Independent Association of Angiogenesis Index with Outcome in Prostate Cancer

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ABSTRACT

New molecular factors have been characterized that are associated with the prognosis of prostate carcinoma patients, including p53 status and angiogenesis. We reported recently that mutant p53 (mp53) was associated with decreased expression of an endogenous inhibitor of angiogenesis, thrombospondin-1 (TSP-1), and increased microvessel density in melanoma and breast cancer. In this study, we performed a similar analysis on primary prostate carcinoma to determine whether these factors were associated with each other or patient outcomes. Paraffin-embedded specimens of 98 cases of primary prostate carcinoma were obtained and examined to confirm tissue diagnosis and Gleason scores. Carcinoma-specific levels of p53, TSP-1, and tumor angiogenesis were determined using semiquantitative immunohistochemistry (IHC) methods. Acquisition of mp53 was significantly associated with decreased TSP-1 (P = 0.002) and increased angiogenesis (P < 0.0001). An angiogenesis index integrating mp53, TSP-1, and angiogenesis (CD31) scores was found to be an independent predictor of survival in univariate and multivariate analyses that included Gleason score, clinical stage, and patient age. Further validation of the angiogenesis index in prostate carcinoma may provide a new tool to stratify patient risk.

INTRODUCTION

A wide variety of clinical and pathological features are currently used to assess risk and tailor treatment strategies for patients with nonmetastatic prostate cancer, including patient age, comorbid conditions, primary tumor stage, prostate-specific antigen level, and Gleason score (1, 2). Clinical tumor stage and Gleason scores have been important predictive factors for local tumor control, as well as disease-free and overall survival. However, molecular prognostic markers that could further stratify prostate carcinoma patients into high-, intermediate-, or low-risk categories for dissemination and survival are lacking. Integration of molecular profiles with conventional pathological staging techniques may provide prognostic information enabling the clinician to accurately predict a given patient’s risk of dissemination. Such information could refine the range of treatment options most suited to an individual patient’s recurrence risk.

Mutation of the p53 tumor suppressor gene has been implicated in the tumorigenesis of various neoplasms, including prostate cancer (3–6). Clinically relevant functions of wt p53 include cell cycle regulation and modulation of apoptosis. Recent studies suggest that p53 may also be involved in up-regulating the expression of TSP-1, a cell matrix adhesion glycoprotein that acts as an inhibitor of tumor angiogenesis and metastasis. Qian et al. (7) showed an inverse correlation between expression of TSP-1 in the stroma of pancreatic adenocarcinoma and that of mp53. Grossfeld et al. (8) demonstrated that bladder carcinoma outcomes were related to mp53, TSP-1, and angiogenesis, and that the three factors were interrelated. We have found a similar correlation between acquisition of mp53, loss of TSP-1, and increased angiogenesis in melanoma and breast cancer specimens (9, 10). In this study, we evaluated 98 cases of primary prostate carcinoma for expression of mp53, TSP-1, and angiogenesis to determine whether there was a significant association between these markers and stage, Gleason score, or clinical outcome. We report here that an AI integrating mp53, TSP-1, and angiogenesis scores was strongly associated with survival in both univariate and multivariate analysis that included stage, Gleason score, treatment status, and patient age. The AI was also associated with the degree of tumor differentiation as measured by Gleason scores.

MATERIALS AND METHODS

Patient Material. Paraffin blocks of prostatectomy (radical or transurethral) or needle biopsy specimens obtained from the pathology departments of the University of California, San Francisco and Western Medical Center were evaluated. Serial blocks for 98 cases that included a broad clinical spectrum of patients were evaluated (Table 3). The median patient age was 70 years. Survival data from the time of diagnosis were obtained from the tumor registry or patient chart review. Median follow-up was 7.5 years. A pathologist blinded to clinical charac-

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2 The abbreviations used are: wt, wild type; TSP-1, thrombospondin-1; mp53, mutant p53; IHC, immunohistochemistry; AI, angiogenesis index; HScore, histoscore.
teristics and clinical outcome examined all tissue sections to confirm the diagnosis and to determine the Gleason score. The 1992 American Joint Committee on Cancer staging system was applied to these cases for outcomes analysis.

