Virchows Arch (1998) 432:445–450

ORIGINAL ARTICLE

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Expression of bcl-2 oncoprotein in transitional cell carcinoma of the upper urinary tract

Received: 3 July 1997 / Accepted: 29 October 1997

Abstract We investigated the immunoreactivity for bcl-2 oncoprotein in 154 cases of transitional cell carcinoma of the upper urinary tract (TCC-UUT) and its relation with the immunoreactivity for p53 oncoprotein and proliferating cell nuclear antigen (PCNA) immunoreactivity. Immunohistochemically, bcl-2 oncoprotein was recognized as positive in 29.2% of the samples. The immunoreactivity for bcl-2 oncoprotein was significantly ($P < 0.05$) correlated only with stage, though there was a borderline correlation ($P = 0.050$) with PCNA immunoreactivity. Furthermore, in invasive TCC the immunoreactivity for bcl-2 oncoprotein was associated with PCNA immunoreactivity ($P < 0.041$). The 5-year disease-free and overall survival rates were 55.7% and 71.5%, respectively. A univariate analysis of survival revealed that stage, grade, pattern of growth, immunoreactivity for p53 oncoprotein, and PCNA immunoreactivity each had a significant effect on disease-free and overall survival rates, whereas the immunoreactivity for bcl-2 oncoprotein had no significant effect on either rate. In the final models of the multivariate analysis, stage was found to be the only prognostic factor for disease-free survival and for overall survival. Detection of immunoreactivity for bcl-2 oncoprotein appears to be of no real value in deciding the prognosis of TCC-UUT.

Key words Transitional cell carcinoma · Upper urinary tract · bcl-2 Oncoprotein

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Introduction

The *bcl-2* proto-oncogene was originally identified at the breakpoint site of the t (14:18)(q32-p21) chromosomal translocation, which occurs in most human follicular lymphomas [28]. It encodes a 26-kDa protein localized to mitochondrial membranes, the endoplasmic reticulum, and the nuclear envelope and is associated with prolonged cell survival by virtue of its role in the inhibition of programmed cell death (apoptosis) in a number of physiological and pathologic conditions [26]. In normal human tissues, it is expressed in cells with a self-renewal capability, such as haemotopoietic progenitor cells, in the replicative compartment of stratified and glandular epithelia, in the epithelia of hormonally responsive organs (including breast, prostate gland, endometrium, and thyroid), and in some long-living cells, such as central and peripheral neurons [2].

Recent studies have revealed a close relationship between the immunoreactivity for bcl-2 oncoprotein and the clinicopathological findings and clinical outcome in a variety of lymphomas [22] and epithelial malignancies, including carcinomas of the lung [25], thyroid [30], breast [4], stomach [17], and ovary [15]. However, the molecular mechanism underlying the expression of bcl-2 oncoprotein in cells without t (14:18) is still unknown. A disturbance of the post-translocational regulation of the *bcl-2* gene may be present in carcinomas [3]. In non-small-cell lung carcinoma [25], breast carcinoma [4], and gastric carcinoma [17], immunoreactivity for bcl-2 oncoprotein has been correlated with favourable prognosis. However, few studies have investigated the prognostic importance of immunoreactivity for bcl-2 oncoprotein in transitional cell carcinoma of the upper urinary tract (TCC-UUT) [10, 14].

Other important oncogenes and tumour-suppressor genes also play roles in the regulation of apoptosis. For instance, wild-type p53 has been demonstrated to induce apoptosis [11]. The *bcl-2* gene is a transcriptional target for wild-type p53, which decreases bcl-2 oncoprotein levels both in vitro and in vivo [20]. Furthermore, mutant

p53 induces down-regulation of bcl-2 at both mRNA and protein levels in breast cell lines [12]. The current study was aimed at ascertaining whether immunoreactivity for bcl-2 oncoprotein is correlated with various clinicopathological data, with proliferating cell nuclear antigen (PCNA) immunoreactivity, with immunoreactivity for p53 oncoprotein, and/or with clinical outcome in TCC-UUT patients. We used an immunohistochemical approach to examine formalin-fixed, paraffin-embedded, tumour-tissue sections from 154 patients with TCC-UUT.

