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Negative p53/Positive p21 Immunostaining Is a Predictor of Favorable Response to Chemotherapy in Patients with Locally Advanced Bladder Cancer

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The relationship between clinical response to DNA-damaging drugs and p53 and p21 status in patients with locally advanced transitional cell carcinoma (TCC) of the bladder was assessed. The response to intraarterial chemotherapy (IAC) comprising 100 mg/m^2 of cisplatin (CDDP) and 40 mg/m^2 of pirarubicin (THP) and the prognosis were assessed in 23 patients (the mean follow-up period was 19 months). The *p53* gene status of tumors was analyzed at exons 5–8 using polymerase chain reaction-single strand conformation polymorphism analysis in 19 patients, and paraffin-embedded tumor sections were immunostained for p53 and p21 in 23 patients. The overall objective response rate (incidence of good responders) was 70%. The negative p53 group (*n*=17) showed a significantly higher objective response rate than the positive p53 group (*n*=6) (82% vs. 33%; *P*=0.045). The *p53* gene status or p21 staining status was not significantly associated with responsiveness. When the p53 and p21 immunostaining results were combined, good responders were more accurately predicted than by p53 staining status alone; the negative p53/positive p21 group (*n*=12) showed an objective response rate of 92%, which was significantly higher than that of the positive p53 and/or negative p21 group (45%, *n*=11) (*P*=0.027). Cause-specific survival of the negative p53 group was significantly superior to that of the positive p53 group (*P*=0.015). Negative p53/positive p21 immunostaining is a possible predictor of favorable chemotherapeutic response in patients with TCC of the bladder.

Key words: p53 — p21 — Chemosensitivity — Transitional cell carcinoma — Bladder

Although radical cystectomy remains the standard treatment for muscle-invasive ($\geq T_2$) bladder cancer, a variety of adjuvant treatments have been tried to induce downstaging of the tumor, treat micrometastases, and ultimately improve long-term survival.[1–4] Neoadjuvant chemotherapy including intraarterial chemotherapy (IAC), administered prior to surgery, reportedly lowers the stage of the tumor and consequently allows the bladder to be preserved by transurethral resection (TUR) or partial cystectomy in 25 to 50% of patients with invasive bladder cancer.[1, 3–5] However, this therapy is potentially harmful as well as unnecessary for patients whose tumor will not respond to the treatment. Accordingly, it is important to identify the characteristics associated with responses to the treatment.

The wild type (wt) p53, a tumor suppressor protein, results in several outcomes including growth arrest, cell death and DNA repair.[6] The p21 protein, the product of a downstream target gene for wt p53 protein, also induces growth arrest, cell death and DNA repair through inhibiting cyclin-dependent kinases and proliferating cell nuclear antigen (PCNA).[7–10] DNA-damaging drugs up-regulate wt p53 and p21; the latter could be induced even in cells bearing mutant type (mt) *p53* gene through a p53-independent pathway.[11–13] As expected, p53 and p21 status may affect chemosensitivity. Concerning the roles of p53 and p21 in drug sensitivity, mutually exclusive opinions have been put forward: (1) wt p53 and p21 increase sensitivity by favoring induction of apoptosis[11–14] and (2) wt p53 and p21 decrease sensitivity by enhancing DNA repair.[15, 16]

Herein, we investigated the relationship between p53 and p21 status in pretreatment biopsied tumor tissue and clinical response to IAC composed of two DNA-damaging drugs, cisplatin (CDDP) and pirarubicin (THP), in patients with locally advanced transitional cell carcinoma (TCC) of the bladder.

MATERIALS AND METHODS

Case materials Between May 1995 and May 1998, 23 patients (4 females and 19 males) diagnosed as locally invasive ($T_{\geq 2}N_0M_0$) TCC of the bladder or $T_{1b}N_0M_0$ disease with multiple large tumors considered incurable by TUR alone, received one or two courses of IAC at the Univer-

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sity Hospital, Dokkyo University School of Medicine. Staging was based on the 1997 TNM system.[17] The median age of patients was 67 years (range 42–87).

