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Nuclear Overexpression of bcl-2 Oncoprotein during the Progression of Human Stomach Cancer

We unexpectedly observed strong nuclear overexpression of bcl-2 protein in advanced stomach cancer. As far as we know, such expression has not yet been reported. To investigate the significance of nuclear expression of bcl-2 protein in gastric carcinoma, we immunohistochemically analyzed bcl-2 overexpression in a gastric carcinogenic sequence, including 19 tubular adenomas (TA), 20 early carcinomas (EGC), and 20 advanced carcinomas (AGC). While TA displayed a specific granular and supranuclear cytoplasmic staining pattern, adenocarcinomas showed a strong nuclear staining pattern. Nuclear staining of bcl-2 was observed in 50% of AGC, 30% of EGC, and 10% of TA; cytoplasmic staining, on the other hand, was observed in all TA, 5% of EGC, and 10% of AGC. Nuclear bcl-2 overexpression differed according to the histologic type of AGC, occurring in 67% of the diffuse type and 25% of the moderately-to-well differentiated type. In the diffuse type, nuclear bcl-2 positive AGC predominated. In metastatic lesions, the pattern of bcl-2 immunostaining was almost identical to that seen in primary tumor. These results suggest that nuclear expression of bcl-2 may be related to malignant transformation in the stomach and is frequently associated with diffuse type advanced gastric adenocarcinomas.

Key Words : *Stomach neoplasms; Gastric adenoma; Genes, bcl-2; Nuclear expression; Immunohistochemistry*

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INTRODUCTION

Bcl-2 is a mitochondrial oncogene which has been recently recognized to be involved in the regulation of cell death (1, 2). The role of this proto-oncogene protein has been associated with prolonged cell survival through the inhibition of apoptosis (1). Bcl-2 proto-oncogene was cloned from the break-point region of t (14;18) chromosomal translocation, which was frequently recognized in follicular lymphoma (3). The expression of bcl-2 protein has been detected in various nodal (4-6) and extranodal (7) lymphomas as well as in non-neoplastic lymphoid tissue (8), and its occurrence in epithelial tissue has been reported (9-11). In gastrointestinal (GI) mucosa, the protein was immunohistochemically detected in the proliferative zone, evidently to protect the renewal potential of the mucosa (8, 12). Previous reports on the expression of bcl-2 in neoplastic and non-neoplastic tissue sections have described cytoplasmic staining by immunohistochemistry (4-11). Nuclear expression of bcl-2 protein observed in G2 or early prophase cells during mitosis in human carcinoma cell lines has been detected by fluorescence immunohistochemistry using monoclonal antibodies (13).

Using a mouse monoclonal antibody, we unexpectedly observed immunohistochemical evidence of strong nuclear overexpression of bcl-2 protein in advanced stomach cancer. To investigate the significance of the nuclear expression of this protein in such cases, we immunohistochemically analyzed its overexpression in the gastric carcinogenic sequence and histologically characterized, namely, tubular adenoma with or without cellular atypia, early gastric carcinoma and advanced carcinoma.

MATERIALS AND METHODS

Tissue specimens

In this study, we selected cases in which the tumor was in different stages; they comprised 19 gastric tubular adenomas, 20 early gastric adenocarcinomas [10 mucosal (m) type EGCs and 10 submucosal (sm) type EGCs], and 20 advanced gastric adenocarcinomas (AGC) from among the pathology records at Korea Cancer Center Hospital. In order to compare primary and metastatic tumors, all

AGCs in this study were lymph node-positive cases. Except for ten endoscopic biopsies of tubular adenomas, all the others were surgically resected specimens; all had been routinely fixed in 10% formalin and embedded in paraffin wax. One or two representative paraffin blocks from both primary stomach lesions and lymph nodes were selected for immunohistochemical study.

Material (antibody)

Bcl-2, a mouse monoclonal antibody (cat # sc-509, Lot No. J175) was obtained from Santa Cruz Biotechnology, Inc. (California, USA). It was derived from the fusion of mouse myeloma cells with spleen cells from a mouse immunized with bcl-2 protein of human origin. The other immunochemicals were from Research Genetics (Ala., USA).

