Determining Prostate Cancer-Specific Death through Quantification of Stromogenic Carcinoma Area in Prostatectomy Specimens

We previously reported that reactive stroma grading in prostate cancer (PCa) is predictive of biochemical recurrence in prostatectomies and biopsies. In this study, we tested whether quantifying the percentage of reactive stromal grade 3 (RSG 3; stromogenic carcinoma pattern) in the entire tumor is predictive of PCa-specific death. Whole-mount prostatectomies operated by a single surgeon obtained between 1983 and 1998 were reviewed. Reactive stroma was evaluated as described previously, and areas of RSG 3 in the entire tumor were registered as percentages of total tumor. Statistical analysis was performed using Spearman, Kaplan-Meier, and Cox analyses. In all, 872 cases were evaluable. Quantification of RSG 3 percentage was an independent predictor of biochemical recurrence, analyzed as a continuous or grouped variable. Patients with higher RSG 3 percentages (larger tumor areas with RSG 3) had a significantly decreased biochemical recurrence-free survival than those with a lower RSG 3 percentage, even within the Gleason score 7 subset of patients. A nomogram introduced this new variable to the model. Furthermore, quantification of RSG 3 percentage was significantly predictive of PCa-specific death. Quantification of the RSG 3 (stromogenic carcinoma) area in PCa provides additional novel information on prognosis. These data substantiate the concept that the tumor microenvironment holds significant predictive information, as well as biological significance. (Am J Pathol 2011, 178:79–87; DOI: 10.1016/j.ajpath.2010.09.042)}
terized it into four grades: RSG 0, ≤5% RS; RSG 1, 6%–15%; RSG 2, 16%–50%; and RSG 3, at least a 1:1 ratio between RS and epithelial cancer. We demonstrated that PCa with either RSG 0 or RSG 3 showed significantly reduced recurrence-free survival. Although some information from RSG overlaps with Gleason score (GS), the predictive ability of RSG is independent of GS and other clinicopathological parameters. The greatest degree of overlap occurs between RSG 0 and high GS. Hence, the most novel predictive information is found in RSG 3, which can be found in all Gleason grades.

Prostate cancer is highly heterogeneous, and several Gleason grades can be found within a single focus. The Gleason scoring system uses the two most common grades in the radical prostatectomy specimens. During our studies, we also identified heterogeneity of reactive stroma grades within tumor foci. This prompted us to explore whether quantification of tumor RSG 3 area (stromogenic carcinoma) on H&E whole-mount slides of prostate cancer specimens is a stronger predictor of recurrence than current predictive models. This RSG-based system attempts to measure the percentage (area) of an aggressive tumor type (stromogenic carcinoma) within the cancer foci. It is important to clarify that the present study does not quantify the amount of reactive stroma in the tumor, but rather the volume of tumor that has a stromogenic pattern (RSG 3).

Our results indicate that quantification of RSG 3 area is a strong continuous and independent marker for BCR-free survival in PCa, as well as for time to PCa-specific death. Patients with prostate cancer tumors with higher percentage area of RSG 3 had significantly reduced time to BCR and to PCa-specific death. This is also true in the Gleason 7 subset of patients, even when considering Gleason 0 and high GS. Hence, the most novel predictive information is found in RSG 3, which can be found in all Gleason grades.

Principles of Stroma Grading

Specimens were fixed in 10% buffered formalin, embedded in paraffin, cut at 4-μm thickness, and routinely stained with H&E. These slides were reviewed by a single pathologist (B.M.), who had been trained by G.E.A. A whole-mount section of a radical prostatectomy exhibiting four different RSG grades is shown in Figure 1. We have demonstrated that RSG 0 and RSG 3 are the most aggressive phenotypes, but because of the potential overlap between high Gleason grades and RSG 0, we decided to include only RSG 3 in this study.

In accord with previous reports, the index cancer was designated as the largest and/or the most aggressive tumor in the patient’s prostate (presumed to be clinically meaningful), as defined with the help of other clinicopathological factors. Subsequently, each index cancer was analyzed and graded in terms of total area of RSG 3.

