Original Article
Expression of NF-κB and PCNA in gastric mucosa-associated lymphoid tissue lymphoma and its clinical significance

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Abstract: Objective: To investigate the expression of nuclear transcription factor κB (NF-κB) and PCNA in gastric mucosa-associated lymphoid tissue (MALT) lymphoma, and analyze its relationship with clinicopathological characteristics. Methods: The NF-κB and PCNA protein expression in 40 cases of gastric MALT lymphoma tissues and paired adjacent tissues were determined by immunohistochemistry. The relationship between NF-κB and PCNA expression and clinicopathological characteristics were analyzed. Results: The positive rate of NF-κB and PCNA protein expression in gastric MALT lymphoma node were 82.5% (33/40) and 52.5% (21/40), significantly higher than 10.0% (4/40) and 47.5% (19/40) in reactive hyperplasia of normal lymph node (P<0.05). The level of NF-κB and PCNA protein expression was correlated with Ann-Arbor clinical stage and histological grade (P<0.05), but its expression was no correlated with patient’s Gender, age and Lymphnode metastasis (P>0.05). Conclusion: NF-κB and PCNA play an important role in the development of gastric MALT lymphoma. Expression of NF-κB and PCNA may be involved in the occurrence and development of gastric mucosa-associated lymphoid tissue lymphoma. They can be used as a prognostic indicator of patients with gastric MALT lymphoma.

Keywords: NF-κB, PCNA, gastric mucosa-associated lymphoid tissue (MALT) lymphoma, immunohistochemistry, significance

Introduction

Gastric mucosa-associated lymphoid tissue lymphoma (MALT) is the primary means in the stomach into the sky from the submucosal lymphoid tissue malignancies, accounting for 40% of extranodal lymphomas [1]. In 2000 the WHO new classification will be named mucosa associated lymphoid tissue type marginal zone B cell lymphoma [2]. MALT lymphoma is a subtype of non-Hodgkin’s lymphoma with it’s own specific pathology, histology and clinical features. It is distinct because it involves lymphoid proliferation in mucosa-associated lymphoid tissue (MALT) rather than lymph nodes. Nuclear transcription factor (NF-κB) is a kind of widely present in a variety of cell nuclei of eukaryotic transcription factors, studies have shown that it is involved in tumorigenesis, development and immunity, inflammation. However, PCNA, and cell proliferation antigen, its DNA replication regulation plays an important role. PCNA synthesis and expression associated with cell proliferation, is a reflection of the main indicators of biological cells. In this study, the expression of NF-κB and PCNA in gastric MALT lymphoma was detected by immunohistochemical method, as well as correlating it with clinicopathological parameters and their clinical significance.

Materials and methods

Clinical information

40 gastric mucosa-associated lymphoid tissue (MALT) lymphoma specimens and paired adjacent tissues were obtained from surgical resection. The patients underwent surgery at Gansu Province People’s Hospital between August...
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2012 and May 2015. The mean age of the gastric mucosa-associated lymphoid tissue lymphomas group was 52.7 years (aged 29 to 70 years) and there were 22 males and 18 females. According to the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 2008 [3]. All tissue specimens were over 10% neutral form alin-fixed, paraffin-embedded sections, sliced thick 4 um, HE and immunohistochemical staining.

Reagents

Mouse anti-human NF-κB and PCNA monoclonal antibody was purchased from Santa Cruz Biotechnology, Envision immunohistochemistry kit was purchased from Beijing Zhongshan Golden Bridge Biotechnology company, Envision two-step immuno-histochemical staining according to kit instructions, NF-κB antibody was 1:100 and PCNA antibody was 1:50, all the slices are using microwave repair antigen. In PBS instead of primary antibody as a negative control.

Immunohistochemistry

The 4 um paraffin dewaxing to water, 3% hydrogen peroxide 10 min block endogenous peroxidase, pancreatic repair 20 min, 10% goat serum closed at room temperature 20 min, NF-κB and PCNA antibody (1:100) in a humified chamber in 4°C refrigerator overnight, dropping two anti, anti next three incubated at room temperature in a humid chamber each 20 min, DAB color, hematoxylin, dehydrated, mounted.

