MIR-200B AFFECTS THE PROLIFERATION, MIGRATION AND INVASION OF BREAST CANCER CELLS BY REGULATING THE PI3K/AKT SIGNALING PATHWAY THROUGH THE REGULATION OF FUT4

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ABSTRACT

Objective: To analyze the effect of microRNA-200b (miR-200b) on the proliferation, migration and invasion of breast cancer cells by regulating the phosphatidylinositol 3 kinase/protein kinase B (PI3K/Akt) signaling pathway through the regulation of fucosyltransferase 4 (FUT4).

Methods: 35 cases of breast cancer tissues and adjacent normal tissues were randomly selected for surgical treatment in our hospital, and the expressions of miR-200b and FUT4 in breast cancer tissues and adjacent normal tissues were determined by real-time quantitative PCR. Human breast cancer cells (MCF-7) were cultured and transfected to obtain overexpressed miR-200b (miR-200b overexpressed group) and control cells (control group). Real-time quantitative PCR was used to determine the expression of miR-200b and FUT4 in the control group and the miR-200b overexpression group. Cell proliferation in each group was determined by CCK-8 and clonogenesis assay. The cell migration ability of each group was determined by scratch test. Transwell cell invasion assay was used to determine the change of cell invasion ability in each group. The expression of PI3K and Akt in each group was determined by western blot.

Results: Compared with normal adjacent tissues, the expression level of miR-200b in breast cancer tissues was significantly decreased, and the expression level of FUT4 was significantly increased (P<0.01). Compared with the control group, the expression level of miR-200b was significantly increased in the miR-200b overexpression group, and the expression level of FUT4 was significantly decreased (P<0.05). With the extension of time, both groups of cells showed an upward trend. There was no significant difference in cell proliferation capacity between the two groups at 1d and 2d (P>0.05). Starting from the 3rd day, the proliferation capacity of cells in the miR-200b overexpression group was significantly lower than that in the control group (P<0.05 or P<0.01). Compared with the control group, the miR-200b overexpression group had significantly reduced cell clone formation, migration ability, invasion ability, and PI3K and Akt expression levels (P<0.05).

Conclusion: miR-200b may inhibit the activation of PI3K/Akt signaling pathway by down-regulating FUT4 expression, thereby inhibiting the proliferation, migration and invasion of breast cancer cells.

Keywords: miR-200b, FUT4, PI3K/Akt signaling pathway, breast cancer, cell proliferation, migration, invasion.

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Introduction

Breast cancer is one of the most common malignancies in women worldwide. With the development of society and economy, changes in people’s lifestyle and diet structure, the incidence of breast cancer is increasing year by year, which has seriously affected women’s health and quality of life. The pathogenesis of breast cancer is more complicated, and is not clear at present. It is believed that its pathogenesis may be related to factors such as heredity and gene mutation(1). According to relevant statistics, more than 1.2 million women develop breast cancer every year, among whom more than 500,000 die of breast cancer annually, and the mortality rate of breast cancer has become the highest among all female malignant tumors(2). Therefore, it is of great significance to search for specific molecular mechanisms related to the diagnosis, occurrence and development of breast cancer and to provide a relevant theoretical basis for
clinical diagnosis and treatment. MicroRNAs (miRNAs) are a class of endogenous non-coding small RNA that can regulate gene expression by binding to the 3' end of its target mRNA, leading to mRNA degradation or inhibiting translation, and can also participate in biological engineering such as regulating cell proliferation, cell apoptosis and cell metabolism. MicroRNA-200b (miR-200b) is a member of the miRNA family. Studies have found that miR-200b plays an important role in the development of many diseases, especially malignant tumors, as well as the development of embryos.

Fucosyltransferase 4 (FUT4) belongs to α-1,3 Fucosyltransferase and is one of the candidate target genes of miR-200b. It has been reported that FUT4 is closely related to the proliferation, apoptosis, metastasis and invasion of tumor cells. FUT4 can be used as an index for early diagnosis and prognosis of tumors. Some studies have found that FUT4 can promote breast cancer cell proliferation, migration and invasion, but the relationship between miR-200b and FUT4 and its role and mechanism in breast cancer is unclear. To investigate whether miR-200b can affect the biological process and mechanism of breast cancer cells by regulating FUT4, the following is reported.

Materials and methods

Experimental tissues and cells
Breast cancer tissue and normal tissues adjacent to the cancer (35 cases of breast cancer tissues and normal tissues adjacent to the cancer that were surgically removed in our hospital were randomly selected); MCF-7 human breast cancer cells (Procell Life Science & Technology Co., Ltd.).

