Correlation of serum GDF-15 and MIF contents with the malignant behaviors of cancer cells in patients with NSCLC

Tian-Ming Zheng 1, Li-Juan Lin 2

1. Introduction

Lung cancer is the most common malignancy in China, and non-small cell lung cancer is the most common pathological type of lung cancer. At present, the early diagnosis rate of lung cancer is low, and the poor sensitivity and specificity of lung cancer-related markers in serum are the important factors that affect the early diagnosis of lung cancer[1-2]. Growth differentiation factor 15 (GDF-15) and macrophage migration inhibitory factor (MIF) are non-small cell lung cancer-related markers discovered in recent years, and both have been confirmed to be closely related to the proliferation, invasion and migration of a variety of malignant tumor cells[3-4]. Non-small cell lung cancer-related in vitro cell research has confirmed that GDF-15 and MIF can promote the in vitro proliferation and invasion of lung cancer cells, but it is not yet clear about the relationship of serum GDF-15 and MIF contents in patients with non-small cell lung cancer with the cancer cell proliferation, invasion and other malignant behaviors within tumor lesions. In the following studies, we specifically analyzed the correlation of serum GDF-15 and MIF contents with the malignant behaviors of cancer cells in patients with non-small cell lung cancer.

2. Clinical information and research methods

2.1 Clinical information of research subjects

Patients with non-small cell lung cancer who underwent surgical resection in Kashgar Prefecture First People’s Hospital in the Xinjiang Uygur Autonomous Region between January 2015 and November 2017 were selected as the lung cancer group for the research, and healthy volunteers who received physical examination in Kashgar Prefecture First People’s Hospital in the Xinjiang Uygur Autonomous Region during the same period were selected as the control group. Serum was collected from the lung cancer group before surgery and from the control group during physical examination respectively to determine the contents of GDF-15 and MIF; lung cancer tissue and adjacent tissue were collected from lung cancer group after surgery to determine the expression of tumor suppressor genes, proliferation genes and invasion genes. Results: Serum GDF-15 and MIF contents of lung cancer group were significantly higher than those of control group; TCF21, Bax, GRPC5A and PTEN mRNA expression in lung cancer tissue were significantly lower than those in the adjacent tissue whereas Bcl-2, AQP4, c-myc, CyclinD1, SIRT1, CatL, MMP9, N-cadherin and Vimentin mRNA expression were significantly higher than those in adjacent tissue; serum GDF-15 and MIF contents of patients with lung cancer were negatively correlated with TCF21, Bax, GRPC5A and PTEN mRNA expression, and positively correlated with Bcl-2, AQP4, c-myc, CyclinD1, SIRT1, CatL, MMP9, N-cadherin and Vimentin mRNA expression in lung cancer tissue. Conclusion: The abnormal increase of GDF-15 and MIF in patients with NSCLC is closely related to the abnormal proliferation and invasion of cancer cells.
November 2017 were selected as the lung cancer group for the research, all patients were diagnosed with non-small cell lung cancer by postoperative tissue pathology, and there were a total of 45 cases, including 29 men and 16 women who were 39-62 years old; healthy volunteers who received physical examination in Kashgar Prefecture First People’s Hospital in the Xinjiang Uygur Autonomous Region during the same period were selected as the control group for the research, they were without history of malignant tumor or autoimmune disease, and there were a total of 50 cases, including 28 men and 22 women who were 36-64 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 index detection
3-5 mL of cubital venous blood was collected from lung cancer group before surgery and 3-5 mL of cubital venous blood was collected from control group during the physical examination. The contents of GDF-15 and MIF were determined by enzyme-linked immunosorbent assay kit.

2.2.2 Gene expression detection
After surgery, lung cancer tissue and adjacent tissue were collected from lung cancer group, the kit was used to extract RNA for fluorescence quantitative PCR reaction, and the PCR curve was referred to calculate the TCF21, Bax, GRPC5A, PTEN, Bcl-2, AQP4, c-myc, CyclinD1, SIRT1, CatL, MMP9, N-cadherin and Vimentin mRNA expression.

2.3 Statistical methods
SPSS 20.0 software was used to input the data, the differences in measurement data between two groups were analyzed by t test, the correlation was analyzed by Pearson test and the differences in test results were statistically significant if P<0.05.

3. Results

3.1 Serum GDF-15 and MIF contents
Serum GDF-15 and MIF contents of lung cancer group were (1.42±0.18) ng/mL and (18.93±2.86) ng/mL respectively, and serum GDF-15 and MIF contents of control group were (0.75±0.09) ng/mL and (10.12±1.47) ng/mL respectively. After t test analysis, serum GDF-15 and MIF contents of lung cancer group were significantly higher than those of control group.

