USP39 and EphA2 expression in breast cancer before and after trastuzumab treatment and their correlation with the pathological characteristics of tumor

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OBJECTIVE: To study the USP39 and EphA2 expression in breast cancer before and after trastuzumab treatment and their correlation with the pathological characteristics of tumor.

METHODS: Breast cancer tissues before and after trastuzumab treatment between May 2013 and September 2016 were collected, the expression levels of USP39 and EphA2 as well as anti-proliferation genes and pro-invasion genes in breast cancer were determined, and the quartile of USP39 and EphA2 expression after treatment were calculated. RESULTS: USP39, EphA2, Sam68 and Gab2 mRNA expression in breast cancer after treatment were significantly lower than those before treatment while ALEX1, PTPN14 and ARID1A mRNA expression were significantly higher than those before treatment; ALEX1, PTPN14, ARID1A, Sam68 and Gab2 mRNA expression in breast cancer with different USP39 and EphA2 expression were significantly different; the higher the USP39 and EphA2 mRNA expression, the lower the ALEX1, PTPN14 and ARID1A expression, and the higher the Sam68 and Gab2 expression. CONCLUSION: Trastuzumab therapy can influence the malignant biological behavior and pathological characteristics of tumor through inhibiting the expression of USP39 and EphA2.

1. Introduction

Breast cancer is a female malignant tumor with the highest incidence, radical resection is a major means of clinical treatment, and postoperative radiotherapy and chemotherapy is still needed to kill cancer cells and furthest prevent tumor recurrence. Human epidermal growth factor receptor-2 (HER-2) is a kind of membrane receptor that plays an important role in the development and change of breast cancer, HER-2 has high expression in lesions of about a quarter of patients with breast cancer and the higher the HER-2 expression, the greater the risk of long-term breast cancer recurrence[1,2]. Trastuzumab is the human recombinant monoclonal antibody targeting HER-2, has high affinity to HER-2 and can antagonize the biological effects of HER-2. In recent years, trastuzumab combined with chemotherapeutics is increasingly used in the treatment of breast cancer, and numerous studies have confirmed the positive value of trastuzumab for treatment of breast cancer[3,4]. Ubiquitin-specific protease 39 (USP39) and Eph receptor tyrosine kinase A2 (EphA2) are the important molecules regulating the malignant biological behavior of breast cancer cells[5,6], and there is no report whether trastuzumab adjusts the expression of USP39 and EphA2 in breast cancer lesions to influence the proliferation and invasion of cancer cells in lesions. In the following study, USP39 and EphA2 expression in breast cancer before and after trastuzumab treatment and their correlation with the pathological characteristics of tumor were analyzed.

2. Materials and methods

2.1 Clinical materials

Breast cancer tissues between May 2013 and September 2016 before and after trastuzumab treatment were collected, all patients
were diagnosed clearly with breast cancer and planned to receive preoperative neoadjuvant chemotherapy, a total of 76 patients were included, and neoadjuvant chemotherapy for breast cancer was as follows: paclitaxel 175 mg/m\(^2\), by intravenous drip, on day 1, trastuzumab 8 mg/kg, by intravenous drip, on day 2, 21 d as one cycle, for 1 cycle; paclitaxel 175 mg/m\(^2\), by intravenous drip, on 1 d, trastuzumab 6mg/kg, by intravenous drip, on day 2, 21 d as one cycle, for 3 cycles.

2.2 Experimental materials

Trizol lysis buffer was purchased from Invitrogen Company, first-strand cDNA synthesis kits were bought in Promega Company, and fluorescence quantitative PCR kits were bought from Beijing Tiangen Company.

