Original Article

Expressions of p53, KAI1, PTEN and their use as prognostic markers in urothelial carcinoma of the bladder

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ABSTRACT

Objectives: To determine the expression levels of p53, KAI1, PTEN and their relations with the clinicopathologic parameters in patients with urothelial carcinoma of the bladder

Design: Retrospective study

Setting: Department of Pathology, Ankara Diskapi Yildirim Beyazit Education and Research Hospital, Ankara, Turkey

Subjects: Seventy-eight patients with a diagnosis of urothelial carcinoma of the bladder at the Pathology Department of Ankara Diskapi Yildirim Beyazit Education and Research Hospital and who were followed up for two years

Intervention: Archived pathology materials of all patients were reviewed and clinical data were collected from medical database records retrospectively.

Main outcome measures: We considered the following parameters: age, gender, survivals and disease-free survivals of the patients, tumor-stage, lymphovascular-angiovascular invasions, expressions of KAI1, PTEN and p53 in tumorous tissues.

Results: In non-metastatic-patients, 55.2% were p53 negative; however, 45.8% of the metastatic-patients had diffuse-strong p53-expressions. Hence, p53 positivity indicates that the tumor will metastase.

In transurethral resection (TUR) materials, patients with positive KAI1-expression had better survival (p=0.0285). The tumor seemed to be more aggressive and invasive in patients with decreased KAI1-expressions because there was no KAI1 expression in 50% of the metastatic-patients, although it wasn't statistically significant (p=0.550).

For PTEN expression, no statistically-significant relation was found with the tumor-stage (p=0.34) and survival/disease-free-survival (TUR:p=0.9599/0.7576, radical cystectomy :p=0.8219/0.5790).

Conclusions: The presence of metastasis dramatically decreases the overall survival in patients with urothelial carcinoma. Identification of these patients as early as possible would effect their therapies, follow-up intervals and surveys. p53 and KAI1 seem to be especially better indicators of the prognosis than PTEN.

KEYWORDS: KAI1, PTEN, p53, urinary bladder, urothelial carcinoma

INTRODUCTION

Urothelial malignancy of the bladder is the fifth most common malignancy occurring worldwide. In the genitourinary tract, it is the second most common malignancy. Approximately 90% of bladder tumors are of epithelial origin and the majority of them are the urothelial carcinomas of the bladder (UCB)[1,2]. The ratio of men to women is approximately 3:1[3,4].

About 25% of patients with UCB present with advanced (metastatic or muscle-invasive) disease[3]. The gold standard of treatment for patients with advanced and refractory non-muscle-invasive UCB is radical cystectomy (RC) with bilateral pelvic lymphadenectomy (BPL) with or without perioperative chemotherapy and radiotherapy. In approximately 50% of patients with muscle-invasive UCB, the recurrence of disease and even death from the disease can be seen, in spite of advances in surgical techniques and perioperative chemotherapy[3,5,6].

The standard prognostic, predictive factors and the risk factors for recurrence and progression of UCB can be listed as; multifocality, tumor size of >3 cm,

Table 1: The sex, mean age and pathological stage (pTstage) distribution of the patients without lymph node/distant metastasis and with lymph node/distant metastasis of the urothelial carcinoma of the bladder.[Number of the patients-(% distribution)].

Parameter	Non-metastatic patients	Metastatic patients	Total
Sex (p = 0.614)			
Male	39 (56.5)	30 (43.5)	69 (100)
Female	5 (55.6)	4 (44.4)	9 (100)
Total number (%)	44 (56.4)	34 (43.6)	78 (100)
Mean age (p = 0.031)	60.04 ± 9.1	55.02 ± 9.06	
pT stage in Transurethral Resection materials			
T1	10 (66.7)	5 (33.3)	15 (100)
T2	19 (55.9)	15 (44.1)	34 (100)
Total number (%)	29 (59.2)	20 (40.8)	49 (100)
pT stage in Radical Cystectomy materials			
T0	2 (100)	-	2 (100)
Ta	1 (100)	-	1 (100)
T1	2 (100)	-	2 (100)
T2a	5 (50.0)	5 (50.0)	10 (100)
T2b	1 (100)	-	1 (100)
T3a	12 (50.0)	12 (50.0)	24 (100)
T3b	1 (3.4)	1 (4.0)	2 (100)
T4a	5 (45.5)	6 (54.5)	11 (100)
Total number (%)	29 (54.7)	24 (45.3)	53 (100)

