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Effect of miR-200c on migration and proliferation of breast cancer MDA-MB-231 cells and BT-549 cells and the possible mechanism

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Abstract. – OBJECTIVE: This study was designed to investigate the effects of miR-200c on the migration and proliferation of breast cancer MDA-MB-231 cells and BT-549 cells and the possible mechanisms.

MATERIALS AND METHODS: The effects of miR-200c on the proliferation and migration of highly invasive human breast cancer MDA-MB-231 and BT-549 cells were investigated by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) MTT and wound-healing assay. Effects of miR-200c on the expression of adhesion molecules in cells of 2 cell lines were examined by Western blot.

RESULTS: MTT assay showed that miR-200c could inhibit the proliferation of MDA-MB-231 and BT-549 cells. Wound-healing assay results showed that miR-200c could inhibit the migration of MDA-MB-231 and BT-549 cells. Western blot results showed that miR-200c up-regulated the expression of E-cadherin protein and down-regulated the expression of Vimentin protein.

CONCLUSIONS: The results showed that miR-200c can inhibit the proliferation of highly invasive human breast cancer cells and may inhibit cell migration by up-regulating the expression of E-cadherin protein and down-regulating the expression of Vimentin protein.

Word

miR-200c, Triple negative, Vimentin.

Introduction

Triple negative breast cancer (TNBC) is a clinically common type. Compared with other types of breast cancer, the survival rate and cure rate of TNBC are significantly lower[1]. Clinically, TNBC is defined as breast cancer lacking the expression of three receptors: progesterone receptor (PR), estrogen receptor (ER) and human epidermal growth factor receptor-2 (HER2)[2]. At present, the treatment of TNBC is still limited to surgical treatment and drug treatment. Although the survival of TNBC patients has been improved with the application of natural drug extracts such as paclitaxel and vinblastine, some patients still develop tumor metastasis. Therefore, the identification of targets for TNBC therapy is a hot research field in breast cancer treatment.

Scholars[3] have shown that genes and signal pathways that are abnormally expressed in tumor cells may be potential targets for treating tumors or inhibiting tumor metastasis. The expression of microRNA (miR) is usually altered during the occurrence and development of cancers. In fact, miR-590-5p could inhibit the metastasis of breast cancer cells by targeting SOX2[4]; miR-4282 could inhibit the proliferation, invasion, and migration of breast cancer by targeting Myc[5], etc. The clinical research showed that the expression of miR-200c was significantly down-regulated in breast cancer tissues. Therefore, the upregulation of miR-200c may be a novel target for the treatment of TNBC[6]. However, potentials of miR-200c in the treatment of TNBC and related molecular mechanisms are not clear. In this work, we investigated the effects of miR-200c on the proliferation and migration of highly invasive human breast cancer MDA-MB-231 and BT-549 cells by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) MTT and wound-healing assay, respectively. The expression of adhesion molecules after miR-200c overexpression in cells of two cell line was detected by Western blot. The mechanism of action of miR-200c in the treatment of TNBC was preliminarily explored in this study.

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Materials and Methods

Main Reagents

MDA-MB-231 and BT-549 human breast duct cancer cells were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences. Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Hangzhou Sijiqing Bioengineering Materials Co., Ltd. (Hangzhou, Zhejiang, China). Trypsin for cell digestion was purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). MTT was purchased from Sigma-Aldrich (St. Louis, MO, USA). B-actin, E-cadherin, and Vimentin antibodies were purchased from Cell Signaling Technology (Boston, MA, USA). The fluorescent secondary antibody was purchased from Beyotime Biotechnology (Shanghai, China).

Cell Culture and Transfection

Cell culture: MDA-MB-231 and BT549 cells were cultured in DMEM medium containing 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37°C in a 5% CO_2 incubator. Subculture was performed when confluence reached 70-80%.

