Immunohistochemical Level of Unsulfated Chondroitin Disaccharides in the Cancer Stroma Is an Independent Predictor of Prostate Cancer Relapse

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Abstract

The glycosaminoglycan chondroitin sulfate is significantly increased in the peritumoral stroma of prostate tumors compared with normal stroma and is an independent predictor of prostate-specific antigen (PSA) relapse following radical prostatectomy. In this study, we determined whether specific alterations in the sulfation pattern of glycosaminoglycan chains in clinically organ-confined prostate cancer are associated with PSA relapse. Immunoreactivity to distinct glycosaminoglycan disaccharide epitopes was assessed by manually scoring the staining intensity in prostate tissues from patients with benign prostatic hyperplasia (n = 19), early-stage cancer (cohort 1, n = 55 and cohort 2, n = 275), and advanced-stage cancer (n = 20). Alterations to glycosaminoglycans in benign and malignant prostate tissues were determined by cellulose acetate chromatography and high-pressure liquid chromatography. Glycosaminoglycan disaccharide epitopes were localized to the peritumoral stroma of clinically localized prostate cancer. The level of immunostaining for unsulfated disaccharides (C0S) in the peritumoral stroma, but not for 4-sulfated (C4S) or 6-sulfated disaccharides (C6S), was significantly associated with the rate of PSA relapse following radical prostatectomy. High levels of C0S immunostaining were determined to be an independent predictor of PSA relapse (1.6-fold, P = 0.020). Advanced-stage prostate cancer tissues exhibited reduced electrophoretic mobility for chondroitin sulfate and increased unsulfated disaccharides when compared with benign prostatic hyperplasia tissues, whereas the sulfated disaccharide levels were unaffected. The level of C0S immunostaining in the peritumoral stroma is an independent determinant of PSA failure in clinically localized prostate cancer. Specific alterations to chondroitin sulfate side chains occurring during tumor development may be a crucial step for disease progression in prostate cancer.

Introduction

Prostate cancer is the most frequently diagnosed invasive solid cancer in men in the Western world. With the advent of better diagnostic tools [e.g., serum prostate specific antigen (PSA) measurement and transrectal ultrasound guided core biopsy], the majority of men are now diagnosed with early-stage disease, which is potentially curable by surgery or radiotherapy (1). However, up to 20% to 30% of men with presumed organ-confined prostate cancer who undergo curative treatments ultimately relapse with disseminated disease (2). Furthermore, the mortality rate for prostate cancer has only shown a slight decline during this period (3). Despite recent advances with chemotherapy treatment, the main treatment option for men with metastatic prostate cancer remains androgen ablation therapy (4). Clinical outlook following androgen ablation therapy is poor, with reemergence of tumor after an initial stabilization of disease in a form that is no longer responsive to this treatment (5). The poor treatment outcomes for both early and advanced prostate cancer, it is critical that new approaches to understanding prostate cancer progression and metastasis be developed.

Proteoglycans are structural components of cell membranes and extracellular matrix and are important modifiers of cellular proliferation and differentiation, playing leading roles in tissue growth and development. Proteoglycans are involved in both normal and neoplastic growth by participating in cell-cell and cell-matrix adhesion and cell migration and proliferation (6). Many
of the functions of proteoglycans are associated with their attached glycosaminoglycan side chains. Glycosa-
mimglycans are unbranched polysaccharide chains composed of repeating disaccharide units that are linked to
the proteoglycan protein core, and are highly negatively charged due to the presence of sulfate and/or
 carboxyl groups. The composition of these disacchar-ide units and their sulfation patterns determines distinct
glycosaminoglycan types. Chondroitin sulfate is a gly-
cosaminoglycan side chain component of several distinct proteoglycans (e.g., versican) and has a linear polymer
structure that possesses repetitive, sulfated disaccharide units containing glucuronic acid and N-acetylgalactos-
amine (GalNAc) joined by β(1-4) and β(1-3) linkages with sulfate residues at either the C-4 or C-6 position of the
disaccharide moiety.

