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ARTICLE

Expression of Bcl-2 and c-ErbB-2 in Colorectal Neoplasia

Ay•e DURSUN, Aylar POYRAZ, Özlem SÜER, Cem SEZER, Gülen AKYOL

Department of Pathology, Gazi University Medical School Beþ evler, Ankara, Turkey

Several studies have been demonstrated the value of c-ErbB-2 and Bcl-2 in predicting the biological behaviour of tumors. The aim of this study was to investigate Bcl-2 and c-ErbB-2 expression in colorectal carcinomas and the correlation between their presence and other clinicopathologic parameters. Eighty-six colorectal carcinomas and 17 adenomas were stained with Bcl-2 and c-ErbB-2 immunohistochemically. Staining patterns were assessed semiquantitatively and correlated with tumor size, Duke's classification, tumor differentiation, mucinous characteristic and anatomic locations. We detected Bcl-2 expression in 10 of 17 adenomas (58.8 %) and 31 of 86 carcinomas (36.04 %). Positive staining in normal mucosa was observed only in the compartment of cryptic cells. However neither the difference in the rates of Bcl-2 positivity in adenoma and carcinoma groups, nor the correlation with other mentioned clinicopathological parameters, were found statistically significant. Bcl-2 expression was found to be significantly high in mucinous carcinomas. Expression of c-ErbB-2 was observed in 12 of 86 (13.95 %) carcinomas. It was not detected in adenomas and normal mucosa. Although the incidence of c-ErbB-2 in nonmucinous carcinoma was higher than that of mucinous carcinoma, this was not significant. In addition we were unable to show any significant relation between c-ErbB-2 expression and other clinicopathologic features. Our result suggest that c-ErbB-2 protein expression in colorectal carcinomas, is not very frequent event. There is no correlation between c-ErbB-2 expression and malignant potential of colorectal carcinomas. Higher expressions of Bcl-2 in adenomas than carcinomas suggest us a possible role of Bcl-2 in early carcinogenesis of colon. However since we were unable to find any significant correlation between Bcl-2 expression and other parameters the impact of this gene on biological behavior is still unclear for us. (Pathology Oncology Research Vol 7, No 1, 24–27, 2001)

Keywords: Bcl-2, c-ErbB-2, colorectal neoplasia

Introduction

Bcl-2 is a protooncogene which codes 26 kd protein that blocks apoptosis and rescues cells from apoptosis.[14] Reduction in the capacity of apoptotic cell turnover could be an important step in the development of neoplasia.[2,13] C-ErbB-2 gene encodes a 185 kd transmembrane protein and involve in cell growth and differentiation. Although Bcl-2 and c-ErbB-2 protein expressions have been shown in colorectal neoplasia, their possible impact on the biologic behavior of the colorectal carcinomas is still controversial. In the present study we evaluated Bcl-2 and c-ErbB-2 expression in colorectal neoplasia and looked for a possible correlation between clinicopathologic parameters.

Materials and Methods

Eighty-six cases with colorectal carcinomas and 17 adenomas who had undergone colectomy and/or polypectomy were included. The clinicopathologic parameters like tumor size, anatomic location, Duke's stage, tumor differentiation and histopathologic type were evaluated as prognostic indicators. Histological classification of the tumors was done according to the WHO system. The anatomic localizations were grouped as proximal colon meaning the distance from the cecum up to the splenic flexura and as distal colorectum beginning from the descending colon to the rectum.

Sections of formalin fixed, paraffin embedded tissues were re-examined and stained immunohistochemically with anti Bcl-2 (Mab124, Dako) and anti c-ErbB-2 (CB11, Biogenex)

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Correspondence: Ay•e DURSUN, Turgut Reis Caddesi 16/8 Mebusevleri-Tando•an, 06580 Ankara/Türkiye; Tel: 00 90 312 213 74 94; Fax: 00 90 312 212 99 08;
E-mail: aylarpoyraz@superonline.com

