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The expression of bcl-2 and bcl-6 protein in normal and malignant transitional epithelium

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Abstract The bcl-2 proto-oncogene plays a key role in cell longevity by preventing apoptosis. Bcl-2 is important in developing and maintaining the normal function of lymphoid and epithelial tissues. The bcl-6 protein is a 96 kDa nuclear protein selectively expressed in mature B cells within normal germinal centers as well as in their transformed counterparts in diffuse large B cell lymphoma. Recently, the bcl-6 protein has also been reported to be expressed in normal skin and epidermal neoplasms. In this study, 47 cases of transitional cell carcinomas (TCCs) were immunohistochemically studied for bcl-2 and bcl-6 protein expression. The results showed that bcl-2 was expressed only on basal layer cells, whereas bcl-6 expression was restricted to the superficial layers in the normal transitional epithelium. Von Brunn's nests showed strong immunostaining to bcl-2, but were negative to bcl-6. Among 47 TCCs, 15 (32.6%) and 29 (61.7%) cases were positive for bcl-2 and bcl-6, respectively. Compared with the normal transitional epithelium, the expression of bcl-2 was significantly decreased, whereas bcl-6 expression was significantly increased in TCCs. Additionally, the strong expression of bcl-6 had a positive correlation with the histopathologic grade of TCC. In conclusion, bcl-2 and bcl-6 proteins may play a role in the pathogenesis of TCCs, and bcl-6 expression reflects histopathologic grade.

Keywords Bcl-2 · Bcl-6 · Immunohistochemistry · Transitional cell carcinoma

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Introduction

The bcl-2 proto-oncogene was initially discovered at the t(14,18) (q32,q21) breakpoint. It is the cytogenetic hallmark of human follicular lymphoma, and constitutes one of the regulators of apoptosis. It encodes for a 26 kDa protein that can block apoptosis induced by several stimuli without affecting cell proliferation [4, 14, 16, 20]. The bcl-2 protein is mainly located in mitochondrial membranes and to a lesser extent in the endoplasmic reticulum and nuclear envelope. It is overexpressed in various human cancers, such as carcinomas of the lung, breast, and uterine cervix. Some studies have also shown that bcl-2 expression is associated with a better postoperative outcome [5, 10, 14, 18].

The bcl-6 gene is known to be located on chromosome 3q27 and has the function of a transcriptional repressor. Several reports demonstrate that the functions of the 5′ noncoding region of the bcl-6 gene are potential mechanisms for deregulating bcl-6 expression; bcl-6 protein is essential for germinal center formation, and it is expressed in germinal center B cells and their neoplastic counterparts [3, 16, 17, 19].

Recently, Yoshida et al. [21] and Kanazawa et al. [8] reported that bcl-6 protein is expressed in normal squamous skin cells and their neoplastic counterparts, suggesting that it may also play a prominent role in the differentiation of epithelial cells. However, few reports have described bcl-6 protein expression in transitional epithelium. In this study, we detected bcl-2 and bcl-6 protein expression in normal and malignant transitional epithelium by immunohistochemistry, to demonstrate a possible etiologic role in urothelial malignancy.

Materials and methods

Materials

A total of 47 cases of transitional cell carcinomas of the urinary bladder, kidney and urethra, and 20 cases of normal transitional epithelia including 19 cases adjacent to the TCCs and one of normal

urothelial tissue from the bladder neck from a patient treated by radical prostatectomy were selected from the pathology file in the Department of Pathology, Anam Hospital. The tissues were routinely processed with 10% buffered formalin fixation and paraffin embedding. The H-E stained slides were retrieved and appropriate blocks were selected for immunohistochemical staining.

