

ARTICLES

Effect of p21^{WAF1/CIP1} Expression on Tumor Progression in Bladder Cancer

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Background: Altered expression of p53 protein is an important predictor of progression in bladder cancer. The action of p53 on cell cycle regulation is mediated, in part, through expression of the cyclin-dependent kinase inhibitor p21^{WAF1/CIP1} (p21). Loss of p21 expression may, therefore, contribute to tumor progression. We sought to determine the relationship between p21 expression in bladder cancer and disease progression. **Methods:** Tumor specimens were obtained from 242 patients who underwent cystectomy for bladder cancer. Median follow-up was 8.5 years (range, 0.1–11.8 years). Nuclear p21 status was determined by immunohistochemistry and was then analyzed in relationship to the probability of tumor recurrence, overall survival, and tumor p53 status. Reported *P* values are two-sided. **Results:** Nuclear p21 expression was detected in the tumors of 156 (64%) of the 242 patients. Patients with p21-positive tumors had a decreased probability of tumor recurrence ($P < .00001$) and an increased probability of overall survival ($P < .00001$) in comparison with patients with p21-negative tumors. In a multivariable analysis, p21 expression was an independent predictor of tumor recurrence ($P = .0017$) and of survival ($P = .006$) when assessed with tumor grade, tumor stage, lymph node status, and p53 status. p21 expression was associated with p53 status ($P < .001$); 56% of tumors with p53 alterations showed loss of p21 expression, whereas 79% of tumors expressing wild-type p53 were p21 positive. Patients with p53-altered/p21-negative tumors demonstrated a higher rate of recurrence and worse survival compared with those with p53-altered/p21-positive tumors ($P < .0001$). Patients with p53-altered/p21-positive tumors demonstrated a similar rate of recurrence and survival as those with p53-wild type tumors. **Conclusion:** Loss of p21 expression is a statistically significant and independent predictor of bladder cancer progression. Maintenance of p21 expression appears to abrogate the deleterious effects of p53 alterations on bladder cancer progression. [J Natl Cancer Inst 1998;90:1072–9]

The optimal management of bladder cancer requires accurate determination of the tumor's biologic potential. The ability to identify tumors of greater malignant potential would facilitate treatment selection for patients who may benefit from adjuvant

therapy as well as identification of patients requiring less aggressive treatment strategies. Recent efforts to improve the biologic assessment of bladder cancer have explored the role of tumor suppressor genes in tumor progression. We and others (1,2) have demonstrated that p53 nuclear accumulation is highly associated with tumor recurrence and overall survival in patients with bladder cancer.

One of the primary functions of p53 is as a cell cycle regulatory protein (3–7). p53 mediates its effects on the cell cycle (in part) through regulation of the expression of p21^{WAF1/CIP1} (p21) (5,7–9). p21 binds to, and universally inhibits, the cyclin/cyclin-dependent protein kinase complexes, thereby preventing phosphorylation of the retinoblastoma protein (pRB) and, thus, inhibiting cell cycle progression (7). The regulation of pRB phosphorylation by the cyclin/cyclin-dependent kinase complexes is a key event in the control of the cell cycle (10). Recently, loss of expression of another cyclin-dependent protein kinase inhibitor, p27^{KIP1} (p27), was shown to be associated with disease progression in patients with colorectal and breast cancer (11–13).

Alterations in p53 function can result in loss of p21 expression and may be one of the mechanisms by which altered p53 influences tumor progression (7–9,14). However, it has been demonstrated that p21 expression can also be mediated through p53-independent pathways (15–18). This important finding suggests that, despite the presence of a p53 alteration, p21 expression (and therefore cell cycle control) can be maintained.

In this study, we sought to determine whether p21 expression is an important factor in predicting the clinical outcome of bladder cancer.

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Patients, Materials, and Methods

Patient Population

This study encompasses 242 patients who underwent radical cystectomy (total removal of the bladder), pelvic lymphadenectomy, and urinary diversion for primary transitional cell carcinoma of the bladder at the Kenneth Norris Jr. Comprehensive Cancer Center from April 1983 through December 1988. The median age of all patients was 63 years (range, 49–83 years), with 186 (77%) men and 56 (23%) women. Patients with pure adenocarcinoma, squamous cell carcinoma, or small cell carcinoma were excluded. A comprehensive computerized database provided clinical information and follow-up data for all patients. The median follow-up was 8.5 years (range, 0.1–11.8 years), with 90% of living patients having at least 5 years follow-up. Patient follow-up consisted of evaluations every 3 months for the first year, every 4 months during the second year postoperatively, and annually thereafter. Follow-up in all cases included a serum biochemical profile (serum electrolyte and complete blood cell count), chest radiography, and a physical examination. Further radiographic studies, with computerized tomography or bone scan, were performed in suspected cases of recurrent disease. Clinical follow-up data, including disease recurrence, survival, and cause of death, were entered into the database. Tumor samples from the cystectomy specimens were preserved as archival paraffin-embedded tissue blocks and were available in all cases. This study was approved by the USC/Norris Comprehensive Cancer Center Institutional Review Board.

