ARTICLE INFO

Article history:
Received 7 Jul 2016
Received in revised form 17 Jul 2016
Accepted 12 Jul 2016
Available online 24 Jul 2016

Keywords:
Non-small cell lung cancer
Macrophage inhibitory cytokine
Insulin-like growth factor binding protein
Silent information regulator-1
Epithelial-mesenchymal transition

ABSTRACT

Objective: To study the correlation of serum Macrophage inhibitory factor (MIF) and insulin-like growth factor binding proteins (IGFBPs) levels with the cancer cell viability in the focus of patients with early lung cancer.

Methods: 38 patients with early non-small cell lung cancer who received surgical resection in our hospital between May 2013 and August 2016 were selected as the lung cancer group, and 60 healthy volunteers who received physical examination during the same period were selected as the control group. The serum was collected from two groups of subjects to determine the MIF and IGFBPs levels, and the lung cancer tissue and the tissue adjacent to carcinoma were collected from the lung cancer group to determine the mRNA expression of SIRT-1-related molecules.

Results: Serum MIF level of lung cancer group was significantly higher than that of control group (P < 0.05), IGFBP-3 level was significantly lower than that of control group (P < 0.05), and the IGFBP-2 and IGFBP-5 levels were not significantly different from those of control group; SIRT-1, Vimentin and N-cadherin mRNA expression in lung cancer tissue were significantly higher than those in the tissue adjacent to carcinoma (P < 0.05) while ZO-1 and E-cadherin mRNA expression were significantly lower than those in the tissue adjacent to carcinoma (P < 0.05); SIRT-1, Vimentin and N-cadherin mRNA expression were higher while ZO-1 and E-cadherin mRNA expression were lower in focus tissue of lung cancer patients with high MIF levels and those with low IGFBP-3 levels.

Conclusion: Serum MIF level increases abnormally while IGFBP-3 level decreases abnormally in patients with early lung cancer, and the change of MIF and IGFBP-3 levels is correlated with the change of cancer cell growth process mediated by SIRT-1.

1. Introduction

Non-small cell lung cancer (NSCLC) is a kind of malignant tumor with high prevalence, and the common means for the treatment of I-IIIa NSCLC is surgical resection combined with postoperative adjuvant chemotherapy is. In clinical practice, the diagnostic timing of NSCLC is an important factor to determine prognosis, the prognosis of patients with I and II early NSCLC is ideal, and the total 5-year survival rate is about 60%–90%[1,2]. But at the moment, the early diagnostic rate of NSCLC is low, and the lack of effective early screening indexes is the important reason that influences the early diagnostic rate of NSCLC. Macrophage inhibitory factor (MIF) and insulin-like growth factor binding protein (IFGBP) are the cell growth-regulating molecules discovered in recent years. Studies have shown that abnormal expression and secretion of MIF and IFGBPs are related to the occurrence and development of lung cancer[3,4], but it is not yet clear about the value of serum MIF and IGFBPs levels for diagnosing early lung cancer and evaluating the illness. In the following study, the correlation of serum MIF and IGFBPs levels with the cancer cell viability in the focus of patients with early lung cancer was analyzed.

2. Materials and methods

2.1. Research subjects
38 patients with early non-small cell lung cancer who received surgical resection in our hospital between May 2013 and August 2016 were selected as the lung cancer group of the research, postoperative pathology biopsy confirmed that all patients were without lung cancer invasion or distant lymph node metastasis, TNM stage was I-II, and they included 25 male cases and 13 female cases that were 36–56 years old; 60 healthy volunteers who received physical examination during the same period were selected as the control group of the research, were without previous history of respiratory disease, and included 38 male cases and 22 female cases that were 32–55 years old. The two groups of subjects were not significantly different in general data \((P>0.05)\).

2.2. Research methods

2.2.1. Serum MIF and IGFBPs level detection methods

5 mL of peripheral venous blood was collected from patients with lung cancer during admission, 5 mL of peripheral venous blood was collected from the control group of volunteers during physical examination, the blood was let stand at room temperature for 20–30 min, then naturally coagulated and centrifuged for 20 min in centrifuge at a speed of 3 000 r/min to separate serum, and enzyme-linked immunosorbent assay kits were used to detect MIF, IGFBP-2, IGFBP-3 and IGFBP-5 levels.