concurrent carcinoma in situ, tumor extension beyond the bladder on bimanual examination, infiltration of the ureteral orifice, lymph node metastases, presence of systemic dissemination, histological grade, stage, lymphatic and/or vascular invasion, specific subtypes or histological variants of urothelial carcinomas (such as small cell carcinoma, sarcomatoid carcinoma, nested variant, micropapillary carcinoma and lymphoepithelioma-like carcinoma have worse prognosis), margin status after cystectomy and the pattern of tumor growth (pushing or infiltrative)[7].

However, all these factors always do not adequately show the individual biological potential and clinical behaviour of the tumor.

Therefore, it is important to find out the molecular events to explain the clinical heterogeneity of these tumors in order to help clinicians apply individualized adjuvant therapy according to individualized prognostic and predictive factors[8-10]. Some of these controversial molecular events are antibodies like PTEN, KAI1 and p53.

PTEN

PTEN (MMACI-The phosphatase and tensin homolog mutated on chromosome ten) is a tumor suppressor gene located on chromosome 10 that inhibits cell proliferation and tumorogenesis by inducing apoptosis and by arresting G1 phase of cell cycle[4]. PTEN is known to be mutated or deleted in many human cancers, including gliomas, breast, prostate, lung, thyroid and endometrial carcinomas. This gene is also reported to show loss of heterozygosity in bladder cancer and more frequently in muscle-invasive urothelial carcinomas[11-14].

KAI1

KAI1 protein (also named as R2, C33, IA4, 4F9, CD82), located on chromosome 11, is a cell surface glycoprotein initially detected as a specific tumor metastasis suppressor gene in prostate cancer[14]. KAI1 inhibits cell migration and attenuates epidermal growth factor signaling[15-16]. Many experimental evidences have shown that expression of KAI1 is down-regulated or lost in the advanced stages of many different human cancers (breast, stomach, colon, lung cancers, melanoma), indicating that a loss of KAI1 function may be an important step in disease progression, invasiveness and metastasis[17-20].

p53

p53 is a tumor suppressor gene located on chromosome 17 that plays a key role in regulating cell cycle progression and apoptosis. It blocks the damaged cell entrance to the S phase of cell cycle until its DNA is repaired and it sends specific damaged cells to apoptosis. p53 is the most commonly mutated gene in human cancers[3,21,22].

In the present study, we analyzed the expressions of PTEN, KAI1 and p53 in materials of the patients with UCB and compared with pathological parameters to determine the prognostic significance of these proteins in the UCB.

