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STRUCTURAL CHARACTERIZATION AND DISTRIBUTION OF ELASTIC SYSTEM FIBERS IN THE HUMAN PROSTATE AND SOME PROSTATIC LESIONS

Patricia S. L. Vilamaior[1], Sueli Suzigan[2], Hernandes F. Carvalho[1] and Sebastião R.Taboga[3]

[1]Department of Cell Biology, Institute of Biology, State University of Campinas (UNICAMP), Campinas, SP,
[2]Department of Morphology, School of Medicine of São José do Rio Preto (FAMERP), São José do Rio Preto, SP,
[3]Laboratory of Microscopy and Microanalysis, Department of Biology, Institute of Biosciences, Letter and Sciences (IBILCE), Paulista State University "Júlio de Mesquita Filho" (UNESP), São José do Rio Preto, SP, Brazil.

ABSTRACT

Prostatic disorders are accompanied by extensive but poorly understood modifications of the cells and surrounding extracellular matrix. In this study, we examined the distribution of the elastic system fibers in prostatic disorders compared to normal tissue. Sections of prostatic transurethral resections and/or radical prostatectomies were examined after staining with hematoxylin-eosin plus fluorescence microscopy and after Weigert´s staining for elastic fibers. Transmission electron microscopy was used to examine the ultrastructure of tissues from radical prostatectomy. A concentric fibrous extracellular matrix and smooth muscle cells were observed surrounding normal acini. The elastic fibers were thin and inconspicuous. In benign prostatic hyperplasia (BPH) the elastic components were of variable thickness and formed a three-dimensional network at the base of the epithelium. Conversely, increased variability in the elastic fiber distribution was observed in adenocarcinomas, depending on the tumor grade. In adenocarcinomas with little differentiation, in some hyperplasic acini, and in the stroma adjacent to tumoral mass, ruptured and residual elastic fibers indicative of matrix degradation or remodeling were seen. In more undifferentiated tumors, a pre-elastic network, perhaps indicative of a new extracellular matrix microenvironment was seen. These results indicate that prostate cancer cell invasion involves extensive remodeling of the fibers of the elastic system.

Key words: Elastic system, extracellular matrix, histochemistry, prostate cancer, stroma

INTRODUCTION

The prostate stroma is an important element in the growth and differentiation of the normal prostate [11,22,41] and is involved in the occurrence of benign prostatic hyperplasia (BPH) and cancer [41]. The stroma influences epithelial function by producing growth factors and by establishing a proper environment with the production of a structurally and compositionally adequate extracellular matrix [37]. The smooth muscle cells and fibroblasts in the prostate produce a number of autocrine and paracrine factors that contribute to organ homeostasis. They also produce extracellular matrix components such as collagen [32], elastic fibers [6], proteoglycans [33,34] and microfibrils [4]. All of these components contribute to the response of the prostate to the circulating and intraprostatic androgen levels [16,27].

There is a progressive decline in the circulating androgen concentration during aging. A decrease in androgen hormone production results in age-related prostatic disorders such as prostatitis, BPH, prostatic intraepithelial neoplasias and prostatic carcinomas [14,23,30]. An altered stromal microenvironment is a common feature of most neoplastic lesions. The stroma may be affected directly (as in BPH) or may react to primary lesions in the epithelium (as in adenocarcinomas) [15,35]. Taboga and Vidal [32] recently described modifications in the collagen fibers of the human prostatic stroma and their reorganization and/or degradation associated with BPH and adenocarcinomas of different grades.

The fibers of the elastic system are particularly important for the structural integrity and function of the prostate, which undergoes cycles of contraction and relaxation during the elimination of accumulated secretion. The elastic system consists of a complex group of proteins, which includes elastin, fibrillin and other microfibrillar proteins [19]. Most of the elastic properties of elastic fibers result from the unique

This paper is dedicated to the memory of our colleague Prof. Gregorio Santiago Montes.

Correspondence to: Dr. Sebastião Roberto Taboga
Laboratório de Microscopia e Microanálise, Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas (IBILCE), Universidade Estadual Paulista "Júlio de Mesquita Filho (UNESP), Rua Cristóvão Colombo, 2265, Jardim Nazareth, CEP 14054-000, São José do Rio Preto, SP, Brasil. Tel: (55) (17) 221-2386; Fax: (55) (17) 221-3390, E-mail: taboga@bio.ibilce.unesp.br

properties of polymeric elastin. However, the ability of elastin-rich structures to deform and subsequently recover also depends on the interactions of elastin with other fibrillar components and with proteoglycans and non-collagenous glycoproteins [7,40].

Cancer cells can interact specifically with elastin via two elastin-binding proteins and galectin-3 [35]. Various studies have suggested that there is a positive correlation between tumor progression and the presence of elastic fibers in the tumor stroma [20]. Although a chemotactic effect of elastin peptides has been demonstrated [20], there is still some controversy as to whether elastin can influence the proliferation of tumor cells.

Many studies have used biochemical and immunocytochemical approaches to characterize the extracellular matrix proteins, glycosaminoglycans and adhesion proteins in prostatic lesions [24,26]. Microscopic and morphometric analyses of prostatic lesions have also been reported [18,25], but have generally not focused on the fibrillar components of the extracellular matrix. Since little is known about the arrangement of the fibrillar components of the stroma and their possible correlation with the biology of prostatic tumors, we have used histochemical and ultrastructural methods to examine the elastic system fibers in human prostatic lesions based on a retrospective analysis of archival biopsy material.