2.2.2. Detection methods of gene mRNA expression in lung cancer tissue and tissue adjacent to carcinoma

The lung cancer tissue and tissue adjacent to carcinoma were collected after surgical resection, the tissue nature was confirmed by pathological examination, RNA extraction kits were used to separate the total RNA in lung cancer tissues and tissues adjacent to carcinoma, and then the cDNA first-strand synthesis kits were used for reverse transcription from total RNA into cDNA; the cDNA after reverse transcription was collected, fluorescence quantitative PCR kit instructions were used to configure the reaction system, the primers were for SIRT-1, ZO-1, E-cadherin, Vimentin and N-cadherin mRNA expression, and the PCR amplification curves were used to calculate SIRT-1, ZO-1, E-cadherin, Vimentin and N-cadherin mRNA expression.

2.3. Statistical analysis

SPSS20.0 software was used to input serum indexes and gene mRNA expression, the median of serum MIF and IGFBP-3 of lung cancer group were calculated, patients with the levels higher than the median were judged as those with high MIF and IGFBP-3 levels, and patients with the levels lower than the median were judged as those with low MIF and IGFBP-3 levels. Analysis of the differences in serum indexes and gene mRNA expression between two groups was by \(t\) test and \(P<0.05\) indicated statistical significance in differences.

3. Results

3.1. Serum MIF and IGFBPs levels of lung cancer group and control group

Analysis of serum MIF, IGFBP-2, IGFBP-3 and IGFBP-5 levels between lung cancer group and control group was as follows: serum MIF level of lung cancer group was significantly higher than that of control group, IGFBP-3 level was significantly lower than that of control group, and the IGFBP-2 and IGFBP-5 levels were not significantly different from those of control group. Differences in serum MIF and IGFBP-3 levels were statistically significant between two groups of subjects \((P<0.05)\) (Table 1).

3.2. SIRT-1-related molecule expression in lung cancer tissue and tissue adjacent to carcinoma

Analysis of SIRT-1-related molecules SIRT-1, ZO-1, E-cadherin, Vimentin and N-cadherin mRNA expression in lung cancer tissue and tissue adjacent to carcinoma was as follows: SIRT-1, Vimentin and N-cadherin mRNA expression in lung cancer tissue were significantly higher than those in the tissue adjacent to carcinoma while ZO-1 and E-cadherin mRNA expression were significantly lower than those in the tissue adjacent to carcinoma. Differences were statistically significant in SIRT-1, ZO-1, E-cadherin, Vimentin and N-cadherin mRNA expression in lung cancer tissue and tissue adjacent to carcinoma \((P<0.05)\) (Table 2).

3.3. Correlation between serum MIF levels and SIRT-1-related molecule expression

Analysis of SIRT-1-related molecules SIRT-1, ZO-1, E-cadherin, Vimentin and N-cadherin mRNA expression in lesion tissue of lung cancer patients with different MIF levels was as follows: SIRT-1, Vimentin and N-cadherin mRNA expression in focus tissue of lung cancer patients with high MIF levels were significantly higher than those of lung cancer patients with low MIF levels while ZO-1 and E-cadherin mRNA expression were significantly lower than those of lung cancer patients with low MIF levels. Differences were statistically significant in SIRT-1, ZO-1, E-cadherin, Vimentin and N-cadherin mRNA expression in focus tissue of lung cancer patients with different MIF levels \((P<0.05)\) (Table 3).

3.4. Correlation between serum IGFBP-3 levels and SIRT-1-related molecule expression

Analysis of SIRT-1-related molecules SIRT-1, ZO-1, E-cadherin, Vimentin and N-cadherin mRNA expression in lesion tissue of lung cancer patients with different IGFBP-3 levels was as follows: SIRT-1,
Vimentin and N-cadherin mRNA expression in focus tissue of lung cancer patients with high IGFBP-3 levels were significantly lower than those of lung cancer patients with low IGFBP-3 levels while ZO-1 and E-cadherin mRNA expression were significantly higher than those of lung cancer patients with low MIF levels. Differences were statistically significant in SIRT-1, ZO-1, E-cadherin, Vimentin and N-cadherin mRNA expression in focus tissue of lung cancer patients with different IGFBP-3 levels ($P<0.05$) (Table 4).