Materials and Methods

Definition of Reactive Stroma in PCa

In contrast to other organs, in the prostate the majority of the stroma consists of smooth muscle cells. Normal prostate smooth muscle cells are dense, eosin-staining cells that are uniform in size and shape, and already have ample cytoplasm and rounded nuclei. In contrast, RS cells lose the majority of their ample eosinophilic cytoplasm, and there is deposition of collagen fibrils and extracellular matrix. Characteristically, the well-organized band pattern of smooth muscle is replaced by a disorderly pattern. The fibers are irregular in length and thickness, and there is a delicate fibrillar background.

Cohort Selection

As of July 2010, there was information on >9500 patients with PCa in the Baylor College of Medicine (BCM) Prostate Cancer Program Tumor Bank and Database (IRB H-11436). This cohort is similar to the one used by Ayala et al. in a study that first identified RSG as predictive for BCR in PCa. The most important different lies in the utilization of the entire radical prostatectomy specimens, rather than tissue microarray dots, and in the quantification of percentage of the tumor involved by RSG 3.

Entry criteria to create a radical prostatectomy tissue array included the following: (1) no preoperative treatment, (2) operation performed by a single surgeon (P.T.S.) between 1983 and 1998, and (3) radical prostatectomy specimen in the tissue bank. In all, 877 totally embedded whole-mount radical prostatectomies were reviewed. Prostate specific antigen (PSA) recurrence was defined as a PSA >0.4 ng/ml on two consecutive measurements. Clinical stage of all radical prostatectomy specimen tumors was determined according to the American Joint Committee on Cancer (AJCC) pT classification.
Quantification of Area Tumor with High-Risk RSG 3 in Whole-Mount-Embedded Radical Prostatectomy Specimens

Previous studies have demonstrated that PCa is heterogeneous, even within a single focus of cancer, and several GS grades can be identified within a single tumor.30,31 Similarly, we found heterogeneity of RSG within a single tumor and commonly discovered several stromal grades within the tumor foci. To determine whether the tumor load of aggressive RSG 3 (stromogenic carcinoma) was predictive of BCR-free survival, we mapped the areas of RSG 3 within the tumor in each whole-mount section of the entire radical prostatectomy (Figure 2). Subsequently, we determined the quantity of RSG 3 in percentile fashion for the entire tumor. The percentage of RSG 3 was recorded as a continuous increase in 5% increments in each index tumor area.

The predictive value of the RSG 3 range in a subset of patients with a GS of 7 was analyzed separately. In this subset, we identified a total of 415 patients.

PNI Diameter

A pathologist assessed all foci of PNI in any part of the radical prostatectomy specimen, including foci of tumor outside the prostate. The diameter of the focus estimated to be the largest was then measured with an ocular micrometer. The diameter of the PNI tumor focus was taken perpendicular to the long axis of the Schwann cell nuclei in the nerve.

Histopathological Parameters

The following histopathological parameters were included in the statistical analysis: lymph node (LN) metastasis, extracapsular extension (ECE), seminal vesicle invasion (SVI), radical prostatectomy GS, and positive surgical margins (SM).

Statistical Analysis

The correlation of stromal grading with the patients’ clinical and pathological factors was analyzed by the Spearman correlation method. For survival analysis, the endpoint was the biochemical recurrence of the cancer, defined as serum PSA level of >0.4 ng/ml on two successive measurements. Time to recurrence was defined as the time interval between the date of surgery and the date of identification of biochemical recurrence. Time to death was defined as the time interval between the date of surgery and the date of identification of death due to prostate cancer.

