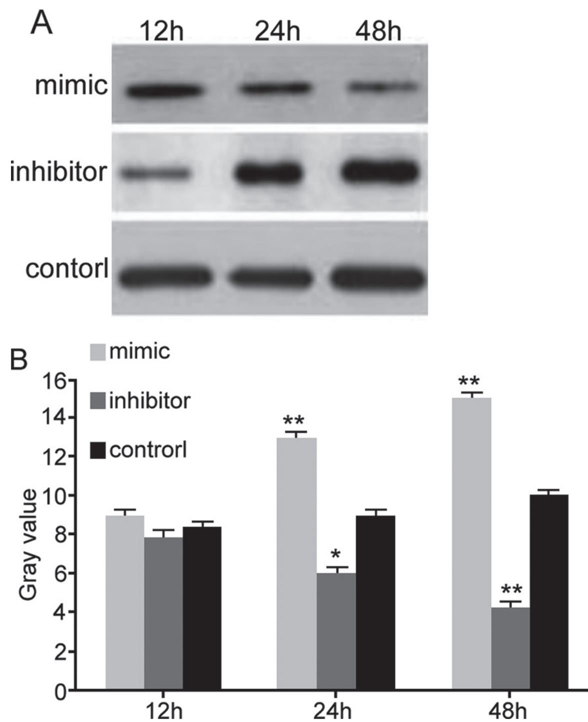

Figure 2. OCT4 protein expression in breast cancer cells transfected by miR-145. *$p < 0.05$, compared with control; **$p < 0.01$, compared with control.

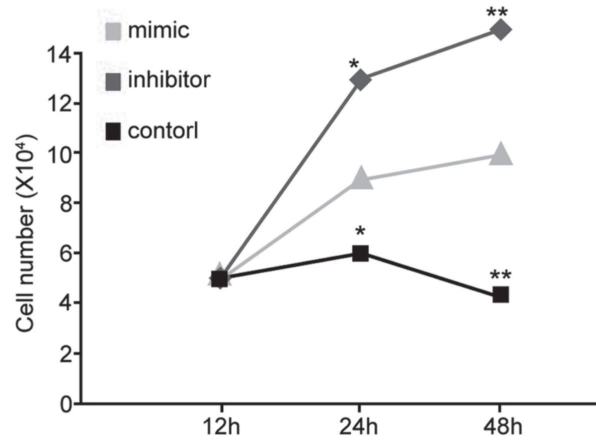

Figure 3. The impact of OCT4 on BS524 cell proliferation. *$p < 0.05$, compared with control; **$p < 0.01$, compared with control.

Table I. Cell migration distance.

	Mimic group	Inhibitor group	Control
12h	2.1 ± 0.65	4.89 ± 1.01	3.13 ± 0.92
24h	2.4 ± 0.64*	14.89 ± 3.43*	5.13 ± 0.92
48h	4.32 ± 8.13**	24.42 ± 5.13**	13.85 ± 2.23

*$p < 0.05$, compared with control; **$p < 0.01$, compared with control.