Specimens were biopsied transurethrally in each patient for histopathological diagnosis based on the guideline of the Japanese Urological Association.[18] Residual tumor volume after biopsy was large enough to assess IAC response. The therapeutic effect of IAC on the tumor and p53 and p21 immunoreactivity in pretreatment tumor specimens were evaluated in 23 patients. In addition, the *p53* gene status was investigated in 19 patients (Table I).

IAC regimen Using Seldinger's technique, each balloon catheter (5F) was placed in the contralateral internal iliac artery and angiography was carried out to identify tumor vessels. Following balloon inflation, 100 mg/m^2 of CDDP and 40 mg/m^2 of THP were administered for 30 and 15 min, respectively, using infusion pumps. The dose for each side was determined according to the clinical tumor location and the degree of tumor stain on angiography. In the case of poor general status or renal function, the dose given was reduced.

Response to IAC Approximately 4 weeks after the last course of IAC, the therapeutic effect was assessed pathologically in 14 patients undergoing total or partial cystectomy, and only clinically in the other 9 patients. Pathological and clinical response criteria were as follows, based on the guideline of the Japanese Urological Association[18]:

Pathological response: Grade 3, absence of any tumor tissue or viable tumor cells on pathological examination of the cystectomized specimens. Grade 2, presence of viable cells in less than 1/3 of tumor tissue. Grade 1-b, presence of viable cells in 1/3 or more but less than 2/3 of tumor tissue. Grade 1-a, presence of viable cells in 2/3 or more of tumor tissue. Grade 0, no morphological therapeutic change.

Clinical response: cCR—clinical complete response, disappearance of tumor based on clinical and cytopathological examination. cPR—clinical partial response, 50% or more reduction in the size of tumor masses according to two-dimensional measurement but cytopathological evidence of tumor. cNC—clinical no change, less than 50%

Table I. Clinicopathological Characteristics, *p53* Gene Status, p53 and p21 Immunoreactivity, Response to IAC and Prognosis

Case no.	Age/sex	Structure	Grade	Stage	*p53* gene	p53	p21	No. IAC	Clinical response	Pathological response	Tx	Time to PD (m)	Outcome	Follow-up (m)
1	71/M	N	G3	$\geq pT_2$	wt	0	0	1	cCR	grade 3	TC	—	NED	30
2	75/M	P	G1>2	$\geq pT_2$	wt	0	2+	1	cPR	grade 2	TC	20	DOD	26
3	48/M	P	G2	$\geq pT_2$	wt	0	2+	2	cPR	grade 2	TC	—	NED	42
4	61/M	P	G1>2	$\geq pT_2$	wt	0	0	1	cNC	grade 1b	TC	—	DOO	3
5	64/M	P	G2>1	$\geq pT_{1b}$	wt	0	0	1	cNC	grade 1b	TC	8	DOD	12
6	79/M	N	G3	$\geq pT_2$	wt	0	0	2	cCR	grade 3	PC	—	NED	39
7	76/M	N	G2	$\geq pT_2$	wt	0	2+	1	cCR	—	—	—	DOO	7
8	71/F	P	G2	$\geq pT_{1b}$	wt	0	3+	2	cCR	—	—	21	AWD	23
9	77/F	P	G3	$\geq pT_2$	wt	2+	0	2	cNC	—	TUR	6	DOD	13
10	67/M	N	G2	$\geq pT_{1b}$	wt	0	3+	1	cPR	—	TUR	16	AWD	19
11	63/M	N	G3	$\geq pT_2$	wt	2+	3+	1	cNC	grade 0	TC	—	NED	20
12	87/M	N	G3	$\geq pT_{1b}$	wt	2+	3+	1	cPR	—	TUR	7	AWR	7
13	61/M	N	G3	$\geq pT_2$	mt	2+	2+	2	cPR	grade 2	TC	3	DOD	5
14	60/M	N	G2>3	$\geq pT_2$	mt	3+	2+	2	cNC	grade 0	TC	9	DOD	15
15	73/M	N	G3	$\geq pT_2$	mt	3+	0	1	cNC	grade 0	TC	—	DOO	3
16	76/M	N	G3	$\geq pT_2$	mt	0	2+	1	cPR	grade 2	TC	—	NED	26
17	50/F	N	G3	$\geq pT_{1b}$	mt	0	3+	1	cNC	grade 0	TC	3	AWD	14
18	83/F	P	G3	$\geq pT_{1b}$	mt	0	2+	2	cPR	—	TUR	11	AWR	15
19	67/M	P	G2	$\geq pT_{1b}$	mt	0	2+	1	cPR	—	TUR	—	NED	14
20	63/M	N	G3	$\geq pT_2$	ND	0	2+	1	cPR	grade 2	TC	6	DOD	10
21[a]	73/M	P	G2>3	$\geq pT_{1b}$	ND	0	2+	2	cPR	—	TUR	18	NED	32
22	42/M	P	G1>2	$\geq pT_{1b}$	ND	0	0	2	cPR	—	TUR	—	NED	40
23	61/M	N	G2	$\geq pT_{1b}$	ND	0	3+	2	cPR	grade 2	TC	—	NED	22

