Value of dynamic contrast-enhanced MRI for evaluating the cell proliferation, angiogenesis and lymphangiogenesis in lung cancer

Shen Cao, Li Cai

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ABSTRACT

Objective: To explore the value of dynamic contrast-enhanced MRI for evaluating the cell proliferation, angiogenesis and lymphangiogenesis in lung cancer. Methods: A total of 519 patients with pulmonary nodules who underwent surgical treatment in the hospital between August 2016 and October 2017 were divided into NSCLC group (n=410) and benign lesion group (n=109) according to postoperative pathological results. The differences in the levels of preoperative dynamic contrast-enhanced MRI parameters of the lesions as well as the expression of proliferation genes, angiogenesis-related genes and lymphangiogenesis-related genes in the lesion tissue were compared between the two groups. Pearson test was used to evaluate the correlation between dynamic contrast-enhanced MRI parameter levels of the lesions and tumor cell malignancy in patients with NSCLC. Results: Dynamic contrast-enhanced MRI parameters $K_{trans}$ and $K_{ep}$ levels in lesions of NSCLC group were higher than those of benign lesion group; proliferation genes Axin and FHIT mRNA expression in the lesion tissue of NSCLC group were lower than those of benign lesion group whereas RACK1, HMGN5 and MIF mRNA expression were higher than those of benign lesion group; angiogenesis-related genes VEGF, COX-2, EGFR and MACC1 mRNA expression in the lesion tissue of NSCLC group were higher than those of benign lesion group; lymphangiogenesis-related genes Akt1, HIF-1α, Prox-1 and PTTG mRNA expression in the lesion tissue of NSCLC group were higher than those of benign lesion group. Correlation analysis showed that dynamic contrast-enhanced MRI parameters $K_{trans}$ and $K_{ep}$ levels of NSCLC group were directly correlated with cell proliferation, angiogenesis and lymphangiogenesis in the lesions. Conclusion: Dynamic contrast-enhanced MRI parameter levels of the lesions of patients with NSCLC change abnormally and are closely related to the tumor cell malignancy.

1. Introduction

Non-small cell lung cancer (NSCLC) is the most common clinical lung tumor disease, it is only manifested as the ground-glass opacity or nodule in early stage, it is difficult to identify tumor nature, and it is not conducive to the follow-up treatment. Early diagnosis of NSCLC is the focus of current clinical research, and dynamic contrast-enhanced MRI is considered to be a reliable method to identify the nature of lung tumor before surgery[1,2].

It injects contrast agent in the blood vessels for conducts continuous scanning, assesses the microvascular distribution and dynamic changes in blood supply in the lesions, and further gets quantitative parameters through the related software to evaluate mass properties[3-5]. At present, there is not much research about the value of dynamic contrast-enhanced MRI for early diagnosis of NSCLC, the differences in preoperative dynamic contrast-enhanced MRI parameter levels were compared between patients with NSCLC and those with benign pulmonary lesions in this study, and the correlation of MRI parameter levels with tumor cell proliferation, angiogenesis, lymphangiogenesis and other malignant behaviors was further determined to clarify the value of dynamic contrast-enhanced MRI for identifying the lung tumor nature and judging the tumor malignancy and provide a reference for future clinical practice.
2. Materials and methods

2.1. Case information

A total of 519 patients with pulmonary nodules who underwent surgical treatment in the hospital between August 2016 and October 2017 were divided into NSCLC group (n=410) and benign lesion group (n=109) according to postoperative pathological results. There were 190 males and 220 females in NSCLC group, and they were 32-69 years old; there were 50 males and 59 females in the benign lesion group, and they were 30-71 years old. There was no significant difference in the gender and age distribution between the two groups of patients with lung lesions, the follow-up data were comparable, and the study plan was approved by the hospital ethics committee.

