DETECTION OF APOPTOTIC CELLS AND IMMUNOHISTOCHEMICAL STUDY OF bcl-2 AND p53 GENE PROTEIN IN PRIMARY GASTRIC MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT) LYMPHOMA

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ABSTRACT

Objective: To identify the apoptotic cells in gastric MALT lymphoma and its relationship between bcl-2 and p53 gene expression. Methods: TdT-mediated dUTP biotin Nick End labeling (TUNEL) and immunohistochemistry ABC method were used to display apoptotic cells and the gene protein expression of bcl-2 and p53 independently. Results: Apoptotic indices (AI) in high-grade MALT lymphomas were significantly higher than in mixed-grade group and low-grade group ($P<0.05$). Bcl-2 was expressed in 83% of low-grade tumors, 61.6% of the median-grade tumors and 43.7% of high-grade tumors. An inverse correlation was observed between the expression of bcl-2 and apoptotic indices. Only 27 cases were p53 positive. The frequency of p53 positivity was significantly increased as the histologic grade advanced ($P<0.05$). There was also an inverse correlation between the expression of bcl-2 and p53. Conclusion: Apoptosis may be important in tumors development and transmission. p53 and bcl-2 were important regulatory genes of apoptosis and may be associated with transformation from low- grade to high-grade lymphomas.

Key wods: Apoptosis, Immunohistochemistry, Genes, p53, bcl-2, Mucosa-associated lymphoid tissue lymphoma

In recent years, the study of apoptosis has become one of the hot subjects for cancer research. Recent research revealed that apoptosis might be associated with tumor initiation and development, cancer therapy and prognosis. A lot of genes have been found to involve in the apoptotic process, especially the p53 and bcl-2 gene. The mucosa associated lymphoid tissue type lymphoma (MALToma) is a sort of extra-nodal low-grade malignant lymphoma, which is different from primary nodal lymphomas in pathogenesis, clinical features, microscopic findings and biologic behaviors. The purpose of this report is to study the apoptosis in gastric MALTomas and its relationship with bcl-2 and p53 gene protein expression.

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MATERIALS AND METHODS

Clinical Materials

Eighty-six patients with gastric MALT lymphoma were treated in our hospital from 1985 to 1999. From them, 65 surgical specimens and 21 biopsies were obtained. Of the patients, 45 are male and 41 female with an age ranging from 32 to 78 (mean=58). All the specimens were fixed with 10% formalin and embedded with paraffin, then a section 3um in thickness was made. All diagnosis and sub-classification were based upon a combination of routine morphology and immunohistochemistry by two experienced pathologist. There were 41 cases with low-grade MALToma, 13 with combined high grade and low grade MALToma (also called mixed-grade type), and 32 with high-grade MALT according to the classification recommended for gastrointestinal lymphomas by Issacson et al..[1]

Antibodies and Main Regents

Mouse anti-human monoclonal antibody bcl-2 and p53 (Do-7) were the products of Santz Cruz Company. Immunohistochemical detection kit and TdT-mediated *in situ* cell death detection kit were purchased from Mannheim Cooperation.

Immunohistochemical staining

All specimens were fixed with 10% formalin, embedded in paraffin and then sectioned. Immunohistochemical staining was performed on 3um sections using a standard avidin biotin complexes (ABC) peroxides technique. The staining process was performed according to the manufacturer's manual. The endogenous peroxidase activity was blocked with 0.8% H_2O_2 in methanol for 30 min. PBS was substituted for the primary antibody as the negative control.

Apoptosis Detection

For the in situ detection of apoptotic cells, terminal deoxynucleotidyl transaferase (TdT)-mediate digoxi-genin-dUTP nick end labeling (TUNEL) was used. The working procedures are performed according to the manufacturer's manual. A rat thymus was taken into the reaction as a positive control, and the TdT enzyme in the working-strength TdT was replaced by aquadest as a negative control.

The Judging Standard of the Results

For the evaluation of p53, bcl-2 and TUNEL, light microscopic evaluation was performed at a magnification of ×400 with the aid of a counting grid. In all evaluations, fields with the highest concentrations of positive cells were examined. Membrane and cytoplasmic staining in >10% of the tumor cells with bcl-2 antibodies was considered a positive result: The positive granule of p53 protein was mainly localized in nuclei of the tumor cells. TUNEL positive nuclei were counted in 10 microscopic fields to a total of 2000–4000 tumor cells. The apoptotic index (AI) was determined by the ratio of TUNEL positive neoplastic cells to the total number of neoplastic cells.