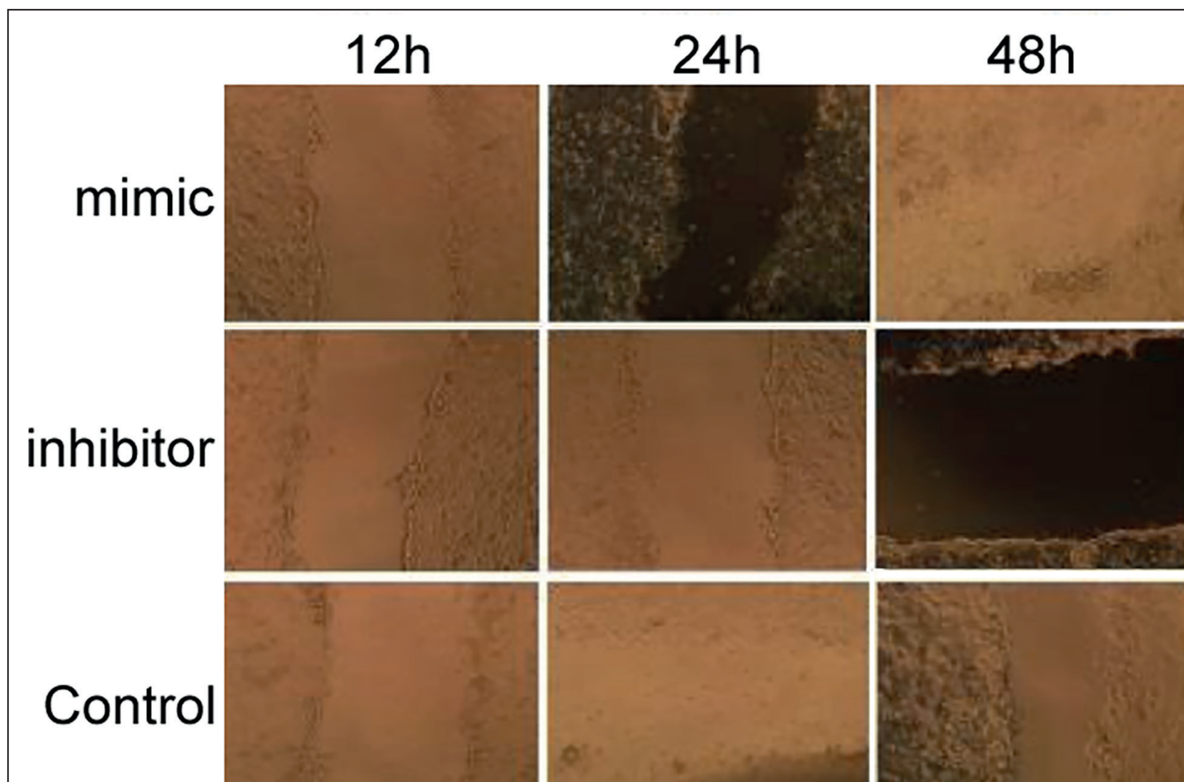

Figure 4. Scratch assay detection of cell migration.

The Influence of OCT4 on BS524 Cell Infiltration

Transwell assay was applied to assess the influence of OCT4 on BS524 cell infiltration. OCT4 upregulation accelerated BS524 cell infiltration, whereas its downregulation markedly restrained cell infiltration (Figure 5).

Discussion

The occurrence of breast cancer is a complicated process accompanied by the characteristics of familial heredity. Cancer cell proliferation and migration seriously affect the treatment of breast cancer[15-17]. Tumorigenesis often accompanies by