**IHC.** Immunohistochemical detection of p53, TSP-1, and microvessel counts was performed (7). Five-μm tissue sections of fixed, paraffin-embedded specimens were cut, mounted on poly-l-lysine slides (VWR Superfrost Plus), and then deparaffinized in Histoclear. Specimens were rehydrated by sequential washing in ethanol solutions. Antigen retrieval for CD31 used Pronase digestion for 20 min or microwave boiling in citrate buffer for 15 min for p53. No antigen retrieval was necessary for TSP-1 detection. Slides were then incubated in 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity and rinsed in tap water, followed by distilled water. Each slide was subsequently incubated in 100 μl of goat serum (Protein Block; Biogenex, San Ramon, CA) for 10 min at room temperature. Excess blocking buffer was shaken off; the slide was incubated with primary antibody solution for 30 min at room temperature and then rinsed twice in PBS for 5 min. Tissue sections were then incubated in biotinylated goat anti-mouse immunoglobulin for 20 min at room temperature in a humidified chamber. After rinsing for 5 min in PBS, each section was exposed to peroxidase-conjugated streptavidin and incubated for 20 min at ambient room temperature, rinsed in PBS, and exposed to diaminobenzidine for 3 min. Slides were then rinsed in PBS for 5 min, exposed to hematoxylin for 1 min, rinsed for 10 min in tap water, dehydrated in ascending ethanol series, cleared in xylene, coverslipped in Permount, and viewed at ×40. All IHC procedures were optimized in preliminary experiments. All antibody reagents were commercially available; p53 was detected with antibody DO1 (Santa Cruz Biotechnology, Santa Cruz, CA); TSP-1 was detected with clone p12 (Immunotech, Marseille, France), and CD31 was detected with clone JC/70A (Dako, Carpinteria, CA). Paraffin-embedded MCF-7 40F and MCF-7 wt breast cancer cells were used as positive controls for p53 and TSP-1 IHC staining, respectively. Titrations were performed for all antibody reagents to insure minimal background staining and optimal antigen detection. Invasive breast carcinoma specimens that stained negative for p53 or TSP-1 were used as negative controls. Control cell line expression status was confirmed previously by Western blot techniques (9). Positive p53 immunostaining has been reported to be related to p53 mutations that increase p53 protein half-life, leading to intracellular accumulation (5). We therefore refer to immunodetectable p53 as mp53 in this report. However, the sensitivity and specificity of IHC for detection of mutant forms of p53 is ~80%, indicating that not all mutant forms can be detected and that not all cases of detectable overexpression of p53 protein are related to mutations. Entire tissue sections were evaluated for intensity and percentage of tumor cells staining positively. Only the malignant component was scored for each case. The intensity of p53 staining was scored as follows: negative when <5% of tumor cells displayed staining; 1+ when intensity was mild; 2+, moderate; 3+, when intensity was equal to the positive control; and 4+ when intensity was greater than the positive control (9, 11). The 5% cutoff used to delineate a positive staining result was based on the previously published cutoff values by Grant et al. (9) in melanoma, and Poller et al. (11) in mammary ductal carcinoma in situ and on observations that tumors showing only weak focal p53 protein expression had expression of p53 equivalent to that sometimes seen in normal cutaneous basal cells. HScores were assigned on the basis of multiplying the percentage of cells staining positive by the intensity of staining plus 1 [HScore = % positive × (intensity + 1)]. HScores are an objective measurement of tumor heterogeneity reflecting the variability in percentage and intensity of staining within a tumor and provide a single unit of measure for the amount of marker present in the field examined, as originally described in early work to quantitate estrogen receptor content in breast cancer by IHC (12). All procedures were performed by pathologists who had no previous knowledge of the clinical outcomes for this series of cases (A. K., H. K., and R. S.). Statistical analysis was performed independently (C. M., K-T. L., T. K.).

Angiogenesis controls with intermediate vessel counts were run in parallel with each series of slides to insure appropriate CD31 staining. An invasive breast carcinoma specimen with high microvascular staining was used for the positive control, and the non-staining areas in the same tumor tissue were used for the negative control. Light microscopy was used to identify three regions within or immediately adjacent to the tumor that contained the greatest microvessel density (‘hotspots’). Microvessel counts were then performed using a ×200 field within the designated hotspot (9, 13, 14). Of the three areas where the highest number of discreet microvessels were stained, the area of greatest counts was chosen for scoring. Any immunoreactive endothelial cell that was separate from adjacent microvessels was considered a “countable” vessel.

