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WJG, 1999 February; 5(1):15-17
World Journal of Gastroenterology
 ISSN 1007-9327

Expression and significance of proapoptotic gene *Bax* in gastric carcinoma *

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Subject headings stomach neoplasms/ pathology; Bax gene; gene expression; immunohistochemistry

Abstract

AIM **To study the expression of proapoptotic gene Bax in human gastric carcinoma and its significance.**

METHODS **Using immunohistochemistry methods, the Bax protein expression in 57 specimens of gastric carcinoma and its relationship with clinical status and pathomorphological parameters were observed.**

RESULTS **Thirty-three (57.9%) cases were positive for Bax pr otein staining which was mainly located in the cytoplasm of tumor cells. The rate of Bax protein expression was not correlated with the tumor size, lymph node metastasis, depth of invasion, clinical stages of tumors and age and sex of patie nts ($P<0.05$), but strongly associated with the morphological type and diff erentiation degree of tumors. It was significantly higher in intestinal type and well or moderately differentiated gastric carcinoma than in diffuse type and poorly differentiated gastric carcinoma ($P<0.05$ and $P<0.01$).**

CONCLUSION **The proapoptotic gene Bax is differently expressed in most of gastric carcinoma and may take part in the modulation of apoptosis i n gastric carcinoma. The expression of Bax might be associated with the occurrence of intestinal type gastric carcinoma and the differentiation of gastric carci noma.**

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***Key project of the 9th 5-year plan for Medicine and Health of Army, No.96Z047.**

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Received 1998-10-08

INTRODUCTION

Recent investigations have demonstrated that apoptosis plays a significant role in the pathogenesis of tumors[1,2]. Emphasis has been laid on the mechanisms that regulate apoptosis pathways. Bcl-2 associated X protein (Bax), which has extensive amino acid homology with Bcl-2, can form heterodimers with Bcl-2 *in vivo*. Overexpressed Bax can counter the death repressor activity of Bcl-2, and accelerate apoptotic cell death[3]. To determine whether proapoptotic gene Bax plays a role in the regulation of apoptosis in gastric carcinoma , an immunohistochemical study of Bax protein expression in gastric carcinoma an d its relation to clinical status, pathomorphological parameters were carried out.

MATERIALS AND METHODS

Histological specimens

Fifty-seven cases of surgically resected gastric carcinomas (male 39, female 16; mean age 58.6 years) were collected from the files of the Department of Pathology of our hospital. All blocks were fixed in 10% formalin and embedded in paraffin. Serial sections were cut from each block in 4μm, stained with hematoxylin and eosin and confirmed pathologically.

Immunohistochemical methods

Immunohistochemical staining for Bax protein was performed using SP technique wi th the following procedure: ① slides were deparaffinized in xylene for 10 minutes each and then were hydrated in decreasing concentrations of ethanol and rinsed in phosphate-buffered saline. Endogenous peroxidase was blocked by 30 mL/L H_2O_2 in methanol for 5 minutes, and then incubated for 1 0 minutes at room temperature in normal goat serum (1:20). ② Slides were incubated with a 1:50 dilution of the primary rabbit antihuman Bax polyclonal antibody (Santa Cruz, USA) for 30 minutes at 37℃. A biotin-streptavidin detection system was employed with diaminobenzidine as the chromogen. ③ Slides were washed twice wit hphosphate-buffered saline and incubated with the linking reagent (biotinylate d antiimmunoglobulin) for 10 minutes at 37℃. After rinsing in phosphate-buff ered saline, the slides were incubated with the peroxidase-conjugated streptavidin

label for 10 minutes at37℃,and incubated with diaminobenzidine and H_2O_2 for 10 minutes in the dark, the sections were then counterstained with hematoxylin. With each batch of test samples, a positive control consisting of a tissue section from tonsil was evaluated. In addition, a negative control was prepar ed for each sample using an irrelevant antibody of the same isotype as the prima ry antibody.