Pathological Evaluation

All tumor specimens were primary transitional cell carcinoma, with a minority of specimens demonstrating some glandular (3%) or squamous (2%) differentiation. Histologic grading (grades 1–4) was performed on the basis of the Bergkvist grading system (19), and the pathologic stage was classified according to the tumor–node–metastasis (TNM) system shown in Table 1 (20). All tumor specimens were evaluated independently by two investigators (J. P. Stein, R. J. Cote) without knowledge of patient survival, tumor recurrence, or tumor p53 status.

Monoclonal Antibodies and Immunohistochemistry

Five-micron sections from archival formalin-fixed, paraffin-embedded tissue were placed on positively charged slides (Probe-On Plus, Fisher Scientific Co., Pittsburgh, PA). Deparaffinization was accomplished with iodine-xylene (7.5 g iodine in 750 mL xylene), and tissue was rehydrated with 95% ethanol. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide/methanol (1 : 4 [volume : volume]). Antigen retrieval was performed with a citrate buffer (0.1 M, pH 6.0) for a total of 10 minutes (two separate 5-minute intervals) in a standard microwave unit (21). Tissue was then cooled and incubated with 5% horse serum in phosphate-buffered saline (PBS; NaCl 123 mM, NaH₂PO₄ 19 mM, K₂HPO₄ 43 mM, pH 7.2–7.4) for blocking purposes. All further incubations were performed at room temperature. The anti-p21 monoclonal antibody (clone designation-Waf1(Ab1), dilution 1 : 20, Oncogene Research Products, Cambridge, MA) was applied to the tissue in an overnight incubation. We confirmed by western blot analysis that this antibody recognizes and is specific for the p21 protein (data not shown). PBS was used to wash the tissue for two 5-minute intervals followed by incubation with a biotinylated horse anti-mouse secondary antibody (dilution 1 : 200 in PBS). Visualization of immunoreactivity with an elite avidin–biotin complex immunoperoxidase system (Vector Laboratories, Inc., Burlingame, CA) was performed according to the manufacturer's recommendations. Aminoethyl carbazol was used as the chromogen, and hematoxylin was used as the counterstain.

The human breast carcinoma cell lines MCF-7 and MDA-MB-453 served as p21-positive and p21-negative controls, respectively. MCF-7, a cell line expressing a wild type p53, has been shown to express substantially higher levels of p21 messenger RNA than MDA-MB-453, a cell line that expresses a mutant p53 (22). In addition, normal bladder urothelium served as an internal negative control in the specimens and did not demonstrate immunoreactivity, although occasional endothelial cell nuclei showed p21 immunoreactivity, particularly those associated with granulation tissue. The extent of p21 nuclear reactivity was ultimately classified as p21-negative or p21-positive (Fig. 1). The p21-negative group consisted of those tumors with no detectable [(-) or no tumor cells showing p21 expression] or only very low levels of p21 nuclear immunoreactivity [(±), generally <10% of tumor cells showing p21 expression]. The p21-positive group consisted of those tumors with moderate levels [(1+), generally 10%–50%

of tumor cells showing p21 expression] or high levels [(2+), generally >50% of tumor cells showing p21 expression] of p21 nuclear immunoreactivity. Optimal cutoff levels for p21 expression will depend on the specific antibody and technique employed as well as the method for evaluating immunoreactivity. All cases were independently read by two investigators (J. P. Stein, R. J. Cote). All readings were performed blinded to clinical outcome and to the results obtained by the co-reader. To assess the reproducibility of the immunohistochemical test for p21 expression, 32 cases were randomly chosen for retesting. Only one tumor produced a discordant result, which was resolved with further testing. On the basis of a 95% exact two-sided confidence interval (for a binomial proportion) (23), we conclude that agreement will occur 89% of the time or more between tests run on two different occasions.

The technique, the extent and classification, and the results of p53 immunohistochemical staining of these specimens have been described previously (1). Tumor samples demonstrating greater than 10% p53 nuclear reactivity were considered to be p53 positive (p53-altered phenotype), whereas tumor samples demonstrating 10% or less p53 nuclear reactivity were considered to be p53 negative (p53 wild-type phenotype). Of the 242 tumors tested, 141 (58%) were p53 negative and 101 (42%) were p53 positive, as reported previously (1).

Statistical Analysis

The clinical outcomes analyzed in this study were the time to first recurrence of bladder cancer and overall survival. Time to recurrence was calculated from the time of cystectomy to the date of the first documented clinical recurrence or the date of the last follow-up visit; patients who died free of disease before any recurrence were censored at the time of death for the analysis of recurrence. Overall survival was calculated from the time of cystectomy to the date of death or the date of the last follow-up visit. Deaths from any cause were considered treatment (surgical) failures. For the purpose of statistical analysis, nuclear accumulation of p21 was classified as either positive or negative.

