Ki-67, p53, Bcl-2 and Bax Expression in Urothelial Carcinomas of Urinary Bladder

Mesanenin Ürotelyal Karsinomlarında Ki-67, p53, Bcl-2 ve Bax Ekspresyonu

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This study has been supported by PAUBAP (project no: TPF 11).

ABSTRACT

Objective: The most important predictive parameter for the biological behavior of urothelial carcinomas except depth of invasion is the histological grade of the tumor. However, crucial discordances arise between pathologists because of subjectivity in histological grading schemes and these discordances cause difficulties especially in low-grade tumors. The number of studies aiming to predict the risk of recurrence and progression in urothelial carcinomas has therefore increased in recent years. Our aim in this study was to evaluate the relation of histopathological and clinical characteristics of urothelial carcinomas with proliferation and apoptosis markers and to determine predictive parameters for their biological behavior.

Material and Method: This study included 84 previously diagnosed cases of urothelial carcinoma of the urinary bladder. Immunohistochemical expressions of Ki-67, p53, Bcl-2, and Bax were examined in each case.

Results: Expressions of Ki-67 and p53 determined a significant relationship between pathological stage and histological grade of the cases. There was no significant relationship between the other apoptotic markers (Bcl-2, and Bax) and clinical or morphological parameters.

Conclusion: We concluded that the evaluation of Ki-67 and p53 expression combined with pathological stage and histological grade may give more accurate information about the biological behavior of urothelial carcinomas of the urinary bladder.

Key Words: Bladder cancer, p53, Ki-67, Bcl-2, Bax

ÖZ

Amaç: Ürotelyal karsinomlarda invazyon özelliği dışında, bu tümörlerin biyolojik davranışlarını önceden tahmin etmemizi kolaylaştıracak en önemli parametre, tümörün histolojik derecesidir. Ancak histolojik derecelendirme modellerinde, subjektiviteye açık oluşları nedeniyle patologlar arasında ciddi uyumsuzluklar ortaya çıkmakta ve özellikle düşük dereceli tümörlerde bu uyumsuzluk sorun oluşturmaktadır. Bu nedenle, ürotelyal karsinomların rekürrens ve progresyon risklerini öngörmeye yönelik olarak farklı parametre arayışları son yıllarda artarak sürmektedir. Bu çalışmada amacımız, ürotelyal karsinomlarda morfolojik ve klinik verilerle hücre proliferasyonu ve apoptozis ilişkili belirleyiciler arasındaki korelasyonu araştırarak tümörlerin biyolojik davranışına yönelik parametreler ortaya koymaktır.

Gereç ve Yöntem: Histopatolojik olarak değerlendirilerek mesanede ürotelyal karsinom tanısı almış 84 olgu çalışmaya alınmıştır. Her olguda immünhistokimyasal yöntemle Ki-67, p53, Bcl-2 ve Bax ekspresyonları değerlendirilmiştir.

Bulgular: Olguların patolojik evre ve histolojik derecesi ile Ki-67 ve p53 ekspresyonları arasında anlamlı ilişki olduğu saptanmıştır. Diğer apoptotik belirleyiciler ile klinik ve morfolojik parametreler arasında anlamlı ilişki gözlenmemiştir.

Sonuç: Ürotelyal karsinomlarda patolojik evre ve histolojik derece ile Ki-67 ve p53 ekspresyonunun kombine edilerek değerlendirilmesinin tümörün biyolojik davranışı konusunda daha doğru bilgi verebileceği sonucuna varılmıştır.

Anahtar Sözcükler: Mesane kanseri, p53, Ki-67, Bcl-2, Bax

INTRODUCTION

Approximately 80% of bladder urothelial carcinomas (UC) are noninvasive (pTa) tumors. These lesions recur frequently but have a good prognosis. The remainder consists of invasive (pT1-4) tumors with high rate of recurrence and progression as well as aggressive clinical behavior (1,2). It is therefore quite important to decide on whether UCs have invasive features to determine their clinical behavior and the choice of treatment. In addition, if noninvasive tumors that will not show progression may be identified the number of cases not included in the clinically aggressive group will increase and these patients will avoid

Received : 22.06.2009
Accepted : 29.07.2009

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unnecessary treatment. The greatest expectation from the large number of studies in recent years is the identification of parameters with proven predictive value that can define the risk of progression and recurrence.

