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Correlation between Bcl-2 Protein Expression and Some Clinicopathological Features of Oral Squamous Cell Carcinoma

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Bcl-2 inhibits most kinds of programmed cell death and provides a selective survival advantage to various cell types. The biological significance of Bcl-2 expression for the development and progression of oral cancer has still to be evaluated. The aim of our study was to estimate possible correlations between the Bcl-2 protein expression and some clinicopathological features of oral cancer. The study was conduced on 129 patients treated surgically for oral squamous cell carcinoma. The statistically significant relationships were observed between oral squamous cell cancer Bcl-2 expression and higher tumor grading ($p<0.005$), higher tumor mitotic index ($p<0.005$), higher index of atypical mitoses ($p<0.001$) as well as microfocal pattern of tumor invasive margin ($p<0.001$). The results suggest that positive Bcl-2 expression may be a valuable factor supplementing the established unfavorable histopathological features of oral squamous cell carcinoma.

Introduction

The neoplastic transformation is a result of disordered mechanisms that control cell growth and mitosis. Neoplastic cells escape the mitotic control. Death of cells, like mitosis, may be an active process subjected to genetic control. It is then defined as apoptosis as opposed to necrosis. Whether a cell initiates the program of death depends not only on external factors but also on its ability to express genes which activate and inhibit apoptosis [7, 19]. The oral cavity is continually exposed to various traumas due to the effect of thermal, mechanical and chemical stimuli, which when accompanied by inflammatory states may promote the growth of neoplastic changes. Numerous studies have revealed a correlation between the expression of Bcl-2 proteins and the progression of neoplastic disease. It cannot be excluded that this protein acts as a marker of a neoplastic transformation threatening in precancerous states or the already existing neoplastic transformation.

The Bcl-2 oncogene was first identified as the site of reciprocal translocation of chromosome 18 in follicular lymphoma and encodes a membrane associated protein that is present in the endoplasmic reticulum, nuclear and outer mitochondrial membranes. Enhanced expression of Bcl-2 in human neoplasms, such as lymphoma, prostatic carcinoma, breast carcinoma, and carcinoma of the lung, suggests a role for this oncoprotein in the pathogenesis of neoplasia [2, 5, 8, 12]. The biological significance of Bcl-2 expression for the development and progression of oral cancer has still to be evaluated [11, 17]. The aim of our study was to estimate possible correlations between the Bcl-2 expression and some clinicopathological features of oral cancer.

Material and Methods

The study was conducted on 129 patients treated for oral squamous cell carcinoma at the Departments of Maxillofacial Surgery and Otolaryngology, Medical Academy, Białystok. Tissue blocks were collected immediately after tumor removal, fixed in 10% buffered formaldehyde solution and embedded in paraffin at 56˚C. The slides were stained with HE and immunohistochemically with the anti-human Bcl-2 antibody (DAKO/Bcl-2, No M0887). The immunolocalization of Bcl-2 protein was performed using the labelled streptavidin biotin (LSAB) method. To visualize the reaction, 3,3'-diaminobenzidine (DAB) was used. The Bcl-2 expression was assessed semiquantitatively in neoplastic cells and defined as follows: (0) - positive reaction presented in less than 10% of tumor cells, (1) - >10% and <30% cells, (2) - >30% and <60% of tumor cells, (3) positive reaction presented in more than 60% of cells. The percentage of Bcl-2 positive cells was calculated independently by two pathologists. The tumor mitotic index and index of atypical mitoses was assessed semiquantitatively and defined as follows: (0) lack of mitoses, (1) 1 - 5 mitoses, (2) 6 - 10 mitoses, (3) more than 10 mitoses. The mitotic ratio was evaluated in at least 100 neoplastic cells per sample, using a light microscope at x 400 magnification. Tumor invasive margin type was evaluated as a microfocal (MIF), macrofocal (MAF) and mixed microfocal/macrofocal pattern (MIX).

