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Bax to Bcl-2 Ratio and Ki-67 Index are Useful Predictors of Neoadjuvant Chemoradiation Therapy in Bladder Cancer

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Objective: In this study, locally advanced bladder cancer was treated by radiation combined with cisplatin therapy and a retrospective analysis was conducted to predict the clinical response to chemoradiotherapy (CRT) based on the immunohistochemistry of apoptosis-related proteins.

Methods: Sixty-two patients (median age, 68 years; range, 45–89 years) with transitional cell carcinoma of the bladder (pT1G3–pT4M0) treated with CRT (median dose: 40.5 Gy of radiation and 230 mg of cisplatin) were studied. Mucosal biopsy was performed before and after CRT. Paraffin-embedded tumor specimens were examined with TUNEL and were immunostained for Ki-67, p53, Bcl-2 and Bax; the Bax/Bcl-2 ratio and apoptosis index (AI) were calculated. Clinical features of the patients and response to CRT were compared with data obtained from examination of the tumors.

Results: The 62 patients had a median follow-up period of 34 months (range, 3–84 months). Responses to CRT were as follows: CR, 34%; PR, 45%; NC, 21%. The survival rate of patients with Ki-67-positive tumors was significantly lower than those of patients with Ki-67-negative tumors ($P < 0.05$). No significant correlation was observed between the expression of any protein, the AI and the clinical response. However, the Bax/Bcl-2 ratio showed a significant association with the CR rate ($P = 0.0289$).

Conclusions: The results of this study suggest that the combined assessment of Bcl-2 and Bax protein expression may be used to predict a clinical response to CRT based on the Bax/Bcl-2 ratio determined before therapy. The Ki-67 index may be a useful predictor of prognosis in patients treated by CRT.

Key words: bladder cancer – chemoradiotherapy – apoptosis – immunohistochemistry – Bax/Bcl-2 ratio

INTRODUCTION

The initial treatments for invasive bladder cancer can be divided into bladder-sparing therapy and radical cystectomy. Although radical cystectomy is the standard treatment, a variety of adjuvant therapies have been proposed to induce the down-staging of tumors and to improve survival. During the last three decades, the most commonly used bladder-sparing treatment has been external beam radiation therapy (1). Studies on systemic chemotherapy combined with radiation therapy prior to surgery have shown that it can achieve bladder preservation by transurethral resection (TUR) or partial cystectomy in 52 to 70% of patients with invasive bladder cancer (2–6). In this study, locally advanced bladder cancer was treated by radiation combined with cisplatin using a modified version of the method developed by Shipley et al. (1). Although several tumors have shown a good response to this treatment, several others have been resistant. This therapy could be potentially harmful as well as unnecessary for patients with non-responding tumors. Thus, predicting the likely response of bladder cancer to this treatment would be helpful in selecting the best treatment option for each patient.

Several studies have shown that apoptosis is an important mechanism of cell death after exposure to DNA-damaging agents such as cisplatin and radiation (7–12). As a transcriptional activator, p53 increases the transcription of several downstream genes, and its regulation of transcription is critical in determining the cellular response to DNA damage (13). It is known that Bax and Bcl-2 regulate apoptosis downstream of p53 (14). Bcl-2 blocks cell death following various stimuli, demonstrating a death-sparing effect (15); however, overexpression of Bax has a pro-apoptotic effect and Bax also counters the anti-apoptotic activity of Bcl-2 (16). It has been proposed that the ratio of Bcl-2 to Bax or other members of the

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Table 1. Patient characteristics

Characteristics	No. of patients	(%)
All patients	62	
Sex		
Male	47	(75.8)
Female	15	(24.2)
Age		
Median	68	
Range	45–89	
Histopathology		
Transitional cell carcinoma	62	(100)
Pathological grade		
G2	15	(19.4)
G3	53	(80.6)
Clinical stage		
T1, G3	12	(19.4)
T2	20	(32.2)
T3	25	(40.3)
T4	5	(8.1)
Lymph node metastasis		
N(+)	1	(1.6)
N(–)	61	(98.4)
Median follow up period (months)	34	

Bcl-2 family may govern the sensitivity of cells to apoptotic stimuli (17,18). Ki-67 recognizes a nuclear protein forming part of the DNA replicase complex (19) and is widely used to determine the proliferative activity of tumors.