The immunostaining of Bax protein was visually classified into negative and positive groups by observing 1000 tumor cells in the areas of the sections: no staining present in any of tumor cells or less than 10% tumor cells with staining (-); more than 10% tumor cells with positive staining. The classification was done by two senior pathologists who did not know the clinicopathological data.

Statistics

Analysis of data was accomplished using Chi-square test. *P* values less than 0.05 were considered to be statistically significant.

RESULTS

Expression of Bax protein in gastric carcinoma

Thirty-three (57.89%) of the fifty-seven gastric carcinomas showed immunoreac tivity for Bax protein in gastric carcinoma cells. The Bax protein immunoreactivity appeared brown or dark brown, which was mainly located in the cytoplasm (Figure 1), and a few specimens simultaneously expressed Bax protein in the cell nuclear of tumor cells. Some of the mature lymphocytes infiltrating in the stroma of gastric carcinomas also had Bax protein expression.

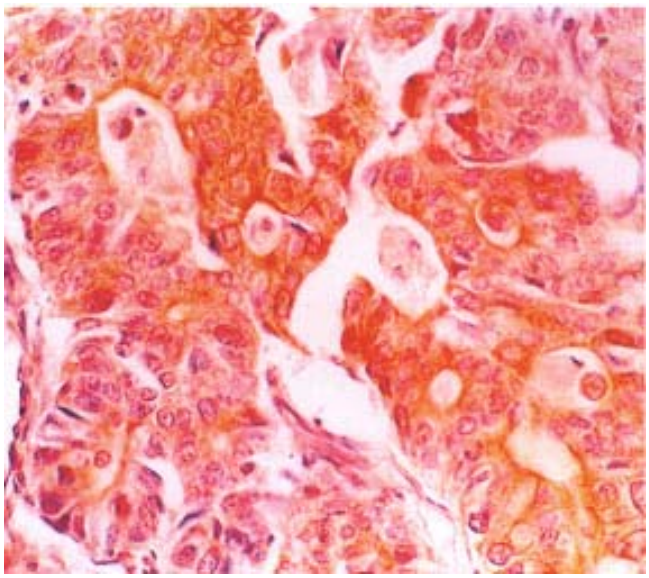

Figure 1 Bax immunoreactivity was detected in cytopl asm of gastric carcinoma cells. SP×200

Correlation between Bax protein expression and clinicopathological parameters of gastric carcinomas

Correlation between Bax protein expression and clinical pathological data of gastric carcinoma is illustrated in Table 1. The rate of Bax protein expression was not correlated with patient age, sex, tumor size, lymph node metastasis, depth of invasion and clinical stages ($P>0.05$). The immunoreactivity of Bax wa s significantly associated with morphologic phenotype and grades of differentiation of gastric carcinoma. 20 (73.3%) of 30 gastric carcinomas of intestinal morphologic phenotype were immunoreactive versus 11 (40.7%) of 27 diffuse gastric carcinomas ($P<0.05$). 17 (81.0%) of 21 well and moderately differentiated gastric carcinomas were immunoreactive versus 10 (38.5%) of 26 poorly differentiated gastric carcinomas($P<0.01$).

Table 1 Correlation between Bax protein expression and clinicopathological parameters of gastric carcinomas

Parameters	*n*	Bax protein expression -	Bax protein expression +	Positive rate (%)
Age (year)				
≤59	39	22	17	56.41
≥60	18	11	7	61.11
Sex				
M	39	24	15	61.54
F	18	9	9	50.00
Type				
Intestinal	30	22	8	73.33[a]
Diffuse	27	11	16	40.74
Grade of differentiation				
Well/moderate	21	17	4	80.95[b]
Poor	26	10	16	38.46
Mucoid	10	6	4	60.00
Tumor size				
<5cm	35	21	14	60.00
≥5cm	22	12	10	54.55
Lymph-node metastasis				
Negative	23	13	10	56.52
Positive	34	20	14	58.82
Serosal invasion				
Absent	27	14	13	51.85
Present	30	19	11	56.67
Clinical stages				
I and II	34	20	14	58.82
III and IV	23	13	10	56.52

[a]$P < 0.05$, $\chi = 6.193$, *vs* diffuse-type gastric carcinoma, [b]$P < 0.01$, $\chi = 8.580$, *vs* poorly differentiated gastric carcinoma.

