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NUCLEAR p53 OVEREXPRESSION IN PATHOLOGICAL T1 BLADDER CANCER: A MARKER FOR TUMOR RECURRENCE AND TUMOR PROGRESSION

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Abstract In order to investigate the malignant potential of stage T1 bladder tumors the status of p53 overexpression and mucosal change of grossly normal bladder mucosa, respectively, were studied regarding tumor recurrence and tumor progression. A total of 30 cases with stage T1 bladder tumor were enrolled and selected site mucosal biopsies (SSMB) were taken of randomly selected 17 cases. All cases were treated by transurethral resection and followed for a median period of 39 months. Six of the 30 cases (20%) showed p53 overexpression. Five of the 17 (29%) had mucosal changes (carcinoma in situ;1, dysplasia;4). Out of 6 cases with p53 overexpression, 3 (50%) showed tumor progression, in contrast to only 2 (8%) of 24 cases without p53 overexpression ($p<0.05$, Fisher's test). Statistically significant differences were found in recurrence-free and progression-free survival between patients whose tumor had p53 overexpression and those whose tumor did not (both $p<0.01$, Log Rank test). Significant correlations were not found between mucosal change of grossly normal bladder mucosa and tumor recurrence, or progression. These results suggest that p53 overexpression in the main tumor might be of useful prognostic marker for tumor recurrence as well as tumor progression.

Introduction

Mutation of the p53 gene is possibly the most common genetic defect in human tumors.[1] Identification of nuclear p53 overexpression has been reported to correlate with p53 mutation in many types of cancer.[2-5] The p53 gene functions as a tumor-suppressor gene and more specifically as a cell-cycle regulator.[6] Levels of p53 protein increase in response to damage to DNA, arresting the cell cycle and allowing time for the repair of DNA.

Mutation of the p53 gene occurs in a high percentage of invasive transitional cell carcinomas of the bladder.[7] Approximately 80% of patients with transitional cell carcinoma of the bladder are diagnosed with superficial disease. Following transurethral resection 70-80% of these patients have tumor recurrence, and 10-20% of those patients develop tumor

Table 1 Patient characteristics, status of p53 overexpression and histopathological change of grossly normal mucosa.
* : p53 overexpression, N: negative, P: positive
** : -: not done
*** : Figures in parenthese represent periods from operation to recurrence of tumor

Pt. No.	Age / Sex	Grade	Adjuvant herapy	p53*	Mucosal change **	Recurrence *** (months)	Progression (months)
1	82/ M	1	Yes	N	–	Yes(94)	Yes(94)
2	83/ F	3	Yes	N	–	No	No
3	46/ M	2	Yes	P	–	Yes(5)	Yes(38)
4	73/ M	2	Yes	N	–	No	No
5	72/ M	2	Yes	N	–	No	No
6	57/ M	1	No	N	–	No	No
7	58/ M	2	Yes	N	–	No	No
8	60/ M	2	No	N	–	No	No
9	68/ M	2	No	N	–	No	No
10	87/ M	3	Yes	N	–	Yes(12)	No
11	41/ M	1	Yes	N	–	No	No
12	75/ F	3	Yes	P	–	Yes(5)	No
13	75/ M	1	No	N	–	Yes(6)	Yes(6)
14	79/ M	2	Yes	P	Yes	Yes(9)	No
15	74/ M	2	No	N	No	No	No
16	52/ M	2	Yes	N	Yes	No	No
17	66/ M	2	Yes	P	Yes	Yes(7)	Yes(7)
18	76/ M	2	Yes	P	Yes	Yes(12)	Yes(27)
19	61/ M	2	No	N	No	No	No
20	77/ M	2	Yes	P	No	No	No
21	58/ M	1	Yes	N	No	No	No
22	71/ F	2	Yes	N	No	No	No
23	79/ M	2	Yes	N	Yes	No	No
24	82/ M	2	Yes	N	No	No	No
25	70/ M	3	Yes	N	No	Yes(4)	No
26	71/ M	1	Yes	N	No	No	No
27	85/ M	1	Yes	N	No	No	No
28	67/ F	2	No	N	No	Yes(5)	No
29	37/ M	2	Yes	N	No	Yes(7)	No
30	76/ M	2	Yes	N	No	No	No