Recent studies have suggested that the indexes for p53, Bcl-2, Bax and Ki-67 may correlate with the grade, stage or prognosis of bladder cancer (20). Although these markers are correlated with the response to radiation therapy or chemotherapy in various types of cancers, the relationship between these markers and the clinical response to radiation combined with cisplatin is unknown for bladder cancer.

In this study, we immunohistochemically investigated the relationship between p53, Bcl-2, Bax or Ki-67 expression by bladder tumors and the clinical response to chemoradiotherapy (CRT) in patients with transitional cell carcinoma (TCC) of the bladder.

SUBJECTS AND METHODS

Clinical Characteristics and Tissue Samples

The cohort that was studied consisted of 62 patients (median age, 68 years; range, 45–89 years) who underwent preoperative chemoradiotherapy (CRT) for locally invasive (T2-4N0M0) or high-risk superficial (pT1G3) bladder cancer at the Department of Urology, Yamaguchi University School of Medicine, between November 1994 and August 2000 (Table 1). Prior to treatment, we performed a cystoscopy, random mucosal biopsy and a systemic work-up for tumor staging on CT scanning for all patients. Tumors were histopathologically graded as per the WHO classification and were staged as per the TNM staging system of the UICC (1992). All the patients received preoperative chemotherapy with cis-diamino-dichloro-platinum (CDDP) intravenously and radiotherapy. The regimen (based on the method by Shipley et al.) was CDDP (70 mg/m^2) on day 1, with radiation administered from day 2 by Liniac to the true pelvis at 1.8 grays (Gy) per fraction on five consecutive days per week. This therapy was repeated at 14-day intervals, and between one and three cycles of therapy. On the completion of one cycle of CRT, the patients were treated with 70 mg/m^2 of CDDP and 16.2 Gy of irradiation to the target tumor cells. Although we attempted to perform three cycles of CRT when possible, we stopped the CRT treatment for those patients who received one or two cycles of the therapy and suffered from persistent side effects such as nausea, vomiting, diarrhea, pancytopenia, etc. for 2 weeks or for those patients who refused to continue with CRT. At 4 weeks after the completion of CRT, the patients were assessed for response and stage by cystoscopy, random biopsy or transurethral resection (TUR) and CT scanning. Patients in whom no change was observed or those with residual tumor cells in the muscle layer were referred for radical or partial cystectomy with lymph node dissection, while patients with responding tumors underwent complete resection of the tumor by TUR. Six patients with only residual carcinoma *in situ* (CIS) were followed up with intravesical instillation of Mitomycin C (MMC) or BCG. The clinical response to CRT was classified as follows: CR, no residual tumor in the bladder (pCR) and no evidence of nodal or visceral metastasis; PR, down-staging of the tumor with a greater than 50% decrease in the bulk of the initial invasive tumor with no evidence of distant or nodal spread on CT scanning; NC, persistent invasive disease that was not downsized by CRT with or without evidence of nodal or distant spread. Treatment-related toxicities were evaluated as per the WHO criteria. Clinical examination of each patient was performed every month for the first 2 years, every 3 months from the third to fifth year and 6 months thereafter. Cystoscopic examination of each patient was carried out every 3 months and complementary examinations, including chest X-rays and/or CT scans, were carried out every 6 months. Recurrence was observed in nine patients after pCR and local recurrence (CIS developed cases) was observed in three patients. Two patients were followed up by BCG instillation and one was followed up by MMC. While other cases had progressive disease (local invasive disease or distant metastases), pelvic exenteration was performed in one patient, M-VAC chemotherapy was administered in two patients, nephroureterectomy was performed for one patient and no treatment was administered in two patients.

