Correlation of NSE and ProGRP levels in patients with small cell lung cancer before and after chemotherapy with cancer cell apoptosis caused by chemotherapy

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Abstract: To study the correlation of NSE and ProGRP levels in patients with small cell lung cancer (SCLC) before and after chemotherapy with cancer cell apoptosis caused by chemotherapy. Methods: Patients with SCLC accepting EP chemotherapy in our hospital between August 2014 and October 2016 were selected, serum was collected before chemotherapy and after 3 cycles of chemotherapy respectively to determine NSE and ProGRP levels, the quartile of serum NSE and ProGRP levels after chemotherapy were referred to divide the patients into NSE and ProGRP-1 group, -2 group, -3 and -4 group from low to high; tumor lesions were collected to determine apoptosis molecule mRNA expression. Results: After chemotherapy, serum NSE and ProGRP levels of patients with SCLC were significantly lower than those before chemotherapy, and Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 mRNA expression in tumor lesion were significantly higher than those before chemotherapy; Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 mRNA expression in lesions of NSE and ProGRP-1 group, -2 group and -3 group were significantly higher than those of NSE and ProGRP-4 group, Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 mRNA expression in lesions of NSE and ProGRP-1 group and -2 group were significantly higher than those of NSE and ProGRP-3 group, and Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 mRNA expression in lesions of NSE and ProGRP-1 group were significantly higher than those of NSE and ProGRP-2 group. Conclusion: Serum NSE and ProGRP levels decrease significantly in patients with small cell lung cancer after chemotherapy, and they are closely related to the cancer cell apoptosis caused by chemotherapy.

1. Introduction

Lung cancer is the malignant tumor with the highest incidence around the world, and the deaths from lung cancer rank the first of all kinds of cancer. Small cell lung cancer (SCLC) is a type of lung cancer with high malignant degree, and compared with non-small cell lung cancer, SCLC has the characteristics of low degree of differentiation, strong cancer cell proliferation and invasion activity as well as prone to distant metastasis, and is with intractable clinical treatment and poor prognosis. Intravenous chemotherapy is the main treatment for advanced SCLC, small cell cancer cell is sensitive to a variety of chemotherapeutic drugs, and the intravenous chemotherapy can effectively kill cancer cells, shrink tumor volume and prolong patients’ survival time[1,2]. During intravenous chemotherapy of patients with SCLC, accurate evaluation of chemotherapy effect has positive value for the formulation and adjustment of clinical chemotherapy regimens[3,4]. Serum tumor marker detection is a common method for clinical diagnosis of malignant tumors and assessment of the chemotherapy effect, neuron-specific enolase (NSE) and pro-gastrin-releasing peptide (ProGRP) are the SCLC markers with high sensitivity and specificity, and there is no related research about the value of NSE and ProGRP for evaluation of SCLC chemotherapy effect[5,6]. In the following study, the correlation of NSE and ProGRP levels in patients with small cell lung cancer before and after chemotherapy...
with cancer cell apoptosis caused by chemotherapy was analyzed.

2. Subjects and methods

2.1. Research subjects

Patients with small cell lung cancer who accepted intravenous chemotherapy in our hospital between August 2014 and October 2016 were selected as the research subjects, and all the patients were diagnosed with small cell lung cancer through pathological examination, were informed of the research items, then signed informed consent and intended to accept EP chemotherapy. A total of 97 patients were selected, including 32 male cases and 65 female cases that were 44–71 years old. EP chemotherapy regimens were as follows: etoposide 100 mg/d was by intravenous drip on the 1–5 d, cisplatin 25 mg/m^2 was by intravenous drip on the 1–3 d, 3 weeks was one cycle of treatment, and all patients accepted over 3 cycles of chemotherapy.

2.2. Experimental materials

Serum NSE and ProGRP enzyme-linked immunosorbent assay kits were bought from CanAg Company in Sweden, and the animal tissue RNA extraction kits, cDNA first-strand synthesis kits and fluorescence quantitative PCR kits were from Beijing ComWin Biotech Company; automatic microplate reader was from Bio-Kinetics company in the United States and the fluorescence quantitative PCR apparatus was from Bio-tek Company in the United States.