IAC, intraarterial chemotherapy; No. IAC, number of IAC given; Tx, treatment after IAC; Time to PD, time to disease progression from initiation of IAC; N, non-papillary tumor; P, papillary tumor; wt, wild type; mt, mutant type; ND, not determined; TC, total cystectomy; PC, partial cystectomy; TUR, transurethral resection; NED, no evidence of disease; DOD, death of disease; DOO, death of other causes; AWD, alive with metastatic disease; AWR, alive with local recurrence; m, months.

a) Case 21 underwent total cystectomy for recurrent T_2 disease at 26 months after IAC.

reduction or less than 25% increase in size of tumor. For convenience, CR, PR and NC represented grade 3 pathological effect and cCR, grade 2 effect and cPR, and grade 0–1 effect and cNC, respectively. Furthermore, the patients were divided into 2 groups; good responders (CR or PR) and poor responders (NC).

Subsequent treatments after IAC In principle, good responders to the first cycle of IAC underwent the second cycle and poor responders to the first cycle of IAC underwent subsequent surgical treatments. Subsequent surgical modalities including total cystectomy, partial cystectomy and TUR were selected considering patients' general status and request.

***p53* gene status** DNA extracted from biopsied fresh tumor tissue was amplified from exons 5 to 8 of the *p53* gene using polymerase chain reaction (PCR). PCR conditions consisted of 40 cycles of 95°C for 1.5 min (denaturation), 48°C for 1.5 min (extension) and 70°C for 2 min (annealing). The PCR products underwent single strand conformation polymorphism (SSCP) analysis. The DNAs in which mutations were detected by SSCP analysis were then sequenced.

Immunohistochemical staining Immunohistochemical staining was performed to study p53 overexpression and p21 expression in tumor tissues. Five-micrometer formalin-fixed, paraffin-embedded tissue sections were mounted on sialinized slides. The sections were deparaffinized in xylene and rehydrated in a graded alcohol series. Following treatment with 3% hydrogen peroxide in methanol to exhaust endogenous peroxidase activity, the sections for p21 staining were pretreated in a microwave oven three times for 5 min at 500 W in 10 m*M* citrate buffer. After blocking of nonspecific binding with 10% rabbit serum, sections were incubated with 5 μg/ml of mouse monoclonal antibody against p53 protein (PAb1801, Oncogene Science, Cambridge, MA) or p21 protein (EA10, Oncogene Research Products, Cambridge, MA) at 4°C overnight followed by two washes with Tris-buffered saline (TBS). The sections were incubated with biotinylated rabbit anti-mouse IgG at room temperature for 50 min followed by two washes with TBS, then reacted with 3,3′-diaminobenzidine tetrahydrochloride (DAB)/hydrogen peroxide solution for 30 min at room temperature. Finally, the sections were washed in water, counterstained with hematoxylin for 1 min, dehydrated and mounted.