The concentration of chondroitin sulfate is greatly
increased over normal tissue levels in several different
diseases (7-10), including prostate cancer (11-13).
Our previous studies illustrated that patients treated by
radical prostatectomy for early stage (cT1c-cT3) cancer
who showed high chondroitin sulfate concentration in
the periurethral stroma of the prostate had a significantly
higher incidence of PSA failure than patients with low
chondroitin sulfate concentration (14). Chondroitin sul-
fate levels in advanced (cT4) prostate cancer tissues
are very similar to the levels present in those early-stage
prostate tumors that ultimately progressed,12 suggesting
that metastasis could be associated with increased
chondroitin sulfate levels. Although chondroitin sulfate
proteoglycans are widely distributed both on the
surfaces of most cells and in the extracellular matrix,
the structure and function of chondroitin sulfate side
chains in malignant tissues have not been thoroughly
investigated. Immunologic studies using monoclonal
antibodies indicated that the sulfation profile of chon-
droitin sulfate chains varies according to specific
spatiotemporal patterns in embryologic tissues, suggest-
ing that chondroitin sulfate isoforms differing in sulfa-
tion position and degree perform distinct functions in
embryologic development (15). Variance in chondroitin
sulfate structure is largely determined by the specificities
of the glycosyltransferases and sulfotransferases in-
volved in chondroitin sulfate synthesis (16). Studies by
Kitagawa et al. (17) showed that the ratio of CS4 to CS6
chondroitin sulfate chains in the embryonic chick brain
changes with development and that the levels of specific
sulfotransferase activities are closely coordinated with
changing levels of individual sulfated disaccharides.
Alterations in chondroitin sulfate structure have been
observed in many different cancer types (7-10, 18, 19).
Importantly, Dietrich et al. (8) showed an increased
amount of unsulfated disaccharides in the urine of
patients with different malignancies, including prostate
cancer.

The aim of this study was to investigate the sulfation
pattern of glycosaminoglycan side chains in clinically
localized and advanced prostate cancer and to determine
whether specific alterations in disaccharide epitopes are
associated with PSA relapse.

Materials and Methods

Patient Cohorts. Samples of prostate tissue were
collected from patients undergoing retropubic radical
prostatectomy for clinically organ confined prostate
cancer (Early Stage Pilot Cohort 1, n = 55 and Early
Stage Cohort 2, n = 275) and from men undergoing
transurethral resection of the prostate for voiding
function [n = 39; 19 from men with advanced
metastatic disease, i.e., cT4N0M1 and 20 from men with
benign prostatic hyperplasia (BPH)]. All tissue samples
were surplus to diagnostic requirements and were
obtained either through the Repatriation General Hospi-
tal Tissue Bank or the Garvan Institute of Medical
Research with approval from the Flinders Medical
Centre, Repatriation General Hospital, University of
Adelaide, and the St. Vincent’s Hospital (Sydney)
Human Ethics Committees. Tumors were staged using
the International Union against Cancer system (20). The
presence of micrometastatic disease at the time of
surgery for patients was determined by a PSA failure,
that is, a return to measurable serum PSA levels on two
sequential measurements subsequent to a postoperative
level below the sensitivity threshold of the assay
(<0.2 ng/mL). Early Stage Cohort 1 (Repatriation General
Hospital cohort), Advanced Cancer Cohort, and BPH
Cohort consisted of whole sections of tissues mounted
on microscope slides, whereas Early Stage Cohort 2 (St.
Vincent’s Hospital Campus Prostate Cancer Group
tissue microarray cohort) consisted of sections of arrayed
prostate tissue cores mounted on microscope slides. In
statistical analyses, the Early Stage Pilot Cohort 1 was
used as a training set and the Early Stage Cohort 2 was
used as a validation set. The cohort profiles are detailed
in Table 1.