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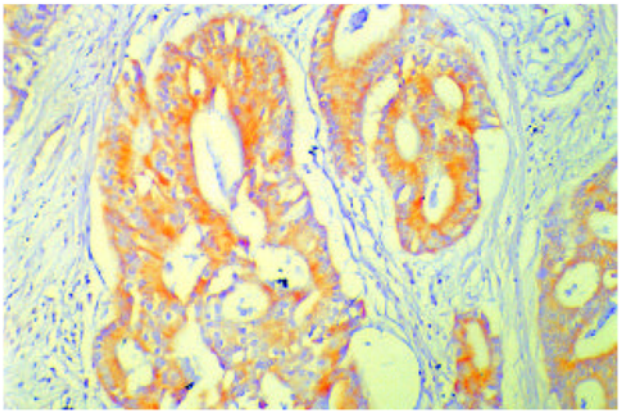

Figure 1. *Bcl-2 immunoreactivity in colonic carcinoma (SAB-peroxidase-DABx200)*

monoclonal antibodies. The streptavidin biotin indirect method was employed along with DAB (3 3' diaminobenzidine tetrahydroclorid) as chromogen. The sections were counterstained with hematoxylin. Cytoplasmic staining was accepted as positive for Bcl-2 whereas only membranous staining was noted for c-ErbB-2 expression. Staining patterns were assessed semiquantitiavely. To define the ratio of positivity, each slide was studied under high power magnification (400x). The entire slide was examined and cells showing distinct membranous reaction with cErbB-2 and cytoplasmic reaction with Bcl-2 were counted. Those areas with the highst immunoreaction were selected for analysis. 10 high power field were counted than frequency scored. Moderate and strong reaction were included. The percentage of cytoplasmic positivity for Bcl-2 was graded as follows negative (0-10 %), + (11-30 %), ++ (31-50 %), +++ (51-100 %).

Statistical analysis was performed using the chi-square test.

Results

In the normal colorectal mucosa adjacent to carcinomas, Bcl-2 staining was observed only in the bases of the cryptic epithelial cells. Few ganglion cells also showed immunoreactivity with Bcl-2. We detected Bcl-2 expression in 10 of 17 adenomas (58.8 %) and 31 of 86 carcinomas (36.04 %) (***Figure 1***). Bcl-2 positivity was not found to be correlated with the tumor size, anatomic location, Dukes stage and tumor differentiation, only the mucinous differentiation was related to Bcl-2 expression (***Table 1***).

Expression of c-ErbB-2 was observed in 12 of 86 (13.95 %) carcinomas ***(Figure 2)*** and it was not detected in adenomas or normal mucosa. Although the incidence of c-ErbB-2 in non-mucinous carcinomas was higher than that of mucinous carcinomas, the difference was not statistically significant. Furthermore we were unable to show any significant correlation between c-ErbB-2 expression and other clinicopathologic parameters. (***Table 2***).

Discussion

Multiple protooncogenes, oncogenes, regulatory factors and tumor suppresor genes appear to have a dominant role in the pathogenesis of colorectal carcinoma. Bcl-2 is a protooncogene that is involved in the regulation of cell death by inhibiting apoptosis. It is known to be expressed in colorectal neoplasia.[4] Increased Bcl-2 expression has been demostrated in gastrointestinal neoplasia and also implicated in carcinogenesis.[10] Pathogenetically, an adenoma-carcinoma sequence can be postulated for most of the colorectal carcinomas. Many molecular genetic changes like loss of tumor supressor gene on 18q and loss of p53 gene are said to be correlated with histologic stages of this sequence.[5] Another one concerns cell proliferation which is abnormally increased in the premalignant epithelial lesions and is found to be associated with increased Bcl-2 expression and decreased progression.[6] It is strongly expressed in most colorectal adenomas but diminishies after conversion to carcinoma.