The predictive value of quantification of RSG 3 for recurrence-free survival and PCa-specific death was evaluated using the Kaplan-Meier actuarial analysis and the log-rank test. The differences between the survival curves of these groups were tested for statistical signifi-
cance using the log-rank test. Minimum $P$-value method was used to group the RSG 3 quantification. The Cox univariate and multivariate proportional hazard models were used to determine the hazard ratios. In the multivariate analysis, the model included LN, SM, SVI, GS, ECE, and the clinical stage (UICC/AJCC), and preoperative PSA levels. The hazard ratio (HR) and its 95% confidence interval (95% CI) were recorded for each marker. Preoperative markers were compared univariately and multivariately, adjusting for postoperative variables: LN, SM, SVI, ECE, and UICC/AJCC using Cox proportional hazard models. Also, the Cox model was used to evaluate the predictive value of RSG 3 area in presence of other preoperative information. The same methodology was used to analyze the predictive ability of stromal grading in the GS 7 subgroup. P-values of <0.05 were considered statistically significant in all analyses. All analyses were performed with the SPSS 11.0 statistical software package (SPSS, Chicago, IL).

Postoperative Nomogram

Univariate and multivariate Cox regression models were fitted and proportional hazards model assumption was checked. To identify the potential predictors that explain the majority of the variation of BCR-free survival, a full multivariate Cox model was fitted to include all potential existing predictors. A nomogram was plotted to visually obtain the prediction of the survival probability from the final model.

Results

Clinical Characteristics

In the present study, a total of 872 patients fulfilled the described entry criteria. Of those, 845 patients had available clinical follow-up for biochemical recurrence and 872 patients for PCa-specific death. Their clinicopathological characteristics are summarized in Table 1. The AJCC pT-stage classification was applied to all carcinoma cases in radical prostatectomy specimens to determine their clinical stage.25

Quantification of RSG 3 Correlates with Clinicopathological Parameters in PCa

Increased percentage of areas with RSG 3 significantly correlated with higher stage of disease (UICC/AJCC) ($P = 0.31$; $P < 0.0001$), positive LN metastasis ($P = 0.19; P < 0.0001$), positive extracapsular extension (ECE) ($P = 0.37; P < 0.0001$), seminal vesicle invasion (SVI) ($P = 0.30; P < 0.0001$), and radical prostatectomy GS ($P = 0.43; P < 0.0001$). It also correlated weakly with positive surgical margins (SM) ($P = 0.10; P = 0.0031$), preoperative PSA ($P = 0.10; P = 0.0031$).

Quantification of RSG 3 Area Independently Predicts Biochemical Recurrence-Free Survival

By univariate Cox proportional hazard analysis, the RSG 3 percentage was a significant continuous predictor of BCR (hazard ratio HR = 1.12, 95% CI = 1.09–1.15, $P < 0.0001$) (Table 2). Consequently, with every 5% increase in total RSG 3 area, the risk of clinical BCR estimated by the hazard ratio increases by 12%.

In multivariate analysis, RSG 3 area was shown to be an independent significant predictor of time to recurrence (HR = 1.08, 95% CI = 1.05–1.11, $P < 0.0001$) (Table 2). Thus, with every 5% increase in RSG 3 the estimated comparative risk increases by 8% when other clinicopathological parameters are taken into account. Minimum $P$-value method was used for identifying major groups to combine patients with similar risk, based on our available data. We identified the following groupings: 0%–5% ($n = 457$), 6%–40% ($n = 262$), 41%–70% ($n = 75$), 71%–85% ($n = 43$), and 86%–95% ($n = 8$). These groupings were significantly univariately statistically different from each other in their ability to predict time to BCR.

### Table 1. Selected Clinicopathological Characteristics for the Study Cohort of Prostate Cancer Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Patients available for follow up</td>
<td>845</td>
</tr>
<tr>
<td>Clinical follow-up (months)$^*$</td>
<td>46.58 ± 34.12</td>
</tr>
<tr>
<td>Age at radical prostatectomy (years)$^{+}$</td>
<td>62 ± 6.83</td>
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<tr>
<td>Preoperative prostate-specific antigen (ng/ml)$^{+}$</td>
<td>10.06 ± 11.19</td>
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<tr>
<td>Gleason score (% patients)</td>
<td></td>
</tr>
<tr>
<td>&lt;7</td>
<td>45.6</td>
</tr>
<tr>
<td>7</td>
<td>48.5</td>
</tr>
<tr>
<td>&gt;7</td>
<td>5.9</td>
</tr>
<tr>
<td>Tumor features, [no. (%)]</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastases</td>
<td>59 (6.9)</td>
</tr>
<tr>
<td>Extracapsular extension</td>
<td>355 (41.5)</td>
</tr>
<tr>
<td>Surgical margins positive</td>
<td>118 (13.8)</td>
</tr>
<tr>
<td>Seminal vesicle involvement</td>
<td>116 (13.6)</td>
</tr>
<tr>
<td>Biochemical recurrence</td>
<td>152 (18)</td>
</tr>
</tbody>
</table>

*Values expressed as mean ± standard deviation.