Immunodetection of Glycosaminoglycan Epitopes.
Sections of paraffin-embedded prostate tissue (4 μm)
from the Early Stage Cohort 1 (pilot cohort) were
immunostained with a panel of commercially available
monoclonal antibodies to the specific glycosaminoglycan
disaccharide epitopes that remain after digestion with
chondroitinase enzymes (21), that is, 2B6 monoclonal
antibody detects 4-sulfated disaccharide (CS4, 1/1000,
Seikagaku Corporation), 3B3 monoclonal antibody
detects 6-sulfated disaccharide (CS6 1/200, Seikagaku
Corporation), and 18S monoclonal antibody detects
unsulfated disaccharide (CS0, 1/400, Seikagaku Corpo-
ration) in chondroitin sulfate and dermatan sulfate
glycosaminoglycan side chains. Positive COS immuno-
reactivity in cancer and nonmalignant prostate tissues was
detected following digestion with chondroitinase ABC
[0.1 unit/mL in 100 mmol/L Tris acetate buffer (pH 7.5)
containing 0.1% bovine serum albumin, 1 h at 37°C,
Sigma Chemical Co.] and with chondroitinase AC [0.1
units/mL in 50 mmol/L sodium acetate buffer (pH 6.0)
containing 0.1% bovine serum albumin, 1 h at 37°C,
Sigma Chemical]. No immunoreactivity for COS was
detected using chondroitinase B [0.1 unit/mL in 100
mmol/L Tris acetate buffer (pH 8.0) containing 0.1%
bovine serum albumin, 1 h at 37°C, Sigma Chemical].
These findings indicate that the COS disaccharides are

12 C. Ricciardelli, unpublished observation.
Table 1. Summary of clinical and pathology data for the four cohorts used in this study

| Early Stage Pilot Cohort 1* | Advanced Cancer Cohort | BPH Cohort | Patients (n) 55 Median age at diagnosis (y) 64 (range 51-73) Median preoperative serum PSA (ng/mL) 9.0 (range 0.3-47.7, n = 50) Median follow-up (mo) 79.0 (range 36.0-154.0) Gleason score (n)° 2-4 14 5-6 29 ≥7 12 PSA failure rate (n) † 22/55 (40%) |
|----------------------------|------------------------|------------|-------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| Early Stage Cohort 2²      |                        |            | Patients (n) 275 Median age at diagnosis (y) 63 (range 44-76) Median preoperative serum PSA (ng/mL) 9.2 (range 0.2-191.0, n = 260) Median follow-up (mo) 108.5 (range 5.6-260.4) Gleason score (n) 2-4 23 5-6 134 ≥7 115 Unknown 3 PSA failure rate (n) 124/275 (45%) |

*Repatriation General Hospital cohort. 
°Gleason score determined by a pathologist (JS or JK). 
†The presence of micrometastatic disease at the time of surgery for patients was determined by a PSA-failure, i.e. a return to measurable serum PSA levels on two sequential measurements subsequent to a postoperative level below the sensitivity threshold of the assay (0.2 ng/mL). 
²St. Vincent’s Hospital Campus Prostate Cancer Group (SVCPCG) TMA cohort.

present in chondroitin sulfate but not in dermatan sulfate chains. In addition, the predominance of 2B6 staining in tissues treated with chondroitinase B indicated that dermatan sulfate regions in prostate tissues are mostly 4-sulfated (data not shown). The levels of COS disaccharides were also assessed in tissue sections from an independent cohort of patients treated by radical prostatectomy (Early Stage validation Cohort 2), following digestion with chondroitinase ABC. Visualization of the glycosaminoglycan disaccharide epitopes for both cohorts 1 and 2 was achieved with a standard streptavidin immunoperoxidase reaction using biotinylated secondary antibody (Dako Corp.) and diaminobenzidine tetrahydrochloride (Sigma Chemical) to yield an insoluble brown deposit. Negative controls included no pretreatment with chondroitinase enzyme or replacement of the primary antibody with PBS. The immunostaining of each disaccharide epitope was scored manually by two independent observers blinded to clinical outcome in areas identified by urologic pathologists (J.S. and J.K.). The immunostaining intensity in the stroma was scored as strong (3+), moderate (2+), weak (1+), or negative.

