Reactive Stroma as a Predictor of Biochemical-Free Recurrence in Prostate Cancer

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ABSTRACT

Extensive scientific literature data point to reciprocal interactions between prostate stromal cells and prostate cancer cells that likely regulate tumor progression. To investigate whether these intratumoral-reactive stromal cells in human prostate cancer are predictive of survival, tumor stroma volume and specific stroma markers were quantitated by using tissue microarrays (index tumors of 847 patients), and the results were analyzed relative to the recurrence-free survival data set for these patients. Tumor tissue was evaluated with Masson's trichrome stains and by immunohistochemistry with antibody probes to smooth muscle α-actin, desmin, vimentin, pro-collagen type I, and calponin. The relative volume of intratumor stroma (5% stroma, grade 0; 5–15%, grade 1; 15–50%, grade 2; >50%, grade 3) and the expression index of stromal marker (staining intensity grade × percentage of positive cells per field) were quantitated and analyzed. Interpretable data were obtained from 545 patients. Statistical analysis of the survival data set showed that the volume of reactive stroma in the tumor was a significant predictor of disease-free survival. Stroma volume was most optimal as an independent predictor in tumors containing stroma, defined as Gleason 7 and lower grades. Of interest, tumors with either little to no stroma or tumors with abundant stroma each showed reduced recurrence-free survival. For specific stromal markers, reduced desmin and smooth muscle α-actin were hallmarks of cancer-associated reactive stroma relative to normal fibromuscular stroma. Quantitative analysis of desmin and smooth muscle α-actin expression showed both to be significant and independent predictors of recurrence-free survival. This is the first study to demonstrate that nonepithelial-reactive stroma elements in prostate cancer tumors can be used as prognostic indicators. These data also add to the concept that tumors are not purely epithelial and the tumor-reactive stroma must be considered an important biological component of the cancer.

INTRODUCTION

Prostate cancer is the most common male cancer in the United States (1). There are currently few suitable prostate cancer biomarkers that distinguish between tumors with a high potential for recurrence and tumors that will not recur. Survival and predictive markers in prostate cancer are of high importance, not only because of the mortality associated with prostate cancer, but also because of the morbidity associated with current forms of therapy. Accordingly, numerous ongoing trials are trying to define markers to discriminate between patients who require immediate therapeutic intervention and patients who are candidates for watchful waiting observation.

Attempts at finding combinations of clinical and pathological parameters as well as serum markers have yielded positive results (2, 3). These are limited in their scope, however, and heavily dependent on markers of epithelial differentiation of cancer (Gleason score). The difficulty in predicting behavior is particularly true for patients with Gleason score 7. This is a significant problem because a large proportion of patients with prostate cancer fall within this category.

To address novel predictive markers for prostate cancer recurrence, we have focused on the tumor-associated stroma within the cancer. The distinguishing element in the prostate is that the smooth muscle phenotype is already present in the normal tissue. A simple trichrome stain makes the process of distinguishing normal stroma and reactive stroma more obvious. The large muscle fibers of the prostatic stroma stain red and show orientation of fibers. In contrast, the reactive stroma in prostate cancer evolves an architecture in which the fibers become disorganized, are much smaller, and present as a mix of red and blue, with predominance of the latter.

It is well established that reciprocal interactions between prostate stromal cells and prostate epithelial cells are central to mechanisms of prostate gland development and differentiation (4, 5). Less clear is the potential contribution of stromal biology to prostate cancer progression. Because of the apparent lack of a typical desmoplastic stromal response as assessed with morphological examination of H&E-stained sections, there is very little information regarding characterization of stromal response in human prostate cancer. To address this, our recent studies have shown that a clear and defined stromal response does occur during prostate cancer progression (6). These studies showed

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that the stroma in the prostate responds to cancer development with a generic type of a wound repair-type process (6, 7). The observed stromal phenotype evolves from a normal tissue stroma (primarily smooth muscle) to a myofibroblastic phenotype. This process is essentially similar in most mammalian tissues (8, 9). The wound repair response is typified by a stromal phenotype that is more plastic, responsive to the microenvironment, and capable of migration, proliferation, and production of matrix and growth factors to effect a repair process.

This reactive stroma is characterized by fundamental alterations in stromal cell phenotypes and expression of extracellular matrix. Prostate cancer-reactive stroma is composed of a myofibroblast/fibroblast mix with a significant decrease or complete loss of fully differentiated smooth muscle, whereas normal prostate stroma is predominantly smooth muscle. This is accompanied by an elevation in expression of collagen type I, tenascin, and fibroblast activation protein (6). These investigations suggest that a fundamental alteration in stromal cell biology is associated with prostate cancer progression and that this reactive stroma is likely to regulate the rate of tumor progression. It stands to reason that markers of stromal biology may be suitable diagnostic and prognostic markers in the assessment of clinical prostate cancer. The present report extends these studies and shows statistically that these markers are useful in prostate cancer prognostics.

To investigate whether reactive stroma markers in human prostate cancer are predictive of survival, we have quantitated alterations in tumor stromal components by using tissue microarrays and have analyzed the results relative to a recurrence-free survival data set. We also tested the predictive ability of specific stromal markers involved in the myofibroblast transformation of cells in reactive stroma of prostate cancer. We report here that both the volume of reactive stroma in prostate cancer and altered expression of desmin and smooth muscle α-actin are each independent and significant predictors of prostate cancer recurrence.

MATERIALS AND METHODS

Cohort Enrollment and Follow-up. As of March 2002, there was information on 6201 patients with benign prostatic hyperplasia or cancer in the Baylor Medical Informatics Core Specialized Programs of Research Excellence Database. More than 3900 of these patients underwent radical prostatectomies at one of the Baylor College of Medicine affiliated institutions and willingly provided tissues (IRB[2] H-1158). Of these patients, 1291 were operated by a single surgeon (P. T. S.) between 1983 and 1998 without any previous form of adjuvant therapy, such as radiation or hormonal therapy. The Baylor IRB (IRB H-11436) approved this study.

Entry criteria for this retrospective cohort study to create a radical prostatectomy tissue array included: (*a*) no preoperative treatment; (*b*) operated by a single surgeon (P. T. S.) between 1983 and 1998; (*c*) radical prostatectomy specimen in the tissue bank; and (*d*) prostate cancer present in the surgical specimen and large enough to be cored (2-mm cores) for microarrays. A total of 847 patients fulfilled the above-mentioned criteria and were cored to produce a large outcomes tissue array.

Radical prostatectomy specimens from these patients were processed using whole mount slides according to procedures published previously (10). A single pathologist (T. M. W.) performed the pathological analysis that included staging, pathological stage, margins, capsular penetration, seminal vesicle invasion, biopsy and prostatectomy primary and secondary Gleason grades, lymph node status, tumor volume, and geographic location. The clinical and pathological data of patients who met the entry criteria were available for analysis in the Baylor Prostate Specialized Programs of Research Excellence data bank. The clinical follow-up data include PSA recurrence (defined as PSA >0.4 ng or two consecutive rises), clinical metastasis, and death.