Inclusion criteria: (1) the lesion tissue nature was determined by pathological examination; (2) patients received pulmonary surgery for the first time; (3) patients themselves or the authorized family members signed the informed consent form. Exclusion criteria: (1) combined with pulmonary inflammatory disease; (2) combined with systemic infectious diseases; (3) combined with severe coagulation dysfunction; (4) combined with primary malignant tumor diseases of other tissue viscera.

2.2. Dynamic contrast-enhanced MRI examination

Both groups of patients underwent dynamic contrast-enhanced MRI examination before operation, the trocarvia was indwelt via the right cubital vein puncture, Siemens nuclear magnetic resonance spectrometer was adopted for regular plain scan at first, 0.1 mmol/L contrast agent gadopentetate dimeglumine was injected at 2 mL/s when the third scan started, and then 20 mL saline was injected for sealing up. The obtained images were processed by Siemens syngo workstation, and the quantitative parameters Ktrans and Kep levels were calculated.

2.3. Detection of related gene expression in lesion tissue

Pulmonary lesion tissue was kept during operation, fluorescence quantitative PCR was adopted to determine the target gene expression in it, and the specific genes included proliferation genes Axin, RACK1, HMGN5, MIF and FHIT, angiogenesis -related genes VEGF, COX-2, EGFR and MACC1 as well as lymphangiogenesis -related genes Akt1, HIF-1α, Prox-1 and PTTG. The expression curves of the above genes were obtained from computer PCR software, and the mRNA expression levels of corresponding genes were calculated.

2.4. Statistical methods

Dynamic contrast-enhanced MRI parameters, proliferation genes, angiogenesis-related genes and lymphangiogenesis-related genes were input in SPSS 25.0 as measurement data, and the statistic P was calculated, and differences were statistically significant if \( P < 0.05 \).

3. Results

3.1. Dynamic contrast–enhanced MRI parameter levels

Comparison of dynamic contrast-enhanced MRI parameters Ctrans and Cep levels in lesions between two groups of patients was as follows: Ctrans level of NSCLC group was \((0.43±0.06) \) min\(^{-1}\), and they were both significantly higher than those of benign lesion group. Differences in dynamic contrast-enhanced MRI parameters Ctrans and Cep levels in lesions were statistically significant between two groups of patients (\( P < 0.05 \)), shown in Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Ktrans</th>
<th>Kep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign lesion</td>
<td>109</td>
<td>0.06±0.01</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>NSCLC group</td>
<td>410</td>
<td>0.22±0.04</td>
<td>0.43±0.06</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>8.192</td>
<td>11.263</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 1. Comparison of dynamic contrast-enhanced MRI parameter levels between two groups of patients (min\(^{-1}\)).

3.2. Proliferation gene expression

Comparison of proliferation genes Axin, RACK1, HMGN5, MIF and FHIT mRNA expression in lesion tissue between two groups of patients was as follows: Axin and FHIT mRNA expression in

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Axin</th>
<th>RACK1</th>
<th>HMGN5</th>
<th>MIF</th>
<th>FHIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign lesion</td>
<td>109</td>
<td>90.37±10.12</td>
<td>84.36±9.50</td>
<td>118.36±13.58</td>
<td>75.48±9.12</td>
<td>132.18±15.36</td>
</tr>
<tr>
<td>NSCLC group</td>
<td>410</td>
<td>70.11±7.58</td>
<td>117.52±13.49</td>
<td>150.51±18.32</td>
<td>92.17±10.49</td>
<td>90.46±10.17</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 2. Comparison of proliferation gene expression in lesion tissue between two groups of patients.
Comparison of angiogenesis-related gene expression in lesion tissue between two groups of patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>VEGF</th>
<th>COX-2</th>
<th>EGFR</th>
<th>MACC1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign lesion</td>
<td>109</td>
<td>103.28±14.32</td>
<td>90.37±9.21</td>
<td>114.38±13.25</td>
<td>75.11±8.52</td>
</tr>
<tr>
<td>NSCLC group</td>
<td>410</td>
<td>141.29±15.48</td>
<td>121.28±14.19</td>
<td>139.41±15.48</td>
<td>91.27±10.93</td>
</tr>
<tr>
<td>t</td>
<td>15.381</td>
<td>10.998</td>
<td>12.351</td>
<td>10.993</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></td>
</tr>
</tbody>
</table>

