Correlation of transvaginal color Doppler ultrasound blood flow parameters of ovarian cancer with angiogenesis and cancer cell proliferation activity

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ABSTRACT

Objective: To study the correlation of transvaginal color Doppler ultrasound blood flow parameters of ovarian cancer with angiogenesis and cancer cell proliferation activity. Methods: A total of 41 cases of stage I-II ovarian cancer, 27 cases of stage III-IV ovarian cancer and 35 cases of benign ovarian lesions surgically removed in Obstetrics and Gynecology Department of Changshu No. 1 People’s Hospital Affiliated to Suzhou University between June 2014 and October 2016 were collected as clinical research samples, transvaginal color Doppler ultrasound was conducted to detect blood flow parameters, and enzyme-linked immunosorbent assay experiment was used to determine the protein expression of angiogenesis genes, cell proliferation genes and tumor suppressor genes. Results: RI and PI of stage I-II ovarian cancer and stage III-IV ovarian cancer were significantly lower than those of benign ovarian lesions, and the RI and PI of stage III-IV ovarian cancer were significantly lower than those of stage I-II ovarian cancer; VEGF165, NRP-1, SphK1, SphK2, YAP, CTGF, Gli2, TNFAIP8 and LSD1 protein expression in stage I-II ovarian cancer and stage III-IV ovarian cancer lesions were significantly higher than those in benign ovarian lesions while PTEN, MFN2 and ST7L protein expression were significantly lower than those in benign ovarian lesions; VEGF165, NRP-1, SphK1, SphK2, YAP, CTGF, Gli2, TNFAIP8 and LSD1 protein expression in III-IV ovarian cancer lesions were significantly higher than those in stage I-II ovarian cancer lesions and negatively correlated with RI and PI while PTEN, MFN2 and ST7L protein expression were significantly lower than those in I-II and ovarian cancer lesions and positively correlated with RI and PI. Conclusion: The decrease of transvaginal color Doppler ultrasound blood flow parameters RI and PI of ovarian cancer is closely related to the increase of angiogenesis and the enhancement of cancer cell proliferation activity.

1. Introduction

Ovarian cancer is one of the most common gynecological malignant tumors, it is with low early diagnosis rate, and it is also short of noninvasive means to assess the degree of malignancy. Angiogenesis, reduced blood flow resistance and increased blood flow within ovarian cancer lesions are the pathological basis to cause cancer cell growth, and accurate assessment of blood flow conditions in tumor lesions can provide the basis for judgment of tumor malignancy[1,2]. Color Doppler ultrasound is a common clinical method to assess tissue blood flow state, and the quantitative evaluation of the blood flow parameters can quantitatively reflect the blood flow within local tissue. Studies have shown that the ultrasonic blood flow parameters RI and PI of the ovarian cancer lesion significantly reduce[3], but the relationship of the RI and PI with the tumor malignancy is not clear. In the following studies, the correlation of transvaginal color Doppler ultrasound blood flow parameters RI and PI of ovarian cancer with angiogenesis and cancer cell proliferation activity was analyzed.
2. Clinical sample and research methods

2.1 Clinical sample information

The ovarian cancer lesions and benign ovarian lesions surgically removed in Obstetrics and Gynecology Department of Changshu No. 1 People’s Hospital Affiliated to Suzhou University between June 2014 and October 2016 were collected as clinical research samples, and the nature of all lesions were confirmed by postoperative pathological examination. There were 68 cases of ovarian cancer lesions, including 41 cases of I-II ovarian cancer and 27 cases of III-IV ovarian cancer; there were 35 cases of benign ovarian lesions. The information of patients corresponding to I-II ovarian cancer sample was as follows: they were 37-59 years old with BMI (22.9±3.5) kg/m$^2$; information of patients corresponding to III-IV ovarian cancer sample was as follows: they were 41-60 years old with BMI (22.1±3.4) kg/m$^2$; the information of patients corresponding to benign ovarian lesions was as follows: they were 34-58 years old with BMI (23.1±3.2) kg/m$^2$. The information of patients corresponding to different clinical samples was not statistically different ($P$ >0.05).

