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BRIEF ARTICLE

Vascular endothelial growth factor, *p53*, and the *H-ras* oncogene in Egyptian patients with bladder cancer

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Abstract

AIM: To evaluate the relationship between vascular endothelial growth factor (VEGF), *p53*, and the *H-ras* oncogene and different clinicopathological parameters in Egyptian patients with Schistosoma-associated transitional cell carcinoma of the bladder.

METHODS: The study included 50 patients with transitional cell carcinoma for whom radical cystectomy and urinary diversions were carried out. VEGF and p53 protein expressions were evaluated with an immunohistochemical staining method, and *H-ras* oncogene mutations were analyzed with a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.

RESULTS: High grade tumors revealed higher p53 immunostaining than low grade tumors (P = 0.016). p53 and VEGF protein expressions, as well as *H-ras* oncogene mutations, had an insignificant impact on patient outcomes (P = 0.962, P = 0.791, and P = 967, respectively). Cancer extension to regional lymph nodes was associated with poor outcomes (P = 0.008).

CONCLUSION: VEGF, *p53* and the *H-ras* oncogene have no relation to patient survival and outcome in Schistosoma-associated transitional cell carcinoma.

Key words: Bladder cancer; Transitional cell carcinoma; Vascular endothelial growth factor; *p53*; *H-ras* oncogene

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INTRODUCTION

Carcinoma of the bladder is the most common cancer of the urinary tract. It is the most common solid tumor in men in Egypt. Squamous cell carcinoma accounted for 59.3% of cases, transitional cell carcinoma for 22.2% of cases and adenocarcinoma for 11.4% of cases in radi-

cal cystectomy specimens studied at the Mansoura Urology and Nephrology Center[1]. Squamous cell carcinomas are common due to associated schistosomiasis[2].

To date, there is no reliable method to identify those patients at risk for recurrence and those who will develop more aggressive disease[3]. The histopathological parameters, such as tumor grade, stage, and lymph node involvement, are the most important parameters in evaluation of the biological behavior of bladder cancer[1,4].

However, there are multiple studies that have shown that clinical, as compared to pathological staging, is inaccurate[5]. The overall error in clinical staging was 32.9%, and, in the majority of cases, the error was due to clinical underestimation of the extent of the disease[1,5]. Therefore, there is continued evaluation of staging systems for bladder cancer due to variable responses of histologically similar tumors to similar treatments[6]. Furthermore, inter- and intra-individual inconsistency seems to be unavoidable in grading bladder tumors[7].

A significant relationship was found between tumor angiogenesis and the presence of lymph node metastasis[8] and prognosis[9,10] in patients with superficial and invasive bladder carcinoma. In contrast, Stavropoulos *et al*[11] found no correlation between angiogenesis and survival of patients with negative lymph node metastasis treated by radical cystectomy. Also, there was no significant correlation between angiogenesis and clinical outcome in patients with stage pT1 disease[12].

The aim of this retrospective study was to clarify the relation between vascular endothelial growth factor (VEGF), *p53*, and the *H-ras* oncogene and different clinical and pathological parameters such as age, sex, histologic cell type, tumor grade, stage, and lymph node status. Also, the goal was to define the relationship between VEGF and p53 in bladder carcinoma that was treated by radical cystectomy and the disease-free survival rate, aiming to detect the significant prognostic factor(s) that can affect patient survival.

MATERIALS AND METHODS

This study was conducted on 50 patients with transitional cell carcinoma for whom radical cystectomy and urinary diversions were performed between years 2002 and 2003. The patients of this study were selected on the basis of the availability of complete archival material. Indications for surgery were failure of endoscopic control of superficial bladder tumors despite adjuvant intravesical chemotherapy and/or immunotherapy, and invasive tumors without evidence of distant metastasis. At the time of cystectomy, all specimens were examined according to the same pathological protocol. Tissue sections were obtained from the tumor, bladder wall, and regional lymph nodes. Tissues were studied from the seminal vesicle and prostate in men and from the ovaries and uterus in women. Tumors were graded using the World Health Organization (WHO) classification[13], and the TNM classification of the International Union against Cancer (UICC) was used for pathological staging of the tumors[14].