Tissue Array Construction. Slides from all 847 radical prostatectomy specimens were reviewed and mapped. The index tumor, defined as the largest and/or highest Gleason (most likely to be clinically significant) tumor was identified on the slide, and areas representative of the highest and most common Gleason rate were circled. The circled areas of tumor were then transferred onto the blocks, and 2-mm cores were punched and transferred to a recipient block. The tissue microarrays were built using a manual tissue arrayer (Beecher Instruments, Silver Spring, MD). Internal controls were placed at a preestablished pattern throughout each one of the blocks to assess adequacy of the stain throughout the sections. A database was built for every block produced, including the coordinates of each core and the area and case of origin. The final tissue array set consisted of 18 blocks with a combined total of approximately 950 cores, including the controls.

A smaller test array was built using a set of 100 patients. Entry criteria for this retrospective cohort study to create a radical prostatectomy tissue array included: (*a*) no preoperative treatment; (*b*) operated by a surgeon other than P. T. S.; (*c*) radical prostatectomy specimen in the tissue bank; (*d*) prostate cancer present in the surgical specimen and large enough to be cored (2-mm cores) for microarrays; (*e*) follow-up of at least 5 years; and (*f*) 50 patients with and 50 patients without biochemical recurrence, chosen randomly.

Histochemical and Immunohistochemical Stains. Slides from the large array set were later stained using Masson's trichrome stain. Each slide was digitized using an automated imaging system that produced an image of every dot and also informed the dot coordinates on the slide. This permitted tracking down each dot to origin and subsequent correlation with the clinical outcome database.

Reactive stroma was analyzed for expression of vimentin, smooth muscle α-actin, calponin, and desmin and pro-collagen type I using immunohistochemical procedures for these markers that we have published previously (6). Calponin, as well as desmin, are markers of late-stage smooth muscle differentiation. Desmin is muscle specific. Smooth muscle α-actin is a smooth muscle and myofibroblast marker, whereas vimentin is a marker in fibroblasts and myofibroblasts. Coexpression of vimentin and α-actin in a calponin-negative background is indicative of myofibroblasts. Pro-collagen I is expressed in wound repair response stroma and prostate cancer-reactive stroma myofibroblasts/

[2] The abbreviations used are: IRB, Institutional Review Board; PSA, prostatic-specific antigen; HR, hazard ratio.

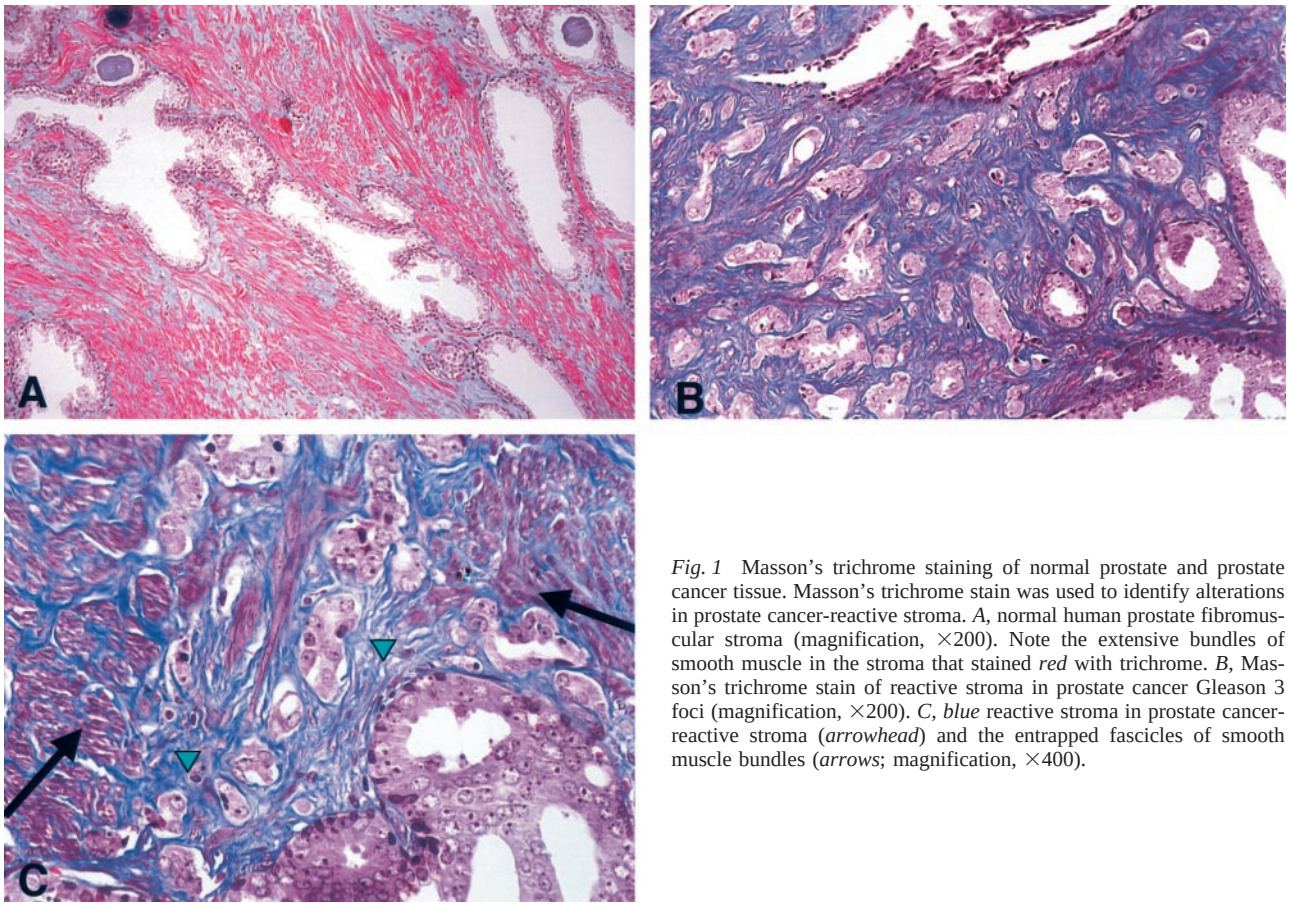


Fig. 1 Masson's trichrome staining of normal prostate and prostate cancer tissue. Masson's trichrome stain was used to identify alterations in prostate cancer-reactive stroma. *A*, normal human prostate fibromuscular stroma (magnification, ×200). Note the extensive bundles of smooth muscle in the stroma that stained *red* with trichrome. *B*, Masson's trichrome stain of reactive stroma in prostate cancer Gleason 3 foci (magnification, ×200). *C*, *blue* reactive stroma in prostate cancer-reactive stroma (*arrowhead*) and the entrapped fascicles of smooth muscle bundles (*arrows*; magnification, ×400).

fibroblasts (6, 9). To test whether altered expression of these stromal markers was predictive of recurrence in human prostate cancer, we used the smaller tissue array.

