

Original article

Apoptosis and gastric carcinogenesis: Immunohistochemical analysis of Bax and Bcl-2 proteins

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SUMMARY

Aim: Our aim was to evaluate the role of Bax and Bcl-2 apoptosis-related proteins in the carcinogenesis of the stomach. **Materials and Methods:** 80 biopsy specimens and 50 surgical specimens of chronic gastritis, atrophic gastritis, intestinal metaplasia, gastric dysplasia and gastric cancer were studied. Routine hematoxyline and eosin sections were evaluated and classified according to the latest diagnostic criteria. Immunohistochemical methods were used for detection of Bax and Bcl-2. **Results:** The expression of Bax in precancerous lesions and in gastric cancer was distributed as follows: chronic gastritis 100% (80/80 cases), atrophic gastritis 60% (39/65 cases), intestinal metaplasia 55% (28/50 cases), dysplasia 25% (10/40 cases), intestinal type adenocarcinoma 40% (12/30 cases), and diffuse type adenocarcinoma 15% (3/20 cases). Bcl-2 expression was as follows: chronic gastritis 0%, atrophic gastritis 35% (23/65 cases), intestinal metaplasia 90% (45/50 cases), dysplasia 100% (40/40 cases), intestinal type adenocarcinoma 83.3% (25/30 cases), and diffuse type adenocarcinoma 70% (14/20 cases). **Conclusions:** Our findings demonstrate the high expression of Bcl-2 in precancerous lesions (intestinal metaplasia, dysplasia) and in gastric cancer; at the same time the suppression of Bax in these diseases is evident. This highlights the major role of Bcl-2 and the contradicting role of Bax in early carcinogenesis.

Key Words: apoptosis, gastric carcinogenesis, Bax, Bcl-2

INTRODUCTION

The development of gastric carcinoma involves a multistep process from chronic gastritis to atrophic gastritis, intestinal metaplasia and gastric dysplasia. It has been shown that atrophic gastritis with intestinal metaplasia and dysplasia significantly increased the risk of gastric carcinoma. Cell proliferation and apoptosis are essential events in the cellular turnover of gastric tissues.¹ The excessive cell proliferation and/or decreased cell apoptosis seem to be the biological basis of gastric carcinogenesis. Apoptosis is a physiological suicide mechanism and was first described by Kerr et al.² It is the programmed death of cells by fragmentation of DNA, cell shrinkage, and dilation of endoplasmic reticulum, followed by cell fragmentation and formation of membrane vesicles called apoptosis bodies.²⁻⁴ Abnormal apoptosis contributes to the onset, development, and progression of cancer.⁵

The Bcl-2 family proteins are important and critical regulators of apoptosis. The Bcl-2 family consists of 15 mammalian family members, which are divided into three subfamilies: i) Bcl-2 subfamily (pro-survival): Bcl-2, Bcl-XL, Bcl-w, Mcl-1 and A1, ii) Bax subfamily (pro-apoptotic): Bax, Bak, Bok, and iii) BH3 subfamily (pro-apoptotic): Bad, Bid, Bik, Blk, Hrk, BNIP3 and BimL. The bcl-2 gene codes for a 25KDa protein. The C terminal 21 aminoacids encode a stretch of hydrophobic aminoacids that are important in membrane docking: Bcl-2 resides on the cytoplasmic face of the mitochondrial outer membrane, the nuclear envelope, and the endoplasmic reticulum. Most Bcl-2 homologs have this hydrophobia C terminal domain, though they are not necessarily located on membranes, but are cytosolic. When homologs of Bcl-2 have been identified, it becomes apparent that the Bcl-2 family can be defined by the presence of conserved motifs, known as Bcl-2 homology domains (BH1 to BH4).⁶ Bcl-2 prevents cells from death through a variety of mech-

