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The Accumulation of Versican in the Nodules of Benign Prostatic Hyperplasia

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Abstract

Background—Proteoglycans, a complex group of extracellular matrix (ECM) molecules, are elevated in benign prostatic hyperplasia (BPH). Versican is a stromal proteoglycan present in prostate tissue. Versican expression is elevated in tissues with increased proliferation. Based on these observations, we determined the extent and distribution of versican expression in prostates with BPH.

Methods—The involvement of versican in BPH nodules was compared with levels in non-nodular transition (TZ) and peripheral zone (PZ) tissues from 18 human prostate glands using immunohistochemistry, Northern blots and/or QRT-PCR to localize versican and quantify versican mRNA transcript levels, and Western blots to assess gene product levels.

Results—Increased versican immunoreactivity was observed in the stroma of BPH nodules. Higher steady state levels of versican variants V0, V1, and V3 mRNA transcript and gene product were detected in the nodular tissues than in the non-nodular TZ or PZ parenchyma.

Conclusions—These results suggest that versican may play a role in nodule formation in BPH.

Keywords

Prostate hyperplasia; proteoglycans; extracellular matrix; versican

INTRODUCTION

Benign prostatic hyperplasia (BPH), which is a significant cause of morbidity in men, is an anatomic abnormality that is characterized by the formation of nodules that are primarily located in the transition zone (TZ) of the prostate (1). These nodules enlarge the prostate,

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and, in some patients, play a major role in the clinical syndrome termed Lower Urinary Tract Syndrome. This syndrome is characterized by abnormalities in urination, i.e., frequency, nocturia, urgency, and dribbling (2,3).

The pathogenesis of these nodules is unknown. Morphometric analyses of the cell composition of these nodules of BPH have demonstrated an increase in the stromal:epithelial cell ratio and in the extracellular matrix (ECM) compared to the surrounding, non-nodular parenchyma (4–6). Studies of developing urogenital tissue by Cunha (7) have demonstrated that the morphology and cytodifferentiation of the epithelial components of the prostate are induced by the urogenital sinus mesenchyme. These observations support the hypothesis that nodule formation results from abnormalities in control of proliferation of the stromal cells and that epithelial proliferation is a secondary phenomenon.

The role of the ECM in the initiation and/or progression of nodule formation has not been extensively investigated. Proteoglycans are an important component of the ECM. This class of extracellular molecules is present in the stroma of the prostate (8,9). Expression of proteoglycans in the prostate increases in response to testosterone (10,11). Recent studies have also shown that glycosaminoglycans are increased in BPH (12). However, the specific nature of the proteoglycans that are increased in BPH is not known.

Versican is a large extracellular matrix proteoglycan and a member of a family of molecules that form large aggregates with hyaluronan (13). In adults, versican is found in the stroma of most tissues and in some epithelia, i.e. the basal cells of skin (14). Versican levels are increased in tissues with metabolically active cells, such as the developing heart (15,16) and in the stroma of a variety of tumors (17–21). In particular, versican is increased in the stroma of prostate cancer (9,22,23). Recent studies show that transforming growth factor β (TGF β) increases versican expression by cultured BPH stromal cells (24).

Based on the observations described above, we postulated that versican may be overexpressed in the nodules of BPH. This prediction was confirmed by immunohistochemical, Northern blot and Western blot studies on a series of human prostates.

MATERIALS AND METHODS

Tissue Acquisition

Transverse sections were made of 18 radical prostatectomy specimens that were received directly from the operating room. Each prostatectomy was assigned an anonymized case identifier number. One to four separate fragments of grossly nodular parenchyma in the TZ of each prostate were removed by gross dissection. In addition, fragments of adjacent non-nodular TZ tissue and separate fragments of PZ tissue were excised. Each tissue fragment was divided into two parts. One portion was immediately processed for possible Northern and Western blot analyses by freezing in liquid nitrogen. In 8 cases fragments intended for Northern and QRT-PCR analyses were placed immediately in RNeasy Lysis Buffer (Qiagen Inc., Austin TX). The second portion of tissue from 10 cases was fixed in methyl Carnoy's

fixative for histology and for immunohistochemical localization of versican. Sections of all fixed tissue samples were histologically screened for the presence of cancer; samples with cancer were rejected from this study. The collection of these anonymized tissue samples was undertaken with approval of the University of Washington Institutional Review Board.