Mi-R200c transfection: MDA-MB-231 and BT549 cells in the logarithmic growth phase were digested with trypsin at 37°C for 3 min. Then, the appropriate amount of medium was added to terminate digestion and the cell suspension was transferred to a centrifuge tube, followed by centrifugation at 1000 r/min for 5 min. The supernatant was discarded and the fresh medium was added. Cell suspensions were prepared and were transferred to a 6-well plate with (4×10^5 cells/well). The DMEM medium was added to a final volume of 2 mL. After 24 h of cell culture, mi-R200c expression vectors purchased from GenePharma Co. Ltd., Shanghai, China were transfected using cationic liposome Lipofectamine™ 2000. The specific procedure was as follows: the excess medium in the 6-well plate was aspirated and cells were washed three times with phosphate-buffered solution (PBS). Next, 50 μL DMEM containing mi-R200c vectors (0.4 pmol/μl) was added. After that, 6 μL Lipofectamine™ 2000 was added. Transfection was achieved after 6 h of incubation.

MTT Assay

MDA-MB-231 cells, BT549 cells, MDA-MB-231 cells transfected with the mi-R200c plasmid, and BT549 cells transfected with mi-R200c plasmid were seeded in 96-well plates (about 2000 cells per well). 20 μL of MTT solution (5 mg/mL) was added into each well at 24 h, 48 h, and 72 h after inoculation. Cells were cultivated for an additional 4 h and the purple crystals formed were dissolved in dimethyl sulfoxide (DMSO), followed by measurement of OD values at 549 nm after shaking for 15 min.

Wound-Healing Assay

MDA-MB-231 cells, BT549 cells, MDA-MB-231 cells transfected with mi-R200c plasmid, and BT549 cells transfected with mi-R200c plasmid were inoculated into 6-well plates (1×10^5 cell/well) and incubated for 24 h. A 200 μL tip was used to scratch the plates. After that, plates were washed several times with PBS to remove the exfoliated cells. The plates were placed in an incubator to continue the culture and the scratch healing was observed at different time points.

Western Blot

Cells (2×10^6) were washed with PBS at about 80% confluence. 4 mL of cell lysate containing 100 μL phenylmethanesulfonyl fluoride (PMSF)/1 mL was added and cells were kept on ice for 30 min to achieve lysis. After that, cells were transferred to a centrifuge tube, followed by centrifugation at 12000 r/min for 5 min. The concentration of protein in the supernatant was determined using a bicinchoninic acid (BCA) quantification kit. After denaturing, cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by gel transfer. The quantified protein sample was boiled for 10 min with the buffer solution before electrophoresis. After the electrophoresis, the separation gel was removed and the sample was transferred to a polyvinylidene difluoride (PVDF) membrane. Membranes were incubated with primary and secondary antibodies. Signals were developed using enhanced chemiluminescence (ECL). An appropriately sized X-ray photographic film is cut according to the size of the PVDF film to perform exposure. The exposed film was placed in a gel imaging analysis system, and the molecular weight and optical density values of the target bands were analyzed using Quantity One image analysis software.

Statistical Analysis

All data were analyzed using SPSS 17.0 statistical software. Comparisons of data between the two groups were performed by Student's *t*-test.

Results were expressed as mean ± standard deviation. The difference was statistically significant when $p<0.05$.

Results

Cell Proliferation Detected by MTT Assay

Effect of mi-R200c on the cell proliferation of MDA-MB-231 cells and BT-549 cells was detected by the MTT assay. The OD values of the four groups of cells showed an increasing trend during cell culture. At 24 h, mi-R200c had no significant effect on the OD of MDA-MB-231 cells and BT-549 cells. At 48 h and 72 h, OD values of MDA-MB-231 cells and BT-549 cells transfected with mi-R200c were significantly lower than those of MDA-MB-231 cells and BT-549 cells without transfection ($p<0.05$). It is proved that mi-R200c can inhibit the proliferation of MDA-MB-231 cells and BT-549 cells (Figure 1).