Comparison of lymphangiogenesis-related gene expression in lesion tissue between two groups of patients (x±s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Akt1</th>
<th>HIF-1α</th>
<th>Prox-1</th>
<th>PTTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign lesion</td>
<td>109</td>
<td>99.48±10.17</td>
<td>104.31±12.49</td>
<td>85.26±9.17</td>
<td>100.18±12.31</td>
</tr>
<tr>
<td>NSCLC group</td>
<td>410</td>
<td>129.47±14.21</td>
<td>153.22±17.19</td>
<td>109.45±13.38</td>
<td>140.46±15.29</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></td>
</tr>
</tbody>
</table>

Comparison of angiogenesis-related genes VEGF, COX-2, EGFR and MACC1 mRNA expression in lesion tissue between two groups of patients was as follows: VEGF, COX-2, EGFR and MACC1 mRNA expression in the lesion tissue of NSCLC group were significantly higher than those of benign lesion group. Differences in angiogenesis-related genes VEGF, COX-2, EGFR and MACC1 mRNA expression in lesion tissue were statistically significant between two groups of patients (P<0.05), shown in Table 3.

Comparison of lymphangiogenesis-related genes Akt1, HIF-1α, Prox-1 and PTTG mRNA expression in lesion tissue between two groups of patients was as follows: Akt1, HIF-1α, Prox-1 and PTTG mRNA expression in lesion tissue were statistically significant between two groups of patients (P<0.05), shown in Table 4.

3.4. Lymphangiogenesis-related gene expression

Comparison of lymphangiogenesis-related genes Akt1, HIF-1α, Prox-1 and PTTG mRNA expression in lesion tissue between two groups of patients was as follows: Akt1, HIF-1α, Prox-1 and PTTG mRNA expression in lesion tissue were statistically significant between two groups of patients (P<0.05), shown in Table 4.

3.5. Correlation analysis

Analysis of the correlation between dynamic contrast-enhanced MRI parameter levels and tumor cell malignancy in patients with NSCLC was as follows: dynamic contrast-enhanced MRI parameters Ktrans and Kep levels in lesion tissue of patients with NSCLC were negatively correlated with proliferation genes Axin and FHIT mRNA expression and positively correlated with RACK1, HMGN5 and MIF mRNA expression; they were positively correlated with angiogenesis-related genes VEGF, COX-2, EGFR and MACC1 mRNA expression; they were positively correlated with lymphangiogenesis-related genes Akt1, HIF-1α, Prox-1 and PTTG mRNA expression (P<0.05), shown in Table 5.

4. Discussion

The new blood vessels in malignant tumors are relatively immature, and their vascular permeability is greater than that of normal vessels, which leads to the abnormality of related parameters under dynamic contrast-enhanced MRI. Contrast agent flow is bidirectional in the vessel lumen and extravascular intercellular space, it spreads from within the lumen to extracellular space at first, and then enters the blood vessels from the outside of vessel lumen after a certain time, and dynamic contrast-enhanced MRI can reflect the process in real time and transform the specific blood flow signal into quantitative parameters[6]. Ktrans reflects the dose of the contrast agent that goes from the vessel lumen to the outside of the vessel within unit volume tissue in the unit time, and the Kep reflects the dose of the contrast agent that goes from the vessel lumen to the outside of the vessel within unit time[7]. In this study, the differences in above dynamic contrast-enhanced MRI parameter levels were compared between the two groups of patients, and the results showed that compared with those in benign lesion group, Ktrans and Kep levels in NSCLC group were higher, showing that the vascular permeability is higher in malignant tumor tissue. The inner link between dynamic contrast-enhanced MRI parameter levels and tumor malignancy will be elaborated from three aspects of proliferation genes, angiogenesis genes and lymphangiogenesis genes in the following text.

