p53 Nuclear Protein Expression Is an Independent Prognostic Marker in Clinically Localized Prostate Cancer Patients Undergoing Radical Prostatectomy

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ABSTRACT

Immunohistochemical (IHC) staining for p53 protein nuclear expression was evaluated in archival paraffin-embedded radical prostatectomy specimens from 139 patients with clinically localized prostate cancer followed up from 1 to 8 (mean, 4) years. Elevated nuclear p53 protein expression was detected in 85 (61%) of 139 patients, being heterogeneous and focal in the majority of specimens. Only four specimens displayed homogeneous nuclear accumulation of p53 protein. Disease progression, most commonly prostate-specific antigen elevation, was noted in 46 (33%) patients, with 39 (85%) having positive p53 protein IHC stains. Conversely, 93 (67%) of 139 have not recurred, with 46 (49%) having positive p53. Of all 54 p53-negative patients, 47 (87%) have had no disease recurrence. An increased p53 protein IHC stain was associated with a higher pathological stage (T1 and T2, 51% versus ≥T3a, 69%) and Gleason score (2–4, 17%; 5–7, 72%; and 8–10, 87.5%). Despite these associations, p53 IHC staining was an independent predictor of disease-free survival in a multivariate analysis of p53, age, race, stage, and grade.

This study revealed that a majority of clinically localized prostate cancers heterogeneously express elevated nuclear levels of p53 protein in at least a subset of malignant cells, and that this expression is an independent predictor of disease progression in prostate cancer patients after radical prostatectomy.

INTRODUCTION

Prostate cancer is the most common solid tumor in U.S. males and the second leading cause of cancer deaths (1). It has been estimated that by the year 2000 there will be almost double the current incidence and a one-third increase in annual mortality (2). Over the last decade there has been a dramatic increase in the use of radical prostatectomy as treatment for clinically localized prostate cancer (3). Despite this, approximately half of these patients will have non-organ-confined disease, and 30–40% will eventually have disease progression (4). Grade and stage, the traditional prognostic markers, are useful, but for individual patients it is difficult to predict outcome. With recent advances in molecular biology, the concept of oncogenes and tumor suppressor genes has dominated basic science research of tumorigenesis and evaluation of these genes, and their protein products may provide new prognostic markers (5).

Mutations of the p53 tumor suppressor gene are the most common genetic defects, thus far, identified in human tumors (6). The p53 tumor suppressor gene has been shown to regulate cell growth by arresting cells in G1 (7). The p53 protein increases in response to damaged DNA, causing a cellular arrest to allow for possible DNA repair or to start the sequence of programmed cellular death, apoptosis (8, 9). Point mutations that lead to the loss of tumor suppressor genes have been implicated in uncontrolled cellular growth leading to tumor formation (10–12). Mutations of p53 have been found in a variety of cancers, including urological neoplasms.

Other studies have implicated p53 protein expression as an independent prognostic factor in carcinomas of the breast, stomach, colon and rectum, and lung (13). Multiple studies have been conducted on prostate cancer specimens, citing frequencies of p53 alterations in localized prostate cancers ranging from 4 to 50% and, with more advanced hormone refractory disease, frequencies as high as 94% (5, 14). These alterations were determined using a variety of techniques, including p53 protein IHC3 staining, SSCP, and DNA sequencing; p53 protein IHC staining exploits the increased half-life of the inactive or altered p53 protein produced by a mutated p53 gene. Normal wild-type protein has a half-life of 6–30 min and generally will not accumulate to high enough levels for detection by IHC staining (15). With the exception of testicular cancers and a subset of breast cancers, a significant correlation exists between IHC

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3 The abbreviations used are: IHC, immunohistochemical; SSCP, single-strand conformational polymorphism analysis; PSA, prostate-specific antigen.
detectable levels of accumulated nuclear p53 protein and a corresponding defect in the p53 gene (14, 16–18). Our group has shown recently that in advanced prostate cancer, 10 (77%) of 13 specimens and 9 (82%) of 11 patients had corresponding p53 mutations when the p53 protein IHC staining was detectable (14). In this study, 8 of 13 specimens had a p53 score of 1+ (1–25% of cells expressing p53). Of these eight specimens, six demonstrated p53 alterations with SSCP analysis. However, Brookstein et al. (18) was unable to find consistent corresponding p53 mutations in specimens with low levels of p53 expression (1–20% of cells expressing p53 with IHC staining). Grosse et al. (19) recently developed a new approach, which can detect p53 mutations at exceedingly low frequencies by analyzing SSCP patterns of TA clones. In their study of three metastatic prostate cancer specimens with positive IHC stains and confirmed p53 alteration, they were able to demonstrate the same corresponding p53 alteration in the primary tumors. The primary tumors, however, had a much lower frequency of p53 alteration (1–14%), and in one specimen p53 expression was detectable by IHC staining. These results suggest that heterogeneous low frequency p53 mutations in a primary tumor are capable of clonal expansion at distant metastatic sites (19).