Materials and methods

The material used comprised 154 surgically resected specimens from patients with primary TCC-UUT; these specimens had been obtained at the Mutual Aid Associations' Hospital, Tachikawa, and the National Defense Medical College Hospital, Tokorozawa, between 1970 and 1995. Another study in these same patients has been reported elsewhere [23, 24]. Histopathological stage was determined according to the criteria proposed by the International Union Against Cancer (UICC) [29]. Tumour cells were divided into two grades histopathologically, using the criteria for urinary bladder tumours laid down by the Armed Forces Institute of Pathology (AFIP) [21].

For immunohistochemistry, we used the avidin–biotin-peroxidase complex (ABC) method on deparaffinized sections, employing mouse monoclonal antibodies against bcl-2 oncoprotein (1:50, Dako, Glostrup, Denmark), and PCNA (PC-10, 1:100, Dako). The sections were pretreated with boiled water for 60 min for immunohistochemistry against bcl-2 oncoprotein, and with boiled 0.01 M citrate buffer, pH 6.0, for 30 min for immunohistochemistry against PCNA. For the analysis of the immunoreactivity for bcl-2 oncoprotein, tumours in which stained tumour cells made up more than 25% of the tumour were graded as positive. This criterion was used whether bcl-2 staining was restricted to the basal cell layer or was present in all cell layers of the tumour, both patterns being seen in the present study. This is in keeping with the practice used by other workers [9, 30]. We used normal lymph nodes as positive control tissues for the above two antibodies. For negative controls, the primary antibody was omitted. For the analysis of PCNA, and on the basis of the immunoreaction in at least 1000 tumour cells, the percentage of nuclei with a positive immunoreaction was determined (PCNA index). The PCNA index was classified as high if it was over 69%, which represented the median value for the carcinomas. p53 oncoprotein was evaluated immunohistochemically; the technique used and the results in these same patients have been reported elsewhere [23].

For statistical analysis, disease-free and overall survival rates were the two main dependent variables tested in this study. 'Disease-free survival' was defined as the period between the initial radical operation and the subsequent appearance of recurrence or metastasis. In this study, recurrence was defined as the occurrence of TCC anywhere in the genitourinary tract. The end-point was either recurrence or metastasis of TCC or the closing date of the study, whichever came first. 'Overall survival' was defined as the interval between surgery and death; the end-point for this variable was either death or the closing date of the study.

Disease-free and overall survival curves for all of the univariate analyses were assessed using the Kaplan–Meier method. Comparisons between two or more survival curves were assessed using Wilcoxon and log-rank tests. Multivariate analysis of the clinicopathological parameters was performed using the Cox stepwise-regression model. The above analyses were performed using the SAS statistical software package (SAS Institute, Carey, N.C.) [27]. Other comparisons were performed using the Chi-square analysis.

Results

The clinicopathological features of these same patients have been reported elsewhere [24]. Briefly, the patients' age at diagnosis was in the range 34 to 84 years, with a median age of 66 years. Forty-one of the 154 patients died of their tumours 1–132 months after surgery. The 154 cases were 64 (41.5%) of tumour in the renal pelvis or calyces, 61 (39.6%) of tumour in the ureter, and 29 (18.8%) of multicentric tumour disease. There were 15 patients with simultaneous bladder tumours at the time of diagnosis, 40 with subsequent bladder tumours, and 10 who had had an antecedent bladder tumour. In all, 55 patients (35.7%) had an associated bladder neoplasm; 10 of these had such a neoplasm at more than one of the above times and thus appear in more than one group.