Immunohistochemistry

Tissues that had been fixed in methyl Carnoy's solution were dehydrated in sequential alcohols and embedded in paraffin. Six-micron thick sections mounted on silane-coated slides were deparaffinized, rehydrated, and stained for versican using a primary polyclonal anti-versican antibody (25) and an indirect avidin-biotin-peroxidase detection method. The sections were sequentially incubated, with interval washes in phosphate-buffered saline, in solutions of polyclonal antibody specific for versican that was diluted 1:500 in phosphate buffered saline, biotinylated goat anti-rabbit IgG, and avidin-biotin-peroxidase complex, respectively. This antibody was raised against human recombinant versican core protein and purified on a versican fusion protein Sepharose column. The antibody does not cross react with other known chondroitin sulfate proteoglycans. It recognizes two principal high molecular weight bands on Western blots that have are identical in size to the calculated V0 and V1 forms of versican from cDNAs.

The signal was detected with a diaminobenzidine tetrahydrochloride hydrogen peroxide solution. The sections were counterstained with hematoxylin. Versican immunoreactivity was assessed by image analysis using Image-Pro Plus v 4.1 (Media Cybernetics, Silver Spring, MD). Twenty-fold final magnification images of all immunostained sections were digitized at one setting with constant lamp illumination to minimize variance consequent to illumination. The images were converted to 256 level grey scale images. The average optical density (OD) of each immunostained section was determined. Mean optical density of all sections of separate nodules from each case was compared with the optical density of the PZ and TZ samples for each case. The average intensity of versican immunoreactivity in the stroma of both nodular BPH and non-nodular samples from both TZ and PZ was semi-quantified by one of the investigators (LDT) on a three point scale – focal (< 10% of the stroma of the nodule stained), partial (between 10% and 60% of the stroma stained), and uniform (> 60% of the stroma stained). A negative control consisted of omission of the primary antibody.

Analysis of Cell Composition

The ratio of stromal cells to epithelial cells in tissues from nodules and from non-nodular parenchyma was determined by counting types of cells in hematoxylin and eosin stained sections. The categories of cells that were separately tabulated were: epithelial, fibromuscular stromal, and hematolymphoid cells. These cell types were enumerated microscopically at 100X magnification in sequential two-millimeter diameter fields until the running mean of the variance of the average number of stromal cells was less than 5%. Since specialized stromal cells such as nerve sheath cells, ganglia, vascular endothelial cells and smooth muscle cells of the vascular media – represented < 1% of all cells, they were not separately tabulated.

Northern Blot Analysis

The frozen samples were first pulverized with a Bio-pulverizer™ (Research Products International Corp., Mt. Prospect, Illinois) and then added to Trizol (Life Technologies Inc. (GIBCO BRL), Rockville, MD). Samples that had been put directly into RNAlater were subsequently put into Trizol. All of these samples were immediately homogenized using a Polytron and frozen at -70°C . These samples were subsequently thawed, chloroform added, and the preparation vortexed for 15 sec. After 2 to 3 min, the tubes were centrifuged and the aqueous phase reserved. This phase was precipitated with isopropanol and washed with 75% ethanol. After air drying, the pellet of RNA was solubilized in RNasecure (Ambion, Austin Texas) and incubated at 60°C for 20 min. The purified total RNA was run on a 1.0% formaldehyde agarose gel, alkali denatured in 50 mM NaOH and 10 mM HCl, neutralized and transferred to a ZetaProbe nylon membrane (Bio-Rad Laboratories, Inc., Hercules, CA). The blots were hybridized with cDNA probes for versican: clone C7, which is specific to the β -GAG region; and clone F, which is specific to the HABR region, were generous gifts from Dr. Erkki Ruoslahti (Burnham Institute for Medical Research, La Jolla, CA). The probes were labeled with ^{32}P -dCTP according to the protocol described by the manufacturer (Ambion Inc., Austin TX). For quantization of mRNA, autoradiograms were normalized to the amount of 28S ribosomal RNA revealed by ethidium bromide stains.

Slot Blot Analysis

A slot blot assay was also used to quantitate the levels of versican mRNA. Total RNA was isolated as described above. Five μg of total RNA was spotted onto a nylon membrane using a 48-well slot blotter (Bio-Rad Laboratories, Inc., Hercules, CA) by the method of Maniatis (26). The RNA was crosslinked to the membrane and then hybridized to the ^{32}P -labeled versican probes described above. For quantization, the blots were stained with methylene blue. Relative levels of the means of versican expression in the following samples were compared using a Student's paired, two-tailed t -test: nodules vs. non-nodular PZ tissue, nodules vs. non-nodular TZ tissue, and non-nodular PZ tissue vs. non-nodular TZ tissue.

