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Function of apoptosis and expression of the proteins *Bcl*-2, *p*53 and C-*myc* in the development of gastric cancer

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INTRODUCTION

In China, the incidence and mortality of gastric cancer rank the second among all cancers. Recent studies indicate apoptosis could play a role in the development of cancer[1-20]. The aim of this study was investigat the insight of apoptosis and *bcl*-2, *p*53 and C-*myc* protein expression in the development of gastric cancer.

MATERIALS AND METHODS

Materials

All 122 specimens were collected by gastroscopy or surgical resection. Among these, 32 were chronic active gastritis, 29 were gastric ulcer, 17 were mild non-classic proliferation, 8 were severe non-classic proliferation, 6 were early gastric cancer, and 30 were progressive gastric cancer. The average age among those types of samples were 49.2, 46.3, 45.8, 50.3, 49.3 and 51.0, while the ratio of men to women was 20/12, 21/8, 11/6, 5/3, 5/1 and 21/9, respectively. There was no significant regularity among those samples after analyzed by statistics.

Reagents and methods

Apoptosis was detected using the TUNEL technique as reported by Ishida[1]. Cells in which the nuclear or cytoplasm was dyed brown were identified to be undergoing apoptosis. We observed five visual fields for each spechmen, and 100 nuclei were observed in each visual field. The average ratio of apoptosis cell was apoptosis index. Proteins *bcl*-2, *p*53, and C-*myc* were dyed by the ABC immunohistochemical method. Cells with obvious brown or deep brown after dying were defined to be positive.

RESULTS

The apoptosis index increased stepwise from chronic active gastritis to gastric ulcer and decreased from non-classic proliferation to early gastric cancer and progressive gastric cancer (Table 1). The expression of protein *bcl*-2 and C-*myc* increased progressively as follows: chronic active gastritis, gastric ulcer, mild non-classic proliferation, severe non-classic proliferation, and early gastric cancer. The expression of protein *bcl*-2 decreased when it developed into progressive gastric cancer while that of C-*myc* increased continually. Protein *p*53 was expressed only in severe non-classic proliferation gastric mucosa and gastric cancer.

The apoptosis index, C-*myc* and *p*53 expression of intestinal type were higher than that of diffuse type ($P<0.05$), while the *bcl*-2 expression was lower ($P<0.05$). The two types had the opposite outcomes (Table 2).

DISSCUSION

Apoptosis, programmed cell death, was first described by Kerr *et al*[21]. 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[21-24]. Recent investigations have demonstrated that apoptosis plays a significiant role in the pathogenesis of tumors[25-30]. Screnhst have began to have realize that apoptosis, in concert with cell proliferation, is an important mechanism towards healthy tissues. Abnormal apoptosis contirbutes to the onset, development, and progression of cancer[2,31]. Stomach carcinoma is estimated to be one of the most frequent cancers worldwide[32-34].

According to Lauren, stomach cancer can be divided into adenocarcinomas of diffuse and intestinal types[35]. Ishida *et al*[1] reported the presence of apoptosis in gastric cancer tissue by using terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling. They pointed out that apoptosis played a decisive function in pre-cancer changes and participated in the

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development of cancer, including epithelial hyperplasia which occurrs 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 developes, infiltrates and transfers. Kasagi *et al*[10] studied the apoptosis indexes of various levels of differentiation. There was some difference between tumor tissue of high and low differentiations (apoptosis indexes were 10.9% and 4.0%, respectively, $P<0.01$). The difference indicated gastric cancer which had a low differentiation was less likely to undergo apoptosis. Non-classic proliferation of gastric mucosa was considered to be a precancerous change. Mijic *et al* [36] reported that numeric densities of apoptosis cell are associated with tumor progression in human gastric carcinogenesis. We found that the apoptosis index decreased from mild non-proliferation to severe non-proliferation, early gastric cancer, and progressive gastric cancer. This indicated that during the development of gastric cancer, apoptosis was 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 gene are involved in the process of apoptosis, including *p*53, *bcl*-2, *myc*, *ras*, Bax and the Fas/Fas system[37-41]. Bax protein expression has been identified in various human malignant tissues[7,15,42,43]. Research has indicated that *bcl*-2 is an inhibitor of apoptosis. Li *et al*[44] reported that abnormal *c-myc* and *bcl*-2 expression is an important factor in biological behavior of gastric carcinoma and can regulate apoptosis. Sundblad *et al*[8] found that the expression of *bcl*-2 increased in cells of gastric cancer. *Bcl*-2 appears to not only inhibit apoptosis, but the protein 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. Our results indicates that when non-classic proliferation occurrs in gastric mucosa, the expression of *bcl*-2 increases significantly. Expression of *bcl*-2 reached the top at the early stage of gastric cancer and decreased in the progressive gastric cancer. *bcl*-2 might do some work both in the triggering of gastric cancer and developing of early gastric cancer. Although *bcl*-2 was a strong inhibitor to apoptosis, it could not induce the cancer alone. However, cancer has been associated with *bcl*-2 in combination with C-*myc*[45].

