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Original Article

Stereological evaluation of fibronectin in the periurethral region of the transitional zone from normal human prostates compared with benign prostatic hyperplasia

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Abstract: The aim of this study was to assess the volumetric density (Vv) of the fibronectin in the periurethral region of patients with benign prostatic hyperplasia (BPH) and compare with a control group. Prostatic periurethral tissue samples were obtained from ten patients (age range 65 to 79 years, mean 66) with clinical symptoms of bladder outlet obstruction who had undergone open prostatectomy. The control group samples (periurethral tissue samples from the transitional zone) were collected from prostates obtained during autopsy of accidental death adults of less than 25 years. The volumetric density (Vv) of the fibronectin was determined with stereological methods from 25 random fields per sample using the point-count method with an M-42 grid test system. The quantitative data were analyzed using the Kolmogorov-Smirnov and Mann-Whitney U tests. The Vv in the control and BPH groups was 21.9% ± 1.5% and 29.1 % ± 1.2% in the fibronectin, respectively. BPH tissues presented a significant increase of fibronectin in prostatic periurethral region in the transitional zone that may cause lengthening of the prostatic urethra.

Keywords: Prostate, BPH, fibronectin, immunohistochemistry, stereology

Introduction

The transitional zone (TZ) is particularly relevant for prostate pathology as it is thought to be the main region (periurethral) of the gland which enlarges in benign prostatic hyperplasia (BPH) [1]. This urological disorder, the commonest in men aged ≥ 50 years [2], is associated with complex and not well understood interactions between acinar epithelial cells [3] and their supportive stroma [4].

Several autocrine and paracrine stimuli are involved in these interactions, which ultimately modulate cell proliferation and the expression of stromal extracellular matrix molecules [5]. Epithelial-stromal interactions and stromal extracellular matrix (ECM) components also play key roles in normal prostate physiology and in tumor growth [6, 7].

However, ECM turnover during tumor development can vary according to prostate region, as the TZ expresses fewer metalloproteinases than the peripheral zone [7]. Studies on the pathophysiology of prostate disorders should therefore consider these heterogeneous cellular responses and focus the analyses on specific regions of the gland. This approach is further warranted by findings showing that the different zones of the normal gland have distinct histological and physiological features [9-11].

Therefore, to properly evaluate changes in a given region of the prostate, the control that should be used is the corresponding region of the normal gland. Indeed, if comparisons are not made against these controls, any interpretation or results might be erroneous [11]. Indeed, it was shown recently that BPH nodules caused a significant decrease of elastic system fibers and collagen in prostatic urethra [12]. Despite this evidence showing the different remodeling of the ECM and of the internal glandular heterogeneity, there are few studies on

the structural organization of the human prostatic stroma [14, 15], of which none has specifically analyzed the TZ and its periurethral region. Thus, little is known about the normal morphological organization of the fibronectin in this critical portion of the prostate. In the present study we used stereology to analyze the volumetric density of the fibronectin.

Materials and methods

Ethics procedures

This study complies with the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh, 2000). Our Internal Review Board approved study guidelines. Also, the protocol received approval by the Ethics Committee on Human Research of the State University of Rio de Janeiro. All patients provided written informed consent for the use of prostatic tissue material for research prior to inclusion in the study.

Groups and samples

BPH tissue samples of the TZ with periurethral tissue were obtained from ten patients without symptoms of bladder outlet obstruction (BOO) who did not undergo any treatment for symptomatic BPH. Each patient had undergone open prostatectomy (retropubic or transvesicular). All patients studied had prostates larger than 40g (mean 60), an urodynamic flow rate of 15 ml/s-voiding pressure above the maximum flow rate of 40 cm H_2O, Schaffer nomogram with average degree of obstruction of 4.5 and an International Prostate Symptom Score (IPSS) of 18. Patient age range was 63 to 79 years [mean 66] and all patients had shown pathological diagnosis of BPH with no foci of prostatic carcinoma.

The control samples were obtained from autopsies of young males ranging in age from 18 to 30 years (mean 24), who died of causes unrelated to the urogenital system. After removing the entire prostate cadaver, the fragments (1 × 1 × 0.3 cm) were obtained through longitudinal incisions, parallel to the prostatic urethra in the transition zone (TZ) and median incision in the periurethral region of the prostatic urethra. The methods described in detail previously were respected [13]. The time elapsed between death and fixation of the excised controls was less than six hours.