2.3 Experimental methods

Breast cancer tissues before and after treatment were taken and added in Trizol lysis buffer to extract total RNA, then first-strand cDNA synthesis kits were used for reverse transcription from total RNA in the tissue to cDNA, and the cDNA samples were saved in a -20 ℃ refrigerator. Specific primers for USP39, EphA2, ALEX1, PTPN14, ARID1A, Sam68 and Gab2 were designed, cDNA samples + specific primers + PCR reaction mixture system was configured for fluorescence quantitative PCR amplification, and the amplification curve was referred to calculate USP39, EphA2, ALEX1, PTPN14, ARID1A, Sam68 and Gab2 mRNA expression.

2.4 Statistical methods

SPSS 22.0 software was used for statistical processing, the quartile of USP39 and EphA2 mRNA expression in breast cancer after trastuzumab treatment was calculated, and the quartile was used to divide the USP39 and EphA2 mRNA expression into USP39-Q1, -Q2, -Q3 and -Q4 groups as well as EphA2-Q1, -Q2, -Q3 and -Q4 groups from low to high; measurement data comparison between two groups was by t test and comparison among four groups was by variance analysis. \( P < 0.05 \) was the standard of statistical significance in differences.

3. Results

3.1 USP39 and EphA2 expression in breast cancer before and after treatment

Before and after trastuzumab treatment, USP39 and EphA2 expression in breast cancer were as follows: before treatment, USP39 and EphA2 mRNA expression in breast cancer were (1.05±0.17) and (1.02±0.15) respectively; after treatment, USP39 and EphA2 mRNA expression in breast cancer were (0.42±0.07) and (0.35±0.06) respectively. After t test, USP39 and EphA2 mRNA expression in breast cancer after treatment were significantly lower than those before treatment, and differences in USP39 and EphA2 mRNA expression in breast cancer were statistically significant before and after treatment (\( P < 0.05 \)).

3.2 Proliferation and invasion gene expression before and after treatment

Before and after trastuzumab treatment, analysis of anti-proliferation genes ALEX1, PTPN14 and ARID1A as well as pro-invasion genes Sam68 and Gab2 expression in breast cancer was as follows: ALEX1, PTPN14 and ARID1A mRNA expression in breast cancer after treatment were significantly higher than those before treatment while Sam68 and Gab2 mRNA expression were significantly lower than those before treatment. Differences in ALEX1, PTPN14, ARID1A, Sam68 and Gab2 mRNA expression in breast cancer were statistically significant before and after treatment (\( P < 0.05 \)).

3.3 Correlation of USP39 and EphA2 with proliferation and invasion genes

After treatment, analysis of the correlation of USP39 expression in breast cancer with anti-proliferation genes ALEX1, PTPN14 and ARID1A as well as pro-invasion genes Sam68 and Gab2 expression was as follows: ALEX1, PTPN14 and ARID1A mRNA expression in breast cancer of USP39-Q2, -Q3 and -Q4 groups were significantly lower than those of USP39-Q1 group while Sam68 and Gab2 mRNA expression were significantly higher than those of USP39-Q1 group;
ALEX1, PTPN14 and ARID1A mRNA expression in breast cancer of USP39-Q3 and -Q4 groups were significantly lower than those of USP39-Q2 group while Sam68 and Gab2 mRNA expression were significantly higher than those of USP39-Q3 group; ALEX1, PTPN14 and ARID1A mRNA expression in breast cancer of USP39-Q4 groups were significantly lower than those of USP39-Q3 group while Sam68 and Gab2 mRNA expression were significantly higher than those of USP39-Q3 group.

