Relationship of serum CCL20 and PCDGF levels with recurrence of non-small cell lung cancer after thoracoscopic radical operation

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ABSTRACT

Objective: To study the relationship of serum CCL20 and PCDGF levels with recurrence of non-small cell lung cancer after thoracoscopic radical operation. Method: A total of 93 patients with non-small cell lung cancer who received thoracoscopic radical operation in our hospital were selected, followed up for 2 years and divided into recurrence group and non-recurrence group, serum was collected before operation as well as 30 d and 90 d after operation to determine CCL20 and PCDGF levels, chest CT perfusion imaging was carried out after recurrence and the perfusion parameters were measured, and recurrent tumor tissue was collected to determine apoptosis molecule levels. Results: Before operation as well as 30 d and 90 d after operation, serum CCL20 and PCDGF levels of recurrence group and non-recurrence group were not significantly different; 180 d after operation, serum CCL20 and PCDGF levels of recurrence group were significantly higher than those of non-recurrence group, and there were significant differences between two groups. Patients with recurrence were grouped according to serum CCL20 and PCDGF levels 180 d after operation, BF, BV and PS of recurrent patients with high CCL20 and PCDGF levels were significantly higher than those of recurrent patients with low CCL20 and PCDGF levels while MTT and TTP as well as Caspase-3, -8, -9, pULK, Beclin1, PICKC3, Atg21 and Atg24 levels in tumor tissue were lower than those of recurrent patients with low CCL20 and PCDGF levels. Conclusion: Monitoring of postoperative serum CCL20 and PCDGF levels can provide reference for the evaluation of postoperative non-small cell lung cancer recurrence, and serum CCL20 and PCDGF levels 180 d after operation are closely related to tumor tissue perfusion and cell apoptosis.

1. Introduction

Non-small cell lung cancer (NSCLC) is the most common malignant tumor in our country, the 5-year survival rate of patients with I or II-stage NSCLC is about 40%-50% after surgical removal, and tumor recurrence is the main factor affecting the long-term prognosis in patients with NSCLC. Malignant tumor recurrence is associated with the biological behaviors such as cell proliferation, growth and migration, and a variety of cytokines are involved in the regulation of malignant biological behaviors of tumor. Chemokine CCL20 and PC cell-derived growth factor (PCDGF) are associated with cell proliferation, growth, migration and so on[1,2], and monitoring the change of serum CCL2 and PCDGF levels can predict tumor recurrence and metastasis. In the following study, the relationship of serum CCL20 and PCDGF levels with recurrence of non-small cell lung cancer after thoracoscopic radical operation was analyzed.

2. Subjects and methods

2.1. Research subjects

A total of 93 patients with non-small cell lung cancer who received thoracoscopic radical operation in our hospital from January 2010
to December 2012 were selected, diagnosed with I-II stage through preoperative evaluation and postoperative pathology, and followed up for 2 years after operation, 5 cases lost follow-up in two years, 3 cases died and the remaining 85 cases were included in the study. Patients were grouped according to postoperative recurrence, 33 cases were with recurrence and included in recurrence group, included 20 male cases and 13 female cases, and were (59.3±7.4) years old; 52 cases were without recurrence and included in non-recurrence group, included 32 male cases and 20 female cases, and were (60.5±7.8) years old.

2.2. Serum specimen collection and detection methods

Before operation as well as 30 d and 90 d after operation, fasting peripheral venous blood was collected from two groups of NSCLC patients in the morning, let stand for 20 min at room temperature and then centrifuged for 10 min at 3 000 r/min, serum was separated, and then enzyme-linked immunosorbent assay was used to determine CCL20 and PCDGF levels.

2.3. CT perfusion imaging

CT perfusion imaging was carried out after making sure there were recurrent lesions in patients, contrast agent was injected, then perfusion scanning was carried after 2-4 s of delay, the dose of contrast agent was 50 mL, injection flow rate was 6 mL/s, the scanning parameters were as follows: voltage 100 kV, current 150 mA, scanning slice thickness 5 mm, reconstruction thickness 1 mm, and perfusion imaging was obtained and then analyzed to get the parameters, including blood flow (BF), blood volume (BV), time to peak (TTP), mean transit time (MTT) and permeability surface (PS).

2.4. Tumor tissue collection and detection methods

Lung biopsy or transbronchial biopsy was used to obtain recurrent lesions from patients with tumor recurrence, washed with normal saline and then made into tissue homogenate and centrifuge it for 20 min, residue was discarded and supernatant was kept, and enzyme-linked immunosorbent assay kit was used to determine Caspase-3, -8, -9, pULK, Beclin1, PICKC3, Atg21 and Atg24 levels.

2.5. Statistical methods

SPSS 20.0 software was used to input and analyze data, measurement data between two groups was analyzed by t test, and \( P < 0.05 \) indicated statistical significance in differences.