The tumours were divided into four groups (A, B, C, and D) on the basis of stage. There were 2 cases (1.3%) in group A (nonpapillary, noninvasive, pTis), 58 cases (37.6%) in group B (papillary, noninvasive, pTa), 24 cases (15.6%) in group C (invading the submucosa or muscularis, pT1 and pT2), and 70 cases (45.5%) in group D (invading beyond the muscularis or renal parenchyma, or metastasizing to the regional lymph node or a distant site, pT3 and pT4).

Immunohistochemically, bcl-2 oncoprotein was recognized as positive in 29.2% of the patients. The immunoreactivity for bcl-2 oncoprotein was confined to the cytoplasm of tumour cells; it appeared frequently in the basal cell layer of tumours, and less frequently as diffuse

Table 1 Relationship between bcl-2 oncoprotein immunoreactivity and clinicopathological findings in 152 cases[a]

Correlative data	No. of cases	Immunoreactivity for bcl-2 oncoprotein (%)	*P*-value
Age			
<67	74	22.9	0.25
≥67	78	33.3	
Sex			
Male	111	28.8	0.73
Female	41	31.7	
Stage[b]			
Group B	58	37.9	0.044
Group C	24	33.3	
Group D	70	18.6	
Grade			
Low	92	33.6	0.17
High	60	23.3	
Pattern of growth			
Papillary	109	27.5	0.37
Non-papillary	43	34.8	

[a] Two of the 154 cases were excluded because of the difference in clinical course between nonpapillary non-invasive transitional cell carcinoma and other types

[b] The tumours were divided into three groups: Group B (papillary, non-invasive tumours, pTa); Group C (tumours invading the submucosa or muscularis, pT1 and pT2); Group D (tumors invading beyond the muscularis or renal parenchyma, or metastasizing the regional lymph node or a distant site, pT3 and pT4)

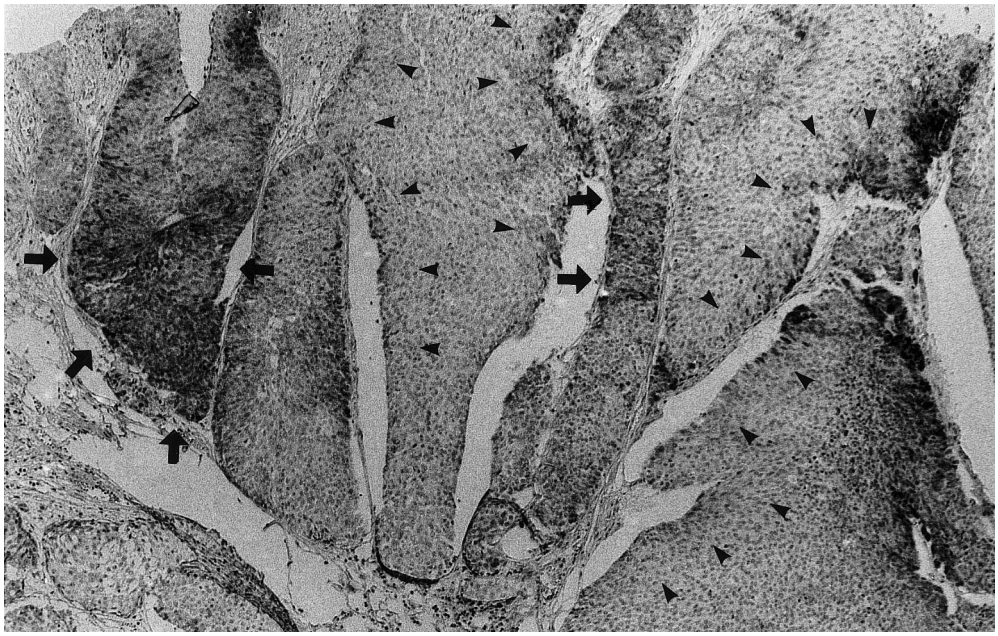

Fig. 1 Immunohistochemical staining for bcl-2 oncoprotein in transitional cell carcinoma of the upper urinary tract. Immunoreactivity for bcl-2 oncoprotein was seen frequently in the cytoplasm of tumour cells in the basal cell layer (*arrowheads*) and less frequently as diffuse staining in all cell layers (*arrows*) of tumours. A papillary, non-invasive tumour (pTa) is shown. Avidin–biotin peroxidase, × 240

