Correlation study between helicobacter pylori infection and tumor malignancy in patients with gastric cancer

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Objective: To study the correlation between helicobacter pylori infection and tumor malignancy in patients with gastric cancer. Methods: A total of 119 patients with gastric cancer treated in our hospital between May 2013 and December 2015 were collected and divided into HP-positive group (n=89) and HP-negative group (n=30) according to the combination of helicobacter pylori infection or not. RIA method was used to detect the serum angiogenesis index levels; fluorescence quantitative PCR (RT-PCR) was used to detect the mRNA expression of autophagy genes and proliferation genes in tumor tissues. Results: Serum angiogenesis indexes bFGF, VEGF and MMP-9 levels of HP-positive group were significantly higher than those of HP-negative groups; autophagy genes Beclin1, BNIP3, pULK and PI3KC3 mRNA expression in tumor tissues of HP-positive group were significantly lower than those of HP-negative group, pro-apoptotic genes Bax and p53 mRNA expression were significantly lower than those of HP-negative group, and anti-apoptotic genes PIK3CD, CIP2A and I2PP2A mRNA expression were significantly higher than those of HP-negative group. Conclusion: Helicobacter pylori infection can promote angiogenesis and cell proliferation in lesions of patients with gastric cancer.

1. Introduction

Gastric cancer is the most common clinical malignant tumor of digestive tract disease, the new cases of gastric cancer in China account for about 35.0% of the current annual 1 million cases in the world, and its incidence is high[1,2]. Numerous studies have shown that helicobacter pylori (HP) infection is an important factor contributing to the pathological change of normal gastric tissue, and the HP infection rate is high in patients with chronic gastritis and gastric ulcer[3,4]. Some scholars believe that HP infection is one of the important causes of gastric cancer, and the study of LIU Kai[5] shows that the pathological features are different between patients with HP-negative and HP-positive gastric cancer, and the theory that HP infection induces and aggravates gastric cancer is developing. At present, there is no clear report about the correlation between HP infection and the malignant degree of gastric cancer. In the following study, the correlation between helicobacter pylori infection and tumor malignancy in patients with gastric cancer was analyzed.

2. Information and methods

2.1 General information

A total of 119 patients with gastric cancer treated in our hospital between May 2013 and December 2015 were included, and all patients received radical operation for gastric cancer and C-14 urea breath test, and were divided into HP-positive group (n=89) and HP-negative group (n=30) according to the combination of helicobacter pylori infection or not. HP-positive group included 49 male cases and 40 female cases, they were 37-80 years old, the
body weight was 47-82 kg and (62.18±9.57) kg in average, and the course of disease was 4-16 months and (8.23±0.97) months in average; HP-negative group included 17 male cases and 13 female cases, they were 35-76 years old, the body weight was 46-79 kg and (60.55±9.86) kg in average, and the course of disease was 3-18 months and (8.91±0.95) months in average. The two groups of patients were not statistically different in the distribution of gender, age and course of disease (P>0.05). All included patients signed the informed consent, and the research process was approved by the hospital ethics committee.

2.2 Inclusion and exclusion criteria

Inclusion criteria: (1) Diagnosed with gastric cancer by histopathology; (2) With primary gastric cancer; (3) ≤80 years old; (4) Never receiving anti-HP treatment before. Exclusion criteria: (1) With secondary gastric cancer; (2) With primary and malignant tumor diseases of other tissues and viscera; (3) With stomach surgery history 6 months prior to admission; (4) With severe heart, liver and kidney dysfunction.

2.3 Observation indexes

2.3.1 Serum angiogenesis indexes

2 mL of fasting peripheral venous blood was extracted from two groups of patients immediately after admission and centrifuged at 3 500 r/min for 10 min to collect supernatant, and RIA method was used to detect the levels of angiogenesis indexes, including basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP-9).

2.3.2 Gene mRNA expression in tissues

Intraoperative tumor specimens were collected from two groups of patients, added in RNA iso, homogenized and centrifuged at room temperature with high speed (12 000 r/min, 5 min) to get supernatant, and the chloroform (Sigma Company in the United States, the article number 372978) and isopropanol (Sigma Company in the United States, the article number 563935) were added in turn to centrifuge it for the second time and get RNA plaque. The plaque was washed with 75% ethanol (Sigma Company in the United States, the article number 56694), dried and dissolved with DEPC water (Sigma Company in the United States, the article number 472565) to take suitable amount of RNA solution. Reverse transcription kit (TAKARA Company in Japan, the article number DRR014A) operation steps were followed to get the complementary DNA strand (cDNA) of messenger RNA, and the fluorescence quantitative PCR kit (Roche Company in the United States, the article number RA118) instructions were further used for mRNA amplification of autophagy genes: Beclin1, BNIP3, pULK and PI3KC3 as well as proliferation genes: Bax, p53, PIK3CD, CIP2A and I2PP2A. The corresponding PCR amplification curves were obtained in a computer to calculate the gene mRNA expression.