Glycosaminoglycan Isolation and Cellulose Acetate Electrophoresis. The limited amount of malignant tissue that is surplus to diagnostic requirements at radical prostatectomy and available for research studies prevented extraction of glycosaminoglycan from early-stage prostate cancer tissues. Glycosaminoglycan was therefore isolated only from archived frozen tissue obtained from men undergoing transurethral prostatic resection for voiding dysfunction (20 from men with advanced cT3N1M1 disease and 19 from men with BPH). Confirmation of tissue pathology for each patient was determined (J.S.) in the adjacent tissue of a portion of resected tissue fragments, which were divided longitudinally following thawing and then formalin fixed and paraffin embedded. For glycosaminoglycan isolation, resected prostate tissues (100-500 mg) were thawed and digested in 20 volumes of 1 mg/mL papain in 0.5 mol/L sodium acetate buffer (pH 5.8) containing 25 mmol/L EDTA and 10 mmol/L cysteine hydrochloride at 60°C for 48 h. Cold (4°C) trichloroacetic acid was then added to a final concentration of 10% and after 1 h the precipitated protein was removed by centrifugation (2,000 × g, 4°C, 15 min). The supernatant was dialyzed against several changes of water for 48 h. The retentate was concentrated by lyophilization and reconstituted in 50 µL water.

Glycosaminoglycans were isolated by precipitation with 4 volumes of cold (4°C) ethanol containing 1% potassium acetate. After 24 h, the glycosaminoglycans were retrieved by centrifugation (2,000 × g, 4°C, 15 min) and reconstituted in water.

Glycosaminoglycans were electrophoretically separated on cellulose acetate membrane (Sepherose II, Pall Corp.) in 0.2 mol/L calcium acetate buffer (pH 7.2) at a constant voltage of 80 V for 105 min. Individual glycosaminoglycans were visualized by staining with Alcian blue [0.2% in 25 mmol/L sodium acetate buffer (pH 5.8), containing 50 mmol/L magnesium chloride and 50% ethanol] for 30 min (22). The separated bands were quantified by laser densitometry. Glycosaminoglycans extracted from prostate tissue were identified by comparison of band migration distances with standard glycosaminoglycans (22). Further identification of individual glycosaminoglycans was facilitated by enzymatic digestion with chondroitinase ABC or hyaluronidase and by nitrous acid hydrolysis.

Characterization of Sulfation Pattern of Glycosaminoglycan Chains. The total glycosaminoglycan extract was batch absorbed to Q-Sepharose and eluted with 2.5 mol/L NaCl to concentrate the glycosaminoglycan preparation. The inherent disaccharide patterns of the chondroitin sulfates and dermatan sulfate components were determined by digestion of 10 to 20 µg glycosaminoglycan with chondroitinase ABC (0.02 units) in buffer containing 50 mmol/L Tris-HCl (pH 8.0) overnight at 37°C (23). Following digestion, four volumes of absolute ethanol was added, and the mixture was placed at −20°C for 4 h before centrifugation at 15,000 × g. The disaccharides remaining in the supernatant (40 µL) were...
separated by high-pressure liquid chromatography on a Partisil-SPAC (5 μm, 25 cm × 4.6 mm id) column, and eluted at a flow rate of 1.0 ml/min, in a mobile phase of 52% acetonitrile, 12% methanol, and 36% aqueous buffer [0.5 mol/L Tris HCl, 0.1 mol/L boric acid (pH 8.0); ref. 24]. This system allows the separation and quantification of disaccharides from chondroitin sulfate and dermatan sulfate. Standard disaccharide preparations of ΔDi-4S, ΔDi-6S, ΔDi-0S, ΔDi-4,6S, ΔDi-HA, and N-acetylgalactosamine, N-acetylgalactosamine-4-sulfate, and N-acetylgalactosamine-6-sulfate were used as controls to characterize the elution profile.