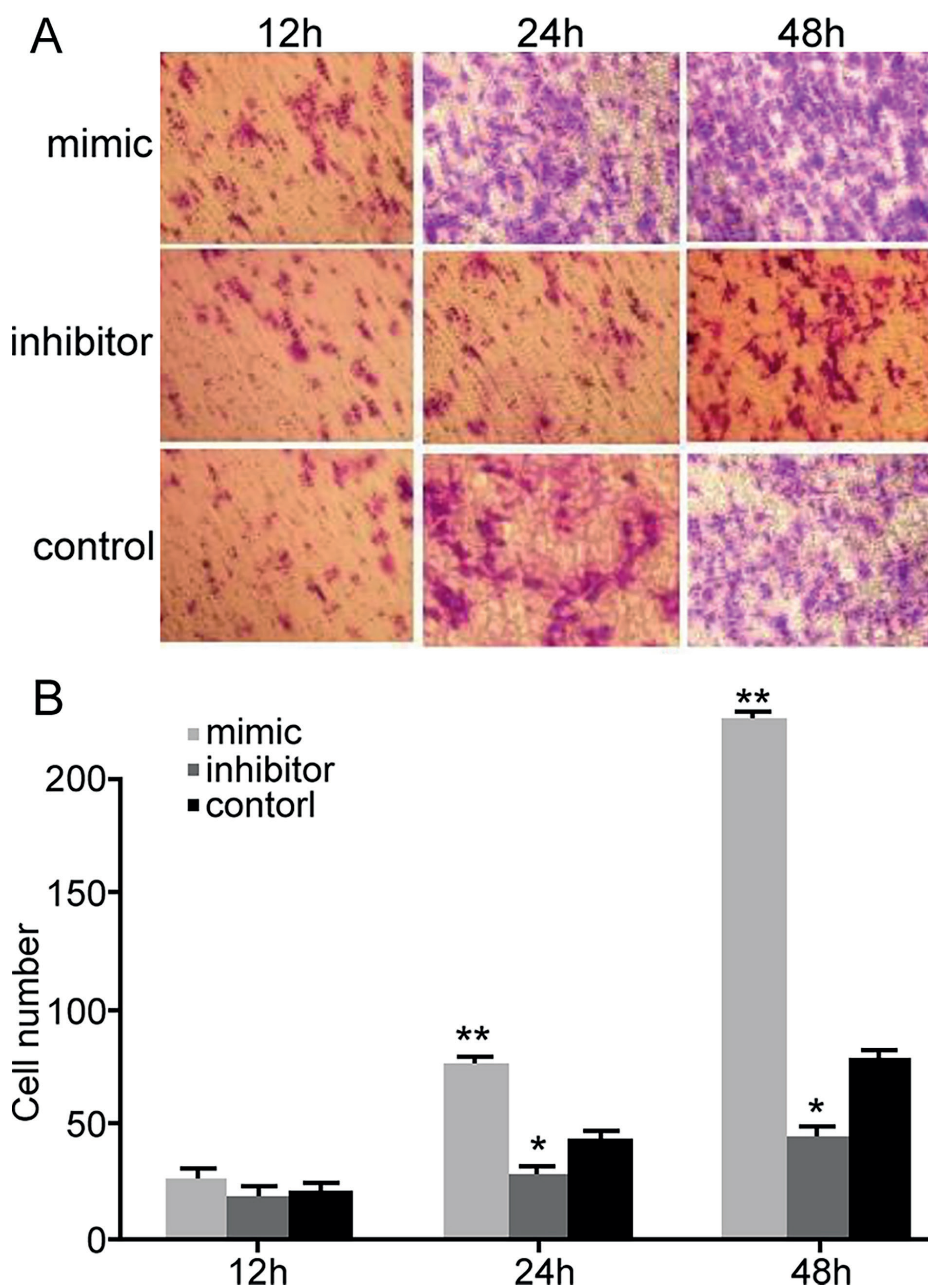

Figure 5. The influence of OCT4 on BS524 cell infiltration. *$p < 0.05$, compared with control; **$p < 0.01$, compared with control.

stemness gene overexpression, such as SOX2, SOX30, Nanog, and OCT4[18,19]. Thus, regulation of stemness gene expression may provide the basis for the selection of a biological target in clinical treatment. MiRNA is closely associated with cancer cell proliferation, apoptosis, and migration. Therefore, miRNA investigation has attracted much attention in tumor treatment[20-22].

As an important transcription factor in POU family, OCT4 is found upregulated in various embryonic germ cells and tumor cells. It was observed that OCT4 cannot only play a stemness role, but also regulate cancer cell proliferation, migration, and infiltration. It exhibits high value in the exploration of tumorigenesis, thus to be treated as a biomarker and treatment target for cancer.

MiRNA is a noncoding RNA at the length of 22 nt that widely exists in various organisms. It promotes or inhibits gene expression by binding with mRNA. It was suggested that miRNA was closely related to multiple diseases. It was reported that miRNAs participate in regulating the biological behavior of cancer cells as tumor suppressor gene. In addition, it was indicated that miR-145 expresses in early embryonic development and plays a regulatory role. Meanwhile, miR-145 may regulate OCT4 gene expression in mouse embryonic stem cells[23]. Urinary tract cancer research found that miRNA can regulate cancer cell proliferation and migration via targeting OCT4. Thus, it has certain clinical value to investigate the regulatory mechanism of miRNA on OCT4.

This study applied bioinformatics to analyze OCT4 to search potential miRNA. Our results showed multiple miR-145 targets on OCT4 gene. It was reported that miR-145 plays a regulatory role in various cancers, such as cervical cancer, renal cancer, and ovary cancer. It was suggested that miR-145 inhibited angiogenesis in breast cancer to reduce the risk of metastasis. MiR-145 was found to facilitate cell apoptosis in cervical cancer cell line SiHa[24,25]. It revealed that miR-145 exhibited regulatory role in a variety of cancers, thus was worthy of further investigation.

This study selected miR-145 mimic and inhibitor to treat BS524 cells to explore whether miR-145 participates in the biological function of breast cancer. It was demonstrated that miR-145 upregulation suppressed OCT4 expression and affected cell proliferation, migration, and infiltration of breast cancer cells. Our results exhibited that miR-145 may affect cell function via targeting OCT4, so it cannot fully represent the complete regulatory mechanism of miR-145. To sum up, miR-145 elevated in multiple cancer cells and regulated cellular biological function. Whether miR-145 could be treated as a new treatment target in most cancers still needs further investigation.

Conclusions

MiR-145 affects breast cancer BS524 cell proliferation, infiltration, and migration via positively regulating OCT4 gene expression.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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