$^*$Median, 43.64 months; maximum, 168 months.

$^+$Minimum, 38 years; median, 63 years; maximum, 81 years.

$^{+}$Minimum, 0.2 ng/ml; median, 7 ng/ml; maximum, 100 ng/ml.

### Table 2. Biochemical Recurrence-Free Survival in All Patients

<table>
<thead>
<tr>
<th>Model</th>
<th>HR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSG (continuous)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Univariate</td>
<td>1.12 (1.09–1.15)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Multivariate*</td>
<td>1.08 (1.05–1.11)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RSG (grouped)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Univariate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6%–40% vs. 0%–5%</td>
<td>2.25 (1.52–3.32)</td>
<td>0.0001</td>
</tr>
<tr>
<td>41%–70% vs. 0%–5%</td>
<td>3.72 (2.27–6.10)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>71%–85% vs. 0%–5%</td>
<td>6.86 (4.06–1.52)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>&gt;85% vs. 0%–5%</td>
<td>17.13 (7.29–0.26)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Multivariate*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;40% vs. 0%–40%</td>
<td>1.99 (1.34–2.96)</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

CI, confidence interval; Cox PH, Cox proportional hazards regression model; HR, hazard ratio; RSG, reactive stromal grade.

The multivariate model included log of the variables PSA (prostate-specific antigen), LN (lymph node) metastasis, ECE (extracapsular extension), SVI (seminal vesicle involvement), positive surgical margins, age at surgery, UICC/AJCC tumor-node-metastasis stage, and Gleason Score.
Patients whose intratumoral RSG 3 fell into a range of 6%–40% (mean recurrence-free survival, 118 months) had a 2.25-fold higher risk (estimated by HR) of BCR, compared with a patient from the 0%–5% group (mean of 146 months) (Table 2). A patient with a range of 41%–70% (mean of 83 months) had a 3.72-fold higher risk, whereas one from the group with 71%–85% of intratumoral RSG 3 (mean of 45 months) had a 6.86-fold higher risk for progression, compared with a patient from the lowest group (0%–5%). The highest risk for progression was seen in the 85%–95% group (mean of 19 months).

Here, the risk for BCR was 17.13-fold higher, compared with the lowest group (0 to 5%) (Table 2 and Figure 3).

We multivariately analyzed this cohort to determine whether the difference between identified groups remained significant after adjustment for all clinicopathological parameters. After adjusting the estimated risk, the two lowest groups (0%–5% and 6%–40%) equalized, as did the risk for the group of patients with RSG 3 percentage of 41%–95%, possibly because of the smaller number of patients in these categories. Therefore, the difference of stroma percentage was a significant multivariate predictor of time to BCR with a binary grouping, 0%–40% and >40% (HR = 1.99, 95% CI = 1.34–2.96, P = 0.0007) (Table 2).

RSG 3 Specifies Prediction of Biochemical Recurrence-Free Survival in Patients with Gleason Score 7

We identified 412 patients in the GS 7 subset with the necessary follow-up information. By univariate Cox PH analysis, the percentage of RSG 3 in the GS 7 subset was a significant continuous predictor of BCR (HR = 1.05, 95% CI = 1.02–1.09, P = 0.0052) (Table 3). Consequently, with every 5% increase in RSG 3 area, the estimated risk of clinical BCR increases by 5%. RSG 3 remained an independent predictor of BCR for this subgroup multivariately as well (HR = 1.84, 95% CI = 1.13–3.00, P = 0.0140) (Table 3), corresponding to an 84% increase in estimated BCR risk with 5% increase in RSG 3 area when all other factors are identical.