Statistical Analysis. Statistical Package for the Social Sciences version 13.0 (SPSS) was used. The Spearman’s correlation and χ² tests were used to determine correlation between chondroitin sulfate epitope expression and clinicopathologic features. The χ² test was used to determine association between the presence of the modified chondroitin sulfate and malignant disease. The Mann-Whitney U test was used to assess differences between the proportion of disaccharides in nonmalignant and cancer tissues. Relapse-free survival was used as the end point in Cox regression and Kaplan-Meier analyses to determine whether the levels of glycosaminoglycan disaccharide epitopes were related to risk and rate of relapse, respectively, in Early Stage Cohort 2. Relapse-free survival was calculated from the date of diagnosis to the date of progression or the date of last follow-up if progression-free. Four patients who died from other causes were censored on the date of death. Statistical significance was set at P < 0.05.

Results

Glycosaminoglycan Epitopes in Prostatic Tissues. C4S, C6S, and C0S disaccharide moieties were localized to the peritumoral stroma of clinically organ confined prostate cancer tissues (Fig. 1A). Strong immunostaining for C4S disaccharide was present in 50.9% (28 of 55) of tumors whereas strong staining for C6S disaccharide was present in 20.0% (11 of 55) of the cancer tissues. Strong immunostaining for C0S disaccharide was present in 32.7% (18 of 55) of the tumors. C4S disaccharide was observed throughout the stroma adjacent to nonmalignant tissue, whereas C6S disaccharide and unsulfated disaccharide were localized to the periglandular stroma of nonmalignant glands (Fig. 1A). Strong immunostaining for C0S disaccharide was observed in 80% (8 of 10) of advanced cancers, whereas 78% (7 of 9) of the BPH tissues examined exhibited weak or moderate staining levels for C0S (Fig. 1B).

Association of Unsulfated Chondroitin Sulfate Level with PSA Relapse. Kaplan-Meier analyses of the rate of PSA relapse with respect to immunostaining in prostate tissue sections of glycosaminoglycan disaccharide epitope for patients in the Early Stage Cohort 1 are shown in Fig. 2. The levels of C4S disaccharide (Fig. 2A; log-rank statistic = 0.69, P = 0.709) and C6S disaccharide (Fig. 2B; log-rank statistic = 2.11, P = 0.349) were not associated with the rate of PSA relapse. In contrast, the level of C0S disaccharide was associated with the rate of PSA relapse (log-rank statistic = 11.12, P = 0.004; Fig. 2C). Patients with moderate or high levels of C0S disaccharide had a significantly higher rate of relapse compared with patients with low levels of C0S disaccharide. The level of C0S disaccharide was also significantly associated with the rate of PSA relapse in the independent Early Stage Cohort 2 (log-rank statistic = 6.617, P = 0.037, Fig. 3A). Because there were only 13 patients with low levels of C0S disaccharide in this cohort, these patients were grouped with patients with moderate C0S disaccharide levels. Patients with high levels of C0S disaccharide (45.7%, 58 of 129) again experienced significantly more relapses than patients with low or moderate levels of C0S disaccharide (33.6%, 48 of 146, log-rank statistic = 6.25, P = 0.012; Fig. 3B).

High levels of C0S were significantly associated with clinical stage C2 (P = 0.021), pathologic stage pT3 (P = 0.006), the presence of seminal vesicle invasion (P = 0.007), and extracapsular extension (P = 0.011) by χ² analysis, whereas no significant relationship was observed between C0S levels and Gleason score (P = 0.393), presence of positive margins (P = 0.399), nor preoperative PSA levels (P = 0.802). High levels of C0S disaccharide were significantly associated with a 1.60-fold increased risk of relapse following radical prostatectomy (Cox univariate analysis, Table 2A). Comparison with the other statistically significant clinical and pathologic variables by multivariate analysis indicated that the level of unsulfated disaccharide predicted PSA relapse (1.59-fold increased risk) independent of pathologic stage, Gleason score, preoperative serum PSA, surgical margins, seminal vesicle involvement, and extracapsular extension (Cox multivariate analysis; Table 2B).