progression. [8]Among superficial bladder tumors, transitional cell carcinoma invading beyond the basement membrane (stage T1) differs from other superficial bladder tumors in showing a higher frequency of tumor recurrence as well as tumor progression, suggesting that T1 tumor has higher malignant potential. Although the status of p53 overexpression has been investigated in stage T 1 bladder cancer, a few investigators have demonstrated that p53 overexpression may be a predictive factor for tumor progression.[4,8]

With regard to histopathological change of concomitant grossly normal mucosa, Eisenberg et al.[9] demonstrated that patients who had dysplastic changes in grossly normal mucosa had a poor prognosis as compared with those who had no mucosal atypia. Although several authors have reported the prognostic significance of intraurothelial dysplasia in patients with superficial bladder cancer, it is, however, still controversial if this change is truly predictive of patient prognosis.[10–12]

The aim of this study was to clarify if immunohistochemistry for p53 alteration and mucosal change of grossly normal bladder mucosa are useful prognostic markers for tumor progression in stage T1 bladder cancer.

Materials and methods

The present subjects comprised 30 patients (26 males and 4 females) with bladder cancer treated in the Department of Urology, Yamaguchi University School of Medicine, between 1986 and 1995. All patients underwent transurethral resection of bladder tumor

(TUR-Bt) and 17 underwent SSMB(Table 1). The median age of the patients was 72 years (range, 37-87 years). The median follow-up was 39 months (range, 5-94 months). The patients were followed up with periodic urinary cytology and cystoscopy. Intravesical instillation of anticancer agents was performed on 23 cases (Mitomycin C; 14, Doxorubicin; 3, Pirarubicin; 2, BCG; 2) as an adjuvant therapy.

Immunohistochemistry

The details of the immunohistochemistry were described elsewhere.[5] In brief, the avidin-biotin peroxidase method was performed on 5-μm-thick tissue sections. After neutralization of internal peroxidase activity with 0.3% H_2O_2, the rabbit polyclonal anti-p53 antibody (NCL-p53-CM1; Novocastra Laboratories, Newcastle, United Kingdom) was used as the antibody against p53 protein at the dilution of 1:750, followed by two hours incubation at 37℃ in a moisture chamber. The detection was carried out with biotinylated anti-rabbit immunoglobulin (1:200; Vector), followed by incubation with peroxidase-conjugated streptavidin (Vecta Elite kit; Vector). The 3,3'-diaminobenzidine tetrahydrochloride substrate (Sigma) was used for coloration. The established breast cancer cell line (MDA231) and a clinically obtained bladder cancer material with strong positivity for anti-p53 antibody were used as positive controls. The specimen was examined by two independent observers (M.T. and K.O.), and judged as positive for immunostaining when the specific brown color was accumulated in nuclei of the cell as compared with surrounding cytoplasms (Fig.1).

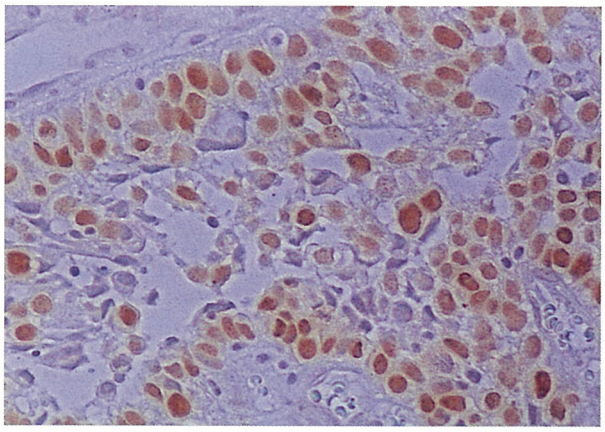

Fig. 1 Immunohistochemical demonstration of p53 in transitional cell carcinoma of the bladder.(magnification × 400)

Selected site mucosal biopsy (SSMB)

The selected sites (lateral to each ureteric orifice, trigon, retrotrigon, posterior wall, dome, anterior wall, bladder neck, prostatic urethra and adjacent to tumor) of the grossly normal urothelium were collected from 17 cases of bladder tumor patients using Storz cold-cup biopsy forceps.