Statistical Analysis

Statistical analysis was performed with the use of SPSS statistical computer package, version 8.0. Correlation between the histologic type of MALToma and other factors, such as macroscopic tumor type, tumor depth, macroscopic tumor size, and expression of bcl-2, p53 and apoptotic cells, were evaluated with the chi-square test. A p value of less than 0.05 was considered significant.

RESULTS

Apoptosis in Gastric MALToma

We detected the apoptotic cells in 20 cases of gastric MALTomas, which were chosen at random. Apoptotic cells were detected in 18 cases and the AI varied from 0.1% to 1.5%. AI in high-grade MALTomas ($1.25\pm0.42\%$) was significantly higher than in mixed-grade group ($0.46\pm0.24\%$) and low-grade group ($0.26\pm0.14\%$).

Immunohistochemical Findings Bcl-2 Immunoreactivity

Of the 86 specimens examined, bcl-2 protein was expressed in 54 cases (62.8%). In 28% of the positive cases, the immunoreactivity was heterogeneous within the tumor area. Negtive or weakly stained cells frequently constituted the larger elements, such as centroblasts or centrocyte-like cells. Among all the 86 cases of MALTomas, bcl-2 was expressed in 34 of 41 low-grade tumors (83%); in 6 of 13 mixed-grade low-grade with a focal high-grade component) tumors (43.7%). Among these three groups, the frequency of positivity was significantly decreased as the histologic grade advanced ($P<0.05$). Significant difference in the bcl-2 positivity were also found between the two groups based on the macroscopic type and two groups based on the depth of invasion ($P<0.05$). (Table 1). However, bcl-2 expression didn't show a correlation with the size of the tumor, nor with the patient's age or sex.

P53 Immunoreactivity

The positivity of p53 protein was observed in 27 of 86 specimens examined (30%). It showed a diffuse and single pattern. Among the 86 cases of MALTomas, p53 was expressed in 3 of 41 low-grade tumors (7.3%), and 3 of mixed-grade tumors (23%), and 21 of 32 high-grade tumors (65%). The frequency of positivity was increased as the histologic grade advanced. The p53 positivity also correlated with the macroscopic type and the depth of invasion ($P<0.05$). No significant correlation was found between the p53 expression and size, age or sex (Table 1).

Relationship between bcl-2 and p53 expression

Of the 86 specimens, the co-expression of both

p53 and bcl-2 was observed in only 8 cases (9.3%). There was an inverse correlation existed between the expression of bcl-2 and p53 in all 86 specimens ($P<0.05$).

Table 1. The expression of Bcl-2 and p53 in primary gastric MALT lymphomas

	bcl-2-positive specimen (%)	P	p53-positive specimen (%)	P
All patients (n=86)	54 (63.8)		27 (30)	
Histological grade for MALT lymphoma				
Low (n=41)	34 (83)		3 (7.3)	
Mixed (n=13)	6 (61.6)		3 (23)	
High (n=32)	14 (43.7)	$P<0.05$	21 (65)	$P<0.05$
Macroscopic type				
SS type (n=45)	39 (72.2)		7 (15.5)	
Other type (n=41)	15 (27.8)	$P<0.05$	20 (84.5)	$P<0.05$
Depth of invision				
Not beyond SM (n=48)	40 (74.0)		10 (20.8)	
Beyond SM (n=38)	14 (26.0)	$P<0.05$	17 (79.2)	$P<0.05$

Abbreviations: SS= superficial spreading; SM= submucosa

P53 and bcl-2 Expression and AI

In Table 2, the correlation with p53, bcl-2 and AI is shown. Bcl-2 negative cases had significantly higher AI compared with bcl-2 positive cases ($P<0.05$). Due to the low number of p53 positive cases in 20 specimens involved in apoptosis detection, we could not establish a relation between p53 expression and AI.