Evaluation of staining

Counting the double-blind method. The results were evaluated by double blind method is determined that two senior pathologists. Cell counts in high-power field (×400) under, each randomly selected five horizons slice, each field counted 100 cells, each specimen count three slices. NF-κB and PCNA: the nucleus and (or) significant brown granules in the cytoplasm of positive cells. The percentage of positive cells: ≤5% and 0 points, 6%-25% 1 point, 26%-50% 2 points, ≥51% 3 points; no positive cell count of 0; staining intensity: uncolored count 0, weak staining (light yellow) 1 point, moderate staining (gold) 2 points, strong staining (brown) 3 points. Multiplied by two scores: 0 divided negative (-), divided 1-9 as positive, including 1-3 into weakly positive (+), 4-6 into moderately positive (++), divided 7-9 strong positive (+++).

Statistics

SPSS 21.0 statistical software was used for analysis, and count data used the χ² test with a significance level α=0.05, P<0.05 indicates significant difference.

Results

**NF-κB expression in gastric MALT lymphoma**

Observation of pathological changes in cell morphology and tumor cells under a microscope (high-power field ×400). NF-κB expression mainly located in the nucleus and cytoplasm, brownish yellow or brown. Immunohis-
Expression of NF-κB and PCNA in gastric MALT lymphoma

Table 1. NF-κB expression in gastric MALT lymphoma (n, %)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (n)</th>
<th>-</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>Negative (n, %)</th>
<th>Positive (n, %)</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal adjacent tissues</td>
<td>40</td>
<td>36</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>36 (90)</td>
<td>4 (10)</td>
<td>42.288</td>
<td>0.000</td>
</tr>
<tr>
<td>Gastric MALT lymphoma</td>
<td>40</td>
<td>7</td>
<td>5</td>
<td>10</td>
<td>21</td>
<td>7 (17.5)</td>
<td>33 (82.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Representative NF-κB staining (Envision ×400). A: Expression of PCNA in Normal adjacent tissues; B: Expression of PCNA in gastric MALT lymphoma.

Table 2. PCNA expression in gastric MALT lymphoma (n, %)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (n)</th>
<th>-</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>Negative (n, %)</th>
<th>Positive (n, %)</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal adjacent tissues</td>
<td>40</td>
<td>37</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>37 (92.5)</td>
<td>3 (7.5)</td>
<td>19.286</td>
<td>0.000</td>
</tr>
<tr>
<td>Gastric MALT lymphoma</td>
<td>40</td>
<td>19</td>
<td>7</td>
<td>6</td>
<td>8</td>
<td>19 (47.5)</td>
<td>21 (52.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

tochemical results showed that the positive rate of NF-κB in the 40 tumor tissues was 82.5% (33/40). It was significantly higher than in the corresponding normal tissues (10.0%, 4/40) and the difference was statistically significant (\( \chi^2=42.288, P<0.05 \)) (Figure 1; Table 1).

PCNA expression in gastric MALT lymphoma

Observation of pathological changes in cell morphology and tumor cells under a microscope (high-power field ×400). PCNA expression mainly located in the nucleus and cytoplasm, brownish yellow or brown. Immunohistochemical results showed that the positive rate of PCNA in the 40 tumor tissues was 52.5% (21/40), It was significantly higher than in the corresponding normal tissues (7.50%, 3/40) and the difference was statistically significant (\( \chi^2=19.286, P<0.05 \)) (Figure 2; Table 2).

Correlation of clinicopathological parameters with NF-κB and PCNA expression in gastric MALT lymphoma. NF-κB protein was closely related to expression of PCNA in gastric MALT lymphoma, \( P=0.002 \) (Table 3).