VEGF and p53 with an immuno-histopathologic staining technique

Four-micron thick sections from retrieved tumor blocks were placed at 60℃ for 15 min, incubated in xylene at RT for 15 min, and then transferred sequentially into graded alcohols (100%, 95%, 70%, and 50%) for 4 min at RT. Sections were rinsed in deionized water and stored in PBS. Antigen sites were unmasked using a microwave antigen retrieval technique in Coplin jars containing 0.1 mol/L Tris-HCl buffer containing 5% urea, pH 9.0 and heated in a microwave oven for 10 min (two times)[15,16]. After cooling, the slides were rinsed with phosphate buffered saline (PBS). Endogenous peroxidase activity was removed by submersing the slides in blocking solution that was prepared by adding one part of 30% H_2O_2 to nine parts of methanol. Non-specific binding was blocked with blocking serum (reagent A), which was left for 10 min in contact with the slides. After removing the excess blocking serum, the primary monoclonal antibody (Zymed, USA) was applied at a dilution of 1:50 in PBS and incubated for 60 min. The slides were rinsed well with PBS for 3 min. Biotinylated secondary antibody solution (reagent B) was incubated with the slides for 20 min. This was followed by a further wash in PBS. Enzyme conjugate solution (ready to-use streptavidin-peroxidase HRP (Reagent C) (reagent A, B, and C from Histostain®-plus bulk kit, Zymed, USA) was applied to the slides for 10 min. The slides were washed with buffer solution followed by the final steps of this procedure; i.e. addition of chromogen substrate di-amino-benzidine (DAB) (Sigma chemical company, UK) and light counter-stain with hematoxylin.

VEGF and p53 protein expressions were assessed in areas with solid tumor morphology away from any artifact or necrosis, and without prior knowledge of the patient outcome. Assessment of protein expression was performed in three areas where the highest density of expression was found (hot spots). Low power light microscopy at × 40 magnification was used to scan the heterogenous tumor sections for identification of these areas. At × 250 magnification, examination was made in all distinct brown-staining endothelial cells. Protein expression found in unaffected areas, adjacent to tumor infiltrated tissues, was not evaluated, but used as an internal control in assessing quality.

Detection of point mutations at codon 12 of the H-ras oncogene by PCR-RFLP

DNA extraction was done using Clontech kits (UK). The mutant specific PCR-RFLP method is as follows. Amplifications with Taq polymerase were done in 25 μL reaction mixtures containing 21 μL of Taq PCR Master Mix (Qiagen, USA), 2 μL of extracted DNA and 1 μL of each primer (Outer primers A and B). The reaction mixtures were subjected to amplification cycles of 95℃ for 1 min, 57℃ for 1.5 min and 72℃ for 2 min for a total

of 14 cycles. Then, a restriction enzyme was used to cleave the amplified DNA sequence, namely Nae I (its cleavage site is GCCGGC). This restriction enzyme can cleave wild type but not mutant *H-ras* genes. The reaction mixture for the digestion with this restriction enzyme contained 8 μL amplified DNA, 1 μL of restriction enzyme and 2 μL of 10 X buffer and 9 μL of distilled water in a total volume of 20 μL. This reaction mixture was incubated at 37℃ for 2 h. Then, a second PCR of 16 cycles was performed and 2 μL of the first digest was used as a template for the second PCR in which other 2 inner primers, C and D, were used to amplify the non-cleaved mutant sequence. The products of the second PCR were incubated with the restriction enzyme as previously mentioned for 2 h. Finally, the PCR products were resolved by electrophoresis in 2% agarose gels with 120 V for 45 min and were visualized by staining with ethidium bromide. The restriction enzyme-resistant DNA fragment is diagnostic of a mutant sequence at codon 12 of the *H-ras* gene. The primer sequence was list in Table 1.

Follow-up

Patients were kept under regular clinical review. Follow-up data, including mortality data, were obtained for all patients. Patients were followed regularly and examined for treatment failure depending on clinical and radiological findings or histopathological evidence. The mean follow-up was 20.4 ± 8.3 mo for transitional cell carcinoma.