SUBJECTS AND METHODS
Patients and tissue samples

We analyzed 78 patients (69 males and 9 females) retrospectively who underwent RC with BPL (53 patients) and/or transurethral resection (TUR) (49 patients) who were diagnosed as urothelial carcinoma
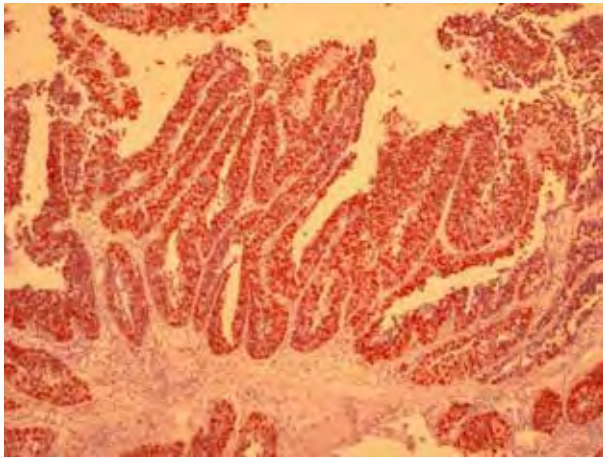
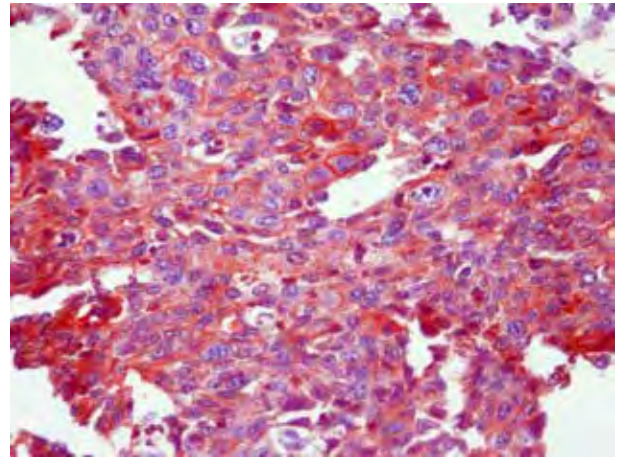**Fig 1:** Nuclear p53 positive staining (p53 x 100)

Fig 2: Cytoplasmic KAI1 positive staining (KAI1 x 200)

between 2007 and 2008 and followed up for 2 years (Table 1). We obtained informed consents from all patients and certification from Ankara Dışkapı Yıldırım Beyazıt Education and Research Hospital Ethics Committee about the relevance of the study (Ethics Committee no: 17/19-03.11). We followed up all the patients after the 3rd month control with intervals of 4 months for 2 years.

The pathology slides of RC and TUR materials of the patients were reviewed and representative sections and paraffin blocks of each tumor were selected.

The histological grade of the bladder tumors were determined according to the 2004 World Health Organization classification[7]. Tumor staging was done using Tumor Node Metastasis system according to the American Joint Committee on Cancer[23].

The patients were seperated into two groups as;

Group 1 (non-metastatic group) : Composed of the patients (n = 44) with no lymph node metastasis present at the time of cystectomy and no distant organ metastasis determined on the follow-up.

Group 2 (metastatic group): Composed of the patients (n = 23) who had lymph node metastasis present at the time of cystectomy and the patients (n = 11) who did not have any lymph node metastasis present at the time of cystectomy, but distant organ metastasis occured on the follow-up.

The age, gender, survivals and disease free survivals of the patients, stage of the tumors, lymphovascular-angiovascular invasions, expressions of KAI1, PTEN and p53 in TUR and RC materials were statistically evaluated in two groups (non-metastatic and metastatic).

Immunohistochemistry

For immunohistochemical staining, 4μm sections of selected tumor blocks were cut and mounted on poly-L-lysine–coated slides. Following deparaffinization
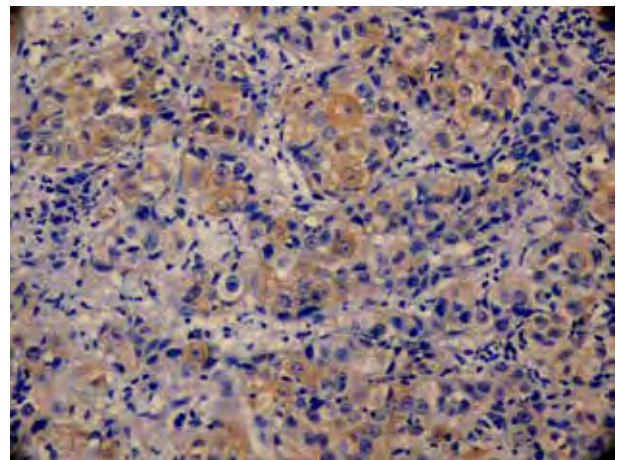