Cell Migration Tested by Wound-Healing Assay

Migration abilities of MDA-MB-231 cells, BT-549 cells, and MDA-MB-231 cells transfected mi-R200c and BT-549 cells were evaluated wound-healing assay. The results are shown in F ure 2. Two days after scratching, the und-hea ing of MDA-MB-231 cells and B trans fected with mi-R200c was sig ntly w se than that of MDA-MB-231 and B c demonstrated that mi-R2 inh the migration ability of cancer o a certa xtent.

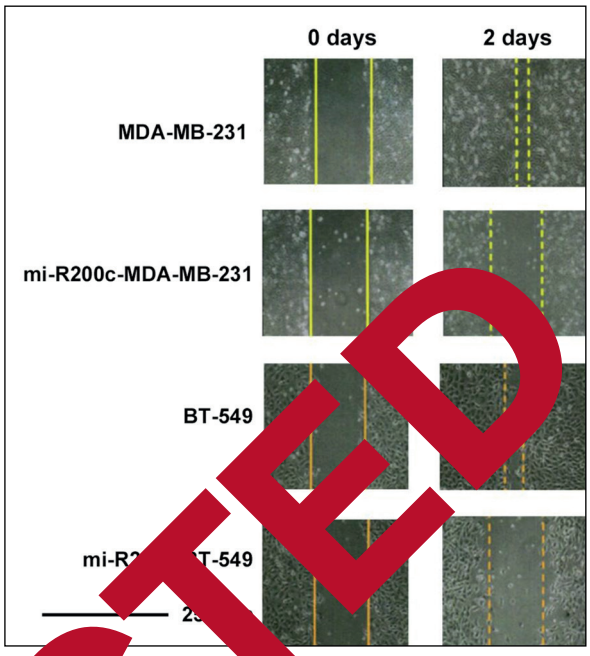

Figure 2. Wound-healing results of different groups of A-MB-213, R200c-MDA-MB-213, BT-549, and mi-c- BT-549 .

stern Blot Detection of ed Adhesion Factors

Western blot results are shown in Figure 3. Relative expression levels of E-cadherin in BT-549 cells and MDA-MB-231 cells were 100% and 98%, respectively, which were significantly lower than those in BT-549 cells (241%) and MDA-MB-231 (265%) with mi-R200c transfection. In

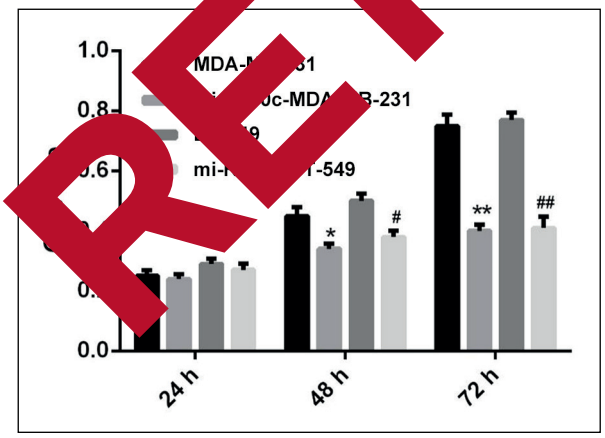

Figure 1. OD values of different groups of MDA-MB-213, mi-R200c-MDA-MB-213, BT-549 and mi-R200c- BT-549 cells at 24 h, 48 h and 72 h. Higher OD values indicate more cells. Notes: *$p<0.05$, **$p<0.01$, compared to MDA-MB-231 cells; $^{\#}p<0.05$, $^{\#\#}p<0.01$, compared to BT-549 cells.