Immunohistochemistry (IHC)

Biopsy and TUR specimens were selected from the main tumors (eligible for pathological findings and imaging by CT scanning). Immunohistochemistry was performed on routinely processed, formalin-fixed paraffin-embedded tissues using an avidin–biotin complex immunoperoxidase technique. Serial tissue sections (5 μm) were cut from selected blocks of representative tumor tissue and mounted on poly-L-lysine-coated slides, baked at 50°C for 1 h, dewaxed with xylene and rehydrated through a series descending from alcohol to distilled water. These sections were then immersed into a 10 mM citrate buffer (pH 6.0) and heated in a water bath at 98°C for 30 min. The heated sections were allowed to cool at room temperature for 20 min and then washed in running water. After the target retrieval, the sections were treated with 3% hydrogen peroxide in methanol for 15 min for blocking endogenous peroxidase activity. After blocking with horse serum at room temperature for 10 min, the primary antibodies were applied at 4°C in a humidified chamber. Ki-67, p53 and Bcl-2 protein expression were detected with undiluted rabbit polyclonal anti-human Ki-67 antibody and mouse monoclonal antibodies (DO-7 and clone 124) (Dako Corporation, Carpentaria, CA). For the detection of Bax protein, a rabbit polyclonal anti-human Bax (N-20) antibody was employed at a dilution of 1:100 (Santa Cruz Biotechnology, Santa Cruz, CA). After washing, immunostaining was performed using the Vectastain Universal Quick Kit (Vector Laboratories, Burlingame, CA) and diaminobenzidine (Dako Laboratories, Carpentaria, CA) as a chromogen. Sections were counterstained with Mayer's hematoxylin. A p53-positive prostate cancer that was known to show a positive nuclear reaction for p53 was used as the positive control. A normal tonsil was used as a positive control for Bcl-2 and Bax. Immunostaining of the duplicate sections was used as a negative control in the absence of the primary antibody.

Detection of Apoptosis

Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) was performed for the detection of apoptotic cells using an Apoptosis *in situ* Detection Kit (Wako, Osaka, Japan) as per the manufacturer's instructions. Slides were developed in the chromogen substrate and counterstained with methyl green. Sections that were treated with DNase prior to the TdT reaction were used as the positive control, and sections processed in the absence of TdT were used as the negative control. Two observers (H.M. and T.W.) examined all the immunostained and TUNEL sections blinded to the clinical data. The entire section was screened in order to locate the area with the highest staining intensity, and any nonspecific staining was avoided. Specimens were considered positive for p53 IHC when more than 20% (20) of the nuclei were positively stained, while specimens were reported positive for Bcl-2 or Bax when more than 1% (20) or 20% of tumor cells were positively stained, respectively. Infiltrating lymphocytes served as an internal positive control for Bcl-2 or Bax staining. The apoptotic index (AI) and the Ki-67 labeling index (KI) were calculated as the percentage of positive tumor nuclei divided by the total number of tumor cells examined. At least 1 000 tumor cells per specimen were examined in five randomly selected fields by light microscopy (×400). For the statistical analysis, tumors with a KI value of 21% or more were classified as having a high KI value and tumors with a KI value lower than 21% were classified as having a low KI value. The cut-off value of 21% was based on the median value for KI. For AI, a cut-off value of 1% was set based on the same criteria.

Statistical Analyses

Correlations of p53, Bcl-2 and Bax immunoreactivity or the KI and AI values with tumor grade, stage and response to CRT were assessed using the chi-square test, Fisher's exact test or the Kruskal–Wallis rank test. The Bax/Bcl-2 ratio was compared with the response to CRT using the Mann–Whitney U test or Student's *t*-test (two-sided) by analysis of covariance. Survival after CRT was analyzed by the Kaplan–Meier method with the log-rank test as the univariate analysis. In the Bax/Bcl-2 ratio, the survival rate analyzed by two categories that consisted of values either higher or lower than the median value (8.23) of the Bax/Bcl-2 ratio (range, 0.9–178) were calculated. Multivariate analysis was performed with regard to p53, Bcl-2 and Bax immunoreactivity or the KI and AI values and the Bax/Bcl-2 ratio using Cox's proportional hazard regression test in a step-wise mode. Data were processed using JMP 4.0 statistical software with a $P < 0.05$, thus indicating statistical significance.