Contingency tables, Pearson's chi-squared test, and logistic regression (24) were used to evaluate the association between p21 nuclear accumulation with clinical prognostic variables—primary histologic grade, pathologic stage, and lymph node status of the tumor. Kaplan–Meier plots (25) and the logrank test (26) were used to evaluate the relationship between these three standard clinical prognostic variables as well as the relationship between the expression of p21 and overall survival; to evaluate time to recurrence, the logrank test and cumulative incidence curves were used (27). Greenwood's formula (26) was used to estimate the standard errors of the Kaplan–Meier estimates, and the delta method (27) was used to estimate the standard errors of the cumulative incidence curves. To determine whether nuclear accumulation of p21 provided any prognostic information beyond the standard clinical parameters for the patients with this group of bladder tumors, both a stratified logrank test and the Cox proportional hazards model were used (26). Estimates of relative risk and the associated 95% confidence intervals were based on regression coefficient estimates and their associated standard errors (26). p21 status was also used to stratify the p53 status of the bladder tumors. All reported *P* values are two-sided.

Results

Association of p21 Nuclear Reactivity With Tumor Grade, Pathologic Stage, and Lymph Node Status

Normal urothelium consistently demonstrated no p21 immunoreactivity, providing a negative internal control for each specimen. The nuclei of endothelial cells, especially those associated with granulation tissue, were occasionally p21 positive. Of the 242 bladder tumors, 86 (36%) were p21 negative and 156 (64%) were p21-positive (Fig. 1). There was no significant association between p21 expression and tumor grade (*P* = .3) (Table 1). However, p21 expression was significantly associated with pathologic tumor stage/lymph node status (*P* = .001) (Table 1); lower stage disease was associated with higher p21 expression. Interestingly, in tumors with detectable p21 immunoreactivity [p21 (1+) or (2+)], the highest levels of expression [p21 (2+)] tended to be associated with more advanced stage disease [p21 (1+) versus p21 (2+) comparing patients with organ-confined