Statistical analysis

The data were initially analyzed by taking one factor at a time and testing for any relationship with survival. Kaplan-Meier survival curves were plotted and log-rank tests were used to determine statistical differences between life table curves[17]. The period of disease-free survival was defined as the time between the date of surgery and death (from cancer) or the development of local recurrence or distant metastasis. Death from unknown cause was considered death from cancer. Censored survival values represent patients who were alive without clinical evidence of disease at the time of last follow-up. To simplify the statistical analysis, the patients were divided into two groups according to their age (≤ 50 years and > 50 years groups) and also, pathologic tumor stage was further subdivided into organ confined tumors (stages pT1 and pT2) and non-organ confined tumors (stages pt3 and pT4). χ^2 tests were performed to evaluate the relationship between p53 as well as VEGF immunostaining, ras gene mutations and different clinicopathological parameters. Statistical analyses were performed using SPSS statistical software packages (SPSS inc., Chicago, IL, USA). $P < 0.05$ was consider significant.

RESULTS

An insignificant relationship was observed between p53 immunostaining and age, sex, stage, and lymph node metastasis. A statistical significance was noticed between p53 immunostaining and tumor grade ($P = 0.016$). Higher grade tumors showed higher expression of p53 protein than low grade tumors (Table 2).

Table 1 Sequence of oligonucleotide primers

Primer	Sequence (5'→3')
Outer primers	
Sense (A)	AGGAGCGATGACGGAATATAAGC
Antisense (B)	GGCTCACCTCTATAGTGGGGTCGTATT
Inner primers	
Sense (C)	AATATAAGCTGGTGGTGGTGGGCGC
Antisense (D)	GGGGTCGTATTCGTCCACAAAATG

Table 2 Clinicopathological characteristics of the transitional cell carcinoma group and their relation to p53 immunostaining *n* (%)

Variable	*n*	P53		*P* value
		Positive	Negative	
Age (yr)				
≤ 50	11 (22)	7 (14)	4 (8)	0.851
> 50	39 (78)	26 (52)	13 (26)	
Sex				
Male	42 (84)	27 (54)	15 (30)	0.558
Female	8 (16)	6 (12)	2 (4)	
Grade				
G2	18 (36)	8 (16)	10 (20)	0.016
G3	32 (64)	25 (50)	7 (14)	
Stage				
pT1	8 (16)	3 (6)	5 (10)	
pT2	28 (56)	21 (42)	7 (14)	0.079
pT3	7 (14)	6 (12)	1 (2)	
pT4	7 (14)	3 (6)	4 (8)	
Node status				
Positive	6 (12)	2 (4)	4 (8)	0.072
Negative	44 (88)	31 (62)	13 (26)	

No correlation was found between VEGF immunostaining, *H-ras* oncogene mutation and age, sex, tumor grade, stage, and lymph node status (Tables 3 and 4).

The 3-year survival rate of the transitional cell carcinoma group was 68%. Most treatment failures occurred during the first two years of follow-up after cystectomy. Node negative patients had better survival compared to those with lymph node metastasis. The 3-year survival rate was 75% for node negative cases, *vs* 16.7% for those with lymph node metastasis. Extension of cancer to the regional lymph nodes was associated with poor outcome ($P = 0.008$).

No impact was noticed for age and sex on patient outcome ($P = 0.487$), however, females had better survival rates than males but without a statistically significant difference ($P = 0.126$). There was no relationship between p53 positivity, VEGF protein expression or the presence of *H-ras* oncogene mutations and patient survival ($P = 0.962$, $P = 0.791$, and $P = 0.967$, respectively; Table 5).

There was a statistically insignificant difference between grades 2 and 3 regarding survival ($P = 0.128$). There was no statistical difference between pT2, pT3, and pT4 tumors regarding prognosis ($P = 0.601$). Further division of transitional cell carcinoma groups into organ-confined (pT1 and pT2; 36 cases) and non-organ confined tumors (pT3 and pT4; 14 cases) was carried out.

