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Objective : *To determine prognostic indicators that would identify the subgroup with a low likelihood of disease recurrence.*

Methods : *Review of medical records and pathological tissues of patients with Ta and T1 transitional cell carcinoma. Twenty-five patients were followed for at least 1 year or until recurrence of tumor. Pathological tissue was reanalyzed and strained for p53 protein by the same pathologist. Age, multiplicity, grade, stage, p53 staining and time of recurrence were analyzed.*

Results : *Nuclear staining for p53 was found in 20 of 25 tumors (80%). More than 10% nuclear staining was used as overexpression because this cut-off point achieved the highest sensitivity (90.9%) and specificity (64.3%) in predicting the tumor recurrence. After analysis, only p53 overexpression had significant effects on first year recurrence. Nine patients (90%) who had not p53 overexpression had not tumor recurrence in the first year after completed endoscopic resection but ten patients (67%) who had p53 overexpression had tumor recurrence in the first year after completed resection.*

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Conclusion : *p53 is likely to remain as the most useful aid for management decisions regarding superficial bladder.*

Key words : *Superficial transitional cell carcinoma of urinary bladder, p53, recurrence.*

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วัตถุประสงค์ : *เพื่อหาปัจจัยเสี่ยงที่สามารถแยกผู้ป่วยที่มีโอกาสเกิดโรคซ้ำหลังการรักษาได้ต่ำกับผู้ป่วยที่มีโอกาสเกิดโรคซ้ำหลังการรักษาได้สูง*

วิธีการวิจัย : *ศึกษาผู้ป่วยมะเร็งของกระเพาะปัสสาวะชนิดที่อยู่ที่เยื่อบุผิวที่ทำการรักษาในโรงพยาบาลจุฬาลงกรณ์ย้อนหลัง 10 ปี ที่ติดตามการรักษาอย่างน้อย 1 ปีหรือจนกว่าจะมีการเกิดซ้ำของโรค และไม่เคยได้รับการใส่ยารักษาในกระเพาะปัสสาวะ โดยดูประวัติเก่าและนำชิ้นเนื้อทางพยาธิวิทยามาทำการย้อมสี H&E ใหม่และย้อม p53 ปัจจัยที่นำมาศึกษามีดังนี้ อายุ จำนวนตำแหน่งของมะเร็ง ลักษณะของเซลล์มะเร็ง ระยะของโรค การติดสีของ p53 และเวลาการเกิดซ้ำของโรค*

ผลการศึกษา : *พบผู้ป่วยที่อยู่ภายใต้เงื่อนไขจำนวน 25 คน ย้อมติด p53 จำนวน 20 คน จากการวิเคราะห์พบว่าการย้อมติดสี p53 เพียงปัจจัยเดียวเท่านั้นที่มีนัยสำคัญทางสถิติ โดยชิ้นเนื้อที่เซลล์มะเร็งติดสีของ p53 มากกว่า10 % มีผลต่อการเกิดโรคซ้ำได้บ่อยกว่าที่ติดสีน้อยกว่า 10 % อย่างมีนัยสำคัญ*

สรุป : *p53 อาจมีส่วนช่วยในการตัดสินใจในการเลือกระยะเวลาที่เหมาะสมเพื่อการติดตามการเกิดซ้ำของโรคมะเร็งของกระเพาะปัสสาวะ*

Though the superficial transitional cell carcinoma (TCC) of the urinary bladder-that is, a tumor confined to the mucosa (Ta, Tis) or submucosa (T1) - can be successfully treated by endoscopic procedure, it is difficult to be sure what the subsequent condition of that bladder will be. On the one hand, the bladder may remain free of tumors. On the other hand, there may be recurrences at frequent or infrequent intervals. Scheduled periodic cystoscopy (every 3 months in first year, every 6 months in second year, and annually thereafter) has been the only means of surveillance. [1] It is an invasive procedure and may not need to be performed as often as traditionally recommended for some patients whose tumors may never recur or recur infrequently.

Several characteristics of TCC have been documented to herald a shorter disease-free period and /or progression, including (a) endoscopic features (multiple tumors,[2-6] dysplasia elsewhere in the bladder[2]); (b) cytologic features (histologic grade,[2,7,8] positive urinary cytology, DNA aneuploid[9]); and (c) biologic markers (absence of blood group antigens from the tumor cell surface,[10,11] increased epidermal growth factor receptors,[12] and p53 overexpression).