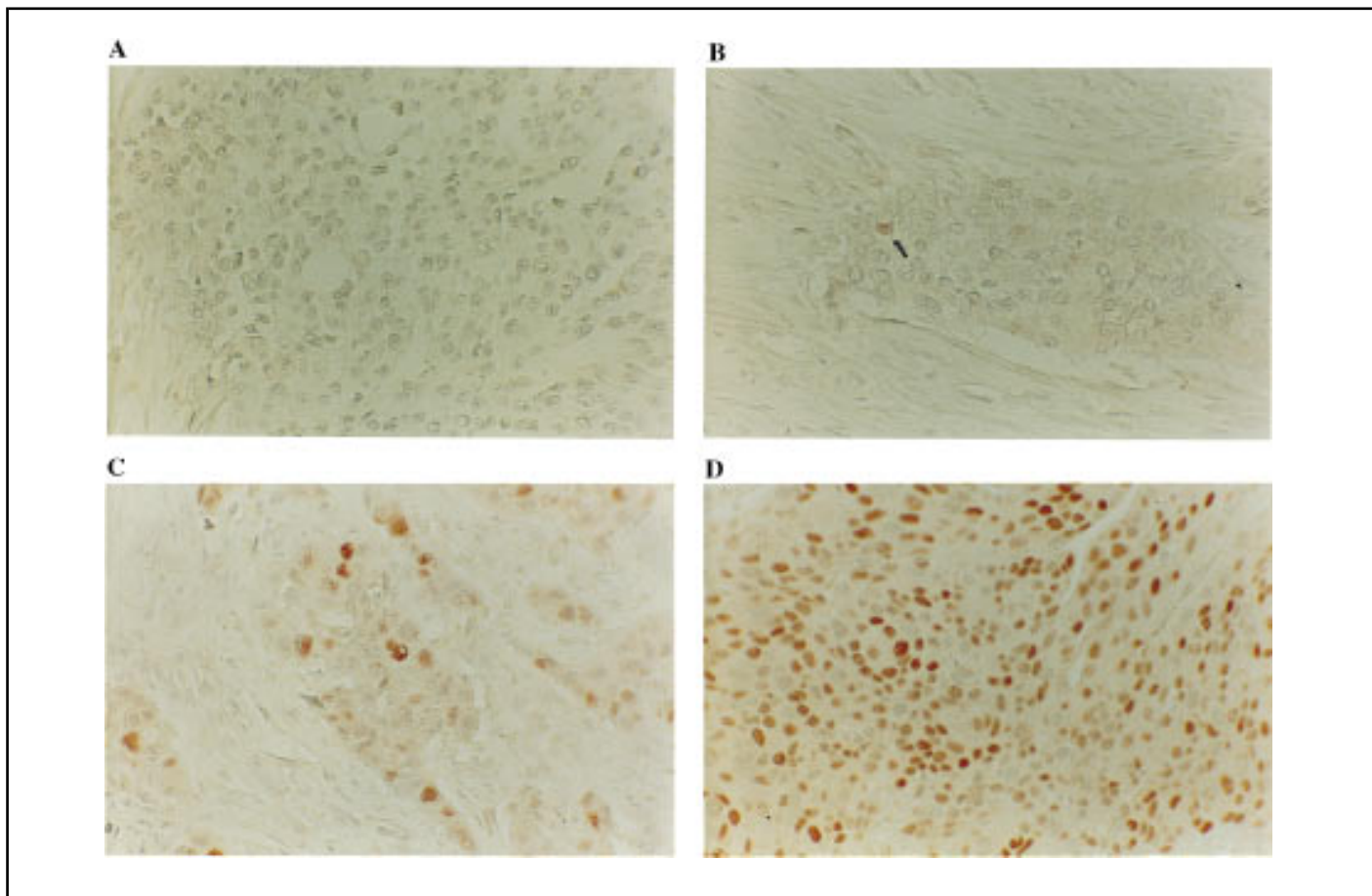


Fig. 1. Immunohistochemical detection of nuclear p21 protein in transitional cell carcinoma of the bladder by use of an anti-p21 monoclonal antibody [Waf(Ab1), dilution 1:20; (Oncogene Research Products)]. The hematoxylin counterstain is intentionally faint to better contrast nuclear immunoreactivity. **A)** No detectable nuclear reactivity [p21 (-)]; **B)** low-level nuclear reactivity [p21 (±)]; single tumor cell nucleus shows p21 immunoreactivity (arrow); **C)** moderate nuclear reactivity [p21 (1+)]; and **D)** high-level nuclear reactivity [p21 (2+)]. All photomicrographs were taken at 200× magnification. See "Patients, Materials, and Methods" for more extensive definitions of p21 expression. Tumors with p21 (-) or (±) immunoreactivity were considered p21 negative. Tumors with p21 (1+) or (2+) immunoreactivity were considered p21 positive.

(tumor stages—P_a, P_{is}, P₂, P_{3a})/lymph node-negative disease versus patients with extra-vesicle (tumor stages P_{3b}, P₄) and/or lymph node-positive disease; $P = .075$) (Table 1).

Association of p21 Nuclear Reactivity With Tumor Recurrence and Overall Survival

A total of 145 patients died. A total of 105 patients developed a documented recurrence, while 44 patients died before a documented recurrence. Loss of p21 expression was significantly associated with an increased probability of recurrence ($P < .00001$) and decreased probability of overall survival at 5 years ($P < .00001$) (Table 2, Figs. 2 and 3). When level of p21 expression was taken into account, patients with p21 (1+) and (2+) tumors had significantly longer recurrence-free intervals than did patients with p21 (-) and (±) tumors ($P < .00001$) (Fig. 4). However, patients with tumors showing the highest levels of p21 expression [p21 (2+)] had a slightly worse outcome than patients with tumors showing moderate levels of p21 [p21 (1+)]. This difference was statistically significant by univariate analysis ($P = .034$), but not by multivariate analysis ($P = .069$), and was largely reduced when stage and grade of disease were taken into account, as higher p21 expression was associated with higher disease stage. Loss of p21 expression was significantly

associated with increased recurrence and decreased survival across all pathologic stages (Table 2).

As shown in Table 3, in addition to p21 status, tumor stage and grade and tumor p53 status were significantly associated with recurrence and survival in a univariate analysis. p21 remained strongly associated with outcome after controlling for stage, grade, and p53 status.

A multivariable analysis demonstrated that p21 status was independent of tumor grade, tumor stage, lymph node status, and tumor p53 status in predicting bladder cancer recurrence ($P = .0017$) and overall survival ($P = .006$).

Association of p21 Nuclear Reactivity and p53 Status

A statistically significant association was found between p21 expression and p53 status; among the p53 wild-type tumors, 79% (112 of 141) were p21 positive compared with 44% (44 of 101) of the p53-altered tumors ($P < .001$).

Association of p21 Nuclear Reactivity and p53 Status With Recurrence and Survival

Patients with p53-altered tumors that maintained p21 expression showed similar rates of tumor recurrence and overall survival compared with patients with p53 wild-type tumors (Fig. 5).

Table 1. Association of p21 immunoreactivity with histologic tumor grade, pathologic stage, and lymph node status in patients with bladder cancer

	No. of patients	No. of patients with p21 nuclear reactivity (%)				Two-sided <i>P</i>
		p21 positive*			p21 negative, all	
		All	1+	2+		
All patients	242	156 (64)	94	62	86 (36)	
Tumor grade†						
2	13	10 (77)	7	3	3 (23)	
3	153	102 (67)	59	43	51 (33)	
4	76	44 (58)	28	16	32 (42)	.3§
Tumor stage‡						
Lymph node negative						
–Pa, Pis, P1, P2, P3a (confined to bladder)	115	89 (77)	59	30	26 (23)	
–P3b, P4 (not confined to bladder)	61	38 (62)	19	19	23 (38)	
Lymph node positive	66	29 (44)	16	13	37 (56)	.001¶

*Any p21 positive (p21 1+ and 2+, see “Patients, Materials, and Methods”) and p21 1+ versus 2+ (moderate vs high p21 expression, see “Patients, Materials, and Methods”).

†According to the method of Bergkvist (19).

