Relationship of serum PCT content with the invasive growth of cancer cells and angiogenesis in patients with small cell lung cancer

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ABSTRACT
Objective: To study the relationship of serum procalcitonin (PCT) content with the invasive growth of cancer cells and angiogenesis in patients with small cell lung cancer. Methods: Patients who were diagnosed with small cell lung cancer in Jiaoling People's Hospital in Meizhou between March 2015 and June 2017 were selected as the SCLC group of the research, and healthy volunteers who received physical examination during the same period were selected as the control group. The serum was taken to determine the PCT content, and the tumor lesion tissue and adjacent lesion tissue for biopsy were taken to detect the expression of proliferation genes, invasion genes and angiogenesis genes. Results: Serum PCT content of SCLC group was significantly higher than that of control group; HOXA13, CyclinD1, MDM2, MMP9, Vimentin, N-cadherin, VEGF, VE-cadherin, EGFL8 and Nestin mRNA expression in tumor lesion tissue were significantly higher than those in adjacent lesion tissue whereas Nocti3, Rb, Bin1 and E-cadherin mRNA expression were significantly lower than those in adjacent lesion tissue; serum PCT content of patients with SCLC was positively correlated with HOXA13, CyclinD1, MDM2, MMP9, Vimentin, N-cadherin, VEGF, VE-cadherin, EGFL8 and Nestin mRNA expression in tumor lesion tissue, and negatively correlated with Nocti3, Rb, Bin1 and E-cadherin mRNA expression. Conclusion: The abnormal increase of serum PCT content in patients with small cell lung cancer is closely related to the infiltrative growth of cancer cells and angiogenesis in the lesion.

1. Introduction
Lung cancer is the most common malignancy in the world, and can be divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) according to the biological behaviors. SCLC accounts for about 20%, which is characterized by high malignancy and high metastasis rate, has mostly developed to the middle and advanced stage when diagnosed, and is mainly treated through radiotherapy and chemotherapy(1,2). At present, the early stage of SCLC is relatively difficult, it is short of clinical effective diagnostic markers, the common SCLC markers NSE and ProGRP have certain diagnostic value, but the sensitivity is poor. SCLC tumor lesions have neuroendocrine characteristics, and can synthesize NSE, ProGRP and various other molecules and secrete them into blood circulation(3,4). Procalcitonin (PCT) is a clinical marker for the diagnosis of infectious diseases. In recent years, it has been confirmed that it can be synthesized by SCLC lesions, so it is expected to become the marker for early diagnosis of the disease(5). In the following studies, we specifically analyzed the relationship of serum PCT content with invasive growth of cancer cells and angiogenesis in patients with small cell lung cancer.

2. Clinical information and research methods
2.1 General case information
Patients who were diagnosed with small cell lung cancer in Jiaoling People's Hospital in Meizhou between March 2015 and June 2017 were selected as the SCLC group of the research, they were diagnosed with SCLC by biopsy and pathological examination of local tissue and signed informed consent, and the patients
combined with infectious diseases or autoimmune diseases were ruled out. Healthy volunteers receiving physical examination during the same period were selected for the control group, and those with history of infectious diseases in previous 1 month were ruled out. There were 62 cases in the SCLC group, including 38 males and 24 females who were 39-62 years old; there were 54 cases in the control group, including 32 males and 22 females who were 36-60 years old. There was no significant difference in general data between the two groups ($P>0.05$).

2.2 Research methods

2.2.1 Serum PCT content detection

3-5 mL of peripheral blood was collected from SCLC group before biopsy, 3-5 mL of peripheral blood was collected from control group during physical exams, the blood was centrifuged to separate serum, and enzyme-linked immunosorbent assay kit was adopted to determine PCT levels.

2.2.2 Tissue gene expression detection

Tumor lesion tissue and adjacent lesion tissue from biopsy were taken, the RNA was extracted with the kit, then the primers of HOXA13, CyclinD1, Noth3, MDM2, Rb, Bin1, MMP9, Vimentin, N-cadherin, E-cadherin, VEGF, VE-cadherin, EGF-8 and Nestin were designed, fluorescence quantitative PCR reaction was conducted, and the reaction curve was referred to calculate the mRNA expression corresponding genes.

2.3 Statistical methods

Software SPSS 20.0 was used to input data, the measurement data between two groups underwent t test, the correlation was by Pearson test and $P<0.05$ was used to judge the statistical significance in differences in test results.

3. Results

3.1 Serum PCT content

Serum PCT content of SCLC group was (0.29±0.06) ng/mL, and serum PCT content of control group was (0.06±0.01) ng/mL. The t test analysis of the differences in serum PCT contents between SCLC group and control group showed that serum PCT content of SCLC group was significantly higher than that of control group ($P<0.05$).

3.2 Proliferation gene expression in tumor lesions

Analysis of proliferation genes HOXA13, CyclinD1, Noth3, MDM2 and Rb expression in tumor lesion tissue and adjacent lesion tissue was as follows: HOXA13, CyclinD1 and MDM2 mRNA expression in tumor lesion tissue were significantly higher than those in adjacent lesion tissue whereas Noth3 and Rb mRNA expression were significantly lower than those in adjacent lesion tissue. Pearson test showed that serum PCT content of patients with SCLC was positively correlated with HOXA13, CyclinD1 and MDM2 mRNA expression, and negatively correlated with Noth3 and Rb mRNA expression in tumor lesion tissue.