Chondroitin Sulfate Exhibits Altered Mobility in Advanced Prostate Cancer. Cellulose acetate chromatography indicated that a physicochemical modification to the structure of chondroitin sulfate is present in the majority of advanced prostate cancer tissues compared with the form isolated from BPH tissues (16 of 20 advanced cancers compared with 5 of 19 BPH tissues, P = 0.0001, χ² test; Fig. 4A). The relative mobility coefficient of chondroitin sulfate for advanced-stage cancers was 0.58 compared with 0.69 for BPH tissues. The relative mobility coefficient of dermatan sulfate was unaltered in BPH and cancer tissues (range, 0.48-0.52).

Characterization of Glycosaminoglycan Sulfation Pattern in Advanced Prostate Cancer. The lower migration rate on cellulose acetate chromatography of chondroitin sulfate from advanced prostate cancer tissues suggested a differential sulfation pattern compared with nonmalignant prostate tissues. To investigate this further, isolated glycosaminoglycan chains were digested with chondroitinase ABC and analyzed by high-pressure liquid chromatography (Fig. 4B). The level of ΔDi-0S disaccharides was significantly increased (P = 0.016) in prostate cancer tissues (mean, 7.5%; range, 4.3-16.7%) compared with BPH tissues (mean, 3.7%; range, 3.4-4.2%; Fig. 4C). The proportion of ΔDi-4S disaccharides was reduced in prostate cancer tissue compared with BPH tissues; however, this difference was not statistically significant. The ΔDi-6S disaccharide levels were not altered in prostate cancer compared with BPH tissues (Fig. 4C). Interestingly, an additional unknown peak, which eluted earlier than the ΔDi-0S disaccharide, was observed in all prostate tissues examined.
cancer tissues examined (Fig. 4B), but was absent in BPH. The identity of this peak is unknown as it did not correspond to the elution profile of any of the following additional glycosaminoglycan structures examined, ΔDi-4,6S, ΔDi-HA, GalNAc-4-S, GalNAc-6-S, and GalNAc (data not shown).

Discussion

The mechanisms underlying the progression of prostate cancer from organ confined to locally invasive and ultimately metastatic disease are poorly understood. Previous studies from this laboratory have shown increased levels of chondroitin sulfate and the chondroitin sulfate proteoglycan versican in the peritumoral stromal matrix of prostate cancer patients who relapse after surgical treatment for presumed organ-confined disease (13, 14, 25). Those studies showed that measurement of chondroitin sulfate level may assist in predicting patient outcome following surgery, especially when combined with other clinical and pathologic features such as preoperative serum PSA levels. The present study investigated distinct glycosaminoglycan epitopes and showed that COs disaccharide levels, but not C4S or C6S disaccharide levels, in the peritumoral stroma are significantly associated with rate of PSA failure in
clinically localized prostate cancer. The level of immunostaining for COS disaccharide was found to be a predictor of PSA relapse, independent of the standard clinical and pathologic predictors. In addition, we showed that chondroitin sulfate but not dermatan sulfate glycosaminoglycan chains had a reduced electrophoretic mobility in advanced-stage prostate cancer tissues, probably due to a reduced charge density. These findings suggest that specific alterations to chondroitin sulfate side chains, in particular a decrease in overall sulfation attributed to an increased proportion of unsulfated chondroitin side chains, may be a crucial step for promoting disease progression in prostate cancer.

Figure 2. Kaplan-Meier product limit plots illustrating the relationship between glycosaminoglycan epitope levels and PSA relapse in clinically localized prostate cancer (Early Stage Cohort 1). A. (-----) low C4S (0 or 1+ score), n = 3; (-----) moderate C4S (2+ score), n = 26; or (-----) strong C4S disaccharide immunostaining (3+ score), n = 26, log-rank statistic = 0.69, P = 0.709. B. (-----) low C6S (0 or 1+ score), n = 27; (-----) moderate C6S (2+ score), n = 15 or (-----) strong C6S disaccharide immunostaining (3+ score), n = 29; log-rank statistic = 2.11; P = 0.349. C. (-----) low unsulfated (0 or 1+ score), n = 19; (-----) moderate unsulfated (2+ score), n = 18; or (-----) strong unsulfated disaccharide immunostaining (3+ score), n = 18; log-rank statistic = 11.12; P = 0.004.