### 4. Discussion

The early diagnosis of NSCLC is of positive value for improving the prognosis and increasing the survival rate, and exploring the screening indexes for early diagnosis of NSCLC has been the research focus in the respiratory and oncology [5,6]. MIF is a member of the transforming growth factor β superfamily, and a variety of malignant tumor cells in epithelium can synthesize and secrete MIF [7]. Basic study on the MIF confirms that the molecule has antagonizing effect on tumor suppressor gene p53, has promoting effect on the cell growth effects mediated by ERK/MAPK, and has supplementary effect on the angiogenesis mediated by COX/PGE2 [8,9]. The clinical study of domestic scholar Shen et al [10] has shown that serum MIF levels significantly increases in patients with lung cancer and is associated with the TNM staging of tumor. In the study, in order to define the serum MIF value for early diagnosis and screening of lung cancer, the serum MIF levels in patients with early lung cancer were analyzed, and results showed that serum MIF level of lung cancer group was significantly higher than that of control group ($P<0.05$). This means that serum MIF levels has significantly increased in the early lung cancer, and to detect the serum MIF levels has a certain value for screening for early lung cancer.

IGFBPs are a group of molecules that regulate cell growth in insulin-like growth factor-dependent way, and the IGFBP-2, IGFBP-3 and IGFBP-5 in the family are often studied. IGFBP-2, IGFBP-3 and IGFBP-5 can be combined with the IGF-1 and IGF-2 in serum and local tissue to form complexes and then antagonize the combination of IGF-1 and IGF-2 with membrane receptors and the activation of downstream signaling pathways, thereby inhibiting the cell growth process mediated by IGF-1 and IGF-2 with membrane receptors and the activation of downstream signaling pathways, thereby inhibiting the cell growth process mediated by IGF-1 and IGF-2 [11]. The study of domestic scholars Zhang and Feng [12] confirms that the IGFBP-3 in IGFBPs family is an independent risk factor to judge non-small cell lung cancer chemosensitivity, and NSCLC patients with low IGFBP-3 content in serum have poor sensitivity to chemotherapy. In the study, in order to define the serum IGFBP-3 levels for early diagnosis and screening of lung cancer, serum

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**Table 1**
Serum MIF and IGFBPs levels of lung cancer group and control group (μg/L, $\bar{x}$±s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>MIF</th>
<th>IGFBP-2</th>
<th>IGFBP-3</th>
<th>IGFBP-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung cancer group</td>
<td>38</td>
<td>1542.65±224.21</td>
<td>396.82±52.64</td>
<td>1044.51±154.36</td>
<td>567.52±78.65</td>
</tr>
<tr>
<td>Control group</td>
<td>60</td>
<td>658.69±86.85</td>
<td>399.12±58.91</td>
<td>2236.54±31.69</td>
<td>573.11±72.85</td>
</tr>
<tr>
<td>$t$</td>
<td></td>
<td>14.855</td>
<td>0.395</td>
<td>12.185</td>
<td>0.448</td>
</tr>
<tr>
<td>$P$</td>
<td></td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
<td>&lt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

**Table 2**
Comparison of SIRT-1-related molecule expression in lung cancer tissue and tissue adjacent to carcinoma ($\bar{x}$±s).

<table>
<thead>
<tr>
<th>Tissue nature</th>
<th>n</th>
<th>SIRT-1 $\bar{x}$±s</th>
<th>ZO-1 $\bar{x}$±s</th>
<th>E-cadherin $\bar{x}$±s</th>
<th>Vimentin $\bar{x}$±s</th>
<th>N-cadherin $\bar{x}$±s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung cancer tissue</td>
<td>38</td>
<td>2.32±0.48</td>
<td>0.45±0.08</td>
<td>0.39±0.07</td>
<td>1.97±0.32</td>
<td>2.56±0.48</td>
</tr>
<tr>
<td>Tissue adjacent to cancer</td>
<td>60</td>
<td>1.05±0.16</td>
<td>1.02±0.18</td>
<td>1.07±0.20</td>
<td>0.98±0.13</td>
<td>1.04±0.17</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>

**Table 3**
Comparison of SIRT-1-related molecule expression in lesion tissue with different serum MIF levels ($\bar{x}$±s).