RESULTS

The 62 patients who received CRT and had a median follow-up period of 33.8 months (range, 2.9–84.2 months) were evaluated. The average total dose of CDDP and radiation was 230 mg (range, 75–375 mg) and 40.5 Gy (30–61.2 Gy), respectively. Treatment-related toxicity was mild, and over grade 3 anemia, leucocytopenia, thrombocytopenia, nausea, vomiting and diarrhea were not observed. The response to CRT was CR in 21 patients (34%), PR in 28 (45%) and NC in 13 (21%). Recurrence was noted in 21 (34%) patients, including 10 patients with progressive disease. Unfortunately, 15 patients had cancer related deaths. The treatment response was evaluated in order to ascertain whether CR was achieved and whether the tumor stage and grade were related to the response.

Although positive p53 IHC, which was identified in 21 patients (34%), was associated with the tumor grade ($P = 0.005$) or stage ($P = 0.0454$) (Table 2), it did not correlate with the response ($P = 0.8964$) (Table 3) or recurrence within 1 year after treatment ($P = 0.3608$).

Positivity for Bcl-2 was detected in 33 cases (53%); however, it was not related to tumor grade ($P = 0.4666$), tumor stage ($P = 0.4304$) or the response to treatment ($P = 0.6008$). In

Table 2. Correlation to histopathological grade and clinical stage with immunohistostaining of p53, Bcl-2, Bax, Ki-67 index, apoptotic index and Bax/Bcl-2 ratio in pretreatment specimens

Parameter[1]	Category	Grade			Stage				
		G2	G3	*P* value[2]	T1	T2	T3	T4	*P* value[2]
p53	Positive	0	21		0	8	11	2	
	Negative	12	28	0.005	12	12	13	3	0.0454
Bcl-2	Positive	8	38		7	15	20	4	
	Negative	4	11	0.4666	5	5	4	1	0.4304
Bax	Positive	2	25		6	12	9	0	
	Negative	9	24	0.0908	6	8	14	5	0.0958
Ki-67 index (KI)	Positive	3	29		3	10	16	3	
	Negative	9	20	0.0522	9	10	8	2	0.116
Apoptotic index (AI)	Positive	3	21		4	7	11	2	
	Negative	9	28	0.3337	8	13	13	3	0.858
Bax/Bcl-2 ratio				0.012					0.0159

[1]61 or 60 cases were studied. The cases of difficult evaluation for p53, Bcl-2, Bax immunostaining or KI and AI were excluded; [2]p53, Bcl-2, Bax, Ki-67 index, apoptotic index: chi-square test. Bax/Bcl-2 ratio: Kruskal–Wallis test or Mann–Whitney U test; *P* value less than 0.05 indicating statistical significance.

the case of Bax expression, Bax positivity was observed in 27 (44%) cases and was not associated with tumor grade (P = 0.0908), stage (P = 0.0958) or response (P = 0.0534). In 60 patients in whom both Bcl-2 and Bax could be evaluated, neither Bcl-2 nor Bax expression correlated with recurrence within 1 year after CRT.