Figure 3. Kaplan-Meier product limit plots illustrating the relationship between chondroitin sulfate epitope levels and PSA progression in clinically localized prostate cancer in an independent cohort derived from an independent institution (Early Stage Cohort 2). A. (-----) low unsulfated (0 or 1+ score), n = 12; (-----) moderate unsulfated (2+ score), n = 129 or (-----) strong unsulfated disaccharide immunostaining (3+ score), n = 119; log-rank statistic = 6.617; P = 0.037. B. (-----) Low or moderate unsulfated (0, 1+, or 2+ score), n = 146 or (-----) strong unsulfated disaccharide immunostaining (3+ score), n = 129; log-rank statistic 6.625; P = 0.012.
Increased proportions of ΔDi-0S disaccharides have been observed in laryngeal (26), rectal (19), colon (7), liver (27), pancreatic (10), and head and neck tumor tissues (9) compared with normal tissue. ΔDi-0S disaccharide is the predominant disaccharide found in pancreatic carcinoma (10) and is increased 4-fold in gastric carcinoma compared with normal tissue (28). Furthermore, gastric carcinomas exhibit increased ΔDi-0S and ΔDi-6S disaccharides with a parallel decrease in ΔDi-4S disaccharides. The current study suggests that increased levels of ΔDi-0S disaccharides also occur in prostate cancer. Thus, a common feature of several cancer types seems to be the presence of chondroitin sulfate harboring specific alterations in disaccharide sulfation.

As sulfation is a nonrandom, biosynthetically regulated process, differences between the sulfation pattern of chondroitin sulfate attached to proteoglycans within normal and neoplastic tissues suggest that tumor factors may affect the synthesis and metabolism of chondroitin sulfate by the cells within the peritumoral stroma. Several growth factors that are secreted by prostate cancer cells at increased levels compared with normal prostate epithelial cells, such as transforming growth factor-β1 and platelet-derived growth factor, have been shown to induce structural modification to chondroitin sulfate chains, while simultaneously modulating proteoglycan core protein expression in arterial smooth muscle cells (29). These structural modifications include increases in chain length and changes in sulfation resulting in altered charge density. In addition, insulin-like growth factor-1 is known to be increased in prostate cancer cells compared with normal prostate epithelial cells and to stimulate synthesis of undersulfated proteoglycans by the peritubular cells of the testis (30).

Chondroitin chains are synthesized by chondroitin synthase and chondroitin N-acetylgalactosaminyl transferase. Chain elongation requires GalNAc transferase II and β-glucuronic acid transferase II activities, whereas chain initiation requires GalNAc transferase I activity. Although little is known as to whether these enzymes are differentially expressed in cancerous tissues, α1-3 GalNAc transferase has been shown to be activated during induced pancreatic carcinogenesis in the hamster (31). Several mechanisms may thus be used by prostate cancer cells to regulate both the levels and pattern of sulfation of chondroitin sulfate proteoglycans by stromal cells in the prostate and it is unlikely that observed changes are serendipitous. These mechanisms may comprise down-regulation of enzymes involved in chondroitin sulfate sulfation, such as chondroitin 4-O-sulfotransferase, chondroitin 6-O-sulfotransferase, and chondroitin 4-sulfate 6-O-sulfotransferase by either cancer cells or peritumoral stromal cells under the direction of cancer cells, and result in increased amounts of GalNAc residues that are unsulfated (16).