<table>
<thead>
<tr>
<th>MIF level</th>
<th>n</th>
<th>SIRT-1 $\bar{x}$±s</th>
<th>ZO-1 $\bar{x}$±s</th>
<th>E-cadherin $\bar{x}$±s</th>
<th>Vimentin $\bar{x}$±s</th>
<th>N-cadherin $\bar{x}$±s</th>
</tr>
</thead>
<tbody>
<tr>
<td>High level</td>
<td>19</td>
<td>3.09±0.59</td>
<td>0.33±0.06</td>
<td>0.28±0.05</td>
<td>2.68±0.45</td>
<td>3.15±0.52</td>
</tr>
<tr>
<td>Low level</td>
<td>19</td>
<td>1.62±0.29</td>
<td>0.60±0.10</td>
<td>0.51±0.09</td>
<td>1.35±0.21</td>
<td>1.87±0.25</td>
</tr>
<tr>
<td>$t$</td>
<td></td>
<td>8.948</td>
<td>9.172</td>
<td>8.583</td>
<td>10.208</td>
<td>7.8534</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>

**Table 4**
Comparison of SIRT-1-related molecule expression in lesion tissue with different serum IGFBP-3 levels ($\bar{x}$±s).

<table>
<thead>
<tr>
<th>IGFBP-3 level</th>
<th>n</th>
<th>SIRT-1 $\bar{x}$±s</th>
<th>ZO-1 $\bar{x}$±s</th>
<th>E-cadherin $\bar{x}$±s</th>
<th>Vimentin $\bar{x}$±s</th>
<th>N-cadherin $\bar{x}$±s</th>
</tr>
</thead>
<tbody>
<tr>
<td>High level</td>
<td>19</td>
<td>1.59±0.27</td>
<td>0.63±0.11</td>
<td>0.48±0.08</td>
<td>1.42±0.23</td>
<td>1.71±0.23</td>
</tr>
<tr>
<td>Low level</td>
<td>19</td>
<td>3.14±0.63</td>
<td>0.29±0.04</td>
<td>0.30±0.06</td>
<td>2.58±0.41</td>
<td>3.34±0.59</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>
IGFBPs levels in patients with early lung cancer were analyzed, and the results showed that serum IGFBP-3 level of lung cancer group was significantly lower than that of control group \((P<0.05)\) while IGFBP-2 and IGFBP-5 levels were not significantly different from those of control group. It means that the abnormal synthesis and secretion of IGFBP-3 in IGFBPs family participate in the occurrence and development of lung cancer, the serum IGFBP-3 levels have significantly reduced in the early lung cancer, and detecting serum IGFBP-3 levels has a certain value for screening for early lung cancer.

After it was confirmed that serum MIF and IGFBP-3 levels had screening value for early lung cancer, the correlation of MIF and IGFBP-3 levels with early lung cancer was further analyzed in the study. Strong proliferation and invasion vitality is characteristic of early lung cancer, and SIRT-1 signaling pathway is the important mechanism to regulate early lung cancer proliferation and invasion vitality\(^{[13,14]}\). SIRT-1 promotes epithelial-mesenchymal transition during the occurrence and development of malignant tumor\(^{[15,16]}\). In vitro study of domestic Yu et al.\(^{[17]}\) confirms that SIRT-1 can inhibit the epithelial marker molecules ZO-1 and E-cadherin expression and increase the mesenchymal marker molecules Vimentin and N-cadherin expression in lung cancer cells so as to lower intercellular polarity and promote cell growth and migration. In the study, analysis of SIRT-1-related molecule expression in lung cancer tissue showed that SIRT-1, Vimentin and N-cadherin mRNA expression in lung cancer tissue were significantly higher than those in the tissue adjacent to carcinoma \((P<0.05)\) while ZO-1 and E-cadherin mRNA expression were significantly lower than those in the tissue adjacent to carcinoma \((P<0.05)\). Further analysis of the correlation of serum MIF and IGFBP-3 levels with SIRT-1-related molecule expression in lung cancer tissues confirmed that SIRT-1, Vimentin and N-cadherin mRNA expression were higher while ZO-1 and E-cadherin mRNA expression were lower in focus tissue of lung cancer patients with high MIF levels and those with low IGFBP-3 levels. This means that the MIF and IGFBP-3 content changes in patients with early lung cancer are associated with the cell growth process mediated by SIRT-1, and high content of MIF and low content of IGFBP-3 can promote epithelial-mesenchymal transition mediated by SIRT-1, and then promote cell proliferation and invasion.

Serum MIF levels abnormally increase while IGFBP-3 levels abnormally decrease in patients with early lung cancer, and the MIF and IGFBP-3 content changes are associated with the cancer cell growth process mediated by SIRT-1. In follow-up study, basic research can be conducted to further confirm the regulating effect of MIF and IGFBP-3 on SIRT-1-related molecule expression in lung cancer cells as well as the effect of SIRT-1 molecules on MIF and IGFBP-3 expression.

References