Table 2 Relationship between bcl-2 oncoprotein immunoreactivity and proliferating cell nuclear antigen (PCNA) index and p53 oncoprotein immunoreactivity in 152 cases[a]

Correlative data	All tumours (*n*=152)			Noninvasive tumours (*n*=58)			Invasive tumours (*n*=94)		
	No. of cases	Immunoreactivity for bcl-2 oncoprotein (%)	*P*-value	No. of cases	Immunoreactivity for bcl-2 oncoprotein (%)	*P* value	No. of cases	Immunoreactivity for bcl-2 oncoprotein (%)	*P*-value
PCNA index									
<69%	76	36.8	0.050	36	41.6	0.45	40	32.5	0.041
≥69%	76	22.3		22	31.8		54	14.8	
p53 oncoprotein									
positive	42	30.9	0.82	10	40.0	0.88	32	28.1	0.33
negative	110	29.0		48	27.5		62	19.3	

[a] Two of the 154 cases were excluded because of the difference in clinical course between nonpapillary noninvasive transitional cell carcinoma and other types

staining in all cell layers (Fig. 1). In normal urothelium, bcl-2 immunoreactivity was restricted to the basal cell layer. In the analysis of the relationship between immunoreactivity for bcl-2 oncoprotein and clinicopathological findings, two cases of nonpapillary noninvasive TCC (carcinoma in situ: CIS) were excluded because of the difference between CIS and the other types of TCC in terms of clinical course.

The relationship between immunoreactivity for bcl-2 oncoprotein and clinicopathological findings was significant only for stage (Table 1). In fact, the proportion showing immunoreactivity for bcl-2 oncoprotein decreased progressively with stage, there being 37.9% in group B, 33.3% in group C, and 18.6% in group D ($P < 0.05$). However, the immunoreactivity for bcl-2 oncoprotein did show a borderline correlation with PCNA immunoreactivity ($P = 0.050$) (Table 2). Furthermore, when immunoreactivity for bcl-2 oncoprotein was analysed according to whether the tumour was invasive or noninvasive, immunoreactivity for bcl-2 oncoprotein was found to be correlated with the PCNA index in the invasive tumours ($P < 0.041$).

The rates for 5-year disease-free survival and for 5-year overall survival were 55.7% and 71.5%, respectively. The analysis of disease-free survival involved 145 patients without CIS who had no metastasis at surgery and in whom the malignant tumour was totally excised by surgery were included in this analysis. Overall survival was analysed in 152 patients without CIS. Univariate analyses of disease-free and overall survival rates in the above patients revealed that stage, grade, pattern of growth, immunoreactivity for p53 oncoprotein, and PCNA index all had a significant effect on the two survival rates, whereas the immunoreactivity for bcl-2 oncoprotein had no significant effect (Table 3). When immunoreactivity for bcl-2 oncoprotein was analysed according to whether the tumour was invasive or noninvasive, immunoreactivity for bcl-2 oncoprotein was found to be have no significant effect in either case. Next, only those variables that appeared significant in the univariate ana-

Table 3 Univariate analysis in 152 cases[a]