Slides were independently reviewed by two of the authors (K. F. and A. K.) without knowledge of clinical information. Staining intensity for p53 and p21 was graded semiquantitatively into four categories by evaluating the percentage of nuclear staining of tumor cells. Categories of 0, 1+, 2+ and 3+ corresponded to negative staining (no nuclear reactivity), minor focally positive staining (1–9% of cells), heterogeneously positive staining (10–49% of cells) and homogeneously positive staining (50–100% of cells), respectively. Only samples demonstrating at least 10% nuclear reactivity (2+) were considered to be positive. We based this criterion on the report by Esrig *et al*. in which mutations in the *p53* gene strongly correlated with the accumulation of p53 protein in 10% or more of the tumor-cell nuclei when the anti-p53 monoclonal antibody PAb1801 was used.[19)]

Known tumor sections of a TCC case with *p53* mutations and normal bladder mucosa without *p53* mutation were used as positive and negative controls, respectively, and normal colonic mucosa was used as a p21 positive control (positive nuclei in superficial epithelium). Normal bladder epithelia showed negative staining (0) for p21. Representative samples of p53 and p21 immunostaining are shown in Fig. 1.

Statistics Fisher's exact test was used to assess the association between patients' characteristics and responses to IAC. Probabilities of survival from the first cycle of IAC were estimated using the Kaplan-Meier method, and univariate tests of significance were performed using the log-rank test. Statistical analyses were performed with the statistical analysis systems package (StatView J-4.5, Abacus Concept, Inc., Berkley, CA). Differences were considered significant when the *P* value was less than 0.05.

RESULTS

Results of mutation analysis and immunohistochemistry, and response to IAC Of 19 patients, seven (37%) had mt *p53* gene (Table I). Of 7 cases with mt *p53* gene, 5 cases had missense mutations and the remaining 2 cases had a point mutation in the 3′ splice site of exon 7 and a 17-base pair deletion in exon 7. Case 16 showed both missense mutations of exon 7 and a 1-base pair deletion in the 3′ splice site of exon 8 (Table II).

Six (26%) of the 23 patients showed positive staining for p53. The mt *p53* genes were detected in 3 of 6 cases with positive p53, and in 4 of 13 cases with negative p53 (Table I). There was no significant association between *p53* gene status and p53 immunoreactivity (P=0.61).

p21 expression was detected in 16 patients (70%) (Table I). The p21 immunoreactivity had no association with histological grade, *p53* gene status or p53 immunoreactivity.

Thirteen patients received one cycle of IAC while 10 patients received 2 cycles. The overall objective response rate (incidence of good responders) and CR rate were 70% (16 of 23) and 17% (4 of 23), respectively. The overall response rate, incidence of grade 3 effect, CR rate, and incidence of grade 0 effect had no significant association with histological structure, grade or the number of IAC given.

Response to IAC according to *p53* gene status or p53 or p21 immunoreactivity Patients with the wt *p53* gene and negative staining for p53 tended to show a better