**Image Analysis.** On the basis of the intracellular and extracellular localizations of TSP-1, tumor expression levels of TSP-1 were measured by image analysis as described previously to integrate overall tissue expression levels (9). Briefly, we used a CAS 2000 two-color system (Becton Dickinson, San Jose, CA) that used a light microscope attached to an interactive microcomputer capable of high-speed digital image processing for cell measurements. Image channels were matched to two-component immunohistochemical staining to enhance the image of one stain in each channel. One channel was used to identify all components in the tissue counterstained with methyl green (i.e., all nuclear components), and the other channel was used to calculate the density of brown stain (diaminobenzidine) per tissue area to identify the proportion of cells stained with antibodies to TSP-1. Image analysis-based units of staining are reported as absorbance (A). A negative control accompanying each specimen was used to set the antibody threshold such that nonspecific background staining was eliminated from the study measurement. A minimum of 10 fields with varying intensities were examined for all radical prostatectomy, transurethral prostatectomy, or biopsy specimens.

**Statistical Analysis.** HScore results were correlated with Gleason score, stage, and survival. Disease-specific survival was calculated from the date of tissue diagnosis to the date of death attributable to prostate cancer or the date of the last follow-up, by the Kaplan-Meier method, and survival of the two subgroups was compared by the log-rank test (15). Deaths attributable to unknown events were considered an event for the analysis presented, although a second analysis performed with censoring of unknown deaths showed similar statistical signif-
cancer patients whose specimens had HScores, IHC scores, image analysis scores, and CD31-based microvessel assumed to be continuous variables with stepwise progression. Case (9, 10). Biomarker IHC or image analysis scores were generation of an AI by integrating these three factors for each determined by Spearman correlation coefficient prompted the genesis and the inverse association between mp53 and TSP-1, then categorized into six equal domains, with p53 HScores with survival was determined to be 30 for mp53 HScores and mor mp53 HScores.

Table 1. Favorable AI scores were assigned positive numbers, corresponding to the mp53 HScore, TSP-1 absorbance and vessel counts. For example, a case expressing an mp53 HScore of 90, a TSP-1 absorbance of 10, and a vessel count of 85 would have an overall AI value of \(-7\) (\(-2\) plus \(-3\) plus \(-2\)).

<table>
<thead>
<tr>
<th>Score</th>
<th>mp53 HScore</th>
<th>TSP-1 OD</th>
<th>Vessel counts per (\times 200) Field</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0–29</td>
<td>(\geq 30)</td>
<td>0–29</td>
</tr>
<tr>
<td>0</td>
<td>30–59</td>
<td>25–29</td>
<td>30–69</td>
</tr>
<tr>
<td>(-1)</td>
<td>60–89</td>
<td>20–24</td>
<td>70–84</td>
</tr>
<tr>
<td>(-2)</td>
<td>90–119</td>
<td>15–19</td>
<td>85–99</td>
</tr>
<tr>
<td>(-3)</td>
<td>120–149</td>
<td>10–14</td>
<td>100–122</td>
</tr>
<tr>
<td>(-4)</td>
<td>(\geq 150)</td>
<td>0–9</td>
<td>(\geq 123)</td>
</tr>
</tbody>
</table>

Overall, AI was derived from the sum of scores (+1 to \(-4\)) corresponding to the mp53 HScore, TSP-1 absorbance and vessel counts. For example, a case expressing an mp53 HScore of 90, a TSP-1 absorbance of 10, and a vessel count of 85 would have an overall AI value of \(-7\) (\(-2\) plus \(-3\) plus \(-2\)).

RESULTS
Representative photomicrographs of immunohistochemical staining of p53, TSP-1, and microvessel density (CD31) are shown in Fig. 1. When p53, TSP-1, and microvessel density (CD31) were measured in the same prostate cancer specimens, a highly significant association was found between acquisition of mp53 and increased angiogenesis (\(P < 0.0001\)). There was also an inverse relationship between mp53 and TSP-1 absorbance units (\(P = 0.002\)) and between TSP-1 and angiogenesis (\(P < 0.0001\)), as depicted in Table 2.