Fig 3: Cytoplasmic PTEN positive staining (PTEN x 400)

and rehydration, antigen retrieval was performed using citrate buffer (pH 6.0) at 121 °C for 10 minutes. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for five minutes. Later, the sections were incubated with antibodies against KAI1 (Santa Cruz, sc-17752, G-2), p53 (Novocastra, NCL-L-p53-D-07) and PTEN (Neomarker, ser370, RB-10620-P1). 3,3'-diaminobenzidine and 3-amino-9-ethylcarbazole were performed as chromogenes and finally slides were counterstained with hematoxylin. Breast carcinoma tissue was used as positive controls for p53 and PTEN. Prostate carcinoma tissue was used as a positive control for KAI1. Cases not treated with primary antibodies served as negative controls.

Immunostaining was evaluated by two pathologists. Nuclear staining for p53 (Figure 1) and cytoplasmic staining for KAI1 (Figure 2) and PTEN (Figure 3) was accepted as positive. The 'expression rate', the extent of staining for each antibody, was scored according to the number of cytoplasmic or nuclear stained carcinoma cells in 100 tumor cells. Less

Table 2: p53, KAI1 and PTEN expressions of the urothelial carcinoma of the bladder in transurethral resection materials.

Antibody expressions	Number of non-metastatic patients-(%)	Number of metastatic patients-(%)	p-value
P53 expression			0.755
0	9 (31.0)	5 (25.0)	
1	10 (34.5)	6 (30.0)	
2	10 (34.5)	9 (45.0)	
KAI1 expression			0.550
0	16 (55.2)	10 (50.0)	
1	5 (17.2)	6 (30.0)	
2	8 (27.6)	4 (20.0)	
PTEN expression			0.611
0	12 (41.4)	12 (60.0)	
1	3 (10.4)	2 (10.0)	
2	9 (31.0)	4 (20.0)	
3	5 (17.2)	2 (10.0)	

Table 3: p53, KAI1 and PTEN expressions of the urothelial carcinoma of the bladder in radical cystectomy materials.

Antibody expressions	Number of non-metastatic patients-(%)	Number of metastatic patients-(%)	p-value
P53 expression			0.036
0	16 (55.2)	8 (33.4)	
1	9 (31.0)	5 (20.8)	
2	4 (13.8)	11 (45.8)	
KAI1 expression			0.832
0	15 (51.7)	13 (54.2)	
1	6 (20.7)	6 (25.0)	
2	8 (27.6)	5 (20.8)	
PTEN expression			0.514
0	18 (64.3)	19 (79.2)	
1	2 (7.1)	2 (8.3)	
2	1 (3.6)	1 (4.2)	
3	8 (25.0)	2 (8.3)	

than 5% positive cells were accepted as negative, while ≥5% positive cells were being accepted as positive.

Scoring for p53 and KAI1:
0 (negative): <5% positive cells;
1+ (positive): 5-50% positive cells;
2+ (positive): >50% positive cells.

Scoring for PTEN:
0 (negative): <5% positive cells;
1+ (positive): 5-25% positive cells;
2+ (positive): 26-50% positive cells;
3+ (positive): >50% positive cells.

Statistical analysis

Statistical evaluation was performed by using Chi-square test, Mann-Whitney test or Fisher's Exact test. The survival and the factors that have effects on disease free survival were evaluated with Log Rank survival analysis. The patients who died within the first month after the operation were excluded during the survival analysis. P-values less than 0.05 were considered to be statistically significant.

RESULTS

Age and gender of the patients

Patients included 69 males and 9 females. The relation of sex with the status of metastasis was not statistically significant. The mean age was lower in metastatic group and this was statistically significant (Table 1).

TUR materials

In TUR materials, there was no statistically significant relation between p53, KAI1, PTEN expressions and the metastatic, non-metastatic groups (Table 2). Also, there were no statistically significant relations between p53, KAI1, PTEN expressions and pT stages of the tumors in metastatic and non-metastatic groups (p = 0.248, p = 0.755, p = 0.059 respectively).