Pathological Evaluation

Samples were fixed in formalin and embedded in paraffin. The sections stained with hematoxylin-eosin were analyzed by one pathologist (M.T.). They were graded and staged according to the WHO-classification and the pTNM staging system. Specimens of SSMB

Table 2 Tumor recurrence and tumor progression in relation to p53 overexpression and mucosal change.

	Tumor Recurrence			Tumor Progression		
	Yes	No	p value	Yes	No	p value
p53 overexpression (evaluated in 30 cases)						
Positive	5 (16.7%)	1 (3.3%)	<0.01	3 (10%)	3 (10%)	<0.05
Negative	6 (20%)	18(60%)		2 (6.7%)	22(73.3%)	
Total	11(36.7%)	19(63.3%)		5 (16.7%)	25(83.3%)	
Mucosal change (evaluated in 17 cases)						
Yes	3 (17.6%)	2 (11.8%)	N.S.	2 (11.8%)	3 (17.6%)	N.S.
No	3 (17.6%)	9 (53.0%)		0 (0 %)	12(70.6%)	
Total	6 (35.2%)	11(64.8%)		2 (11.8%)	15(88.2%)	

were graded in terms of urothelial atypia as described by Nagy et al.[13] Tumor progression was defined as being present when the recurrent tumor had a higher tumor grade and/or stage compared with the parental tumor.

Statistical analysis

Fisher's exact probability test and Kaplan-Meier plot with Log-Rank test were used to calculate the frequency and progression-free survival of each parameter. A p-value less than 0.05 was considered significant.

Results

Tumor recurrence and tumor progression were observed in 11 and 5 cases, respectively (Table 2).

Six of the 30 cases (20%) showed p53 overexpression. Out of the 6 cases, 5 (83%) had tumor recurrence, in contrast to 6 (25%) of 24 cases without p53 overexpression (Table 2). There was a statistical correlation between p53 overexpression and tumor recurrence ($p < 0.05$). As for relation between tumor progression and p53 overexpression, 3 (50%) of 6 cases with p53 overexpression showed tumor progression, but only 2 (8%) of 24 cases without p53 overexpression.

Five of the 17 (29%) had mucosal changes (carcinoma in situ; 1, dysplasia; 4). Out of the 5 cases, tumor recurrence and tumor progression were found in 3 (60%) and 2 (40%) cases, respectively (Table 2). There was no statistical relationship between mucosal change of grossly normal bladder mucosa and tumor recurrence or tumor progression.

A Kaplan-Meier plot demonstrated that patients with p53 overexpression had statistically shorter recurrence-free and progression-free survivals than those without p53 overexpression ($p<0.01$, Log-Rank test) (Fig. 2).

No significant bias was found in the frequency of instillation therapy between the two groups.