Immunohistochemical staining for bcl-2 and bcl-6 proteins

For immunohistochemical staining with DAKO LSAB kit (DAKO A/S, Denmark), 4-μm thick tissue sections were deparaffinized and rehydrated. Endogenous peroxidase activity was eliminated by incubation with 3% H_2O_2 in methanol for 15 min. The antigen was retrieved at 103 kPa for 2 min by placing the slides in 0.001 M EDTA buffer (pH 8.0). The slides were then incubated with the primary monoclonal antibody for bcl-2 (1:50, DAKO A/S, Denmark) and bcl-6 (1:10, DAKO A/S, Denmark) for 1 h at room temperature. After incubation with biotinylated link for 30 min, the sections were incubated with streptavidin-peroxidase complex at room temperature for 30 min. Immunostaining was visualized by using 3,3′diaminobenzidine. The sections were counterstained with hematoxylin. As a negative control, 0.1 M Tris buffer (pH 7.6) replaced the primary antibody, and tonsil tissue was used as a positive control.

Interpretation for immunostaining

The staining for bcl-2 was cytoplasmic, but the nucleus staining was considered to be positive for bcl-6. The expression of bcl-2 and bcl-6 was analyzed in normal and malignant transitional cells. The

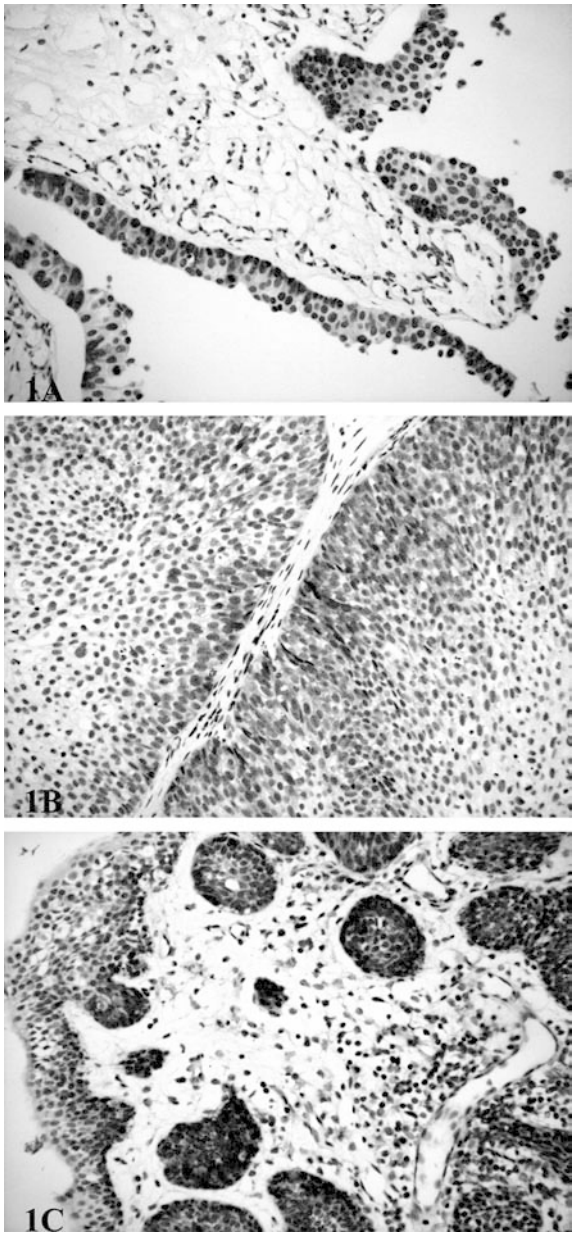

Fig. 1 Bcl-2 immunoreactivity in normal transitional epithelium and TCC. **A** Normal transitional epithelium. The cytoplasm of the basal layer is positive. **B** TCC (grade 2). Tumor cells are positive, especially adjacent to the basal layer. **C** TCC (grade 3). The tumor cells are strongly positive, and the lymphocytes were also positive