Using the minimum P-value method, we divided patients of the GS 7 subset with RSG 3 into two risk groups, low (0%–40%, n = 329; mean BCR-free survival, 119 months) and high (>40%, n = 83; mean BCR-free survival, 76 months) (Figure 4). Grouped RSG 3 is a significant predictor of BCR for this subgroup both univariately (HR = 1.83, 95% CI = 1.20–2.78, P = 0.0047) and multivariately (HR = 1.99, 95% CI = 1.23–3.22, P = 0.0051) (Table 3). Thus, among any two patients with GS 7 and identical preoperative PSA, LN, ECE, SVI, SM, age, and UICC/AJCC, the one with >40% intratumoral area of RSG 3 is estimated to have, in effect, twice the risk of BCR as one with a lesser intratumoral area of RSG 3 (Table 3).

<table>
<thead>
<tr>
<th>Table 3. Biochemical Recurrence-Free Survival in the Gleason 7 Subset</th>
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<tbody>
<tr>
<td>Cox PH model</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>RSG (continuous)</td>
</tr>
<tr>
<td>RSG (grouped)</td>
</tr>
<tr>
<td>&gt;40% vs. 0%–40%</td>
</tr>
<tr>
<td>&gt;40% vs. 0%–40%</td>
</tr>
</tbody>
</table>

CI, confidence interval; Cox PH, Cox proportional hazards regression model; HR, hazard ratio; RSG, reactive stromal grade.

*The multivariate model included log of the variables PSA (prostate-specific antigen), LN (lymph node) metastasis, ECE (extracapsular extension), SVI (seminal vesicle involvement), positive surgical margins, age at surgery, UICC/AJCC tumor-node-metastasis stage, and Gleason Score.
RSG 3 Is Independent of GS 4 + 3 and 3 + 4 in GS 7 Patients

Considering the known heterogeneity of a GS 7, we subdivided the GS 7 subset into a 3 + 4 group (n = 323) and a 4 + 3 group (n = 88). The multivariate analysis, which included the 3 + 4 vs. 4 + 3 indicator variable along with all other clinicopathological parameters as previously described, established that RSG 3 remained an independent significant predictor for time to BCR (HR = 1.84, 95% CI = 1.13–2.00, P = 0.014) (Table 3). We note that the difference between the two GS 5 groups was not a significant predictor of time to BCR (HR = 1.47, 95% CI = 0.94–2.28, P = 0.088) in this model. These data are similar to those obtained in our previous biopsy study. Therefore, we concluded that the heterogeneity of GS 7 of a PCA does not affect the predictive ability of RSG 3 in this subgroup.

RSG 3 Is a Significant Predictor of Prostate Cancer-Specific Mortality

We identified 872 patients who met the criteria for follow-up information. By univariate Cox proportional hazard analysis, the RSG 3 percentage was a significant continuous predictor of PCa-specific death (HR = 1.2, 95% CI = 1.13–1.27, P < 0.001). Consequently, with every 5% increase in total RSG 3 area, the risk of PCa-specific death estimated by HR increases by 20%.

In multivariate analysis, RSG 3 area was shown to be an independent significant predictor of time to PCa-specific death (HR = 1.10, 95% CI = 1.02–1.18, P = 0.016). Thus, with every 5% increase in RSG 3 the estimated comparative risk of PCa-specific death increases by 10% when other clinicopathological parameters are taken into account.

Minimum P-value method was used for identifying major groups to combine patients with similar risk based on our available data. We identified the following risk groupings: None, 0% intratumoral RSG 3 (n = 463; mean time to PCa-specific death, 218 months); Low, <15% intratumoral RSG 3 (n = 356; mean time to PCa-specific death, 204 months); and High, >15% intratumoral RSG 3 (n = 53; mean time to PCa-specific death, 143 months). These groups were statistically significantly univariately different from each other in their ability to predict time to PCa-specific death.