Immunohistochemistry

The samples of prostatic periurethral tissue were immediately fixed in 4% phosphate buffered formalin solution for 24 hours and then embedded in paraffin. All samples were initially diagnosed by a pathologist (not a coauthor) and then reviewed by a second pathologist to detect any foci of carcinoma and to exclude samples with artifacts.

Tissue sections (3-μm thick) were immunostained using the labeled streptavidin–biotin peroxidase complex system (LSAB2) in a Dako Autostainer (DakoCytomation, Carpinteria, CA, USA). Heat-induced antigen retrieval was carried out for fibronectin (FN) (Dako's Target Retrieval solution, pH 6.1, steaming for 300 at 941C) and sections were incubated with primary antibody (Polyclonal, A0245-at 1:600 dilution - Dako, Carpinteria, CA, USA) for 300-600 at room temperature. After primary antibodies, all sections were blocked for endogenous avidin and biotin by incubating with avidin solution for 200 followed by biotin solution for 200 (Dako's avidin/biotin blocking system, × 0590). Positive controls were normal tonsil for FN. Appropriate negative controls by substituting primary antibody with isotype-matched rabbit IgG were also included.

Stereology

From each prostate, five different samples of the periurethral tissue were taken from the transitional zone. From each sample, five different sections were selected. Five random fields were evaluated from each section, resulting in the analysis of 25 test areas from each periurethral tissue, totaling 250 fields that were analyzed for fibronectin in each group. For the stereological analysis, the analyzed fields were then digitized to a final magnification of × 400 using a video camera coupled to a light microscope (BH-2 Olympus). The selected histological areas were then quantified by applying a test-grid system (M42) on the digitized fields on the screen of a color monitor (Sony). From stereologic principles and methods have been described in detail previously [14, 15].

Statistical analysis

The data were analyzed using the Kolmogorov-Smirnov test to verify normal distribution

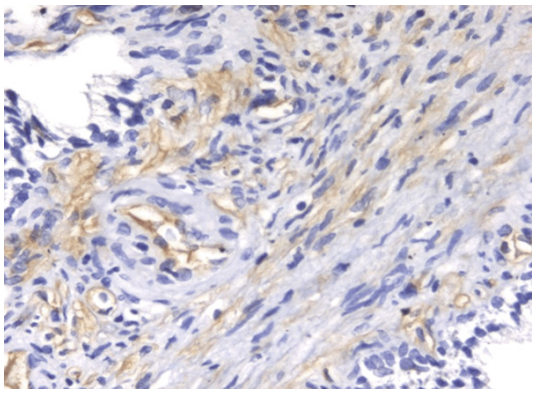

Figure 1. Photomicrograph of a TZ periurethral tissue section from BPH group illustrating intense expression in periacinar area, and dispersed in the stroma. Original magnification × 40.

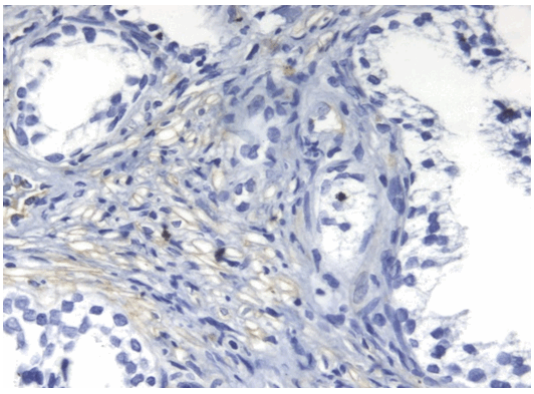

Figure 2. Photomicrograph of a control group showing the fibronectin showed more dispersed immunoreactivity. Original magnification × 40.

(Gaussian) and variance of data, as well as the Mann-Whitney U Tests System to demonstrate whether the Vv differences were statistically significant. $P \leq 0.05$ was considered statistically significant.

Results

Immunohistochemical expression of fibronectin was observed in the interstitial ECM of all prostatic tissues for two groups. In the periurethral tissue of BPH samples showed an intense expression in periacinar area, and dispersed in the stroma (**Figure 1**). In turn, the periurethral tissue of the control group samples showed more dispersed immunoreactivity (**Figure 2**).

The volumetric density (Vv) of the fibronectin in the periurethral tissue of the transition zone of the controls and patients with BPH groups was 21.9% ± 1.5% and 29.1 % ± 1.2% in the fibronectin, respectively (differences statistically significant, P < 0.0001; **Figures 1**, **2**). When compared with the control group, the fibronectin volumetric density (%) was significantly increased in the BPH group (32,8%; $P < 0.0001$).