Discussion

Mucosa-associated lymphoma is the most common extranodal B-cell lymphoma, first proposed in 1983 by Issacson and Wright, its clinical behavior and histological unique, performance limitations, inert growth and “homing” features [4]. The gastric MALT lymphoma most common, seen in all ages, but is more common in older patients, and with the growth of the incidence of age are on the rise. There are many symptoms of H. pylori according prompt for having induced gastric MALT lymphoma Disease resistance. Gastric MALT lymphoma H pylori detection rate, a set of reports
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Table 3. Correlation of clinicopathological parameters with NF-κB and PCNA expression in gastric MALT lymphoma

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total</th>
<th>NF-κB Positive</th>
<th>NF-κB Negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>10 (45.5%)</td>
<td>12 (54.5%)</td>
<td>0.725</td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>9 (50.0%)</td>
<td>9 (50.0%)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;45</td>
<td>19</td>
<td>9 (47.4%)</td>
<td>10 (52.6%)</td>
<td>0.752</td>
</tr>
<tr>
<td>≥45</td>
<td>21</td>
<td>11 (52.4%)</td>
<td>10 (47.6%)</td>
<td></td>
</tr>
<tr>
<td>Histological Stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-II</td>
<td>18</td>
<td>9 (50.0%)</td>
<td>9 (50.0%)</td>
<td>0.004*</td>
</tr>
<tr>
<td>III-IV</td>
<td>22</td>
<td>2 (9.0%)</td>
<td>20 (90.9%)</td>
<td></td>
</tr>
<tr>
<td>Histological Grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>12</td>
<td>8 (66.7%)</td>
<td>4 (33.3%)</td>
<td>0.006*</td>
</tr>
<tr>
<td>High</td>
<td>28</td>
<td>6 (21.4%)</td>
<td>22 (78.6%)</td>
<td></td>
</tr>
<tr>
<td>Lymphnode metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21</td>
<td>2 (9.5%)</td>
<td>19 (90.5%)</td>
<td>0.001*</td>
</tr>
<tr>
<td>No</td>
<td>19</td>
<td>11 (57.9%)</td>
<td>8 (42.1%)</td>
<td></td>
</tr>
<tr>
<td>PCNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+)</td>
<td>21</td>
<td>21 (100%)</td>
<td>0 (0.00%)</td>
<td>0.002*</td>
</tr>
<tr>
<td>(-)</td>
<td>19</td>
<td>12 (63.2%)</td>
<td>7 (36.8%)</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05.

gastric MALT lymphoma H pylori detection rate of over 90% [5, 6], far higher than other diseases [7, 8]. MALT accumulates within gastric mucosa as a result of long-standing H pylori infection in a subset of infected patients, and from this acquired MALT, low-grade B cell MALT lymphoma may eventually develop [8-10]. H pylori can be demonstrated in the gastric mucosa of the majority of cases of gastric MALT lymphoma [11-13]. Additionally, eradication of H pylori was reported to result in the complete regression of the majority of these tumors [14-16]. However, the exact mechanism responsible for the development of MALT lymphoma still remains obscure.

Tumorigenesis and metastasis are common oncogenes and tumor suppressor genes involved in regulating a complex process, the occurrence of metastasis-related genes overexpressed by the tumor, as well as participate in a series of gene products of the whole process of tumorigenesis and metastasis to regulate. NF-κB is widespread in eukaryotic transcription regulatory factor, for the first time in 1986 by Sen and Baltimor it found a capable κ light chain immunoglobulin gene enhancer κB binding sequence-specific nuclear protein factor. Rel family known as NF-κB family, including five family: RelA (P65), RelB, NF-κB1 (P50), NF-κB2 (P52), Rel (c-REL), of which P50/P65 composed of two mers most common. Studies have shown that, NF-κB to P50/P65 dimer binding when active, MEKKI and NF-κB inducible enzyme (NIK) by regulation, stimulating factor in the activation of NF-κB from the cytoplasm to the nucleus so that [17], and then combined with the NF-κB binding sites on the target gene sequence to promote transcription of target genes, and thus participate in the regulation of apoptosis in a variety of genes and transcription proliferation related genes in tumor occurrence and development plays an important effect. In the unstimulated state cells, NF-κB inhibitor IKB and its binding to a cytosolic form not activated when the cells are damaged and other stimuli, IKB degradation activity of NF-κB is released and transferred to a cell nucleus, regulating transcription activity of the target gene expression NF-κB protein so that the nucleus can be considered a sign of NF-κB activation [18-20]. Activation of NF-κB transcription can promote apoptosis genes, leading to tumor cell proliferation. Most studies suggest that, NF-κB may regulate apoptosis through the following ways: (1) by regulating the cell cycle S, indirectly affect cellular response to apoptotic signals; (2) by a number of protein interactions and apoptosis; (3) apoptotic genes directly regulated by regulating the cell cycle S, indirectly affect cellular response to apoptotic signals [21].

