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P53 May Still a Reliable Marker in Determining the Prognosis of Non-Muscle Urothelial Carcinomas

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Abstract

Objectives: We aimed to analysis immunostaining of p53 regarding to grade, stage, recurrence and progression in non-muscle urotelial cancers (pTa and pT1 papillary tumors).
Methods: A total of 60 patients undergoing their first transurethral resection for bladder cancer and newly diagnosed papillary urothelial carcinoma (pTa and pT1) were included in retrospective study. Immunohistochemically, all tissue sections were stained with p53. The patients were divided into two groups based on recurrence and progression.
Results: Using Spearman's correlation test, p53 was significantly positively correlated with tumor stage and tumor grade ($r = 0.372$ and 0.443, respectively; $p < 0.001$). Based on a multivariate analysis, p53 staining was found to be the only independent predictive factor for recurrence (OR = 12.01, 95% CI = 1 16.6) and no independent predictive factor for progression was found.
Conclusions: p53 may be useful in predicting recurrence of pTa and pT1 urothelial cancers.

Keywords: p53; prognosis; urothelial carcinoma.

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Introduction

Transitional cell carcinoma is the most common type of cancer of the bladder and constitutes approximately 90% of bladder cancer cases. The biological behavior of these tumors is highly variable (1). Non-invasive superficial tumors of bladder (pTa) are usually low grade tumors and their recurrence rate is approximately 34-72%. The risk of progression into lamina propria and muscle is low in pTa tumors. However pT1 tumors usually have papillary structures which show lamina propria invasion, may be low or high grade and these tumors generally have a poorer prognosis than pTa tumors.

Approximately 15-70% of pT1 tumors relapse within a year after transurethral resection and 7-44% of these tumors are likely to progress into muscle in five years (2).

Although grade and stage are the most important prognostic parameters in urothelial carcinomas, the tumors having the same stage and grade may show different clinical courses. Therefore varied genetic mechanisms may play a role in the development and the clinical outcome of urothelial carcinoma (3).

In the past and recent years mutation of cell cycle proteins have been investigated in many cancers; p53 is one of these proteins which frequently undergoes mutations in cancers (4-6). p53 plays a key role in controlling transition to the G1/S phase in cell cycle, DNA repair and apoptosis. When there is DNA damage, p53 arrests the cell cycle in G1 and it saves time to repair the DNA. If the damage cannot be repaired the *cell* undergoes *apoptosis* (7, 8). Wild type p53 is normally present at low levels in the cell and has a short half-life. Loss of function of wild type p53 causes loss of cell growth control and removal of the cell-death control (9). Mutated p53 gene encodes a metabolically stable protein with a long half-life. It generally accumulates in high levels in the tumor nuclei and can be detected by standard immunohistochemical methods (10).

Numerous studies have evaluated association of abnormalities and mutations of p53 and prognosis in bladder cancers. Some previous studies have suggested that p53 alteration was associated with poor prognosis in urothelial bladder cancer (11-18). On the other hand many studies have shown that p53 expression was not related to high risk of tumor recurrence or progression (19-23).

In this study we aimed to analyze immunostaining of p53 regarding to grade, stage, recurrence and progression in non-muscle urothelial cancers (pTa and pT1 papillary tumors).

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Table 1: Comparison of patients according to the presence of recurrence and progression.

Parametreler	Referans aralığı	RECURRENCE			PROGRESSION		
		Present N=40	Absent N=20	P	Present N=11	Absent N=49	P
P 53	< 10	7 (%17.5)	11 (%55.0)	**0.003**	1 (%9.1)	17 (%34.7)	0.094
	≥ 10	33 (%82.5)	9 (%45.0)		10 (%90.9)	32 (%65.3)	
Age	< 65	13 (%32.5)	7 (%35)	0.846	5 (%45.5)	15 (%30.6)	0.345
	≥ 65	27 (%67.5)	13 (%65)		6 (%54.5)	34 (%69.4)	
Gender	Female	3 (%7.5)	4 (%20)	0.155	1 (%9.1)	6 (%12.2)	0.768
	Male	37 (%92.5)	16 (%80)		10 (%90.9)	43 (%87.8)	
Smoking	No	3 (%10.3)	8 (%53.3)	**0.002**	0 (%0)	11 (%32.4)	**0.038**
	Yes	26 (%89.7)	7 (%46.7)		10 (%100)	23 (%67.6)	
Number of Tumor	One	21 (%52.5)	17(%46.7)	**0.014**	3 (%27.3)	35 (%71.4)	**0.006**
	Multipl	19 (%47.5)	3 (%15)		8 (%72.7)	14 (%28.6)	
Tumor Size	< 3 cm	16 (%41)	18 (%90)	**< 0.001**	4 (%36.4)	30 (%62.5)	0.114
	≥ 3 cm	23 (%53)	2 (%10)		7 (%63.6)	18 (%37.5)	
Stage	pTa	19 (%47.5)	15 (%75)	**0.043**	5 (%45.5)	29 (%59.2)	0.406
	pT1	21 (%52.5)	5 (%25)		6 (%54.5)	20 (%40.8)	
Grade	Low	19 (%47.5)	16 (%80)	**0.016**	3 (%27.3)	32 (%65.3)	**0.021**
	High	21 (%52.5)	4 (%20)		8 (%72.7)	17 (%34.7)	