These associations were similar to those observed previously in melanoma and breast cancer, and their integration into an AI was performed and applied to the cases of prostate cancer studied here (9, 10). The results of Fisher’s exact test for association between AI and clinical variables are presented in Table 3. No statistically significant difference in age, stage, or radiation and hormone treatment was found when patient characteristics were compared between the two groups stratified by AI \(\leq -6\) (\(n = 18\)) and AI \(\geq -5\) (\(n = 80\)). The statistically significant associations were between AI and Gleason score (\(P = 0.0003\)), TSP-1 (\(P = 0.003\)), CD31 (\(P = 0.014\)), and p53 (\(P = 0.0001\)).

As shown in Table 4, univariate analysis demonstrated that patient age (\(P < .0007\)), stage (3–4 versus 0–2; \(P = 0.0003\)), Gleason score (\(\geq 7\) versus 2–6; \(P = 0.005\)), mp53 (\(\geq 60\) versus \(<60\); \(P = 0.003\)), CD31-based microvessel counts (\(\geq 65\) versus \(<65\); \(P < 0.017\)), and Al (\(\leq -6\) versus \(\geq -5\); \(P = 0.002\)) were significantly associated with patient survival. As expected, hormonal treatment status was significantly associated with poor survival (\(P = 0.0001\)), because this form of treatment is usually given to patients with advanced stage of disease. TSP-1 and prostatic bed radiation treatment status were not significant in univariate analysis. p53 and microvessel counts individually were not statistically significant prognostic factors after adjustment for age, stage, Gleason score, and hormonal treatment in multivariate analysis (data not shown).

Patients with an AI of \(-5\) or greater showed significantly longer disease-specific survival (\(P = 0.002\)) compared with patients with AI of \(-6\) or less in univariate analysis (Fig. 2A). In addition, multivariate analysis showed the AI to be independently associated with survival (\(P < .005\)) after adjustment for age and hormonal treatment status (Fig. 2B). A 3.2-fold increase in mortality was noted for patients with an AI of \(-6\) or less, compared with those with an AI of \(-5\) or greater in multivariate analyses (Table 5). After adjusting for hormonal treatment status, both stage and Gleason score lost significance, because stage and Gleason score dictated treatment, whereas AI remained an independent predictor of disease-specific survival.

We noted a significant association between an increasing Gleason score and an increasing percentage of patients having mutant p53, lower TSP-1, higher CD31, and an AI less than or equal to \(-6\). This was demonstrated by Cochran’s test for linear trend (Table 6). The association between stage, CD-31, and TSP-1 demonstrated significance, whereas associations between stage, mp53, and AI did not reach statistical significance by Cochran’s test for linear trend (Table 7).

DISCUSSION
This study of 98 prostate cancer specimens revealed a correlation between IHC-based detection of p53 protein, decreased levels of the angiogenesis inhibitor TSP-1, and in-
increased angiogenesis. Furthermore, integrating IHC staining results for mp53, TSP-1, and vessel counts into an AI resulted in a scoring system strongly associated with Gleason score and with prostate cancer patient survival. Our current study applied the AI scoring system validated previously using breast cancer patients to prostate cancer patients (9). In this study, we found that patients with AI index scores \( \leq -6 \) compared with patients with AI index scores \( \geq -5 \) demonstrated poor survival in both univariate and multivariate analyses. These data support the concept that integrating multiple markers related to the regulation of angiogenesis may be a more robust predictor of clinical outcome than when single biomarkers are applied to determine prognosis (9, 10, 13).

Angiogenesis has been shown to be associated with poor prognosis in many tumor types, including prostate and breast carcinomas (16–18). Angiogenesis results from disruption of the homeostatic balance between factors that stimulate endothelial cell proliferation and those that inhibit neovascular formation. The relationship between the loss of p53 function, decreased expression of TSP-1, and increased angiogenesis was initially demonstrated by Bouk et al. (19), who studied Li-

Fig. 1 Photomicrographs depicting representative IHC staining patterns for prostate carcinoma. A, mp53 negative; B, mp53 positive; C, high TSP-1; D, low TSP-1; E, low CD31; F, high CD31. ×200.