Table 3 Clinicopathological characteristics of the transitional cell carcinoma group and their relation to VEGF immunostaining *n* (%)

Variable	*n*	VEGF		*P* value
		Positive	Negative	
Age (yr)				
≤ 50	11 (22)	5 (10)	6 (12)	0.364
> 50	39 (78)	12 (24)	27 (54)	
Sex				
Male	42 (84)	16 (32)	26 (52)	0.161
Female	8 (16)	1 (2)	7 (14)	
Grade				
G2	18 (36)	6 (12)	12 (24)	0.941
G3	32 (64)	11 (22)	21 (42)	
Stage				
pT1	8 (16)	3 (6)	5 (10)	0.935
pT2	28 (56)	9 (18)	19 (38)	
pT3	7 (14)	3 (6)	4 (8)	
pT4	7 (14)	2 (4)	5 (10)	
Node status				
Positive	6 (12)	1 (2)	5 (10)	0.339
Negative	44 (88)	16 (32)	28 (56)	

VEGF: Vascular endothelial growth factor.

Table 4 Clinicopathological characteristics of the transitional cell carcinoma group and their relation to *H-ras* mutations *n* (%)

Variable	*n*	*H-ras* mutation		*P* value
		Positive	Negative	
Age (yr)				
≤ 50	11 (22)	1 (2)	10 (20)	0.479
> 50	39 (78)	7 (14)	32 (64)	
Sex				
Male	42 (84)	7 (14)	35 (70)	0.768
Female	8 (16)	1 (2)	7 (14)	
Grade				
G2	18 (36)	4 (8)	14 (28)	0.368
G3	32 (64)	4 (8)	28 (56)	
Stage				
pT1	8 (16)	1 (2)	7 (14)	0.156
pT2	28 (56)	4 (8)	24 (48)	
pT3	7 (14)	3 (6)	4 (8)	
pT4	7 (14)	0 (0)	7 (14)	
Node status				
Positive	6 (12)	0 (0)	6 (12)	0.254
Negative	44 (88)	8 (16)	36 (72)	

The organ-confined group showed better outcomes than the non-organ confined group, however, this difference did not reach a statistically significant level ($P = 0.3$). The 3-year survival rate for the organ-confined group was 85.7% *vs* 42.8% for the non-organ confined group.

DISCUSSION

Carcinoma of the urinary bladder is the most common solid tumor in men in Egypt. It represents 30.3% of all cancer cases treated at the National Cancer Institute in Cairo[18]. Recently, Gouda *et al*[19] reported a significant decline of the relative frequency of bladder cancer to 11.7%, Bilharzia association from 82.4% to 55.3%, and squamous cell carcinomas from 75.9% to 28.4%. At the same time, this decline was associated with a significant rise in transitional cell carcinomas from 16.0% to 65.8%, an increase in the median age of patients from 47.4 years to 60.5 years, and a decrease of the male: female (M/F) ratio from 5.4 to 3.3.

Table 5 Kaplan-Meier estimates of 3-year disease-free survival in relation to patient and tumour characteristics in the transitional cell carcinoma group

Characteristic	*n*	3-year survival rate (%)	*P* value
Total No.	50	68.0	
Age (yr)			
≤ 50	11	71.8	0.487
> 50	39	54.5	
Sex			
Male	42	64.3	0.126
Female	8	87.5	
Grade			
G2	18	72.2	0.128
G3	32	65.6	
Stage			
pT1	8	100	
pT2	28	71.4	0.601
pT3	7	57.1	
pT4	7	28.6	
Node status			
Positive	6	16.7	0.008
Negative	44	75.0	
p53			
Positive	33	63.6	0.962
Negative	17	76.5	
VEGF			
Positive	17	64.7	0.791
Negative	33	69.7	
H-ras			
Positive	8	66.7	0.967
Negative	42	75.0	

The age range of this study group was 41-87 years (59.0 ± 9.9). In western countries, the median age is 65 years, while in Egyptian series, it was 46 years[19]. However, Koraitim *et al*[20] noticed a marked shift in the age-related incidence curve for Schistosoma-associated bladder carcinoma towards an older age group approximating that in non-Schistosomal cases.

Despite the use of advanced imaging techniques, regional or distant metastases may go unnoticed until surgery or at follow-up. There is ample documentation that clinical compared to pathological staging is inaccurate in one third of cases, with a tendency for underestimation of the extent of the disease. Therefore, there is a need for additional objective information on the aggressiveness of bladder tumors[21].