3. Results

3.1. Serum CCL20 and PCDGF levels before and after operation

Before operation as well as 30 d and 90 d after operation, serum CCL20 and PCDGF levels of recurrence group and non-recurrence group were not significantly different \((P>0.05)\); 180 d after operation, serum CCL20 and PCDGF levels of recurrence group were significantly higher than those of non-recurrence group, and there were significant differences between two groups \((P<0.05)\). Serum CCL20 and PCDGF levels of both groups 30 d and 90 d after operation were significantly lower than those before operation \((P<0.05)\); serum CCL20 and PCDGF levels of recurrence group 180 d after operation were significantly higher than those before operation \((P<0.05)\), and serum CCL20 and PCDGF levels of non-recurrence group 180 d after operation were significantly lower than those before operation \((P<0.05)\).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Operation</th>
<th>CCL20(pg/mL)</th>
<th>PCDGF(ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrence</td>
<td>Before operation</td>
<td>44.28±6.59</td>
<td>23.59±4.86</td>
</tr>
<tr>
<td></td>
<td>30 d after operation</td>
<td>35.92±5.52</td>
<td>14.41±2.24</td>
</tr>
<tr>
<td></td>
<td>90 d after operation</td>
<td>36.15±6.18</td>
<td>17.03±2.19</td>
</tr>
<tr>
<td></td>
<td>180 d after operation</td>
<td>112.38±17.80</td>
<td>46.31±6.63</td>
</tr>
<tr>
<td>Non-recurrence</td>
<td>Before operation</td>
<td>46.04±7.14</td>
<td>22.78±3.50</td>
</tr>
<tr>
<td></td>
<td>30 d after operation</td>
<td>33.28±4.95</td>
<td>13.78±2.09</td>
</tr>
<tr>
<td></td>
<td>90 d after operation</td>
<td>35.56±6.06</td>
<td>15.52±2.44</td>
</tr>
<tr>
<td></td>
<td>180 d after operation</td>
<td>38.23±7.25</td>
<td>16.69±1.95</td>
</tr>
</tbody>
</table>

\*: compared with before operation, there were significant differences, \( P < 0.05 \); #: compared with non-recurrence group, there were significant differences, \( P < 0.05 \).

3.2. CT imaging of recurrent patients with different CCL20 and PCDGF levels

CT scan images of recurrent patients with different CCL20 and
PCDGF levels showed that the tumor volume was bigger and peripheral invasion was more significant in recurrent patients with high CCL20 and PCDGF levels. Analysis of perfusion parameters was shown in Table 2: BF, BV and PS of recurrent patients with high CCL20 and PCDGF levels were significantly higher than those of recurrent patients with low CCL20 and PCDGF levels while MTT and TTP were lower than those of recurrent patients with low CCL20 and PCDGF levels.

### 3.3. Cell apoptosis in recurrent patients with different CCL20 and PCDGF levels

Analysis of Caspase levels in tumor tissue was shown in Table 3: Caspase-3, -8 and -9 levels in tumor tissue of recurrent patients with high CCL20 and PCDGF levels were significantly lower than those of recurrent patients with low CCL20 and PCDGF levels; analysis of autophagy gene levels in tumor tissue was shown in Table 4: pULK, Beclin1, PI3Kc3, Atg21 and Atg24 levels in tumor tissue of recurrent patients with high CCL20 and PCDGF levels were significantly lower than those of recurrent patients with low CCL20 and PCDGF levels.

Table 3.

<table>
<thead>
<tr>
<th></th>
<th>Caspase-3 (μg/mL)</th>
<th>Caspase-8 (μg/mL)</th>
<th>Caspase-9 (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High CCL20 level</td>
<td>1.86±0.26</td>
<td>252.38±42.47</td>
<td>304.52±41.38</td>
</tr>
<tr>
<td>Low CCL20 level</td>
<td>3.34±0.57</td>
<td>451.34±61.37</td>
<td>513.72±68.14</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>High PCDGF level</td>
<td>1.72±0.28</td>
<td>264.27±39.23</td>
<td>289.31±44.12</td>
</tr>
<tr>
<td>Low PCDGF level</td>
<td>3.71±0.49</td>
<td>424.29±66.51</td>
<td>536.73±61.25</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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</table>

### 4. Discussion

CCL20 is the chemokine able to interact with CCR6 receptor, and a wide variety of tumor cells can express CCL20/CCR6, and promote tumor growth through receptor-ligand binding. PC cell-derived growth factor (PCDGF), also known as TDGF1 and Cripto1, has stronger tumorigenicity, and can participate in various biological processes of malignant tumors through MAPK, PI3K and FAK signaling pathways. Study has shown that PCDGF can promote cancer cell proliferation and migration, and can also degrade extracellular matrix and induce angiogenesis, and is associated with the occurrence and development of many kinds of malignant tumors. In the study, the relationship of serum CCL20 and PCDGF levels with postoperative recurrence of non-small cell lung cancer was studied. Serum CCL20 and PCDGF levels of recurrence group and non-recurrence group 30 d and 90 d after operation significantly decreased and were not different, and serum CCL20 and PCDGF levels of recurrence group 180 d after operation significantly increased and were significantly higher than those of non-recurrence group. Thus it confirmed that monitoring of serum CCL20 and PCDGF levels after operation for NSCLC could provide reference for the evaluation of tumor recurrence, and if postoperative serum CCL20 and PCDGF levels significantly increased, recurrence of tumor should be paid attention to.