Materials and Methods

Patient and tumor characteristics

In total, 60 consecutive patients undergoing their first transurethral resection (TUR) in Abant Izzet Baysal University urology clinic for bladder cancer and newly diagnosed papillary transitional cell carcinoma (pTa and pT1) between 2005 and 2013 were included. Clinical data (age, gender, grade, stage, smoking, tumor size and number, presence of CIS, re-TUR, postoperative immunotherapy, and chemotherapy) of the patients were collected. Pathological specimens of the patients were evaluated by a single pathologist (GÖ) from the pathology department of the same university. The pathologist was blinded to the patient characteristics. All tumors were graded in accordance with World Health Organization (WHO)/International Society of Urological Pathology (ISUP) (2004). Tumor staging was performed

according to the Union for International Cancer Control (UICC) 2009 TNM classification.

Patient follow-up

Recurrence was defined as the relapse of transitional cell carcinoma of the bladder at any pathological stage after the initial surgery. Histopathologically confirmed progression to a higher stage or metastasis, was deemed progression. Patients having carcinoma in situ, undergoing a second TUR, or receiving intracavitary chemotherapy and immunotherapy were excluded from the study.

Immunohistochemistry

Immunohistochemistry (IHC) was performed using a streptavidin-biotin-peroxidase technique (Bond fully integrated IHC and In situ Hybrizadition (ISH) system; Leica) with a monoclonal antibody to p53 (clone: DO-7, Novocastra, ready to use). First, 5-µm sections from paraffin wax-embedded samples were cut and placed on poly-L-lysine-coated slides, deparaffinized with xylene, and then rehydrated. For antigen retrieval, the slides were treated by microwave heating in citrate buffer (pH 6.0) for 10 min. Next, 3% hydrogen peroxide was used to block endogenous peroxidase activity.

The sections were incubated with primary antibodies, including those against p53 for 1 h at room temperature. After washing in phosphate-buffered saline, the samples were incubated with a biotin-conjugated secondary antibody and then with a streptavidin-biotin system for 30 min at room temperature. The reactions were visualized by immersion of the specimens in diaminobenzidine tetrahydrochloride. The sections were counterstained with hematoxylin, then rinsed and mounted.

Immunohistochemical scoring system

Only strong nuclear staining was taken into account when scoring p53. Weak and moderate intensity of nuclear staining was ignored. Breast tissue was used as a positive control for assessment of p53 staining. The cut-off value was accepted 10% for p53. Tumors expressing below 10% were regarded negative while those expressing above 10% were regarded as positive (24-26).

Statistical Methods

Statistical analyses were performed using IBM Statistical Package for the Social sciences (SPSS) Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY). Continuous variables were tested for normality by the Kolmogorov–Smirnov test. Normally distributed data were presented as means ± standard deviation. The rates and proportions of discrete variables were determined using the chi-squared test. The median with data range (minimum to maximum) was used for non-normally distributed data. Correlations between tumor grade, stage, smoking, number of tumor, tumor size and staining of p 53 were evaluated using Spearman's rank correlation coefficient. Potential predictors of recurrence and progression in individual patients with superficial transitional cell cancer of the bladder were initially compared, and variables that showed a p value of <0.05 were included in a logistic regression model. Results were expressed as odds ratio (OR) and 95% confidence interval (CI). The two-sided p value of <0.05 was considered to indicate statistical significance. Relation between p 53 staining with recurrence time was evaluated with log rank test. Recurrence rates were determined with Kaplan Meier analysis. A %5 type-1 error level was used to infer statistical significance.

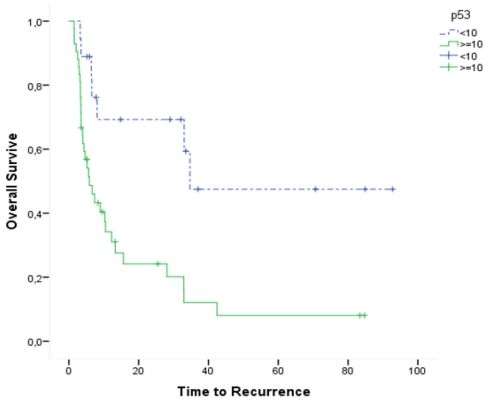


Figure 1: The relationship between with p53 and recurrence time.