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anisms, whereas overexpression of Bax protein increases the susceptibility of cells to apoptosis. Although they have different functions, they share similar structures. Bax as a homolog protein of Bcl-2 possesses two conserved regions, BH1 and BH2 that appear to be important for Bax/Bcl-2 binding. Bax and Bax-like proteins are associated with mitochondria-related apoptosis. While cell survival – promoting molecules Bcl-2 and Bcl-x, localised at the outer mitochondrial membrane, prevent translocation of cytochrome c from the mitochondria, induced expression or enforced dimerisation of Bax results in mitochondrial dysfunction leading to cytochrome c release.⁷ Bax and Bcl-2 may form homodimers (Bax/Bax, or Bcl-2/Bcl-2) or heterodimers (Bax/Bcl-2).⁶ The ratio of Bcl-2 to Bax proteins appeared to control the relative sensitivity of resistance of many types of cells to apoptotic stimuli.⁸ The Bcl-2 protein has been shown to prevent apoptosis and may play an important role in the regulation of gastric cancer growth. Also, it was discovered as a protooncogene, and is found at the breakpoints of t(14:18) chromosomal translocations in low grade B-cell lymphoma.⁹

In this study, we examined the immunohistochemical expression of a proapoptotic protein (Bax) and an antiapoptotic protein (Bcl-2) in precancerous lesions and in gastric cancer for determining their role in gastric carcinogenesis.

MATERIALS AND METHODS

Eighty endoscopic biopsy specimens and fifty surgical specimens (from our archives, Department of Pathology, Areteion University Hospital) of chronic gastritis, atrophic gastritis, intestinal metaplasia, gastric dysplasia, and gastric carcinoma were examined. The biopsy specimens were obtained from patients who underwent upper gastrointestinal endoscopy due to dyspeptic symptoms in our institution during 2005 and 2006. The surgical specimens were obtained from patients who underwent preoperative upper gastrointestinal endoscopy and were diagnosed as gastric cancer. All specimens were fixed in formalin and embedded in paraffin wax by using routine methods. Among 130 patients, 75 were male and 55 were female with median age 57.5 years. 5 samples for biopsy were taken from each patient: 2 were taken from gastric antrum, 2 from corpus, and 1 from incisura. The patients with preoperative diagnosis of gastric cancer underwent total gastrectomy.

Routine H&E sections of chronic active gastritis, chronic atrophic gastritis, and intestinal metaplasia were diagnosed by using the Sydney classification.¹⁰ Dysplasia

was diagnosed according to the Padova International classification system.¹¹ Gastric carcinoma was diagnosed according to the WHO classification of 2000.¹² For the immunohistochemical analysis, we used the EnVision System and the monoclonal antibodies for Bax (Dako Denmark 1:500) and Bcl-2 (Dako Denmark 1:40). Briefly, 5- μ m histological sections were dewaxed in xylene, rehydrated using a graded alcohol series, immersed in 10 μ mol and 0.5 mol/L ethylenediamine tetraacetic acid (EDTA) (pH 9.0), and microwaved. Subsequently, the sections were incubated with 0.3% H₂O₂ for 30 min to block endogenous peroxidase activity. Detection was made by using the EnVision System kit (Dako). Moreover, in certain specimens Giemsa histochemical stains were performed for the detection of *Helicobacter pylori*. The expression of Bax and Bcl-2 in epithelial cells was cytoplasmic and was graded according to the classification of Koshida et al as follows: 0 negative, 1 (very weak) <5% of cells stained, 2 (weak) <20% of cells stained, 3 (moderate) 20-50% of cells stained, 4 (intense) >50% of cells stained.¹³ Lymphocytes and small vessels were used as positive controls for Bcl-2 and Bax immunoreactivity.

RESULTS

The immunohistochemical findings in gastric lesions were distributed as follows: [Table]

Chronic gastritis

Bax was expressed in all cases (80/80 cases, 100%) in epithelial cells. The cytoplasmic stain was intense in 80% and moderate in 20% of the cases. Bcl-2 was not expressed. *H. pylori* (HP) was detected in 67/80 cases (83.7%) and all cases were Bax positive (100%).