Gastric carcinogenesis is a gradually developed process which result from the sequential alteration of multigenes[1,46-48]. Gene *p*53 is an oncogenetic repressor. Its anti-cancer function has been realized by triggering apoptosis. If gene *p*53 is inhibited, apoptosis can not be induced. Ikeda *et al*[49] observed that the progression of gastric cancer is defined by a gradual increase of proliferation activity and constant occurrence of apoptosis. Furthermore, Ikeda reported that the naturally occurring apoptosis is induced predominantly via a *p*53-gene-independent pathway. The half life of the mutant protein *p*53 is prolonged when gene *p*53 is mutated. It is easy to detect by immunohistochemical methods. We observed that expression of *p*53 was mainly in progressive gastric cancer tissue. No *p*53 was observed in the tissue of benign stomach diseases and mild non-classic proliferation. This indicated that the mutation of *p*53 might be an event in the late gastric cancer. The cooperation of *bcl*-2 and C-*myc* could inhibit the biological function of *p*53 to suppress cell growth by keeping it in the cytoplasm. Meanwhile, the co-expression of *p*53 and C-*myc* would lead to cell apoptosis and inhibit oncogenesis[50,51]. The co-expression of *bcl*-2 and C-*myc* in the same carcinoma tissue indicates a higher level of malignancy and a lack of sensitivity to chemical therapy and radiotherapy. The prognosis is not favorable. On the other hand, the co-expression of *C-myc* and *p*53 indicates a low level of malignancy, less sensitivity to chemical therapy and radiotherapy, and a favorable prognosis. Therefore *p*53 status and the expression of *bcl*-2 by tumor cells might be good indicators of sensitivity to chemotherapy for patients with gastric cancer[52].

Table 1 Relationship between apoptosis, protein expression of *bcl*-2, *p*53,C-*myc* and each kind of gastric diseases

	Chronic active gastritis	Gastric ulcer	Non-classic proliferation		Gastric cancer	
			Mild	Severe	Early	Progressive
Sample numbers	32	29	17	8	6	30
Apoptosis index %	16.8±12.3	24.1±20.0[a]	19.3±16.4	15.7±15.2[c]	10.1±9.1[d]	6.3±6.0[e]
Bcl-2 positive	3	8	9	6	5	14
Numbers(%)	(9.4)	(27.6)[a]	(52.9)[b]	(75.0)[c]	(83.3)	(46.7)[e]
C-myc positive	5	6	6	4	3	20
Numbers(%)	(15.6)	(20.7)	(35.3)[b]	(50.0)	(50.0)	(63.3)[e]
P53 positive	0	0	0	2	2	19
Numbers(%)				(25.0)[c]	(33.3)	(63.3)[e]

*Compared with left itim: [a]$P<0.05$, gastric ulcer *vs* chronic active gastritis; [b]$P<0.05$, mild non-classic proliferation *vs* gastric ulcer; [c]$P<0.05$, severe non-classic proliferation *vs* mild non-classic proliferation; [d]$P<0.05$, early gastric cancer *vs* severe non-classic proliferation; [e]$P<0.05$, early gastric cancer *vs* progressive gastric cancer.

Table 2 Relationship between apoptosis, protein expression of *Bcl*-2, *P*53, *C-myc* and Lauren typing of gastric cancer

Lauren typing	Sample numbers	Apoptosis index %	Bcl-2 positive numbers(%)	C-myc positive numbers(%)	P53 positive numbers(%)
Intestinal	18	8.3±7.2	7(38.9)	13(77.7)	15(83.3)
Diffuse	12	5.1±4.9[a]	7(58.3)[a]	6(50.0)[a]	4(33.3)[b]

[a]$P<0.05$, Diffuse *vs* Intestinal; [b]$P<0.01$, Diffuse *vs* Intestinal.

Different pathological types of gastric cancer are associated with different physiological mechanisms. Intestinal gastric cancer had a higher level of differentiation and a closer relationship with gastric epithelial metaplasia as compared with diffuse type of gastric cancer. We observed intestinal type cancer more frequently among men and among older patients. Intestinal type cancer had a more favorable prognosis than diffuse type cancer. Also, the index of intestinal type of gastric cancer was higher than that of diffuse type ($P<0.05$), with lower expression of *bcl*-2 and higher expression of *C-myc* and *p*53. These results indicate that the pathogenic mechanism might be different between those two types of gastric cancer. Vollmers *et al*[53] had similar results, reporting that the gene expression modulation of apoptosis and the apoptosis indexes were different between type intestinal and diffuse gastric cancer. The apoptosis mechanism was different between those two types of gastric cancer.

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