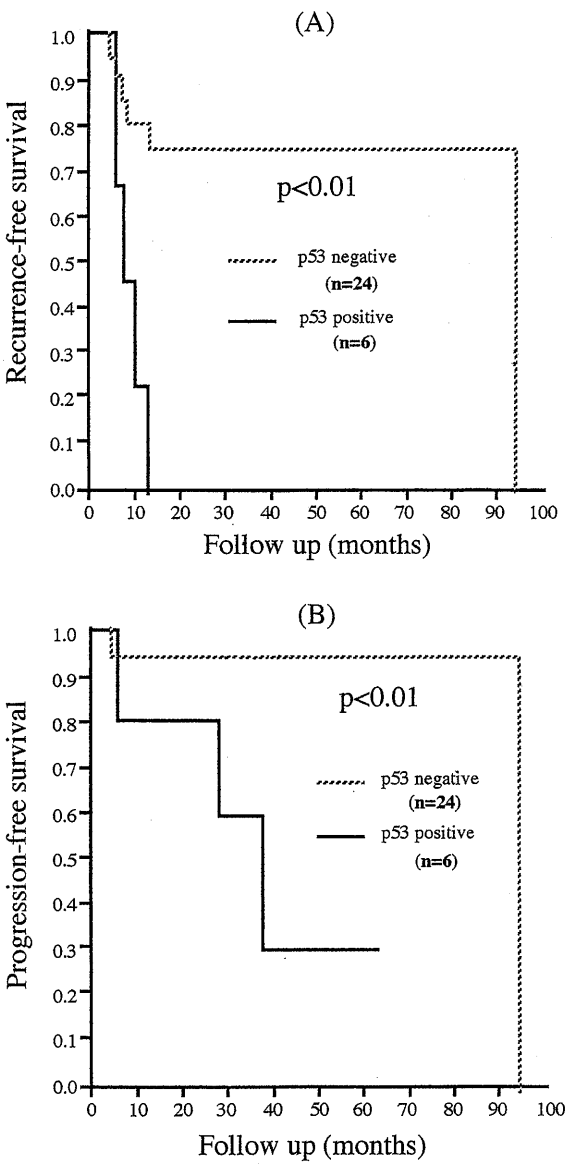

Fig. 2 Kaplan-Meier analysis showing recurrence-free (A) and progression-free (B) survivals between cases with p53 positive and negative expression.

Significantly shorter period to tumor recurrence and tumor progression were observed in patients expressing p53 positivity in the tumor as compared with patients with negative p53 staining in the tumor.

Discussion

Tumor stage, grade, multicentricity and tumor size have been reported as being the most important predictive factors of patient outcome in superficial bladder cancer.[14] However, few factors have been recognized as reliable parameters to identify those patients at increased risk of tumor recurrence and tumor progression.

Mutation of p53 gene usually leads to a protein with an altered configuration, often associated with prolonged half-life and higher intracellular levels compared with the wild-type protein, thereby allowing its immunohistochemical detection.[8] The specificity of the immunohistochemical staining reaction for p53 protein was confirmed by comparison of the results with the detection of p53 mutations.

Moch et al. reported that there was a great difference in the frequency of p53 overexpression between Ta bladder cancer and T1 bladder cancer, whereas there was no difference in p53 overexpression between T1 bladder cancer and T2-4 bladder cancer.[15] This suggests that T1 bladder cancer may harbour a genetic change of malignant potential similar to that of invasive bladder cancer in terms of p53 alteration. Several immunohistochemical studies have revealed p53 overexpression to be well correlated with poor survival[16], or with tumor progression[4] in bladder tumor. Also in stage T1 bladder cancer, p53 overexpression has been reported to predict disease progression.[4,17] In our study, cases with p53 overexpression had a significantly higher recurrence rate (83% vs. 25%) and shorter progression-free survival than those without p53 overexpression. All progressed cases proved to have p53 overexpression. These results are consistent with other reports[4,8], leading to the assumption that p53 overexpression may be a predictive factor for patient outcome in T 1 bladder cancer.

It has been reported that concomitant dysplastic changes occur often in glossly normal bladder mucosa from patients with bladder cancer, although the clinical relevance of this finding is still controversial.[10–12] Our results failed to confer the mucosal change taken from SSMB to prognostic significance for tumor recurrence and tumor progression. This may result from the limited number of cases with SSMB.

Although this is a preliminary study, it is likely that mutant p53 overexpression in T1 bladder tumor may be a useful prognostic marker for tumor recurrence as well as tumor progression. A large number of cohort and prospective studies with longer follow-up will be needed to confirm these preliminary results.

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