Discussion

It is well established that the different macromolecules of the ECM combine in various compositions and/or proportions to form connective tissues that vary widely in morphological structure and function. In the human prostate, investigations using different methods showed that the stromal ECM contains collagen types I, III, IV and V, fibronectin, laminin, chondroitin sulphate and heparan sulphate proteoglycans, and elastic fibers [16-18]. This composition is thought to be highly organ-specific [19], and factors from the prostate stroma can, indeed, induce non-prostate epithelial cells to differentiate into a prostatic phenotype [20].

Although apparently homogeneous under light microscopy, the prostate stromal ECM around acini is organized as different structures, *e.g.* fibrous sheets and spongy septa that are made up of dense lamellae, as was particularly evident in the present acellular preparations [21]. The distinct conformations and locations of these structures imply different functions, e.g. supportive scaffold, retention of soluble factors, and regulation of diffusion [22], which should result from locally different proportions of ECM molecular components. The stroma of the periurethral tissue (TZ) of the normal human prostate should therefore be spatially heterogeneous. Moreover, this condition, which facilitates the formation of microenvironments, might be enhanced in disease.

For example, a scanning electron microscopy (SEM) investigation on the density of collagen fibers in the prostatic stroma as a whole showed differences among normal, hyperplastic and malignant tissues, although the anatomical locations of the samples that were examined were not indicated [23]. Also, in BPH the stromal distribution of cytokines differs from that in the normal gland [24], while the periacinar and interstitial stroma in this disease have markedly distinct ECM compositions [16]. Interestingly, this latter finding can be explained by our results on the three-dimen-

sional organization of the normal prostate stroma.

In BPH, acinar epithelial cells are known to release growth factors and cytokines that can exert various effects on stromal cells, including enhanced synthesis of ECM molecules [5, 25]. These paracrine effects depend on diffusion of the factors from the acinar epithelium to the stromal cells through the ECM, which itself acts as a barrier to such diffusion [22]. As stromal lamellae surrounding acini contain elastic fibers [17] and are composed of dense connective tissue, as shown in the present results, diffusion of factors would be more limited, and only stromal cells that are more adjacent to the epithelium should be affected.

Our previous findings therefore support this hypothesis, as we showed that in BPH the expression of chondroitin sulphate proteoglycans is selectively and conspicuously increased in the periacinar region, with little or no labeling in the remainder of the stroma, compared with the TZ of the normal prostate [16]. A denser matrix also makes it more difficult for cells to migrate [26] and as such might contribute to the nodular organization of stromal smooth muscle cells in BPH [27].

The majority of the TZ prostatic urethral injuries have a compressive origin from the BPH nodules [1, 12, 13, 27]. BPH nodular growth occurs in the TZ in > 70% of the cases and compresses the prostatic urethra resulting in BOO [1, 13]. Morphological and quantitative features of BPH nodules are known [3, 4, 17]. It is well known that BOO in BPH results from mechanical obstruction by the enlarged prostate and the tone of the prostate smooth muscles in the prostatic tissue [2, 12, 21].

Presumably, BPH nodules increase urethral resistance, resulting in “pressure” of tissue expansion to the urethra and leads to an increase in outflow resistance, accompanied by characteristic lengthening of the prostatic urethra [2, 13]. As a consequence of these changes, lower urinary tract symptoms (LUTS) may occur.

Our results show that, in the TZ periurethral tissue of the human prostate, dense fibronectin around acini act as a diffusion barrier that might enhance local cellular responses and events that are known to occur in disorders such as BPH. The periacinar stroma also includes a distinct fibronectin, and this supports the notion of high structural variability in this region of prostate.

Indeed, the connective tissue growth factor is clearly implicated in the pathogenesis of progressive BPH. There is much to learn about the production, function, and mechanism of action of connective tissue growth factor. Elucidating the signal transduction pathways activated by connective tissue growth factor will also definitely help to clarify other actions of connective tissue growth factor which may be independent of transforming growth factor-beta [28]. Because of the inflammatory and immunosuppressive properties of transforming growth factor-beta, connective tissue growth factor seems to be an attractive alternative therapeutic target for combating BPH.

In conclusion, BPH caused a significant increase of fibronectin in the TZ periurethral tissue of the human prostate.

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Disclosure of conflict of interest

None.

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