We then assessed whether the Bax/Bcl-2 ratio was correlated with clinical parameters and positive p53 IHC in the 60 patients with complete IHC data. Significant correlations were observed between Bax/Bcl-2 ratio and CR rate (P = 0.0289) (Table 3), as well as histological grade (P = 0.0120) or clinical stage (P = 0.0159) (Table 2). Among the 60 patients, a high (>8.23) and low (<8.23) Bax/Bcl-2 ratios were considered to be 30 (50%) and 30 (50%), respectively. Thirteen of the 30 patients (43%) with positive p53 IHC showed a higher Bax/Bcl-2 ratio, while eight patients (27%) showed a lower Bax/Bcl-2 ratio. Among the 39 patients without p53 IHC, higher and lower Bax/Bcl-2 ratios were 17 (44%) and 22 (56%), respectively. The Bax/Bcl-2 ratio did not have a significant relationship with p53 IHC (P = 0.3017). Moreover, a higher Bax/Bcl-2 ratio combined with negative p53 IHC showed no significant correlation with the CR rate (P = 0.2182).

Table 3. Correlation to pathological response with immunohistostaining of p53, Bcl-2, Bax, Ki-67 index, apoptotic index and Bax/Bcl-2 ratio in pretreatment specimens

Parameter[1]	Category	CR	PR + NC	*P* value[2]
p53	Positive	7	14	
	Negative	14	26	0.8964
Bcl-2	Positive	15	31	
	Negative	6	9	0.6008
Bax	Positive	13	14	
	Negative	8	25	0.0534
Ki-67 index (KI)	Positive	13	19	
	Negative	8	21	0.2844
Apoptotic index (AI)	Positive	6	18	
	Negative	15	22	0.2120
Bax/Bcl-2 ratio				0.0289

[1]61 or 60 cases were studied. The cases of difficult evaluation for p53, Bcl-2, Bax immunostaining or KI and AI were excluded; [2]p53, Bcl-2, Bax, Ki-67 index, apoptotic index: chi-square test. Bax/Bcl-2 ratio: Kruskal–Wallis test or Mann–Whitney U test; *P* value less than 0.05 indicating statistical significance.

A higher AI value (>1%) was observed in 24 (39%) tumors; however, it was not associated with tumor grade (P = 0.3337), stage (P = 0.8580) or response (P = 0.2120). A higher KI (>21%) was noted in 32 (53%) tumors, and this showed a weak correlation with tumor grade (P = 0.0522), stage (0.1160) and response (P = 0.2844) (Table 2 and Table 3). No significant correlations were noted between the expression of any protein (p53, Bcl-2 or Bax) combined with AI or KI and the clinical response.

The cause-specific survival rate of Ki-67 positive patients was significantly lower than that of negative patients (P = 0.0148) (Fig. 1). However, no significant difference was noted between the cause-specific survival rate of patients with positive p53 IHC (P = 0.8754), Bcl-2 expression (P = 0.7338), Bax expression (P = 0.7144), AI (P = 0.5507) and Bax/Bcl-2 ratio (P = 0.9347). Clinical grade and stage showed no significant correlation with the cause-specific survival rate. After follow up, no significant difference was noted in cause-specific survival depending on the response to treatment (CR vs non-CR, P = 0.1850). We performed a multivariate analysis regarding the immunohistochemical parameters using Cox proportional

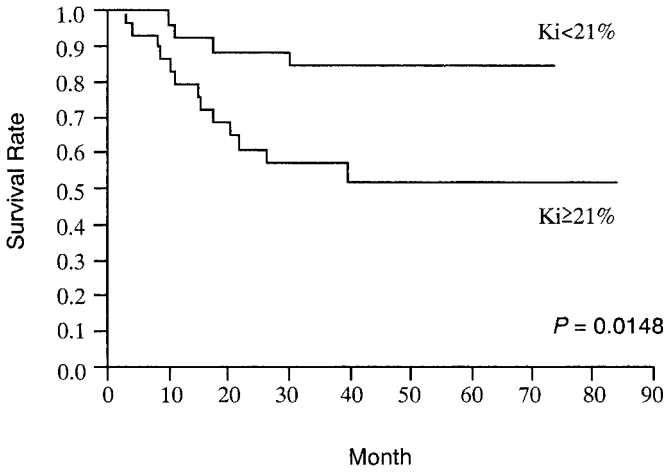

Figure 1. The cause-specific survival rate of Ki-67 index (KI). Survival rate was analyzed using the Kaplan–Meier method with Log rank test as univariate analysis. *P* value less than 0.05 indicating statistical significance.

hazards tests. No strong correlations were observed among the parameters used. The KI (hazard ratio: 5.76, 95% CI: 1.42–38.44, $P = 0.0126$) was an independent predictive factor for cancer-specific survival. The Bax/Bcl-2 ratio was not available as a predictor of survival (data not shown).