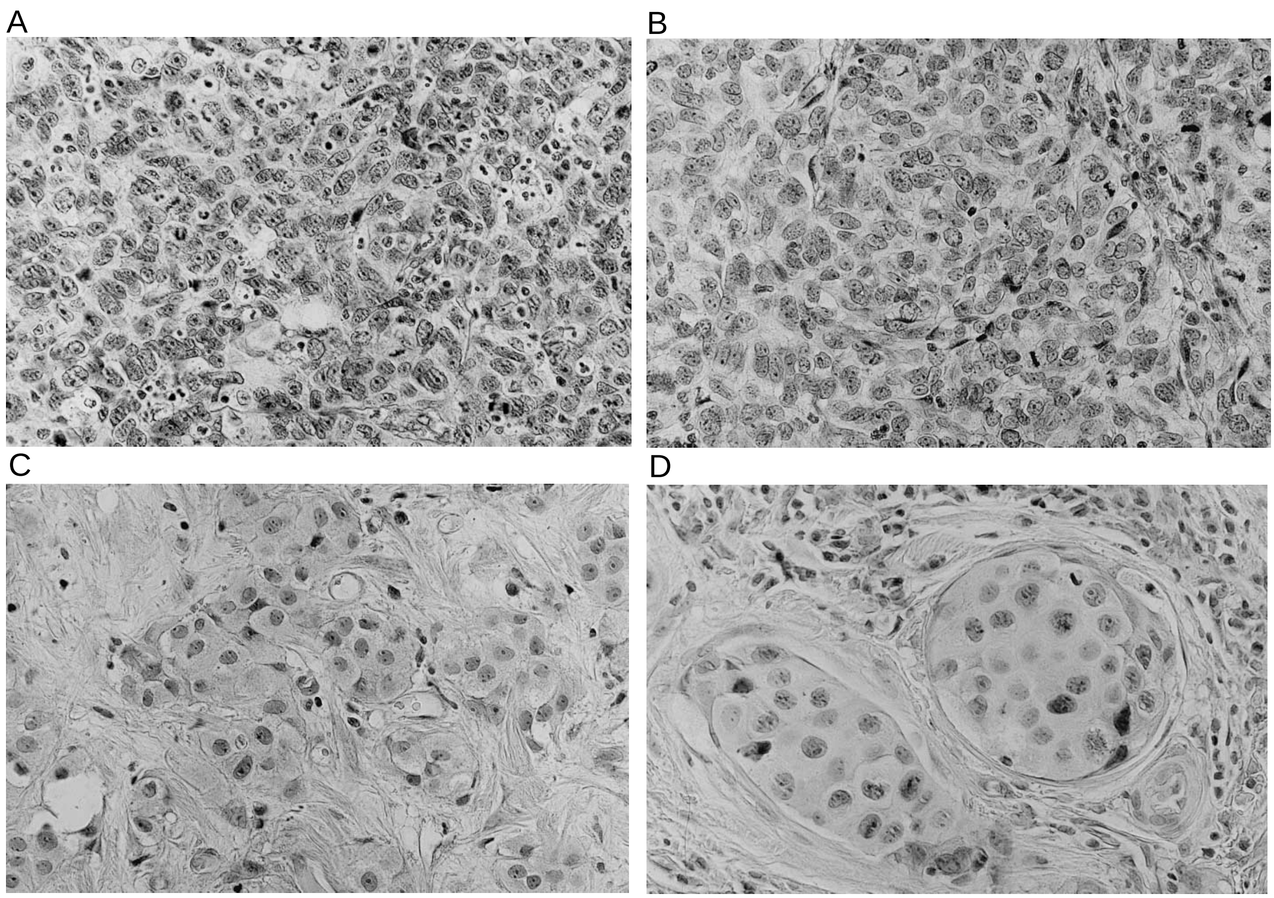

Fig. 1. Strongly positive staining (3+) for p53 (A) and negative staining (0) for p21 (B) in case 15, and negative staining (0) for p53 (C) and moderately positive staining (2+) for p21 (D) in case 20 (reduced from ×400).

response to IAC compared to patients with the mt *p53* gene and positive staining for p53 (Table III). None of the 9 patients with p53 alterations (mt *p53* gene and/or positive p53) achieved CR (Table III), and all of the 4 patients showing grade 0 effect had p53 alterations (Table I).

The patients with negative p53 showed a significantly higher objective response rate and lower incidence of grade 0 effect than patients with positive p53 (82% vs. 33%; P=0.045 and 10% vs. 75%; P=0.041, respectively). However, *p53* gene status had no significant association with IAC response (Table III).

There was no significant association between p21 immunoreactivity and IAC response; the objective response rate was 81% in patients with positive p21 and 43% in those with negative p21 (P=0.14). Patients with positive p21 showed a CR rate of 13% and an incidence of grade 0 effect of 33% while those with negative p21 showed a CR rate of 29% and an incidence of grade 0 effect of 20% (P=0.56 and >0.99, respectively) (Table III).