Immunohistochemistry

Sections of paraffin-embedded tumor samples were collected on organosilane-coated glass slides. Following microwave antigen retrieval in 0.01 mol/L citrate buffer, pH 6.0 (14) for 10 minutes, a routine ABC immunoperoxidase method was used. Primary antibody, bcl-2, was applied overnight at a dilution of 1:200 and at 4℃, and the chromogen used was diaminobenzidine tetrahydrochloride. Normal mouse sera were used as a negative serum control, and positive tissue control was included in every staining.

RESULTS

Interestingly, the three neoplastic stages of gastric lesion (tubular adenomas, EGCs, and AGCs) showed different patterns of bcl-2 expression. All tubular adenomas displayed a specific granular and supranuclear cytoplasmic staining pattern, which was consistent with the findings of previous reports (15, 16) (Fig. 1, 2). Adenocarcinomas including EGCs and AGCs, on the other hand, showed strong nuclear staining in most positive cases (Fig. 3, 4); as far as we know, this is a new finding. Positive bcl-2 staining in normal gastric epithelium was limited to the epithelial regenerative compartments and gastric mucous neck region. Epithelial staining also showed regularly a granular cytoplasmic pattern. Adjacent to the epithelial lesion, mucosa-associated lymphoid tissue (MALT) showed nuclear membrane staining in mantle zone lymphocytes; the germinal center of MALT lacked bcl-2 protein expression.

The results of bcl-2 protein immunostaining in 59

Table 1. Bcl-2 expression in gastric adenoma and adenocarcinoma

Gastric lesion	No. of cases	Cases showing bcl-2 expression (%)	
		Cytoplasmic	Nuclear
Tubular adenoma	19	19 (100)	2 (11)
Early gastric carcinoma	20	1 (5)	6 (30)
Advanced carcinoma	20	2 (10)	10 (50)

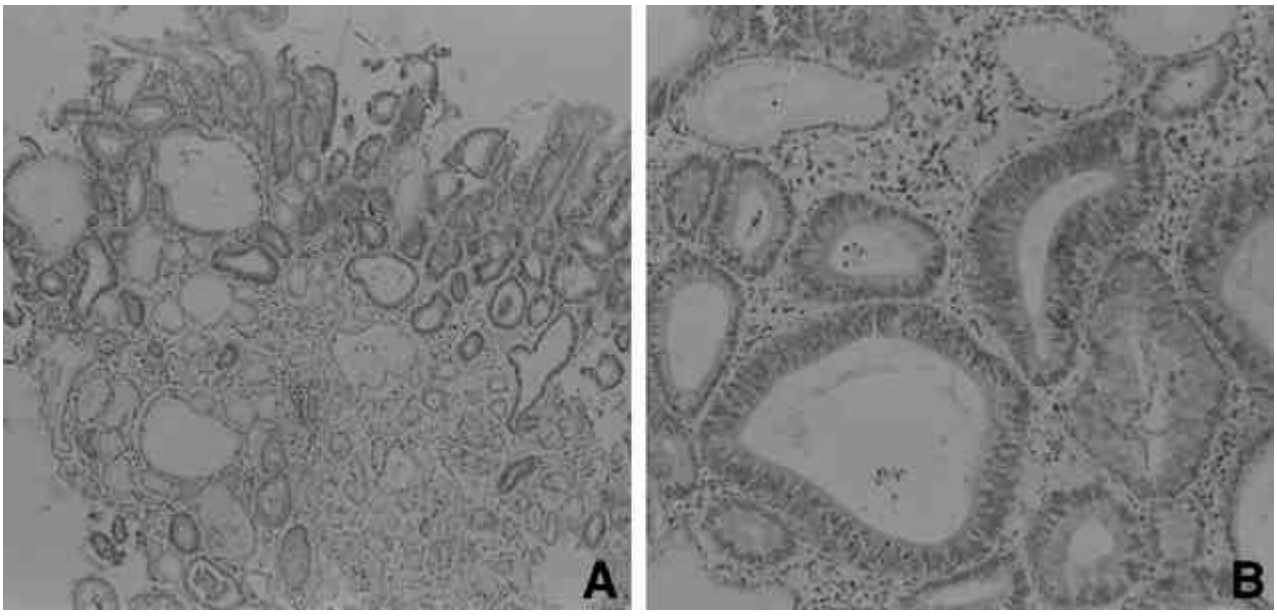

Fig. 1. Bcl-2 expression with cytoplasmic immunostaining in gastric tubular adenoma. A: low power view, B: high magnification of supranuclear cytoplasmic staining pattern (A, ×16; B, ×40).

