Altered Expression of p27 and Skp2 Proteins in Prostate Cancer of African-American Patients¹

Marija Drobnjak, Jonathan Melamed, Samir Tanema, Kate Melzer, Rosemary Wieczorek, Benjamin Levinson, Anne Zeleniuch-Jacquotte, David Polsky, Jay Ferrara, Roman Perez-Soler, Carlos Cordon-Cardo, Michele Pagano, and Iman Osman

Department of Pathology [M. D., C. C-C.], Memorial Sloan-Kettering Cancer Center, New York, New York, Departments of Pathology [J. M., R. W., M. P.], Urology [S. T., J. F., I. O.], Environmental Medicine [B. L., A. Z-J.], Medicine [R. P-S.], and Dermatology [K. M., D. P., I. O.], Kaplan Comprehensive Cancer Center [S. T., A. Z-J., R. P-S., M. P., I. O.], New York University School of Medicine, New York, New York

ABSTRACT

Purpose: The purpose is to investigate the clinical relevance of altered patterns of p27 and Skp2 expression in African-American patients with localized prostate cancer. The abundance of p27, an inhibitor of cell proliferation, is controlled by Skp2-dependent proteolysis.

Experimental Design: A well-characterized cohort of 162 African-Americans who underwent radical prostatectomy at the Veterans Affairs Medical Center of New York between 1990 and 2000 was studied. We analyzed p27 and Skp2 expression by immunohistochemistry. Altered expression of p27 (defined as <40% tumor cells expressing the protein) and Skp2 (defined as ≥20% tumor cells expressing the protein) were correlated with clinicopathological parameters and time to prostate-specific antigen (PSA) recurrence.

Results: Altered expression of p27 and Skp2 was observed in 112 of 162 (69.1%) and 93 of 162 (57.4%) cases, respectively. Inverse patterns of Skp2 and p27 protein expression were seen in 87 of 162 (53.7%) cases. A marginally significant association was found between Skp2 overexpression and extracapsular extension (P = 0.065). Moreover, patients with Skp2 overexpression had a 2.77 years decreased median time to PSA recurrence compared with patients with low Skp2 expression; however, the difference was not statistically significant. In multivariate analysis, only tumor grade and stage independently predicted PSA recurrence in this cohort.

Conclusions: Our data suggest a role for Skp2 overexpression in prostate cancer pathogenesis that might not be exclusively related to p27 degradation. More studies are needed to determine the mechanistic role of Skp2 in prostate cancer.

INTRODUCTION

Several epidemiological studies have demonstrated that African-American ethnic origin is an important determinant of prostate cancer risk, incidence, and disease progression (1–3). Differences in socioeconomic levels as well as medical care access have been implicated as causative factors for the ethnic differences in the clinical behavior of prostate cancer (4, 5). However, several groups have reported that African-American patients treated for localized prostate cancer had a higher recurrence rate compared with Caucasian patients diagnosed with the same stage (6, 7). Recently, a study of 1468 patients demonstrated that African-American ethnicity is an independent predictor of disease recurrence in community patients undergoing radical prostatectomy (8).

We previously reported our analyses of the alterations affecting the p53 and retinoblastoma pathways in a well-characterized cohort of prostate cancer patients treated at Memorial Sloan-Kettering Cancer Center (New York, NY; Refs. 9–13). Interestingly, loss of p27 protein expression was found to be the most clinically informative marker of all cell cycle regulators studied. Specifically, the data revealed that primary prostate carcinomas expressing lower levels of p27 were clinically more aggressive, based on their independent association with time to PSA³ recurrence after radical prostatectomy (13). Several groups have reported similar observations (14–18).

In prostate carcinoma cells, the regulation of p27 expression has been shown to be at the posttranscriptional level (13). The Skp2 protein specifically recognizes p27 in a phosphorylation-dependent manner and degrades it via ubiquitination (19–21). This interaction can be significantly relevant from a therapeutic standpoint because a small molecule capable of inhibiting Skp2 should lead to an increase in the cellular abundance of p27 and a subsequent block in cell cycle proliferation.