3.3 Invasion gene expression in tumor lesions

Analysis of invasion genes Bin1, MMP9, Vimentin, N-cadherin and E-cadherin expression in tumor lesion tissue and adjacent lesion tissue was as follows: Bin1 and E-cadherin mRNA expression in tumor lesion tissue were significantly lower than those in adjacent lesion tissue whereas MMP9, Vimentin and N-cadherin mRNA expression were significantly higher than those in adjacent lesion tissue. Pearson test showed that serum PCT content of patients with SCLC was negatively correlated with Bin1 and E-cadherin mRNA expression, and positively correlated with MMP9, Vimentin and N-cadherin mRNA expression in tumor lesion tissue.

Table 1.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>$n$</th>
<th>HOXA13</th>
<th>CyclinD1</th>
<th>Noth3</th>
<th>MDM2</th>
<th>Rb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor lesion</td>
<td>62</td>
<td>2.77±0.36</td>
<td>3.29±0.52</td>
<td>0.32±0.06</td>
<td>2.03±0.36</td>
<td>0.43±0.07</td>
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<td>Adjacent lesion</td>
<td>62</td>
<td>1.04±0.17</td>
<td>1.01±0.14</td>
<td>0.97±0.15</td>
<td>1.05±0.15</td>
<td>1.02±0.16</td>
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<tr>
<td>$t$&lt;br&gt;$p$</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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<td>&lt;0.05</td>
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</tr>
</tbody>
</table>

Table 2.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>$n$</th>
<th>Bin1</th>
<th>MMP9</th>
<th>Vimentin</th>
<th>N-cadherin</th>
<th>E-cadherin</th>
</tr>
</thead>
<tbody>
<tr>
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<td>62</td>
<td>0.37±0.06</td>
<td>2.84±0.42</td>
<td>2.55±0.34</td>
<td>3.46±0.51</td>
<td>0.28±0.06</td>
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<td>Adjacent lesion</td>
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<td>1.03±0.15</td>
<td>1.01±0.16</td>
<td>0.97±0.14</td>
<td>0.92±0.13</td>
<td>1.04±0.17</td>
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<tr>
<td>$t$&lt;br&gt;$p$</td>
<td></td>
<td>20.98</td>
<td>16.676</td>
<td>13.474</td>
<td>23.490</td>
<td>27.657</td>
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<tr>
<td>$p$&lt;br&gt;$p$</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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</tbody>
</table>
Table 3.
Comparison of angiogenesis genes in tumor lesion tissue and adjacent lesion tissue.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>VEGF</th>
<th>VE-cadherin</th>
<th>EGFL8</th>
<th>Nestin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor lesion</td>
<td>62</td>
<td>2.95±0.45</td>
<td>2.24±0.37</td>
<td>1.89±0.24</td>
<td>3.05±0.41</td>
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<tr>
<td>Adjacent lesion</td>
<td>62</td>
<td>1.04±0.17</td>
<td>0.98±0.13</td>
<td>1.00±0.14</td>
<td>0.97±0.15</td>
</tr>
<tr>
<td>t</td>
<td></td>
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<td>&lt;0.05</td>
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</tr>
</tbody>
</table>

3.4 Angiogenesis gene expression in tumor lesions

Analysis of angiogenesis genes VEGF, VE-cadherin, EGFL8 and Nestin expression in tumor lesion tissue and adjacent lesion tissue was as follows: VEGF, VE-cadherin, EGFL8 and Nestin mRNA expression in tumor lesion tissue were significantly higher than those in adjacent lesion tissue. Pearson test showed that serum PCT content of patients with SCLC was positively correlated with VEGF, VE-cadherin, EGFL8 and Nestin mRNA expression in tumor lesion tissue.

4. Discussion

SCLC is a malignant tumor with neuroendocrine characteristics, and the cancer cells within the lesion are highly malignant, have strong infiltrative growth performance and are prone to distant metastasis[6]. At present, the early diagnosis indexes of SCLC are short and the early diagnosis rate is low in clinical practice, the disease has developed to the middle and advanced stage when diagnosed and the overall prognosis is poor[7,8]. NSE and Pro-GRP are the molecules synthesized and secreted by SCLC, they are the common diagnostic markers of SCLC in clinic, but the diagnostic sensitivity is not good. It has been found in recent years that PCT is the calcitonin precursor that can be secreted by SCLC, it was first used in the diagnosis of bacterial infectious diseases, and recent studies have confirmed the SCLC cell lines cultured in vitro can massively express PCT[9,10]. Analysis of the changes in serum PCT contents in patients with SCLC showed that serum PCT content of SCLC group was significantly higher than that of control group. This indicates that PCT secretion significantly increase in the course of SCLC, and the serum PCT content can provide the basis for the diagnosis of SCLC.