Experimental instruments and reagents
Biological microscope (Shanghai Precision Instruments Co., Ltd., model: XSP-8C); real-time quantitative PCR (Jinan Guangyao Medical Equipment Co., Ltd., model: TL-988-IV); low-temperature high-speed centrifuge (Huawei Kechuang Technology Co., Ltd., model: SIGMA 3-18K); ultra-low-temperature refrigerator (Zhejiang Jiesheng Low-temperature Equipment Co., Ltd., model: DW-86W300); autoclave (Shanghai Boxun Medical Biological Instrument Corp., model: BXM-50VE); DMEM medium [Thermo Fisher Scientific (China) Co., Ltd.]; phosphate buffer solution (Shanghai Jingke Chemical Technology Co., Ltd.); fetal bovine serum (Hangzhou Qiannuo Biotechnology Co., Ltd.); CCK-8 reagent (Beijing Think-Far Technology Co., Ltd., specification: 500t); rabbit anti-human FUT4 monoclonal antibody (Shanghai Xuanling Biotechnology Co., Ltd.); rabbit anti-human PI3K monoclonal antibody (Shanghai Xiyuan Biotechnology Co., Ltd.); rabbit anti-human Akt monoclonal antibody (Shanghai Kemin Biotechnology Co., Ltd.).

Experimental methods and observation indicators
Real-time quantitative PCR was used to determine the expression of miR-200b and FUT4 in breast cancer tissues and adjacent normal tissues.

Cell culture
The MCF-7 human breast cancer cell was cultured at 37 °C, 5% CO2, 10% fetal bovine serum, and 90% humidity. The medium was changed in time during the culture process, and when the cells grew to the logarithmic growth phase, cells were passaged.

Cell transfection
2,000 cells/well were inoculated into 6-well plates in a good growth state and in the logarithmic growth phase, and the cells were transfected when they grew to about 70%. 5 μL of 100 pmol/L miR-200b mimic was taken and transfected into MCF-7 cells for culture to obtain over-expressed miR-200b. Four duplicate wells were set up in each group, and the expression of miR-200b and FUT4 in the control and miR-200b overexpression groups was determined by real-time quantitative PCR.

Cell proliferation
Cell proliferation in each group was determined by CCK-8 method. The cells in good growth state and in the logarithmic growth phase were made into cell suspension and inoculated into 6-well plate with 2000 cells/holes, and were then put into the incubator for culture. After 1d, 2d, 3d, 4d, 5d and 6d, 10 μl CCK-8 solution was added to each well, and culture was continued for 3 h. The absorbance value at 470 nm was determined by enzyme-labeled instrument.

Cell clone formation experiment
Cells in a good growth state and in the logarithmic growth phase were made into cell suspension and seeded in a cell culture dish at an appropriate density for culture. Once the cells in the dish were visible to the naked eye (there were about 50 cells per clone) the cells’ culture was terminated. The Giemsa staining solution was used for staining, and
after washing and drying, the clones were counted under a microscope. When more than 50 cells were counted, they were photographed and stored to calculate the colony formation rate. Colony formation rate = (number of clones/number of inoculated cells) × 100%.

**Cell migration**

The cell migration ability of each group was measured by scratch test. The cells in good growth state and in logarithmic growth phase were made into cell suspension, which was spread in the cell culture dish with appropriate density. When the growth reached about 85%, the cells were put in the cell incubator for culture, and the liquid was changed after 8 h. During liquid change, vertical scratches were made with the nozzle head, and photos were taken at 0 h and 24 h.

**Cell invasion**

Transwell cell invasion assay was used to determine the change of cell invasion ability in each group. 200 μL of mixed cells were added to the upper compartment of the Transwell compartment, and 800 μL of DMEM/F12 medium containing 10% FBS was added to the lower compartment of the Transwell compartment to avoid bubbles during the operation. They were cultured in a cell incubator for 24 h. Crystal violet dye was used for dyeing, phosphate buffer was rinsed, and photos were taken under the microscope. 5 fields were randomly selected for counting, and each group of experiments was repeated several times.

The expression of PI3K and Akt in each group of cells was determined by western blotting.

**Statistical methods**

The comparison of count data in this group of studies was performed using χ² comparison, expressed as [n (%)]. Measurement data comparisons were performed using independent sample t-tests, expressed as (x̅±s). Real-time quantitative PCR was used to determine the expression of miR-200b and FUT4 in breast cancer tissues and adjacent normal tissues. Real-time quantitative PCR was used to determine the expression of miR-200b and FUT4 in the control and miR-200b overexpression cells.