2.3. Experimental methods

2.3.1. Clinical sample collection methods

Before chemotherapy and after 3 cycles of chemotherapy, 5 mL of cubital venous blood was extracted and centrifuged to separate serum and store it in -80°C cryogenic refrigerator; on the same day of the peripheral blood extraction, pathology biopsy was performed, moderate amount of biopsy tissue was collected, washed with saline and then frozen with liquid nitrogen, RNA extraction kits were used to extract total RNA in lung cancer tissue, cDNA first-strand synthesis kits were used to reverse-transcribe the RNA into cDNA, and the cDNA samples were preserved in -80°C cryogenic refrigerator.

2.3.2. Clinical index detection methods

Serum specimens were collected, and enzyme-linked immunosorbent assay kits were used to determine the NSE and ProGR levels; cDNA were collected, and fluorescent quantitative PCR kits were used to amplify target genes Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 as well as reference gene GAPDH, and Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 mRNA expression were calculated.

2.4. Statistical methods

SPSS 16.0 software was used for statistical processing, analysis before and after treatment was by paired-sample t test, analysis among groups was by variance analysis and P<0.05 indicated statistical significance in differences.

3. Results

3.1. Serum SE and ProGRP level change before and after chemotherapy

Before chemotherapy, serum NSE and ProGRP levels patients with SCLC were (49.51±6.23) ng/mL and (1.36±0.17) ng/mL respectively; after chemotherapy, serum NSE and ProGRP levels of patients with SCLC were (15.63±1.78) ng/mL and (0.35±0.07) ng/mL respectively. Analysis of serum SE and ProGRP levels before and after chemotherapy was as follows: after chemotherapy, serum NSE and ProGRP levels were significantly lower than those before chemotherapy; differences in serum SE and ProGRP levels were statistically significant before and after chemotherapy (P<0.05).

3.2. Apoptosis molecule expression change in tumor lesions before and after chemotherapy

Analysis of apoptosis molecules Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 mRNA expression in tumor lesions before and after chemotherapy was as follows: after chemotherapy, serum NSE and ProGRP levels were significantly lower than those before chemotherapy; differences in serum SE and ProGRP levels were statistically significant before and after chemotherapy (P<0.05).

3.3. Relationship between serum NSE level and apoptosis molecule expression after chemotherapy

The quartile of serum NSE level after chemotherapy was referred

| Table 1
| Comparison of apoptosis molecule expression in tumor lesions before and after chemotherapy.
<table>
<thead>
<tr>
<th>Chemotherapy</th>
<th>n</th>
<th>Fas</th>
<th>FasL</th>
<th>Caspase-8</th>
<th>Caspase-3</th>
<th>pULK</th>
<th>PI3KC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before chemotherapy</td>
<td>97</td>
<td>1.04±0.12</td>
<td>1.08±0.12</td>
<td>0.97±0.11</td>
<td>0.95±0.13</td>
<td>1.02±0.08</td>
<td>1.05±0.14</td>
</tr>
<tr>
<td>After chemotherapy</td>
<td>97</td>
<td>2.76±0.36</td>
<td>2.38±0.32</td>
<td>2.21±0.28</td>
<td>3.04±0.42</td>
<td>2.89±0.37</td>
<td>1.89±0.21</td>
</tr>
<tr>
<td>t</td>
<td>15.682</td>
<td>12.452</td>
<td>14.293</td>
<td>22.395</td>
<td>18.392</td>
<td>9.185</td>
<td></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>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>
to divide the patients into NSE-1 group, -2 group, -3 and -4 group from low to high, and analysis of apoptosis molecule expression in lesions among the four groups was as follows: Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 mRNA expression in lesions of NSE-1 group, -2 group and -3 group were significantly higher than those of NSE-4 group, Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 mRNA expression in lesions of NSE-1 group and -2 group were significantly higher than those of NSE-3 group, and Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 mRNA expression in lesions of NSE-1 group were significantly higher than those of NSE-2 group. Differences in pair-wise comparison of Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 mRNA expression in lesions were statistically significant among NSE-1 group, -2 group, -3 and -4 group (P<0.05) (Table 2).