Conflicting results have been obtained regarding the effect of Ki-67, p53, Bcl-2 and Bax proteins on the prognosis of bladder UCs due to the lack of standards in patient selection, staining protocol and threshold values (3-8). There is also limited information on the expression of Ki-67, p53, Bcl-2 and Bax in UCs graded according to the 2004 World Health Organization (WHO) classification (1,8).

Our aim in this study was to evaluate the relationship between the histopathological as well as clinical characteristics of UC (pathological stage, histological grade, multifocality, tumor size, papillary framework, and presence of carcinoma in situ) and proliferation marker Ki-67 and apoptosis markers p53, bcl-2, bax and to find out whether these parameters can be used independently or interdependently to predict the biological behavior of these tumors.

MATERIAL and METHOD

A total of 84 cases that had been investigated histopathologically at the Pamukkale University Medical Faculty Department of Pathology between April 1996 and June 2003 and received a diagnosis of "UC" were included in the study. Hematoxylin-&eosin stained sections of all cases were re-evaluated and graded and staged according to the 2004 WHO classification and 2002 TNM revision, respectively (2). They were also re-evaluated whether papillary framework or carcinoma in situ were present. The clinical information of the patients was obtained from their pathology reports and patient charts.

One paraffin block best represented the tumor tissue was selected in all cases and 4-5 µm-thick sections were obtained on poly-L-lysine coated slides for immunohistochemical staining. The endogenous peroxidase activity of deparaffinized and rehydrated sections was suppressed by 3% hydrogen peroxide solution. The sections were put in a pH=7.3, 10 mM citrate buffer solution into a microwave (700 Watt) 3 times, 10 minutes each, for Ki-67 and bcl-2, and 6 times, 10 minutes each, for p53 and bax. The sections taken from the microwave were washed for 10 minutes in PBS solution. All the following procedures were performed with the automatic method (Ventana, USA). The primary antibodies used for immunohistochemical staining (with clone, manufacturer, dilution, incubation period and positive control) were as follows: Ki-67 (MB67, Neomarkers, USA, 1:100, 30 minutes, tonsil), p53 (DO-7, Neomarkers, USA, 1:100, 60 minutes, high grade urothelial carcinoma), Bcl-2 (100/D5, Neomarkers, USA, 1:100, 30 minutes, follicular lymphoma), Bax (2D2, Neomarkers, USA, 1:25, 30 minutes, Hodgkin's lymphoma).

When immunohistochemical staining was evaluated, the whole section was scanned at the 10x magnification of the microscope (Nikon E200) in each case and the tumor areas with the densest positive staining and the thinnest section were chosen. 40x magnification was then used to calculate the rate of positive staining cells in 1000 tumor cells. Nuclear staining was considered for Ki-67 and p53, and cell membrane and cytoplasmic staining for bcl-2 and bax. The threshold values used for statistical analysis were as follows: Ki-67, 13% (negative: <13%; positive: ≥13%); p53, 20% (negative: <20%; positive: ≥20%); bcl-2, 1% (negative: <1%; positive: ≥1%); Bax, 20% (negative: <20%; positive:≥20%)

Statistical analysis of the data was performed by the SPSS 10.0 statistical package software (SPSS Inc., Chicago, IL, USA) using the Chi-square test.

RESULTS

The age range was 40 to 84 (mean±SD: 65.15±11.36) with 75 males and 9 females. All specimens were from transurethal resections (TUR). Re-grading according to the 2004 WHO classification revealed 2 cases of papillary urothelial neoplasm of low malignant potential, 26 low-grade noninvasive UCs, 2 high-grade noninvasive UCs, 10 low-grade invasive UCs and 44 high-grade invasive UCs. Pathological staging revised according to the 2002 TNM revision revealed 30 noninvasive (pTa) and 54 invasive (32 of pT1 and 22 of pT2) cases. Grouping the cases in high- and low-grade histological grades revealed 38 low-grade and 46 high-grade UCs. Of the noninvasive (pTa) cases, 28 were low-grade and 2 high-grade, while of the invasive (pT1 and pT2) cases 10 were low-grade and 44 high-grade. The number of tumors was known in 77 cases with 32 single and 45 multiple tumors. The tumor size was determined in only 42 cases; 25 were smaller than 3 cm and 17 were 3 cm or larger. 43 cases displayed papillary appearance while 41 were non-papillary. All non-papillary cases had a solid growth pattern. Accompanying foci of carcinoma in situ were present in 9 cases. One low grade and 8 high grade invasive carcinomas (5 pT1 and 4 pT2) harbored carcinoma in situ. One was papillary and 8 were non-papillary in appearance.