In this study, we expanded our work on p27 expression of prostate cancer to study the clinical relevance of altered patterns of p27 and Skp2 protein expression in African-American patients treated for localized prostate cancer.

¹ The abbreviations used are: PSA, prostate-specific antigen; VAMC, Veterans Affairs Medical Center.

Received 5/13/02; revised 11/5/02; accepted 1/6/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ Supported by NIH/National Cancer Institute Grants CA-01713 (to I. O.), SPORE-92629 and DK-47650 (to C. C-C.), CA-76584 and GM-57587 (to M. P.), and Irma T. Hirschl Scholarship (to M. P.). Also supported, in part, by the use of facilities at the Manhattan Veterans Affairs Medical Center.

² To whom requests for reprints should be addressed, at New York University Medical Center, 350 First Avenue (H-100), New York, NY 10016. Phone: (212) 686-7500, x3522; Fax: (212) 951-3214; E-mail: Iman Osman@med.nyu.edu.
PATIENTS AND METHODS

Patient Characteristics and Tissues. Patients were identified through review of the Department of Urology database at the VAMC/New York University School of Medicine. This prospective database enrolled patients with prostate cancer from 1990 to the present, documenting patient demographics, including ethnic origin, stage, and grade of the primary tumor. After Institutional Review Board approval and activation of the protocol, we retrospectively reviewed all relevant clinical information, PSA values, imaging studies, and pathology parameters, and entered this information into a database. Representative H&E-stained tissue sections were examined by two attending pathologists (J. M., R. W.) to evaluate the histopathological characteristics of each case. Of the 180 African-American patients whose tumors were resected at the VAMC, representative tissue blocks of formalin-fixed, paraffin-embedded primary tumors were obtained from 162 patients. Patient selection was based on the availability of both adequate clinical follow-up and representative-archived pathological materials for immunohistochemical analysis. Clinicopathological parameters included pretreatment PSA, pathological stage, and Gleason score. Cases were grouped as either low Gleason score (<7, n = 79) or as high Gleason score (≥7, n = 83). Cases were also grouped according to pathological stage, into either early organ-confined tumors (pt2, n = 97), or advanced tumors extending beyond the prostatic capsule (pt ≥ 3, n = 65). The response variable, time to PSA relapse, was defined as the time from radical prostatectomy to the time of the first detectable (nonzero) PSA measurement. To confirm PSA relapse, three consecutive increases of PSA were required; however, the time of relapse was defined as the time from radical prostatectomy to the time of the first detectable PSA measurement. Although exclusively nuclear staining was the predominant pattern represented for p27 immunoreactivity, cytoplasmic staining was found to be the main pattern of Skp2 reactivity. p27 immunostaining in surrounding normal prostate glands as well as in the lymphocytes was used as a positive internal control for each slide. Tonsil (germinal proliferating center) and squamous (suprabasal cells) tissues were used as positive controls for Skp2. Results were recorded accordingly as the percentage of tumor cells displaying positive p27 and Skp2 immunoreactivity. The cut point for p27 was set at <40% because this level was previously determined to be clinically relevant in our previous study of p27 expression in prostate cancer (13). The cut point for Skp2 of ≥20% was based on data published by other groups correlating Skp2 immunoreactivity with clinicopathological parameters of poor prognosis (22). We also examined Skp2 altered expression using different cut points (10 and 25%) to reveal any possible association between Skp2-altered expression and clinical outcome.

Statistical Analyses. The χ² test and χ² test for trend were used to explore associations between the immunophenotypic variables p27 and Skp2 and the clinicopathological parameters PSA, Gleason score, tumor stage, and age of specimens. The Kaplan-Meier method was used to estimate time to PSA recurrence. Recurrence-free survival was calculated from date of treatment to date of PSA relapse or last follow up date, whichever the earliest; patients who died without PSA relapse were censored at the date of death. The Log-Rank test was used to assess the prognostic value of the immunophenotypic variables and clinicopathological parameters on time to PSA recurrence. The Cox proportional hazards model was used to assess the relationship between the immunophenotypic variables p27 and Skp2 (both as dichotomous and continuous variables) and time free of disease recurrence controlling for Gleason score and tumor stage. The variables p27 and Skp2 were entered into the model separately and jointly. All P values were two-sided. All statistical analyses were done using SAS release 8.2 (SAS Institute, Inc., Cary, NC).

