Effect of zhenwu decoction combined with neoadjuvant chemotherapy on malignant characteristics of cancer cells in ovarian cancer

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Objective: To explore the effect of zhenwu decoction combined with neoadjuvant chemotherapy on malignant characteristics of cancer cells in ovarian cancer. Methods: A total of 80 patients with ovarian cancer who underwent surgical treatment in this hospital between March 2015 and January 2017 were divided into the control group (n=42) who received preoperative neoadjuvant chemotherapy and the zhenwu decoction group (n=38) who received preoperative zhenwu decoction combined with neoadjuvant chemotherapy. Intraoperative ovarian cancer tissue was kept to detect the expression of proliferation genes, invasion genes and autophagy genes through fluorescence quantitative PCR method. Results: Proliferation genes AKT, LSD1, SIRT1 and NOB1 mRNA expression in ovarian lesion tissue of zhenwu decoction group were lower than those of control group; invasion gene DACT1 mRNA expression was higher than that of control group whereas MTA1 and GRP78 mRNA expression were lower than those of control group; autophagy gene Beclin1 mRNA expression was higher than that of control group whereas LC3-II and Atg5 mRNA expression were lower than those of control group. Conclusion: Preoperative zhenwu decoction combined with neoadjuvant chemotherapy can effectively inhibit the proliferation and invasion activity of tumor cells and regulate their autophagy function in patients with ovarian cancer.

1. Introduction

Ovarian cancer is a disease with extremely high clinical malignancy, and most patients have no discomfort in the early stage, leading to disease progression and poor prognosis[1,2]. For patients who have developed middle-advanced ovarian cancer at diagnosis, how to furthest optimize the treatment outcome is a focus of the present clinical research, and the application of neoadjuvant chemotherapy in recent years has made many patients who have missed the chance of operation regain operation qualification and has greatly improved the treatment dilemma brought by the difficulty in early diagnosis[3,4]. Postoperative recurrence rate of ovarian cancer is high, and it is associated with preoperative small metastases, so some scholars recommended neoadjuvant chemotherapy combined with TCM preparation therapy in order to furthest kill the preoperative invisible metastases and increase the effectiveness of surgical treatment. Zhenwu decoction is a Chinese patent medicine that warms yang to promote diuresis and has succeeded in the treatment of patients with liver cancer ascites, it was introduced into the preoperative treatment of patients with ovarian cancer in the research, and the change in tumor malignancy after the application of therapy was discussed in order to provide reference for the follow-up treatment of ovarian cancer.

2. Data and methods

2.1 Clinical data

A total of 80 patients with ovarian cancer who underwent surgical treatment in this hospital between March 2015 and January 2017 were selected as the research subjects and divided into the control group (n=42) who received preoperative neoadjuvant chemotherapy and the zhenwu decoction group (n=38) who received preoperative zhenwu decoction combined with neoadjuvant chemotherapy after...
their therapies were reviewed. Control group were 45-71 years old; zhenwu decoction group were 43-72 years old. The difference in age distribution was not statistically significant between the two groups, and the study plan was discussed and then approved by the members of the hospital ethics committee.

Inclusion criteria: (1) pathologically diagnosed with primary ovarian cancer; (2) in accordance with the standard of radical operation for ovarian cancer; (3) those who or whose family members signed the informed consent. Exclusion criteria: (1) with cachexia and unable to tolerate surgery; (2) combined with primary malignant tumor diseases in other tissue viscera; (3) severely allergic to chemotherapy drugs; (4) combined with significant autoimmune system dysfunction.

2.2 Therapy

Both groups underwent radical operation for ovarian cancer, and control group received preoperative neoadjuvant chemotherapy, which was as follows: paclitaxel, by intravenous drip, 150 mg/m², on d1; carboplatin, by intravenous drip, 200 mg/m², on d1, 21d as one course of treatment, for 2 courses of treatment in a row. The operation was performed after two weeks of rest.

Zhenwu decoction group received preoperative zhenwu decoction combined with neoadjuvant chemotherapy, specifically as follows: poria cocos 20 g, peony 15 g, ginger 15 g, aconite 10 g, atractylodes 15 g and water 200 mL were decocted with boiled water to 150 mL, which was divided into two and taken in the morning and evening for 8 weeks in a row.

2.3 Malignant molecule expression in lesion tissue

Ovarian cancer specimens were collected during ovarian tumor resection and stored in liquid nitrogen tanks for test. Fluorescence quantitative PCR was used to detect the expression of malignant molecules in lesion tissues, including proliferation genes AKT, LSD1, SIRT1 and NOB1, invasion genes DACT1, MTA1 and GRP78 as well as autophagy genes Beclin1, LC3-II and Atg5, and the testing kits and chemical reagents were purchased from Sigma Company in the United States. The PCR curve was obtained from computer software and the corresponding gene expression was read.