Table II. Mutation Sites of the *p53* Gene

Case no.	PCR-SSCP	Codon	Nucleotide	Amino acid
13	exon 5	175	CGC → CAG	Arg → His
14	exon 5	132	AAG → GAG	Lys → Glu
15	exon 6	193	CAT → CGT	His → Arg
16	exon 7	243	ATG → ATT	Met → Ile
	exon 7	244	GGC → TGC	Gly → Cys
	exon 8	3′ splice site	1 bp deletion	→
17	exon 7	248	CGG → CAG	Arg → Gln
18	exon 7	3′ splice site	G → C	→
19	exon 7	250–255	17 bp deletion	→

Response to IAC according to p53/p21 immunoreactivity Based on both p53 and p21 immunohistochemical findings, 23 patients were divided into 4 groups; negative p53/positive p21 (12 patients), negative p53/negative p21

(5 patients), positive p53/positive p21 (4 patients) and positive p53/negative p21 (2 patients) (Table IV). The negative p53/positive p21 group showed an objective response rate of 92%, which was significantly higher than the positive p53 and/or negative p21 groups (45%; P=0.027). Both patients of the positive p53/negative p21 group showed NC while 24% of patients of the negative p53 and/or positive p21 groups showed NC; the positive p53/negative p21 group had a strong trend for NC compared to the negative p53 and/or positive p21 groups (P=0.083). Nine patients in the negative p53/negative p21 and positive p53/positive p21 groups had no apparent trend in IAC response (Table IV).

Follow-up data Patients were stratified by *p53* gene status, p53 immunoreactivity, p21 immunoreactivity, p53/p21 immunoreactivity, responses to IAC and post-IAC therapeutic modalities, and assessed for differences in cause-specific and progression-free survival. For patients whose bladders were preserved after IAC, disease progression was defined as the recurrence of muscle-invasive ($\geq T_2$) bladder cancer as well as the development of extravesical diseases. The mean follow-up period from the initial IAC therapy was 19 months (3–42 months). Within this period, disease progression was observed in 12 patients (6 of them belonged to the bladder preservation group) and 6 patients died of the disease. Three other patients died of other causes. Of 9 good responders whose bladders were preserved, 3 patients developed recurrent muscle-invasive disease and 2 other patients relapsed in distant sites. Estimated 1.5-year cause-specific and progression-free survival rates of patients stratified by each factor are shown in Table V. The negative p53 group showed a significantly longer cause-specific survival period (P=0.015) and a strong trend for a longer progression-free survival period (P=0.057). The good responders also showed a trend for longer cause-specific and progression-free survival periods (P=0.076 and 0.094, respectively). The other factors did not affect survival.

DISCUSSION

Following DNA damage, p53 protein levels rise dramatically in cells bearing the wt *p53* gene. The p53 protein transcriptionally regulates expression of the downstream effector genes including *p21/WAF1/CIP1* (*p21*), *bax*, *bcl-2* and *Fas*, and eventually allows DNA repair or triggers cell death by apoptosis.[6] Missense mutations and deletions in exons 5–8 of the *p53* gene, which encode the DNA-binding domain of the p53 protein, usually abolish wt p53 function, prolong the half-life of the protein from minutes to hours and result in nuclear accumulation of the p53 protein allowing its detection by immunohistochemistry.[6]

Table III. IAC Response according to p53 Immunoreactivity, *p53* Gene Status, and p21 Immunoreactivity

	p53		*p53* gene		p21	
	Negative (%)	Positive (%)	wt (%)	mt (%)	Positive (%)	Negative (%)
Grade 3 effect	2/10 (20)	0/4 (0)	2/7 (29)	0/5 (0)	0/9 (0)	2/5 (40)
	P>0.99		P=0.47		P=0.11	
CR	4/17 (24)	0/6 (0)	4/12 (33)	0/7 (0)	2/16 (13)	2/7 (29)
	P=0.54		P=0.25		P=0.56	
CR+PR	14/17 (82)	2/6 (33)	8/12 (67)	4/7 (57)	13/16 (81)	3/7 (43)
	P=0.045		P>0.99		P=0.14	
Grade 0 effect	1/10 (10)	3/4 (75)	1/7 (14)	3/5 (60)	3/9 (33)	1/5 (20)
	P=0.041		P=0.22		P>0.99	

CR: grade 3 effect+cCR. PR: grade 2 effect+cPR.

