Correlation study between CT perfusion parameters of non-small cell lung cancer and angiogenesis, cell proliferation as well as tumor load

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ABSTRACT

Objective: To study the correlation between CT perfusion parameters of non-small cell lung cancer and angiogenesis, cell proliferation as well as tumor load. Methods: Patients diagnosed with non-small cell lung cancer in our hospital between May 2012 and December 2015 were selected, CT perfusion was used to measure the blood volume (BV), blood flow (BF) and time to peak (TTP) of lung cancer lesions and unaffected-side lung tissue, and the lung cancer tissue and para-carcinoma tissue were collected to determine the expression of angiogenesis and cell proliferation molecules. Results: BV and BF of non-small cell lung cancer tissue were significantly higher than those of unaffected-side lung tissue while TTP was significantly shorter than that of unaffected-side lung tissue; PCDGF, bFGF, FGFR, VEGF, VEGFR, TCF3, Skp2, Livin and Survivin expression in non-small cell lung cancer tissue were significantly higher than those in para-carcinoma tissue, positively correlated with BV and BF, and negatively correlated with TTP. Conclusion: CT perfusion parameters BV, BF and TTP are closely related to the expression of angiogenesis molecules and cell proliferation molecules in non-small cell lung cancer lesions, and are valuable for the assessment of angiogenesis and cell proliferation.

1. Introduction

Lung cancer is the malignant tumor with the highest incidence in our country, non-small cell lung cancer is its most common pathological type, and massive new blood vessels within the tumor tissue are able to provide the nutrients necessary for cancer cell metabolism. In the development of non-small cell lung cancer, the accurate assessment of angiogenesis within lesions can provide reference and basis for the judgment of disease prognosis. CT perfusion (CTP) is a newly developed imaging method that combines histomorphology and functional imaging, and can quantitatively evaluate tissue perfusion\(^1,2\). In recent years, more and more clinical studies have found that the CT perfusion parameters are valuable for the assessment of blood perfusion within lung cancer lesions\(^3,4\), but the relationship between CT perfusion parameters of lung cancer and lung cancer angiogenesis as well as cell proliferation and invasion is not yet clear. In the following study, the correlation between CT perfusion parameters of non-small cell lung cancer and angiogenesis, cell proliferation as well as tumor load was analyzed.

2. Subjects and methods

2.1 Research subjects

Patients diagnosed with non-small cell lung cancer in our hospital between May 2012 and December 2015 were selected as the research subjects, all patients were diagnosed with non-small cell lung cancer by pathological biopsy and received CT perfusion examination before the pathological biopsy, and patients complicated with pulmonary lymphadenectasis and distant organ metastasis were excluded. A total of 65 patients were enrolled, there were 43 male cases and 22 female cases, they were 44-73 years old and 61 years old in average, and the lesion diameter was 1.4-7.4 cm.
and (3.4±0.5) cm in average.

2.2 CT perfusion examination methods

GE spiral CT was used for thin-layer chest CT scanning, 50 mL of iohexol was injected under high pressure via elbow vein before scanning, the speed was 5 mL/s, scanning was started 10 s after injection, scanning slice thickness was 3 cm, voltage was 120 kV, current was 80 mA, the images was obtained, then software was used for 3D reconstruction, the lesion parts were selected as region of interest to measure blood volume (BV), blood flow (BF) and time to peak (TTP), and meanwhile, the areas corresponded to unaffected-side lung were selected to measure BV, BF and TTP.

2.3 Detection methods of protein content in tissues

Lung cancer tissue and para-carcinoma tissue were collected, frozen with liquid nitrogen, added in protein lysis buffer, fully grinded and then centrifuged for 20 min at 4 ℃ with 12 000 r/min, the precipitation after centrifuged was abandoned, the supernatant was kept, and enzyme-linked immunosorbent assay kits were used to determine PCDGF, bFGF, FGFR, VEGF, VEGFR, TCF3, Skp2, Livin and Survivin levels.