### 3.4. Relationship between serum ProGRP level and apoptosis molecule expression after chemotherapy

The quartile of serum ProGRP level after chemotherapy was referred to divide the patients into ProGRP-1 group, -2 group, -3 and -4 group from low to high, and analysis of apoptosis molecule expression in lesions among the four groups was as follows: Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 mRNA expression in lesions of ProGRP-1 group, -2 group and -3 group were significantly higher than those of ProGRP-4 group, Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 mRNA expression in lesions of ProGRP-1 group and -2 group were significantly higher than those of ProGRP-3 group, and Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 mRNA expression in lesions were statistically significant among ProGRP-1 group, -2 group, -3 and -4 group (P<0.05) (Table 3).

### 4. Discussion

Small cell lung cancer (SCLC) in lung cancer is the type of lung cancer with high malignant degree and poor prognosis, and chemotherapy is the main method for clinical treatment of SCLC.[7,8] Although small cell lung cancer has higher sensitivity to chemotherapy, the drug resistance during chemotherapy can affect the effect of chemotherapy, so accurately assessment of chemotherapy effect is required to adjust the treatment plan. Serum tumor marker detection is the most common method for clinical screening of SCLC and evaluation of the tumor load, and the NSE and ProGRP are the most widely applied SCLC markers. SCLC lesions are with neuroendocrine properties, the NSE secreted is acid protease specifically expressed by neurons and neuroendocrine cells, and ProGRP is a precursor of GRP and mainly exists in the nerve tissue and lung tissue.[9-11] But at present, the value of serum NSE and ProGRP levels for evaluating the chemotherapy effect of patients with SCLC is still not clear. In the study, in order to define the value of serum NSE and ProGRP levels for evaluating the chemotherapy effect of patients with SCLC, serum NSE and ProGRP levels were analyzed before and after chemotherapy, and the results showed that serum NSE and ProGRP levels of patients with SCLC after chemotherapy were significantly lower than those before chemotherapy. It means that chemotherapeutics drugs can not only kill small cell lung cancer cells, but also significantly reduce the serum NSE and ProGRP levels, and it is expected that serum NSE and ProGRP levels can be used as the auxiliary examination indexes to assess the effect of chemotherapy in patients with SCLC.

The killing effect of intravenous chemotherapy on cancer cells is mainly achieved through inducing cell apoptosis. Fas, FasL, Caspase-8, Caspase-3, pULK, PI3KC3 and other molecules play an important regulating role in the process of cell apoptosis. Fas/ Fasl are the upstream molecules that regulate cell apoptosis through death receptor pathway, Fas is a member of tumor necrosis factor superfamily, FasL is its nature-ligand, and the combination of the two can form death-inducing complexes, split inactive Pro-Caspase-8 into active Caspase-8, then start the cascade reaction, eventually activate Caspase-3 and cause apoptosis.[12,13] PULK and PI3KC3 are the signaling molecules that regulate cell apoptosis through the

### Table 2

<table>
<thead>
<tr>
<th>NSE level</th>
<th>n</th>
<th>Fas</th>
<th>FasL</th>
<th>Caspase-8</th>
<th>Caspase-3</th>
<th>pULK</th>
<th>PI3KC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>NSE-1</td>
<td>24</td>
<td>3.98±0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.13±0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.49±0.48&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.21±0.57&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.93±0.45&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.18±0.42&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>NSE-2</td>
<td>24</td>
<td>3.07±0.38&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.57±0.34&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.83±0.35&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.42±0.45&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.31±0.41&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.45±0.32&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>NSE-3</td>
<td>24</td>
<td>2.52±0.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.02±0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.89±0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.65±0.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.78±0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.91±0.23&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>NSE-4</td>
<td>25</td>
<td>1.89±0.22</td>
<td>1.74±0.22</td>
<td>1.44±0.16</td>
<td>2.10±0.28</td>
<td>2.21±0.28</td>
<td>1.52±0.17</td>
</tr>
</tbody>
</table>

<sup>a</sup>: compared with NSE-2 group, P<0.05; <sup>b</sup>: compared with NSE-3 group, P<0.05; <sup>c</sup>: compared with NSE-4 group, P<0.05.