Table IV. IAC Response according to p53/p21 Immunoreactivity

	CR		PR		NC			Total	CR+PR(%)
	Grade 3 effect	cCR	Grade 2 effect	cPR	Grade 1 effect	cNC	Grade 0 effect		
Negative p53/positive p21	0	2	5	4	0	0	1	12	92
Negative p53/negative p21	2	0	0	1	2	0	0	5	60
Positive p53/positive p21	0	0	1	1	0	0	2	4	50
Positive p53/negative p21	0	0	0	0	0	1	1	2	0

Good responders: negative p53/positive p21=92% (11/12), positive p53 and/or negative p21=45% (5/11). P=0.027.

Table V. Estimated 1.5-year Cause-specific and Progression-free Survival Rates in Patients Stratified by Clinicopathological Factors

Factors	1.5-year cause-specific survival rate (%)	*P* value	1.5-year progression-free survival rate (%)	*P* value
wt *p53* gene (*n*=12)	78	0.74	61	0.35
mt *p53* gene (*n*=7)	63		36	
Negative p53 (*n*=17)	87	0.015	60	0.057
Positive p53 (*n*=6)	27		21	
Positive p21 (*n*=16)	79	0.79	46	0.36
Negative p21 (*n*=7)	60		60	
Negative p53/positive p21 (*n*=12)	91	0.18	53	0.95
Positive p53 and/or negative p21 (*n*=11)	51		46	
Good responders (*n*=16)	87	0.076	60	0.094
Poor responders (*n*=7)	40		21	
Total cystectomy (*n*=13)	64	0.15	56	0.81
Bladder preservation (*n*=10)	88		41	

Although abrogation of wt p53 function affects the normal cell response to DNA damage, the relationship between p53 status and chemosensitivity remains controversial.

The *p21* gene is an important downstream target for the p53 protein to generate growth suppression, DNA repair and apoptosis.[7–10] *p21* gene alterations are rare in human cancers, indicating that the *p21* gene is not associated with oncogenesis.[20, 21] The *p21* gene product, p21 protein, inhibits cell cycle progression and DNA replication through binding to cyclin-dependent kinases and PCNA.[7–10] Overexpression of p21 alone results in not only growth suppression, but also G1-cell cycle arrest and apoptosis *in vitro*.[7–10] Accordingly, p21 is considered to prevent progression of tumors. Actually, clinical studies have shown that p21 expression is a significant factor associated with good prognosis in some malignancies, including bladder cancer.[22] Besides the functions of p21 as an apoptosis-inducer and a growth suppressor, p21 contributes to DNA repair in DNA-damaged cells.[6] Therefore, it remains controversial whether p21 contributes to a favorable result of cytotoxic chemotherapy or not.

The relationship between chemosensitivity and wt p53 and p21 is currently considered from two mutually exclusive points of view: (1) cells bearing wt p53 and p21 are chemosensitive because after exposure to DNA-damaging drugs they undergo apoptosis through cell cycle arrest[11–14] and (2) cells bearing wt p53 and p21 are chemoresistant because following DNA damage they are protected from cell death through cell cycle arrest and DNA repair.[15, 16] Waldman *et al*. have supported the latter viewpoint; they have shown that following DNA damage, cells lacking wt p53 or p21 can undergo and often complete DNA synthesis in the absence of mitosis (uncoupling of synthesis phase and mitosis) due to loss of the G1-cell cycle checkpoint, acquire deformed, polyploid nuclei and subsequently die through apoptosis.[16] In the clinical setting, Cote *et al*. have reported that following radical cystectomy, adjuvant chemotherapy including the DNA-damaging agents cisplatin and doxorubicin improves survival only in patients with p53-altered bladder cancer, suggesting that abrogation of wt p53 functions increases chemosensitivity.[23]