QRT-PCR (Quantitative Real-Time Reverse Transcriptase PCR)

DNA-free RNA was obtained from approximately 100 mg tissue homogenized at 15,000 RPM for 30 – 40 sec in lysis buffer using the Total RNA Isolation Kit from Agilent Technologies (Wilmington, DE) following manufacturer's directions. cDNA was prepared by reverse transcription of 1 μg RNA in a 40 μl reaction mix using random primers with the High Capacity cDNA Archive Kit from Applied Biosystems (Foster City, CA). PCR was carried out using an ABI Prism 7900HT Sequence Detection System and TaqMan Fast Universal PCR Master Mix Reagents from ABI as directed by the manufacturer. Copy number of mRNA was determined using the Absolute Standard Curve Method (Applied Biosystems). Standard curves were generated from human versican clones spanning 400–700 base pairs across the respective splice junctions of the variants. Background signal from the other splice variants and non-specific nucleic acid was tested and found to be $< 1.0\%$. For each run, standard curves generated R2 values near maximum efficiency (> 0.9778). Each tissue sample was run in triplicate with all probes on at least two different occasions. Copy number was normalized to total RNA since normal housekeeping genes can be

inappropriate for normalization of hyperplastic tissues (27). However, 18S probes were run for all sets and normalization was similar to total RNA. ABI Gene Expression Assays used are as follows: Versican V0 Hs01007944_m1; Versican V1 Hs01007937_m1; Versican V3 Hs01007941_m1, 18S Hs99999901_s1. Splice junctions for ABI probes are indicated in Figure 1.

Versican Core Protein Analysis

The tissues from six different patients were weighed, diced into small pieces and extracted with 4 M guanidine buffer (4 M guanidine, 0.5 M sodium acetate, pH 5.8) with protease inhibitors (5 mM benzamidine, 100 mM 6-aminohexanoic acid, and 50 mM phenylmethylsulfonyl fluoride (PMSF)) as described previously. The extracts were first dialyzed against 8 M urea buffer (8 M urea, 2 mM EDTA, 250 mM NaCl, 50 mM Tris-HCL, and 5% TX-100 detergent, pH 7.4) and then concentrated and purified by ion exchange chromatography on DEAE Sephacel (Sigma Aldrich, St. Louis, MO) in 8 M urea buffer and eluted with 8 M urea buffer containing 3 M NaCl. Equal amounts of tissue based on wet weight were precipitated in 74% ethanol and digested with chondroitin ABC lyase. Proteins in the samples were resolved by electrophoresis in a 4 to 12% SDS-PAGE gel using a Hoefer SE600 series electrophoresis unit (Hoefer, Inc., San Francisco, CA). Core proteins were identified by staining with Coomassie Blue. Western blot analysis was performed by transferring to 0.2 μ M nitrocellulose membranes (Schleicher and Schell, Keene, NH) using a BioRAD Transblot semi-dry transfer apparatus. The transferred proteins were then detected with a polyclonal rabbit antibody made against recombinant human versican (25) using enhanced chemiluminescence (Western-light Chemiluminescent Detection System with CSPD subcontract; Tropix, Bedford, MA).

RESULTS

General

Of the 18 cases, high quality RNA was obtained from at least one nodule and from both TZ and PZ samples in 16 cases. In one case, the RNA of the TZ sample was degraded, as was the RNA of the PZ sample of another case. If the RNA was not of high quality, versican immunostaining was not undertaken since comparison between mRNA levels, determined by slot blots, and protein levels, determined by immunohistochemistry, could not be undertaken.

Cell Composition of Tissue Samples

The observed epithelial:stromal cell ratio in the nodules ranged from 2:1 to 1:5. This range of ratios overlapped with that observed in the non-nodular TZ tissue (1.5:1 to 1:4) and in the non-nodular PZ tissues (2.5:1 to 1:3.5). There was no evidence of a correlation between the ratios of cell types and the intensity of versican immunoreactivity in the stroma or in the levels of versican mRNA or gene product as determined by the Northern and Western blots, respectively. Sections of nodules could not be histologically distinguished from non-nodular samples based on cell composition or on the histologic features of the epithelial cells in the glands. The histology of the epithelium in glands from non-nodular and

nodular tissues displayed a similar histologic spectrum – from atrophic to hyperplastic (Figure 2).