‡Performed according to the tumor–node–metastasis system (20).

§Based on Pearson’s chi-squared test (24).

||In cases with detectable p21 immunoreactivity, [p21 (1+) or (2+)] high level (2+) p21 expression was associated with more advanced disease comparing patients with organ-confined (tumor stages—Pa, Pis, P1, P2, P3a)/lymph node-negative disease versus patients with extra-vesicle (tumor stages—P3b, P4) and/or lymph node-positive disease ($P = .075$).

¶Based on the likelihood-ratio test for logistic regression (24).

In contrast, patients with p53-altered tumors that lost p21 expression showed significantly higher rates of tumor recurrence ($P < .00001$) and worse overall survival ($P < .00001$); only 12% of patients with p53-altered/p21-negative tumors remained recurrence free at 5 years compared with 64% of patients with p53-altered/p21-positive tumors. In patients with p53 wild-type tumors, differences in p21 status did not result in significant differences in recurrence and overall survival (Fig. 5).

Discussion

In this study, we demonstrate that expression of the cyclin-dependent kinase inhibitor p21 provides important prognostic information in patients with transitional cell carcinoma of the bladder. Patients with tumors that maintained p21 expression demonstrated a statistically significant decreased rate of tumor recurrence and a statistically significant increased overall sur-

vival in comparison with those whose tumors had lost expression of p21. A statistically significant association between p21 expression and tumor progression was observed at all pathologic stages, including organ-confined disease. Finally, p21 appears to play a critical role in the p53 pathway of bladder cancer progression; patients with p53-altered tumors that maintained p21 expression had similar rates of recurrence and overall survival as patients with p53 wild-type tumors. In contrast, patients with p53 altered tumors that had lost p21 expression had significantly higher rates of recurrence and worse overall survival, with most patients experiencing a tumor recurrence and dying of their disease in 5 years. Thus, p21 is an independent and highly significant predictor of disease progression.

One of the primary functions of p53 is regulation of cell cycle events at the G₁ phase to S phase transition (3,6,7). This process is mediated through transcriptional activation of the p21 gene by wild-type p53 protein (5,7–9,13,14). p21 regulates cell

Table 2. Estimated probability of tumor recurrence and overall survival at 5 years in patients with bladder cancer according to p21 status and pathologic stage*

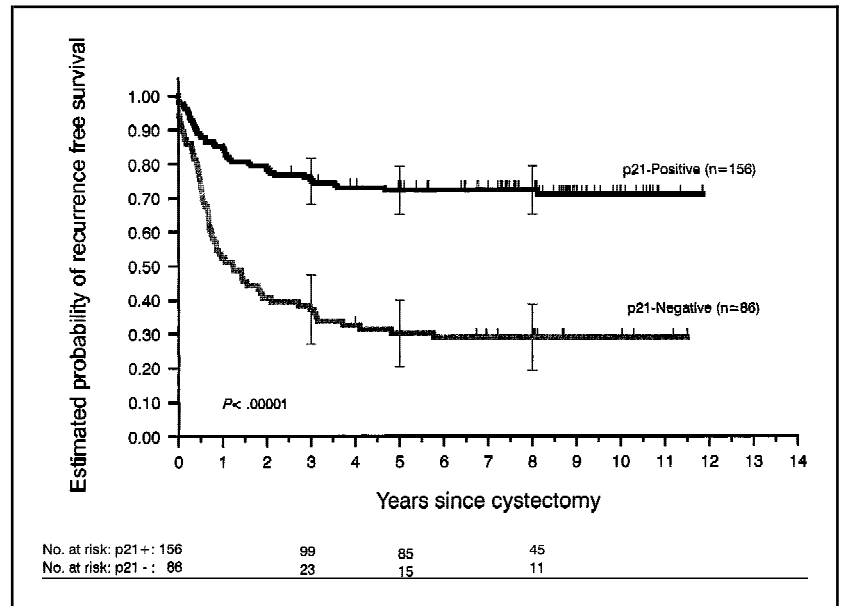
Group and stage†	No. of patients	% probability of tumor recurrence at 5 years		Logrank <i>P</i> ‡	% probability of overall survival at 5 years		Logrank <i>P</i> ‡
		p21 positive (n = 156)	p21 negative (n = 86)		p21 positive (n = 156)	p21 negative (n = 86)	
All patients	242	30 (±4)	76 (±5)	<.00001	63 (±4)	25 (±5)	<.00001
Lymph node negative							
–Pa, Pis, P1, P2, P3a (confined to bladder)	115	13 (±4)	49 (±11)	.001	81 (±4)	50 (±10)	.002
–P3b, P4 (not confined to bladder)	61	48 (±9)	79 (±9)	.006	48 (±8)	26 (±9)	.03
Lymph node positive	66	63 (±10)	94 (±4)	.02	28 (±8)	8 (±4)	.02

*Probability of overall survival based on Kaplan–Meier estimates (25), probability of recurrence estimates are based on cumulative incidence curve estimates. Plus-minus values in parentheses are estimates of the standard error calculated with Greenwood’s formula for overall survival (26) and with a delta method for tumor recurrence (27).