Table 2 Association between TSP-1, mp53, and vessel counts

<table>
<thead>
<tr>
<th>Correlation coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSP-1 vs. Vessel Counts</td>
<td>0.465</td>
</tr>
<tr>
<td>mp53 vs. Vessel Counts</td>
<td>0.533</td>
</tr>
<tr>
<td>TSP-1 vs. mp53</td>
<td>(-0.302)</td>
</tr>
</tbody>
</table>

* Spearman correlation.
Fraumeni fibroblasts carrying p53 mutations. Bouk’s group was able to demonstrate that mutation in the p53 gene resulted in the translation of a defective p53 protein that was unable to up-regulate TSP-1, resulting in a proangiogenic state. Transfecting wt p53 into defective cells resulted in increased TSP-1 production and restoration of the antiangiogenesis state associated with wt p53. Further, Guo et al. (20) have demonstrated that TSP-1 induces apoptosis of endothelial cells, defining one mechanism of its antiangiogenesis effects. On the basis of these observations, we evaluated whether there was a relationship between p53, TSP-1, and angiogenesis in malignant tissues. We subsequently noted that these factors were interrelated in melanoma and breast carcinoma specimens (9, 10). Others have recently reported similar associations in bladder and colorectal carcinoma (8, 21, 22). In contrast, in a small series, Kawahara et al. (23) were unable to find a relationship between p53 and TSP-1 in cholangiocarcinoma or hepatocellular carcinoma. These results can be explained, in part, by the fact that TSP-1 expression may be influenced by c-jun or other factors independent of p53 (24, 25). Hsu et al. (26) have demonstrated that loss of an allele on chromosome 10 may mediate loss of TSP-1 up-regulation independent of p53 in human glioblastomas. Similarly, Fontanini et al. (27) examined the relationship between mutations in p53 and TSP-1 cDNA copy numbers in 19 cases of non-small cell lung cancer, finding no relationship between p53 and TSP-1. However, they did note an inverse association between TSP-1 cDNA and fibroblast growth factor protein levels (27).

Bleuel et al. (28) have suggested that TSP-1 is a potential tumor suppressor gene based on their observation that TSP-1 gene deletion secondary to loss of chromosome 15 was associated with the development of a highly vascularized tumor phenotype that could be reversed by TSP-1 transfection. These studies indicate that TSP-1 regulation is multifactorial, and that it may differ, in part, depending on the tissue under investigation. Data presented in this paper suggest that TSP-1 is significantly asso-

<table>
<thead>
<tr>
<th>Prognostic features</th>
<th>n</th>
<th>Comparison of survival curves&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (≥69 vs. ≥70)</td>
<td>91</td>
<td>0.0324</td>
</tr>
<tr>
<td>Stage (0–2 vs. 3–4)</td>
<td>95</td>
<td>0.0003</td>
</tr>
<tr>
<td>TSP-1 (≥20 vs. ≥20)</td>
<td>98</td>
<td>0.2243</td>
</tr>
<tr>
<td>Vessel counts (&lt;65 vs. ≥65)</td>
<td>98</td>
<td>0.0169</td>
</tr>
<tr>
<td>mp53 (&lt;60 vs. ≥60)</td>
<td>98</td>
<td>0.0034</td>
</tr>
<tr>
<td>Gleason score (&lt;7 vs. ≥7)</td>
<td>98</td>
<td>0.0053</td>
</tr>
<tr>
<td>AI (≥5 vs. ≤6)</td>
<td>98</td>
<td>0.0022</td>
</tr>
<tr>
<td>Radiation treatment (Yes vs. No)</td>
<td>91</td>
<td>0.7722</td>
</tr>
<tr>
<td>Hormone treatment (Yes vs. No)</td>
<td>92</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

<sup>a</sup> P determined by the log-rank test.
associated with both p53 status and microvessel density in prostate carcinoma.

Many investigators who have evaluated the prognostic significance of biomarkers in prostate cancer patients have suggested that p53 and angiogenesis are relevant to outcomes. Theodorescu et al. (6) found that mp53 and Rb reactivity were independently predictive of disease-specific survival in 71 patients who underwent radical prostatectomy for stage A1 to B2 prostate carcinoma. Moreover, these two factors were stronger predictors of survival than conventional pathological parameters. Similarly, Silberman et al. (29) found a correlation between angiogenesis and progression after radical prostatectomy in patients with Gleason scores of 5 to 7. Bettencourt et al. (30) used a monoclonal antibody directed against CD34 to determine angiogenesis levels to analyze the link between microvessel density and recurrence in 149 patients who had undergone radical prostatectomy. They found that the rate of recurrence-free survival for 5 years was 71% for patients with counts <90 vessels/microfield and 51% for patients with counts >90. Ofersen et al. (31) used factor VIII staining of prostate carcinoma specimens to determine whether maximal microvessel density was associated with outcomes. They found that the maximal microvessel density was significantly associated with survival in both univariate and multivariate analyses. Taken together, these studies suggest that levels of angiogenesis may provide prognostic information about clinical outcomes in prostate cancer.