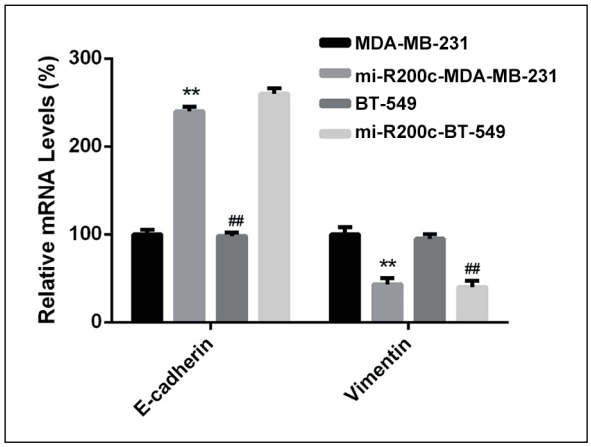

Figure 3. Western blot analysis of MDA-MB-213, mi-R200c-MDA-MB-213, BT-549, and mi-R200c- BT-549 cells. Notes: compared with MDA-MB-231 cells, *$p<0.05$, **$p<0.01$; compared with BT-549 cells, $^{\#}p<0.05$, $^{\#\#}p<0.01$.

addition, relative expression levels of Vimentin BT-549 cells and MDA-MB-231 cells were 100% and 95%, respectively, which were significantly lower than those in BT-549 cells and MDA-MB-231 with mi-R200c transfection. Results suggested that mi-R200c could up-regulate the expression of E-cadherin, a key protein of epithelial-associated cell adhesion, and down-regulate the expression of interstitial-associated adhesion protein Vimentin.

Discussion

MiR is a group of non-coding RNAs composed of only 18 to 25 nucleotides and they were first discovered in nematodes[7]. MiR can form a complementary pair with a specific part of the target mRNA, which can directly lead to the degradation of target mRNA, maintaining the level of mRNA within a normal range[8]. A miR may regulate the expression of hundreds of target mRNAs. Authors[9-11] have shown that a variety of tumor sites have an abnormal expression of miR, such as lung cancer, colon cancer, and breast cancer. MiRs may not only promote but inhibit tumor development. Iorio et al[12] found that expression levels of miR-145, miR-125b and miR-10b decreased, while expression levels of miR-155 and miR-21 increased in breast cancer tissues compared with those in surrounding tissues, indicating that different miRs may have different roles in the occurrence and development of tumors. Therefore, miR provides a new research direction for tumor therapy.

TNBC is a subtype of breast cancer with the highest degree of tumor deterioration. At present, there is no effective targeted therapy for this disease. Patients with TNBC usually develop tumor recurrence and metastasis. The pathogenesis of TNBC is not clear. It has been found that expression levels of certain miRs in TNBC tissues change significantly. Castilla et al found that the miR-200 family members were expressed to different levels in different breast cancer subtypes, and the expression level of miR-200c in TNBC was significantly lower than that in other breast cancer subtypes, indicating that miR-200c may play an important role in the development of TNBC. In this work, the miR-200c plasmid was transfected into highly invasive human breast cancer cells MDA-MB-231 and BT-549, which enabled the high expression of miR-200c in both cells. The MTT assay showed that mi-R200c had no significant effect on the proliferation of MDA-MB-231 cells and BT-549 cells within 24 h. But at 48 h and 72 h, proliferation rates of MDA-MB-231 cells and BT-549 cells transfected with mi-R200c were significantly lower than those of MDA-MB-231 cells and BT-549 cells without transfection. In addition, the wound-healing assay also showed that the overexpression of mi-R200c inhibited cell migration.

E-cadherin and Vimentin play important roles in the process of tumor invasion. Downregulation of E-cadherin and upregulation of Vimentin enable cancer metastasis[14]. E-cadherin is a member of the cadherin family that can promote cell-to-cell adhesion. In contrast, Vimentin promotes the mobility of tumor cells[16]. This research showed that mi-200c overexpression led to significantly upregulated expression of E-cadherin and downregulated the expression of Vimentin. Therefore, miR-200c may inhibit TNBC by interacting with these 2 factors.

Conclusions

This study detected that miR-200c can inhibit the proliferation and migration of highly invasive human breast cancer cells. The actions of miR-200c in TNBC are likely mediated by the downregulation of E-cadherin and upregulation of Vimentin. This research provided new insights for the treatment of TNBC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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