After treatment, analysis of the correlation of EphA2 expression in breast cancer with anti-proliferation genes ALEX1, PTPN14 and ARID1A as well as pro-invasion genes Sam68 and Gab2 expression was as follows: ALEX1, PTPN14 and ARID1A mRNA expression in breast cancer of EphA2-Q1 group was significantly higher than those of USP39-Q3 group while EphA2-Q1 group while Sam68 and Gab2 mRNA expression were significantly higher than those of USP39-Q3 group; ALEX1, PTPN14 and ARID1A mRNA expression in breast cancer of EphA2-Q3 and -Q4 groups were significantly lower than those of EphA2-Q1 group while Sam68 and Gab2 mRNA expression were significantly higher than those of EphA2-Q1 group; ALEX1, PTPN14 and ARID1A mRNA expression in breast cancer of EphA2-Q3 and -Q4 groups were significantly lower than those of EphA2-Q2 group while Sam68 and Gab2 mRNA expression were significantly higher than those of EphA2-Q2 group; ALEX1, PTPN14 and ARID1A mRNA expression in breast cancer of EphA2-Q4 groups were significantly lower than those of EphA2-Q3 group while Sam68 and Gab2 mRNA expression were significantly higher than those of EphA2-Q3 group.

### 4. Discussion

The product encoded by USP39 gene is the key enzyme to regulate ubiquitin process, USP39 itself does not have the function of debiquitination, but its combination with USP4 can remove the inhibitory effect of Ubi1 domain on debiquitination catalytic activity of USP4 so as to strengthen the debiquitination process mediated by USP4. Increased USP39 gene expression and enhanced debiquitination process in breast cancer lesions can cause the changes in the expression of a variety of proliferation and invasion-related genes, and then promote the infiltrative growth of cancer cells[7]. To clarify the trastuzumab influence on USP39 expression in breast lesions, USP39 expression in breast cancer lesions was analyzed before and after trastuzumab treatment, and the results showed that USP39 mRNA expression in breast cancer after treatment was significantly lower than that before treatment. This means that trastuzumab can inhibit the expression of USP39 within the breast lesions, and thus it is speculated that trastuzumab can inhibit breast cancer cell proliferation and invasion mediated by USP39.

The protein encoded by EphA2 gene is a type of glycoprotein located in the cell membrane, and its combination with the corresponding ligand EphrinA1 can cause both ligand and receptor tyrosine phosphorylation, thus produce two-way signal transduction and start the cascade activation of downstream signaling pathways. Within the breast cancer lesions, high expression of EphA2 will cause excessive activation of signaling pathway cascade mediated by EphA2/EphrinA1, cause changes in the expression of a variety of target genes, and thus accelerate the cancer cell growth, movement and infiltration[8,9]. To clarify the trastuzumab effect on EphA2 expression in breast cancer lesions, EphA2 expression in breast cancer lesions was analyzed before and after trastuzumab treatment, and the results showed that EphA2 mRNA expression in breast cancer after treatment was significantly lower than that before treatment. It means that trastuzumab can inhibit the expression of EphA2 inside the breast cancer lesions, and thus it is speculated that trastuzumab can inhibit breast cancer cell proliferation and invasion mediated by EphA2.

The reduction of USP39 and EphA2 expression in breast cancer lesions before and after treatment is closely related to the changes of malignant biological behavior of cancer cells. Malignant proliferation is the prominent malignant biological behavior of breast cancer cells, and the expression deletion of ALEX1, PTPN14, ARID1A and other anti-proliferation genes is an important factor causing malignant breast cancer cell proliferation. ALEX1 is a member of the Arm repeat protein family that can increase the expression of Bax and Caspase-3 and start the mitochondrial pathway apoptosis[10,11]; PTPN14 is with tyrosine phosphatase activity and has inhibitory effect on cell cycle progress[12,13]; ARID1A is the chromosome

### Table 2.

USP39 correlation with proliferation and invasion genes.

<table>
<thead>
<tr>
<th>USP39</th>
<th>n</th>
<th>Proliferation genes</th>
<th>Invasion genes</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ALEX1</td>
<td>PTPN14</td>
</tr>
<tr>
<td>Q1</td>
<td>19</td>
<td>3.77±0.59</td>
<td>4.02±0.61</td>
</tr>
<tr>
<td>Q2</td>
<td>19</td>
<td>2.82±0.39</td>
<td>3.32±0.44</td>
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<tr>
<td>Q3</td>
<td>19</td>
<td>2.31±0.32</td>
<td>2.56±0.37</td>
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<tr>
<td>Q4</td>
<td>19</td>
<td>1.78±0.25</td>
<td>1.94±0.24</td>
</tr>
</tbody>
</table>

*: compared with USP39-Q1, **: compared with USP39-Q2, ***: compared with USP39-Q3, ****: compared with USP39-Q4, P<0.05.

