Role of $p21^{WAF1}$ and $p27^{KIP1}$ in Predicting Biochemical Recurrence for Organ-confined Prostate Adenocarcinoma

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Background: Both $p21^{WAF1}$ and $p27^{KIP1}$ have been reported as prognostic markers predicting biochemical failure for prostate cancers. We examined the expression and prognostic significance of $p21^{WAF1}$ and $p27^{KIP1}$ in organ-confined (pT2) prostate cancer patients.

Methods: The medical records of 53 pT2 prostate adenocarcinomas were analyzed retrospectively. Radical prostatectomy specimens were stained using anti-$p21^{WAF1}$ and anti-$p27^{KIP1}$ antibodies. Biochemical relapse was defined as 2 consecutive elevations in serum prostate specific antigen (PSA) level > 0.2 ng/mL with an interval of more than 3 months. The prognostic significance of $p21^{WAF1}$ and $p27^{KIP1}$ expression was assessed.

Results: $p21^{WAF1}$ immunoreactivity was found in 19 patients (35.8%). Twenty-nine tumors (54.7%) had decreased $p27^{KIP1}$ expression. Both markers were not associated with Gleason scores ($p = 1.00$ for both). At a median follow-up of 49 months, 15 patients (28.3%) experienced biochemical recurrence. Both p21 and p27 had no prognostic significance in log-rank test ($p = 0.98$ and $p = 0.64$, respectively).

Conclusion: $p21^{WAF1}$ and $p27^{KIP1}$ expression have no role in predicting biochemical relapse for stage pT2 prostate cancers. [*J Chin Med Assoc* 2007;70(1):11–15]

Key Words: p21, p27, prognosis, prostate neoplasms

Introduction

Knowledge of prognostic factors is essential for oncology practice because it helps physicians in consulting patients and to select appropriate treatment for individual patients.[1] For prostate adenocarcinoma, some of the prognostic factors have been well established, such as initial serum prostate specific antigen (PSA), Gleason score and pathologic stage.[2] However, all of these factors are hard to apply to the individual patient. With recent developments in molecular biology, we may now be able to identify new marker(s) for predicting clinical outcome of cancer patients.

Cyclins and cyclin-dependent kinases (CDK) are necessary for the induction of DNA replication.[3] $p21^{WAF1}$ is an inhibitor of CDK, and appears to be activated by wild-type p53 protein.[4,5] Through both p53-dependent and p53-independent pathways, $p21^{WAF1}$ blocks cell cycle progression at the G1 phase.[5–9] $p21^{WAF1}$ expression has been found to be correlated with advanced stage, higher Gleason score and shortened biochemical-free survival in prostate cancer, although the exact mechanism remains unknown.[10–13] In 1 study, expression of $p21^{WAF1}$ was identified as a poor prognostic marker for Caucasian but not African-American prostate cancer patients.[11] Since the $p21^{WAF1}$ gene is regulated transcriptionally by p53,[5,11] and our previous study illustrated that p53 mutation was less common and without prognostic significance in Taiwanese prostate cancers,[14] it would be interesting to further examine the expression of p21 in Taiwanese prostate cancer patients.

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Cell proliferation is inhibited by $p27^{KIP1}$ by binding and inactivating the cyclin-CDK complex, thereby blocking the transition from G-1 to S-phase.[3,15–17] Decreased $p27^{KIP1}$ expression has recently been associated with high grade tumor and poor clinical outcomes in prostate cancers. However, the data are inconsistent.[16–21]

This study focused on the significance of $p21^{WAF1}$ and $p27^{KIP1}$ expression in predicting PSA recurrence in stage pT2 prostate cancers treated by radical prostatectomy alone.

Methods

Between January 1991 and December 2003, a total of 275 patients underwent radical prostatectomy in our institute for clinically localized prostate cancers. Among them, 153 (55.6%) were pT2 disease. Medical records were reviewed, and patients who had neoadjuvant or adjuvant therapy were excluded. To avoid the possibility of understaging, only patients with postoperative PSA < 0.01 ng/mL on the PSA-RIACT (CIS Bio International, Cedex, France) assay were enrolled.

