Elevated Levels of Peritumoral Chondroitin Sulfate Are Predictive of Poor Prognosis in Patients Treated by Radical Prostatectomy for Early-Stage Prostate Cancer

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Abstract

The disease course of localized prostate cancer is highly variable, and patients potentially curable by aggressive management are not readily identified by current clinical practice. Chondroitin sulfate (CS) glycosaminoglycan is a candidate biomarker as elevated levels of CS in peritumoral stroma of prostate cancer have been associated with prostate-specific antigen (PSA) failure. Immunoactive CS was measured using image analysis of archived radical prostatectomy tissue sections, obtained from 157 men with a median of 47 months (range, 16–111 months) clinical follow-up. CS level, Gleason score, and preoperative serum PSA levels were independent predictors of PSA failure by Cox’s multivariate analysis. Patients with low CS levels had significantly fewer PSA failures after radical prostatectomy than patients with high levels of CS (Kaplan-Meier plot; 32% PSA failures at 5 years for CS mean integrated absorbance cut point <7.0, 50% for CS ≥7.0, P = 0.0001). In the subgroup of patients with preoperative serum PSA levels <10 ng/ml, CS was particularly useful in discriminating retrospectively those patients most suited for surgery (Kaplan-Meier plot; 14% PSA failures at 5 years for CS mean integrated absorbance cut point <7.0 versus 47% for CS ≥7.0, P = 0.0001). We conclude that measurements of CS level can assist in predicting patient outcome after surgery. Additionally, our data suggest that the combination of CS and PSA measurements may improve outcome prediction for patients with intermediate Gleason scores.

Introduction

The widespread use of serum PSA measurement to detect prostate cancer combined with public awareness programs has resulted in increased numbers of men being diagnosed with clinically organ-confined disease, for which potentially curative therapeutic options including surgery and radiotherapy are available. However, 20–30% of patients treated by radical prostatectomy relapse with disseminated disease because of inaccurate staging of a subset of men with preexisting micrometastases or direct tumor extension beyond the surgical margin (1, 2). The clinical determination of organ-confined disease is currently based on low serum PSA values (<10 ng/ml), the histological grade of the tumor from TRUS-guided core biopsies, and the absence of metastases as defined by negative bone scans. Despite the value of Gleason score and serum PSA measurements at initial diagnosis, better markers are required to assess the extent of disease preoperatively (3, 4).

We recently reported that increased levels of CS-GAG in the peritumoral stroma of clinically localized prostate cancers was predictive of PSA failure (5). In a subsequent report, we proposed that the proteoglycan responsible for the association of CS with disease progression was versican (6). Versican is known to antagonize cell adhesion to pericellular matrix components, which suggests that increased deposition of this proteoglycan in the stroma may contribute to the progression of prostate cancer. This study demonstrates in a large cohort (n = 157) of patients treated by radical prostatectomy for clinically localized prostate cancer that the measurement of CS levels in the peritumoral stroma can predict disease progression.

Materials and Methods

Prostatic Tissue. Formalin-fixed tissue sections from peripheral zone tumors of prostates from 170 men undergoing radical prostatectomy for clinically organ-confined prostate cancer were obtained retrospectively from two independent sources, 60 through members of the Collaborative Center for Prostate Health, Adelaide, South Australia and 110 through members of the St Vincent’s Campus Prostate Cancer Group, Sydney, New South Wales. These tissue sections were individually confirmed by a pathologist (W. A. R.) as containing sufficient prostatic cancer for immunostaining and image analysis in 157 cases. Prostate cancers were graded in this study on the radical surgery specimen according to the system of Gleason (7) by the reporting pathologists. Twenty-four patients were graded as Gleason score 2–4, 100 patients as Gleason score 5–6, 32 as Gleason score 7, and 12 as Gleason score 8–10.