Prognostic indicator	All tumours				Non-invasive tumours				Invasive tumours			
	Overall survival (*n*=152)		Disease-free survival (*n*=145)		Overall survival (*n*=58)		Disease-free survival (*n*=58)		Overall survival (*n*=94)		Disease-free survival (*n*=87)	
	Wilcoxon	Log-rank	Wilcoxon	Log-rank	Wilcoxon	Log-rank	Wilcoxon	Log-rank	Wilcoxon	Log-rank	Wilcoxon	Log-rank
bcl-2	0.11	0.33	0.13	0.34	0.89	0.52	0.39	0.98	0.32	0.65	0.4	0.68
p53	0.10	0.023	0.016	0.0067	0.14	0.34	0.25	0.30	0.78	0.34	0.20	0.13
PCNA index	0.013	0.013	0.11	0.047	0.43	0.98	0.35	0.92	0.0067	0.077	0.41	0.22
Stage	0.0001	0.0001	0.0001	0.0001	–[b]	–[b]	–[b]	–[b]	0.044	0.035	0.19	0.12
Grade	0.0006	0.0024	0.0012	0.0026	0.59	0.59	0.57	0.57	0.090	0.22	0.14	0.22
Pattern of growth	0.0012	0.014	0.0048	0.023	–[b]	–[b]	–[b]	–[b]	0.34	0.93	0.56	0.90

[a] Two of the 154 cases were excluded because of the difference in clinical course between nonpapillary noninvasive transitional cell carcinoma and other types
[b] *P*-value was not determined because of one factor

lyses of overall survival (152 cases) and disease-free survival (145 cases), (i.e. stage, grade, pattern of growth, immunoreactivity for p53 oncoprotein, and PCNA index) were entered in the final models of the multivariate analysis. Only stage was a prognostic factor for either disease-free or overall survival (disease-free survival: risk ratio 3.35, $P < 0.0001$, overall survival: risk ratio 3.69, $P < 0.0001$).

Discussion

Although carcinoma of the upper urinary tract is a relatively rare neoplasm and accounts for only 2–6% of all urothelial tumours [1, 13], the stage and grade of this type of carcinoma have been reported to correlate with survival rate, as well as with the presence of urinary bladder cancer. The purpose of our investigation was to look for a possible relationship between the immunoreactivity for bcl-2 oncoprotein and clinicopathological findings or clinical outcome in TCC-UUT. Moreover, the correlation of this variable with PCNA index was to be tested, as was its prognostic significance. We observed a positive relationship between the immunoreactivity for bcl-2 oncoprotein and tumour stage. Furthermore, the correlation between the immunoreactivity for bcl-2 oncoprotein and PCNA index showed borderline significance ($P = 0.050$). In invasive TCC, immunoreactivity for bcl-2 oncoprotein was associated with PCNA immunoreactivity ($P < 0.041$). However, detection of the immunoreactivity for bcl-2 oncoprotein appears to be of no real value in determining the prognosis of TCC-UUT.

In the present study, cytoplasmic immunoreactivity for bcl-2 oncoprotein in more than 25% of the tumour cells was present in 29.2% of TCC-UUT patients. This incidence accords well with the figure (22.3%) given in a previously published report by Furihata et al. [10]. However, there are some discrepancies in the literature concerning the relationships between immunoreactivity for bcl-2 oncoprotein and clinical and clinicopathological findings. With regard to the relationship between the immunoreactivity for bcl-2 oncoprotein and clinicopathological findings in TCC, in one report dealing with 45 TCCs of the urinary bladder it was noted that the immunoreactivity for bcl-2 oncoprotein was significantly higher in poorly differentiated tumours (grade III, 2.02 ± 0.34 immunoreactive cells per 100 malignant cells) than in lower grade tumours (grade I, 1.11 ± 0.26; grade II, 0.86 ± 0.14) [14]. Furthermore, in the same study the number of apoptotic cells was investigated using in situ end-labelling of fragmented DNA, and the incidence of apoptosis was shown to increase with increasing tumour grade. On this basis, the authors suggested that the elevation of immunoreactivity for bcl-2 oncoprotein in poorly differentiated tumours pointed to a potential role for this apoptosis-suppressor protein in the progression of bladder cancer. However, this finding is in contrast with our finding that immunoreactivity for bcl-2 oncoprotein was present in 33.3% of patients (31 of 93) with low-grade TCC, whereas it was found in only 23.0% (14 of 61) of those with high-grade TCC. Although there is a significant difference between the two studies in terms of the way the immunoreactivity for bcl-2 oncoprotein was quantified, the reason for this discrepancy is not clear.

