Effect of Xiaoaiping combined with neoadjuvant chemotherapy on the pro-proliferation molecule expression and immune function in patients with breast cancer

Xue-Gang Li, Bing-Xiong Liu

General Surgery Department No. 3, Hanchuan People’s Hospital in Hubei Province, Hanchuan City, Hubei Province, 431600

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ABSTRACT

Objective: To investigate the effect of Xiaoaiping combined with neoadjuvant chemotherapy on the pro-proliferation molecule expression and immune function in patients with breast cancer.

Methods: A total of 98 patients with primary breast cancer who were diagnosed and treated in the hospital between December 2015 and February 2017 were collected and divided into control group and Xiaoaiping group by random number table, each with 49 cases. Control group received neoadjuvant chemotherapy + surgery + postoperative chemoradiotherapy, and Xiaoaiping group received Xiaoaiping + neoadjuvant chemotherapy + surgery + postoperative chemoradiotherapy. The differences in pro-proliferation gene expression in intraoperative breast cancer tissue as well as the differences in serum levels of Th1/Th2 cytokines and Th17/Treg cytokines before chemotherapy started (T0) and 1 week after neoadjuvant chemotherapy ended (T1) were compared between the two groups of patients.

Results: MTA2, NRP-1, PKM2, TM4SF1 and ZIC1 mRNA expression in breast cancer tissue of Xiaoaiping group were lower than those of control group. At T1, serum IFN-γ and TNF-α levels of Xiaoaiping group were higher than those of control group whereas IL-4, IL-10, IL-17, IL-22, IL-35 and TGF-β levels were lower than those of control group.

Conclusion: Xiaoaiping combined with neoadjuvant chemotherapy can effectively improve the curative effect of preoperative chemotherapy, and also significantly inhibit the proliferation activity of breast cancer cells and balance the immune function of the body.

1. Introduction

Breast cancer is the most common malignant tumor disease in women at present. The incidence of breast cancer is on the rise in recent years, and the radical operation for breast cancer is the most reliable method for the disease[1,2]. For some breast cancer patients with larger mass size or local metastasis, the complete resection of tumor is difficult and with relatively high risk of recurrence, and neoadjuvant chemotherapy, as the systemic intravenous chemotherapy before the operation, can kill cancer cells, shrink the tumor volume and restrain the growth of the small metastases, has been widely used for the preoperative treatment of breast cancer, and can provide good conditions for subsequent surgery[3,4]. Traditional Chinese medicine also has a long history of treating malignancy, Xiaoaiping has been successfully applied in the adjuvant treatment of esophageal cancer, colorectal cancer and so on, and it can effectively improve the body's sensitivity to the subsequent intravenous chemotherapy. At present, there is not much research about the value of Xiaoaiping for treatment of breast cancer, it was used together with neoadjuvant chemotherapy for clinical treatment of patients with breast cancer, and the effects of Xiaoaiping on the tumor cell malignancy and the body’s immune function as well as the possible mechanisms were discussed in the study.
2. Information and methods

2.1 Case information

A total of 98 patients with primary breast cancer who were diagnosed and treated in this hospital between December 2015 and February 2017 were collected as research subjects and divided into control group and Xiaoaiping group by random number table, each with 49 cases. Control group were 39-71 years old and Xiaoaiping group were 37-75 years old. The difference in age was not significant between the two groups, and the research plan was approved by the hospital ethics committee.

2.2 Inclusion and exclusion criteria

Inclusion criteria: (1) pathologically diagnosed with breast cancer; (2) diagnosed for the first time, and receiving no relevant treatment before; (3) the patients themselves/family members signed informed consent; (4) completing all scheduled courses successfully and cooperating with related inspections.

Exclusion criteria: (1) combined with chemotherapy drugs and (or) Xiaoaiping allergy; (2) with history of autoimmune diseases; (3) combined with systemic infectious diseases; (4) combined with primary malignant tumor diseases in other tissue viscera.

2.3 Therapy

Control group received neoadjuvant chemotherapy + surgery + postoperative radiochemotherapy, and the specific neoadjuvant chemotherapy regimens were shown as follows: docetaxel 75 mg/m², by intravenous injection, on d 1; cyclophosphamide 600 mg/m², by intravenous injection, on d 1; epirubicin 75 mg/m², by intravenous injection, on d 1; 21 d of above therapies was as 1 course of treatment, conducted for 4 courses in a row and followed by surgery. Xiaoaiping group accepted Xiaoaiping + neoadjuvant chemotherapy + surgery + postoperative radiochemotherapy, and Xiaoaiping usage and dosage were as follows: Xiaoaiping treatment was conducted when neoadjuvant chemotherapy began, 60 mL of Xiaoaiping injection was added in 250 mL of 5% glucose liquid, which was slowly intravenously dripped, 1 time/d, for same duration as neoadjuvant chemotherapy.