DISCUSSION

Apoptosis is a highly regulated form of programmed cell death defined by distinct morphological and biochemical features. Apoptosis plays a major role in development, embryogenesis, regulation of the immune system, and carcinogenesis, as well as in the

maintenance of tissue homeostasis. Various protein molecules or oncogenes and suppressor genes are involved in the process of apoptosis, including *p53*, *myc*, *ras*, Bcl-2, Bax and the Fas/Fas ligand system[4]. In recent studies, Bax protein expression has been identified in various human malignant tissues, including the prostate, colon, breast, testis and ovary[5-8]. But, little is known about Bax protein expression and its relationship with the biological behavior of human gastric carcinoma.

In this study, we found that the positive rate of Bax protein staining in gastric carcinoma was 57.9%. The proapoptotic gene Bax can express to various degrees in most kinds of the gastric carcinoma and may take part in the regulation of apoptosis of gastric carcinoma. Our findings concerning the relationship between Bax protein expression and the pathological characteristics of gastric carcinoma showed that Bax expression was associated with morphologic phenotype and grades of differentiation of gastric carcinomas. The difference in the Bax protein expression in the intestinal and diffuse types demonstrated that aberrant Bax protein expression was preferentially associated with development of intestinal type gastric carcinoma, indicating once more the different biologic mechanisms involved in the development of these two histologic subtypes. The difference in the Bax protein expression between poorly differentiated and well/ moderately-differentiated gastric carcinomas demonstrated that aberrant Bax protein expression was associated with differentiation or growth speed of gastric carcinomas. There was no significant relationship between Bax protein expression and tumor size, lymph node metastasis, serosal invasion or clinical stages. Therefore, Bax prote in expression might play an important role in the early development and phenotypic differentiation of gastric carcinomas, but not in tumor progression.

REFERENCES

1 Que FG, Gores GJ. Cell death by apoptosis: basic concepts and disease relevance for the gastroenterologist. *Gastroenterology*,1996;110:1238-1243
2 Solary E, Dubrez L, Eymin B. The role of apoptosis in the pathogenesis and treatment of diseases. *Eur Respir J*, 1996;9:1293-1305
3 Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell*, 1993; 74:609-621
4 Hale AJ, Smith CA, Sutherland LC, Stoneman VEA, Longthorne VL, Culhane AC. Apoptosis: molecular regulation of cell death. *Eur J Biochem*,1996;236: 1-26
5 Krajewski S, Blomqvist C, Franssila K, Krajewska M, Wasenius VM, Niskanen E.Reduced expression of proapoptotic gene Bax is associated with poor response rates tocombination chemotherapy and adenocarcinoma.*Cancer Res*,1995;55:4471-4478
6 Krajewska M, Moss SF, Krajewski S, Song K, Holt PR, Reed JC. Elevated expression of Bcl-x and reduced Bak in primary colorectal adenocarcinomas. *Cancer Res*,1996;58:2422-2427
7 Chresta CM, Masters JRW, Hickman JA. Hypersensitivity of human testicular tumors to etoposide-induced apoptosis is associated with functional p53 and a high Bax: Bcl-2 ratio. *Cancer Res*, 1996;56:1834-1841
8 Krajewska M, Krajewski S, Epstein JI, Reed JC, Franssila K. Immunohistoche-mical analysis of Bcl-2, Bax, Bcl-x and mcl-1 expression in prostate cancers. *Am J Pathol*, 1996;148:1567-1576

Edited by MA Jing-Yun