Athrophic gastritis

Bax was expressed in 39 out of 65 cases (60%). The stain was weak in 50% of cases, moderate in 42%, and intense in 8 % of all cases. Bcl-2 was expressed in 23 out of 65 cases (35%) with very weak stain in 86% and weak

Table Bax and Bcl-2 expression

	Bax (% of cases)	Bcl-2 (% of cases)
Chronic gastritis	100%	0%
Atrophic gastritis	60%	35%
Intestinal metaplasia	55%	90%
Dysplasia	25%	100%
Intestinal type adenocarcinoma	40%	83.3%
Diffuse type adenocarcinoma	15%	70%

in 14% of cases. HP was detected in 41/65 cases (63%) of atrophic gastritis. Bax was positive in 28/41 cases with HP (68.3%), while Bcl-2 was positive in 14/41 cases with HP (34.15%).

Intestinal metaplasia

Intestinal metaplasia was observed in 50 out of 80 cases of precancerous lesions, which are classified as follows: 14 cases were diagnosed as incomplete intestinal metaplasia type IIB and 8 were type IIA. The other 28 cases were diagnosed as complete intestinal metaplasia. Also, type IIB intestinal metaplasia was observed in 25 out of 50 cases of gastric cancer. Bax was expressed in 55% (28/50) of intestinal metaplasia cases, equally expressed in type IIA and IIB. (Fig 1). The staining was very weak in 55% and weak in 45%. Bcl-2 was expressed in 90% of the cases, with greater expression in type IIB intestinal metaplasia. (Fig. 2) The staining was very weak in 55%, weak in 33%, and moderate in 12% of the cases. HP (+) cases were 28 (56% with no significant difference in three types). Bax positivity was observed in 19/28 cases with HP (67.8%) and Bcl-2 in 23/28 cases with HP (82.1%).

Dysplasia

High grade dysplasia was observed in 15 cases of biopsy specimens and low grade dysplasia was observed in 25 cases. Bax was expressed in 10 low grade dysplasia cases out of a total of 40 cases (25%), and Bcl-2 was expressed in 100% of all cases. (Fig. 3) Bax and Bcl-2 expression was very weak in 25%, weak in 45%, and moderate in 30% of the cases. HP (+) cases were 17 (42.5%). Bax was expressed in 6/17 cases with HP (35.3%) and Bcl-2 in 17/17 cases with HP (100%).

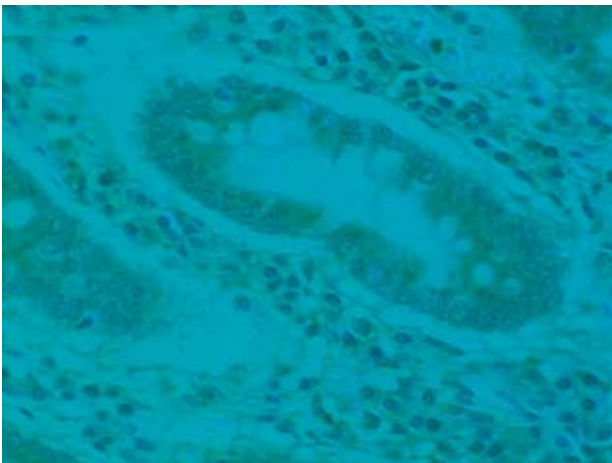


Fig. 1. Bax expression in intestinal metaplasia (x250).