RESULTS

We successfully retrieved 162 of 180 (90%) registered cases in the database of the Department of Urology at the...
VAMC. The remaining 18 cases lacked clinical information (n = 10) or did not have enough tissue for immunohistochemical analyses (n = 8). The median age at the time of surgery was 65 years (range, 51–78 years). The median follow-up was 5.5 years (range, 0.5–11.4 years). Fifty-nine (39.1%) patients had PSA recurrence during the follow-up period.

Altered expression of p27, defined as <40% tumor cells displaying nuclear immunoreactivities, was observed in 112 of 162 (69.1%) cases. Skp2 overexpression, defined as ≥20% tumor cells showing immunostaining, was observed in 93 of 162 (57.4%) cases. Data analyses included the stratification of tumor specimens by their accession year to ensure that immunohistochemical results were not influenced by the duration of storage or changing methods of tissue preservation. The percentage of samples displaying p27 and Skp2 positivity was calculated for each year. There was no trend linking expression of the markers with the accession year, leading us to conclude that expression profile was not affected by these variables (data not shown).

Table 1 summarizes data relating p27 and Skp2 immunophenotype to clinicopathological parameters, including pretreatment PSA, tumor stage, and grade. The association with pretreatment PSA was assessed in 145 cases. The remaining 17 patients were not included because PSA testing did not become available on a routine basis at the VAMC until 1991, after the test was approved by the Federal Drug Administration. There was no statistically significant association between p27, Skp2, and baseline characteristics; however, a marginally statistically significant association was observed between Skp2 overexpression and local progression of the tumor: 43 of 65 patients (66%) with advanced stage were Skp2 overexpressors, as compared with 50 of 97 (52%) with early stage (P = 0.065). In addition, we explored the correlation between altered patterns of p27 and Skp2 with pretreatment PSA, Gleason score and tumor stage as continuous variables. No significant association was observed, confirming the lack of association seen when results were analyzed as discrete categories using different cut points.

Recent publications by other groups, reporting on Skp2 expression levels in different tumor types (19–21), took into account only nuclear Skp2 immunostaining. In this study, we observed that Skp2 exhibited a primarily cytoplasmic pattern of expression. To ascertain the specificity of this expression profile and to eliminate the possibility of nonspecific antibody binding, we used Skp2 antibody preadsorbed with a purified recombinant Skp2 protein. Both the nuclear reactivity seen in control tissues (proliferating germinal center of the tonsil and suprabasal squamous epithelium) as well as the cytoplasmic reactivity seen in the prostate cancer glands were successfully abolished, thereby confirming the specificity of Skp2 immunostaining (Fig. 1).

In univariate analysis, PSA at baseline, tumor stage, and grade significantly correlated with time to PSA recurrence after surgery (Table 2). There was no association between altered expression of p27 or Skp2 and time to PSA recurrence. These results were consistent with analyses of p27 and Skp2 expression in subgroups defined by stage (<3, ≥3) and by Gleason score (<7, ≥7; data not shown). In multivariate analysis, tumor stage and grade remained the most significant predictors of treatment outcome (Table 3).

Inverse patterns of protein expression of Skp2 and p27 were observed in 87 of 162 (53.7%) cases. These include 65 patients who showed Skp2 overexpression and low p27 (Fig. 2, bottom panel), as well as 22 patients who showed low Skp2 expression and normal p27 levels (Fig. 2, top panel). However, the inverse pattern between the expression of p27 and Skp2 was not seen in the remaining 75 cases. These include 47 (29.0%) cases showing low expression of both p27 and Skp2. In these cases, we observed normal expression of p27 and very low expression of Skp2 in the surrounding normal prostate tissues, which we considered an internal control. The remaining 28 (17.3%) cases showed protein overexpression of both p27 and Skp2.