CCK-8 method and clone formation experiments were used to determine the cell proliferation in each group. The scratch migration test was used to determine the cell migration ability of each group. Transwell cell invasion assay was used to determine the cell invasion ability of each group. The expression of PI3K and Akt in each group of cells was determined by western blotting. In this group of studies, SPSS 21.0 software was used for statistical data analysis, and the statistical result P<0.05 was considered to be statistically significant.

**Results**

**Comparison of miR-200b and FUT4 expression in breast cancer tissues and adjacent normal tissues**

Compared with normal tissues adjacent to cancer, the expression level of miR-200b in breast cancer tissues was significantly reduced, and the expression level of FUT4 was significantly increased (P<0.01). See Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases (n)</th>
<th>miR-200b</th>
<th>FUT4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer tissues</td>
<td>35</td>
<td>0.0004±0.0001</td>
<td>0.0028±0.0003</td>
</tr>
<tr>
<td>Adjacent normal tissue</td>
<td>35</td>
<td>0.0012±0.0002</td>
<td>0.0019±0.0002</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>15.875</td>
<td>14.767</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 1: Comparison of miR-200b and FUT4 expression in breast cancer tissues and adjacent normal tissues (x̅±s).

**Comparison of miR-200b and FUT4 expression in the control group and miR-200b overexpression group cells**

Compared with the control group, the expression level of miR-200b in the miR-200b overexpression group was significantly increased, and the expression level of FUT4 was significantly reduced (P<0.05). See Table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>miR-200b</th>
<th>FUT4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0005±0.0001</td>
<td>0.0003±0.0001</td>
</tr>
<tr>
<td>miR-200b overexpression</td>
<td>0.0010±0.0003</td>
<td>0.0001±0.0001</td>
</tr>
<tr>
<td>t</td>
<td>3.162</td>
<td>2.828</td>
</tr>
<tr>
<td>P</td>
<td>0.020</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Table 2: Comparison of miR-200b and FUT4 expression in the control group and miR-200b overexpression group (x̅±s).

**Comparison of cell proliferation**

With the extension of time, the cells in both groups showed an upward trend. There was no significant difference in cell proliferation capacity between the two groups at 1 d and 2 d (P>0.05). Starting from the 3rd day,
the proliferation capacity of cells in the miR-200b overexpression group was significantly lower than that of the control group. See Table 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>1 d</th>
<th>2 d</th>
<th>3 d</th>
<th>4 d</th>
<th>5 d</th>
<th>6 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.39±0.01</td>
<td>0.53±0.06</td>
<td>0.61±0.05</td>
<td>0.79±0.08</td>
<td>0.96±0.09</td>
<td>1.14±0.10</td>
</tr>
<tr>
<td>miR-200b overexpression</td>
<td>0.38±0.02</td>
<td>0.42±0.07</td>
<td>0.52±0.04</td>
<td>0.61±0.06</td>
<td>0.72±0.04</td>
<td>0.79±0.06</td>
</tr>
<tr>
<td>t</td>
<td>0.894</td>
<td>2.386</td>
<td>2.811</td>
<td>3.600</td>
<td>4.874</td>
<td>6.003</td>
</tr>
<tr>
<td>P</td>
<td>0.406</td>
<td>0.054</td>
<td>0.031</td>
<td>0.011</td>
<td>0.003</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 3: Comparison of cell proliferation (x̅±s).

**Experimental results of clone formation**

Compared with the control group, the miR-200b overexpression group showed significantly decreased cell cloning and formation ability (P<0.05), as shown in Figure 1.

**Changes in cell migration ability in each group**

Compared with the control group, the cell migration ability of the miR-200b overexpression group was significantly decreased (P<0.01). See Figure 2 and Table 4.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cell migration capacity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.94±2.53</td>
</tr>
<tr>
<td>miR-200b overexpression</td>
<td>7.11±2.45</td>
</tr>
<tr>
<td>t</td>
<td>6.150</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 4: Comparison of cell migration capacity of each group (x̅±s).

**Cell invasion ability changes in each group**

Compared with the control group, the invasion ability of miR-200b overexpression group was significantly reduced (P<0.01). See Figure 3, Table 5.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cell invasion ability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>300.17±43.66</td>
</tr>
<tr>
<td>miR-200b overexpression</td>
<td>126.37±41.54</td>
</tr>
<tr>
<td>t</td>
<td>5.768</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Table 5: Comparison of cell invasion ability of each group (x̅±s).