In gastric lymphoma [22] and in carcinomas of the lung [25], thyroid [30], breast [6] and stomach [17], a relative absence of immunoreactivity for bcl-2 oncoprotein has been found in poorly differentiated tumours compared with well-differentiated ones. This suggests that down-regulation of the oncoprotein occurs with progression in these tumours. In prostate cancer, however, immunoreactivity for bcl-2 oncoprotein was associated with high stage, poor prognosis and hormone-refractory disease [5, 19]. Similarly, in neuroblastoma, it was associated with poorly differentiated, high-grade histological-type tumours [6].

The role of bcl-2 oncoprotein is to protect stem cells and the renewal and repair capabilities of the epithelium [2, 26]. In the gastric mucosa, bcl-2 oncoprotein has been detected immunohistochemically in the proliferat-

ing zone [16]. In the urinary tract, bcl-2 oncoprotein is restricted in its topographical distribution to the basal layer of transitional cell epithelium. Furthermore, Furihata et al. reported that, in coexistent dysplastic lesions (premalignant lesions) adjacent to TCC-UUT, immunoreactivity for bcl-2 oncoprotein was detected more frequently in cases with a bcl-2 oncoprotein-positive tumour (66.7%) than in cases with a bcl-2 oncoprotein-negative tumour (50.7%) [10]. Therefore, they suggested that, while the immunoreactivity for bcl-2 oncoprotein may assist in the early detection of premalignant lesions, it is not specific to malignancy. In the present study, however, the immunoreactivity for bcl-2 oncoprotein was detected more frequently in the early stages than in the more advanced stages ($P < 0.05$).

Recent studies have associated both the *p53* gene and the *bcl-2* gene with the process of apoptosis. Furthermore, an inverse relationship between immunoreactivity for bcl-2 oncoprotein and immunoreactivity for p53 oncoprotein has been shown in malignant lymphoma [22] and in breast carcinoma [18]. In the present study, the immunoreactivity for bcl-2 oncoprotein was not related to the immunoreactivity for p53 oncoprotein.

Although there have been a number of studies on proliferative activity in TCC of the urinary bladder, there are only two reports [7, 8] on proliferative activity in TCC-UUT, and the results in them are mutually conflicting. One of them [7] was a study of proliferative activity carried out using antibodies against PCNA (PC10) and Ki-67 (MIB-1) in quite a large group of 58 TCC-UUT patients with relatively long follow-up periods (mean follow-up 7.4 years). That study concluded that proliferative activity was not an independent prognostic factor (as also implied by the present study). However, the other report [9], which dealt with 67 TCC-UUT patients, concluded that Ki-67 (MIB-1) was an independent prognostic factor.

In conclusion, immunoreactivity for bcl-2 oncoprotein was associated with favourable prognostic factors, such as early stage and low PCNA index at presentation. However, the detection of immunoreactivity for bcl-2 oncoprotein appears to be of no real value in determining the prognosis of TCC-UUT. Since bcl-2 oncoprotein is one of the apoptosis-related proteins, future studies should examine the relationship between apoptosis-related proteins and apoptosis.

Acknowledgments The authors are indebted to Dr. K. Kageyama, Dr. I. Hirano, Dr. I. Nagakubo and Dr. T. Fujioka, Mutual Aid Associations' Hospital, Tachikawa, and Dr. S. Tamai, the Department of Laboratory Medicine, National Defense Medical College for providing materials; to Mr. H. Kasamatsu, the Central Research Laboratory, National Defense Medical College for statistical analysis of prognoses; and to Dr. R. Timms for correcting the English.

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