The Cell Cycle Inhibitors $p21^{WAF1}$ and $p27^{KIP1}$ Are Associated with Survival in Patients Treated by Salvage Prostatectomy after Radiation Therapy[1]

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ABSTRACT

We evaluated $p27^{KIP1}$ and $p21^{WAF1}$ expression in 52 patients treated by salvage radical prostatectomy and bilateral pelvic lymphadenectomy for biopsy-proven locally persistent or recurrent prostate cancer after external beam radiation therapy. We defined low and high expression based on the median value observed in our sample. Five-year distant metastasis-free survival and cancer-specific survival were 71 and 82%, respectively, for patients with low expression of p21 (≤5%), compared with 94 and 100%, respectively, for those with high expression of p21 (>5%; *P* = 0.02 and 0.01, respectively). Five-year distant metastasis-free survival and cancer-specific survival were 71 and 82%, respectively, for patients with low expression of p27 (<50%), compared with 88 and 96%, respectively, for those with high expression of p27 (≥50%; *P* = 0.06 and 0.01, respectively). These findings indicate that p21 and p27 expression levels are significant predictors of survival for patients selected for salvage prostatectomy for recurrent prostate cancer.

INTRODUCTION

Locally persistent or recurrent prostate cancer after RT[4] may be associated with a poor prognosis (1). Although the need for and nature of therapy for such patients remains controversial, "salvage" prostatectomy may be used in an attempt to provide a second opportunity for long-term disease control (2, 3). The identification of factors associated with subsequent distant disease relapse and cancer-specific mortality after salvage prostatectomy may aid in patient selection for this procedure or the consideration of adjuvant systemic therapy.

$p21^{WAF1}$ and $p27^{KIP1}$ are members of the KIP family of cell cycle proteins, which inhibit several cyclin-dependent kinase complexes (4). Functional loss of the cycle-dependent inhibitors has been implicated in carcinogenesis and cancer progression (5, 6). We and others have demonstrated that loss of $p27^{KIP1}$ expression in prostatic (7–10) and nonprostatic malignancies (11–14) may be associated with a more aggressive phenotype. Loss of $p21^{WAF1}$ function has been implicated in the failure of irradiation response (15–18), and p21 has been shown to be an independent prognostic factor in prostate carcinoma (19, 20). The present study investigated the levels of p21 and p27 protein expression and their association with survival in patients treated by salvage prostatectomy for locally persistent prostate cancer after RT.

MATERIALS AND METHODS

The study population consisted of 52 patients treated by salvage radical prostatectomy and bilateral pelvic lymphadenectomy between October 1967 and March 1996 for biopsy-proven locally persistent or recurrent prostate cancer after RT (2). The original cohort consisted of 86 patients (2), who were selected based on the availability of tissues for the study. There were no differences in age, preoperative prostate-specific antigen concentration, pathological stage, surgical margins, Gleason score, cancer volume, DNA ploidy, distant metastasis-free survival, cancer-specific survival, and all-cause survival between the study population (52 patients) and those excluded (34 patients).

The interval from RT to cancer recurrence ranged from 7 months to 17 years (mean, 3.8 years). Patient age at the time of surgery ranged from 51 to 75 years (mean, 65 years), and the mean follow-up duration after surgery was 5.7 years (range, 1.0–13.0 years). Thirteen patients received adjuvant hormonal therapy within 90 days of surgery; and 12 patients received orchiectomy at the time of surgery. Postoperatively, patients were generally evaluated every 3 months for the first

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[4] The abbreviation used is: RT, radiation therapy.

Table 1 Clinical and pathologic characteristics of 52 patients who underwent salvage prostatectomy for recurrent cancer after RT

The preoperative PSA concentration was not available in 14 patients, the highest postirradiation PSA concentration was not available in 13 patients, and cancer volume was not available in 2 patients.

Characteristics	No. of patients (%)
Age at surgery (years)	
<55	3 (5.8)
55–64	20 (38.5)
≥65	29 (55.8)
Preoperative PSA levels (ng/ml)	
<4	9 (23.7)
4–9	17 (44.7)
≥10	12 (31.6)
Highest postirradiation PSA levels (ng/ml)	
<4	8 (20.5)
4–9	15 (38.5)
≥10	16 (41.0)
Interval from RT to surgery (years)	
<3	25 (48.1)
≥3	27 (51.9)
Pathologic stage	
T2	17 (32.7)
T3	24 (46.1)
TxN+	11 (21.2)
Surgical margins	
Negative	30 (57.7)
Positive	22 (42.3)
Gleason score	
<7	3 (5.8)
7	33 (63.5)
8–10	16 (30.8)
Cancer volume (cm^3)	
<5	23 (46.0)
5–9	11 (22.0)
≥10	16 (32.0)
DNA ploidy	
Diploid	13 (25.0)
Tetraploid	34 (65.4)
Aneupoid	5 (9.6)

2 years, biannually for the next 3 years, and annually thereafter (2). Ten patients (19%) developed distant metastasis, 8 (15%) died of prostate cancer, and 6 (12%) died of other causes.