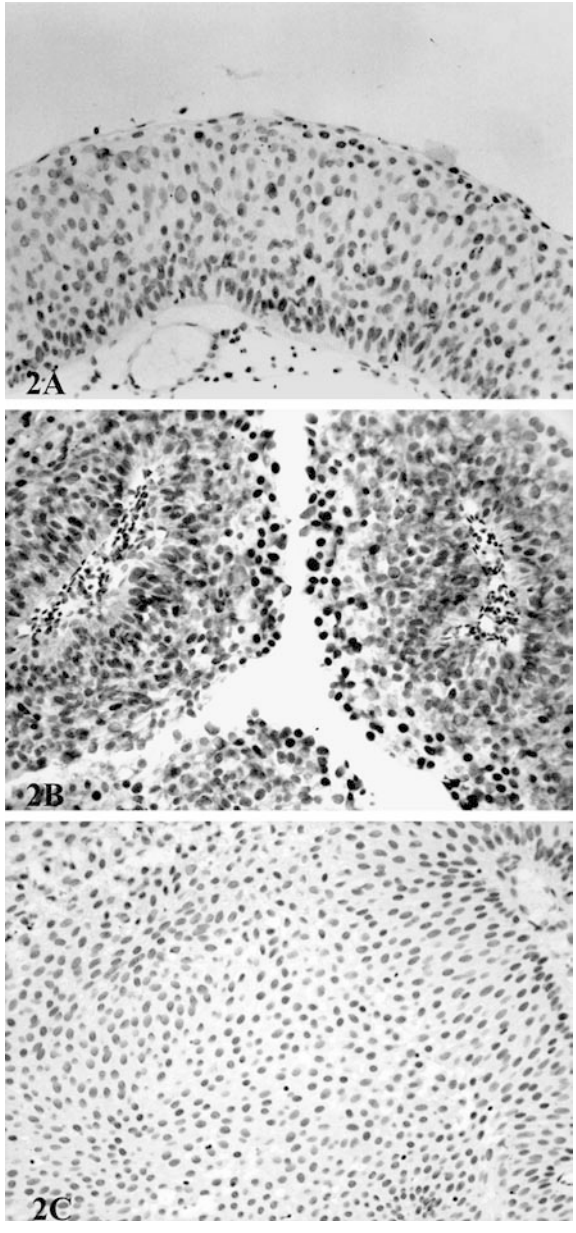

Fig. 2 Bcl-6 immunoreactivity in normal transitional epithelium and TCC. **A** Normal transitional epithelium. The superficial cells are positive in nucleus. **B** TCC (grade 1). Tumor cells on the surface are positive. **C** TCC (grade 3). The tumor cells are negative

immunohistochemical results were evaluated as (+++) when more than 75% of the epithelial cells were stained, (++) when 50–75% of the cells were stained, (+) when 20% of the cells were stained, and (−) when less than 5% of the epithelial cells were stained.

Statistics

Analysis of data was accomplished using χ-square tests. $P < 0.05$ was considered to be statistically significant.

Results

Bcl-2 and bcl-6 protein expression in normal transitional epithelium

Bcl-2 was positive in the basal layer of normal transitional epithelium with a rate of 90.6%. In contrast, bcl-6 was negative in most normal transitional epithelium with a positive rate was only 10%; it was mainly distributed in the superficial layer of transitional epithelium. In von Brunn's nest, bcl-2 was strongly positive, but bcl-6 was negative (Figs 1, 2).

Bcl-2 protein expression in TCC

The positive rate of bcl-2 protein expression was 32.6% in TCC, which was significantly lower than that in normal transitional epithelium (90.6%) (Table 1). Bcl-2 protein expression was increased in grade 3 compared to grade 1 TCC, but this was not significant.

Bcl-6 protein expression in TCC

The positive rate of bcl-6 protein expression was 61.7% in TCC, which was significantly higher than that in normal transitional epithelium (10%). The expression of bcl-6 protein at (++ or +++) was seen in 13/19 (68.4%) cases of grade 1, 6/13 (46.2%) cases of grade 2, and 2/15 (13.3) cases of grade 3 TCC. The positive rate for grade 3 of TCC was significantly lower than that for grade 1 and 2 (Table 2) ($P < 0.05$).