All patients were advised to have PSA follow-up every 3 months in the first year after operation and at least biannually thereafter. Biochemical relapse was defined as 2 consecutive PSA measurements > 0.2 ng/mL with an interval of more than 3 months. PSA progression-free survival time was defined as the time from radical prostatectomy to the first follow-up date showing PSA > 0.2 ng/mL or until the last follow-up.

All original pathologic slides were reviewed by 1 pathologist (JSW) to reassign the Gleason scores and pathologic stage. The 1997 AJCC TNM staging system was used. A representative section containing the poorest tumor grade in the radical prostatectomy specimen was selected for immunohistochemical study. Serial 4 μm sections from corresponding archival blocks were dewaxed, rehydrated, and microwave heat retrieved. Sections were incubated with anti-$p21^{WAF1}$ (SX118) and anti-$p27^{KIP1}$ (SX53G8) antibodies (DakoCytomation, Denmark) according to manufacturer recommendations.

Two investigators (JSW and TTLW) examined all immunostained sections while blinded to the clinical data. Any amount of tumor nuclei demonstrating $p21^{WAF1}$ immunoreactivity was categorized as positive.[11] $p27^{KIP1}$ nuclear staining was assessed on a continuous scale from 0% to 100% by estimating a positive-to-total ratio. Different cutoffs have been recommended.[17,18] Based on the study of Vis et al,[18] an optimal cutoff point of 25% (for pT2 disease) was selected for statistical analysis.

Table 1. Patient characteristics

Median age, yr (range)	70 (57–75)
Initial prostate specific antigen	
Median, ng/mL (range)	10.0 (2.4–52.8)
≤ 4.0, *n* (%)	2 (3.8)
4.1–10.0, *n* (%)	25 (47.2)
10.1–20.0, *n* (%)	17 (32.1)
> 20.0, *n* (%)	9 (17)
Surgical Gleason score	
Median (range)	6 (2–9)
2–6, *n* (%)	43 (81.1)
7, *n* (%)	8 (15.1)
9, *n* (%)	2 (3.8)
Pathologic stage, *n* (%)	
T2a	17 (32.1)
T2b	36 (67.9)

Statistical analysis was performed using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA). Association between Gleason score on radical prostatectomy specimens with $p21^{WAF1}$ and $p27^{KIP1}$ expression was calculated using Fisher's exact test. Kaplan-Meier survival curves and the log-rank test were used to assess the prognostic significance of $p21^{WAF1}$ and $p27^{KIP1}$ in predicting biochemical failure. All reported p values were 2-sided, and $p < 0.05$ was considered statistically significant.

Results

A total of 53 patients were enrolled; their basic demographic data are listed in Table 1. At a median follow-up of 49 months (range, 10–117 months), 15 patients (28.3%) experienced biochemical recurrence. The 5- and 10-year PSA progression-free probabilities were 69% and 62.7%, respectively.

We identified $p21^{WAF1}$ immunoreactivity in 19 patients (35.8%). Twenty-nine tumors (54.7%) had decreased $p27^{KIP1}$ expression (Figure 1). No association of these immunohistochemical markers with Gleason score was identified ($p = 1.000$ for both; Table 2). Kaplan-Meier survival analysis and log-rank test disclosed no association of $p21^{WAF1}$ or $p27^{KIP1}$ expression with PSA progression-free survival ($p = 0.988$ and $p = 0.641$, respectively; Figure 2). Among 15 patients who experienced biochemical failure, 5 (33.3%) overexpressed $p21^{WAF1}$ and 7 (46.7%) had decreased $p27^{KIP1}$ expression ($p = 1.000$ and $p = 0.54$, respectively, Pearson's χ^2 test).