The radical prostatectomy and bilateral pelvic lymphadenectomy specimens were examined as described previously (2). The pathological stage was T2a in 10 patients (19%), T2b in 7 (13%), T3 in 24 (46%), and TxN+ in 11 (21%). The Gleason score was <7 in 3 patients (6%), 7 in 33 (63%), and >7 in 16 (31%; Table 1). Immunostaining was performed on 6-μm formalin-fixed paraffin-embedded sections, using the avidin-biotin complex technique. Primary monoclonal antibodies were used for immunostaining for p21 (dilution 1:500; Transductin Laboratory, Lexington, KY) and for p27 (dilution 1:1000; Transductin Laboratory). Nuclear immunoreactivity was evaluated on a 5% incremental scale, ranging from 0 to 90%, as described previously (7–10). At least 1000 cells were analyzed in each case. All immunostains were evaluated without knowledge of the clinical outcome.

The Cox proportional hazards model was used to evaluate the association of p21 and p27 expression with distant metastasis-free survival and cancer-specific survival, respectively, after salvage radical prostatectomy. The nuclear immunoreactivity measures were evaluated as dichotomous measures by stratifying patients into low- and high-expression groups based on the median values (21). Survival curves were estimated based on the Kaplan-Meier method. $P < 0.05$ was considered significant, and all P values were two-sided.

RESULTS AND DISCUSSION

$p21^{WAF1}$ nuclear immunoreactivity of cancer cells was detected in 39 (75%) of 52 patients (median of nuclear immunoreactivity, 5%; mean, 12%; range, 0–80%), and $p27^{KIP1}$ nuclear immunoreactivity was detected in all 52 patients (median degree of nuclear immunoreactivity, 50%; mean, 49%; range, 5–90%). We defined low and high expression based on the median value observed in our sample. Five-year distant metastasis-free survival and cancer-specific survival were 71 and 82% for patients with low expression of p21 (≤5%), compared with 94 and 100% for those with high expression of p21 (>5%; $P = 0.02$ and 0.01, respectively; Fig. 1, *A* and *B*). Five-year distant metastasis-free survival and cancer-specific survival were 71 and 82% for patients with low expression of p27 (<50%), compared with 88 and 96% for those with high expression of p27 (≥50%; $P = 0.06$ and 0.01, respectively; Fig. 1, *C* and *D*). There were a total of 14 deaths. Five-year all-cause survival rates were 82 and 100%, respectively, for those with low (≤5%) and high (>5%) expression of p21 ($P = 0.02$). Similarly, 5-year all-cause survival rates were 82 and 96%, respectively, for those with low (<50%) and high (≥50%) expression of p27 ($P = 0.09$).

We recently evaluated p53 protein expression, cellular proliferation (Ki-67 labeling index), and glutathione *S*-transferase-π expression in patients who underwent salvage prostatectomy for recurrent cancer after RT (22). We found that the majority of prostate cancers after RT were proliferative (mean Ki-67 labeling index, 7.0) and that p53 protein overexpression was associated with increased cell proliferation. However, we did not find significant correlation between p21 or p27 expression and p53, Ki-labeling index, or glutathione *S*-transferase-π expression (data not shown). The relationship between these markers is inconclusive because of the limited sample size in the present study.

Decreased $p27^{KIP1}$ expression in prostatic carcinoma has been associated with aggressive phenotype, and loss of $p21^{WAF1}$ function has been implicated in the failure of irradiation response. In the present study, the loss of expression of these two cell cycle inhibitors was associated with reduced metastasis-free, cancer-specific, and all-cause survival durations among patients treated with salvage prostatectomy for locally persistent or recurrent prostate cancer after RT. These findings extended previous observations on the prognostic significance of p21 and p27 (7–10, 19, 20). However, this study population differed from other studies of prostate cancer patients in certain aspects. These patients were selected for a second attempt at cancer ablation with surgical salvage after clinically localized prostate cancer recurrence, and patients were evaluated and treated over a 30-year period. Thus, caution is warranted in comparison of these results with other study populations. The use of median values (5% for p21 and 50% for p27) as arbitrary cutoffs may