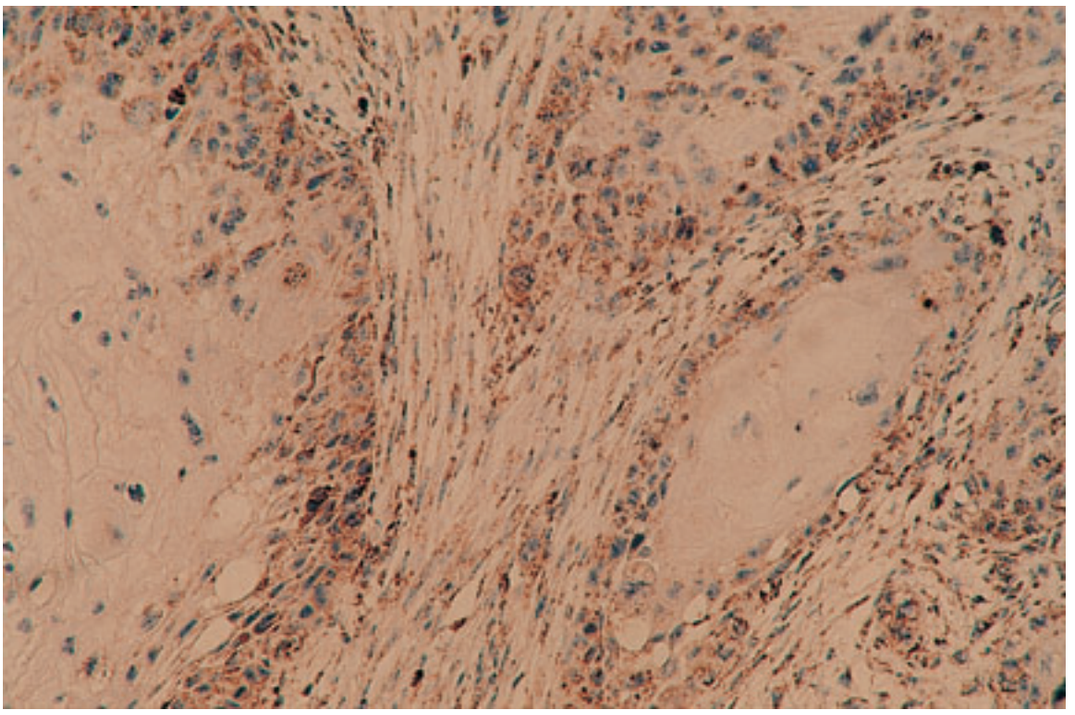

Fig. 1. Marked Bcl-2 expression in the peripheral cells of squamous cell carcinoma focus diminishing toward its center. Magn. 200x.

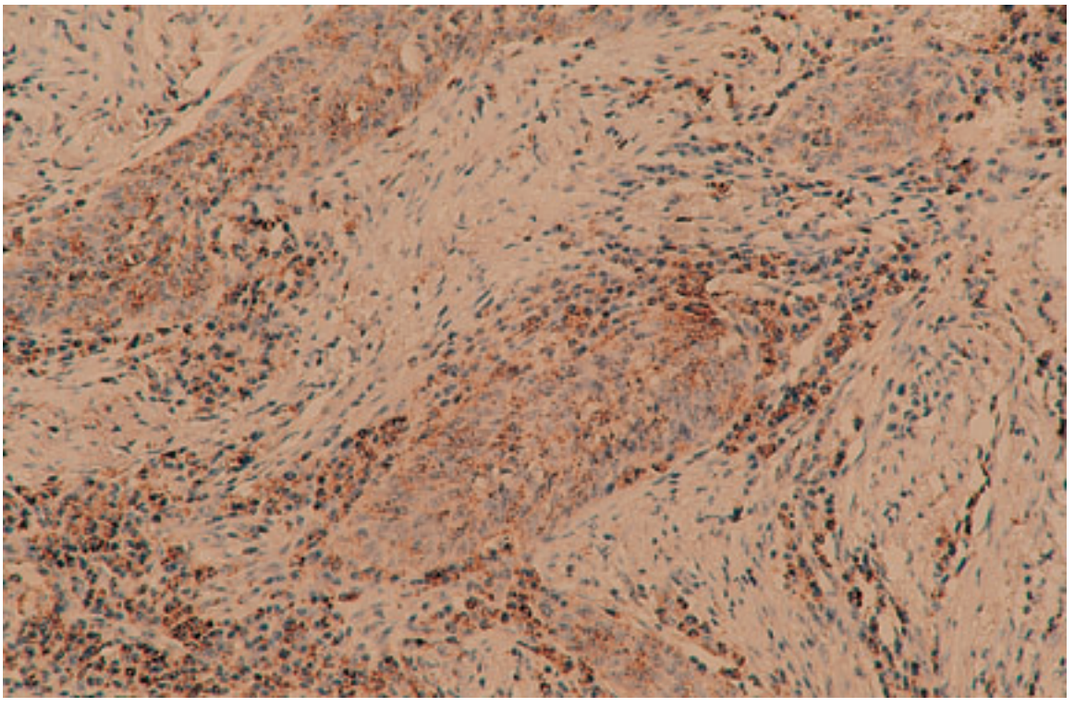

Fig. 2. Strong positive immunohistochemical staining for Bcl-2 in poorly differentiated squamous cell cancer. Magn. 100x.

TABLE 1

Correlation between Bcl-2 expression and oral squamous cell cancer clinicopathological features

Feature	Bcl-2(-)	Bcl-2(+)	χ^2	p
Gender				
M	79	30	0.054	NS
F	15	5		
Age				
≤60	53	21	0.974	NS
≥60	41	14		
Site				
Tongue	58	15		
Oral floor	15	10	0.943	NS
Lip	11	1		
Other regions of oral cavity	10	9		
Histological Tumor Grading				
1	62	8	34.700	<0.005
2	28	12		
3	4	15		
Mitotic Index				
(1)	47	11	14.352	<0.005
(2)	41	13		
(3)	6	11		
Index of Atypical Mitoses				
(0)	32	2	24.478	<0.001
(1)	41	10		
(2)	18	17		
(3)	3	6		
Pattern of Tumor Invasive Margin				
MAF	51	3	25.946	<0.001
MIX	36	21		
MIF	7	11		

Abbreviations: Bcl-2(-) - positive immunohistochemical reaction presented in less then 10% of tumor cells; Bcl-2(+) - positive immunohistochemical reaction presented in more than 10% of cancer cells; NS - not significant; groups (0), (1), (2), (3) for mitotic index and index of atypical mitoses as defined in Material and Methods.