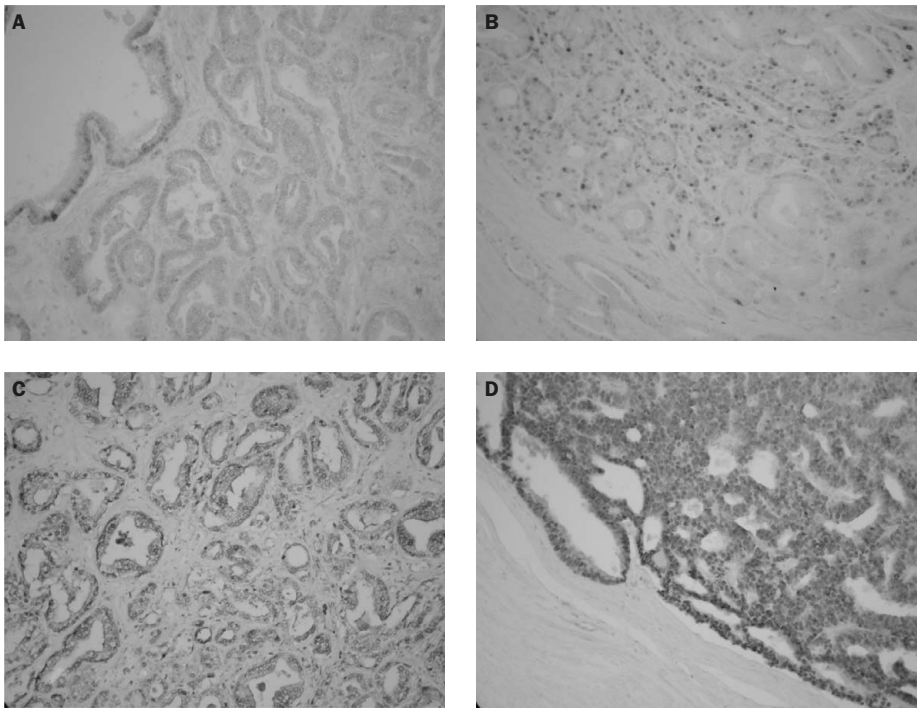

Figure 1. (A) Negative stain for p21; (B) positive nuclear stain for p21; (C) negative stain for p27 (stain of cytoplasm only); (D) strong positive nuclear stain for p27. (Magnification: all 200×.)

Table 2. Association between Gleason scores and p21 and p27 expression (Pearson's χ^2 test)

	p21		p27	
	Negative	Positive	Negative	Positive
Gleason score				
≤6	28 (65%)	15 (35%)	24 (56%)	19 (44%)
≥7	6 (60%)	4 (40%)	5 (50%)	5 (50%)
p	1.000		1.000	

Discussion

With the wide application of PSA tests, most of the prostate cancers diagnosed today are clinically localized diseases and have a good probability of being cured by radical prostatectomy. Preoperative PSA, Gleason score and tumor stage are well established prognostic indicators for prostate cancer.[2] However, there remains significant variation in individual patient outcome when these traditional markers are applied.[11,18] To improve predictive accuracy, many molecular markers are under investigation.[18]

$p21^{WAF1}$ is a product of the WAF1 gene that plays an essential role in the initiation of G1 arrest in response to DNA damage.[4–9,22] As a product of the tumor suppressor gene, $p21^{WAF1}$ should have lower expression in aggressive tumors.[11] However, Adsay et al illustrated a significant correlation between $p21^{WAF1}$ expression and advanced stage, but only a trend for higher Gleason score.[10] In the present study, we did not observe a correlation between $p21^{WAF1}$ expression and Gleason score. As to its prognostic significance, Byrne et al identified no relationship between $p21^{WAF1}$ expression and patient outcome.[23] On the contrary, Lacombe et al found $p21^{WAF1}$ overexpression to be an independent predictor for PSA failure in pT3 patients after radical prostatectomy.[13] In the study of Aaltomaa et al, $p21^{WAF1}$ expression indicated poorer outcome only in clinically localized prostate cancers but not metastatic disease.[12] Interestingly, Sarkar et al found that $p21^{WAF1}$ served as a prognostic marker for Caucasians but not for African-Americans.[11] Our study also found that $p21^{WAF1}$ had no prognostic significance for organ-confined prostate cancers.