Early-Stage Prostate Cancer Patients. The 170 men with prostate cancer had a median age of 65 years (range, 46–78). Clinical staging [IUCC system (8)] based on digital rectal examination, TRUS-guided biopsy, and bone scan was available for 132 of the patients. Fifty-six men were staged at cT1, and 80 at cT2. Additionally, serum PSA levels were routinely measured by a solid-phase, two-site immunoenzymatic assay (Tandem-E PSA, Hybritech, Inc., San Diego, CA). The median for preoperative serum PSA level in 162 men tested was 9.0 ng/ml (range, 0.2–79.0). All of the patients underwent retroperitoneal radical prostatectomy. Patients receiving any form of neoadjuvant or adjuvant endocrine treatment or radiotherapy were excluded from this study. Progression of the disease was assessed by PSA failure, i.e., either a return to measurable serum PSA levels subsequent to a postsurgical level below the sensitivity threshold of the assay (usually <0.3 ng/ml) or, in cases where detectable levels persisted after surgery, a serum PSA level that continued to rise. PSA progression (i.e., PSA relapse, PSA failure) is correlated with subsequent clinical failure (9). Fifty-five of the 170 patients demonstrated PSA relapse. The median follow-up for this cohort of early-stage prostate cancer patients was 47 months (range, 16–111).

Pathological indices for the operative specimen were available for all of the 170 patients. Indices were pT1 for 102 patients and pT2 for 68. The index for...
one patient was pT3N0M0. Extracapsular extension of tumor was present in 67 patients and absent in 103. Seminal vesicle involvement was positive for tumor in 17 patients and negative in 142.

Immunohistochemical Staining of Tissue Sections for CS-GAG. Sections (4-μm) of prostatic tissues were immunostained as described previously (10) using monoclonal antibody 6C3 (kindly provided by Professor B. Carter, Cardiff, Wales, United Kingdom), which recognizes epitopes in native, intact CS-GAG (11, 12). Antibody binding of CS was visualized by a standard streptavidin immunoperoxidase reaction using biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) and DAB to yield an insoluble brown deposit. Immunostaining was accomplished in several batches. To reduce staining variation to a minimum, the immunostaining protocol was strictly adhered to, and a known positive control section was included in each run to monitor any variation in staining level. Deglycosylation of tissue sections by chondroitinase ACH (Seikagaku Corp, Tokyo) treatment was used previously as a negative control for CS staining (5). Other controls performed in previous studies (5) indicated that delay in tissue collection or differences in fixation times between individual prostate specimens or between pathology laboratories, did not contribute to variations of CS epitope.

Image Analysis of Immunohistochemically Stained Tissue Sections. The area and absorbance of DAB deposition were measured using an automated image analysis system (VideoPro 32; Leading Edge P/L, Marion, South Australia) similar to previous reports (5, 6). Color images were collected at a magnification of ×100. Twenty contiguous fields were captured for each prostatic sample, beginning adjacent to a randomly chosen cancer focus. Captured fields included both glandular and stromal tissue areas. Because of the exclusively stromal localization of CS in both malignant and nonmalignant prostates (5), video image measurements were confined to the stromal component, with luminal and epithelial areas including cancer cell foci manually edited from the images. The IOD of DAB deposited in the stroma for each image was determined, and the amount of specific-antibody staining was expressed as the MIOD per unit area of stromal tissue for the 20 images. The level of CS was, therefore, expressed as the MIOD of staining in arbitrary density units per pixel. Because of alterations to the calibration of the video camera system, the recorded measurements of CS staining intensity were significantly lower in this study (median MIOD = 4.87) compared with our previous report (median MIOD = 11.7; 5). Variation in staining intensity between runs was within 10% in all of the instances, as determined by image analysis of identical areas within the control tissue sections, and no adjustment to individual values were made in this study.

Statistical Analysis. Progression-free survival was calculated from the date of diagnosis to the date of progression or the date of last follow-up if progression-free. Survival times for the various components of the cohort were censored between October 1997 and April 1998. Two patients who died from causes other than prostate cancer were censored on the date of their death. One patient died from prostate cancer. Progression-free survival was used as the end point in Cox’s univariate and multivariate analyses (SPSS package, SPSS Inc, Chicago, IL). PSA failure rate with the time after radical surgery for different patient subgroups was compared using the Kaplan-Meier product-limit method and log-rank test. Statistical significance in this study was set at $P < 0.05$.

Results

CS Immunostaining in Tissue Sections of Clinically Localized Prostate Cancer. As reported previously (5), prominent peritumoral staining of stromal tissue was identified in the prostate cancer sections with complete absence of immunoreactivity for the 6C3 epitope within all of the cancer cell foci. The median concentration (MIOD) of CS staining in the peritumoral stroma of the prostatic tissue sections as determined by computer-assisted video image analysis was 4.87 (range, 0.16–24.19).