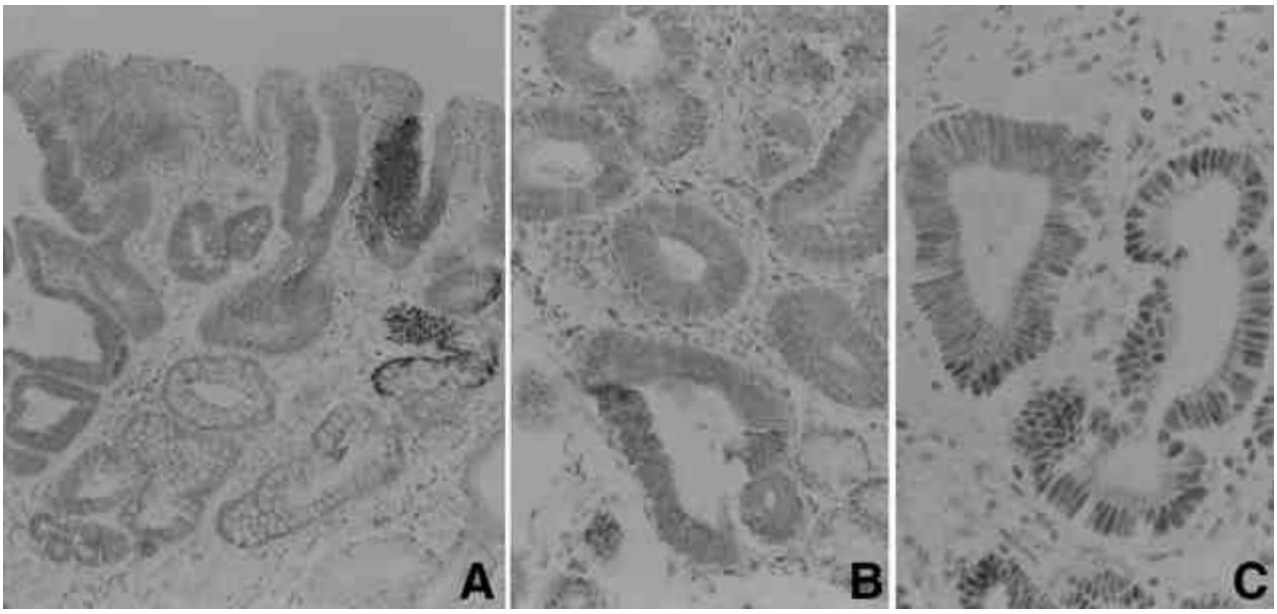

Fig. 2. Tubular adenoma showing diffuse cytoplasmic (B), focal nuclear (A), and strong nuclear staining (C) by bcl-2 immunohistochemistry (A, ×16; B & C, ×50).

cases of gastric lesions are summarized in Table 1. Nuclear or cytoplasmic staining was regarded as positive when more than 50% of tumor cells showed the increased bcl-2 expression. All 19 tubular adenomas with or without atypia displayed cytoplasmic bcl-2 staining (Fig. 1), and two of these 19 also showed nuclear staining. One adenoma with atypia showed nuclear staining in many tumor cells, and the other showed focal nuclear staining in some cells, in addition to diffuse cytoplasmic staining (Fig. 2).