2.4 Statistical methods

Proliferation, invasion and autophagy gene expression were all input in software SPSS 25.0, t test was adopted to calculate the statistic $P$ and $P<0.05$ was set as the standard of statistical significance in differences.

3. Results

3.1 Proliferation genes

Comparison of proliferation genes AKT, LSD1, SIRT1 and NOB1 mRNA expression in ovarian lesion tissue between the two groups of patients was as follows: AKT, LSD1, SIRT1 and NOB1 mRNA expression in ovarian lesion tissue of zhenwu decoction group were greatly lower than those of control group. Differences in proliferation genes AKT, LSD1, SIRT1 and NOB1 mRNA expression in ovarian lesion tissue were statistically significant between the two groups ($P<0.05$), shown in Table 1.

3.2 Invasion genes

Comparison of invasion genes DACT1, MTA1 and GRP78 mRNA expression in ovarian lesion tissue between the two groups of patients was as follows: DACT1 mRNA expression in ovarian lesion tissue of zhenwu decoction group was higher than that of control group whereas MTA1 and GRP78 mRNA expression were lower than those of control group. Differences in invasion genes DACT1, MTA1 and GRP78 mRNA expression in ovarian lesion tissue were statistically significant between the two groups ($P<0.05$), shown in Table 2.

3.3 Autophagy genes

Comparison of autophagy genes Beclin1, LC3-II and Atg5 mRNA expression in ovarian lesion tissue between the two groups of patients was as follows: Beclin1 mRNA expression in ovarian lesion

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>AKT</th>
<th>LSD1</th>
<th>SIRT1</th>
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<td>42</td>
<td>114.38±13.57</td>
<td>92.17±9.63</td>
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<tr>
<td>$t$</td>
<td></td>
<td>12.837</td>
<td>9.473</td>
<td>10.536</td>
<td>8.293</td>
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<table>
<thead>
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<th>Groups</th>
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<th>DACT1</th>
<th>MTA1</th>
<th>GRP78</th>
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<td>102.71±13.42</td>
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<td>74.18±8.54</td>
<td>90.52±9.17</td>
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<tr>
<td>$t$</td>
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<td>13.048</td>
<td>9.172</td>
<td>11.654</td>
</tr>
<tr>
<td>$P$</td>
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</tr>
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</table>
tissue of zhenwu decoction group was higher than that of control group whereas LC3- II and Atg5 mRNA expression were lower than those of control group. Differences in autophagy genes Beclin1, LC3- II and Atg5 mRNA expression in ovarian lesion tissue were statistically significant between the two groups \((P<0.05)\), shown in Table 3.

### 4. Discussion

Ovarian cancer is a malignant neoplastic disease that is quite difficult to treat in clinical practice. Ascites is the first symptom in some patients, which is difficult to undergo surgical treatment. Neoadjuvant chemotherapy is the way that shrinks the tumor volume and kills the small metastases invisible to the naked eye through preoperative intravenous chemotherapy, which creates convenience for follow-up surgery implementation and eventually aims to optimize the operation effect[5-7]. The therapy for middle-advanced ovarian cancer is difficult to make, the implementation of ovarian tumor resection will require the killing of metastases before operation as far as possible, and the role of conventional neoadjuvant chemotherapy might be limited. Zhenwu decoction is a Chinese patent medicine made from poria cocos, peony, ginger, aconite, atractylodes and many other kinds of traditional Chinese medicines, it mainly treats edema syndrome due to Yang deficiency and has been successfully applied in cardiac edema, thyroid hypofunction, and intestinal tuberculosis and other spleen-kidney yung deficiency diseases, and it has been found at present that it can effectively control the condition of liver cancer ascites, reduce the ascites volume and kill the cancer cells in ascites. In view of this, zhenwu decoction combined with neoadjuvant chemotherapy was used for preoperative treatment of patients with ovarian cancer in this research so as to create more favorable conditions for the surgical treatment, and the effectiveness of the therapy will be determined from the malignant molecule expression level in ovarian tumor in this paper.