Table 4. Prostate Cancer-Specific Death

<table>
<thead>
<tr>
<th>Cox PH model</th>
<th>HR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSG (continuous)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Univariate</td>
<td>1.2 (1.13–1.27)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Multivariate*</td>
<td>1.1 (1.02–1.18)</td>
<td>0.0106</td>
</tr>
<tr>
<td>RSG (grouped)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Univariate</td>
<td>&lt;15% vs. 0%</td>
<td>4.22 (1.39–12.85)</td>
</tr>
<tr>
<td></td>
<td>&gt;15% vs. &lt;15%</td>
<td>7.18 (3.37–15.27)</td>
</tr>
<tr>
<td></td>
<td>&gt;15% vs. 0%</td>
<td>30.3 (9.89–92.85)</td>
</tr>
</tbody>
</table>

CI, confidence interval; Cox PH, Cox proportional hazards regression model; HR, hazard ratio; RSG, reactive stromal grade.

*The multivariate model included log of the variables PSA (prostate-specific antigen), LN (lymph node) metastasis, ECE (extracapsular extension), SVI (spermatic vesicle involvement), positive surgical margins, age at surgery, UICC/AJCC tumor-node-metastasis stage, and Gleason Score.

Figure 5. Kaplan Meier curves for prostate cancer (PCa)-specific mortality for percentage of RSG 3. Blue (None) indicates no intratumoral RSG 3, green (Low) indicates <15% of the tumor with RSG 3, red (High) indicates ≥15% of the tumor with RSG 3. Mean time to prostate cancer-specific death: 218 months (N = 463) for None, 143 months (N = 356) for Low, 143 months (N = 53) for High.

Percentage of RSG 3 in radical prostatectomies was grouped as follows: Low versus None (HR = 4.22, 95% CI = 1.39–12.85, P = 0.0112), High versus Low (HR = 7.18, 95% CI = 3.37–15.27, P < 0.0001), and High versus None (HR = 30.3, 95% CI = 9.89–92.85, P < 0.0001) (Table 4 and Figure 5). Thus, patients whose intratumoral RSG 3 area fell into the low range had a 4.22-fold higher risk (estimated by HR) of cancer-specific mortality, compared with a patient who was analyzed to have no intratumoral RSG 3. A patient with a high range of RSG 3 had a 7.18-fold higher risk, compared with a patient with low RSG 3 area. The highest risk for PCa-specific mortality was seen in patients with a high range of RSG 3, compared with patients with no RSG 3. Here, the risk of...
PCa-specific death was 30.3-fold higher, compared with patients who were analyzed not to have any RSG 3.

Postoperative Nomogram with PNI Diameter and Quantification of RSG 3

A postoperative nomogram that includes the quantification of RSG and PNI diameter is shown in Figure 6. Both RSG and PNI diameter were demonstrated to be independent predictors of BCR-free survival. The nomogram relates the commonly used clinicopathological factors of the nomogram model and PNI diameter and RSG 3 percentage to the prediction for 5-year recurrence-free probabilities from the fitted Cox regression model. PNI diameter was obtained as previously described.22,27

The point scores are obtained from each predictor (log preoperative PSA, lymph node status, SM, ECE, Gleason score, stroma percentage quantification of RSG 3 area as stroma percentage, and PNI diameter) and then manually tallied to obtain a total score before reading predicted recurrence-free probability. As the nomogram shows (Figure 6), RSG 3 provides unique information to the model and the information provided has significant weight. We are unable to show an ROC curve of the model and compare it with the previous nomogram, because the PCa nomogram was created using the same dataset and therefore the data are fitted to the model.