The current clinical common way to judge the postoperative disease outcome in patients with NSCLC is CT scan, which, through CT scan and perfusion imaging, can not only make sure the presence of tumor lesions and the scope of lesions, but can also reflect the blood supply of recurrent tumor through measurement of perfusion parameters. Study has shown that blood flow (BF), blood volume (BV), time to peak (TTP), mean transit time (MTT) and permeability surface (PS) are associated with local microvascular density in lung cancer tissue, BF, BV and PS are positively correlated with microvascular density while MTT and TTP are negatively correlated with microvascular density. Angiogenesis is an important part of the postoperative recurrence of NSCLC. CCL20 and PCDGF are involved in the regulation of angiogenesis, and in order to define the relationship of serum CCL20 and PCDGF levels with blood perfusion in recurrent tumor tissue, the perfusion imaging parameters were compared in the study: BF, BV and PS of recurrent patients with high CCL20 and PCDGF levels were significantly higher than those of recurrent patients with low CCL20 and PCDGF levels while MTT and TTP were lower than those of recurrent patients with low CCL20 and PCDGF levels. It meant that serum CCL20 and PCDGF levels could evaluate the degree of blood perfusion in recurrent NSCLC lesions.

NSCLC recurrence is directly related to the proliferation of cancer cells, and apoptosis deficiency can cause that abnormally proliferated cells in local part after operation cannot be cleared in time, thereby causing that cells proliferate constantly and form recurrent lesions. Caspase are the classic molecules regulating apoptosis of mitochondrial pathway and death receptor pathway. Cytochrome C can be released from mitochondria to the cytoplasm and then

Table 4.

<table>
<thead>
<tr>
<th></th>
<th>pULK (μg/mL)</th>
<th>Beclin1 (ng/mL)</th>
<th>PI3Kc3 (μg/mL)</th>
<th>Atg21 (ng/mL)</th>
<th>Atg24 (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High CCL20 level</td>
<td>2.59±0.35</td>
<td>195.52±25.52</td>
<td>5.58±0.71</td>
<td>305.52±46.38</td>
<td>221.48±31.34</td>
</tr>
<tr>
<td>Low CCL20 level</td>
<td>1.14±0.22</td>
<td>110.34±16.58</td>
<td>3.04±0.55</td>
<td>161.14±25.56</td>
<td>110.37±15.27</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>High PCDGF level</td>
<td>2.31±0.40</td>
<td>224.12±30.23</td>
<td>6.14±0.78</td>
<td>331.49±50.23</td>
<td>193.41±28.65</td>
</tr>
<tr>
<td>Low PCDGF level</td>
<td>1.32±0.18</td>
<td>93.59±13.14</td>
<td>2.42±0.33</td>
<td>148.55±22.15</td>
<td>130.21±19.86</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
splice Caspase-9 precursor and activate it, death receptor Fas can be combined with its ligand FasL to activate Caspase-8, and the activated Caspase-8 and -9 activate Caspase-3 by Caspase cascade and perform apoptosis[10-12]. In the study, analysis of Caspase levels in recurrent tumor tissue confirmed that Caspase-3, -8 and -9 levels in tumor tissue of recurrent patients with high CCL20 and PCDGF levels were significantly lower than those of recurrent patients with low CCL20 and PCDGF levels. Autophagy is a newly discovered apoptosis regulation pathway in recent years, which degrades own organelles to remove abnormally proliferated cells[13,14]. pULK is involved in the inducing stage of autophagy, Beclin1 is involved in the initial stage of autophagy, and PICKC3 can be involved in the inducing stage of autophagy, Beclin1 is involved in the inducing stage of autophagy, Beclin1 is involved in the initial stage of autophagy, and PICKC3 can be involved in the induction stage of autophagy with Atg21 and Atg24 in the initial stage of autophagy, and PICKC3 can be involved in the inducing stage of autophagy, Beclin1 is involved in the inducing stage of autophagy, Beclin1 is involved in the initial stage of autophagy, and PICKC3 can be involved in the inducing stage of autophagy with Atg21 and Atg24[15]. In the study, analysis of autophagy gene levels in the recurrent tumor tissue confirmed that pULK, Beclin1, PICKC3, Atg21 and Atg24 levels in tumor tissue of recurrent patients with high CCL20 and PCDGF levels were significantly lower than those of recurrent patients with low CCL20 and PCDGF levels.

To sum up, monitoring of postoperative serum CCL20 and PCDGF levels can provide reference for the evaluation of postoperative non-small cell lung cancer recurrence, and serum CCL20 and PCDGF levels 180 d after operation are closely related to tumor tissue perfusion and cell apoptosis.

References