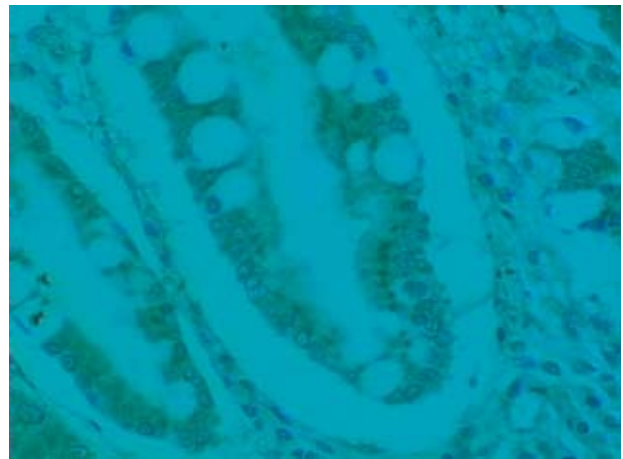


Fig. 2. Bcl-2 expression in intestinal metaplasia (x250).

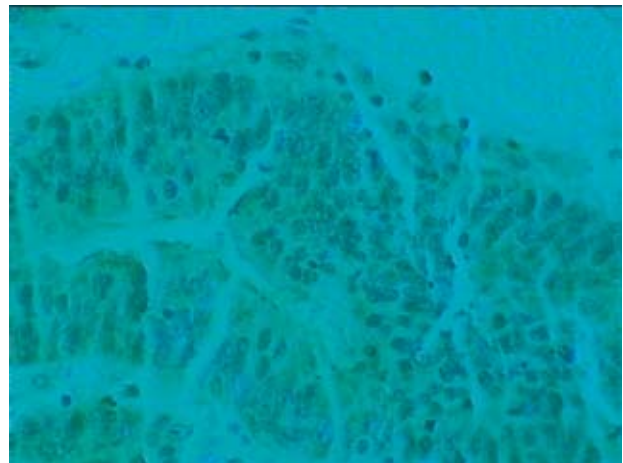


Fig. 3. Bcl-2 expression in high grade dysplasia (x250).

Gastric carcinoma

Bax was expressed in 12 out of 30 cases (40%) of intestinal type adenocarcinoma and in 3 out of 20 cases (15%) of diffuse type adenocarcinoma. The staining was very weak in 10 cases and weak in 2 cases of intestinal type adenocarcinoma and very weak in 3 cases of diffuse type adenocarcinoma. Bcl-2 was expressed in 25 out of 30 cases (83.3%) of intestinal type adenocarcinoma (Fig. 4) and 14 out of 20 cases (70%) of diffuse type adenocarcinoma. The staining was very weak in 2 cases, weak in 5 cases, moderate in 7 cases, and intense in 11 cases of intestinal type adenocarcinoma. The staining was moderate in 5, and intense in 9 cases of diffuse type adenocarcinoma.

DISCUSSION

The balance between cell proliferation and cell loss



Fig. 4. Bcl-2 expression in intestinal type gastric adenocarcinoma (x250).

regulates tumor growth, and therefore apoptosis, which regulates cell death and influences cell proliferation, may play an important role in tumor development. Ishida et al¹⁴ reported the presence of apoptosis in gastric cancer tissue by using terminal deoxynucleotidyl transferase-mediated d UTP-biotin nick end labelling. They pointed out that apoptosis played a decisive function in pre-cancer changes and participated in the development of cancer, including epithelial hyperplasia which occurs in the gastric mucosa. The apoptosis action in sick gastric mucosa cells decreased. Cell life was prolonged, and cells were piled up. This may be the reason why gastric cancer develops, infiltrates and transfers.¹³ Also, Mijic et al¹⁵ reported that numeric densities of apoptosis cell are associated with tumor progression in human gastric carcinogenesis. The apoptosis index decreased from mild non-proliferation to severe non-proliferation, early gastric cancer, and progressive gastric cancer.¹⁶ This indicates that during the development of gastric cancer, apoptosis is inhibited.