†Staging performed according to the tumor–node–metastasis system (20).

‡Two-sided.

Fig. 2. Probability of remaining recurrence free in 242 patients with bladder tumors that were evaluated for p21 expression. 95% Confidence intervals and numbers of patients at risk are provided at 3, 5, and 8 years.



cycle events by binding directly to cyclin-dependent kinases, inhibiting their action, resulting in phase-specific cell cycle arrest. In this regard, the role of p21 is analogous to that of p27^{Kip-1} (p27), another cyclin-dependent kinase inhibitor; loss (or decreased) p27 expression is associated with progression in a number of tumor systems (11–13).

The transcriptional activation of the p21 gene was initially thought to be mediated entirely through p53-dependent pathways. Our results indicate that, in bladder tumors with a p53 wild-type phenotype, p21 expression is generally seen. However, it is now known that there are also p53-independent pathways that mediate p21 expression; certain growth factors, including platelet-derived growth factor, fibroblast growth factor, and epidermal growth factor, have been shown to increase p21 expression (15–18). In fact, it has been shown that p53-null cell lines can maintain p21 expression (28). In addition, p21 expression may be maintained in cells with altered p53 if the p53 product has some functional activity (in this case the continued

ability to transcriptionally activate the p21 gene). Thus, some p53 mutations may be silent, at least in regard to control of p21 expression.

Although we have demonstrated that p53 alteration is a significant predictor of bladder cancer progression (1), not all patients with altered p53 will show recurrence of their bladder cancers. We reasoned that some patients with altered p53 might maintain p21 expression through p53-independent mechanisms; we found that more than 40% of p53-altered tumors maintain p21 expression. We further reasoned that in patients in which p21 expression is maintained, cell cycle control might also be maintained, despite alterations in p53. If loss of cell cycle control is important in tumor progression, then p21 expression in p53 altered tumors might be clinically relevant. Our findings support this hypothesis; p21 expression is a highly significant predictor of recurrence and survival, even in patients with p53-altered tumors; p53-altered tumors that maintain p21 expression behave similarly to tumors with no detectable p53 alterations.

Fig. 3. Probability of overall survival in 242 patients with bladder tumors that were evaluated for p21 expression. 95% Confidence intervals and number of patients at risk are provided at 3, 5, and 8 years.

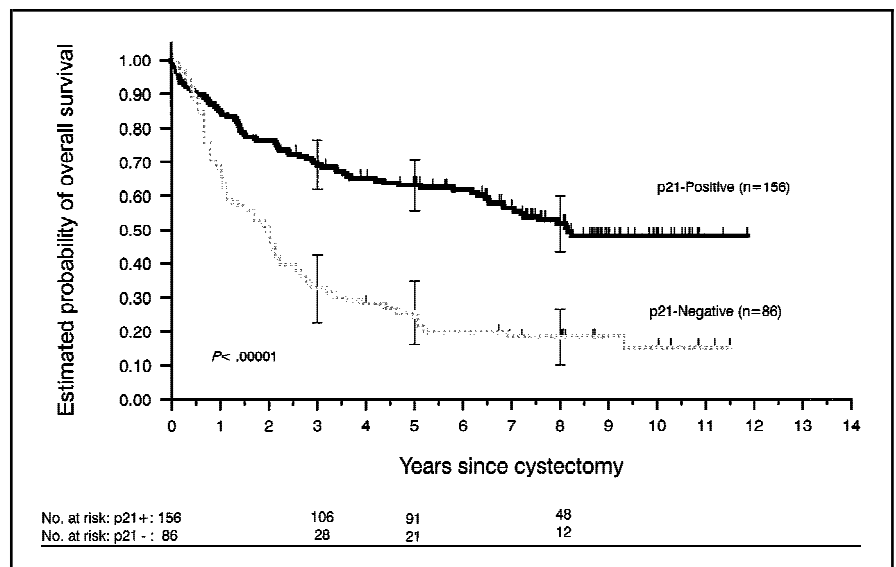
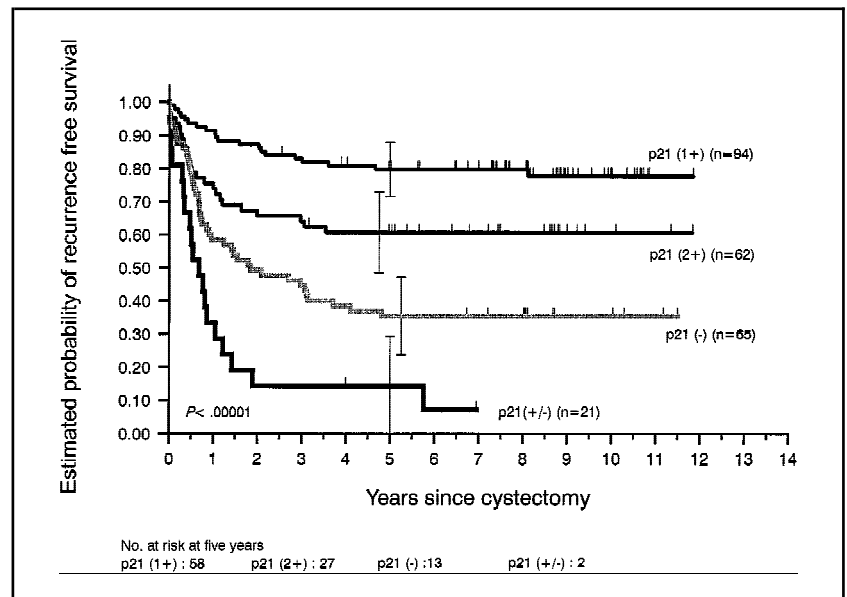