Several investigators have shown that tumor angiogenesis is important for continued tumor growth and progression. It has been suggested that angiogenic capacity is an early marker of preneoplastic and neoplastic lesions of the human bladder, and the development of a vascular network is integral to tumor progression[10]. The formation of a vascular network is not only essential for tumor growth but also provides a route by which tumor emboli may disseminate, resulting in the development of metastatic disease. There appears to

be a quantitative relationship between angiogenesis and prognosis in several human cancers. Moreover, angiogenic activity was correlated with a higher incidence of lymph node metastasis and poor prognosis for patients with transitional cell carcinoma of the bladder[22,23].

Mutational studies of the *H-ras* gene family have demonstrated that an alteration in codons 12 and 61 of the *H-ras* gene occurs in about 20% of bladder cancers[24].

In Schistosoma-associated bladder carcinoma, the incidence of transitional cell carcinoma ranged from 16% to 43.8%[20]. In western countries, transitional cell carcinoma represents 90% of bladder cancers[25]. In the current study, the overall 3-year survival rate of patients with transitional cell carcinoma was 68%. There was no significant impact of patient age or sex on the patient outcome. Similar results have been previously reported[1,26].

In this study, transitional cell carcinomas were of grade 2 (36%) and grade 3 (64%) because most of the patients had invasive transitional cell carcinoma (84%). This study showed that tumor grade did not significantly affect patient outcomes in transitional cell carcinoma according to univariate analysis. The 3-year survival rate of patients with grade 2 tumors was 72.2% *vs* 65.6% for those with grade 3 ($P = 0.128$). Similar results have been previously reported[26,27]. However, other reports demonstrated a significant relationship between tumor grade and patient survival[1].

These contradictory results might be due to the presence of inter- and intra-individual variations in evaluation of tumor grades in patients with bladder tumors[7,28]. On the other hand, Ghoneim *et al*[1] studied a large cohort of patients (1026 patients; 764 men and 262 women) with different types of bladder cancer (Squamous, transitional carcinoma, and adenocarcinoma), with very long follow up times (up to 24 years) and relative higher incidences of regional lymph nodes metastasis (18.3%).

The critical importance of tumor stage had been recognized in several studies[1,29]. In the present study, there was a tendency of survival advantage in low stage tumors compared to high stage tumors. The 3-year survival rates were 100%, 71.4%, 57.1%, and 28.6% for stage pT1, pT2, pT3, and pT4, respectively. However, these differences in survival were not significant ($P = 0.601$). This insignificant relationship between tumor stage and survival might be attributed to the small number of patients in this group (50 patients) and that most of patients in our study had stage pT2 (56%). The small number of patients in other stages rendered statistical analysis of tumor stage in relation to patient survival insignificant. Also, there was no significant difference between the organ-confined group (pT1 and pT2) and the non-organ confined bladder group (pT3 and pT4) in the 3-year survival group ($P = 0.3$).

The incidence of lymph node metastasis for patients with transitional cell carcinoma was 12%. Lymph node metastasis was associated with significantly poor prognosis. Node negative patients had a 3-year survival rate of 75% in comparison to 16.7% for patients with lymph node metastasis. Similar results were reported in previous studies[29-31].

In the present study, tumor cell surface expression of p53 and VEGF proteins as well as the presence of *H-ras* gene mutations had no prognostic value in Schistosoma-associated transitional cell carcinoma of the bladder. Similar results were previously reported either with invasive bladder tumors[32], or with superficial bladder tumors[12,33,34]. Our results were not in agreement with other reports that found a significant relationship between VEGF and p53 markers and prognosis[35], and lymph node metastasis[8] in bladder cancer.

These contradictory results might be attributed to different study population criteria and different methodology. For example, the Lorenzo-Romero *et al*[35] study was done on 115 samples (21 cases without and 94 cases with bladder tumor). In this study, 63.8% of patients had superficial and 37.2% had infiltrative (20% recurrent) transitional cell carcinoma (16% and 84% in the present work, respectively), p53 detection was done at the level of DNA (not tissue expression) by polymerase chain reaction-single strand conformational polymorphism analysis, and lastly, all the cases of our work were Schistosoma-associated bladder carcinoma.

The difference in results between the present work and that of Suzuki *et al*[8] can be explained by differences in patient numbers (87 *vs* 50), longer follow up times (42 mo *vs* 20 mo), higher percentages of nodal involvement (25% *vs* 12%), and immunohistochemistry detection was done for VEGF-C, which is one of the isoforms of VEGF.