In accordance with the observations published recently,[1,7] we detected Bcl-2 expression in 11 of the 17 (58.8

Table 1. **Correlation between Bcl-2 positivity and clinicopathological parameters**

Clinicopathologic Findings		*Bcl-2 staining*				*P value*
		Negative	*Positive*			
			+	++	+++	
Tumor size (cm)	>5	18	5	2	3	NS
	≤5	37	5	8	8	
Anatomic location	Proximal colon	20	4	3	2	NS
	Distal colorectum	35	8	6	8	
Duke's stage	Stage A	8	–	–	1	NS
	Stage B	25	6	4	5	
	Stage C	21	4	5	5	
	Stage D	1	–	1	–	
Tumor differentiation	Well	35	4	8	7	NS
	Moderate	14	6	2	3	
	Poor	6	–	–	1	
Tumor type	Mucinous	15	–	1	1	P = 0.0240
	Non-mucinous	40	10	9	10	

NS = Not significant

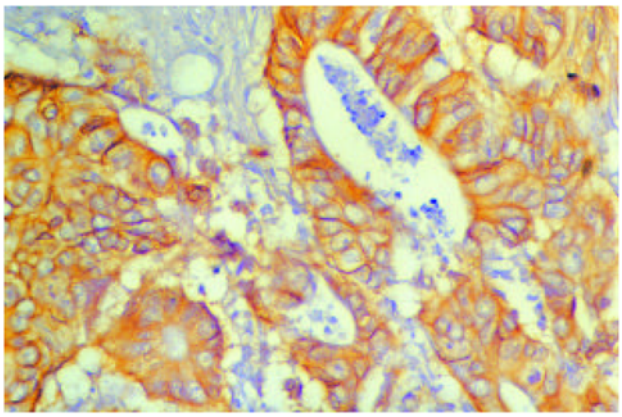

Figure 2. *Strong membranous positivity stained with anti c-ErbB-2 in colonic adenocarcinoma (SAB-peroxidase-DABx400)*

%) adenomas and 31 of the 86 (36.04 %) carcinomas. This finding may indicate the possibility of an early event in colorectal carcinogenesis. The prognostic significance of Bcl-2 expression is still controversial in colorectal carcinoma. In the current study, except for an association between Bcl-2 expression and mucinous tumors, similar to the various studies no correlation was found between other clinicopathologic parameters like tumor size, tumor differantiation, anatomic location and Duke's stage.[3,15] In contrast, Manne et al[11] reported an association between Bcl-2 expression and tumor stage. This finding suggested that the tumors expressing Bcl-2 are less agressive and progress more slowly than the tumors that do not express Bcl-2. It was also reported that Bcl-2 expression was associated with tumor differentiation and tumor size. However the biological role of Bcl-2 in clinical outcome and progression of the tumor is still controversial.

Table 2. **Correlation between c-ErbB-2 positivity and clinicopathological parameters**

Clinicopathologic Findings		*c-ErbB-2 staining*		
		Negative	*Positive*	*P value*
Tumor size (cm)	>5	25	3	NS
	≤5	49	9	
Anatomic location	Proximal colon	22	7	NS
	Distal colorectum	52	5	
Duke's stage	Stage A	9	–	NS
	Stage B	35	5	
	Stage C	296		
	Stage D	1	1	
Tumor differentiation	Well	48	6	NS
	Moderate	20	5	
	Poor	6	1	
Tumor type	Mucinous	16	1	NS
	Non-mucinous	58	11	

NS = Not significant

C-ErbB-2 is a protooncogene which encodes a transmembrane protein with tyrosine kinase activity and closely related to the epidermal growth factor receptor (EGFR) but biologically distinct from it. Amplification and overexpression of the c-ErbB-2 gene has been demonstrated in several tumors such as breast, stomach, lung, urinary bladder tumors. Furthermore c-ErbB-2 has been accepted as an important prognostic indicator in some carcinomas like breast and gastric carcinomas.[12] However several studies suggested that overexpression of c-ErbB-2 in colorectal carcinoma is not a frequent event.[8] Consistent with these studies, we found immunoreactivity of c-ErbB-2 in only 12 of the 86 (13.95 %) colorectal carcinomas and there was no expression in adenomas. Although, Kapitanovic et al[9] found that c-ErbB-2 expression in adenocarcinomas and adenomas were correlated with Duke's stage we were unable to show any correlation between c-ErbB-2 expression and mentioned clinical parameters.

We conclude that Bcl-2 is expressed in colorectal tumors and may appear early in colorectal carcinogenesis, but expression of c-ErbB-2 is an infrequent event in colorectal tumors. This study shows that there is no correlation between c-ErbB-2 or Bcl-2 expression and the malignant potential of colorectal carcinomas.

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