3.2 Tumor suppressor gene expression in tissue
Analysis of tumor suppressor genes TCF21, Bax, GRPC5A and PTEN expression in lung cancer tissue and adjacent tissue was as follows: TCF21, Bax, GRPC5A and PTEN mRNA expression in lung cancer tissue were significantly lower than those in the adjacent tissue. Analysis of the correlation of serum GDF-15 and MIF contents of patients with lung cancer with tumor suppressor genes TCF21, Bax, GRPC5A and PTEN expression in lung cancer tissue was as follows: serum GDF-15 and MIF contents were negatively correlated with TCF21, Bax, GRPC5A and PTEN mRNA expression in lung cancer tissue.

3.3 Proliferation gene expression in tissue
Analysis of proliferation genes Bcl-2, AQP4, c-myc and CyclinD1 expression in lung cancer tissue and adjacent tissue was as follows: Bcl-2, AQP4, c-myc and CyclinD1 mRNA expression in lung cancer tissue were significantly higher than those in adjacent tissue. Analysis of the correlation of serum GDF-15 and MIF contents of patients with lung cancer with proliferation genes Bcl-2, AQP4, c-myc and CyclinD1 expression in lung cancer tissue was as follows: serum GDF-15 and MIF contents were positively correlated with Bcl-2, AQP4, c-myc and CyclinD1 mRNA expression in lung cancer tissue.

3.4 Invasion gene expression in tissue
Analysis of invasion genes SIRT1, CatL, MMP9, N-cadherin and Vimentin expression in lung cancer tissue and adjacent tissue was as follows: SIRT1, CatL, MMP9, N-cadherin and Vimentin mRNA expression...
expression in lung cancer tissue were significantly higher than those in adjacent tissue. Analysis of the correlation of serum GDF-15 and MIF contents of patients with lung cancer with tumor suppressor genes TCF21, Bax, GRPC5A and PTEN expression in lung cancer tissue was as follows: serum GDF-15 and MIF contents were positively correlated with SIRT1, CatL, MMP9, N-cadherin and Vimentin mRNA expression in lung cancer tissue.

4. Discussion

Serum tumor marker is the effective clinical means for early screening for non-small cell lung cancer and assessing the illness, but the common lung cancer markers such as CEA and CYFRA21-1 are without ideal sensitivity or specificity, so the early diagnosis rate of non-small cell lung cancer is low[5]. GDF-15 and MIF are newly discovered tumor markers in recent years, and their biological effects of GDF-15 and MIF are diverse, and they are mainly involved in the regulation of cell proliferation, invasion and migration of cancer cells in tumor lesion[6-8]. The relevant experimental studies in vitro have confirmed that GDF-15 and MIF have promoted the proliferation, invasion and migration of lung cancer cells; relevant clinical studies have confirmed that the serum GDF-15 and MIF contents significantly increase in patients with NSCLC[9,10]. However, it is not clear about the value of GDF-15 and MIF in serum of patients with NSCLC for evaluating the malignant characteristics of cancer cells within the lesion. In the study, in order to define the relationship of GDF-15 and MIF contents in serum with the malignant behaviors of cancer cells within the lesion of patients with non-small cell lung cancer, GDF-15 and MIF contents in serum of patients with non-small cell lung cancer were analyzed in the study, and the results showed that serum GDF-15 and MIF contents of lung cancer group were significantly higher than those of control group. It means that the abnormal secretion of GDF-15 and MIF is closely related to the occurrence of non-small cell lung cancer, and it is speculated based on the biological function of the two kinds of molecules that the abnormally secreted GDF-15 and MIF can participate in the regulation of cancer cell proliferation and invasion in non-small cell lung cancer lesions.

The expression deletion of tumor suppressor genes, such as TCF21, Bax, GRPC5A and PTEN, is an important pathological feature in the occurrence and development of non-small cell lung cancer. TCF21 can increase the expression of KISS1 gene to mediate anticancer activity and induce apoptosis[11]; Bax is located in the mitochondrial membrane and can promote the cytochrome C in the mitochondria to enter the cytoplasm, and thereby initiate the apoptosis mediated by caspase[12]; GRPC5A can antagonize the activation of transcription factor NF-κ B, and thereby inhibit the cell proliferation mediated by NF-κ B and cause apoptosis; PTEN is a negative regulator of the proliferation signaling pathway PI3K/AKT, which mediates the dephosphorylation process to inhibit cell proliferation and induce apoptosis[13,14]. Analysis of the changes in the expression of above tumor suppressor genes in non-small cell lung cancer showed that TCF21, Bax, GRPC5A and PTEN mRNA expression in lung cancer tissue were significantly lower than those in adjacent tissue. This indicates that the expression deletion of tumor suppressor genes is closely related to the occurrence of NSCLC. Further analysis of the relationship between the change of GDF-15 and MIF contents in serum and the change of tumor suppressor gene expression in lesions showed that serum GDF-15 and MIF contents of lung cancer group were negatively correlated with TCF21, Bax, GRPC5A and PTEN mRNA expression in lung cancer tissue. This means that the increase of serum GDF-15 and MIF contents in patients with non-small cell lung cancer is closely related to the decrease of tumor suppressor gene expression, and the determination of serum GDF-15 and MIF contents can reflect the abnormal apoptosis of cancer cells in non-small cell lung cancer lesions.