The infiltrative growth of cancer cells in SCLC is closely related to the excessive proliferation of the cells, and the expression of multiple genes has significantly changed. HOXA13 is a member of the HOX gene family, which can be combined with the promoter region of CyclinD1 gene to promote its expression, and then accelerate the cell cycle and promote cell proliferation through the biological function of CyclinD1[11]; MDM2 is a kind of ubiquitin ligase that can degrade Rb through ubiquitination pathway, reduce the inhibitory effect of negative cell cycle regulator Rb on transcription factor E2F and promote cell proliferation[12,13]; Notch3 is the Notch family member with negative regulatory effect on cell cycle, which can induce cell cycle arrest and inhibit cell proliferation[14]. The analysis of above proliferation gene expression in the SCLC lesions showed that HOXA13, CyclinD1 and MDM2 mRNA expression in tumor lesion tissue were significantly higher than those in adjacent lesion tissue whereas Notch3 and Rb mRNA expression were significantly lower than those in adjacent lesion tissue. It indicates that the high expression of pro-proliferation genes and the low expression of anti-proliferation genes are closely related to the occurrence of SCLC. Further analysis of the correlation between serum PCT content and proliferation gene expression indicated that serum PCT content of patients with SCLC was positively correlated with HOXA13, CyclinD1 and MDM2 mRNA expression, and negatively correlated with Notch3 and Rb mRNA expression in tumor lesion tissue. This indicates that the changes in serum PCT content of patients with SCLC are related to the changes of cell proliferation activity in the lesions, and the proliferation activity of SCLC cells can be evaluated by the determination of PCT content.

On the basis of abnormal proliferation, SCLC cells need to degrade the extracellular matrix to make invasion and then complete the biological process of invasive growth. Bin1 is the upstream regulator of the cell invasion process, and can regulate the expression of the downstream MMPs family molecules and epithelial mesenchymal markers through the NF-κB B[15]. MMP9 is the MMPs family member that is regulated by Bin1. Bin1 can reduce the expression of MMP9 and inhibit the degradation of extracellular matrix and basement membrane to hinder cell invasion[16]. During epithelial-mesenchymal transition, the expression of mesenchymal phenotype markers Vimentin and N-cadherin are inhibited by Bin1 while the expression of epithelial phenotype marker E-cadherin is promoted by Bin1; Bin1 can hinder the epithelial-mesenchymal transition process, enhance intercellular polarity and inhibit cell invasion and migration[17,18]. The analysis of above invasion gene expression in the SCLC lesions showed that Bin1 and E-cadherin mRNA expression in tumor lesion tissue were significantly lower than those in adjacent lesion tissue whereas MMP9, Vimentin and N-cadherin mRNA expression were significantly higher than those in adjacent lesion tissue. This indicates that the high expression of the pro-invasion genes and the low expression of the anti-invasion genes are closely related to the occurrence of SCLC. Further analysis of the correlation between serum PCT levels and invasion gene expression indicated that serum PCT content of patients with SCLC was negatively correlated with Bin1 and E-cadherin mRNA expression, and positively correlated with MMP9, Vimentin and N-cadherin mRNA expression in tumor lesion tissue. This indicates that the changes in serum PCT content of patients with SCLC are related to changes in the invasion activity of the cells in the lesion,
and the invasion activity of SCLC cells can be evaluated by the determination of PCT content.

The proliferation and invasion of cancer cells in SCLC are dependent on the nutrients and energy provided by the new blood vessels, and new blood vessels are also needed to provide pathways for the metastasis of cancer cells. VEGF is the most powerful cytokine inhibiting angiogenesis at present, which can directly affect endothelial cells and promote their proliferation and vascular structure formation[19]; VE-cadherin is a kind of adhesion molecule specifically expressed on the surface of endothelial cells, which can promote the endothelial cell adhesion and migration to tumor lesions and facilitate the formation of new blood vessels in the lesion[20]; EGFL8 is a secretory protein containing EGF-like domain, which has extensive pro-proliferation effect and can maintain the high level of angiogenesis; Nestin is the marker proliferation antigen in endothelial cells, which can reflect the proliferation and growth activity of endothelial cells[21]. Analysis of the above angiogenesis gene expression in SCLC lesions showed that VEGF, VE-cadherin, EGFL8 and Nestin mRNA expression in tumor lesion tissue were significantly higher than those in adjacent lesion tissue. This indicates that the high expression of angiogenesis gene is closely related to the occurrence of SCLC. Further analysis of correlation between serum PCT content and angiogenesis gene expression indicated that serum PCT content of patients with SCLC was positively correlated with VEGF, VE-cadherin, EGFL8 and Nestin mRNA expression in tumor lesion tissue. This indicates that the changes in serum PCT content of patients with SCLC are related to the changes of angiogenesis activity in the lesion, and the determination of PCT content can assess the vitality of angiogenesis in SCLC lesions.

In conclusion, serum PCT content significantly increases in patients with small cell lung cancer; abnormal increase of PCT is associated with the changes in cell proliferation and invasion activity as well as angiogenesis activity within the lesions, and the determination of PCT content can assess the cell proliferation and invasion activity as well as angiogenesis activity within the SCLC lesions.

References