2.4 Observation indexes

2.4.1 Pro-proliferation molecule expression

Breast cancer lesion tissue samples were collected during operation, added in Trizol reagent to crack cells, dissolved by chloroform and then centrifugated at 12 000 r/min for 10-15 min to separate the upper clear water phase, and same volume of isopropyl alcohol was added to precipitate the RNA gel block in it. The RNA gel block was cleaned by 75% alcohol and dried at room temperature to determine its concentration and purity, reverse transcription kit instructions were followed to synthesize the sample cDNA, and the fluorescence quantitative PCR kit instructions were referred to amplify the target pro-proliferation genes: MTA2, NRP-1, PKM2, TM4SF1 and ZIC1. The expression of individual genes was calculated according to the amplification curve in computer software.

2.4.2 Immune function indexes

Before chemotherapy started (T0) and 1 week after neoadjuvant chemotherapy ended (T1), fasting blood samples were obtained via cubital vein puncture, immediately anti-coagulated and then centrifuged at low speed to separate the upper serum, which was cryopreserved in the cryogenic environment. And enzyme-linked immunosorbent assay was used to determine the serum contents of Th1/Th2 and Th17/Treg cytokines, including IL-17, IL-22, IL-35, TGF-β, IFN-γ, TNF-α, IL-4 and IL-10.

2.5 Statistical processing

Data calculation in the study was by SPSS 26.0 and $P<0.05$ was the standard of statistical significance in differences. Pro-proliferation molecules, Th1/Th2 cytokines and Th17/Treg cytokines were measurement data, in terms of mean ± standard deviation (Mean ± SD) and compared by $t$ test.

3. Results

3.1 Pro-proliferation molecule expression

Comparison of pro-proliferation molecules MTA2, NRP-1, PKM2, TM4SF1 and ZIC1 mRNA expression in breast cancer tissue between two groups of patients was as follows: MTA2, NRP-1, PKM2, TM4SF1 and ZIC1 mRNA expression in breast cancer tissue of Xiaoaiping group were lower than those of control group. Differences in pro-proliferation molecules MTA2, NRP-1, PKM2, TM4SF1 and ZIC1 mRNA expression in breast cancer tissue were statistically significant between the two groups ($P<0.05$), shown in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>MTA2</th>
<th>NRP-1</th>
<th>PKM2</th>
<th>TM4SF1</th>
<th>ZIC1</th>
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</thead>
<tbody>
<tr>
<td>Control group</td>
<td>49</td>
<td>99.72±10.84</td>
<td>95.16±10.49</td>
<td>98.57±9.29</td>
<td>101.55±12.48</td>
<td>107.32±14.85</td>
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<td>Xiaoaiping group</td>
<td>49</td>
<td>72.35±8.09</td>
<td>61.53±7.29</td>
<td>77.34±9.05</td>
<td>80.68±9.52</td>
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<td>$P$</td>
<td></td>
<td>&lt;0.05</td>
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</table>
3.2 Th1/Th2 cytokines

At different points in time before and after chemotherapy, comparison of serum Th1/Th2 cytokines IFN-γ, TNF-α, IL-4 and IL-10 levels between the two groups was as follows: serum IFN-γ, TNF-α, IL-4 and IL-10 levels were not significantly different between the two groups at T0 (P>0.05). Serum IFN-γ and TNF-α levels of both groups at T1 were higher than those at T0 whereas IL-4 and IL-10 levels were lower than those at T0; serum IFN-γ and TNF-α levels of Xiaoaiping group at T1 were higher than those of control group whereas IL-4 and IL-10 levels were lower than those of control group (P<0.05).

3.3 Th17/Treg cytokines

At different points in time before and after chemotherapy, comparison of serum Th17/Treg cytokines IL-17, IL-22, IL-35 and TGF-β levels between the two groups was as follows: at T0, serum levels of Th17 cytokines IL-17 and IL-22 as well as Treg cytokines IL-35 and TGF-β were not significantly different between the two groups (P>0.05). At T1, serum levels of Th17 cytokines IL-17 and IL-22 as well as Treg cytokines IL-35 and TGF-β of both groups were lower than those at T0; serum levels of Th17 cytokines IL-17 and IL-22 as well as Treg cytokines IL-35 and TGF-β of Xiaoaiping group were lower than those of control group (P<0.05).