Immunohistochemistry

Some Versican immunoreactivity was localized to the stroma in all of the tissues examined. No epithelial cells expressed versican immunoreactivity. The stroma in the majority of nodules exhibited more intense immunoreactivity than did the stroma in either TZ or PZ samples (Figure 3). The intensity of the staining in the nodules varied to a moderate degree, both within a nodule and in different nodules from the same patient. Evaluation of staining intensity by optical density confirmed this visual impression; median relative optical densities of the BP nodules was 128 (range 119 – 167), of TZ samples was 156 (range 136 – 174) and of PZ samples was 155 (range 132 – 172). A signed-rank test for paired data compared the difference in OD between TZ and mean BP and PZ and mean BP. The difference between TZ and mean BP was positive in all cases except one ($p = 0.012$). The difference between PZ and mean BP was positive in all cases ($p = 0.004$). This is strong evidence that versican staining is more intense in the BP nodules than in the transition and peripheral zone tissues. A signed-rank test for paired data comparing TZ to PZ showed no significant difference in staining intensity ($p = 0.95$) between these zones of the prostate.

Northern and Slot Blot Analyses

Northern blot analyses revealed major mRNA transcripts for versican in the range of 8kb to 12 kb (Figure 5). These findings are consistent with other studies evaluating versican mRNA in vascular tissue (28–30). In samples from nine of ten patients, slot blot analyses demonstrated that the steady state levels of versican mRNA were greater in the samples of nodular tissue than in both the samples from the non-nodular TZ tissue and from the PZ tissue (Figure 6A). This difference of versican mRNA levels was significant at the $P < 0.04$ level using a Student's paired t -test for both pairs of data. Conversely, there was no significant difference in versican expression levels in PZ vs. TZ tissue. Evaluating this data on a patient-by-patient basis revealed that the versican mRNA steady state levels were consistently higher in the nodule samples than in the corresponding TZ and PZ samples (Figure 6B).

QRT-PCR

Versican is synthesized as four distinct isoforms. The most common isoform V1 consists of two globular domains with a large glycosaminoglycan (GAG) attachment region in the middle. V0 is the largest form with two GAG attachment regions. V2 lacks the large β GAG domain and V3 has only the two globular domains and no GAG attachment region (Figure 1). The major versican splice variant found in prostate was V1 and was significantly upregulated in BPH and to a lesser extent in TZ (Figure 7). V0 mRNA was less abundant than V1 in PZ and BPH, respectively. However, V0 expression in BPH distinguished itself significantly from TZ as well as PZ. V3 was expressed at the lowest levels, yet was significantly different in PZ vs. BPH ($P < 0.01$). Detection of V2 mRNA was rare if at all (data not shown).

Versican Core Protein Analysis

Analyses of the Coomassie blue -stained gels revealed two high molecular weight bands, one of which was the size of the V1 variant of versican, in the nodule extract. These bands were absent in the TZ extract (Figure 8a). Western blot analyses of nodules and TZ samples from two representative patients revealed the presence of two high molecular weight bands at 400 kDa and 350 kDa in both nodular and TZ extracts (Figure 8B), consistent with the presence of the V0 and V1 isoforms of versican (21,30–34). In four of the six patients analyzed, western blot immunoreactivity was significantly greater for the nodule extracts. The antibody was also reactive with multiple bands below 350 kDa; these bands most likely represent breakdown products of versican. There were no consistent differences in the amounts of these lower molecular weight bands.

DISCUSSION

In these studies we have demonstrated that the majority of nodules of BPH are characterized by increased steady state levels of versican protein and versican mRNA relative to the surrounding, non-nodular prostatic tissue. Furthermore, the results of our study indicate that the V1 variant form of versican is the major isoform that accumulates in the prostate transition zone and nodules in BPH while the V0 form also accumulates in the nodules, though to a lesser extent. These results indicate that different forms of versican may be differentially regulated in these diseased tissues and could affect the behavior of the resident cells of the tissue in different ways. Additional and expanded studies are needed both to confirm these observations and to ascertain the functional significance of these differences. Accumulation of versican confirms and extends a previous report that described increased levels of glycosaminoglycans in BPH (12) by demonstrating that a specific proteoglycan, versican, may be a contributor to the expanded stroma in the BPH nodules.