There was no relationship between tumor expression of both p53 and VEGF proteins, as well as the presence of *H-ras* gene mutations, and the different clinicopathologic factors, except for the presence of a significant relation between the expression of p53 and tumor grade ($P = 0.01$). High grade tumors had increased incidence of cell surface expression of p53 compared to low grade tumors. Also, invasive bladder tumors (30 out of 42 cases) demonstrated higher expression of p53 protein than superficial bladder tumors (3 out of 8 cases). The observed difference in surface expression of p53 between superficial low grade and invasive high grade bladder tumors in our study may support the idea that there may be two different angiogenic pathways in bladder cancer that are associated with different tumor morphology and behavior[36]. One pathway is related to the superficial low grade bladder tumors with organized branching and the other to the muscle invasive and solid tumors with disorganized vasculature[12].

With Kaplan-Meier analysis, lymph node status was the only significant prognostic factor in transitional cell carcinoma. Patients with lymph node metastasis had an increased risk to die much higher than those with negative lymph nodes.

In conclusion, our results suggest that the degree of VEGF, p53, and *H-ras* oncogene expression has no relation to patient survival and outcome in Schistosoma-associated transitional cell carcinoma. Invasive high grade bladder tumors had more cell surface expression of p53

protein than superficial low grade bladder tumors. Hence, lymph node metastasis could provide more objective tools for better judgment on the patient survival and help in choosing the most convenient therapy for individual patients. Additional studies, with wider prospective series and longer follow up, should address whether angiogenesis predicts prognosis and recurrence in separate and homogenous samples of patients with bladder tumors that equally reflect all categories.

COMMENTS

Background

Carcinoma of the bladder is the most common cancer of the urinary tract. It is the most common solid tumor in men in Egypt. To date, there is no reliable method to identify those patients at risk of recurrence and those who will develop more aggressive disease. The histo-pathological parameters such as tumor grade, stage, lymph node involvement are the most important parameters in evaluation of the biological behavior of bladder cancer. However, there are multiple documents that clinical as compared to pathological staging is inaccurate.

Research frontiers

In bladder tumor, there was a significant relationship between tumor angiogenesis and the presence of lymph node metastasis, and prognosis in patients with superficial and invasive bladder carcinoma. The research hotspot is how to clarify the relation between certain angiogenic and oncogenic markers [vascular endothelial growth factor (VEGF), *p53*, and *H-ras* oncogene] and risk of recurrence and their significance as prognostic markers.

Innovations and breakthroughs

VEGF and p53 protein expression were evaluated by using immunohistochemical staining method, and H-Ras oncogene mutation by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The study showed that high grade tumors revealed higher p53 immunostaining than low grade tumors. p53, VEGF protein expression as well as H-ras oncogene mutation had insignificant impact on patient outcome and cancer extension to regional lymph nodes was associated with poor outcome.

Applications

Lymph node metastasis could provide more objective tools for better judgment on the patient survival and help in choosing the most convenient therapy for individual patient.

Peer review

In this manuscript, the authors examined vascular endothelial cell growth factor, p53, and H-Ras oncogene as prognostic markers in Egyptian patients with transitional cell bladder carcinoma. They concluded that p53, VEGF, and H-ras mutations have no prognostic value in Schistosoma-associated transitional cell carcinoma of the bladder. Only lymph node status provides prognostic information. In general, the manuscript is reasonably well written and addresses a critical issue, the molecular pathogenesis of Schistosoma-associated bladder cancer.

REFERENCES

1 **Ghoneim MA**, el-Mekresh MM, el-Baz MA, el-Attar IA, Ashamallah A. Radical cystectomy for carcinoma of the bladder: critical evaluation of the results in 1,026 cases. *J Urol* 1997; **158**: 393-399

2 **El-Bolkainy MN**, Chu EW, Ghoneim MA, Ibrahim AS. Cytologic detection of bladder cancer in a rural Egyptian population infected with schistosomiasis. *Acta Cytol* 1982; **26**: 303-310

3 **Brausi M**, Collette L, Kurth K, van der Meijden AP, Oosterlinck W, Witjes JA, Newling D, Bouffioux C, Sylvester RJ. Variability in the recurrence rate at first follow-up cystoscopy after TUR in stage Ta T1 transitional cell carcinoma of the bladder: a combined analysis of seven EORTC studies. *Eur Urol* 2002; **41**: 523-531