2.2 Experimental research methods

2.2.1 Transvaginal color Doppler ultrasound blood flow parameter detection methods

Patients with ovarian cancer and patients with benign ovarian lesions received transvaginal color Doppler ultrasonography, probe frequency was set to 7.5-10.5 MHz, routine scan was conducted at first to confirm the focal position, then the section with the most abundant blood flow signals was selected to detect blood flow parameters, the Angle between acoustic beam and blood flow incident should be < 30° during detection, and the blood flow resistance index (RI) and pulsatility index (PI) were measured.

2.2.2 Angiogenesis and cell proliferation–related gene expression detection methods

Ovarian cancer lesions and benign ovarian lesions were taken and added in RIPA lysis buffer to extract the total protein and centrifuge it to get protein suspension, and enzyme-linked immunosorbent assay kit was used to determine VEGF165, NRP-1, SphK1, SphK2, YAP, CTGF, Gli2, TNFAIP8, LSD1, PTEN, MFN2 and ST7L protein expression.

2.3 Statistical processing

SPSS 20.0 software was used for statistical processing, comparison of ultrasonic blood flow parameters and gene expression among three groups was by variance analysis and $P$ <0.05 indicated statistical significance in differences.

3. Results

3.1 Ultrasonic blood flow parameters of ovarian cancer and benign ovarian lesions

Analysis of transvaginal color Doppler ultrasound blood flow parameters RI and PI of I-II ovarian cancer, III-IV ovarian cancer and benign ovarian lesions was as follows: RI and PI of stage I-II ovarian cancer and stage III-IV ovarian cancer were significantly lower than those of benign ovarian lesions, and the RI and PI of stage III-IV ovarian cancer were significantly lower than those of stage I-II ovarian cancer. Differences were statistically significant in pair-wise comparison of RI and PI of I-II ovarian cancer, III-IV ovarian cancer and benign ovarian lesions ($P$ <0.05).

Table 1.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>$n$</th>
<th>RI</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign ovarian lesion</td>
<td>35</td>
<td>0.72±0.09</td>
<td>1.52±0.19</td>
</tr>
<tr>
<td>I-II ovarian cancer</td>
<td>41</td>
<td>0.41±0.08$^*$</td>
<td>0.89±0.12$^*$</td>
</tr>
<tr>
<td>III-IV ovarian cancer</td>
<td>27</td>
<td>0.22±0.05$^*$</td>
<td>0.52±0.08$^*$</td>
</tr>
</tbody>
</table>

*: compared with benign ovarian lesions, $P$ <0.05; $^*$: compared with stage I-II ovarian cancer, $P$ <0.05.

Table 2.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>$n$</th>
<th>VEGF165</th>
<th>NRP-1</th>
<th>SphK1</th>
<th>SphK1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign ovarian lesion</td>
<td>35</td>
<td>1.03±0.17</td>
<td>85.65±9.35</td>
<td>63.95±7.81</td>
<td>112.42±16.86</td>
</tr>
<tr>
<td>I-II ovarian cancer</td>
<td>41</td>
<td>2.29±0.42$^*$</td>
<td>202.52±33.65$^*$</td>
<td>112.34±17.58$^*$</td>
<td>269.87±42.15$^*$</td>
</tr>
<tr>
<td>III-IV ovarian cancer</td>
<td>27</td>
<td>4.18±0.55$^*$</td>
<td>412.58±57.54$^*$</td>
<td>259.76±41.28$^*$</td>
<td>448.97±64.72$^*$</td>
</tr>
</tbody>
</table>

*: compared with benign ovarian lesions, $P$ <0.05; $^*$: compared with stage I-II ovarian cancer, $P$ <0.05.
3.2 Angiogenesis gene expression in ovarian cancer and benign ovarian lesions

Analysis of angiogenesis genes VEGF165 (μg/L), NRP-1 (ng/L), SphK1 (ng/L) and SphK2 (ng/L) expression in I-II ovarian cancer, III-IV ovarian cancer and benign ovarian lesions was as follows: VEGF165, NRP-1, SphK1 and SphK2 protein expression in stage I-II ovarian cancer and stage III-IV ovarian cancer lesions were significantly higher than those in benign ovarian lesions, and VEGF165, NRP-1, SphK1 and SphK2 protein expression in III-IV ovarian cancer lesions were significantly higher than those in stage I-II ovarian cancer lesions. Differences were statistically significant in pair-wise comparison of VEGF165, NRP-1, SphK1 and SphK2 protein expression in I-II ovarian cancer, III-IV ovarian cancer and benign ovarian lesions (P<0.05). Pearson correlation analysis showed that RI and PI of ovarian cancer lesions were negatively correlated with VAP, CTGF, Gli2, TNFAIP8 and LSD1 protein expression in lesions.