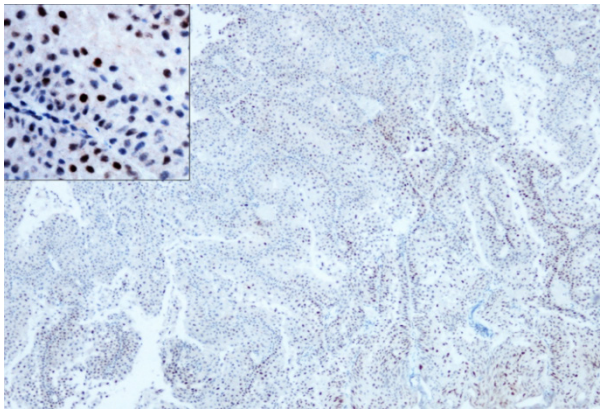


Figure 2: p53 expression is below 10% (5 %) in low grade pTa urothelial carcinoma (p53, x40). (top left: p53, x200).

Table 2: Univariate and Multivariate logistic regression analysis of independent predictive factors for recurrence in non-muscle invasive tumors.

Parameters	Referents	Univariate Analysis			Multivariate Analysis		
		OR	95%CI	P	OR	95%CI	P
P 53	≥ 10	5.76	(1.73-19.14)	**0.003**	12.01	(1.65-87.28)	**0.014**
Smoking		8.44	(2.21-32.19)	**0.001**	7.08	(0.8-62.46)	0.078
Tumor Size	≥ 3 cm	5.65	(2.0-15.97)	**0.001**	5.83	(0.59-57.93)	0.133
Grade	pT1	3.05	(1.20-7.73)	**0.016**	1.4	(0.2-9.74)	0.732

Results

The study population included 53 males and 7 females. The average follow-up time was 32.8 (IQR 36.2-103.6) months.

Using Spearman's correlation test, p53 was significantly positively correlated with tumor stage and tumor grade (r = 0.372 and 0.443, respectively; p < 0.001).

According to chi-square test, while 15 of pTa tumors showed <10 % staining with p53, 19 were ≥10 %. There were 3 pT1 tumors that showed <10 % and 23 had ≥10 % staining with p53. There was statistically significant difference between pTa and pT1 tumors with regard to p53 staining (p=0.006). While 15 of low grade tumors showed <10 %, 20 showed ≥10 % staining with p53. In high grade tumors, <10 % p53 staining was observed in 3 patients and ≥10 % was observed in 22 patients. There was statistically significant difference between low grade and high grade tumors according to p53 staining (p=0.01).

Using Spearman's correlation test, there was a positive correlation between recurrence and p53 (r:0.386, p=0.002), smoking (r:0.409, p<0.001) , number of tumor (r:0.506, p<0.001), stage (r:0.253, p=0.016), grade (r:0.288, p=0.006). A positive correlation was only found between progression and smoking (r:0.037, p=0.037).

According to the univariate analysis, ≥10 % p53 staining, tumor size > 3 cm, multiple tumors, cigarette smoking and high-grade tumor were definite risk factors for recurrence and smoking, multiple tumors and grade were definite risk factors for progression (Table 1). A significant correlation was also found between p53 staining and stage with respect to univariate analysis (p=0.006).

Based on a multivariate analysis, p53 staining was found to be the only independent predictive factor for recurrence (OR = 12.01, 95% CI = 1 16.6) and no independent predictive factor for progression was found. As there was a significant correlation between tumor number and size and between tumor grade and stage, only tumor size and grade were evaluated in the multivariate analysis (Table 2). Since the number of patients who smoke cigarettes and female patients were zero in progression group, gender and smoking were excluded from the statistics while multivariate analysis assessing.

According to Kaplan Meier analysis, while the average recurrence time was 53.22 (31.73-74.70) months in patients who showed <10 % staining with p53, the recurrence time was 16.78 (8.8-24.75) months in patients with ≥10 % p53 staining. There was statistically significant difference between groups according to recurrence time (p=0.001) (Figure 1).

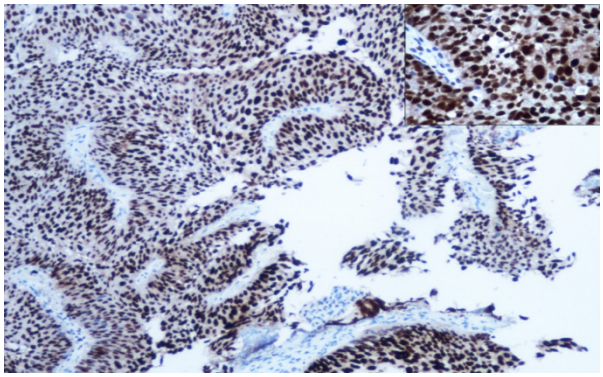


Figure 3: p53 expression is above 10% (80 %) in high grade pT1 urothelial carcinoma (p53, x100) (top right: The nuclear staining is too strong in this figure, p53, x400).