The current study shows that physicochemical alterations to the structure of chondroitin sulfate in the
prostatic stroma occur during prostate cancer progression and may aid prostate cancer metastasis. Changes in chondroitin sulfate structure may contribute to the efficiency with which chondroitin sulfate proteoglycans, such as versican, destabilize tumor cell focal adhesion and promote cancer invasion (32). Other studies have shown that a lowering of the sulfation level altered the ability of chondroitin sulfate to modulate embryonic cell migration on extracellular matrix in vitro (33). Alterations in sulfation pattern of chondroitin sulfate may well reflect modified biological roles of chondroitin sulfate proteoglycans between normal and neoplastic tissues (34). Although the biological and developmental significance of the different chondroitin sulfate epitopes is not well understood, studies have found that the sulfation profile of chondroitin sulfate chains changes with specific spatiotemporal patterns in various tissues during embryologic development, suggesting that chondroitin sulfate isoforms differing in sulfation position and degree perform distinct functions (35, 36). In particular, the ratio of C4S to C6S varies during normal embryonic development (17) and a change in ratio in favor of C6S seems to be associated with cell proliferation (29). Furthermore, knockdown of the chondroitin synthase gene (by RNA interference) revealed that C0S (the only chondroitin synthesized by Caenorhabditis elegans) is required for embryonic morphogenesis and cell division (37). Interestingly, differential sulfation of heparin sulfate proteoglycans directs retinal axons through the chiasm during optic innervation (38). In addition, heparan sulfate and chondroitin sulfate have been shown to interact in a sulfation-dependent manner with various axon guidance proteins, including slit2, netrin1, ephrinA1, ephrinA5, and semaphorin5B (39). These studies lend support to the notions that glycosaminoglycans are sulfated in complex and changing

Figure 4. Cellulose acetate electrophoresis of papain-digested glycosaminoglycans from human prostate tissue (A). Profile produced by BPH and advanced prostate cancer tissue. The leading peak (relative mobility, RM 0.69 in BPH and RM 0.58 in cancer) was composed of chondroitin sulfate, the middle peak (RM 0.48-0.52) was composed of dermatan sulfate, and the trailing peak (RM 0.39-0.41) was composed of heparan sulfate and hyaluronan (A). High-pressure liquid chromatography elution profile of disaccharides from human prostate cancer tissue following chondroitinase ABC digestion (B). Absorbance was measured at 229 nm. Glycosaminoglycan chains were digested with chondroitinase ABC and analyzed by high-pressure liquid chromatography. Proportion of ΔDi-4S, ΔDi-6S, and ΔDi-0S disaccharides from chondroitin sulfate and dermanan sulfate isolated from nonmalignant, that is, BPH, and malignant (M) human prostate tissues (C). *, the level of ΔDi-0S disaccharides were significantly increased in prostate cancer tissues compared with BPH tissues (P = 0.016, Mann Whitney U test).
patterns and that undersulfation of chondroitin sulfate may be involved in fundamental biological processes involving cell migration.

A reduction in the electrophoretic migration of chondroitin sulfate has been observed in the urine of patients with different malignancies, including prostate cancer, compared with patients without cancer (8). The lower electrophoretic migration of chondroitin sulfate from cancer patients was found to be due to an increased amount of ΔDi-0S disaccharide. A study by Dietrich et al. (8) showed a direct correlation between the relative amounts of the ΔDi-0S disaccharide present in chondroitin sulfate and the stage of malignancy of the cancer. The structural change to chondroitin sulfate occurred early in the development of the tumor and, following surgery and chemotherapy, the structure of chondroitin sulfate in the urine of treated patients was found to revert back to a form similar to that secreted by normal subjects. It is therefore possible that measurement of ΔOS disaccharides in biological fluids may be useful in the diagnosis and follow-up of prostate cancer.

In conclusion, this study supports the tenet that specific glycosaminoglycan alterations occur during tumor development and consequent changes in proteoglycan composition may be a critical step for prostate cancer invasion. Although the physiologic significance of the modifications induced by various growth factors for proteoglycan biosynthesis and particularly undersulfation are not understood, we postulate that changes to chondroitin sulfate structure in the peritumoral stroma of prostate cancer may facilitate cancer invasion by destabilizing focal cell adhesions between prostate cancer cells and the surrounding stroma. Further study of the regulation and biological role of chondroitin sulfate in prostate cancer will improve our knowledge of both the prostate tumor cell microenvironment and epithelial-stromal cell interactions.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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