The frequency of loss of $p27^{KIP1}$ expression in our patients (54.2% and 62.7% at cutoffs of 25% and 50%, respectively) was much higher than that reported by Yang et al (16.3% at cutoff of 30%)[17] and Vis et al (38% at cutoff of 50%).[18] Decreased $p27^{KIP1}$ expression has recently been reported to be associated with higher tumor grade.[19,21] Consistent with the findings of Kuczyk et al,[3] we could not correlate $p27^{KIP1}$ expression with Gleason scores. Some investigators reported that low expression of $p27^{KIP1}$ is an independent predictor

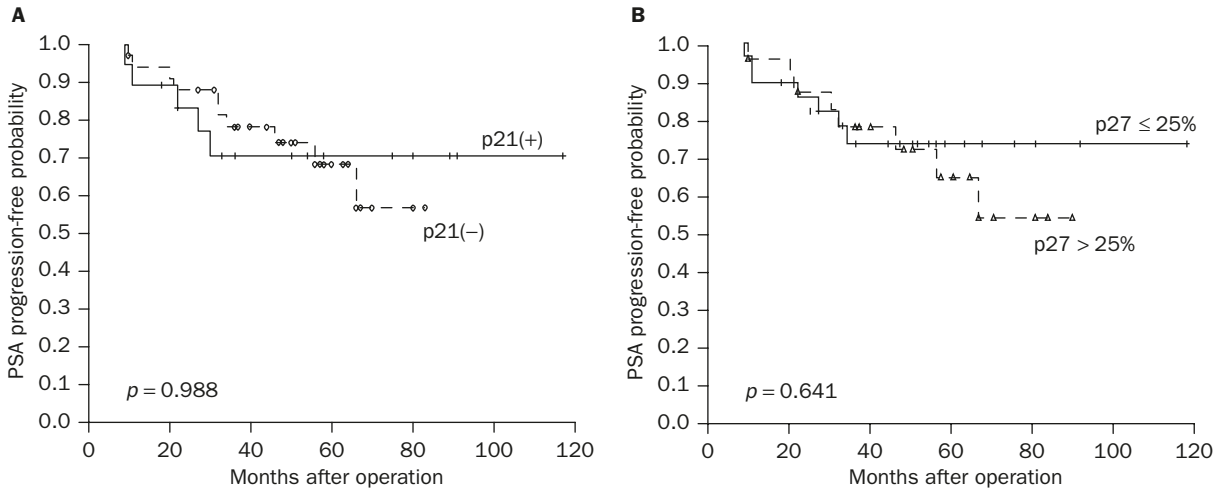

Figure 2. Kaplan-Meier survival curves with log-rank test *p* value: (A) p21; (B) p27. PSA = prostate specific antigen.

for biochemical failure and disease-specific survival in prostate cancers,[3,17,18,20,21] while some could not confirm this correlation.[24] We also failed to establish $p27^{KIP1}$ expression as a prognostic indicator for organ-confined prostate cancers. However, the unusually high frequency of loss of $p27^{KIP1}$ expression in Taiwanese prostate cancer patients might at least partially explain why our patients had a higher incidence of biochemical recurrence compared to Caucasians.

To improve the outcome of management of prostate cancer, identifying individuals at risk of disease recurrence after radical prostatectomy is very important, especially in the subset of patients with organ-confined tumors. Previously, we found that overexpression of bcl-2 and aberrant expression of E-cadherin are adverse prognostic factors.[14] In the current study, we failed to find any association of $p21^{WAF1}$ or $p27^{KIP1}$ with PSA progression-free survival in patients with localized prostate cancers. The main limitations of the present study were the small sample size and short follow-up period.

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