Discussion

Although the development of cancer has a multifactorial etiology, it has been shown that mutations of several cell cycle genes (p16, cyclin D1, Rb, p53) play an important role in development and progression of bladder cancer (24, 27, 28). p53 is one of the cell cycle proteins that act as a tumor suppressor gene and is most frequently mutated in cancer. Over one-half of bladder tumors contain a mutation in the p53 gene and the prognostic significance of p53 expression remains controversial in studies with bladder cancers (29, 30). The current study determined that the predictive value of p53 was extremely high for tumor recurrence in non-muscle invasive bladder cancers.

Even if p53 mutations can be detected by molecular analysis, immunohistochemistry is still the most commonly used method to evaluate the p53 status (31). Protein product of the mutated p53 gene frequently accumulates in the tumor nuclei and can be demonstrated by immunohistochemical methods. However despite mutation of p53, occasionally its protein product cannot accumulate in the tumor nucleus and cannot be shown by immunohistochemistry (12). In our study a significant correlation was found between expression of p53 and grade, however three high grade tumors showed below 10% expression with p53. According to several previous studies that investigated genetic alteration of p53 in sarcomas, some different mutations (like a point mutation) may lead to loss of p53 protein synthesis (32, 33). As a result, some high grade urothelial tumors may not show p53 expression by immunohistochemistry. Nevertheless, p53 staining was statistically high in high grade urothelial carcinomas in current study as indicated by such previous studies (1, 12, 34).

We demonstrated that p53 was also correlated with tumor stage. pTa tumors are less reactive than pT1 tumors regarding p53 staining in our study. Wagner et al detected that p53 expression was high in carcinoma in situ and pTa tumors and they claimed that p53 alteration is a shift in early bladder carcinogenesis (35). Another study demonstrated that p53 defect may play an important role in progression of papillary urothelial neoplasm of low malignant potential (PUNLMP) to low grade urothelial carcinoma (LGUC), because they found significant differences between PUNLMP and LGUC with respect to p53 expression (36). Many studies have shown that increasing stage correlates with accumulation of p53 by immunohistochemistry similar to our results (12, 15, 37, 38).

Prediction of recurrence and progression is very important in terms of selecting patients for early aggressive management in bladder cancer. In our study high p53 staining was an independent predictor for recurrence in multivariate analysis. We didn't find any significant value for p53 staining in predicting progression, likely due to the limited number of patients showing progression. In the literature, predictive value of p53 expression for prognosis in urothelial carcinoma remains controversial. Ogata DC et al found strong correlation between p53 accumulation and progression but they didn't determine any significant results with regard to recurrence (1). Similarly Goebell et al detected that p53 positivity was an important predictive marker for progression in pT1 urothelial carcinomas as well as in advanced bladder cancers, however differently from former study p53 gene defect was not useful for pTa bladder cancer prognosis (39). Esrig et al investigated alteration of p53 with both molecular analysis and immunohistochemistry and have shown that tumors with high p53 accumulation are high grade, stage and at high risk for recurrence (12). Their immunohistochemical results were similar to ours. Few studies reported that using the cell cycle proteins in combination might be useful for prognosis in bladder cancers. Hitchings et al assessed the prognostic value of p53 and p16 combined staining and alterations of both of them were more informative than staining p53 alone (40). Conversely some studies reject the predictive value of p53 mutation analysis and immunostaining for recurrence and progression (22, 30, 41, 42).

The reason of the conflicting results in the literature about p53 expression may be due to a number of factors. The preferred antibody clone is one of them. DO-7 anti-p53 antibody (Novacastra) was used in our study. Masters et al assert that the DO-7 clone is less effective than the pAb1801 antibody (Novacastra) in the determination of pTa and pT1 bladder cancer progression (43). Interobserver inconsistency is one of the other reasons. Only strong nuclear staining was taken into account when scoring p53, weak and moderate intensity nuclear staining was ignored in the current study. However in one study all intensities of staining were considered positive (40). Using the different cut-off points for p53 and heterogeneity of the series (only pT1 carcinomas/ pTa-pT1 tumors/ all stages) may lead to different results.

Conclusion

In conclusion in spite of the controversies in the literature regarding to expression of p53, we believe that p53 is still a reliable marker in prognosis, especially in recurrences of pTa and pT1 urothelial cancers.

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