DISCUSSION

During the last decade, multimodal organ-sparing treatment has become a standard treatment for urological malignancies in many countries (21). External beam radiation does not achieve local control of invasive bladder cancer with a frequency that is high enough to warrant its use as a single modality; however, it can be combined with chemotherapy to achieve greater effectiveness. While it is true that neither transurethral resection of the tumor nor chemotherapy or radiation alone can achieve significant local tumor control, it is clear that the combination of those three treatments is highly effective in carefully selected patients (21). Tri-modality therapy with transurethral resection, radiation and concurrent systemic chemotherapy followed by bladder preservation in patients with complete remission and prompt cystectomy in patients with persistent or recurrent invasive tumors results in a similar overall survival rate to that of immediate cystectomy (21). Certain cytotoxic agents, particularly CDDP and 5-fluorouracil (5-FU), are capable of sensitizing tumor tissues to radiation; thus, increasing cell killing in a synergistic fashion. We recently performed combined therapy of radiation and concurrent intravenous CDDP followed by complete transurethral resection in over 70 patients with invasive bladder cancer. A follow-up study showed a CR rate of approximately 30%, and we achieved relatively good bladder preservation. This figure appears lower than that of other reports in which a high dose of CDDP with 60 Gy of radiation therapy yielded 60–75% of CR (1). Such a discrepancy may result from differences in patient characteristics and pathological background, as well as varying doses of CDDP and radiation. When CR cases were compared with non-CR cases in terms of CDDP and radiation doses, age and concomitant CIS, no significant difference was observed in CDDP and radiation doses and age and concomitant CIS between different responders (data not shown). We considered the reasons for which the response to CRT was not associated with the survival of the patients. First, probably the duration of the observation period was insufficient, and the number of cases in our study was less. We may have achieved a significant difference in the cause-specific survival depending on the response to treatment (CR vs non-CR), if the follow-up period was longer and larger number of cases were examined. Second, in the follow-up period, while we obtained relatively good local disease control for the patients who did not achieve CR, for example, with regard to downstage to T1, it was obtained for the residual CIS only. If the patients did not have fatal metastases, our bladder preservative therapy might have contributed to the extension of survival in patients with dormant tumors. Third, some patients had late recurrences (local or distant) in the CR cases, because the bladder was preserved and there was a possibility of invisible micrometastasis before therapy.

Therefore, we consider that the Bax/Bcl-2 ratio showed no association with survival either. However, it might be possible to show a significant correlation of the Bax/Bcl-2 ratio with survival if three of the above mentioned problems had been overcome.

The pretreatment AI is related to post-treatment programmed cell death, cell survival and tumor control. Recently, several authors have investigated the role of the pretreatment apoptotic index in predicting the clinical response to radiation (22). The M. D. Anderson Group showed that a high pretreatment tumor apoptotic AI was related to a better immediate response to preoperative radiation therapy in patients with bladder cancer (23). However, the pretreatment AI was not significantly associated with the clinical response in our study. There could be two reasons for this discrepancy. First, several tumors with a low AI as well as tumors with a high AI due to the radiosensitizing effect of CDDP may have also shown a good response to CRT. Second, apoptotic cells might have been rapidly destroyed by phagocytes and only some of these cells may have been detected by the TUNEL method.

In recent reports, several immunohistochemical markers such as tumor suppressor gene p53, anti-apoptotic factor Bcl-2, death agonist Bax and proliferation marker Ki-67 have shown to be related to the recurrence of bladder cancer (20,24). Although p53, Bcl-2 and Bax were reported to show a significant relationship with the response to CDDP-based chemotherapy (25), to the best of our knowledge the existence of an association between these proteins and the response of bladder cancer to CRT has not been reported.