In recent years, there is a lot of literature, NF-κB is involved in cell proliferation of a variety of malignant tumors such as leukemia, stomach cancer, prostate cancer, ovarian cancer, breast cancer, and anti-apoptosis, angiogenesis and metastasis [22-24]. Wherein, NF-κB P65, CyclinD1 positive rate in papillary thyroid carcinoma was 58.8% and 89.9% respectively, which were significantly higher than those in adjacent normal thyroid tissue; As well reported in the literature, NF-κB positive rate in esophageal carcinoma 77.5%, and showed a positive rate had significant differences among different pathological type. In gastric cancer
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tissues, the positive expression of NF-κB was related to tumor size, lymph node invasion, invasion depth and peritoneal metastasis [25].

The expression and clinical significance of some scholars NF-κB in cells and tissues of breast cancer, found that NF-kappa B increased in cancer tissue and cell lines, and play an important role in monitoring breast cancer [26]. There are also reports about the gastric mucosa-associated lymphoma, for example, COX-2 and iNOS expressions were significantly higher in gastric MALT lymphoma tissues than those in adjacent normal tissues. The expression of COX-2 was observed in 22 of 32 cases of MALT lymphoma tissues (68.8%). A positive cytoplasmic immunoreactivity for iNOS was detected in 17 of 31 (53.1%) [27].

In this study, The positive rate of NF-κB and PCNA protein expression in gastric MALT lymphoma, reactive hyperplasia of gastric MALT lymphoma node tissues were 82.5% (33/40) and 52.5% (21/40), significantly higher than 10.0% (4/40) and 7.5% (3/40) in reactive hyperplasia of normal lymph node (P<0.05). The level of NF-κB and PCNA protein expression was correlated with Ann-Arbor clinical stage and histological grade (P<0.05), but its expression was no correlated with patient’s Gender, age and Lymphnode metastasis (P>0.05). NF-κB and PCNA positive expression rate of low grade malignant group was lower than the height of the two groups are significantly different, which is consistent with other numerous reports, the higher the degree of malignancy associated gastric lymphoma, value-added activity of their cells will the stronger, the more the tumor cells, thus indirectly show excessive proliferation of tumor cells is a cause gastric lymphoma have one of the mechanisms of low-grade to high-grade transformation [28].

In conclusion, NF-κB is expressed in the majority of gastric MALT lymphoma tissues, and correlates with cellular proliferation and PCNA expression, NF-κB and PCNA may play a synergistic role in the pathogenesis of gastric MALT lymphoma. NF-κB overexpression is closely associated with PCNA accumulation status. The molecular basis for the expression of NF-κB and PCNA and their roles in the evolution of H pylori-associated gastritis to gastric MALT lymphoma requires to be carefully investigated in follow-up studies. Is expected to become a new marker of prognosis judgement of lymphoma

The malignant degree of gastric mucosa associated lymphoma increased, which may be caused by Helicobacter pylori and other induc-
ting factors, such as NF-κB, resulting in the imbalance between cell proliferation and apoptosis, which makes the malignant tumor cells rapidly increased, therefore, to inhibit the activity of NF-κB by gene method, and to open up a new way for clinical treatment of gastric mucosa associated lymphoma.

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Disclosure of conflict of interest

None.

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sures 2008.
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