Several institutional reviews of Ta and T1 tumors utilizing multivariate analysis have demonstrated p53 overexpression status as a statistically significant prognostic factor for progression-free and overall survival.[13, 14]

Objective

This study was carried out to investigate the possibility of using p53 overexpression as a prognostic factor to identify a subgroup of superficial TCC patients with a low likelihood for early disease recurrence.

Material and method

Medical records : Of 236 patients diagnosed with TCC urinary bladder tumor from January 1987 to 1997 at King Chulalongkorn Memorial Hospital, 69 were found to have superficial TCC. Of the remaining 69, after excluding patients with intravesical therapy, there were only 25 patients who had complete medical records with a minimum follow-up time of one year and retievable fixed tumor specimens. The medical records of these 25 patients were reviewed retrospectively for age at first diagnosis; multiplicity, histologic grade, and stage of tumors; and time of first recurrence.

Molecular genetic marker : P53 expressions were studied from newly cut 4mm sections of paraffin wax fixed tumor specimens using the monoclonal antibodies immunohistochemical staining technique. The primary antibody was used to stain the sections: mouse monoclonal antiserum to p53 (Ab6) (oncogene science) at a dilution of 1:100. Following deparafinnization and dehydration, the sections were incubated with primary antibody for 4 hours at room temperature. Following further washing, anti-goat immunoglobulin G (1:200, Vector laboratory) was incubated on the sections for 30 minutes, the section were then washed and incubated with avidin-biotin peroxidase complex for 30 minutes. Peroxidase reaction was detected by addition of diaminobenoiodine tetrahydrochloride. Four high power fields of a section were counted for stained cells and total cells. Valves after stained cells divided total cells were reported as percent of cell straining.

Data and statistic analyses : Data was analyzed via use of the SPSS program (Statistical Package for the Social Sciences, Version 6). Variables examined included age; multiplicity, grade, stage of tumor; and p53 overexpression at presentation. Each factor was

first tested as a single regression variable model. Stepwise multivariate regression was then used to identify the most important predictor variable for disease-free survival.

Results

The mean age of the patients in this group was 64.8±11.84 years (range 43-94). Twenty-three were males and two were females. Twenty patients (80%) had single tumors and 5 (25%) had multiple tumors. Nineteen patients (76%) had low grade and six (24%) had high-grade tested tumors. Thirteen (52%) were stage Ta and twelve (48%) were stage T1 tumors (Table 1). Nuclei staining for p53 was positive in 20 (80%) out of 25 tumors. The degree of staining varied from 1% to 100% of cells (Table 2).

Table 1. General Data of patients.

	Recurrence within 1 year %	No. recurrence within 1 year %	N
Age < 60 year	3 (38%)	5 (62%)	8
Age > 60 year	8 (47%)	9 (53%)	17
Low grade (grade 1,2)	8 (42%)	11 (58%)	19
High grade (Grade 3)	3 (50%)	3 (50%)	6
Stage Ta	4 (31%)	9 (69%)	13
Stage T1	7 (58%)	5 (42%)	12
Single lesions	7 (35%)	13 (65%)	20
Multiple lsions	4 (80%)	1 (20%)	5
Overexpression of p53	10 (67%)	5 (33%)	15
No. overexpression of p53	1 (10%)	9 (90%)	10

Table 2. Degree of p53 staining.

% cell p53 positivity	0	1-10	11-20	21-30	31-50	51-70	71-100
No. of tumors	5	5	3	2	3	6	1

The Receiver Operating Characteristic (ROC) curve plot and ten percent or more of nuclei staining for p53 protein was used as a cut-off point for over-expression to achieve the highest sensitivity (90.9%) and specificity (64.3%) in predicting the tumor recurrence, as shown in Figure 1 and Table 3, overexpression of p53 was found in 15 tumors (60%).

Eleven patients (44%) had tumor recurrence in the first year after complete endoscopic resection. Age, multiplicity of tumors, histologic grade of tumor, stage of tumors and p53 overexpression were tested as single regression variables in the univariate model. If more than one factor was significant, multivariate regression was used to identify the most important predictor (Table 4.) Only p53 had significant effects on the tumor recurrence in the first year after complete endoscopic eradication.