### DISCUSSION

In this study, we investigated the clinical predictive value of altered p27 and Skp2 expression in a well-characterized cohort of African-American patients treated for localized prostate cancer. Our data reveal that altered expression of p27 and Skp2 are common biological events, which suggest that they might have a role in the pathogenesis of prostate cancer in African-American patients; however, their clinical predictive

### Table 1 Correlation between baseline clinicopathological parameters and p27 and Skp2 phenotypes

<table>
<thead>
<tr>
<th>Factor</th>
<th>p27 negative (≤40%), n = 112</th>
<th>p27 positive (≥40%), n = 50</th>
<th>Skp2 negative (≤20%), n = 69</th>
<th>Skp2 positive (≥20%), n = 93</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA ≤4</td>
<td>10 71%</td>
<td>4 29%</td>
<td>5 36%</td>
<td>9 64%</td>
<td>0.57*</td>
</tr>
<tr>
<td>&gt;4–10</td>
<td>40 71%</td>
<td>16 29%</td>
<td>26 46%</td>
<td>30 54%</td>
<td>0.57*</td>
</tr>
<tr>
<td>&gt;10</td>
<td>50 67%</td>
<td>25 33%</td>
<td>32 43%</td>
<td>43 57%</td>
<td>0.57*</td>
</tr>
<tr>
<td>Gleason &gt;7</td>
<td>53 67%</td>
<td>26 33%</td>
<td>37 47%</td>
<td>42 53%</td>
<td>0.57*</td>
</tr>
<tr>
<td>≥7</td>
<td>59 71%</td>
<td>24 29%</td>
<td>32 39%</td>
<td>51 61%</td>
<td>0.57*</td>
</tr>
<tr>
<td>Stage &lt;2</td>
<td>69 71%</td>
<td>28 29%</td>
<td>47 48%</td>
<td>50 52%</td>
<td>0.57*</td>
</tr>
<tr>
<td>≥3</td>
<td>43 66%</td>
<td>22 34%</td>
<td>22 34%</td>
<td>43 66%</td>
<td>0.57*</td>
</tr>
</tbody>
</table>

*a Seventeen cases with unknown baseline PSA.

*2 test for trend.
value appears limited compared with standard of care prognostic criteria. Nevertheless, we observed a marginally significant association between Skp2 overexpression and local progression as well as shorter time to PSA relapse. Although the correlation with both variables did not reach statistical significance, it might still have some clinical relevance that requires additional examination.

Although this study reports a limited predictive value of p27 and Skp2 as prognostic factors in African-American patients with clinically localized prostate cancer, it has important distinctive features. First, the unique resources provided by the VAMC in New York allowed the generation of a clinicopathological database of considerable volume (n = 162) of African-American patients who received standardized care at a single institution compared with studies of smaller sample sizes (23, 24) or those constructed by the pooling of cases from different institutions with different standards of care (25, 26). Second, the strong predictive power of the standard of care parameters observed in this cohort, as well as the considerable number of events (PSA recurrence) that occurred during the follow-up period, are reassuring of the credibility of the conclusions. Third, the high retrieval rate of tissues (90%) minimizes the chance of selection bias, which is usually a major issue in conducting this type of retrospective analyses (27). On the basis of these characteristics, we believe that the present cohort provides a reliable resource for the assessment and validation of
molecular markers in the context of clinical outcome studies for these patients.

The observation that decreased expression of p27 is a common event in prostate cancer of African-American men is comparable with the published reports of p27 in prostate cancer from our group and others. In the present analysis, however, we did not observe the strong correlation between decreased p27 expression and clinical outcome (13–15, 18). Possible explanations include the selection of patients representative of a specific stage in one study (15). Also, our previous study included patients who received neoadjuvant hormone treatment prior to their radical prostatectomy (13) compared with our current study that includes a more homogenous population of patients who were primarily hormone-naïve at the time of surgery. These variables are known to influence time to PSA relapse after surgery. In addition, different methodologies, pathologic material, and cutoff points were used to define p27 expression levels in other studies (14–18). It is not clear whether the ethnic background per se of the patients contributed to the difference in the correlation with treatment outcome results.