In contrast to tubular adenomas, advanced gastric carcinomas (AGCs) showed strong nuclear staining in 50% of cases (10/20) and weak cytoplasmic staining in two cases (10%). According to Lauren's classification (17), the histological type of the 20 AGCs was 12 diffuse and eight intestinal. Nuclear bcl-2 staining was positive in

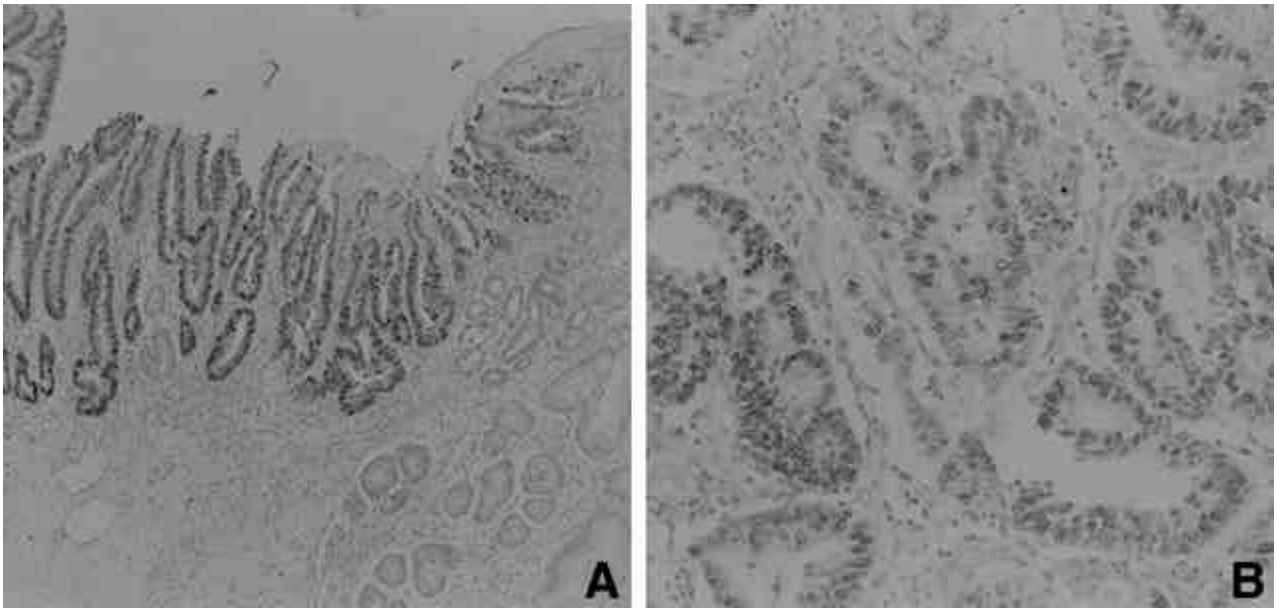

Fig. 3. Nuclear expression of bcl-2 in well differentiated adenocarcinoma. A: Early mucosal-type gastric carcinoma, B: Advanced gastric carcinoma (A, ×13; B, ×50).