2.4 Statistical methods

Data in the study was input in software SPSS 20.0, measurement data was in terms of Mean ± SD, comparison between groups was by t test and P<0.05 indicated statistical significance in differences.

3. Results

3.1 Serum angiogenesis indexes

Comparison of serum angiogenesis indexes bFGF (μg/L), VEGF (ng/L), MMP-9 (pg/mL) levels between two groups of patients was as follows: serum angiogenesis indexes bFGF, VEGF and MMP-9 levels of HP-positive group were significantly higher than those of HP-negative groups. Differences in serum angiogenesis indexes bFGF, VEGF and MMP-9 levels 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>bFGF</th>
<th>VEGF</th>
<th>MMP-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP-positive</td>
<td>89</td>
<td>32.84±4.92</td>
<td>923.27±101.25</td>
<td>325.38±40.27</td>
</tr>
<tr>
<td>HP-negative</td>
<td>30</td>
<td>20.76±2.95</td>
<td>434.29±59.33</td>
<td>215.75±28.64</td>
</tr>
</tbody>
</table>

Table 1.

Comparison of serum angiogenesis index levels between two groups of patients.

3.2 Autophagy gene mRNA expression

Comparison of autophagy genes Beclin1, BNIP3, pULK and PI3KC3 mRNA expression in tumor tissues between two groups of patients was as follows: autophagy genes Beclin1, BNIP3, pULK and PI3KC3 mRNA expression in tumor tissues of HP-positive group were significantly lower than those of HP-negative group. Differences in autophagy genes Beclin1, BNIP3, pULK and PI3KC3 mRNA expression in tumor tissues were statistically significant between two groups of patients (P<0.05), shown in Table 2.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Beclin1</th>
<th>BNIP3</th>
<th>pULK</th>
<th>PI3KC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP-positive</td>
<td>89</td>
<td>54.38±6.09</td>
<td>51.27±6.93</td>
<td>45.27±5.18</td>
<td>40.18±7.53</td>
</tr>
<tr>
<td>HP-negative</td>
<td>30</td>
<td>102.47±12.53</td>
<td>103.28±11.64</td>
<td>99.73±10.18</td>
<td>103.27±13.24</td>
</tr>
</tbody>
</table>

Table 2.

Comparison of autophagy gene mRNA expression in tumor tissues between two groups of patients.
3.3 Proliferation gene mRNA expression

Comparison of pro-apoptotic genes Bax and p53 as well as anti-apoptotic genes PIK3CD, CIP2A and I2PP2A mRNA expression in tumor tissues between two groups of patients was as follows: pro-apoptotic genes Bax and p53 mRNA expression in tumor tissues of HP-positive group were significantly lower than those of HP-negative group while anti-apoptotic genes PIK3CD, CIP2A and I2PP2A mRNA expression were significantly higher than those of HP-negative group. Differences in pro-apoptotic genes Bax and p53 as well as anti-apoptotic genes PIK3CD, CIP2A and I2PP2A mRNA expression in tumor tissues were statistically significant between two groups of patients (P<0.05), shown in Table 3.

Table 3. Comparison of proliferation gene mRNA expression in tumor tissues between two groups of patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Bax</th>
<th>p53</th>
<th>PIK3CD</th>
<th>CIP2A</th>
<th>I2PP2A</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP-positive</td>
<td>89</td>
<td>100.59±11.28</td>
<td>100.47±10.95</td>
<td>99.26±10.15</td>
<td>102.36±13.28</td>
<td>101.63±11.58</td>
</tr>
<tr>
<td>HP-negative</td>
<td>30</td>
<td>192.74±23.73</td>
<td>212.48±23.81</td>
<td>43.28±5.09</td>
<td>39.72±5.15</td>
<td>56.38±6.23</td>
</tr>
<tr>
<td>t value</td>
<td></td>
<td>11.29</td>
<td>12.183</td>
<td>9.283</td>
<td>14.382</td>
<td>9.283</td>
</tr>
<tr>
<td>P value</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>