3.3 Cell proliferation gene expression in ovarian cancer and benign ovarian lesions

Analysis of cell proliferation genes YAP (μg/L), CTGF (μg/L), Gli2 (μg/L), TNFAIP8 (ng/L) and LSD1 (ng/L) expression in I-II ovarian cancer, III-IV ovarian cancer and benign ovarian lesions was as follows: YAP, CTGF, Gli2, TNFAIP8 and LSD1 protein expression in stage I-II ovarian cancer and stage III-IV ovarian cancer lesions were significantly higher than those in benign ovarian lesions, and YAP, CTGF, Gli2, TNFAIP8 and LSD1 protein expression in III-IV ovarian cancer lesions were significantly higher than those in stage I-II ovarian cancer lesions. Differences were statistically significant in pair-wise comparison of YAP, CTGF, Gli2, TNFAIP8 and LSD1 protein expression in I-II ovarian cancer, III-IV ovarian cancer and benign ovarian lesions (P<0.05). Pearson correlation analysis showed that RI and PI of ovarian cancer lesions were negatively correlated with VEGF165, NRP-1, SphK1 and SphK2 protein expression in lesions.

3.4 Tumor suppressor gene expression in ovarian cancer and benign ovarian lesions

Analysis of tumor suppressor genes PTEN (μg/L), MFN2 (ng/L) and ST7L (ng/L) expression in I-II ovarian cancer, III-IV ovarian cancer and benign ovarian lesions was as follows: PTEN, MFN2 and ST7L protein expression in stage I-II ovarian cancer and stage III-IV ovarian cancer lesions were significantly lower than those in benign ovarian lesions, and PTEN, MFN2 and ST7L protein expression in III-IV ovarian cancer lesions were significantly lower than those in I-II and ovarian cancer lesions. Differences were statistically significant in pair-wise comparison of PTEN, MFN2 and ST7L protein expression in I-II ovarian cancer, III-IV ovarian cancer and benign ovarian lesions (P<0.05). Pearson correlation analysis showed that RI and PI of ovarian cancer lesions were positively correlated with PTEN, MFN2 and ST7L protein expression in lesions.

4. Discussion

Angiogenesis is an important pathological feature of ovarian cancer, and also the pathological basis causing abnormal proliferation and invasion of cancer cells. New tumor vessels have the characteristics such as less smooth muscle structure, higher vascular wall permeability and more arteriovenous shunt, and they are able to provide abundant blood perfusion for the lesions[3,4]. Color Doppler ultrasound is the noninvasive examination method for clinical evaluation of tissue blood perfusion characteristics, which can quantitatively evaluate the blood flow characteristics through RI, PI table

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>PTEN</th>
<th>MFN2</th>
<th>ST7L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign ovarian lesion</td>
<td>35</td>
<td>5.29±0.71</td>
<td>325.42±55.65</td>
<td>286.95±42.63</td>
</tr>
<tr>
<td>I-II ovarian cancer</td>
<td>41</td>
<td>2.95±0.42*</td>
<td>185.45±22.39*</td>
<td>133.25±17.79*</td>
</tr>
<tr>
<td>III-IV ovarian cancer</td>
<td>27</td>
<td>1.44±0.18**</td>
<td>112.15±17.94**</td>
<td>89.58±11.25**</td>
</tr>
</tbody>
</table>

*: compared with benign ovarian lesions, P<0.05; **: compared with stage I-II ovarian cancer, P<0.05.
and other parameters\(^{[5,6]}\). In the study, the analysis of color Doppler ultrasound blood flow parameters RI and PI of ovarian cancer lesions showed that RI and PI of ovarian cancer were significantly lower than those of benign ovarian lesions, and the RI and PI of stage III-IV ovarian cancer were significantly lower than those of stage I-II ovarian cancer. It illustrates that the blood flow resistance decreases significantly in ovarian cancer lesions, and the blood flow resistance further reduces with the rise of tumor staging, this is related to the structure of new blood vessels within the tumor lesion, and they can provide sufficient blood perfusion for the growth of ovarian cancer lesions.