Correlates of PSA Progression in Clinically Localized Prostate Cancer. Associations between established tumor features and progression-free survival for this cohort of patients with clinically localized disease were examined using Cox’s univariate model (Table 1). Whereas clinical stage cT1 was not associated with an increased risk of progression compared with cT1, pathological stage pT1 was significantly associated with a 2.5-fold increased risk compared with pT1. Evidence of extracapsular extension of tumor and involvement of seminal vesicles were associated with approximately 3-fold increases in the risk of progression. Patients with tumors of Gleason score 5–6 or Gleason score 7 were 12 times and 17 times, respectively, more likely to progress than patients with tumors of Gleason score 4 or less. Patients with tumors of Gleason score greater than 7 had a relative risk greater than 80-fold that of patients with tumors of Gleason score 4 or less. Elevates preoperative serum PSA levels were predictive of an increased risk of progression. There was a 3% increase in risk per unit increment of serum PSA concentration, and a 3-fold increase in risk when serum PSA concentrations were dichotomized using 10 ng/ml as the cut point.

Increased levels of CS in peritumoral stroma were also significantly associated with an increased risk of progression in clinically localized tumors by Cox’s univariate analysis (Table 1). There was an 8% increase in risk for each unit increment in CS level. When CS was dichotomized, using the mean and median CS levels (MIOD, 6.07 and 4.87, respectively) as initial cut points for this early-stage tumor cohort, patients with tumors of CS level equal to or above the respective cut points had 2.3-fold and 1.8-fold increases, respectively, in the risk of progression compared with patients with tumors of CS level below the cut points. Because of the lack of an established cut point for CS, additional cut points were tested around the mean value at 0.5-intervals (i.e., CS MIOD, 5.0–7.5), and all of them were found to be significant (data not shown). The largest increase in risk (2.7-fold) was observed with CS MIOD = 7.0 (Table 2), which was close to the mean value (6.07).

Stepwise multivariate analyses were then constructed by backward elimination in the Cox’s proportional hazards regression model to test the effect of several potential prognostic factors (Table 2A). Pathological stage, extracapsular extension, Gleason score, and CS levels in the peritumoral stroma of the resected specimen, as well as preoperative serum PSA values, remained independent predictors of disease progression in early-stage prostate cancer patients (Table 2B). A final comparison of the cut points for CS measurement (MIOD = 7.0) and preoperative serum PSA level (10 ng/ml) indicated that these features had similar relative risks for PSA failure (Table 2C).
Kaplan-Meier product-limit curves comparing the rate of PSA relapse for the 157 patient cohort using MIOID = 7.0 as the cut point for CS concentration are shown in Fig. 1A. Early-stage patients with low levels of CS (MIOID, <7.0) in the peritumoral stroma had significantly fewer PSA failures than patients with higher CS levels (33 versus 51% PSA failures at 5 years, respectively; log-rank statistic, 14.34; P = 0.0002). The overall relapse rate for the entire immunostained cohort was 33.1% (52 of 157 patients). In patients with low CS levels, the overall relapse rate was 9.1% (5 of 55), compared with 44 or 56% PSA failures at 5 years, respectively). Patients with high levels of both CS and serum PSA appeared to relapse sooner than the other groups. Log-rank statistics indicated that the difference between the four groups was statistically significant (37 events, log-rank statistic, 18.28; df; 3; P = 0.0004).

Kaplan-Meier plots for the effect of CS level in the patient subgroup in which preoperative serum PSA values were <10 ng/ml are shown in Fig. 1B. Patients with low stromal level of CS (MIOID, <7.0) had significantly fewer PSA failures than patients with higher CS levels (14 versus 47% PSA failures at 5 years, respectively; log-rank statistic, 15.15; P = 0.0001). The overall relapse rate in the cohort of patients with serum PSA level <10 ng/ml was 22.0% (18 of 82 patients). In patients with low CS and low PSA levels, the overall relapse rate was 9.1% (5 of 55), compared with 48.1% (13 of 27) in patients with high CS but low PSA levels. The median follow-up for the subgroups was similar [low CS, 47 months (range, 29–111); high CS, 49 months (range, 28–108)].

Kaplan-Meier plots comparing the PSA relapse rate of patients according to Gleason score are shown in Fig. 2A. Patients with tumors of Gleason score >7 had a considerably increased rate of PSA relapse compared with patients with tumors of Gleason score 2–4 (100 versus 4% PSA failures at 5 years, respectively). Patients with tumors of Gleason score 7 had a slightly increased rate of PSA relapse compared with patients with tumors of Gleason score 5–6 (44 versus 35% PSA failures at 5 years, respectively). Log-rank statistics indicated that the difference between the 4 groups was statistically significant (54 events, log-rank statistic, 52.71; df; 3; P < 0.0001).