The correlations between cancer Bcl-2 expression and patient age, gender, tumor site, histological grading, mitotic index, index of atypical mitoses, invasive margin pattern were subjected to statistical analysis with chi-square test, with $p<0.05$ considered significant.

Results

The group of 129 patients consisted of 109(84%) men and 20(16%) women. The age of patients ranged from 38 to 81 years, median 67. Most of the cancers developed in 50 to 70-year-old patients. Seventy-three (57%) cancers were located in the tongue, 25(19%) in oral cavity floor, 12(9%) in the lip and 19(15%) in other regions of oral cavity including gingival. All cases of lip squamous cell carcinoma developed in men. Poorly differentiated (G3) squamous cell cancers were located more often in the tongue and oral cavity floor than in other regions, although this relationship was not statistically significant.

Positive immunoreactivity for Bcl-2 was present as a coarse or finally granular cytoplasmatic staining, irregularly distributed around nuclei. Marked Bcl-2 expression was observed in the peripheral cells of squamous cell carcinoma focuses, diminishing toward their centers (Fig. 1). Strong positive immunohistochemical staining was also observed in poorly differentiated squamous cell cancers and most cancer microfocuses at the tumor invasive margins (Fig. 2). Relationships between Bcl-2 expression and some clinicopathological features of presumed or established value are presented in Table 1. The statistically significant positive correlations between Bcl-2 expression in tumor cells and higher tumor grading ($p<0.005$), higher mitotic index ($p<0.005$) as well as MIF type of invasive tumor margin ($p<0.001$) and index of atypical mitoses ($p<0.001$) were observed. There was also a statistically significant positive relationship between MIF pattern of cancer invasive margin and mitotic index ($p<0.001$).

Discussion

The identification of molecular markers that provide an insight into the potential behavior or aggressiveness of tumors is a necessary step for the improvement of cancer treatment. Bcl-2 protein product seems to be one of the most promising members of molecular markers to evaluate cancer malignant behavior. The bcl-2 gene is a protooncogene whose protein product inhibits apoptosis. Its role is associated with keeping cells alive, but not by stimulating them to proliferation, as other protooncogenes do. Many authors have shown that increased expression of protein product of bcl-2 gene appears in the early phase of carcinogenesis leading to apoptosis impairment and in consequence to the progression of neoplastic changes [6, 9, 15].

In our study positive immunohistochemical staining for Bcl-2 protein in more than 10% of tumor cells was present in 35(27%) oral cancer cases. Singh et al. [16] reported Bcl-2 expression in 25% of oral cancers whereas Ravi et al. [14] in 87% of the cases. The discrepancies might be attributed to geographic specificity as well as different etiologic factors involved in oral cavity carcinogenesis. Ravi et al. [14] studied the expression of Bcl-2 in various leukoplakia stages in the oral cavity. The authors analysed leukoplakias with and without epithelial dysplasia and squamous cell carcinomas, revealing a positive Bcl-2 expression in 3% of changes without dysplasia, in 12 - 19% with dysplasia and in 60 - 90% of oral squamous cell carcinomas. In our early study [18], a positive expression of Bcl-2 protein was found in 35%

of leukoplakias not accompanied by oral squamous cell carcinomas. The incidence increased to 50% in the leukoplakia-like lesions accompanied by oral carcinomas. The positive immunohistochemical reactivity for Bcl-2 in 27% cases of oral cancer in these studies was much lower than in leukoplakias accompanied by oral carcinomas (50%) in our earlier studies [18]. The results indicate that this may be an early phenomenon in the process of leukoplakia-derived oral squamous cell carcinoma growth. Increased Bcl-2 expression does not seem to be a required factor for the progression of the neoplastic process, but it may play a significant role in early carcinogenesis. This is suggested by the increase in its expression found in certain precancerous lesions and the corresponding decrease in the carcinomas arising from these lesions, e.g. in the uterine cervix, endometrium, stomach and large bowel [4, 13].

Positive expression of Bcl-2 has been correlated with poor prognosis in patients with B-cell lymphomas [12] and urinary tract transitional-cell carcinoma [3]. In contrast, expression of Bcl-2 in colorectal cancers has been demonstrated as being a favorable prognostic factor [1, 10]. In our study a statistically significant positive correlation between Bcl-2 immunohistochemical reactivity and low grade differentiation of tumor, higher mitotic index, higher index of atypical mitoses as well as tumor microfocal invasive margin was observed. The results suggest that positive Bcl-2 expression may be a valuable factor supplementing the established unfavorable histopathological features of oral squamous cell carcinoma.

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