Discussion

Current nomograms attempt to individualize risk assessment, but continue to examine markers in the context of PCa considered as a single disease. However, the heterogeneity of PCa suggests that different marker profiles may exist, based on the distinct nature of PCa in individual patients. PCa heterogeneity has also been demonstrated by examining gene expression profiles32 and patterns of metastasis.33 Thus, diversity in PCa progression is a reflection of different phenotypes.34 Some prostate cancers exhibit an indolent course, even without therapy. Conversely, some prostate cancers are very aggressive and are not affected by current forms of therapy. Recent Swedish studies of the natural history of PCa show that most cases of PCa diagnosed at an early stage have an indolent course. Results of some studies have shown that 10%–26% of nonpalpable cancers detected by PSA screening are “clinically unimportant,” based on pathological criteria.35–36 Nonetheless, many apparently localized prostate cancers are so highly virulent that even aggressive local therapies cannot control them. Tens of thousands of patients subjected to definitive localized therapy (ie, radical prostatectomy or radiation therapy) have subsequently progressed to having increased PSA levels, showing them to be curative failures.39–44 Overall, the reported failure rate, defined as rising PSA levels within 5 years of radical prostatectomy, is 35%.40–43 The dilemma is that current prognostic methods are limited in their ability to predict these individual PCa patterns of progression.

In the present study we have demonstrated that differential alterations in the tumor microenvironment may explain PCa diversity and aggressiveness. Our previous studies characterized RS as an important process in early tumorigenesis, promoting angiogenesis and formation of a wound-repair type of matrix.13–15,45 We demonstrated that carcinoma-associated RS is composed of activated fibroblasts and myofibroblasts that are associated with rapid and elevated angiogenesis. We have also shown that the microenvironment provides significant predictive information that can help define indolent and lethal phenotypes of PCa. Distinguishing indolent PCa from stroma independent and stromogenic PCa can be achieved only by incorporating measures of the stromal response into current predictive clinical tools.

Specifically, in this study, we demonstrate that quantification of RSG 3 area in whole-mount sections PCa is a continuous and independent predictor for time to biochemical recurrence in PCa for both biochemical recurrence and, more importantly, PCa-specific death. Few studies that have analyzed prediction of PCa-specific death,39,46–51 and even fewer have attempted to identify biomarkers of PCa-specific death.52–55 The study reported here was possible because of our unique resources, including a large tissue bank and database with extensive follow-up clinical data for >15 years. Unlike most other cancers, PCa-specific death continues up to the 20th year of follow-up.

Our studies demonstrate that patients are at a higher risk of recurrence if their tumors have higher components of RSG 3, a histological pattern that we previously named stromogenic carcinoma. The risk for progression significantly increases and the time to BCR significantly decreases with increased intratumoral RSG 3 area. Patients with smaller intratumoral loads of RSG 3 had significantly better clinical outcome than those with higher loads (a stromogenic carcinoma pattern). The same was true for the GS 7 subset of patients, even when considering GS 3 + 4 and 4 + 3 in the model. Based on our data, patients with intratumoral RSG 3 > 15% do have a 30.3-fold higher risk of PCa-specific mortality, compared with patients with no intratumoral RSG 3.

Furthermore, with the present study we have demonstrated that the predictive information in RSG 3 area quantification can add significant novel information to the postoperative nomogram. The existing postoperative nomogram can be improved by the addition of both RSG 3 area quantification and PNI diameters. Both markers play a central role in the interaction between the tumor and the host. RSG 3 and PNI diameters provide unique information to the model and that information has significant weight.

More importantly, these clinical data validate an enormous body of literature on the biology of host response to tumors. Basic scientists have long anticipated that the type of response by the host to the tumor would help determine the aggressiveness and progression of tumors. Here, we demonstrate that an excessive stromal response, measured as RSG 3 area, is permissive, or protumorigenic, for PCa progression. Similarly, PNI diameter, another measure of the interaction between
tumor and host, also seems to act as a permissive factor. These and other host elements seem to act in coordination to produce a permissive, progrowth microenvironment for PCa.

This study is novel in demonstrating the biomarker potential of RSG 3 (stromogenic carcinoma) intratumoral area quantification in PCa as an independent predictor of recurrence-free survival as well as of PCAspecific mortality. Therefore, quantification of RSG 3 is a promising tool in predicting BCR-free survival as well as PCAspecific death. These data substantiate the concept that the tumor microenvironment holds significant predictive information, as well as biological significance. Future studies are needed to validate this biomarker and to test its transferability to other populations.

References