In the present study, we hypothesized that because p53 expression is more commonly detected in advanced prostate cancer, detection of clones of malignant cells positive for p53 in localized prostate cancer may predict outcome. We show that IHC staining of the primary radical prostatectomy specimen for p53 expression is an independent prognostic marker for subsequent disease progression. Although most cases demonstrated a relatively small percentage of malignant primary tumor cells harboring p53 expression, these clones seem to affect outcome for men undergoing radical prostatectomy adversely. This is the first study to demonstrate that focal p53 protein expression is an independent prognostic marker in a homogeneous population of clinically localized prostate cancer patients who underwent radical prostatectomy.

**MATERIALS AND METHODS**

**Specimens and Clinicopathological Features.** Between January 1986 and June 1992 (6.5 years), 222 patients underwent radical retropubic prostatectomy at Walter Reed Army Medical Center. From this group, 139 patients had available archival pathological material and accurate clinical follow-up as of January 1995. Clinical information and pathological data were obtained from the Center for Prostate Disease Research Institutional Review Board-approved clinical research database of all prostate cancer patients treated at Walter Reed Army Medical Center. The demographics of the study population, stage, grade, and p53 expression frequency data are listed in Table 1. Follow-up ranged from 1 to 8 years, with a mean of 4 years. Serological recurrence of prostate cancer based on PSA was defined as two successive PSA measurements greater than 0.2 ng/ml (20).

Histopathological grading was done by a single investigator (I. A. S.) according to the Gleason system (21) and the WHO method for nuclear grade and glandular differentiation (22). Staging was based on the modified Whitmore-Jewett system and the TNM system (23).

**IHC Analysis.** Archival paraffin-embedded specimens were analyzed using hematoxylin and eosin of a 4-μm-thick section for the presence of tumor. Sections containing the highest numbers of tumors and the largest tumor volumes were selected for each patient. More than one block per patient was used if the clonal distribution of the tumor cells differed significantly between paraffin blocks. The corresponding tissue blocks were then recut into 4-μm-sections and mounted on silanated slides. The sections were deparaffinized, and endogenous peroxidase was blocked with 0.6% hydrogen peroxide in methanol. Antigen retrieval was accomplished by microwaving for 15 min in 1 mM citrate buffer. Immunological detection was achieved with commercially available polyclonal anti-p53 antibody (Signet Lab; catalog 8640). The avidin-biotin-peroxidase system (Vectastain Elite Kit; Vector Labs) was used to visualize the binding of the antibody. Dilution of a polyclonal antibody (1:80) was noted to be optimal from previous studies (14, 24, 25). Colon carcinoma with known p53 mutation was used as a positive control, and negative controls consisted of benign prostate tissue.

The p53 IHC slides were graded by a single pathologist (I. A. S.) in a similar manner as described previously by our group (14). The p53 grade was assigned to the specimens before the clinical follow-up was obtained; this was done to blind the pathologist (I. A. S.) and primary investigator (J. J. B.) from follow-up results. The majority of specimens showed a very heterogeneous focal distribution; the areas of focal positivity with the highest percentages of nuclei staining positively were used to grade the tumor as: 1+, 1–25%; 2+, 26–50%; 3+, 51–75%; and 4+, 76–100%, rare (occasional) or none. Cytoplasmic staining was not evaluated (Fig. 1, A–D).

**Statistical Analysis and Progression.** Survival was assessed using a univariate Kaplan-Meier survival analysis based on the log rank statistical test. Multivariate statistical analysis