2.4 Statistical methods

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

3. Results

3.1 CT perfusion parameters of non-small cell lung cancer

Analysis of CT perfusion parameters BV, BF and TTP between non-small cell lung cancer tissue and unaffected-side lung tissue was as follows: BV and BF of non-small cell lung cancer tissue were significantly higher than those of unaffected-side lung tissue while TTP was significantly shorter than that of unaffected-side lung tissue. Differences in CT perfusion parameters BV, BF and TTP were statistically significant between non-small cell lung cancer tissue and unaffected-side lung tissue \((P<0.05)\).

3.2 Angiogenesis molecule expression in non-small cell lung cancer lesions

Analysis of angiogenesis molecules PCDGF, bFGF, FGFR, VEGF and VEGFR expression between non-small cell lung cancer tissue and para-carcinoma tissue was as follows: PCDGF, bFGF, FGFR, VEGF and VEGFR expression in non-small cell lung cancer tissue were significantly higher than those in para-carcinoma tissue. Differences in PCDGF, bFGF, FGFR, VEGF and VEGFR expression were statistically significant between non-small cell lung cancer tissue and para-carcinoma tissue \((P<0.05)\). Pearson correlation analysis showed that PCDGF, bFGF, FGFR, VEGF and VEGFR expression in non-small cell lung cancer lesions were positively correlated with BV and BF, and negatively correlated with TTP.

3.3 Cell proliferation molecule expression in non-small cell lung cancer lesions

Analysis of cell proliferation molecules TCF3, Skp2, Livin and Survivin expression between non-small cell lung cancer tissue and para-carcinoma tissue was as follows: TCF3, Skp2, Livin and Survivin expression in non-small cell lung cancer tissue were

### Table 1.
Comparison of CT perfusion parameters between non-small cell lung cancer tissue and unaffected-side lung tissue.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>BV (mg/100 g)</th>
<th>BV [mL/(min•100 g)]</th>
<th>TTP (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung cancer tissue</td>
<td>65</td>
<td>45.42±6.68</td>
<td>51.78±7.61</td>
<td>22.68±3.69</td>
</tr>
<tr>
<td>Unaffected-side lung tissue</td>
<td>65</td>
<td>31.63±4.95</td>
<td>36.54±4.57</td>
<td>34.12±5.58</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td>7.597</td>
<td>8.371</td>
<td>7.142</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

### Table 2.
Angiogenesis molecule expression in non-small cell lung cancer lesions (pg/mL).

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>PCDGF</th>
<th>bFGF</th>
<th>FGFR</th>
<th>VEGF</th>
<th>VEGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung cancer lesion</td>
<td>65</td>
<td>14.48±1.76</td>
<td>11.35±1.57</td>
<td>8.59±0.93</td>
<td>18.67±2.52</td>
<td>13.67±1.85</td>
</tr>
<tr>
<td>Para-carcinoma tissue</td>
<td>65</td>
<td>8.34±0.94</td>
<td>5.52±0.67</td>
<td>4.25±0.64</td>
<td>9.03±1.07</td>
<td>5.68±0.78</td>
</tr>
<tr>
<td>T</td>
<td></td>
<td>8.547</td>
<td>12.038</td>
<td>10.861</td>
<td>10.382</td>
<td>15.872</td>
</tr>
<tr>
<td>P</td>
<td></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>
significant higher than those in para-carcinoma tissue. Differences in TCF3, Skp2, Livin and Survivin expression were statistically significant between non-small cell lung cancer tissue and para-carcinoma tissue \( (P<0.05) \). Pearson correlation analysis showed that TCF3, Skp2, Livin and Survivin expression in non-small cell lung cancer lesions were positively correlated with BV and BF, and negatively correlated with TTP.