4. Discussion

Western medicine treatment of breast cancer is given priority to surgical resection of the tumor and peripheral lymphatic tissue, neoadjuvant chemotherapy is a reliable way to kill the metastases invisible to the naked eye and shrink the tumor volume before operation, and it has been proven to be able to optimize the final treatment outcome of patients with breast cancer[5,6]. TCM holds that the pathogenesis of breast cancer is the stagnation of qi and blood stasis as well as the blood poison generation, the treatment should be given priority to regulating qi and removing blood stasis as well as removing toxins and removing tumor, Xiaoaiping is the TCM preparation extracted from glaucescent fissistigma root and has been successfully applied in the treatment of a variety of malignant tumors, and some scholars suggest the combination of Xiaoaiping and neoadjuvant chemotherapy for preoperative treatment of patients with breast cancer in order to further kill tumor cells or weaken their activity, and lay a foundation for subsequent surgery and postoperative radiochemotherapy[7,8]. In this research, Xiaoaiping and neoadjuvant chemotherapy were used for the preoperative treatment of patients with breast cancer at the same time, and the patients who received combined therapy were compared with those who received conventional neoadjuvant chemotherapy in order to confirm the clinical value of adjuvant TCM treatment and further clarify the approaches or mechanisms for it to play its role.

High expression of pro-proliferation genes is one of the biggest characteristics to differentiate tumor cells from the benign tumor and normal tissue, and the specific expression of pro-proliferation genes can objectively reflect the malignant degree of tumor and evaluate the clinical therapeutic effect. MTA2 is highly expressed in multiple solid tumors. After specific shRNA treatment, MTA2 expression decreases and tumor proliferation activity also decreases significantly[9,10]. NRP-1 is the co-receptor gene of the VEGF that can enhance the combination between VEGF and its receptors and promote mitosis and angiogenesis, it has been found that NRP-1 is highly expressed in many malignant tumor tissues, and it is an important factor to promote tumor cell proliferation and metastasis[11,12]. PKM2 is the rate-limiting enzyme and transcriptional cofactor of glucose metabolism pathway, it is involved

### Table 2.

Comparison of serum IFN-γ, TNF-α, IL-4 and IL-10 before and after chemotherapy (pg/mL).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>T0</th>
<th>T1</th>
<th>T0</th>
<th>T1</th>
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<tr>
<td></td>
<td></td>
<td>IFN-γ</td>
<td>TNF-α</td>
<td>IL-4</td>
<td>IL-10</td>
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<td>TNF-α</td>
<td>IL-4</td>
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<tr>
<td>Control group</td>
<td>49</td>
<td>2.67±0.31</td>
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<td>1.43±0.17</td>
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<td>3.15±0.37</td>
<td>2.15±0.24</td>
<td>2.12±0.13</td>
<td>2.13±0.16</td>
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<td>Xiaoaiping group</td>
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<td>1.76±0.23</td>
<td>1.45±0.18</td>
<td>1.76±0.23</td>
<td>3.96±0.45</td>
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<td>t</td>
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<td>0.263</td>
<td>0.117</td>
<td>0.218</td>
<td>7.397</td>
<td>8.162</td>
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</table>

Note: compared with same group at T0, P<0.05.

### Table 3.

Comparison of serum IL-17, IL-22, IL-35 and TGF-β levels before and after chemotherapy (pg/mL).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>T0</th>
<th>T1</th>
<th>T0</th>
<th>T1</th>
<th>T0</th>
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<tr>
<td></td>
<td></td>
<td>IL-17</td>
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<td>IL-35</td>
<td>TGF-β</td>
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<tr>
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<td>64.29±7.18</td>
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<td>Xiaoaiping group</td>
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</table>

Note: compared with same group at T0, P<0.05.

### 3.2 Th1/Th2 cytokines

At different points in time before and after chemotherapy, comparison of serum Th1/Th2 cytokines IFN-γ, TNF-α, IL-4 and IL-10 levels between the two groups was as follows: serum IFN-γ, TNF-α, IL-4 and IL-10 levels were not significantly different between the two groups at T0 (P>0.05). Serum IFN-γ and TNF-α levels of both groups at T1 were higher than those at T0 whereas IL-4 and IL-10 levels were lower than those at T0; serum IFN-γ and TNF-α levels of Xiaoaiping group at T1 were higher than those of control group whereas IL-4 and IL-10 levels were lower than those of control group (P<0.05).