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographics, stage, and grade data on study cohort and p53 expression (1+ to 4+) combined positivity data</th>
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<tbody>
<tr>
<td>Variables</td>
<td>n (% total patients)</td>
</tr>
<tr>
<td>Age, yr</td>
<td></td>
</tr>
<tr>
<td>&lt;59</td>
<td>32 (23.0)</td>
</tr>
<tr>
<td>60–64</td>
<td>34 (24.5)</td>
</tr>
<tr>
<td>65–69</td>
<td>53 (38.1)</td>
</tr>
<tr>
<td>&gt;70</td>
<td>20 (14.4)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>110 (79.1)</td>
</tr>
<tr>
<td>Black</td>
<td>25 (18.0)</td>
</tr>
<tr>
<td>Others</td>
<td>4 (02.9)</td>
</tr>
<tr>
<td>Stage (pathological)</td>
<td></td>
</tr>
<tr>
<td>pT2b</td>
<td>2 (01.4)</td>
</tr>
<tr>
<td>pT2a</td>
<td>19 (13.7)</td>
</tr>
<tr>
<td>pT2b+c</td>
<td>36 (25.9)</td>
</tr>
<tr>
<td>pT3</td>
<td>81 (58.3)</td>
</tr>
<tr>
<td>pT4, N1 (D1)</td>
<td>1 (01.4)</td>
</tr>
<tr>
<td>Gleason score</td>
<td></td>
</tr>
<tr>
<td>2–4</td>
<td>27 (19.4)</td>
</tr>
<tr>
<td>5–7</td>
<td>98 (70.5)</td>
</tr>
<tr>
<td>8–10</td>
<td>14 (10.1)</td>
</tr>
<tr>
<td>Nuclear grade</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>29 (20.9)</td>
</tr>
<tr>
<td>II</td>
<td>105 (75.5)</td>
</tr>
<tr>
<td>III</td>
<td>5 (03.6)</td>
</tr>
</tbody>
</table>
category, there was a clear progression in recurrence rates (P < 0.001). Table 2 provides the degree of p53 IHC expression related to these, 47 (50%) and 46 (50%) showed negative and positive p53 expression, respectively. Of interest are the results of those patients that had no p53 protein expression; 47 (87%) of 54 were without evidence of recurrence, independent of race, stage, Gleason score, and nuclear grade.

Kaplan-Meier survival analysis for the p53-positive and -negative patients was assessed with the Cox regression method. Estimates of the relative risk between p53-positive and -negative patients was assessed with the Cox regression method. Actuarial analyses were used a Cox regression analysis with backward elimination controlling for age, race, Gleason score, nuclear grade, and stage. The data show equal distribution of p53-positive specimens between black (<5%, 5–15%, 16–20%, and 21–25% of positive nuclei. Table 2 provides the degree of p53 IHC expression related to recurrence. As the p53 expression increased from 0 to the 2–4+ category, there was a clear progression in recurrence rates (P < 0.0001). Table 3 displays the p53 expression compared with the demographics, stage, grade, and recurrence. The data show that race, stage, and Gleason grade were significant, whereas nuclear grade and age were not. Multivariate Cox regression analysis revealed that p53 expression was an independent prognostic factor for disease progression (P < 0.001) controlling for age, race, Gleason grade, nuclear grade, and stage. Race and

Table 2 Univariate analysis of p53 expression and recurrence after radical prostatectomy*  

<table>
<thead>
<tr>
<th>p53 score (n)</th>
<th>No progression, n (%)</th>
<th>Progression, n (%)</th>
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<tbody>
<tr>
<td>0 (26)</td>
<td>23 (88.5)</td>
<td>3 (11.5)</td>
</tr>
<tr>
<td>Rare (28)</td>
<td>23 (82.1)</td>
<td>5 (17.9)</td>
</tr>
<tr>
<td>1+ (56)</td>
<td>35 (62.5)</td>
<td>21 (37.5)</td>
</tr>
<tr>
<td>2–4+ (29)</td>
<td>11 (37.9)</td>
<td>18 (62.1)</td>
</tr>
</tbody>
</table>

* Cox-Mantel-Haenszel statistics (based on table scores). P < 0.0001.
expression in the range of 42-50% (33-35) in localized prostate cancer and long-term clinical outcome have shown conflict-

When cancer will recur, clinicians need to determine whether the p53-positive patients who have not yet recurred, and the p53-negative (none or rare) patients. Fig. 2 Kaplan-Meier disease-free survival curve comparing p53-positive (1-4+) and p53-negative (none or rare) patients.

cancer, with one report as high as 79% (36); however, this expression was noted to be predominantly cytoplasmic. Our results show that p53 expression was found in 61.1% of our clinically localized prostate cancers. Considering the focal nature of nuclear accumulation of the p53 protein that we observed, it is likely that the discrepancy in the frequency of p53 positivity is related to sampling and interpretation variability. The specific specimens used for p53 protein IHC staining in our study were determined by looking at all of the available blocks for each prostate and finding the sections with the highest tumor volumes and greatest varieties of clonally different cell populations. From the literature, it is difficult to determine whether other investigators used random samples or a systematic approach to the entire prostate specimen when performing p53 IHC staining. Also, the definition of a positive p53 sample is not clear and implies a certain percentage of the entire tumor volume; using this approach would have ignored focal p53 positivity, which we suggest does have prognostic value. The above methods may have increased our positivity rate compared with those of other researchers. Had we ignored focal p53 positivity, our cohort would have also shown a low p53 positivity rate, in line with currently reported percentages in localized prostate cancer specimens.