Considering pathological stage and histological grade, histological grade correlated with increasing stage (p=0.000).

Table I summarizes the statistical comparison of Ki-67, p53, Bcl-2 and Bax expressions and the clinical and morphological findings. Ki-67 expression was found in 21 (25%) cases (Figure 1). A correlation was present between Ki-67 expression and pathological stage and histological grade (p=0.010; p=0.005, respectively). Ki-67 expression was significantly higher in non-papillary cases (p= 0.017).

p53 expression was found in 40 (47.6%) cases (Figure 2). We found a correlation between p53 expression and pathological stage and histological grade (p=0.003; p=0.000, respectively). p53 expression was high, in a borderline significant manner, in non-papillary cases (p=0.050).

Bcl-2 expression was found in 2 (2.4%) cases and Bax expression in 45 (53.6%). No significant correlation was detected between Bcl-2 or Bax expression and clinical or pathological data (p>0.05).

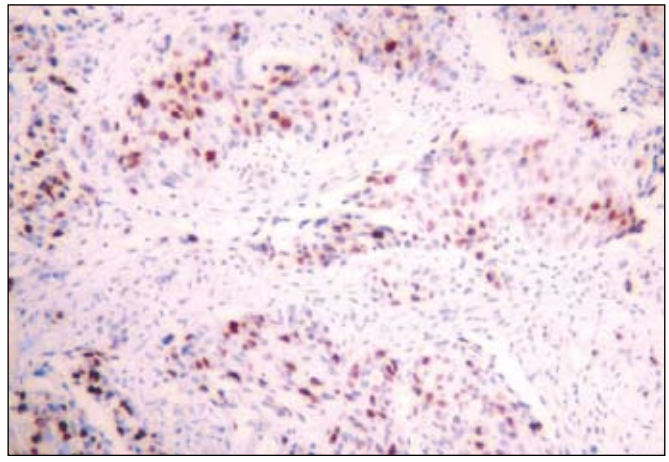

Figure 1: Nuclear Ki-67 expression in high-grade invasive urothelial carcinoma (x200).

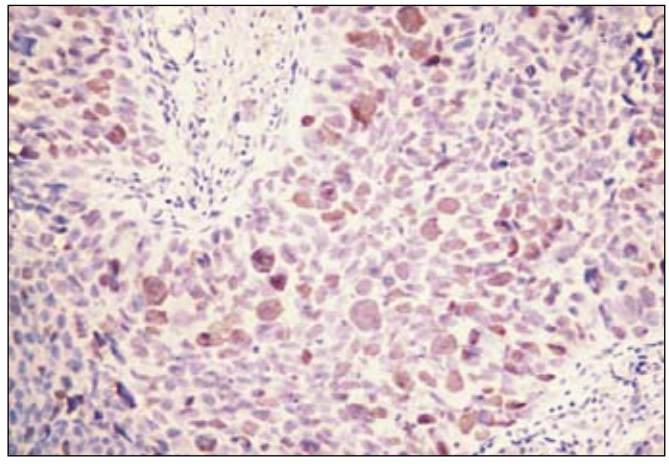

Figure 2: Nuclear p53 expression in high-grade invasive urothelial carcinoma (x400).

DISCUSSION

The 2004 WHO classification classifies UCs that do not invade the basal membrane and stay limited to the epithelium (pTa) as "noninvasive" and those that invade the lamina propria, muscle or deeper tissues (pT1-4) as "invasive" (2). We used the 2004 WHO classification and graded the cases accordingly in our study.

Ki-67 is a nuclear protein coded by a gene localized to chromosome 10 and makes up part of the DNA replicase complex. This protein functions as a cellular proliferation marker and immunohistochemical Ki-67 expression is used to predict proliferative activity, and thus biological aggressiveness of the tumor (9).