Tumor angiogenesis is the pathological basis that reduces blood flow resistance and increases blood perfusion. VEGF165 is an important member of the VEGF family, and its combination with the receptor NPR-1 on endothelial cell and tumor cell membrane can not only promote endothelial cells to generate vascular structures, but can also promote the growth of tumor cells\(^{[7,8]}\). SphK1 and SphK2 are the important catalytic enzymes that catalyze sphingosine phosphorylation into S1P, and the S1P combination with the S1PR on cell membrane surface can start downstream MAPK, PI3K/AKT, Rho and other signal pathways, which promote endothelial cells to proliferate, migrate and form vascular structures\(^{[9]}\). The formation of new blood vessels in ovarian cancer significantly increases under the combined action of VEGF165 and S1P. In order to define the angiogenesis mediated by VEGF165 and S1P in ovarian cancer lesions, the above angiogenesis gene expression levels were analyzed in the study, and the results showed that VEGF165, NRP-1, SphK1 and SphK2 protein expression in ovarian cancer lesions were significantly higher than those in benign ovarian lesions, and VEGF165, NRP-1, SphK1 and SphK2 protein expression in III-IV ovarian cancer lesions were significantly higher than those in stage I-II ovarian cancer lesions. Further analysis of the correlation between angiogenesis gene expression and blood flow characteristics showed that RI and PI of ovarian cancer lesions were negatively correlated with YAP, CTGF, Gli2, TNFAIP8 and LSD1 protein expression in lesions. It means that the reduced blood flow resistance and increased blood perfusion in ovarian cancer lesions can promote the cancer cell proliferation mediated by YAP, CTGF, Gli2, TNFAIP8 and LSD1, and be conducive to the growth of tumor lesions.

In the pathological process of ovarian cancer, abnormal proliferation of cancer cells is not only associated with the high expression of proliferation genes, but also closely related to the expression deletion of a variety of tumor suppressor genes. PTEN is a tumor suppressor gene with dual phosphatase activities, it can exert the activities of tyrosine phosphatase and lipid-specific phosphatase at the same time, and it causes PI3K/AKT signal pathway phosphorylation in the process of cell proliferation to induce cell apoptosis and inhibit cell proliferation\(^{[15,16]}\); MFN2 gene encodes a kind of mitofusin, it participates in the regulation of mitochondrial function and related signal transduction in cells, the coding sequence of the gene contains the Ras signal sequence, and it can inhibit ERK1/2-MAPK signal pathway and the cell cycle development mediated by it through the Ras signal in order to reduce cell proliferation activity\(^{[17]}\); ST7L is a type of new tumor suppressor gene, it positions in chromosome 7 with Wnt2 gene, and overexpression of ST7L can make the cell cycle arrest in the G1 phase so as to inhibit cell proliferation\(^{[18]}\). In the study, the analysis of above tumor suppressor gene expression in ovarian cancer lesions showed that PTEN, MFN2 and ST7L protein expression in ovarian cancer lesions were significantly lower than those in benign ovarian lesions, and PTEN, MFN2 and ST7L protein expression in III-IV ovarian cancer lesions were significantly lower than those in I-II and ovarian cancer lesions. Further analysis of the correlation between tumor suppressor gene expression and blood flow characteristics showed that RI and PI of ovarian cancer lesions were positively correlated with PTEN, MFN2 and ST7L protein expression in lesions. It means that the reduced blood flow resistance and increased blood perfusion in ovarian cancer can abate the
proliferation-inhibiting effect mediated by PTEN, MFN2 and ST7L, and be conducive to the growth of tumor lesions.

To sum up, it is believed that the transvaginal color Doppler ultrasound blood flow parameters RI and PI of ovarian cancer significantly reduced, indicating the decreased blood flow resistance and increase blood perfusion in ovarian cancer lesions; the reduction of RI and PI is closely related to the increased angiogenesis and the enhanced cancer cell proliferation activity mediated by VEGF165 and S1P.

References