Radical cystectomy materials

In RC materials, there was a statistically significant relation between p53 expressions and the groups (non-metastatic and metastatic, Table 3). However, this relation was not significant with KAI1, PTEN. Also, there was no statistically significant relation between

Table 4: Survivals-disease free survivals of the patients with the urothelial carcinoma of the bladder and p53, KAI1 and PTEN expressions in transurethral resection and radical cystectomy materials.

Antibody expressions	Transurethral Resection Materials				Radical Cystectomy materials			
	Survival	p-value	Disease free survival	p-value	Survival	p-value	Disease free survival	p-value
P53		0.9378		0.6581		0.7771		0.7923
- (<5%)	50.00		78.57		40.00		71.43	
+(≥ 5%)	44.12		70.59		38.77		69.23	
KAI1		0.0285		0.7461		0.9808		0.5081
-(<5%)	27.27		69.23		37.50		65.22	
+(≥ 5%)	53.33		77.27		36.66		75.00	
PTEN		0.9599		0.7576		0.8219		0.5790
- (<5%)	45.79		69.13		41.89		66.67	
+(≥ 5%)	54.48		75.34		42.75		73.91	

p53, KAI1, PTEN expressions and pT stages of the tumors in metastatic and non-metastatic groups (p = 0.185, p = 0.723, p = 0.34 respectively).

However, when the lymphovascular vessel invasion was evaluated, it was seen positive only in 2 patients (6.8%) in non-metastatic group and in 11 patients (45.8%) in metastatic group. These rates were statistically quite significant (p <0.001). As the angiovascular invasion was evaluated, it was seen positive in 2 patients (6.8%) in non-metastatic group and in 12 patients (50.0%) in metastatic group. These rates were also statistically quite significant (p <0.001).

When the survival, the disease free survival and p53, KAI1 and PTEN expressions were evaluated in TUR and RC materials; according to TUR materials, there was a statistically significant relation in survival only between the patients with positive and negative KAI1 expressions (Table 4).

DISCUSSION

Bladder carcinoma is the second most common cancer of the genitourinary tract and the incidence of it has been increasing in the last 20 years. UCB is a progressive disease typically seen in older ages. The depth of invasion, grade of the tumor and lymph node metastasis are the major factors used in determining clinical treatment. About 70% of bladder tumors are superficial, 25% are invasive and 5% are metastatic. Also, 30% of the superficial tumors become invasive in the follow-up. Long term survivals are better in patients with organ-confined disease[24-25].

BPL helps to understand the local spread of the disease. The risk of pelvic lymph node metastasis increases with the stage of the disease. The risk of pelvic lymph node metastasis in patients with stage pT3 and pT4 is 30 – 60% respectively, while the risk of lymph node positivity at the time of surgery in patients with stage pT2 is about 10 - 30%. Generally, the patients with positive lymph node metastasis determined postoperatively show simultaneous distant organ micrometastasis. Hence, it is important to predict the tumors that tend to metastise before the surgery in order to determine the apropriate clinical treatment and aproach. Still, there is no reliable indicator to use in predicting the metastatic disease[26-27].

The first notable finding in our study was that, the average age of the metastatic group was lower than the non-metastatic group. This shows that the tumors seen in younger ages are more aggressive.