Assessment and Interpretation. The digitized dot image was interpreted for the amount of tumor stroma using a predetermined scale from 0 to 4. The tumor-specific stroma was defined as stroma found in the invasive component of the cancer and that was not part of the normal preexisting host stroma. As we have reported previously, host stroma stains red with Masson's trichrome stain because of a high composition of smooth muscle and is found in large directional bands (histological architecture; Ref. 6). In contrast, reactive stroma in cancer foci is most frequently blue with Masson's trichrome stain (although small areas or bands of stromal staining red can be seen in some fields) and has no histological structure (Fig. 1, *A* and *B*; Ref. 6).

This grading system was analyzed independent of the epithelial grade of differentiation of the tumor (Gleason or others). Tumors with little or <5% of tumor stroma area relative to total tumor area were given stromal grade 0. Tumors with stroma ranging from 5% to 15% of the tumor were given stromal grade 1. Tumors with stroma ranging from 15% to 50% were given stromal grade 2, whereas those with >50% of the tumor being stroma were given stromal grade 3. The last grade category has at least a 1:1 ratio between stroma and epithelium (Fig. 2). The information collected per dot was transferred to the database and correlated with all clinical and pathological data.

Because of the inherent difficulties in reproducibility of all types of visual semiquantitation, we selected criteria that would be easy to reproduce by others. At the two ends of the spectrum are patients with virtually no stroma (grade 0) or patients with large amounts of stroma in their tumors (grade 3). In the former category, we included patients with insignificant amount of stroma (up to 5%). The pathologist is, therefore, not required to make certain that the tumor is absolutely devoid of stroma. At the other end of the spectrum, we have tumors with large amounts of stroma. To make this reproducible, we selected an internal control. The pathologist would have to make a single discrimination: Is the amount of reactive stroma in the tumor equal to or greater than the malignant epithelium (stromal: epithelial ratio greater than 1)? If so, the tumor would be classified as having stromal grade 3.

Expression of stromal marker proteins were analyzed for immunoreactive staining using a 0–3+ scoring system for staining intensity and by quantitating the percentage of positive cells (labeling frequency) for the specific marker per field. For determination of staining intensity, the grading scale ranged from no detectable signal (score of 0) to strong signal seen at low power (score of 3). A score of 2 corresponded to moderate signal observable at low to intermediate power. A score of 1 corresponded to a weak signal seen only at intermediate to high power. The labeling frequency was scored as 0 (0%), 1 (1–33%), 2 (34–66%), or 3 (67–100%). The overall expression

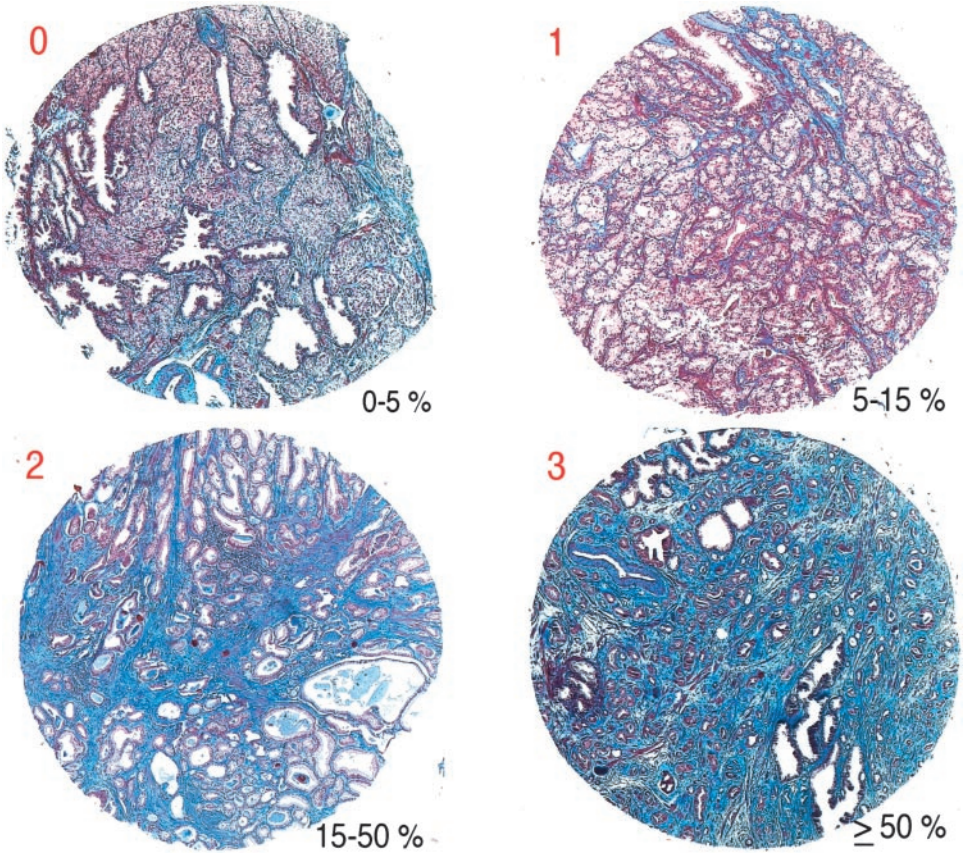


Fig. 2 Use of a tissue array to quantitate reactive stroma in prostate cancer. Shown are representative tissue array cores illustrating the stromal scoring index. The stromal score (shown in *red*) is indicative of the relative percentage area of the tumor composed of reactive stroma, as determined by Masson's trichrome staining. Stromal score 0, 0–5% reactive stroma; score 1, 5–15% reactive stroma; score 2, 15–50% reactive stroma; stromal score 3, >50% reactive stroma.