The growth of lung cancer cells in tumors is not only related to the expression deletion of tumor suppressor genes, but also associated with the pro-proliferation effect mediated by multiple proliferation genes. Bcl-2 is a negative regulator of mitochondrial pathway apoptosis, which can form heterodimer with Bax in the cell membrane and block cytochrome C from entering into cytoplasm to impede the occurrence of apoptosis[15]; AQP4 and c-myc are upstream regulators of the cell cycle, and both can induce the expression of CyclinD1, and then regulate cell cycle progression through the biological effect of CyclinD1[16,17]; CyclinD1 is an important positive regulator in the cell cycle progression, which can form complex with CDK4 and CDK6 to promote the cell cycle to pass through checkpoints, then accelerate cell cycle and promote cell proliferation[18]. Analysis of the changes in the expression of these proliferation genes in non-small cell lung cancer lesion showed that Bcl-2, AQP4, c-myc and CyclinD1 mRNA expression in lung cancer tissue were significantly higher than those in adjacent tissue. This indicates that the increase in proliferation gene expression is closely related to the occurrence of NSCLC. Further analysis of the relationship of serum GDF-15 and MIF contents with proliferation gene expression in lesions showed that serum GDF-15 and MIF contents of lung cancer group were positively correlated with Bcl-2, AQP4, c-myc and CyclinD1 mRNA expression in lung cancer tissue. This means that the increase of serum GDF-15 and MIF contents in patients with non-small cell lung cancer is closely related to the increase of proliferation gene expression, and the determination of serum GDF-15 and MIF contents can reflect the abnormal proliferation of cancer cells in non-small cell lung cancer lesions.

The generation of non-small cell lung cancer is characterized by infiltration, and the infiltration of cancer cells is not only dependent on the abnormal proliferation of cells, but also depends on the abnormal invasion of cells. The invasion process of cancer cells

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<tr>
<th>Tissue origin</th>
<th>n</th>
<th>SIRT1</th>
<th>CatL</th>
<th>MMP9</th>
<th>N-cadherin</th>
<th>Vimentin</th>
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<tr>
<td>Lung cancer tissue</td>
<td>45</td>
<td>2.52±0.37</td>
<td>2.65±0.39</td>
<td>1.89±0.22</td>
<td>5.27±0.52</td>
<td>2.66±0.41</td>
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<td>Adjacent tissue</td>
<td>45</td>
<td>1.04±0.16</td>
<td>1.02±0.15</td>
<td>0.96±0.11</td>
<td>1.00±0.15</td>
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involves the degradation of extracellular matrix mediated by protease and the enhancement of cell movement performance mediated by epithelial mesenchymal transition. SIRT1 is the transcription factor involved in protease expression and epithelial mesenchymal transition regulation in cancer cells[19-20], which can on the one hand, increase the expression of protease CatL and MMP9, and then promote the degradation of extracellular matrix by the hydrolysis of protease on the collagen and elastin in extracellular matrix[21], and on the other hand, increase the expression of mesenchymal phenotype marker molecules N-cadherin and Vimentin to induce epithelial mesenchymal transition, reduce the intercellular adhesion and strengthen the cell movement performance so as to help the cell invasion[22]. Analysis of the changes in the expression of above invasion genes in non-small cell lung cancer lesions indicated that SIRT1, CatL, MMP9, N-cadherin and Vimentin mRNA expression in lung cancer tissue were significantly higher than those in adjacent tissue. This indicates that the increased expression of the invasion genes is closely related to the occurrence of non-small cell lung cancer. Further analysis of the relationship of serum GDF-15 and MIF contents with invasion gene expression in the lesions showed that serum GDF-15 and MIF contents of lung cancer were positively correlated with SIRT1, CatL, MMP9, N-cadherin and Vimentin mRNA expression in lung cancer tissue. This means that the increase of serum GDF-15 and MIF contents in patients with non-small cell lung cancer is closely related to the increase of invasion gene expression, and the determination of serum GDF-15 and MIF contents can reflect the abnormal invasion of cancer cells in non-small cell lung cancer lesions.

Above all, it can be concluded that serum GDF-15 and MIF contents abnormally increase in patients with non-small cell lung cancer and are closely related to the changes of tumor suppressor gene, proliferation gene and invasion gene expression in the lesions; the determination of serum GDF-15 and MIF contents can be used to evaluate the abnormal proliferation and invasion of cancer cells within non-small cell lung cancer lesions.

References