Many studies on bladder UCs have found a significant relationship between the pathological stage and histological grade and Ki-67 positivity (7,10-13). Korkolopoulou et al. and Krouse et al. have shown a significant correlation between histological grade and Ki-67 positivity and observed that pTa and pT1 bladder tumors have lower Ki-67 positivity than pT2-4 bladder tumors (10,13). Quintero et al. studied 164 Ta/T1 UC cases and reported increased mean Ki-67 expression with increasing histological grade and tumor invasiveness (1). We also found markedly higher Ki-67 expression in high grade and invasive cases than low grade and non-invasive cases in our study. Ki-67 proliferation index is expected to be higher in high grade and invasive UC than in low grade and noninvasive UC as proliferation becomes uncontrolled due to the dysregulation of cell cycle with decreased differentiation of the tumor. Since papillary framework in UC is seen mostly with low grade and noninvasive tumors the high Ki-67 expression in our cases without a papillary framework, therefore, correlated with the high Ki-67 expression in invasive cases with high histological grade.

Tumor suppressor gene p53 plays an important role in cell cycle regulation (14). A combination of immunohistochemical p53 protein detection and molecular sequence analysis has shown that p53 protein accumulation correlates with the amount of mutant p53 gene (15). Loss of "wild" type p53 expression results in abnormal cell cycle regulation with continuing proliferation of cells with DNA damage (4). Some studies on the relation between p53 positivity and pathological stage in bladder UC have demonstrated the presence of a significant association between p53 expression and pathological stage and histological grade. Increasing stage and histological grade corralate with increased p53 expression (4,5,7,8,11-13,16-18). Korkolopoulou et al. stated that the observance of p53

Table I: Comparison of Ki67, p53, Blc-2 and Bax expressions in urothelial carcinomas of the bladder with clinical and pathological findings

	Total (N = 84)	Ki-67		p* value	p53		p* value	Bcl-2		p* value	Bax		p* value
		<13% 63 (75%)	≥13% 21 (25%)		<20% 44 (52.4%)	≥20% 40 (47.6%)		<1% 82 (97.6%)	≥1% 2 (2.4%)		<20% 39 (46.4%)	≥20% 45 (53.6%)	
Pathological stage				0.010			0.003			0.099			0.360
pTa	30 (35.7%)	27 (90%)	3 (10%)		22 (73.3%)	8 (26.7%)		28 (93.3%)	2 (6.7%)		16 (53.3%)	14 (46.7%)	
pT1	32 (38.1%)	23 (71.9%)	9 (28.1%)		15 (46.9%)	17 (53.1%)		32 (100%)	0		14 (43.8%)	18 (56.3%)	
pT2	22 (26.2%)	13 (59.1%)	9 (40.9%)		7 (31.8%)	15 (68.2%)		22 (100%)	0		9 (40.9%)	13 (59.1%)	
2004 WHO classification				0.007			0.001			0.066			0.579
PUNLMP	2 (2.4%)	2 (100%)	0		2 (100%)	0		2 (100%)	0		1 (50%)	1 (50%)	
Noninvasive urothelial carcinoma, low grade	26 (31%)	24 (92.3%)	2 (7.7%)		19 (73.1%)	7 (26.9%)		24 (92.3%)	2 (7.7%)		13 (50%)	13 (50%)	
Noninvasive urothelial carcinoma, high grade	2 (2.4%)	1 (50%)	1 (50%)		1 (50%)	1 (50%)		2 (100%)	0		2 (100%)	0	
Invasive urothelial carcinoma, low grade	10 (11.9%)	8 (80%)	2 (20%)		7 (70%)	3 (30%)		10 (100%)	0		3 (30%)	7 (70%)	
Invasive urothelial carcinoma, high grade	44 (52.4%)	28 (63.6%)	16 (36.4%)		15 (34.1%)	29 (65.9%)		44 (100%)	0		20 (45.5%)	24 (54.5%)	
Tumor grade				0.005			0.000			0.115			0.778
LG	38 (45.2%)	34 (89.5%)	4 (10.5%)		28 (73.7%)	10 (26.3%)		36 (94.7%)	2 (5.3%)		17 (44.7%)	21 (55.3%)	
HG	46 (54.8%)	29 (63%)	17 (37%)		16 (34.8%)	30 (65.2%)		46 (100%)	0		22 (47.8%)	24 (52.2%)	
Number of tumors**				0.500			0.358			0.902			0.064
1	32 (38.1%)	25 (78.1%)	7 (21.9%)		15 (46.9%)	17 (53.1%)		31 (96.9%)	1 (3.1%)		18 (56.3%)	14 (43.8%)	
≥2	45 (45.2%)	27 (71.1%)	11 (28.9%)		22 (57.9%)	16 (42.1%)		37 (97.4%)	1 (2.6%)		13 (34.2%)	25 (65.8%)	
Tumor diameter***				0.224			0.302			0.332			0.793
<3 cm	25 (29.8%)	20 (80%)	5 (20%)		10 (40%)	15 (60%)		24 (96%)	1 (4%)		11 (44%)	14 (56%)	
≥3 cm	17 (20.2%)	10 (58.8%)	7 (41.2%)		9 (52.9%)	8 (47.1%)		16 (94.1%)	1 (5.9%)		7 (41.2%)	10 (58.8%)	
Papillary framework				0.017			0.050			0.162			0.373
Present	43 (51.2%)	37 (86%)	6 (14%)		27 (62.8%)	16 (37.2%)		41 (95.3%)	2 (4.7%)		22 (51.2%)	21 (48.8%)	
Absent	41 (48.8%)	26 (63.4%)	15 (36.6%)		17 (41.5%)	24 (58.5%)		41 (100%)	0		17 (41.5%)	24 (58.5%)	
Carcinoma in situ				0.839			0.364			0.620			0.561
Present	9 (10.7%)	7 (77.8%)	2 (22.2%)		6 (66.7%)	3 (33.3%)		9 (100%)	0		5 (55.6%)	4 (44.4%)	
Absent	75 (89.3%)	56 (74.7%)	19 (25.3%)		38 (50.7%)	37 (49.3%)		73 (97.3%)	2 (2.7%)		34 (45.3%)	41 (54.7%)	