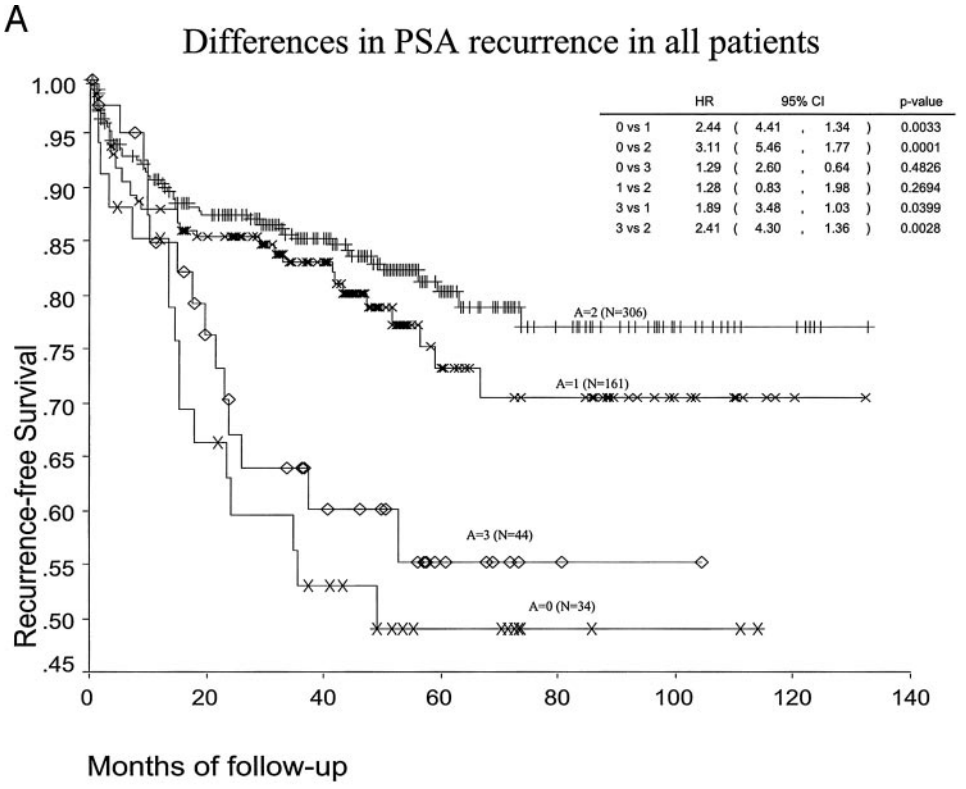
index was then obtained by multiplying the scores of staining intensity and labeling frequency.

Statistical Analysis. The correlation of stromal grading with the patients' clinical and pathological variables was analyzed by the Spearman or Pearson correlation test. The predictive value of stromal quantification for recurrence-free survival was determined using the Kaplan-Meier actuarial analysis and the log rank test. In addition, the Cox proportional hazards regression model was used to analyze the value of using stromal quantification and other pathological and clinical markers to predict the risk of recurrence. The risk ratio and its 95% confidence interval were recorded for each marker. *P*s of <0.05 were considered statistically significant in all of our analyses. All analyses were performed with statistical software (Statview, version 5.0; SAS Institute Inc., Cary, NC).

RESULTS

Reactive Stroma Grade and Recurrence-free Survival. A total of 545 patients had interpretable data for this study. Patients (dots) were excluded because of irregularity in the sections that did not permit interpretation, absent dots in the sections, or insufficient clinical data. Of the 545 patients, 34 were found to have stromal grade 0, 161 had stromal grade 1, 306 had stromal grade 2, and 44 had stromal grade 3. These patients were followed after radical prostatectomy for a time ranging from 0.3 to 167 months (average, 46). One hundred fifteen patients had biochemical recurrence during follow-up, 39 had positive lymph nodes, 240 had some degree of extracapsular extension, 77 had seminal vesicle invasion, and 92 had positive margins. A higher percentage of patients with stromal grades 0 and 3 had a positive digital rectal examination (68.9%) in contrast to those with stromal grades 1 and 2 (60%). This trend did not reach statistical significance ($P = 0.069$).

Survival Analysis. Additional analysis was performed to determine the value of using stromal scoring as a predictive marker for the patients' recurrence-free survival. The actual probability of time remaining free of progression for these patients after surgery was calculated by using the Kaplan-Meier method. The study showed that patients stratified in two major groups. Patients with stromal grade 1 or grade 2 had recurrence-free survival between 70% and 80%, whereas those having stromal grade 3 or grade 0 had a recurrence-free survival between 50% and 60% (Fig. 3*A*). Furthermore, the differences between grades 1 and 2 ($P = 0.2694$) and grades 0 and 3 ($P = 0.4826$) were not statistically significant, whereas those between all other groups were significant. It is evident from this graph and statistical analysis that patients with stromal grades 0 and 3 have similar survivals and that these are statistically different from those with grades 1 and 2. Accordingly, patients were, therefore, clustered into two groups: those with stromal grades 0 and 3 and those with stromal grades 1 and 2. As shown in Fig. 3*B*, the former set (grades 0 and 3) had a mean survival time of



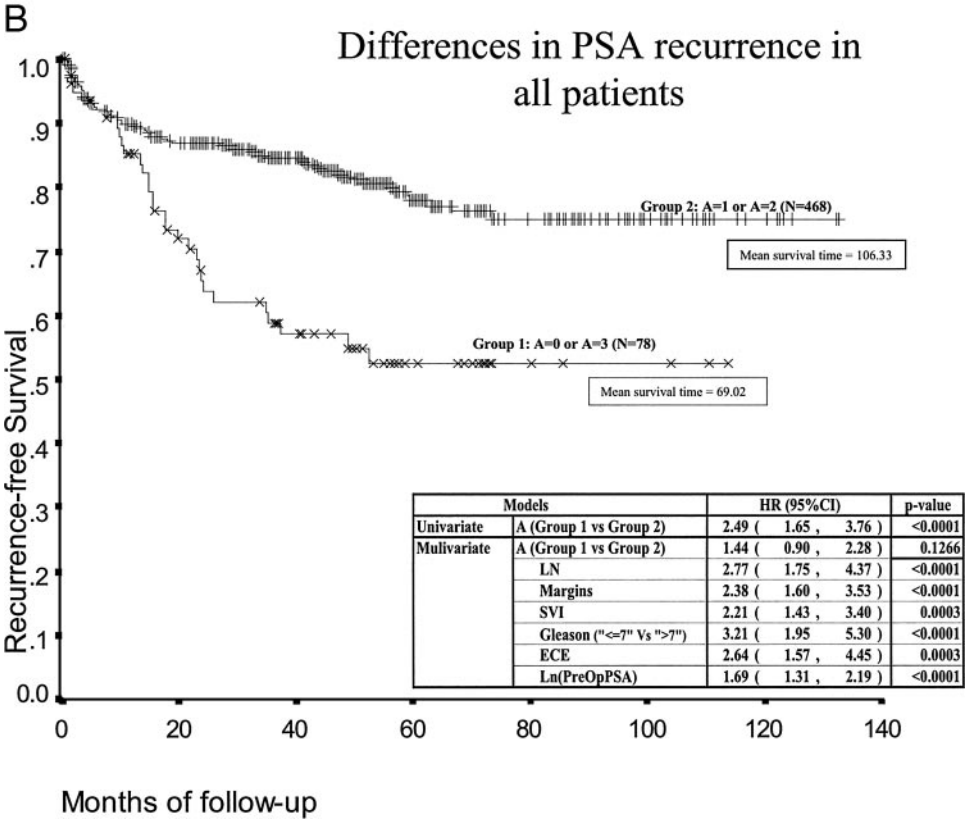


Fig. 3 *A*, differences in PSA recurrence for all patients. Shown is the actual probability of time remaining free of progression as determined by PSA recurrence for all patients in the study, regardless of Gleason score after surgery as calculated by the Kaplan-Meier method. Note the clustering of groups 1 and 2 *versus* groups 0 and 3. The *inset* shows the *P*s for differences between the different groups, demonstrating that 1 *versus* 2 as well as 0 *versus* 3 are not statistically different. *B*, differences in PSA recurrence for all patients. Shown is the actual probability of time remaining free of progression as determined by PSA recurrence for all patients in the study, regardless of Gleason score after surgery as calculated by the Kaplan-Meier method. The *inset* shows the relative HR average and range for all parameters as described in "Materials and Methods" and "Results."