In the present study, patients with p53-negative tumors had a significantly higher objective response rate and lower incidence of grade 0 effect than patients with p53-positive tumors. Moreover, grade 3 effect and cCR were observed only in patients with p53-negative tumors. Our results are consistent with the report of Kakehi *et al*. that p53-negative urothelial carcinomas have responded better to cisplatin-based neoadjuvant chemotherapy than p53-positive carcinomas.[24] These findings suggest that wt p53 protein increases the chemosensitivity of urothelial cancer in the primary sites. As for metastatic urothelial carcinomas, 3 recent studies failed to demonstrate a correlation between the p53 staining status of primary tumors and the effect of chemotherapy on metastatic disease.[24–26] The difference in chemosensitivity between primary and metastatic tumors might be based on cumulative molecular changes of apoptosis-related genes having more critical roles than *p53* gene in metastatic tumors. However, these events in tumors could not explain the strikingly contrasting results to ours in the study of adjuvant chemotherapy targetting micrometastatic disease.[23] A further large-scale study is needed to elucidate whether the relationship of p53 staining status of tumors at the primary sites with chemosensitivity is actually different between the primary, micrometastatic and gross metastatic tumors, or not.

In the study of Esrig *et al*. on bladder cancer, 84% of tumors with evidence of mutation in the *p53* gene by molecular analysis showed p53 nuclear accumulation in

10% or more of the tumor cells using a monoclonal antibody PAb1801.[19] Although we used the same methodology as Esrig *et al.*, 4 of 7 tumors with mt *p53* gene showed negative staining for p53 (p53 accumulation in less than 10% of tumor cells). A high incidence of discordant cases in our study may be mainly due to sampling bias; we used only a small amount of fresh tumor tissue for SSCP analysis while entire biopsy specimens were screened to assess the p53 immunoreactivity. Considering tumor heterogeneity, we believe that p53 staining results reflect tumor characteristics associated with p53 functions rather than SSCP results in the present study.

Concerning the relationship between p21 staining status and responsiveness to chemotherapy, there have been a few clinical studies. In a study of pancreatic adenocarcinoma, adjuvant chemotherapy or radiation significantly improved survival if tumors expressed p21 or did not express p53.[27] In our study, p21 immunoreactivity alone did not significantly affect response to IAC. Therefore, p21 staining status seemed less predictive of chemotherapeutic response than p53 status. However, when patients were stratified by the combination of p53 and p21 staining status, negative p53/positive p21 and positive p53/negative p21 were better predictors of good responders (92%; 11/12) and poor responders (100%; 2/2) than negative p53 alone (82%; 14/17) and positive p53 alone (67%; 4/6), respectively. This suggests that p21 enhances the chemosensitivity of the primary bladder cancer, regardless of p53 status.

The relationship between p53 status and prognosis of patients with invasive bladder cancer has been well established; patients with p53-altered tumors have a poor prognosis.[28, 29] Survival data in the present study are consistent with the study of Sarkis *et al*. in which patients with p53-negative bladder cancer have a significant survival advantage compared to those with p53-positive bladder cancer following neoadjuvant systemic chemotherapy composed of methotrexate, vinblastin, adriamycin and cisplatin.[30] These results may reflect the aggressiveness and chemoresistance of p53-positive tumors. Concerning p21 status, Stein *et al*. reported that positive p21 was a significant and independent predictor of favorable prognosis in patients who underwent cystectomy for bladder cancer.[22] However, in the present study, we found no difference in survival according to p21 staining status. Because of the small sample size and short follow-up duration of the present study, we cannot reach a clear conclusion about the relationship between p21 status and survival in patients with bladder cancer. A large-sized study is required to elucidate the association of p21 status with the prognosis of patients with bladder cancer treated with neoadjuvant chemotherapy.

In conclusion, negative p53/positive p21 immunostaining is a possible predictor of favorable response to IAC in patients with locally advanced transitional cell carcinoma (TCC) of the bladder. Accordingly, wt p53 and p21 protein are likely to increase sensitivity to DNA-damaging drugs by favoring apoptosis, rather than to decrease chemosensitivity by enhancing DNA repair in TCC.

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