We have previously shown that prostatic carcinoma cells regulate p27 expression at the posttranscriptional level (13). More specifically, in colon cancer (28), lymphomas (29), nonsmall cell lung cancer (30), as well as in astrocytoma brain tumor tissues (31), the decrease in p27 protein levels has been shown to be due to an increase in its ubiquitin proteasome-mediated proteolysis. Both in vivo and in vitro, Skp2 has been shown to be a component of the ubiquitin-mediated p27 degradation machinery (32). Several studies have documented this observation in colon, oral squamous carcinoma, lymphoma tissues (19–21) and most recently in prostate cancer (33). This interaction can be significantly relevant from a therapeutic standpoint because, hypothetically, a small molecule capable of inhibiting Skp2 should lead to an increase in the cellular abundance of p27 and a subsequent block in cellular proliferation that could delay and/or prevent disease progression. In fact, several compounds are currently being tested for inhibiting proteasome degradation, and the first compound (PS-341) is presently undergoing Phase II study in patients with different tumors, including prostate cancer (34, 35).

In this study, the reversed pattern of expression of p27 and Skp2 was a common feature, identified in ~53% of cases.
However, almost one-third of cases had decreased levels of p27 expression in the absence of Skp2 overexpression, which could be attributed to the degradation of p27 by a Skp2-independent pathway, a mechanism that has been recently described (36). In addition, a subset of tumors (17%) showed abundant expression of p27 despite accumulation of Skp2, which may be due to a mutation in one of the other members of the ubiquitination-proteasome complex, and suggests a role of Skp2 that might not be directly related to targeting p27 for degradation. In this regard, an oncogenic potential for Skp2 has been recently demonstrated. Skp2 cooperates with activated H-Ras to malignantly transform primary rodent fibroblasts as scored by colony formation in soft agar and tumor formation in nude mice (22). Moreover, Skp2 cooperates with activated N-Ras in a mouse model of lymphomagenesis (21). Skp2 is also a key target of extracellular matrix signaling that controls cell proliferation. Its forced expression induces growth in the absence of cell adhesion both in fibroblast (37) and epithelial cells (38).

We observed that Skp2 exhibited a primarily cytoplasmic pattern of expression in prostate cancer glands. The specificity of the cytoplasmic immunostaining was demonstrated using a blocking experiment with Skp2 antibody preadsorbed with a purified recombinant Skp2 protein. Both the nuclear reactivity seen in two different positive controls as well as the cytoplasmic reactivity seen in the prostate cancer glands were successfully abolished, thereby confirming the specificity of Skp2 immunostaining (Fig. 1). Remaining to be determined is the mechanism that is driving the shift of Skp2 subcellular localization from the nucleus to the cytoplasm in prostate cancer cells. In this regard, a splice variant of Skp2 was shown to be retained in the cytoplasm in uterine cancer cell lines (39). In addition, it is unknown whether this phenotype is peculiar to prostate cancer glands. In fact, available data regarding the expression of Skp2 are limited to a few human cancers, and thus, more work is needed before firm conclusions can be drawn.

In sum, the high frequency of Skp2 overexpression in this population, which was associated with poor prognostic features, suggests a role for Skp2 overexpression in prostate cancer pathogenesis that might not be exclusively related to p27 degradation. More studies are needed to uncover the mechanism and significance of Skp2 cytoplasmic localization seen in this subset of patients. In addition, our data reveal that altered expression of p27 and Skp2 are common biological events in prostate cancer affecting African-American patients, however, their prognostic value appears limited compared with standard of care.

REFERENCES


Altered Expression of p27 and Skp2 Proteins in Prostate Cancer of African-American Patients

Marija Drobnjak, Jonathan Melamed, Samir Taneja, et al.


Updated version  Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/9/7/2613

Cited articles  This article cites 39 articles, 12 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/9/7/2613.full#ref-list-1

Citing articles  This article has been cited by 7 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/9/7/2613.full#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, use this link
http://clincancerres.aacrjournals.org/content/9/7/2613.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.