Fig. 4. Probability of remaining recurrence free in 242 patients with bladder tumor stratified by p21 status; p21 (-), (\pm), (1+), (2+) (see "Patients, Materials, and Methods" for more extensive definitions of p21 expression levels). 95% Confidence intervals and number of patients at risk are provided at 5 years.



This provides further evidence that maintenance of p21 expression in p53 altered tumors may abrogate the deleterious effects of p53 alterations.

One interesting finding of this study was that patients with tumors showing the highest levels of p21 expression [p21 (2+)] had a slightly worse prognosis in comparison with patients whose tumors showed moderate levels of expression [p21 (1+)]. This appears to be due, at least in part, to the fact that, when p21 is expressed, the highest levels are associated with higher stage tumors. While the p53/p21 pathway is clearly important in blad-

der cancer progression, approximately 50% of tumors that metastasize and kill the patient will show no evidence of p53 alterations; these tumors progressed despite an intact p53/p21 pathway. Support for the notion that multiple pathways may be involved in tumor progression comes from our increasing understanding that cancer is a multistep genetic process (29). Furthermore, we and others (30,31) have recently shown that alterations in two different tumor suppressor genes (p53 and Rb) show cooperative and synergistic effects in promoting bladder cancer progression. We postulate that in bladder cancers with an intact p53/p21 pathway, disease progression (most likely due to alterations in the Rb pathway) may be associated with increased p21 expression. This increased expression may be, in effect, the attempt of the tumor cell to apply a "brake" at the G₁ phase to S phase checkpoint. A similar finding has been observed in the case of the Rb pathway; tumors showing loss of Rb demonstrate high levels of expression of the cyclin-kinase inhibitor p16 (INK 4A) (32,33). Thus, increased expression of the tumor suppressor gene product p16 is actually associated with tumor progression in these cases, because the increased expression is the result of downstream dysregulation in cell cycle control. We further postulate that, in tumors for which p53 alterations are not an important pathway of tumor progression, increased levels of p21 expression may be associated with disease progression; p21 expression in these cases may be a manifestation of dysregulation in other pathways.

While p21 expression appears to be an important predictor of bladder cancer behavior, evidence to date suggests that alterations in (i.e., loss of) p21 protein expression are not due to mutations or deletions in the p21 gene, which appear to be rare in human cancers (34,35). Thus determination of p21 functional status will depend on analysis of p21 protein expression.

This study may influence the selection of patients for adjuvant treatment of bladder cancer. Patients with bladder tumors that are p21 negative are at high risk of recurrence and death and may benefit from adjuvant treatment. In contrast, those patients whose bladder cancers are p21 positive have a significantly

Table 3. Relative risk (95% confidence interval) of recurrence and death in 242 patients with bladder cancer according to tumor stage, grade, p53 status, and p21 status

Variable	Recurrence		Overall survival	
	Unadjusted* relative risk	Adjusted† relative risk	Unadjusted* relative risk	Adjusted† relative risk
Stage:‡				
P3b-P4 LN-	3.90	3.19	2.51	2.10
LN+	(2.30-6.64)	(1.81-5.63)	(1.64-3.83)	(1.32-3.34)
Grade§				
4	7.34	4.78	5.11	3.74
	(4.42-12.18)	(2.75-8.30)	(3.42-7.62)	(2.42-5.79)
p53:¶				
Altered	1.83	1.05	1.77	1.11
	(1.24-2.71)	(0.69-1.59)	(1.26-2.47)	(0.77-1.61)
p21:¶¶				
Negative	3.26	1.91	2.47	1.60
	(2.19-4.85)	(1.24-2.94)	(1.77-3.44)	(1.11-2.31)
	3.83	2.21	2.67	1.72
	(2.58-5.68)	(1.44-3.39)	(1.92-3.73)	(1.20-2.49)

*Based on Cox proportional hazards model (26) in a univariate analysis. Confidence interval that does not include the value of 1.00 indicates a significant association with outcome at the $P = .05$ level (two-sided).

†Based on Cox proportional hazards model (26) in a multivariable analysis with all four variables included (tumor stage, grade, p53 status, and p21 status). Confidence interval that does not include the value of 1.00 indicates a significant association with outcome at the $P = .05$ level (two-sided).