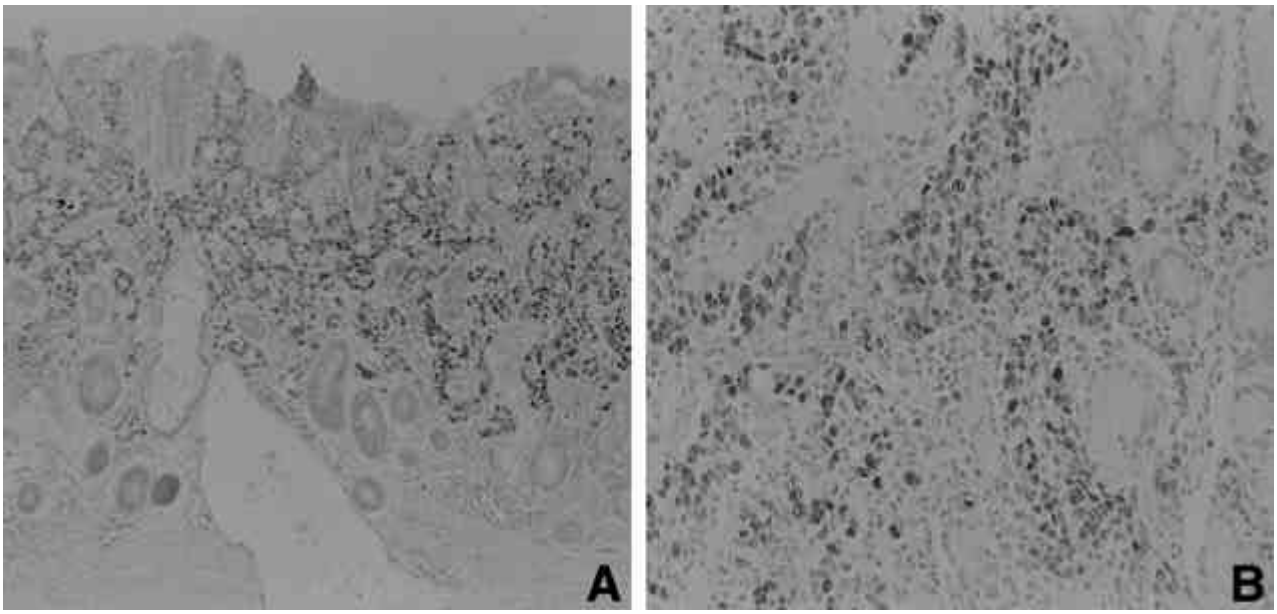

Fig. 4. A: Nuclear expression of bcl-2 in poorly differentiated adenocarcinoma of early gastric carcinoma, B: Nuclear expression of bcl-2 in diffuse type advanced gastric carcinoma (A, ×13; B, ×50).

eight of the 12 diffuse-type cases (67%) and in two of the eight intestinal type (25%) (Table 2). Among the cases of diffuse type, nuclear bcl-2 positive AGCs predominated. All AGCs in this study were lymph node-positive cases. Bcl-2 nuclear staining in metastatic lymph node sections correlated well with primary gastric carcinomas. Ten bcl-2 positive AGCs also showed positive staining in metastatic lymph-node tumor cell nuclei, and one case of cytoplasmic bcl-2 positive AGC showed nuclear staining in a few metastatic lymph-node tumor cells. In cases where primary gastric lesions were bcl-2 negative, metastatic lesions were similarly negative, and this suggests that nuclear expression of bcl-2 might not occur during metastasis.

Six out of 20 early gastric carcinomas (EGCs) displayed strong nuclear staining and one of them showed cytoplasmic staining. Histologically, EGCs were divided into two groups, namely well-to-moderately differentiated (n=10) and poorly differentiated gland-forming adenocarcinomas (n=10); six nuclear-positive cases were well-to-moderately differentiated and the other three were poorly differentiated. Positive rates for nuclear bcl-2 staining were neither different between the two histologic types (Table 2), nor between the mucosal and submucosal type. One case of cytoplasmic bcl-2 positive EGC was a mucosal type and histologically moderately differentiated.

DISCUSSION

Aberrant expression of bcl-2 protein has been reported in lymphomas (4-7) and colon (9), lung (10), and breast carcinomas (11). In the gastrointestinal tract, the protein has been immunohistochemically detected in the proliferative zone, where it is understood to be protecting the renewal potential of the mucosa (8, 12). Aberrant bcl-2 protein expression has also been sequentially

Table 2. Bcl-2 expression in gastric adenocarcinoma, according to histologic type

Gastric lesion	Histologic type	No. of cases	Cases showing bcl-2 expression (%)	
			Cytoplasmic	Nuclear
Early gastric carcinoma	W/D-M/D	10	1 (10)	3 (30)
	P/D	11	0	3 (27)
Advanced carcinoma	Intestinal	8	2 (25)	2 (25)
	Diffuse	12	0	8 (67)

W/D, well differentiated type; M/D, moderately differentiated type; P/D, poorly differentiated type

demonstrated in a gastric carcinogenic sequence (18) and the potential role of bcl-2 protein expression has been recognized in an early stage of a carcinogenic sequence (16, 18). It was expressed in 65% of cases of chronic atrophic gastritis with intestinal metaplasia, in 82% of gastric epithelial dysplasia cases (18), and in 72% of gastric adenocarcinomas (19). Bcl-2 expression in both colonic and gastric adenomas was higher than in their malignant counterparts or non-neoplastic lesions (16, 18). Most studies used monoclonal antibody from DAKO, and all bcl-2 expressions were shown to be cytoplasmic; nuclear staining on tissue section has not been previously reported.