Table 2. The relationship between bcl-2 expression and AI

	Case	Apoptotic indices (AI)	P
bcl-2 positive	11	0.32 ± 0.22 (%)	
bcl-2 negative	9	1.30 ± 0.48 (%)	$P<0.05$

DISCUSSION

Mucosa-associated lymphoid tissue (MALT) type lymphoma is the term first purposed by Issacson et al.,[2] and it belong to primary extra-nodal malignant lymphoma. The stomach is one of the most common sites of extra-nodal lymphomas, with primary lymphomas accounting for approximately 5% of malignancies at this site. Recent research revealed that gastric MALToma had a special pathologic morphology, biological behavior and pathogenesis. For example, most gastric MALTomas appear to arise in MALT acquired as reaction to Helicobacter pylori (Hp) infection; many MALTomas contain some lymphoid follicles and plasma cell differentiation; the neoplastic lymphocytes often infiltrate the epithelium of the stomach (also called "lympho-epithelial lesions"), and they generally have an excellent prognosis and tend to remain localized to the stomach for many years.

Failure to maintain an appropriate balance of cell number is a hallmark of neoplasia.[3] Apoptosis is the counterweight to proliferation, and disruption of apoptosis is a key event in tumorigenesis. Our research revealed a significantly lower AI in the gastric MALTomas than in the normal stomach mucosa. It suggested that the inhibition of apoptosis, accompanied with excessive cell proliferation, might result in unlimited cell accumulation, which may play an important role in tumor's development and malignant progression. We also found AI in high-grade MALTomas was significantly higher than in mixed-grade and low-grade group, which suggested that AI in gastric MALToma might be a useful indicator of tumor's grade and prognosis in gastric MALToma.

The bcl-2 proto-oncogene, which was cloned from the break-point region of t (14; 18) chromosomal translocation, encodes a 26-KD protein associated with prolonged cell survival because of the inhibition of apoptosis. The expression of bcl-2 protein has been detected in various nodal or extra-nodal lymphomas[4] and several epithelial tumors. [5][6] However, few articles have evaluated the expression of bcl-2 protein in primary gastric MALToma.[7,8] Our research revealed that bcl-2 positivity decreased significantly as the histologic grade advanced, and an inverse correlation was observed between the bcl-2 positivity and AI in gastric MALToma. These were in keeping with data indicating that bcl-2 can inhibit apoptosis.[4–8]

p53 gene, a tumor suppressor gene located on the short arm of chromosome 17, is known to mutate frequently in different kinds of tumors. In its mutant form, p53 fails to induce apoptosis. Although transcripts of the p53 gene are usually undetectable at the protein level, point mutation results in accumulation of mutant p53 protein. The Do-7 antibody we used in this study has been shown to be relatively reliable for the detection of p53 gene mutations by immunohistochemistry.[9] In the current study, the p53 positive neoplastic cells were found in 30% of the specimens, which was almost the same as that in the nodal lymphoma.[10] We also found that the p53 positive rate increased as the histologic grade advanced, and the p53 positivity correlated with the macroscopic type and the depth of invasion. These findings were in agreement with a previous study in which the accumulation of p53 mutation was associated with progression of MALToma.[11] We could not find a relationship between p53 and apoptosis in the current study because, due to the small number of cases, a proper analysis could not be performed.

p53 and bcl-2 gene are important regulatory gene of apoptosis, and both of them exist functional association. The wild-type p53 (wtp53) is a tumor suppressor and a death pathway gene, whereas bcl-2 is an antidote to apoptotic cell death. Wtp53 is a central player of DNA damage-induced apoptosis, and it transmits the apoptotic signal by transactivate target gene such as Bax and a series of p53-inducible gene.[12] Once mutated, p53 became a regulatory factor of bcl-2 and thus inhibit apoptosis. A series of studies revealed a controversial relationship between p53 and bcl-2. In a research on bladder cancer, Lu QL et al. found an obvious positive correlation between p53 and bcl-2,[13] but in gastrointestinal cancers, no correlation was found between both of them.[14] In the present study, we notice that the bcl-2 expression inversely correlation with the p53 expression in primary MALToma , as previously observed in malignant lymphoma[7,15] and breast carcinoma.[6, 16]

In conclusion, we found that AI in gastric MALToma increased significantly as the histologic grade advanced, also we observed an inverse correlation between the expression of bcl-2 and p53, and the positivity of both two proteins correlated significantly with the histologic grade of gastric MALToma. All these results suggest that the expression of bcl-2 and p53 may be associated with transformation from low-grade to high-grade MALToma, and apoptosis may be important in tumor's development and transmission.

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