Our results suggest that the AI, which reflects an angiogenic phenotype constructed from the immunostaining results of three different molecular factors influencing proliferation of new blood vessels, can serve as a more robust predictor of survival than individual prognostic markers. In this study, AI was more significant than stage and Gleason score as a predictor of survival, with a 3.2-fold difference in survival times between patients with AI of ≥5 and patients with AI <5. Our study focused on a heterogeneous population of patients comprised of those with prostate-confined cancer undergoing prostatectomy as well as patients with metastatic disease. If the relevance of the AI is confirmed in a more homogeneous group of patients, it may be feasible to stratify patients to expectant versus active treatment at the time of diagnostic biopsy, based on their prognostic marker profile. Although our series of 98 blocks included only seven needle biopsy specimens, all seven were adequate for evaluation of the three biomarkers that comprised the AI. The simultaneous use of three

![Fig. 2 A](image1.png) Kaplan-Meier plot of disease-specific survival comparing AI ≥ −5 versus AI ≤ −6. No adjustment of covariates was made (n = 91). B, comparison of disease-specific survival probability for cases with AI ≥ −5 versus AI ≤ −6 adjusted for covariates using the Fleming-Herrington estimate of survival adjusted for age and hormonal treatment status (n = 90).

<table>
<thead>
<tr>
<th>Prognostic features</th>
<th>$\chi^2$</th>
<th>P</th>
<th>Risk ratio</th>
<th>95% CI</th>
</tr>
</thead>
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<tr>
<td>Hormone treatment (Yes vs. No)</td>
<td>0.0001</td>
<td>8.058</td>
<td>3.585–18.116</td>
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</tr>
<tr>
<td>Al (≥−6 vs. ≤−5)</td>
<td>0.005</td>
<td>3.237</td>
<td>1.435–7.302</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.016</td>
<td>1.052</td>
<td>1.010–1.097</td>
<td></td>
</tr>
</tbody>
</table>

* $n = 90$.

* CI, confidence interval.

<table>
<thead>
<tr>
<th>Marker status</th>
<th>2−3 (n = 10) (10%)</th>
<th>4−6 (n = 50) (51%)</th>
<th>≥7 (n = 38) (39%)</th>
<th>$P^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSP-1</td>
<td>≤20</td>
<td>3</td>
<td>32</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>≥20</td>
<td>7</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Vessel counts</td>
<td>&lt;65</td>
<td>10</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>≥65</td>
<td>0</td>
<td>20</td>
<td>32</td>
</tr>
<tr>
<td>mp53 HScore</td>
<td>&lt;60</td>
<td>10</td>
<td>47</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>≥60</td>
<td>0</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>AI</td>
<td>≤−6</td>
<td>0</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>≥−5</td>
<td>10</td>
<td>46</td>
<td>24</td>
</tr>
</tbody>
</table>

* Cochran-Armitage test for trend.

![Table 6](image2.png) Marker profile of prostate cancer progression (Gleason scores 2–3 vs. 4–6 vs. ≥7)
biologically relevant factors integrated into the AI may partly address the issue of intratumoral heterogeneity of marker expression and should improve the predictive accuracy of prognostic testing. Because our study included only seven needle biopsy specimens, a larger study conducted to correlate clinical outcome with AI determined on pretherapy biopsy material would be an essential adjunct in assessing the role of AI in prostate cancer management. Furthermore, it will be important in future studies to evaluate the concordance of AI scoring in small pretherapy needle biopsy material with markers studied on subsequent prostatectomy specimens to quantify tumor heterogeneity.

Our retrospective study results presented here have defined a study set of HScores for mp53, TSP-1, and angiogenesis that comprise the AI. The simultaneous evaluation of molecular events that are associated with the angiogenic pathway may not only assist in defining the need for aggressive treatment versus observation but may eventually guide treatment with antiangiogenesis compounds. We plan to carry out prospective studies to confirm the value of the AI in prostate cancer management.

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REFERENCES


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