The proliferation-related genes are abnormally expressed in malignant tumor cells, and they directly act on the cell proliferation cycle, prompt it to obtain infinite proliferation activity and inhibit the process of physiological apoptosis. Axin has an independent nuclear localization signal and can travel through the cytoplasm and nucleus, and the β-catenin protein synthesis decreases when its expression increases, which inhibits the expression of Xtwn-lux promoter.
and inhibits the proliferation of lung cancer cells[8,9]. RACK1 can regulate BinEL protein degradation to inhibit the normal apoptosis of breast cancer cells, and it has been found in NSCLC cell research that RACK1 can be combined with MCM7 to promote its phosphorylation and promote cancer cell proliferation[10]. HMGN5 is related to the occurrence of neoplastic diseases such as glioma and endometrioma, and reducing HMGN5 with RNAi technology can effectively reduce the proliferation activity of malignant tumor cells[11]. MIF is a proto-oncogene, many current studies have confirmed its involvement in the occurrence of NSCLC, and the increase of its expression can lead to increased microvascular density in tumors[12]. FHIT is a newly discovered tumor suppressor gene in recent years, its expression is positively correlated with the apoptosis index of tumor cells, and it can promote tumor cell apoptosis and inhibit its proliferation[13]. In this study, the differences in above proliferation gene expression in lesion tissue were compared between the two groups, and the results showed that compared with those of benign lesion group, Axin and FHIT mRNA expression in the lesion tissue of NSCLC group were lower whereas RACK1, HMGN5 and MIF mRNA expression were higher, indicating that there are the lower expression of tumor suppressor genes and the high expression of oncogenes in NSCLC. Further correlation analysis showed that dynamic contrast-enhanced MRI parameters Ktrans and Kep levels in NSCLC group were positively correlated those of benign lesion group. The correlation analysis showed that Ktrans and Kep levels in NSCLC group were positively correlated with Axin and FHIT mRNA expression, confirming that Ktrans and Kep levels can objectively reflect the angiogenesis degree of NSCLC, and indirectly reflect the tumor malignancy.

Akt1, HIF-1 α, Prox-1 and PTGG are the genes closely related to the lymphangiogenesis of malignant tumors, Akt1 is an important subtype of Akt, and Akt signaling pathway activation can stimulate the original lymphatic area to grow new lymphatic capillary in the form of "budding"[19]; HIF-1 α is widely expressed in various malignant tumors of the human body, which has been found to be an important promoter of lymphangiogenesis in recent years, and can significantly increase the lymphatic density in patients with breast cancer and colorectal cancer[20]; Prox-1 plays an important role in the later stage of lymphangiogenesis, which can promote the extension of lymphatic vessels and increase the density of lymphatic vessels[21]; PTGG is an oncogene closely related to tumor cell proliferation and differentiation, it is also regarded as one of the most important stimulators of lymphangiogenesis, and it can promote tumor lymphangiogenesis and prompt the tumor cells to transfer to lymphatic area[22]. The results of this study showed that Akt1, HIF-1 α, Prox-1 and PTGG mRNA expression in the lesion tissue of NSCLC group were higher than those of benign lesion
group. Further correlation analysis showed that Ktrans and Kep levels in NSCLC group were positively correlated with the above lymphangiogenesis-related gene expression, confirming that the dynamic contrast-enhanced MRI parameter levels can objectively reflect lymphangiogenesis degree of NSCLC, and indirectly reflect the malignant degree of tumor.

Thus, it comes to the conclusion that the dynamic contrast-enhanced MRI parameters Ktrans and Kep levels of NSCLC are unusually high, they can objectively reflect the proliferation activity of tumor cells as well as the degree of angiogenesis and lymphangiogenesis, and they can be used as the important means to early identify lung cancer and evaluate the malignant degree, and are worthy of popularization and application in clinical practice in the future.

References