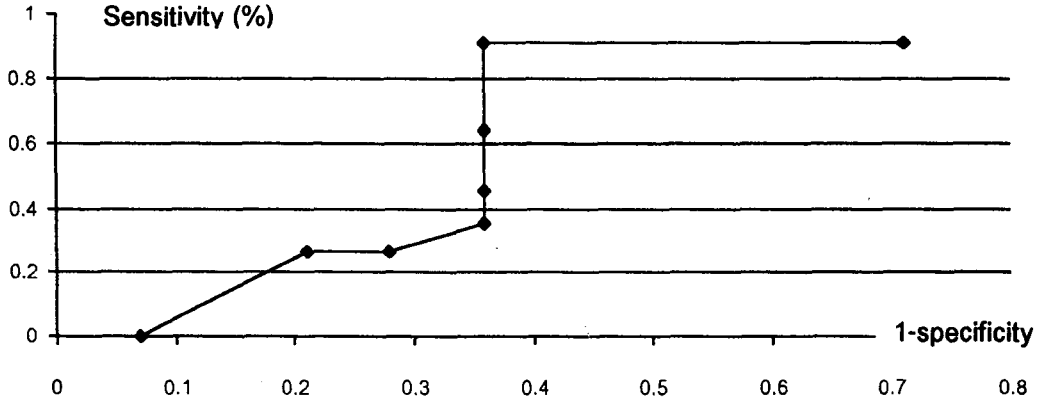

Figure 1. Receiver operating characteristic curve plot (ROC) curves of p53 and time to recurrence.

Table 3. Predictor of recurrence.

Nuclear staining of p53	>0%	>10%	>20%
Sensitivity (%)	90.9	90.9	63.6
Specificity (%)	28.6	64.3	64.3

Table 4. Univariate and multivariate regression for risk factors predictive of the first year recurrence.

	Univariate Model
Factor	**Prognostic Value (p-value)**
Age	No (0.276)
Multiplicity	No (0.098)
Grade	No (0.734)
Stage	No (0.172)
p53	Yes (0.015)

Discussion

The p53 oncogene (17p13-1 locus) codes for a nuclear phosphoprotein whose major role appears to be in transcriptional regulation.[15] p53 is involved in DNA repair or induction of apoptosis for irreversibly damaged DNA. Hence, it is thought to function as a tumor suppressor gene.[16] There are two forms of this gene product - the normal form (wild type, or unaltered type) with a half-life of 6-20 minutes, and the mutant form with a prolonged half-life of up to 6 hours.[17,19] Mutation of the wild type results in the accumulation (over-expression) of mutant p53 protein within the tumor cell nucleus due to the prolonged half-life. This leads to alteration in modulating activity.[20] Mutant p53 can be easily detected immunohiotochemically using monoclonal

antibodies on fresh or fixed tumor specimens.[21] At least 10% nuclei staining of mutant p53 is defined as overexpression by Thomas DJ[22] and 20% or more by Sarkis,[23] and Serth, et al.[24]

Changes in p53 are the most commonly recognized genetic alteration in human malignancy, and they correlate with tumor behavior.[25] Mutations of p53 have been observed in 50% of high stage bladder tumors.[26-29] However, p53 changes and their relationship to tumor behavior in superficial bladder tumor are less clear. Mutation has been observed in up to 65% of biopsies of primary Tis (pTis).[30] Positive for p53 was demonstrated as an independent predictor of progression in a series of 33 pTis patients followed for 124 months.[23] Positive mutant p53 staining has ranged between 8 to 95% for Ta and T1 disease with a strong correlation between positivity and tumor grade.[26,31-35]

By using 10% nuclei staining of p53 as a cut-off point this study demonstrated that tumors with a high level of p53 overexpression had a shorter tumor-free period ($p<0.05$). Therefore, it seemed that patients without over- expression of p53 had a longer period of time before tumor recurrence than patients with overexpression of p53.

Conclusion

The result of this study tends to indicate that overexpression of p53 may be helpful in identifying a subgroup of patients with superficial TCC urinary bladder tumors who may be prone to having early recurrence. These patients should have their first check cystoscopy after one year in selected cases.

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