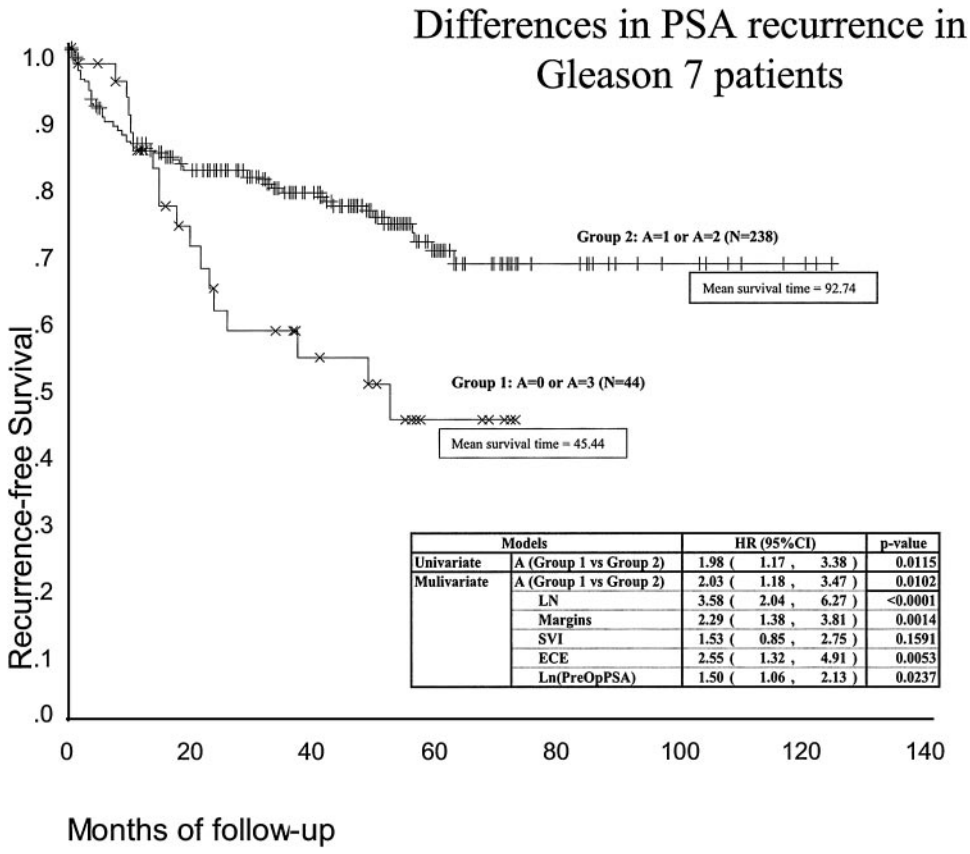


Fig. 4 Differences in PSA recurrence for Gleason 7 patients. Data were analyzed for patients with Gleason 7 score and analyzed by the same method described in Fig. 3.

69.02 months compared with 106.33 months in the latter set (grades 1 and 2). The difference was significant on univariate analysis ($P = 0.0001$) but not in multivariate analysis ($P = 0.12$; Fig. 3), with HRs of 2.49 and 1.44, respectively.

We further analyzed Gleason 7 patients exclusively as shown in Fig. 4. This group was limited to 282 patients. Of these, 14 had stromal grade 0, 75 had stromal grade 1, 163 had stromal grade 2, and 30 had stromal grade 3. Patients were stratified into two groups as defined previously. Patients with stromal grade 0 or grade 3 had a mean survival time of 45.44 months compared with 92.74 months in those with stromal grade 1 or grade 2 ($P = 0.0115$, univariate analysis; $P = 0.0102$, multivariate analysis). Differences were also significant when grouping patients with Gleason scores 6 and 7 (data not shown).

Postoperatively, multivariate Cox models suggest that stromal grading index [HR, 2.0 (1.3–3.1); $P = 0.0012$] is as good a postoperative marker as PSA [HR, 2.1 (1.4–3.2); $P = 0.0005$] and biopsy Gleason score [HR, 1.8 (1.2–2.7); $P = 0.0024$], when information on stage, lymph node metastasis, extracapsular extension, seminal vesicle invention, and margins is already known. However, when examining patients with Gleason score 7 exclusively, multivariate Cox models suggest that stroma A index [HR, 2.1 (1.2–3.6); $P = 0.0092$] is a better postoperative marker than PSA [HR, 1.8 (1.1–3.0); $P = 0.0295$], when information on stage, lymph node metastasis, extracapsular extension, seminal vesicle invention, and margins is already known.

In addition, we have identified that stromal grading could potentially be used in the pretherapy setting (time of diagnosis). The amount of stroma (grouped as "0 or 3" and "1 and 2") was compared with preoperative markers currently used in practice, preoperative PSA and biopsy Gleason score, in the ability to distinguish high-risk patients in the preoperative setting. First, patients were grouped into high PSA(>10) and low PSA (<10) categories and into high biopsy Gleason grade (>6) and low biopsy Gleason grade (<6) categories. Univariate analysis showed that, individually, PSA grouping is the best marker [HR, 4.3 (2.9–6.3)], followed by biopsy Gleason grade [HR, 3.2 (2.2–4.7)] and then by stromal grading [HR, 2.5 (1.6–3.8)], in the ability to distinguish between high-risk and low-risk patients for recurrence. However, these three preoperative markers carry different information and should be used together. Of two patients with identical PSA levels and biopsy Gleason grades, a patient with a stromal score of 0 or 3 has about 2.3 times the risk of having earlier recurrence than one with a stromal score of 1 or 2 [HR, 2.3 (1.5–3.4); $P = 0.0001$]. This demonstrates the potential of stromal grading as a preoperative marker that could be used in biopsies.