p53 located in the nucleus blocks the cell cycle in G1 phase and prevents the cell passing to the S phase. Then it activates the genes that repair DNA and sends the damaged and unrepaired cells to apoptosis. p53 mutations can be seen in many tumors like lung, colon and breast[26]. It was shown that p53 can be used in diagnosing urothelial carcinoma in situ of the urinary bladder and in predicting the progression of the non-invasive pT1 lesions[28]. In a study, Uygur et al evaluated p53 expressions in TUR materials of 31 patients with RC. In 17 patients with p53 positivity (nuclear staining 20% and higher was accepted as positive), 11 had lymph node metastasis. Therefore, patients who had pT2 and pT3a disease with p53 positivity were accepted as high risk group and concluded that early aggressive therapy had to be started as soon as possible[29]. In another study, Esrig et al reported that there was a positive relation between p53 staining and the pathological stage. In that study, they also reported that in p53 positive patients, recurrence of the disease was higher and the survival was lower. Hence, p53 was determined as a prognostic factor independent from the stage and the grade[22]. On the other hand, there are studies reporting that there is no relation between p53 expression and the stage[30]. Also Puzio-Kuter et al reported similar results; that p53 overexpression was seen in invasive bladder carcinoma compared to non-invasive papillary tumors[1]. Goebell et al showed in their international study that p53 positivity was significantly correlated with tumor progression in pT1 disease and advanced bladder cancer and p53 appeared to be predictive in high grade bladder cancer[21].

In our study, we found out that in RC materials; there was a statistically significant relation between p53 expressions and the groups (non-metastatic and metastatic). p53 positivity in metastatic group was higher. We thought that the patients with p53 expression of 50% or higher had more risk to develop metastasis. So, p53 positivity may be a bad prognostic factor and the patients expressing 50% or more p53 may be treated more radically and followed up carefully. However, we could not find this relation in TUR materials and we thought that p53 is not sufficient in predicting lymph node metastasis of the tumor in TUR materials. The reason for this is thought to be that the material obtained by TUR is inadequate and also cautery artefact can limit adequate examination of all material. We also showed that the lymphovascular vessel and the angiovascular invasions were seen more frequently in metastatic group than the non-metastatic group as known before[7]. When we looked at the survivals and disease-free survivals, the increase of p53 expression had negative effects on the survival and disease-free survival. However, this effect was not statistically significant. We think that this effect would be statistically significant in new studies with more patients.

KAI1 was detected in tumors like prostate, stomach, colon, breast carcinomas and the invasiveness and the metastatic potentials of the tumors were shown to increase with the loss of KAI1 expressions[18-20]. In a

study, Su *et al* reported that decreased KAI1 expression was associated with the degree of invasiveness and progression of cancer and was an independent prognostic factor for tumor recurrence in primary pTa and pT1 UCB[16]. We found that according to TUR materials, patients with positive KAI1 expression had better survival than patients with negative KAI1 expression. The tumor was more aggressive and invasive in TUR materials with lower KAI1 expressions. We thought that this was not statistically significant because of the low number of patients.

PTEN inhibits cell proliferation and tumorogenesis by inducing apoptosis and by arresting G1 phase of cell cycle. PTEN was detected in many human cancers, like gliomas, breast, prostate, lung, thyroid and endometrial carcinomas. This gene is also reported to show loss of heterozygosity in bladder cancer, more frequently in muscle-invasive urothelial carcinomas[11-15]. In their study, Lee *et al* reported that loss of PTEN expression was associated with non-papillary histology, high grade and invasive urothelial carcinomas[13].

We only found that the loss of PTEN expression in metastatic group was more than the loss of PTEN expression in non-metastatic group. However, this correlation was not statistically significant.

In UCB, the lymphovascular vessel and the angiovascular invasions are very important as they are indicators of the lymph node or distant metastasis. The presence of lymph node or distant metastasis shortens the surveys. We need more reliable indicators to use at the beginning of the disease before the radical operation to predict the tumors which will be more aggressive, in order to decide the correct therapy, neoadjuvant or early adjuvant therapy. p53, KAI1 and PTEN seem to be good indicators, although we could not reach perfect results. We think that the reason is the limited number of our patients as indicated before. New studies with large series seem to support the usefulness of p53, KAI1 and PTEN.

CONCLUSION

In UCB, it is very important to predict the progression of the disease before the surgery as it frequently relaps and shortens the lives of the patients. It is very important to find new indicators showing the aggressiveness of the disease. The results of this study give the impressions that p53 positivity, KAI1 negativity and PTEN negativity in UCB are good indicators of worse prognosis and aggressiveness.

ACKNOWLEDGMENT

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