The role of versican in the formation of the BPH nodule can only be a matter of speculation at this time. The tendency of this proteoglycan to be associated with actively dividing cells *in vivo* is consistent with a role in the modulation of proliferative activity in some tissues (14). This possibility is further supported by the observations that versican is synthesized and secreted by proliferating cells in culture and is positively regulated by growth factors such as platelet-derived growth factor (PDGF) and TGF β (24,35,36). Overexpression of versican minigene constructs enhances the proliferation of several different cell types, further supporting a role for versican in cell proliferation (37–39). Whether versican plays a pro-proliferative role in the pathology of BPH remains to be determined by both functional studies and expanded analysis of BPH tissues.

The immunohistochemical distribution of this matrix protein in the nodules indicates that it is synthesized by the stromal fibromuscular cells. It is of interest that Sakko et. al. recently demonstrated that versican synthesis by fibroblasts from prostate is enhanced by prostate cancer cell derived TGF β (40). In addition, recent studies indicate that TGF β increases versican expression by cultured BPH stromal cells while decreasing enzymes that degrade versican such as ADAMTS-1,-5, 9 and 15 (24). A microarray study has identified versican as one of 76 genes to be upregulated in BPH (41). Such results suggest that the accumulation of versican may be regulated by a combination of factors that affect synthesis and

degradation. Furthermore, these results indicate that the stromal cells of both BPH nodules and those associated with tumor may in part be regulated by the epithelia of the prostate. It is generally believed that prostatic enlargement and nodule formation primarily results from hyperplasia of the fibromuscular stromal cells. However, this notion is based on indirect evidence. The observations that are most often cited in support of this notion are: (1) that the ratio of stromal:epithelial cells is increased (42); (2) that the stromal mesenchyme induces epithelial proliferation during development (7); and (3) that the levels of growth factors such as fibroblast growth factor (FGF) and PDGF, which regulate stromal cell proliferation, are increased in BPH (43).

Several models can explain the formation of nodules in prostates with BPH. Nodules could result from the proliferation of a small cluster of cells (or possibly a single cell) at a rate that exceeds the rate of proliferation of cells in adjacent tissue. This hyperproliferative state could be caused by localized changes in the extracellular environment. Alternatively, the progenitor cells of the nodule could be a small sub-population of stromal cells that are hyper-responsive to positive growth signals and/or hypo-responsive to negative growth signals. It has been suggested that these hypothetical progenitor cells may be androgen-sensitive stem cells (44). A third, often cited theory, referred to as the “embryonic reawakening hypothesis,” predicts that reactivation of the inductive capacity of the prostatic stroma is the major pathogenetic mechanism of BPH (45). If a population of progenitor (stem) cells could be identified, a central issue in the study of BPH would be to characterize the mechanism by which they become activated. One possibility is the occurrence of age-related changes in the extracellular environment and in the hormonal milieu within the prostate.

Our observation that the steady state levels of versican mRNA and gene product are higher in nodules is consistent with the presence of a fibromuscular cell population in the nodules that is phenotypically distinct from those cells in the surrounding non-nodular tissue and, therefore, with the hypothesis that these BPH nodules are derived from a sub-population of cells within the stromal compartment of the prostate. Demonstrating differences in the level of versican expression *in vitro* would provide further direct evidence that the increased cellular activity resides in the fibromuscular stromal cells and that the stromal cells in the nodular and non-nodular tissues are phenotypically distinct populations.

Other mechanisms may explain the higher levels of activity of the versican gene in the stromal cells of the nodules. For example, the change in versican gene expression levels could be related to the proliferative age of the nodular stromal cells. Normal human cells are capable of a finite number of cell divisions in culture. This phenomenon is viewed by many investigators as a manifestation of cellular senescence (46,47). Changes in the activity of a number of genetic *loci* occur as these cultures approach the limit of their proliferative potential (48). We have initiated stromal cell cultures from nodular and non-nodular tissues from different donors to determine if there are phenotypic differences between these cell populations that are maintained in culture. In our initial characterizations, we have observed a significantly lower proliferative potential in the cultures derived from nodules (unpublished data).—This attenuated growth capacity of nodules *in vitro* could be due to the

consumption of cell doublings during the formation of these nodules. Thus, it is possible that proliferative age could contribute to the elevated versican gene activity in the nodules.

In contrast to some previous reports, we observed that the stromal:epithelial ratio is extremely variable in the series of nodules that we examined. The reason for this difference is unclear. The relative fraction of gland and stromal cells could be a function of the age of the nodule; i.e., a function of time and, possibly, age-related changes in the stromal cell population. In support of this possibility is McNeal's speculation that at the earliest stage of nodule formation the nodule is composed entirely of stromal cells (45). Of possible relevance is a recent report that near-senescent human fibroblasts promote the growth of tumors more efficiently than younger, actively proliferating cells (49).