Correlations. We did not identify any significant direct correlation between stromal grading and clinical pathological parameters when analyzing stromal scoring without grouping. However, after analyzing the groups identified during the survival analysis (0 and 3 compared with 1 and 2), significant correlations were found. A weak but significant inverse corre-

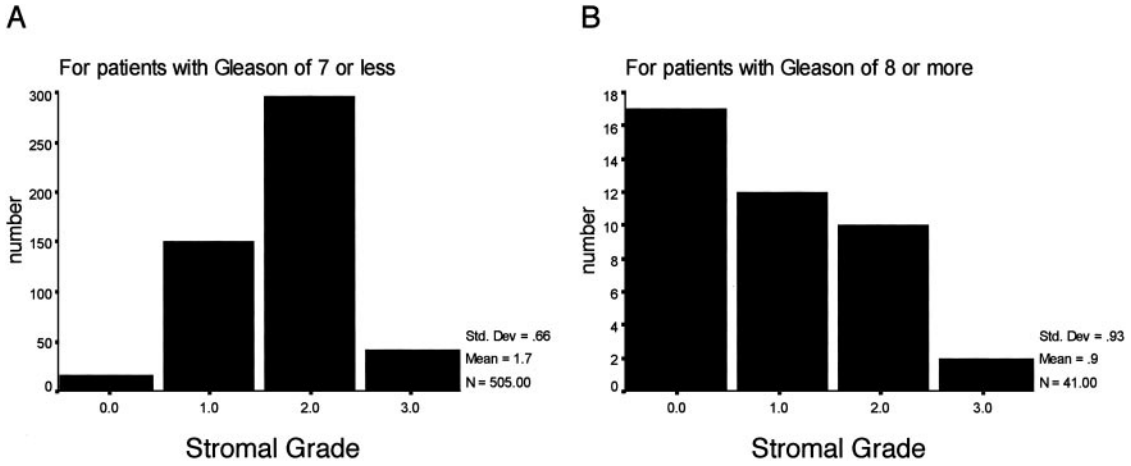


Fig. 5 Distribution of stromal scores within Gleason categories. Distribution A is for patients with Gleason score 7 or less. Distribution B is for patients with Gleason score of 8 or greater.

lation was found between the epithelial grading of tumors (Gleason grade) and the stromal grading groups (correlation coefficient, −0.227; $P = 0.000$). We believe that this correlation is influenced largely by tumors without stroma (grade 0). Within tumors containing some stroma (stromal categories 1, 2, and 3), Gleason grade did not correlate significantly. Of note, most patients with stromal grade 0 had a higher Gleason grade, whereas most patients with stromal grade 3 were Gleason 7, as shown in Fig. 5. Most patients with Gleason score 7 or less had stromal grade 2, whereas those with Gleason score 8 or greater had stromal grade 0 (Fig. 5).

A weak, but significant, negative correlation was also found between stromal grading groups and lymph node status (correlation coefficient, −0.090; $P = 0.036$), staging (correlation coefficient, −0.085; $P = 0.047$), and PSA (correlation coefficient, −0.094; $P = 0.028$). Patients with stromal grade 0 had a mean PSA value of 16.3, and those with stromal grades 1, 2, and 3 had mean values of 11.4, 9.9, and 12.3 respectively. Note the sequential increase in the latter group, with the highest value lower than the former group. No correlation was found between stromal grading and extracapsular extension, seminal vesicle invasion, or surgical margins.

Stromal Immunohistochemical Markers and Recurrence-free Survival. Smooth muscle α-actin, followed by desmin, were the most intensely expressed markers in this group of patients, whereas calponin and pro-collagen I were the least expressed. The Kaplan-Meier method was used to determine the value of using quantitation of immunohistochemical stains as a predictive marker for the patients' recurrence-free survival. Vimentin ($P = 0.4257$), calponin ($P = 0.2914$), and pro-collagen I ($P = 0.2194$) were not significant predictors of recurrence in this data set. Smooth muscle α-actin and desmin seem to be the best predictors of biochemical recurrence. Both the decrease of smooth muscle α-actin and decrease in desmin expression were associated with an increased risk for biochemical recurrence.

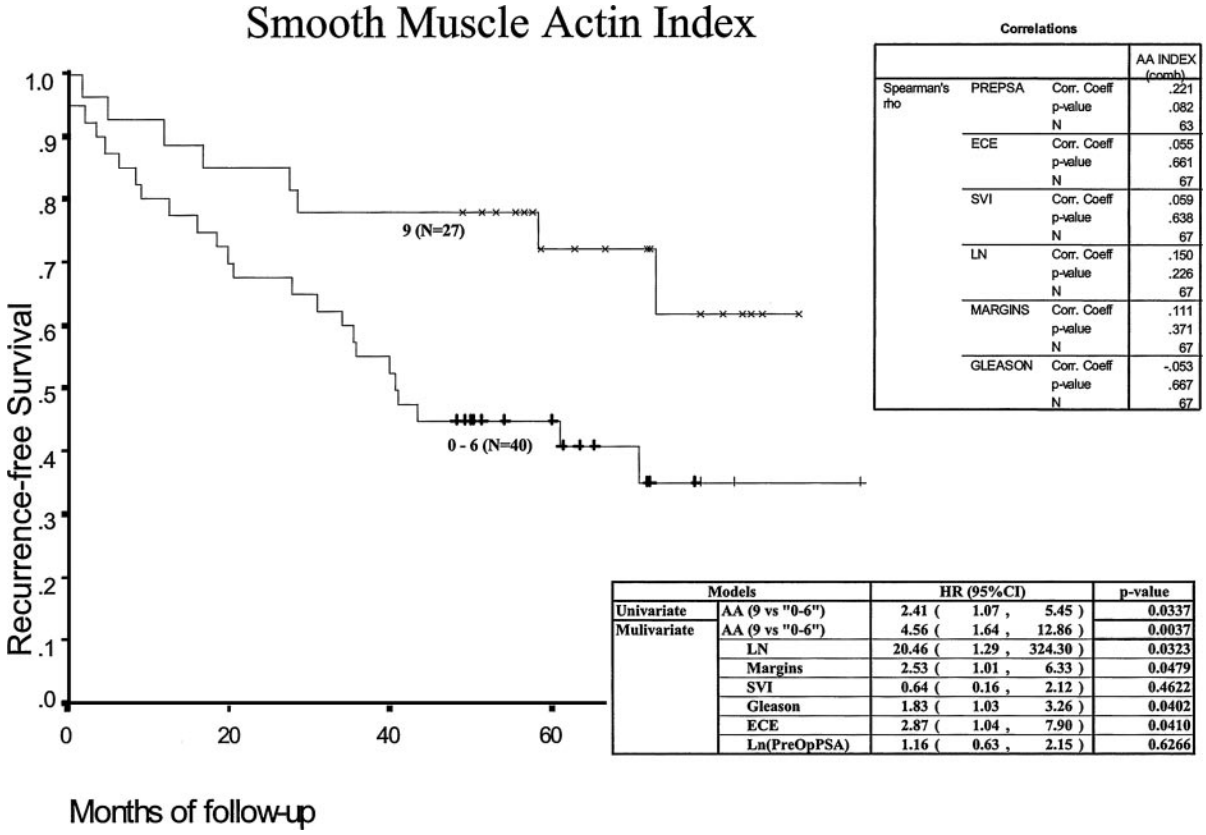
Smooth Muscle α-Actin. As shown in Fig. 6, patients with a smooth muscle α-actin labeling index score of 9 had a mean survival time of 70.22 months, whereas those with indices 0 and 6 had a mean survival time of 52.3 months each. The differences were significant on univariate ($P = 0.03$) and multivariate analysis ($P = 0.003$), with HRs of 2.4 and 4.5 (Fig. 6).