We report the nuclear overexpression of the proto-oncogene bcl-2 in the adenoma-carcinoma of the stomach neoplastic sequence, using a mouse monoclonal antibody from Santa Cruz Biotechnology, Inc. (Calif., USA). The results suggest that bcl-2 nuclear expression may be related to the malignant transformation of adenoma to carcinoma in the stomach, and might be stage-specific. In this study, adenocarcinomas displayed strong nuclear staining in the majority of tumor cells (in 30% and 50% of EGCs and AGCs, respectively), while all adenomas (n=19) showed cytoplasmic bcl-2 expression with additional partial nuclear staining in two adenomas showing cellular atypism. Cytoplasmic bcl-2 expression in adenocarcinomas was, on the other hand, negligible, with one and two cases among 20 EGCs and 20 AGCs, respectively, and their staining intensity was weak to moderate. These results suggested a possible time shift of bcl-2 expression from the cytoplasm in the premalignant or benign stage to the nucleus at the time of malignant transformation. This may be accomplished either by bcl-2 itself, or by binding with another nuclear oncoprotein, or it may to a certain extent be related to the cell cycle. A similar phenomenon was observed in the shift of epidermal growth factor (EGF) receptor from plasma membranes to nuclei during liver regeneration (20). Willingham and Bhalla (13) demonstrated the diffuse nuclear distribution of bcl-2 in human carcinoma cell lines. This was present during the early prophase or late G2 phase, persisted throughout mitosis, and rapidly disappeared during the telophase, in human carcinoma cell lines.

The subcellular distribution of bcl-2 is a matter of controversy. On the base of subcellular fractionation experiments and immunofluorescence confocal microscopy, Hockenbery et al. (1) reported that bcl-2 is found primarily in the inner mitochondrial membrane. Others, however, have observed that bcl-2 resides primarily in the nuclear envelope, endoplasmic reticulum, and outer mitochondrial membranes (15, 21-24).

In AGCs, an association between nuclear expression and the diffuse type of adenocarcinoma has been noted. Eight out of 12 diffuse type AGCs (67%) and two intestinal type adenocarcinomas (25%) were positive for bcl-2 immunostaining. Bcl-2 nuclear expression in EGCs did not, however, differ according to histologic type, though Lauwers et al. (19) reported bcl-2 expression in 72% of 64 gastric adenocarcinomas with cytoplasmic staining and emphasized the association with intestinal type gastric adenocarcinoma. This result contrasts with ours. Our finding of a high rate of association between bcl-2 nuclear expression and diffuse type of AGC was supported by the results of Ayhan et al. (25). They reported a high percentage of loss of heterozygosity (LOH) at the bcl-2 gene locus, and the expression of bcl-2 gene in gastric and colorectal carcinomas. Furthermore, these two events seemed to be independent. The expression of bcl-2 mRNA and protein was observed in poorly differentiated adenocarcinoma and had implications for the development of them in the gastrointestinal tract, whereas LOH at the bcl-2 locus was frequently associated with a well differentiated adenocarcinoma cell line of the stomach and colon (25).

We compared the results of the immunostaining using a mouse bcl-2 monoclonal antibody from DAKO, Denmark (clone 124) and an antibody from Santa Cruz Biotechnology Inc. (cat # sc-509, Lot No. J175). The former did not show nuclear staining in carcinoma cells; but in non-neoplastic mucosa and tubular adenoma, the two antibodies showed very similar staining patterns. The nature of bcl-2 monoclonal antibody from Santa Cruz thus may be different from that of other bcl-2 monoclonal antibodies in character. By means of molecular studies, we are at present investigating the significance of nuclear expression of bcl-2 oncoprotein.

In conclusion, our results suggest that nuclear expression of bcl-2 may be related to malignant transformation in the stomach and is frequently associated with diffuse type advanced gastric adenocarcinomas.

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