Because of the difficulty in predicting the outcome after radical surgery for individual patients with intermediate Gleason score, the value of using both peritumoral CS and serum PSA levels for prognosis was investigated in the patient subgroup with Gleason score 5–7 (Fig. 2B). Patients with low CS and low PSA levels had fewer PSA failures than patients with low levels of either CS or PSA (11% versus 44 or 56% PSA failures at 5 years, respectively). Patients with high levels of both CS and serum PSA appeared to relapse sooner than the other groups. Log-rank statistics indicated that the difference between the four groups was statistically significant (37 events, log-rank statistic, 18.28; df; 3; P = 0.0004).

**Table 2** Multivariate Cox regression analysis of PSA progression in early-stage prostate cancer patients treated by radical prostatectomy

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative risk</th>
<th>95% confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Multivariate analysis of all variables significant by univariate analysis</td>
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<td></td>
<td></td>
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<tr>
<td>Pathological stage (n = 147)*</td>
<td>31.53</td>
<td>3.17–313.25</td>
<td>0.0032</td>
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<tr>
<td>Extracapsular extension (n = 147)</td>
<td>83.61</td>
<td>8.32–839.83</td>
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<td>Seminal vesicle involvement (n = 137)</td>
<td>0.62</td>
<td>0.24–1.58</td>
<td>0.3126</td>
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<tr>
<td>Gleason score (n = 147)</td>
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<td></td>
</tr>
<tr>
<td>Gleason 2–4 (n = 22)</td>
<td>1.00</td>
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<td></td>
</tr>
<tr>
<td>Gleason 5–7 (n = 114)</td>
<td>8.54</td>
<td>1.13–64.33</td>
<td>0.0376</td>
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<tr>
<td>Gleason ≥8 (n = 11)</td>
<td>53.86</td>
<td>6.05–479.29</td>
<td>0.0004</td>
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<tr>
<td>CS7.0 (n = 147)*</td>
<td>2.02</td>
<td>1.08–3.77</td>
<td>0.0286</td>
</tr>
<tr>
<td>PSA10 (n = 147)*</td>
<td>2.81</td>
<td>1.47–5.26</td>
<td>0.0017</td>
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<td>B. Step-wise elimination of nonindependent variables</td>
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<td></td>
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<tr>
<td>Pathological stage (n = 147)</td>
<td>43.12</td>
<td>4.41–421.62</td>
<td>0.0012</td>
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<tr>
<td>Extracapsular extension (n = 147)</td>
<td>91.33</td>
<td>9.07–920.12</td>
<td>0.0001</td>
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<tr>
<td>Gleason score (n = 147)</td>
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<tr>
<td>Gleason 2–4 (n = 22)</td>
<td>1.00</td>
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</tr>
<tr>
<td>Gleason 5–7 (n = 114)</td>
<td>8.40</td>
<td>1.12–63.11</td>
<td>0.0387</td>
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<tr>
<td>Gleason ≥8 (n = 11)</td>
<td>46.24</td>
<td>5.47–397.08</td>
<td>0.0004</td>
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<tr>
<td>CS7.0 (n = 147)</td>
<td>2.17</td>
<td>1.17–4.02</td>
<td>0.0137</td>
</tr>
<tr>
<td>PSA10 (n = 147)</td>
<td>3.30</td>
<td>1.74–6.27</td>
<td>0.0003</td>
</tr>
<tr>
<td>C. Comparison of relative risks of CS and PSA</td>
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<td></td>
<td></td>
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<tr>
<td>CS7.0 (n = 149)</td>
<td>3.22</td>
<td>1.83–5.67</td>
<td>0.0001</td>
</tr>
<tr>
<td>PSA10 (n = 149)</td>
<td>3.14</td>
<td>1.72–5.73</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

* Pathological stage pT3 versus pT4.

† CS level in peritumoral stroma (CS MIOD, pixel density units) cut point <7.0 versus ≥7.0.

‡ Preoperative serum PSA level (ng/ml), cut point <10 versus ≥10.