4 **Hara S**, Miyake H, Fujisawa M, Okada H, Arakawa S, Kamidono S, Hara I. Prognostic variables in patients who have undergone radical cystectomy for transitional cell carcinoma of the bladder. *Jpn J Clin Oncol* 2001; **31**: 399-402

5 **El-Shazli S**, Anwar H, Ramzy S, Al-Didi M. Extended lymphadenectomy to the lower paraaortic nodes during radical cystectomy. *J Egypt Natl Canc Inst* 2004; **16**: 22-28

6 **Hermanek P**, Sobin LH, Wittekind C. How to improve the present TNM staging system. *Cancer* 1999; **86**: 2189-2191

7 **Ooms EC**, Anderson WA, Alons CL, Boon ME, Veldhuizen RW. Analysis of the performance of pathologists in the grading of bladder tumors. *Hum Pathol* 1983; **14**: 140-143

8 **Suzuki K**, Morita T, Tokue A. Vascular endothelial growth factor-C (VEGF-C) expression predicts lymph node metastasis of transitional cell carcinoma of the bladder. *Int J Urol* 2005; **12**: 152-158

9 **Inoue K**, Slaton JW, Karashima T, Yoshikawa C, Shuin T, Sweeney P, Millikan R, Dinney CP. The prognostic value of angiogenesis factor expression for predicting recurrence and metastasis of bladder cancer after neoadjuvant chemotherapy and radical cystectomy. *Clin Cancer Res* 2000; **6**: 4866-4873

10 **Santos L**, Costa C, Pereira S, Koch M, Amaro T, Cardoso F, Guimarães T, Bento MJ, Lobo F, Pinto S, Lopes C. Neovascularisation is a prognostic factor of early recurrence in T1/G2 urothelial bladder tumours. *Ann Oncol* 2003; **14**: 1419-1424

11 **Stavropoulos NE**, Bouropoulos C, Ioachim IE, Michael M, Hastazeris K, Tsimaris I, Kalogeras D, Liamis Z, Stefanaki S, Agnantis NI. Prognostic significance of angiogenesis in superficial bladder cancer. *Int Urol Nephrol* 2004; **36**: 163-167

12 **Sağol O**, Yörükoğlu K, Sis B, Tuna B, Ozer E, Güray M, Mungan U, Kirkali Z. Does angiogenesis predict recurrence in superficial transitional cell carcinoma of the bladder? *Urology* 2001; **57**: 895-899

13 **Mostofi FK**, Sobin LH, Torloni H. Histological typing of urinary bladder tumors. International classification of tumors. No. 10. Geneva: World Health Organization, 1973

14 **Sobin LH**, Wittekind CH, editors. TNM classification of malignant tumors. 5th edition. New York: John Wiley & Sons, Inc., 1997

15 **Kok LP**, Boon ME. Microwave Cookbook for Microscopists: Art and Science of Visualization. 3rd edition. Leiden: Coulomb Press, 1992

16 **Shi SR**, Cote RJ, Young L, Imam SA, Taylor CR. Use of pH 9.5 Tris-HCl buffer containing 5% urea for antigen retrieval immunohistochemistry. *Biotech Histochem* 1996; **71**: 190-196

17 **Kaplan EL**, Meier P. Non-parametric estimation from incomplete observation. *J Am Stat Ass* 1958; **53**: 457-481

18 **Rafla M**, Ibrahim AS, Sherif M, Valleron AJ. A model to predict the outcome of the bilharzial bladder cancer patient after radical cystectomy. *Br J Cancer* 1987; **56**: 830-833

19 **Gouda I**, Mokhtar N, Bilal D, El-Bolkainy T, El-Bolkainy NM. Bilharziasis and bladder cancer: a time trend analysis of 9843 patients. *J Egypt Natl Canc Inst* 2007; **19**: 158-162

20 **Koraitim MM**, Metwalli NE, Atta MA, el-Sadr AA. Changing age incidence and pathological types of schistosoma-associated bladder carcinoma. *J Urol* 1995; **154**: 1714-1716