### 3.3 Th17/Treg cytokines

At different points in time before and after chemotherapy, comparison of serum Th17/Treg cytokines IL-17, IL-22, IL-35 and TGF-β levels between the two groups was as follows: at T0, serum levels of Th17 cytokines IL-17 and IL-22 as well as Treg cytokines IL-35 and TGF-β were not significantly different between the two groups (P>0.05). At T1, serum levels of Th17 cytokines IL-17 and IL-22 as well as Treg cytokines IL-35 and TGF-β of both groups were lower than those at T0; serum levels of Th17 cytokines IL-17 and IL-22 as well as Treg cytokines IL-35 and TGF-β of Xiaoaiping group were lower than those of control group (P<0.05).
in the regulation of cell cycle, and the related research shows that breast cancer cell proliferation activity decreases and apoptosis activity increases after silencing PKM2 expression\cite{13}. TM4SF1 plays an important role in sustaining tumor angiogenesis, and the tumor cell proliferation activity decreases after targeted inhibition of TM4SF1 expression, which is directly related to the reduced activity of tumor angiogenesis and the declined cellular blood supply\cite{14}. ZIC1 is a newly discovered tumor-related gene, which is highly expressed in breast cancer tissues and is closely related to tumor staging\cite{15}. It was found in the study that compared with those of control group, MTA2, NRP-1, PKM2, TM4SF1 and ZIC1 mRNA expression in breast cancer specimens of Xiaoaiping group were lower during operation, confirming that adding Xiaoaiping treatment to neoadjuvant chemotherapy can further the expression of breast cancer-related pro-proliferation genes, showing the effectiveness of Xiaoaiping therapy. Xiaoaiping contains saponins, alkaloids, phenolic acids, polysaccharides and other active components, which are involved in the inhibition of tumor proliferation activity together. Immune dysfunction is one of the important reasons lead to tumorigenesis, the polysaccharide, alkaloids and other components in Xiaoaiping have been proven to be able to strengthen the immunosuppressive effects of immune function on tumor cells it is speculated that this may be the core mechanism for Xiaoaiping to inhibit tumor proliferation activity, but there is no clear research conclusion. Th1/Th2 shifting exists in various malignant tumor diseases, and many studies have shown that Th1 cells play an important role in anti-tumor immune response and can kill tumor cells and inhibit malignant proliferation of tumor cells; Th2 cells have the effect of inhibiting anti-tumor immune response, and the proportion of Th2 cells also reduces in most patients with malignant tumors\cite{16,17}. Th1/Th2 immune imbalance can be figuratively expressed in the changes of the contents of cytokines secreted by them, IFN-γ and TNF-α are mainly secreted by Th1 cells, and they can enhance the phagocytosis and natural killing effect of mononuclear macrophages and NK cells on tumor cells; IL-4 and IL-10 are secreted by Th2 cells, and they can induce Th0 cells to differentiate into Th2 cells and inhibit the function of Th1 cells\cite{18,19}. In this study, it was found that compared with those at T0, serum levels of Th1 cytokines of the Xiaoaiping group increased while the levels of Th2 cytokines decreased at T1; further comparison of the differences in Th1/Th2 cytokines between the two groups showed that serum Th1 cytokines IFN-γ and TNF-α levels of Xiaoaiping group were higher while Th2 cytokines IL-4 and IL-10 levels were lower at T1, confirming that Xiaoaiping combined with neoadjuvant chemotherapy can effectively balance the Th1/Th2 cellular immune function, and this is also one of the important mechanisms for it to inhibit tumor proliferation activity.

Th17/Treg cellular immune response imbalance also participates in the occurrence and development of breast cancer, many studies have confirmed that there are abnormal activation of Th17 cells and inhibition of Treg cell function in patients with inflammatory diseases, but the proportion of Th17/Treg cells are increasing in patients with malignant tumor, and the increase of Treg cells is more prominent\cite{20,21}. Th17 cells as well as the cytokines IL-17 and IL-22 secreted by them can promote inflammation and current study has shown that they have the functions of angiogenesis factors and can also promote the tumor growth\cite{22}. Treg cells are immunosuppressive and can provide the conditions for tumor cells to escape from immune surveillance, and the cytokines IL-35 and TGF-β secreted by them are directly involved in the process\cite{23}. In this study, it was discovered that compared with those at T0, serum levels of Th17 cytokines IL-17 and IL-22 as well as Treg cytokines IL-35 and TGF-β decreased in both groups at T1; further compared with those of control group, serum levels of Th1 cytokines IL-17 and IL-22 as well as Treg cytokines IL-35 and TGF-β were lower in Xiaoaiping group at T1, confirming that Xiaoaiping combined with neoadjuvant chemotherapy can effectively inhibit the Th17/Treg cell function, reduce the immune escape of tumor cells and enhance the patients’ autoimmune function.

Preoperative Xiaoaiping combined with neoadjuvant chemotherapy can effectively inhibit the tumor cell proliferation and balance the immune function in patients with breast cancer, it is expected to further optimize the patients’ treatment outcome, and it is worthy of popularization and application in clinical practice in the future.

References