**Comparison of PI3K and Akt expression in each group**

Western blot results showed that the expression levels of PI3K and Akt in the miR-200b overexpression group were significantly lower than those in the control group (P<0.05). As shown in Figure 4.
Discussion

With the rapid development of China's economy and the acceleration of the pace of work and life, people's social pressure is gradually increasing, and the incidence of breast cancer is gradually rising, becoming one of the most common malignant tumors that seriously threaten women's physical and mental health, and even their lives. Statistics show that the incidence of breast cancer is regional, expanded, urbanized and younger, and the incidence of breast cancer is significantly higher in cities than in rural areas. In clinical practice, breast cancer can be divided into different molecular subtypes according to the different molecular characteristics, with different prognoses. Therefore, early prevention, early detection, early diagnosis and early treatment of breast cancer are effective means to improve the survival rate after treatment.

miRNAs are a class of endogenous non-coding single-stranded RNA, which were first discovered by Lee et al. in nematode worms. They can regulate gene expression after transcription and play an important role in cell proliferation, apoptosis and other processes. In recent years, studies have confirmed that miRNAs can regulate gene expression by targeting specific targets, thus participating in the occurrence and development of diseases. Mir-200 is a member of the miRNA family, which includes miR-200a, miR-200b, miR-200c, miR-141, etc. Studies have found that the expression level of miR-200b is closely related to the proliferation, metastasis, invasion and epithelial-mesenchymal transformation of tumor cells. In gastric cancer, miR-200b expression is down-regulated, and ZEB1 and ZEB2 expression levels are increased, thus promoting tumor invasion and metastasis. In ovarian cancer, the increased expression level of miR-200b can significantly inhibit the invasion and metastasis of tumor cells. In gastric cancer, miR-200b expression is down-regulated, and ZEB1 and ZEB2 expression levels are increased, thus promoting tumor invasion and metastasis. In ovarian cancer, the increased expression level of miR-200b can significantly inhibit the invasion and metastasis of tumor cells. In gastric cancer, miR-200b expression is down-regulated, and ZEB1 and ZEB2 expression levels are increased, thus promoting tumor invasion and metastasis. In ovarian cancer, the increased expression level of miR-200b can significantly inhibit the invasion and metastasis of tumor cells. Fucosyltransferase (FUT) is an enzyme protein that can catalyze the transfer of fucosin to oligosaccharides, glycolipids and other substrates, or to epidermal growth-factor-like repeats, threonine, and encode the glycosylation of complex glycolipids. At present, a total of 13 types of FUT have been discovered, which play an important role in the occurrence and development of tumors. FUT4, a member of the FUT family, is mainly expressed in white blood cells and epithelial cells, and is a key enzyme for LewisY (LeY) oligosaccharide antigen synthesis in tumors derived from epithelial tissues, which can regulate LeY synthesis. The results of this study found that the expression level of miR-200b in breast cancer tissues was significantly lower than that in adjacent normal tissues, and miR-200b could regulate the expression of FUT4 in a targeted manner. miR-200b and FUT4 may be widely used as important indicators in the diagnosis and treatment of breast cancer.

The unlimited proliferation and metastasis of tumor cells is one of the biological characteristics of tumors. The metastasis of cancer is a complicated process, among which the invasion of tumor cells is the most important. Therefore, inhibiting the growth of breast cancer cells and blocking the invasion and metastasis of tumor cells have developed into a treatment of breast cancer. In this study, changes in cell proliferation, migration and invasion ability were analyzed by CCK-8 method, cell scratch experiment and transwell cell invasion experiment. The results showed that miR-200b could significantly inhibit the proliferation, invasion and migration ability of breast cancer cells.

Phosphatidylinositol 3 kinase (PI3K) is an intracellular phosphatidylinositol kinase with lipid kinase activity, namely protein kinase activity. When activated, PI3K can convert the substrate phosphatidylinositol 2-phosphate into phosphatidylinositol 3-phosphate, which is involved in cell proliferation, differentiation, migration and other processes. Protein kinase B (Akt), as the downstream effector of PI3K, can phosphorylate a variety of cell proteins, promote cell survival and circulation. The signaling pathway composed of PI3K and Akt exists widely in cells and participates in a series of physiological processes including cell proliferation, differentiation, metabolism and apoptosis. It has been reported that the PI3K/Akt signaling pathway has an important relationship with tumor lymph node metastasis. The results of this group of studies found that miR-200b can significantly inhibit the expression levels of PI3K and Akt and inhibit the activation of PI3K/Akt signaling pathway, which is consistent with the results of Zheng et al.

In summary, miR-200b may inhibit the activation of the PI3K/Akt signaling pathway by down-regulating FUT4 expression, thereby inhibiting the proliferation, migration and invasion of breast cancer cells.
References


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