The mechanism of apoptosis modulation of gastric-intestinal epithelia is very complicated. Many genes and factors are involved. Various proteins or oncogenes and suppressor genes are involved in the process of apoptosis, including Bcl-2 and Bax. Bax protein expression has been identified in various human malignant tissues.¹⁷⁻²⁰ According to this study, Bcl-2 appears to not only inhibit apoptosis, but it appears to be an antagonist of apoptosis mediated by oncogenesis suppressor genes. When the expression of Bcl-2 increased, cancer cells would resist the apoptosis induced by chemical drugs or γ -radiation during therapy. For this reason overexpression of Bcl-2 has been reported for a variety of human epithelial malignant tumors, including gastric carcinoma.²¹⁻²³ Other studies, too,

indicate that Bcl-2 is upregulated in gastric premalignant lesions and downregulated after malignant change.²⁴⁻²⁵ It has been shown that Bcl-2 expression is confined to only a few regenerative epithelial cells of the mucous neck region. In dysplasia Bcl-2 expression increases and extends over the parabasal and superficial epithelium.²⁶ Thus, Bcl-2 is involved in the progression of premalignant lesions. On the other hand, Bax protein reduces gastric epithelial cell proliferation and apoptosis, thereby decreasing the subsequent risk of gastric carcinogenesis.

In our study Bax was expressed in all cases of chronic gastritis and showed gradually declining expression in premalignant lesions: atrophic gastritis (60%), intestinal metaplasia (55%), and dysplasia (25%). In contrast, according to our study, Bcl-2 was not expressed in chronic gastritis and was poorly expressed in atrophic gastritis (35%). But it had a remarkably high expression in precancerous lesions of intestinal metaplasia (90%) and dysplasia (100%). In both intestinal metaplasia and dysplasia, Bcl-2 expression increased and extended over the parabasal and superficial epithelium and this finding promotes the notion that Bcl-2 is involved in the progression of premalignant lesions.²⁶ In intestinal type adenocarcinoma, Bax was expressed in 40% and in diffuse type in 15% which indicates that Bax is less expressed in poorly-differentiated adenocarcinoma. This finding correlates the reduced expression of Bax with poorer prognosis. On the other hand, Bcl-2 was expressed in 83.3% in intestinal type adenocarcinoma and in 70% of diffuse type. These findings showed that apoptosis-regulating proteins are more commonly expressed in intestinal type adenocarcinoma than in diffuse type adenocarcinoma as suggested in other studies.^{27,28} Another aspect of our study was the *H. pylori* detection in precancerous lesions and its correlation with Bcl-2 family proteins. Chronic and persistent infection with *H. pylori* is considered to be one of the earliest steps in gastric carcinogenesis and it has recently been classified as a group I carcinogen by an International Agency for Research on Cancer Working Group. *H. pylori* infection not only increases gastric epithelial cell proliferation, but also induces apoptosis of gastric epithelial cells.²⁹ The relationship between *H. pylori* infection and Bax protein expression in the precursor lesions of gastric cancer has been well studied.³⁰

In our study, *H. pylori* was detected in 67/80 cases (83.7%) of chronic gastritis, in 41/65 cases (63.1%) of atrophic gastritis, in 28/50 cases (56%) of intestinal metaplasia, and in 17/40 cases (42.5%). This demonstrates the higher expression of HP in chronic gastritis in comparison to the other precancerous lesions. According to these

findings, we support the suggestion that modified gastric mucosa (atrophy, metaplasia, dysplasia) becomes inhospitable for HP. Bax protein was expressed in all HP (+) cases with chronic gastritis (100%), less frequently in atrophic gastritis (68.3%) and intestinal metaplasia (67.8%), and finally demonstrating a lower expression in dysplasia (35.3%). Bcl-2 was positive in 34.15% of H.P. (+) cases with atrophic gastritis and was highly expressed in H.P. (+) cases with intestinal metaplasia (82.15%), and dysplasia (100%). Thus, our results demonstrate that HP infection, which is known to increase apoptosis, increases the expression of Bcl-2, and anti-apoptotic gene, giving chances for gastric carcinogenesis.

In conclusion, this study showed that suppression of Bax and overexpression of Bcl-2 proteins are early events during gastric carcinogenesis. Thus, their immunohistochemical detection might be a useful prognostic indicator in gastric cancer.

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