### Table 3.

EphA2 correlation with proliferation and invasion genes.

<table>
<thead>
<tr>
<th>EphA2</th>
<th>n</th>
<th>Proliferation genes</th>
<th>Invasion genes</th>
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<tr>
<td></td>
<td></td>
<td>ALEX1</td>
<td>PTPN14</td>
</tr>
<tr>
<td>Q1</td>
<td>19</td>
<td>3.81±0.62</td>
<td>3.98±0.64</td>
</tr>
<tr>
<td>Q2</td>
<td>19</td>
<td>2.81±0.40</td>
<td>3.39±0.47</td>
</tr>
<tr>
<td>Q3</td>
<td>19</td>
<td>2.25±0.30</td>
<td>2.60±0.34</td>
</tr>
<tr>
<td>Q4</td>
<td>19</td>
<td>1.74±0.22</td>
<td>1.94±0.22</td>
</tr>
</tbody>
</table>

*: compared with EphA2-Q1, **: compared with EphA2-Q2, ****: compared with EphA2-Q3, ***: compared with EphA2-Q4, P<0.05.
remodeling complex SWI/SNF subunit, has repairing effect on DNA damage, and can inhibit malignant cell proliferation[14]. In the study, analysis of proliferation gene expression in breast cancer lesions before and after treatment showed that ALEX1, PTPN14 and ARID1A mRNA expression in breast cancer lesions after treatment were significantly higher than those before treatment. Further analysis of the relationship of USP39 and EphA2 expression with the proliferation gene expression by quartile method showed that the higher the USP39 and EphA2 expression in breast cancer lesions after treatment, the lower the ALEX1, PTPN14 and ARID1A expression. This means that USP39 and EphA2 have inhibitory effect on the expression of anti-proliferation genes, and trastuzumab treatment can reduce USP39 and EphA2 expression to increase the anti-proliferation genes ALEX1, PTPN14 and ARID1A expression, and thus inhibit the proliferation of breast cancer cells.

On the basis of malignant proliferation, breast cancer cells may show the characteristics of invasive growth, and cell invasion is the biological basis causingy invasive growth. Sam68 is a kind of RNA-binding protein, and it can adjust the expression of N-cadherin, Vimentin and other mesenchymal marker molecules by KH domain so as to promote the epithelial-mesenchymal transition, make the cells transit into mesenchymal phenotype and get a stronger movement performance[15,16]. Gab2 is the Gabs family member, and can be combined with signaling molecules containing SH2 domain to start the downstream signal transduction pathways, further start the expression of MMP2 and MMP9 through PI3K, ERK1/2 and other pathways, then degrade extracellular matrix and promote cell invasion[17]. In the study, analysis of invasion gene expression in breast cancer lesions before and after treatment showed that Sam68 and Gab2 mRNA expression in breast cancer lesions after treatment were significantly lower than those before treatment. Further analysis of the relationship of USP39 and EphA2 expression with the invasion gene expression by quartile method showed that the higher the USP39 and EphA2 expression in breast cancer lesions after treatment, the higher the Sam68 and Gab2 expression. It means that USP39 and EphA2 have promoting effect on the expression of invasion genes, and trastuzumab treatment can reduce USP39 and EphA2 expression to inhibit invasion genes Sam68 and Gab2 expression, and thus inhibit breast cancer cell invasion.

Trastuzumab therapy can inhibit the expression of USP39 and EphA2 in breast cancer lesions; the reduced expression of USP39 and EphA2 can increase the anti-proliferation gene expression and inhibit invasion gene expression so as to affect the malignant biological behavior and pathological features of tumors.

References