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Fig. 1. A. Kaplan-Meier product limit plots. Rate of PSA relapse in early-stage prostate cancer patients treated by radical prostatectomy (n = 157). Cut point of postoperative peritumoral CS level MIOID = 7.0. CS < 7.0, n = 102 (—); CS ≥ 7.0, n = 55 (---); log-rank statistic, 14.34; P = 0.0002. B. Kaplan-Meier product limit plots. Rate of PSA relapse in subgroup of early-stage prostate cancer patients treated by radical prostatectomy in which preoperative serum PSA level < 10 ng/ml (n = 82). Cut point of postoperative peritumoral CS level MIOID = 7.0. CS < 7.0, n = 55 (---); CS ≥ 7.0, n = 27 (—); log-rank statistic, 15.15; P = 0.0001.
Discussion

During the last decade, there has been a dramatic rise in both the diagnosis of clinically localized prostate cancer and the use of radical prostatectomy as one of the preferred treatments. However, many patients with clinically localized disease are understaged preoperatively as indicated by the upstaging from clinical to pathological stage, particularly by the delineation of extracapsular tumor extension. Eventually close to 30% of these patients relapse with metastatic disease (1, 2, 13). A similar relapse rate (32%, 55 of 170) was observed in this historical cohort, in which patients with serum PSA levels ranging up to 10 ng/ml (14) results in a reduction in the overall relapse rate to 22% (18 of 82) in this study. A second important observation of our study was that the relapse rate in patients with low serum PSA levels and low CS levels in the peritumoral stroma is further reduced to 9% (5 of 55).

Currently, Gleason grading is the most important biological feature of the tumor being used to predict patient outcome after surgery. Patients with tumors of Gleason score 2–4 almost invariably fare well after surgery, whereas those with Gleason score >7 usually have a very poor outcome (4 versus 100% relapse at 5 years in this study). Predicting outcome in individual patients with intermediate Gleason score is more difficult because approximately 33% (43 of 132) of this patient group relapse. A further significant finding of our study was that the combination of peritumoral CS level with preoperative serum PSA level is capable of separating patients with tumors of Gleason score 5–7 into 4 groups with differing rates of relapse. Patients with tumors of intermediate Gleason score but low peritumoral CS and serum PSA levels had a substantially lower 5-year relapse rate of 11%, compared with 72% for patients with high CS and PSA levels. An intermediate rate of relapse (44%–56%) was observed when either PSA or CS was elevated.

Our study has, therefore, established that the peritumoral CS level and Gleason score of the resected tumor and the preoperative serum PSA level are independent predictors for PSA failure. In so doing, it establishes the benefit of combined peritumoral CS and serum PSA measurements to predict the outcome after definitive treatment, i.e., radical surgery, in the absence of neoadjuvant or adjvant endocrine therapy or radiotherapy. We speculate that elevated CS promotes decreased cancer cell adhesion to extracellular matrix components of the peritumoral prostatic stroma and results in a more rapid development of extraprostatic extension. We have proposed previously (6) that the risk of relapse from prostate cancer is increased in patients in association with elevated peritumoral levels of versican, a macromolecular proteoglycan that contains CS side chains and has recognized antiadhesive qualities (15, 16).

The findings of this study suggest that CS measurement in the peritumoral stroma of the resected tumor is an important adjunct to currently accepted indicators of disease outcome for determining the prognosis of patients with clinically localized prostate cancer. In a practical sense, however, improvements in outcome will only result from better stratification of patients for a particular treatment, combined with the adoption of new treatment strategies. Consequently, the potential for tumor progression must be estimated before aggressive local therapy or watchful waiting, and evaluation of suitable prognostic indicators for clinically localized prostate cancer must use diagnostic core biopsies. We are currently compiling all of the available preoperative TRUS biopsies for this cohort to determine whether the measurement of peritumoral CS is sufficiently robust to enable prediction of PSA failure before radical surgery.

In conclusion, these studies have confirmed that an increased CS level in the peritumoral stroma of the resected tumor is associated with PSA failure in patients treated by radical surgery alone for clinically localized prostate cancer. We conclude that the measurement of CS level can assist in predicting patient outcome after surgery, especially when combined with other biological features such as preoperative serum PSA measurement and Gleason score. Furthermore, we propose that similar measurements from diagnostic core biopsies could lead to a reduction in the incidence of radical prostatectomy in men who will not benefit from this procedure because of the existence of extracapsular extension or micrometastases.
Acknowledgments

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References

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