Desmin. As shown in Fig. 7, patients with a desmin labeling index score of 6–9 had a mean survival time of 72.81 months; those with indices 1–4 had a mean survival time of 57.25 months, whereas those with no expression (0) had a mean survival time of 16.68 months. These differences were also significant on univariate and multivariate analysis (Fig. 7). This stromal marker seems to be the best predictor of biochemical recurrence among the set of markers tested. It is of note that the HRs are very high [no expression (0) compared with score of 6–9 = 10.10 HR by multivariate analysis] and that, in this model, all other clinicopathological parameters lose multivariate significance.

These results are consistent with our previous published reports showing the emergence of the myofibroblast/fibroblasts-mixed phenotype in prostate cancer-reactive stroma (6, 9). Myofibroblasts exhibit a somewhat decreased expression of smooth muscle α-actin and a near total loss of late-stage smooth muscle markers (calponin and desmin) relative to differentiated smooth muscle. Fibroblasts are negative for smooth muscle α-actin, however, both myofibroblasts and fibroblasts are positive for vimentin. Prostate smooth muscle are vimentin negative (6).

DISCUSSION

This study presents a novel concept in the field of prostate cancer. Our results indicate that reactive stroma is an informative marker of prostate cancer progression. It also represents a new search for novel prognostic indicators that do not rely exclusively on the carcinoma cell for assessing likelihood of prostate cancer progression. This study suggests that specific stromal markers and quantification of reactive stromal grade



Correlations

			AA INDEX (comb)
Spearman's rho	PREPSA	Corr. Coeff	.221
		p-value	.082
		N	63
	ECE	Corr. Coeff	.055
		p-value	.661
		N	67
	SVI	Corr. Coeff	.059
		p-value	.638
		N	67
	LN	Corr. Coeff	.150
		p-value	.226
		N	67
	MARGINS	Corr. Coeff	.111
		p-value	.371
		N	67
	GLEASON	Corr. Coeff	-.053
		p-value	.667
		N	67

Models		HR (95%CI)	p-value
Univariate	AA (9 vs "0-6")	2.41 (1.07 , 5.45)	0.0337
Mulivariate	AA (9 vs "0-6")	4.56 (1.64 , 12.86)	0.0037
	LN	20.46 (1.29 , 324.30)	0.0323
	Margins	2.53 (1.01 , 6.33)	0.0479
	SVI	0.64 (0.16 , 2.12)	0.4622
	Gleason	1.83 (1.03 3.26)	0.0402
	ECE	2.87 (1.04 , 7.90)	0.0410
	Ln(PreOpPSA)	1.16 (0.63 , 2.15)	0.6266

Fig. 6 Smooth muscle α-actin staining index. Staining intensity and percentage of field positive was quantitated as described in "Materials and Methods." Results were analyzed using the Kaplan-Meier method. The *inset* shows the relative HR averages and range.

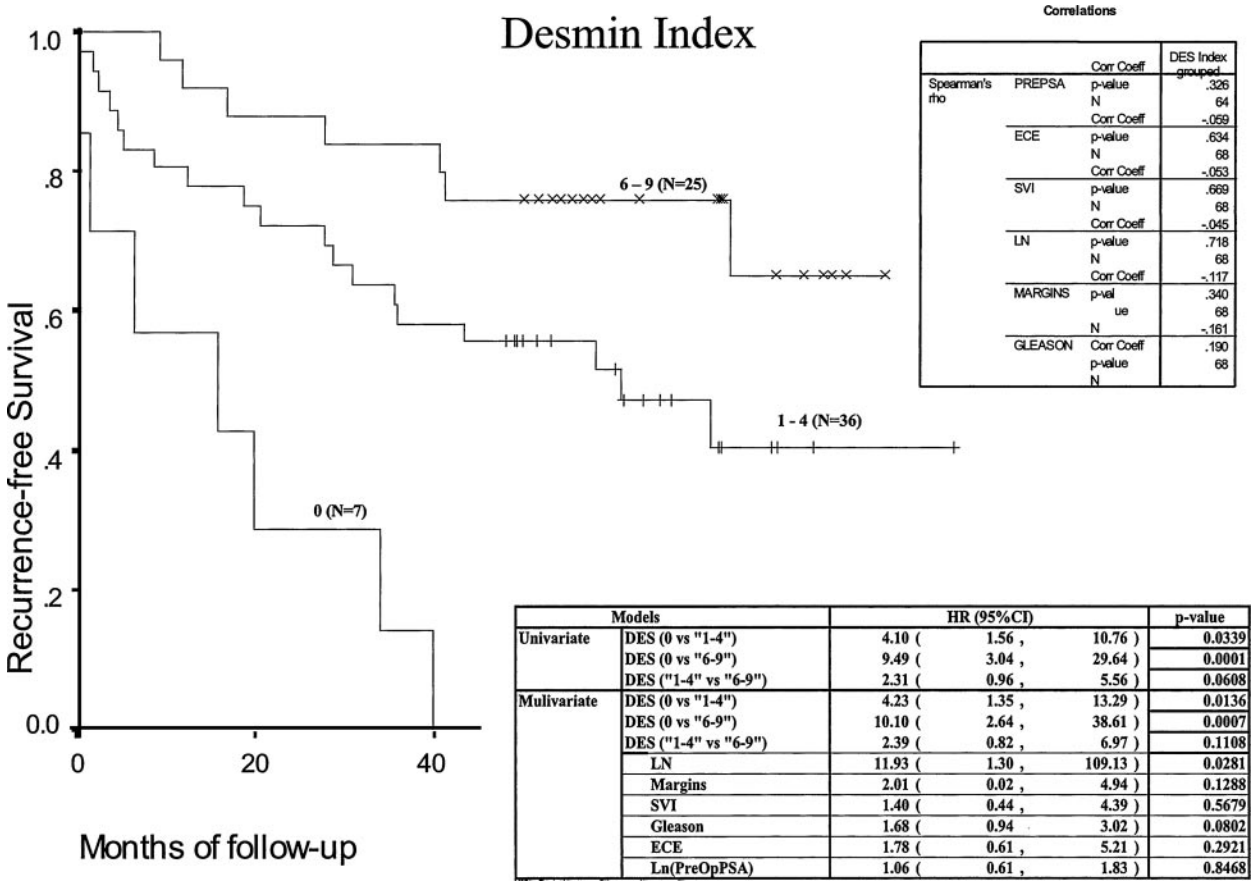
might be of prognostic value in evaluating patients with prostate cancer, particularly in patients with Gleason score 7.