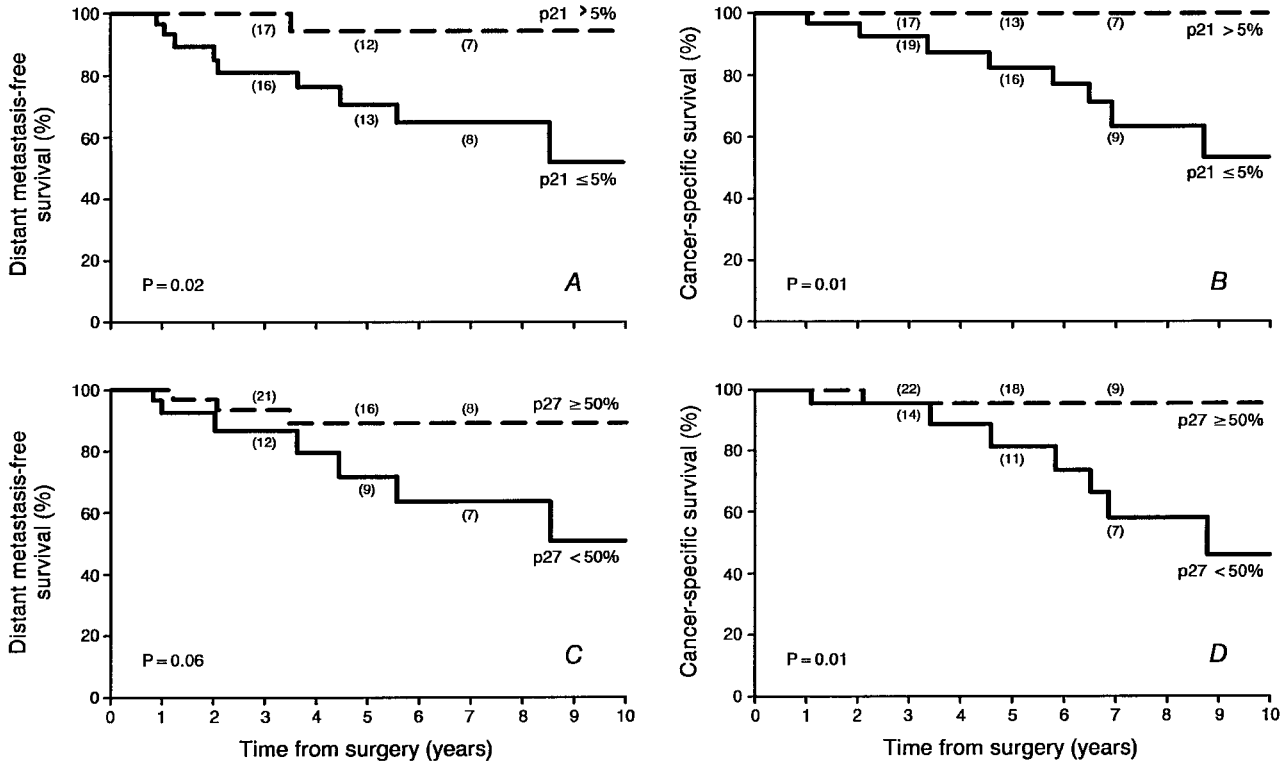

Fig. 1 Kaplan-Meier curves (distant metastasis-free survival and cancer-specific survival) for patients treated by radical prostatectomy for postirradiation local tumor recurrence according to $p21^{WAF1}$ expression (*A* and *B*) and $p27^{KIP1}$ expression (*C* and *D*). The median value of the percentage of nuclear immunoreactivity was used as cutoff to stratify patients into low- and high-expression groups for analysis (21). Numbers in *parentheses* represent numbers of patients under observation at 3, 5, and 7 years.

not be reliable and should be tested in a larger study series. Disease-free survival was not analyzed in this study because a significant proportion of patients were treated prior to the prostate-specific antigen era. Furthermore, the sample size and the number of clinical events were small, which limits the statistical power for evaluating the association with the time-dependent end points. A larger series of patients should be sought to confirm the observations reported herein, and the importance of other prognostic covariates in concert with p21 and p27 requires investigation.

In summary, reduced levels of $p21^{WAF1}$ and $p27^{KIP1}$ expression were predictive of distant metastasis-free, cancer-specific, and all-cause survival for patients selected for salvage prostatectomy for recurrent prostate cancer. Additional studies are required to clarify the causality and significance of these observations.

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