The p53 tumor suppressor gene shows the most common mutation in human cancer, and is recognized as an important component of the pathway leading from DNA damage to apoptosis. Several studies have suggested that p53 is involved in the activation of apoptosis induced by DNA-damaging anticancer agents (13). A recent analysis showed that a lack of p53 over-

expression or negative p53 and positive p21 immunostaining was associated with a favorable response from advanced bladder cancer to CDDP-based neoadjuvant chemotherapy (26). In the present study, negative p53 staining (no mutation) alone was not associated with the response to CRT. This suggests that radiation might activate several apoptotic pathways other than those induced by p53 activation.

Upregulation of Bax through transactivation by p53 could play an important role in apoptosis, although its mechanism remains unclear (27). Bax suppresses Bcl-2 expression and the extent of p53 suppression of Bcl-2 expression may be tissue-specific. Bax is a Bcl-associated protein encoded by the Bcl-2 gene family, including Bcl-XL, Bad, etc. (9, 28). Over-expression of Bax accelerates apoptotic death and Bax also counters the death repressor activity of Bcl-2.

Recent studies have suggested that the Bcl-2 to Bax ratio may be a marker for early relapse and progression of superficial or low grade bladder cancer (24). Our results suggest that the Bax/Bcl-2 ratio could be used to predict the response to CRT by analysis of pretreatment biopsy specimens from bladder tumors. Ye et al. (24) evaluated the expression of Bcl-2 and Bax by superficial bladder cancers treated with intravesical chemotherapy after resection and suggested that the p53 gene status may contribute to the response to chemotherapy and sensitivity to apoptosis by a pathway associated with endogenous Bcl-2/Bax or another unrelated pathway. Han et al. (29) reported that apoptosis can be induced in X-irradiated leukemia HL-60 cells by a p53-independent mechanism at the G2 checkpoint, despite the presence of endogenous Bcl-2. Several anticancer agents induce apoptosis by upregulation of Bax in some cancer cells through a p53-independent pathway (30,31).

The Ki-67 antibody had been reported as a useful marker of proliferative activity and a prognostic indicator in bladder cancer (19,32). A high Ki-67 index implies that the tumor has a high growth fraction and rapid growth. Therefore, tumors with high Ki-67 indexes indicate a poor prognosis. It has been shown that the Ki-67 index in grade 3 tumors was marginally higher than that in the grade 2 tumors ($P = 0.0522$); however, the Ki-67 index was not associated with the clinical stage. Various Ki-67 cut-off values have been used for bladder cancer. Our cut-off value (21%), which was set on the basis of the median value of the Ki-67 positive cells, can be justified by the result that the Ki-67 index was the only prognostic factor for our treatment. The usefulness of Ki-67 for predicting local control after CRT is still largely unknown. Several authors have noted higher local control rates in patients with squamous cell carcinoma of the head and neck or cervix that had a high Ki-67 labeling index (33,34) after radiation therapy. On the contrary, other authors have found better local control rates for slowly proliferating tumors of the head and neck (35). An immediate response to irradiation could be an important factor for achieving local control. Lara et al. obtained better local control with 66 Gy of radiotherapy for bladder cancer showing a low Ki-67 index (36). In our study, the Ki-67 index was not directly correlated with the immediate response of bladder cancer to CRT. With regard to AI, this result may have been obtained due to tumor heterogeneity causing a mixed response to CRT.

In conclusion, two significant correlations were obtained in this study: one between the relative expression of Bcl-2 and Bax proteins and the response to CRT, and the other between the Ki-67 index and cancer-specific survival. These results suggest that the Bax/Bcl-2 ratio and the Ki-67 index may be useful predictors of the effects of CRT and of patient prognosis, respectively, in bladder cancer. Data on a larger number of patients treated with CRT is required in order to validate the preliminary results of our study.

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