21 **Fernández Mena FJ**, Moreno-Torres YC. [Bladder cancer] *Arch Esp Urol* 2001; **54**: 493-510

22 **Canoğlu A**, Göğüş C, Bedük Y, Orhan D, Tulunay O, Baltaci S. Microvessel density as a prognostic marker in bladder carcinoma: correlation with tumor grade, stage and prognosis. *Int Urol Nephrol* 2004; **36**: 401-405

23 **Beecken WD**, Engl T, Hofmann J, Jonas D, Blaheta R. Clinical relevance of serum angiogenic activity in patients with transitional cell carcinoma of the bladder. *J Cell Mol Med* 2005; **9**: 655-661

24 **Saito S**, Hata M, Fukuyama R, Sakai K, Kudoh J, Tazaki H, Shimizu N. Screening of H-ras gene point mutations in 50 cases of bladder carcinoma. *Int J Urol* 1997; **4**: 178-185

25 **Messing ED**, Catalona W. Urothelial tumors of the urinary tract. In: Walsh PC, Retik AB, Vaughan ED Jr, Wein AJ,

editors. Campbell's urology. 7th edition. Philadelphia: WB Saunders Company, 1998: 2327-2410
26 **Dalbagni G**, Genega E, Hashibe M, Zhang ZF, Russo P, Herr H, Reuter V. Cystectomy for bladder cancer: a contemporary series. *J Urol* 2001; **165**: 1111-1116
27 **Krüger S**, Thorns C, Böhle A, Feller AC. Prognostic significance of a grading system considering tumor heterogeneity in muscle-invasive urothelial carcinoma of the urinary bladder. *Int Urol Nephrol* 2003; **35**: 169-173
28 **Yorukoglu K**, Tuna B, Dikicioglu E, Duzcan E, Isisag A, Sen S, Mungan U, Kirkali Z. Reproducibility of the 1998 World Health Organization/International Society of Urologic Pathology classification of papillary urothelial neoplasms of the urinary bladder. *Virchows Arch* 2003; **443**: 734-740
29 **Stein JP**, Lieskovsky G, Cote R, Groshen S, Feng AC, Boyd S, Skinner E, Bochner B, Thangathurai D, Mikhail M, Raghavan D, Skinner DG. Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients. *J Clin Oncol* 2001; **19**: 666-675
30 **Quek ML**, Stein JP, Clark PE, Daneshmand S, Miranda G, Cai J, Groshen S, Cote RJ, Lieskovsky G, Quinn DI, Skinner DG. Microscopic and gross extravesical extension in pathological staging of bladder cancer. *J Urol* 2004; **171**: 640-645
31 **Hong B**, Park S, Hong JH, Kim CS, Ro JY, Ahn H. Prognostic value of lymphovascular invasion in transitional cell carcinoma of upper urinary tract. *Urology* 2005; **65**: 692-696
32 **Hemal AK**, Khaitan A, Dinda AK, Gupta NP, Seth A, Dogra PN, Nabi G. Prognostic significance of p53 nuclear overexpression in patients of muscle invasive urinary bladder carcinoma treated with cystectomy. *Urol Int* 2003; **70**: 42-46
33 **Chow NH**, Liu HS, Chan SH, Cheng HL, Tzai TS. Expression of vascular endothelial growth factor in primary superficial bladder cancer. *Anticancer Res* 1999; **19**: 4593-4597
34 **Gil P**, Allepuz C, Blas M, Borque A, del Agua C, Plaza L, Rioja LA. Significance of protein p53 overexpression in the clinical course of high-risk superficial bladder cancer. *Urol Int* 2003; **70**: 172-177
35 **Lorenzo-Romero JG**, Salinas-Sánchez AS, Giménez-Bachs JM, Sánchez-Sánchez F, Escribano-Martínez J, Segura-Martín M, Hernandez-Millán IR, Virseda-Rodríguez JA. Prognostic implications of p53 gene mutations in bladder tumors. *J Urol* 2003; **169**: 492-499
36 **O'Brien TS**, Smith K, Cranston D, Fuggle S, Bicknell R, Harris AL. Urinary basic fibroblast growth factor in patients with bladder cancer and benign prostatic hypertrophy. *Br J Urol* 1995; **76**: 311-314

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