The malignancy of ovarian cancer is on the one hand, characterized by the strong proliferation activity of cancer cells, and on the other hand, mainly manifested as the abnormal expression of proliferation-related genes in genetics. AKT is an important downstream molecule of PI3K/AKT signal pathway, it has been confirmed in a variety of studies that it is closely associated with the development of ovarian cancer, and the signaling pathway can directly mediate cancer cell proliferation and inhibit normal apoptosis process after it is activated[8,9]. LSD1 is the first discovered histone demethylase that can regulate multiple gene transcription and p53 activity, it is known at present that the gene is abnormally highly expressed in colorectal cancer, breast cancer, liver cancer and other common malignant tumor tissues, and specifically reducing its expression can inhibit tumor cell proliferation and become a new target for malignant tumor treatment[10,11]. SIRT1 is a gene differentially expressed in different tumor tissues, study has shown that it is unusually highly expressed in ovarian cancer, cervical cancer, breast cancer and other gynecological tumor diseases and plays the role of an oncogene, but it is lowly expressed in gastric cancer and bladder cancer and plays the role of a tumor suppressor gene[12]. NOB1 can affect the protease synthesis and assembly in cells at exponential growth phase, and it is found in the cell research that the ovarian cancer cell proliferation activity dramatically reduces after shRNA lentivirus infects cancer cells and leads to the decrease of NOB1 gene expression[13,14]. The study results showed that compared with those of control group, AKT, LSD1, SIRT1 and NOB1 mRNA expression in ovarian cancer tissue of zhenwu decoction group were lower, and the combination of the physiological and pathological roles of above genes indicates that zhenwu decoction combined with neoadjuvant chemotherapy can effectively reduce the proliferation activity of ovarian cancer cells.

Ovarian cancer is prone to invasion and metastasis, the expression levels of pro-invasion and anti-invasion genes are in a relatively balanced state under physiological state, they restrict each other and maintain ovarian cell growth activity, but in the case of pro-invasion gene over-expression or anti-invasion gene dysfunction, tumor invasion is enhanced, and the canceration happens. DACT1 can be combined with Dvl to inhibit Wnt signaling pathway activity by lysosome-dependent pathway and actively reduce the invasion and metastasis activity of ovarian cancer cells, and the abnormal decrease of DACT1 expression in ovarian cancer tissue is one of the signs of poor prognosis of tumor[15]. MTA1 is a newly discovered tumor cell metastasis-associated gene in recent years, it can strengthen stability and activity of HIF-1 and promote the invasion and metastasis of tumor cells and its role has been confirmed in breast cancer, bladder cancer and other malignant tumors[16,17]. GRP78 plays an important role in protein folding and transport, and it has been confirmed that its expression rate is high in metastatic tumors, and is closely related to tumor staging, lymph node metastasis and so on[18,19]. The study results showed that compared with those of control group, DACT1 mRNA expression in ovarian cancer tissue of zhenwu decoction group was higher whereas MTA1 and GRP78 mRNA expression were lower, indicating that preoperative zhenwu decoction combined with neoadjuvant chemotherapy can more effectively inhibit the cancer cell invasion activity in patients with ovarian cancer, and helps eradicate tumor by surgery and early kill small metastases.

Autophagy is bidirectionally related to human tumorigenesis, which can promote the development of some tumors and also inhibit the occurrence of tumors. At present, clear evidence has

<table>
<thead>
<tr>
<th>Groups</th>
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<th>Beclin1</th>
<th>LC3- II</th>
<th>Atg5</th>
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<td>Control group</td>
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<td>86.73±9.12</td>
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<td>79.24±8.65</td>
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<tr>
<td>Zhenwu decoction</td>
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<td>99.84±10.17</td>
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<td>65.12±7.39</td>
</tr>
<tr>
<td>( t )</td>
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<td>11.304</td>
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</tr>
<tr>
<td>( P )</td>
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</table>
shown that autophagy plays a negative regulatory role in the formation of ovarian cancer, and the decline in its marker gene Beclin1 expression is an important cause of the occurrence and development of tumor[20]. LC3-II is a marker molecule specifically expressed in autophagosome membrane, it is not expressed in normal ovarian epithelium, but its expression is abnormally high in ovarian cancer, which enhances the viability of cancer cells in the stress environment, and is also one of the important reasons leading to chemotherapy resistance of the patients[21]. Atg5 plays an important role in the autophagosome elongation. It is a molecular switch of autophagy and apoptosis, and its positive expression in ovarian cancer is much higher than that in normal ovarian tissue[22]. The study results showed that compared with those of control group, Beclin1 mRNA expression in ovarian lesion tissue of zhenwu decoction group was higher whereas LC3-II and Atg5 mRNA expression were lower, confirming that the preoperative zhenwu decoction combined with neoadjuvant chemotherapy can more effectively regulate the autophagy level in patients with ovarian cancer and promote the realization of surgical effect.

To sum up, preoperative neoadjuvant chemotherapy and zhenwu decoction therapy can further inhibit the ovarian cancer cell proliferation and invasion activity, and regulate the autophagy function in patients with ovarian cancer, and it helps to reduce the tumor malignancy, further expand the surgical effect and finally optimize the patients’ treatment outcome.

References