This study supports a correlation between p53 expression, tumor stage, Gleason score, and nuclear grade that has been reported in previous studies (16, 18, 27, 28, 37–39). The expression of p53 was noted to be increased with higher Gleason grade tumors (2–4, 17%; 5–7, 72%; and 8–10, 87.5%) and with more advanced stages (B, 51%; C, 69%). As the nuclear grade increased, so did p53 positivity (I, 37.9%; II, 67.6%; and III, 80%); however, the number of grade III specimens was very small. The percentages of p53 expression between black (60%) and white (63%) patients were surprisingly similar considering that black patients have been shown to have higher grade and stage tumors and poorer prognoses (40).

In the literature, studies on p53 protein expression in prostatic cancer and long-term clinical outcome have shown conflicting results. Visakorpi et al. (41), using univariate statistical analysis, showed that high level p53 accumulation predicted a...
short progression-free interval and poor survival, whereas low level p53 expression had no prognostic significance (18). Berner et al. (39, 42), in two separate studies comparing multiple potential prognostic markers, found no statistically significant correlations between p53 accumulation and time to progression or survival. They did note a weak trend between p53 expression and poor survival using univariate analysis. However, multivariate analysis did not show p53 protein accumulation to be an independent prognostic indicator. Issacs et al. (32), in metastatic prostate cancer specimens, also found no statistically significant correlation between p53 expression and a more aggressive course. Thomas et al. (26) reported on 68 men, using univariate statistical analysis, the influence of p53 expression on longer term clinical outcome for prostate cancer patients to be significant. Their specimens were transurethral resection chips from prostate cancers that required treatment for bladder outflow obstruction. Unlike our study, their patients were older (mean age, 71 years), and both local and distant disease were included; however, most (85%) had locally advanced T1 or T2 tumors, and a large number of the patients [26 (38.2%) of 68] had distant disease. Thirteen percent of their samples exhibited p53 expression. Follow-up data were available on only 45 (66%) of their patients, including 7 whose tumors were p53 positive. Progression was seen in all 7 p53-positive tumors and 29 (78%) of 37 p53-negative tumors. The time to clinical progression and survival correlated well with p53 expression, being associated with poor prognosis in terms of progression and survival. Multivariate analysis controlling for race, stage, and grade was not completed. A more recently published study by Shurbaji et al. (43) on 109 prostate cancer specimens showed an overall p53-positive rate of 21%. These specimens were also obtained from an older population (mean age, >69 years), and there was a heterogeneous mixture of clinical stages (A, 24%; B, 30%; C, 23%; and D, 15%). Their specimens represented a mixture of needle biopsies (36%), transurethral resection specimens (31%), radical prostatectomies (28%), and enucleation specimens (5%). The recurrence rate in this cohort was very low (17%), because PSA recurrences were not included. Their study did show how p53 protein expression to be an independent prognostic marker of disease progression with multivariate analysis. However, the probability of progression-free survival of their p53-positive and -negative patients became less significant as the follow-up time approached 6 years.

Our study was conducted on a younger homogeneous population (mean age, 65 years) with clinically localized prostate cancers who were treated with radical prostatectomy. With the entire prostate specimen available, our sampling error was less than that of needle biopsy or transurethral resection specimens used in the above-noted studies. Furthermore, our study included serological PSA recurrence, the most commonly encountered first recurrence in contemporary clinical practice. Our results in a well-defined clinically relevant population of potentially curable patients demonstrated that p53 protein expression is a powerful independent prognostic marker, and this relationship remains highly significant statistically at relatively long follow-up.

It may be important to distinguish between patients with localized prostate cancer, in whom surgery alone is sufficient treatment, and those who would benefit from both surgery and further therapy to include radiation and early hormonal therapy. Multiple studies, including ours, show that 40–70% of clinically organ-confined diseases after radical prostatectomy have positive surgical margins, extracapsular invasion, or distant disease (44). Many urologists will elect watchful waiting for recurrence and then institute second-line therapy, whereas others empirically treat locally advanced prostate cancer with radiation or early hormonal therapy. Our study shows that a patient with p53 negativity has an 87% chance of not having a recurrence, even if he has pathological stage C disease. Whereas, if the patient was p53 positive, his chances of progression approximate 50% and, therefore, might warrant more aggressive follow-up or adjuvant therapy.

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