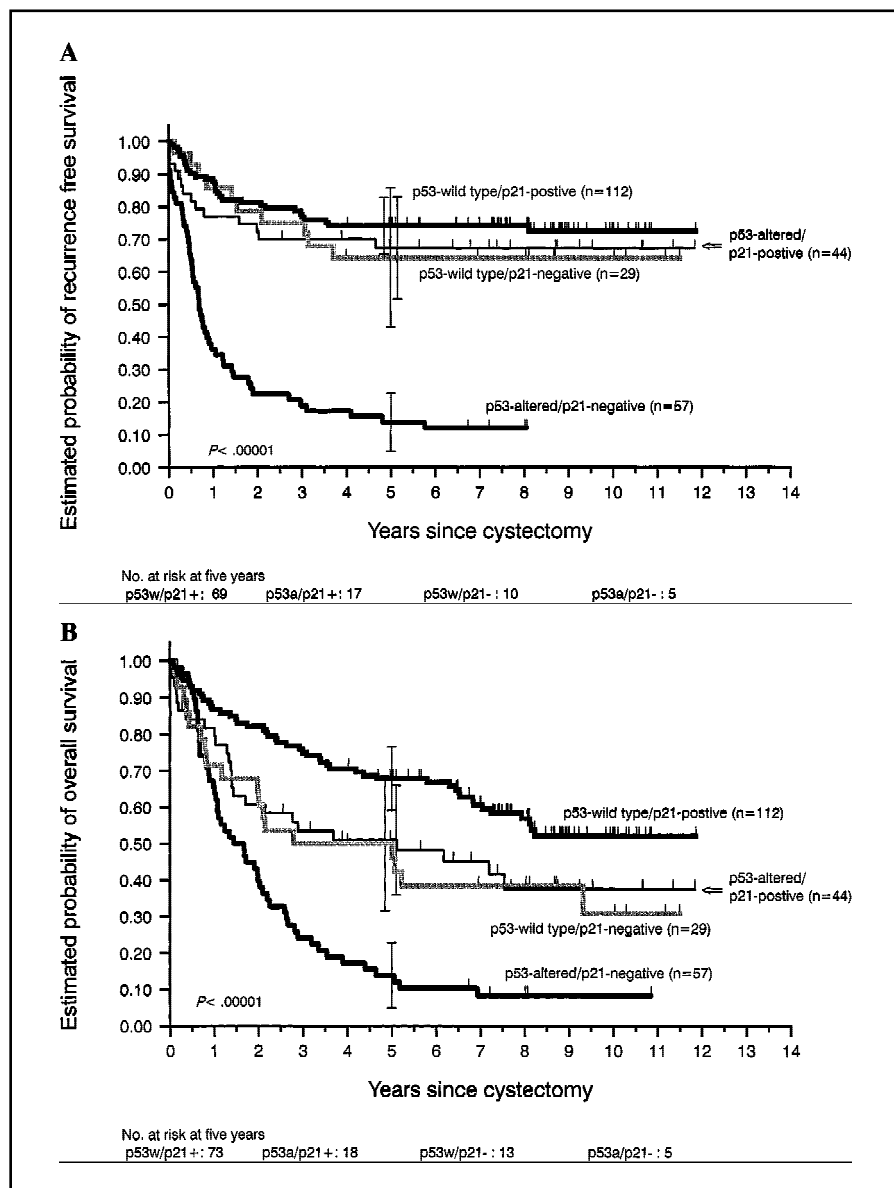
‡Compares the risk of failure to that of patients with organ confined, lymph-node negative (LN-) disease. See (20) for information on tumor staging.

§Compares the risk of failure to that of patients with grade 2 or 3 tumors. See (19) for information on tumor grading.

¶Compares the risk of failure to that of patients with p53 wild-type tumors.

¶¶Compares the risk of failure to that of patients with p21-positive tumors.

Fig. 5. Probability of **A**) remaining recurrence free and **B**) overall survival in 242 patients with bladder tumors stratified by p53 and p21 status: p53 wild-type, no detectable p53 nuclear reactivity (p53 wild-type phenotype); p53 altered, nuclear p53 reactivity detected (p53-altered phenotype); p21-positive, p21 nuclear expression detected; p21-negative, no detectable p21 nuclear expression. (See "Patients, Materials, and Methods" for more details on immunohistochemical staining classifications.) The patients were stratified into four groups on the basis of p53 status and p21 status of their tumors. Patients with p53-altered/p21-negative tumors had a significantly higher rate of recurrence (two-sided $P < .00001$) and worse overall survival (two-sided $P < .00001$) in comparison with other patients. 95% Confidence intervals and number of patients at risk are provided at 5 years. Each tick mark represents a patient who had no evidence of tumor recurrence or who was alive at the time of last follow-up. The logrank test was used to compare the time to tumor recurrence and survival among the patient groups (25).



lower rate of disease progression and may not benefit significantly from adjuvant treatment following cystectomy. This study underscores the need to dissect further pathways that are involved in the control of tumor behavior; knowledge of these pathways will be important in developing more targeted therapeutic strategies.

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Notes

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