### 4. Discussion

CT perfusion is the imageological examination means developed in recent years, which can determine lung tissue perfusion, has the advantages of non-invasion and repeatability, and is suitable for the assessment of dynamic disease change\(^5,6\). In the study, comparison of CT perfusion parameters BV, BF and TTP between the lung lesions and the unaffected-side lung tissue showed that BV and BF of non-small cell lung cancer tissue were significantly higher than those of unaffected-side lung tissue while TTP was significantly shorter than that of unaffected-side lung tissue. All three CT perfusion parameters can reflect the total amount and speed of blood perfusion within the tissue, BV represents the capacity within the vascular system of lesions, and the greater the BV, the more abundant the tumor tissue perfusion; BF represents the blood flow in unit time in lesions, and the greater the BF, the faster the tumor tissue perfusion; TTP represents the time from contrast agent appearance to the peak in tumor lesions, and the shorter the TTP, the greater the blood flow within the tumor tissue and the faster the velocity\(^7,8\). Comparison of CT perfusion parameters between the lung lesions and the unaffected-side lung tissue showed that the blood perfusion in lung cancer lesions was richer, and the perfusion velocity was faster.

Angiogenesis in lung cancer tissue is the pathological basis of rich and fast perfusion within the lesions, and PCDGF, bFGF, VEGF and other cytokines are the main active materials mediating angiogenesis in tumor tissue. Study has reported that serum levels of PCDGF, bFGF, VEGF and other cytokines in patients with lung cancer significantly increase and are closely related to the prognosis of the disease. PCDGF is a member of growth regulator family, and can promote angiogenesis through MAPK and PI3K/Akt signaling pathway\(^9\); the combination between bFGF and its receptor FGFR and the combination between VEGF and its receptor VEGFR can promote endothelial cell proliferation and vascular structure formation, thereby inducing angiogenesis\(^10-12\). In order to further clarify the correlation between CT perfusion parameters and angiogenesis in lung tissue, angiogenesis-related cytokine expression levels in tumor tissue were analyzed in the study, and the results showed that PCDGF, bFGF, FGFR, VEGF and VEGFR expression in non-small cell lung cancer tissue were significantly higher than those in para-carcinoma tissue, positively correlated with BV and BF, and negatively correlated with TTP. This means that CT perfusion parameters can not only directly reflect the blood perfusion within the lung cancer lesions, but are also closely associated with the high expression of angiogenesis-related cytokines, and they can reflect the angiogenesis in lesions.

The blood perfusion increased by new blood vessels in lung cancer tissues can provide the necessary nutrients for cancer cell proliferation, and promote cell proliferation and tumor lesion growth. TCF3, Skp2, Livin and Survivin are the important molecules mediating lung cancer cell proliferation. TCF3 and Skp2 mainly regulate the cell cycle to promote cell proliferation, the former can form transcription complex with LEF and then increase the expression of c-myc, cyclinD1, etc., and accelerate cell cycle progression\(^13\), and the latter ubiquitinates and degrades a variety of phosphorylated protein substrates to promote cell cycle transition and accelerate cell cycle progression\(^14\); Livin and Survivin mainly by inhibit cell apoptosis to promote cell proliferation, and the two can antagonize the biological function of a variety of members in caspase family and inhibit the activation of caspase cascade amplification signal pathways, thereby inhibiting apoptosis and promoting cell proliferation\(^15,16\). In the study, analysis of the expression of these proliferation-related molecules showed that TCF3, Skp2, Livin and Survivin expression in non-small cell lung cancer tissue were significantly higher than those in para-carcinoma tissue, positively correlated with BV and BF, and negatively correlated with TTP. This means that CT perfusion parameters can not only directly show the blood perfusion within the lung lesions, but are also closely related to the high expression of proliferation-related molecules in lesions, and they can reflect the cancer cell proliferation and tumor load within the lesions.

To sum up, it is believed that CT perfusion parameters BV, BF and TTP of non-small cell lung cancer lesions are closely related to the
high expression of angiogenesis molecules and cell proliferation molecules, and BV, BF and TTP can be used to assess the non-small cell lung cancer angiogenesis and cell proliferation.

References