The biological implications of this finding are clear. Previous studies have shown that a reactive stroma response, also termed "a desmoplastic response," is the histological mark of several invasive carcinomas. It is one of the most important criteria to determine invasion of early tumors in many organs, including the cervix, breast, and colon. However, the limitations of light microscopy coupled with the simple examination of H&E staining has precluded a clear understanding of the importance of a stromal desmoplastic response in human prostate cancer. The lack of an easily visible reactive stroma desmoplasia in routine pathology sections is also one of the elements that makes the diagnosis of prostate cancer relatively more difficult compared with other carcinomas, in which a desmoplastic response is clear.

To understand the stromal desmoplastic response in prostate cancer, the cell of origin must be considered. Although the supporting stroma in most tissues is fibroblastic, human prostate stoma is predominantly composed of smooth muscle. Desmoplastic response is defined histologically by larger, plumper stromal cells with increased extracellular fibers and immunohistochemically by transformation of fibroblastic-type cells to a myofibroblastic phenotype. Because prostatic stroma is muscular, it is difficult to detect the myofibroblastic phenotypic change on routine examination of pathology slides. Our previous report has shown that use of Masson's trichrome together with immunohistochemical staining of vimentin, smooth muscle α-actin, calponin, pro-collagen type I, tenascin, and fibroblast activation protein together are able to profile phenotypic changes in stromal cells and a remodeling of the extracellular matrix in prostate cancer-reactive stroma (6).

Our study also adds to the concept of stromal dependency of epithelial cancers. All carcinomas have two major components: the epithelium, which is regarded as the malignant process, and the supporting stroma, which we regard as both a reactive and regulatory process. All available evidence suggests that initial carcinoma growth is stromal dependent, where the stroma is permissive and supporting, and is regulated through paracrine interactions with the carcinoma cells (8, 9). Therefore, it would stand to reason that when stroma is abundant, the ability of the carcinoma to grow and progress is likely to be greater.

The concept of an epithelial-mesenchymal transformation dictates that during the later stages of cancer progression, cancer cells begin to express genes that are normally restricted to the stromal compartment of cells. It is possible that the expression of these genes by the stroma becomes superfluous and, thus, paracrine interactions with stroma are no longer rate limiting. This theory would predict that the cancer cell would become functionally stromal independent. The stroma would then become redundant and may decrease in quantity. This theory is



Correlations

			DES Index grouped
Spearman's rho	PREPSA	Corr Coeff	
		p-value	.326
		N	64
	ECE	Corr Coeff	-.059
		p-value	.634
		N	68
	SVI	Corr Coeff	-.053
		p-value	.669
		N	68
	LN	Corr Coeff	-.045
		p-value	.718
		N	68
	MARGINS	Corr Coeff	-.117
		p-value	.340
	GLEASON	Corr Coeff	68
		p-value	-.161
		N	.190
			68

Models		HR (95%CI)			p-value
Univariate	DES (0 vs "1-4")	4.10 (	1.56 ,	10.76)	0.0339
	DES (0 vs "6-9")	9.49 (	3.04 ,	29.64)	0.0001
	DES ("1-4" vs "6-9")	2.31 (	0.96 ,	5.56)	0.0608
Mulivariate	DES (0 vs "1-4")	4.23 (	1.35 ,	13.29)	0.0136
	DES (0 vs "6-9")	10.10 (	2.64 ,	38.61)	0.0007
	DES ("1-4" vs "6-9")	2.39 (	0.82 ,	6.97)	0.1108
	LN	11.93 (	1.30 ,	109.13)	0.0281
	Margins	2.01 (	0.02 ,	4.94)	0.1288
	SVI	1.40 (	0.44 ,	4.39)	0.5679
	Gleason	1.68 (	0.94	3.02)	0.0802
	ECE	1.78 (	0.61 ,	5.21)	0.2921
	Ln(PreOpPSA)	1.06 (	0.61 ,	1.83)	0.8468

Fig. 7 Desmin staining index. Staining intensity and percentage of field positive was quantitated as described in "Materials and Methods." Results were analyzed using the Kaplan-Meier method. The *inset* shows the relative HR averages and range.

consistent with the observation that once cancers loose stroma (become stromal independent) the histological grading of the epithelial cancer performs very well as a predictive factor. It is also, therefore, understandable that stromal grading predicts best in tumors that contain some degree of stroma, because we propose that these tumors are still stromal dependent. Our data demonstrate that stromal grading becomes an independent predictor only when analyzing tumors that still contain stroma (Gleason 7). This theory and the data presented here add to the concept that carcinoma tumors are not purely epithelial and the stroma must be considered as a biologically relevant part of the tumor.

Our data suggest that the prognostic value of stromal quantification or stromal markers should be evaluated to a greater extent. This study indicates that these markers can be evaluated in all patients with prostate cancer. The poor survival associated with patients with stromal grade 0 is not surprising, given that stromal-independent cancer (cancer with no stroma) is usually high in epithelial grade. However, it was surprising to identify that patients with large amounts of stroma (stromal grade 3) had survival curves that were equivalent to patients with a stromal grade 0. The issue of stromal dependence relative to stromal independence might explain this counterintuitive finding. Significantly, these makers are also useful as independent predictors in patients with Gleason score 7, which are tumors, by our definition, that are still stromal dependent. Stromal quantification can be used to discriminate patients with a higher possibility of recurrence, regardless of the epithelial grade component. To our knowledge, this is the first time that quantitation of reactive stroma has been shown to have prognostic significance in cancer recurrence.

Of potentially further value are the results obtained with immunohistochemical stains. The results are proof of concept that elements in the stroma are significant for prediction of cancer progression. Although some markers were not predictive of survival, smooth muscle α-actin and desmin seem to be good discriminators of biochemical recurrence. Within the limitations of the smaller data set used for these markers, we believe that the results indicate that future studies might identify stromal proteins that are able to better discriminate which patients will progress. The markers used in the present study are markers of stromal cell differentiation. Additional studies that address stromal proteins associated with growth regulation might end up being even more useful to discriminate survival. Candidates' stromal markers, including caveolin and ps20, are currently under study.

Because stroma is an integral component of all carcinoma tumors, the scoring of reactive stroma could be used in pre-

therapy biopsies. Combinations of epithelial and stromal scoring could also become more powerful predictors in all patient categories. Future studies will address these questions in detail.

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