PUNLMP, papillary urothelial neoplasm of low malignant potential; LG, low-grade; HG, high-grade.

* $\chi 2$ ** Not known in 7 cases. *** Not known in 42 cases.

expression in advanced stages supports a crucial role for p53 mutations in bladder cancer progression. Although there is an undisputed relationship between p53 positivity and high histological grade, p53 expression can decrease in retrospective studies as paraffin embedded tissues may lost their immunoreactivity in time (5). Our finding that increased p53 expression with increased histological grade and invasion depth of the tumor also supports the role of p53 mutations in UC progression.

Programmed cell death plays an important role in the cellular response to genotoxic stress. Loss of the apoptotic responses in tumor cells is therefore one of the mechanisms that contribute to malignant progression and cancer relapse. Bcl-2 and Bax are two important genes of the apoptotic pathway (16). The Bcl-2 gene product protein is located in the inner mitochondrial membrane and inhibits programmed cell death, thus prolonging cell life without affecting cellular proliferation in the cells that express this oncoprotein (19). Bcl-2 prolongs cell life by inhibiting apoptosis and leads to slower neoplastic growth than that caused by the oncoproteins that stimulate cellular proliferation. Therefore one may expect Bcl-2 expression to be increased in the less aggressive forms of the disease (7). Investigation of the association between Bcl-2 expression and the pathological stage and histological grade in UCs has shown a significant correlation between Bcl-2 positivity and pathological stage and histological grade in some studies (6,17,18). We found a low degree of Bcl-2 positivity only in noninvasive (pTa) and low-grade cases while none of the invasive (pT1 and pT2) and high-grade UC cases showed Bcl-2 expression. These results, although not statistically significant, indicate that the Bcl-2 expression found in UCs is associated with the less aggressive phenotype.

Bax, Bcl-2 gene family member, is a 21 kDa protein that dimerizes with Bcl-2 and stimulates apoptosis (20). Most studies on Bax expression in UC have not found a correlation between Bax positivity and pathological stage and histological grade (5,6,21). Ong et al. have investigated prognostic factors in 83 UC and found a correlation between increased Bax positivity and increased histological grade (6). Our results also did not reveal a statistically significant relationship between Bax positivity and pathological stage and histological grade in UC cases.

In conclusion, we found that Ki-67 and p53 expressions in bladder UCs increased with pathological stage and histological grade and that it was possible to obtain more accurate information about the biological behavior of UC by evaluating these parameters together with morphological findings. The results also support the observation that the 2004 WHO classification corresponds to the biological behavior of these tumors.

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