4. Discussion

After entering into the digestive tract through the mouth, HP uses its flagellum to cross through the gastric mucus layer, reach the epithelial surface, and then be connected to the gastric epithelial cells through adhesion. HP itself can secrete many antioxidant enzymes (superoxide dismutase, catalase, etc.) to protect it from being killed by gastric neutrophils. HP can also hydrolyze urea to generate specific “ammonia clouds” protective coating and resist acid killing. Through the above mechanism, HP is eventually colonized in the stomach, specifically adheres in gastric lesion mucosa, and releases urease, phospholipids and vacuolating cytotoxin to further damage mucusal barrier and lead to inflammation cell infiltration, massive release of oxygen free radical and malignant transformation of mucosal cells[6,7]. In the study, the patients with gastric cancer were classified according to the combination of HP infection or not, it was found that 119 patients with gastric cancer included 89 HP-positive cases and 30 HP-negative cases, and the HP infection rates were basically identical with those of domestic population.

The angiogenesis and extracellular matrix degradation ability within tumor is the material basis of its proliferation and invasion, cancer cells secrete a lot of materials that promote angiogenesis and matrix degradation, some fall off and are released into the blood circulation, so the levels of above molecules in peripheral blood increase, and they become one of the reliable quantitative indexes to judge tumor malignancy[8]. Basic fibroblast growth factor (bFGF) is a kind of peptide that can promote mesoderm and neuroderm cell division, has been proved to have strong angiogenesis effect, and can promote tumor cell proliferation and migration in experiments in vitro[9]. Vascular endothelial growth factor (VEGF) is the currently known factor with the strongest pro-angiogenesis ability, it is highly expressed in lung cancer, liver cancer, colon cancer and other malignant tumor tissues, and its expression is positively correlated with tumor malignancy[10]. Matrix metalloproteinase (MMP-9) belongs to the matrix metalloproteinase family, it can enhance the ability of tumor cells to degrade extracellular matrix, break vascular basement membrane and enter into the microenvironment as well as prompt tumor cell invasion and metastasis, and MMP-9 can also promote the angiogenesis within tumors. In the study, the levels of above factors in gastric cancer patients with different HP infection were detected, and it was found that compared with HP-negative group, HP-positive group were with higher serum bFGF, VEGF and MMP-9 levels, showing that the angiogenesis in gastric cancer patients with HP infection is more exuberant, and the tumor cell invasion and metastasis are stronger.

Autophagy can both inhibit/promote the development of tumor, and the effect on different autophagy is not the same[11]. The autophagy activity is weakened in the late stage of tumor growth, so inhibiting autophagy activity can promote tumor cell proliferation. Beclin1, BNIP3, pULK and PI3KC3 are the most typical autophagy genes, and the study of IU Chang[12] shows that Beclin1 expression decreases in patients with gastric cancer, and Beclin1 gene expression in gastric cancer specimens is significantly lower than that in para-carcinoma tissues. The study of WU Nan[13] shows that BNIP3, pULK and PI3KC3 genes are lowly expressed in patients with non-small cell lung cancer, and the decreased autophagy activity is the important factor for lung cancer. In the study, the autophagy gene mRNA expression in gastric cancer tissue with different HP infection was analyzed, and it was found that compared with HP-negative group, HP-positive group were with lower autophagy genes Beclin1, PI3KC3 and BNIP3 are the most typical autophagy genes, and its expression is positively correlated with tumor malignancy[14]. Abnormal proliferation gene expression in tumor cells is the root of the malignant tumor progression and metastasis, proliferation gene mRNA expression in tumor tissue specimens of two groups of
patients was further tested in this study in order to make clear the effect of HP infection on tumor malignancy. Bax and p53 are the commonly reported pro-apoptotic genes, their expression is inhibited in malignant tumor cells, and the inhibition degree is consistent with the malignant degree of tumor\cite{14}. Proliferation genes PIK3CD, CIP2A and I2PP2A have anti-apoptotic effect, and it is found that tumor cell proliferation activity decreases after targeted silence of PIK3CD; The study of SHI Hai-rong\cite{15} shows that gastric cancer BGC-823 cell proliferation and invasion activity both decrease after targeting CIP2A; the study of ZHANG Rong\cite{16} shows that decreased I2PP2A expression can mediate the inhibition of gastric cancer cell proliferation. It was found in the study that compared with HP-negative group, HP-positive group were with lower pro-apoptotic genes PIK3CD, CIP2A and I2PP2A mRNA expression in tumor tissues, showing that the gastric cancer tumor cell proliferation activity is better after HP infection, and confirming that HP infection is a pro-proliferation factor.

To sum up, it is concluded as follows: the tumor malignancy is higher and the long-term prognosis is poor in gastric cancer patients with HP infection, and early HP infection treatment is expected to become the adjuvant means to treat gastric cancer.

References