### Table 3

<table>
<thead>
<tr>
<th>ProGRP level</th>
<th>n</th>
<th>Fas</th>
<th>FasL</th>
<th>Caspase-8</th>
<th>Caspase-3</th>
<th>pULK</th>
<th>PI3KC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProGRP-1</td>
<td>24</td>
<td>3.89±0.49&lt;sup&gt;de&lt;/sup&gt;</td>
<td>3.22±0.42&lt;sup&gt;de&lt;/sup&gt;</td>
<td>3.56±0.47&lt;sup&gt;df&lt;/sup&gt;</td>
<td>4.12±0.56&lt;sup&gt;de&lt;/sup&gt;</td>
<td>3.85±0.42&lt;sup&gt;de&lt;/sup&gt;</td>
<td>3.30±0.46&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>ProGRP-2</td>
<td>24</td>
<td>3.11±0.41&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.60±0.35&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.91±0.33&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.55±0.47&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>3.22±0.38&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.37±0.36&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>ProGRP-3</td>
<td>24</td>
<td>2.60±0.32&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.98±0.23&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.82±0.21&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.59±0.29&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.71±0.31&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.98±0.24&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>ProGRP-4</td>
<td>25</td>
<td>1.82±0.20</td>
<td>1.66±0.18</td>
<td>1.47±0.18</td>
<td>1.98±0.23</td>
<td>2.18±0.25</td>
<td>1.44±0.18</td>
</tr>
</tbody>
</table>

<sup>d</sup>: compared with ProGRP-2 group, P<0.05; <sup>c</sup>: compared with ProGRP-3 group, P<0.05; <sup>f</sup>: compared with ProGRP-4 group, P<0.05.
autophagy pathway, pULK can be involved in the induced activation stage of autophagy through Atg6 and Beclin1, and PI3KC3 can be involved in the extension of autophagy through Atg21 and Atg24[14,15]. In the occurrence and development of small cell lung cancer, the death receptor apoptosis pathway mediated by Fas/FasL and the cell autophagy apoptosis pathway mediated by pULK and PI3KC3 are significantly suppressed, and the expression levels of corresponding apoptosis molecules in tumor lesions significantly reduce[16,17]. During the intravenous chemotherapy in patients with SCLC, chemotherapeutics drugs can adjust the expression of apoptosis molecules in tumor lesions to induce cancer cell apoptosis and inhibit tumor lesion growth. In the study, analysis of the expression of above apoptosis molecules in lung cancer lesions before and after chemotherapy showed that after chemotherapy, Fas, FasL, Caspase-8, Caspase-3, pULK and PI3KC3 mRNA expression in tumor lesions were significantly higher than those before chemotherapy. This means that intravenous chemotherapy can increase the expression of apoptosis molecules in SCLC lesions and promote the cancer cell apoptosis mediated by death receptor pathway and autophagy pathway.

In order to further clarify the value of serum NSE and ProGRP levels for evaluating the chemotherapy effect in patients with SCLC, the correlation of serum NSE and ProGRP levels with apoptosis molecules in lesions was analyzed in the study after chemotherapy. Abnormally elevated NSE and ProGRP in blood circulation of in patients with SCLC come from the synthesis and secretion of cancer cells, and intravenous chemotherapy can induce cancer cell apoptosis to significantly reduce the cancer cell viability in lesions, thus decrease NSE and ProGRP synthesis and secretion, and reduce serum NSE and ProGRP levels. Thus it was speculated that the decreased serum NSE and ProGRP levels after chemotherapy are correlated with the increased apoptosis molecule expression in lesions, and in order to verify this theory, the relationship of serum NSE and ProGRP levels after chemotherapy with apoptosis molecule expression was analyzed. The quartile of serum NSE and ProGRP levels were referred to divide the patients with SCLC into four groups that were with NSE and ProGRP levels from high to low, and the analysis results showed that the lower the serum NSE and ProGRP levels, the higher the Fas, FasL, Caspase-3, pULK and PI3KC3 mRNA expression in tumor lesions. This means that the changes of serum NSE and ProGRP levels in patients with SCLC are correlated with apoptosis molecule mRNA expression in tumor lesions, and detecting serum NSE and ProGRP levels after chemotherapy can reflect the degree of cancer cell apoptosis within lesions, and thus evaluate the overall effect of the chemotherapy.

To sum up, it shows that serum NSE and ProGRP levels decrease significantly after patients with small cell lung cancer accept EP chemotherapy; the changes of serum NSE and ProGRP levels after chemotherapy are correlated with the changes of apoptosis molecule expression caused by chemotherapy, and can reflect the degree of cancer cell apoptosis and evaluate the effect of chemotherapy.

References