Table 1 Relationship between bcl-2 protein expression and grade of bladder cancer

Grade	Total	Positive			Positive rate (%)
		(+)	(++)	(+++)	
1	18	3	1	0	22.2
2	13	2	0	1	23.1
3	15	7	1	0	53.3
Total	46	12	2	1	32.6

Table 2 Relationship between bcl-6 protein expression and the grade of bladder cancer. (++) and (+++) were considered to be strongly positive cases

Grade	Total	No. of positive cases			Positive rate (%)	Strongly positive rate (%)
		(+)	(++)	(+++)		
1	19	4	5	8	89.5	68.4**
2	13	0	4	2	46.2	46.2*
3	15	4	0	2	40.0	13.3
Total	47	8	9	12	61.7	44.7

Compared with grade 3 *$P < 0.05$ **$P < 0.01$

Relationship between bcl-2 and bcl-6 protein expression in TCC

The positive rate of bcl-6 protein was higher than that of bcl-2 in TCC, and the expression of the two proteins showed an inverse correlation.

Discussion

The bcl-2 gene product regulates programmed cell death. A number of studies suggest that bcl-2 is involved in the selection and maintenance of cell longevity and prevention of apoptotic cell death, leading to their accumulation in the G_0 phase of the cell cycle [11, 12]. Bcl-2 was reported to be over-expressed in breast and uterine cervical cancers, and associated with an unfavorable prognosis. However, its decreased expression is also associated with a favorable prognosis in gastrointestinal cancer [2, 6, 12]. Lipponen et al. reported that bcl-2 protein was expressed in basal cells in normal transitional epithelium, and 68% of the transitional cell tumors showed bcl-2 positivity in the basal cells. The tumors with bcl-2 positivity in non-basal cells had an unfavorable prognosis, although this was not an independent prognostic factor [11]. However, King et al. reported that bcl-2 expression was significantly lower in the normal transitional epithelium and in well and moderately differentiated tumors (grades 2 and 3), compared with poorly differentiated (grade 3) tumors [9]. In this study, bcl-2 was positive in the basal layer of normal transitional epithelium, suggesting that bcl-2 protein has a role in the maintenance of the growth potential of transitional epithelium. Interestingly, the several layers of the basal portion were also well stained in TCC of grade 1. Although there was no significant difference between the expression of bcl-2 protein and the grade of TCC, bcl-2 protein showed a higher positive rate in grade 3 than that in grade 1. It is highly probable that the over-expression of bcl-2 promotes the accumulation of malignant cells and tumor formation in some tumors by the inhibition of apoptosis.

Bcl-6, also known as LAZ3 or bcl-5, is highly associated with the pathogenesis of some diffuse large B cell lymphomas. Analysis of bcl-6 expression in non-transformed cells suggests that it may play a role in B cell development. In human tonsils and lymph nodes, bcl-6 protein is expressed in germinal center B cells, but not in most mantle zone B cells [1, 2, 7]. Yoshida et al. [21] and Kanazawa et al. [8] reported that bcl-6 protein

was expressed in normal squamous cells of the skin and their neoplastic counterparts, and suggested that bcl-6 protein might play a prominent role in the differentiation of epithelial cells. Until now, the expression of bcl-6 protein in TCC has not been reported. In contrast to bcl-2, bcl-6 protein was expressed intensely in the nuclei of the upper layer cells in the normal transitional epithelium, but not in basal layer cells. This was similar to the results of bcl-6 protein expression in squamous cells, suggesting that bcl-6 protein may play a role in the differentiation of transitional epithelium, as suggested by Yoshida et al. and Kanazawa et al. In TCCs, the expressions of bcl-6 protein showed various staining patterns. Unlike in the normal transitional epithelium, the positive cells were irregularly distributed throughout the layers. In well-differentiated TCCs, bcl-6 protein was strongly positive in the upper layer, as shown for normal transitional epithelium, but the cancer cells in the basal portion were also well stained. In grade 3 TCC, a small number of cases were positive, suggesting that bcl-6 protein expression plays some role in the differentiation but not in the progression of TCC.

In summary, bcl-2 and bcl-6 proteins may participate in the pathogenesis of TCC, and their expression could reflect the histopathologic grade of TCC.

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