CONCLUSIONS

In summary, we believe that the potential significance of the observations reported here are: (1) that versican may be a significant factor in the pathogenesis of nodule formation in BPH and (2) that stromal cells in the nodules are phenotypically distinct from those in the adjacent, non-nodular prostate tissue. Since this is a small study, confirmation on a population basis using large sample numbers is warranted. Experimental confirmation and extension of these observations could provide new insights into the mechanisms of age-associated aberrant cell growth and could identify new therapeutic targets for the treatment of BPH.

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Abbreviations used

BP	nodule of BPH tissue
BPH	Benign prostatic hyperplasia
ECM	Extracellular matrix
kDa	kilodalton
mRNA	messenger RNA
NA	data not available
OD	optical density
PZ	peripheral zone
PDGF	platelet-derived growth factor
QRT-PCR	Quantitative Real-Time Reverse Transcriptase PCR
TZ	transition zone

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Figure 1.

Graphic display of versican isoforms. Exons for V0, V1, V2, and V3 indicate globular domains G1 and G3 and glycosaminoglycan (GAG) attachment domains a and b. Arrows indicate sites of probes for QRT-PCR.

Figure 2.

Cross-section of radical prostatectomy with nodules of BPH. Two nodules in the TZ have been removed. Hematoxylin and eosin stained sections of a portion of each nodule (A, B) and of non nodular parenchyma from both the TZ and the PZ confirm the absence of cancer in these samples and illustrate the variability of cell composition in different tissue samples (microscopic images $40\times$ magnification).

Figure 3.

Immunoreactivity for anti-versican in the stroma of two nodules varied from uniform (A) to partial (B). The non-nodular stroma adjacent to the nodules exhibited focal immunoreactivity (C), as did the stroma of a sample of the PZ (D) (40 × magnification).

Figure 4.

Average relative optical densities of BP nodules compared with paired TZ samples (red circles) and PZ samples (green squares). The range of the OD scale is 1 (darkest) to 200 (lightest).

Figure 5.

Panel (A) is a representative Northern blot of mRNA extracted from a BPH nodule and surrounding non-nodular tissue from the TZ. This blot was scanned and the amount of versican message was normalized to 28 S ribosomal RNA as revealed by ethidium bromide staining. In panel B the dark bars represent data from scans of the 8.12 kb band, and the gray bars represent data from scans of the 5kb transcript.

Figure 6.

Slot bolt analyses of steady state levels of versican message in the indicated tissues from 10 patients using a cDNA probe. These values include all detectable versican isoforms. A: The levels of message from the samples of nodular tissue (median value $\frac{1}{4}$ 0.98 scan units) are significantly higher than those in the non-nodular TZ (median value $\frac{1}{4}$ 0.54 scan units) and the PZ (median value $\frac{1}{4}$ 0.33 scan units) samples. However, the difference in the levels in the non nodular tissues from the TZ and PZ is not significant. B: When these data are

expressed on a per patient basis, the nodules virtually always had higher levels of versican mRNA than the transition zone.

Figure 7.

Expression of versican splice variants V0, V1, and V3 mRNA in PZ, TZ, and BPH. Each point represents a separate tissue sample. Mean values are indicated by horizontal lines. Statistical analysis using one way ANOVA with Tukey's Multiple Comparison Post Test measured significance as follows: V1: PZ versus BPH, $P < 0.001$; TZ versus BPH, $P > 0.05$; and PZ versus TZ, $P < 0.01$. V0: PZ versus BPH, $P < 0.001$; TZ versus BPH, $P < 0.001$; and PZ versus TZ, $P > 0.05$. V3: PZ versus BPH, $P < 0.01$; TZ versus BPH, $P > 0.05$; and PZ versus TZ, $P > 0.05$. Groups were: PZ (n = 12), TZ (n = 13), and BPH (n = 27).

Figure 8.

A: Representative Coomassieblue stained SDS-PAGE gel of proteoglycans isolated from the TZ and from two B PH nodules of prostate after digestion with chondroitin ABC lyase. B: Western blot analysis of proteoglycans extracted from a B PH nodule and from a sample of non-nodular TZ from two different patients. Note more intense staining of versican from the nodule sample.