MATERIAL AND METHODS

Archival paraffin-embedded tissues from transurethral resections and radical prostatectomies obtained from 26 patients with BPH and 25 patients with adenocarcinoma, previously diagnosed by pathologists at the Base Hospital of the Medical School of São José do Rio Preto (FAMERP), were used. The patients ranged in age from 52 to 82 years (mean = 58.2 and median = 62). Only patients who had not received anti-androgen or anti-α-adrenergic therapy were included in the study.

Areas of the prostate free of histological or cytological alterations in 12 cases with BPH were used as controls (Table I). We were aware that submicroscopic alterations could not be avoided during sampling these areas. This study was approved by the institutional (UNESP) Ethics Committee.

Histochemistry

Six to 8 µm thick sections were obtained using a Reichert-Jung microtome. Histological sections were dewaxed in xylene and stained with Harris hematoxylin and eosin/phloxine (HE) (10:1, v:v, 1% solutions) in acidified 70% ethanol and mounted in Canada balsam [1]. The sections were examined with Olympus and Zeiss microscopes equipped with a reflected light fluorescence system and filter sets for green and red fluorescence, according to Carvalho and Taboga [3-5]. Weigert´s resorcin-fuchsin procedure [39] was employed after prior oxidation with 1% oxone to evaluate elaunin and oxytalan pre-elastic fibers. This procedure was applied for comparison with the HE plus fluorescence microscopy technique. The imaging software Image-Pro-Plus, version 4.5 for Windows (Media Cybernetics Inc.) was used to capture images.

For each patient, 30 fields were randomly chosen for examination.

Transmission electron microscopy

Tissue fragments from the different prostatic zones (transition, central, peripheral and anterior fibromuscular stroma) were fixed by immersion for 24 h in a solution of 3% glutaraldehyde and 0.25% tannic acid in Millonig´s buffer according to Cotta-Pereira *et al.* [9]. The fragments were then washed in buffer and post-fixed in 1% osmium tetroxide in the same buffer for 1 h. After additional washes, the material was dehydrated in a graded acetone series and embedded in Araldite resin. Sections 0.5 µm thick were obtained with a glass knife and were stained with toluidine blue. After examination by light microscopy, areas of interest, showing different stages of tumor

Table 1. Patient with benign prostatic hyperplasia (BPH) and adenocarcinomas classified according to Gleason´s combined score [12]. The asterisks indicate patients with normal (control) areas of prostatic tissue.

Lesion classification	Case codes
BPH	B93-5936*, B93-5979*, B93-5994, B93-6141*, B93-6187, B94-363, B94-357, B94-390*, B94-433, B94-459*, B94-503*, B94-715*, B94-742, B94-786, B94-5882*, B94-4891, B94-5921, B94-5926, B94-5987*, B95-026, B95-032, B95-060*, B95-120*, B95-160, B95-163, B95-229*
Adenocarcinomas according to Gleason´s score [23]	
Grade 3.3	B93-6312, B94-710, B94-2397, B94-2880, B942502, B94-5889, B94-6198, B94-7610, B94-6272, B95-469, B95-706
Grade 3.4	B94-2410, B94-7542, B95-046, B95-488
Grade 3.5	B93-6364
Grade 4.4	B93-6476, B94-1602, B95-219, B95-693
Grade 4.5	B93-6458, B94-228, B94-5923
Grade 5.5	B94-1378, B94-2930

progression according to the structural aspects described by Gleason [12] were selected and further sectioned with a diamond knife for transmission electron microscopy. Silver sections were stained with uranyl acetate and lead citrate [36,38]. The sections were analyzed and documented using a Leo 906 transmission electron microscope in the Laboratory for Microscopy and Microanalysis at the "Prof. Dr. Celso Abbade Mourão" Microscopy Center of Paulista State University (UNESP) in São José do Rio Preto, SP.

RESULTS

In normal areas of the prostate, the prostatic acini were surrounded by concentric layers of fibrous extracellular matrix and smooth muscle cells (Fig. 1A). Using the eosin-fluorescence method, the elastic fibers appeared as long, thin fibers, arranged amongst the smooth muscle cells (Fig. 1B). This aspect was confirmed using Weigert´s resorcin-fuchsin procedure (Fig. 1C), although this method was less effective for demonstrating thin elastic fibers.

In areas of BPH, there were more elastic fibers which appeared undulating and shorter than in control areas (Fig. 2A-D). Thick elastic fibers were observed around the epithelial acini (Fig. 2E,F).

The normal distribution of elastic fibers was progressively lost as the epithelium became disorganized during tumor progression. In tumors with Gleason scores of 3.3 and 3.4, elastin was observed as a scaffold amongst small epithelial acini (Fig. 3A-D). In undifferentiated, metastatic tumors with Gleason scores of 4.4 to 5.5, the elastic fibers were interrupted and fragmented, and appeared as short patches (Fig. 3E). After previous oxidation of the sections with oxone, Weigert´s resorcin-fuchsin revealed a three-dimensional network of thin pre-elastic fibers amongst the invading tumor cells (Fig. 3F).

Ultrastructural analysis showed that elastic and elastic-related microfibrils were present in all cases, with the pattern of these fibers being identical in normal and hyperplastic areas (Fig. 4A). However, the elastic fibers in the stroma invaded by tumor cells showed signs of elastin degradation (Fig. 4B).

The results described above for each prostatic lesion were consistent for all of the patients in each category.

DISCUSSION

The dynamics of the prostate have been studied during the development and maintenance of tissue physiology [2,10,35] and during remodeling after castration [6,8]. In both situations, important alterations may occur in the stroma reflecting a finely regulated cross-talk between epithelial and stromal cells. These interactions are disturbed in neoplasms [10]. Genetic damage to epithelial cells adversely affects tissue homeostasis and leads to morphological and

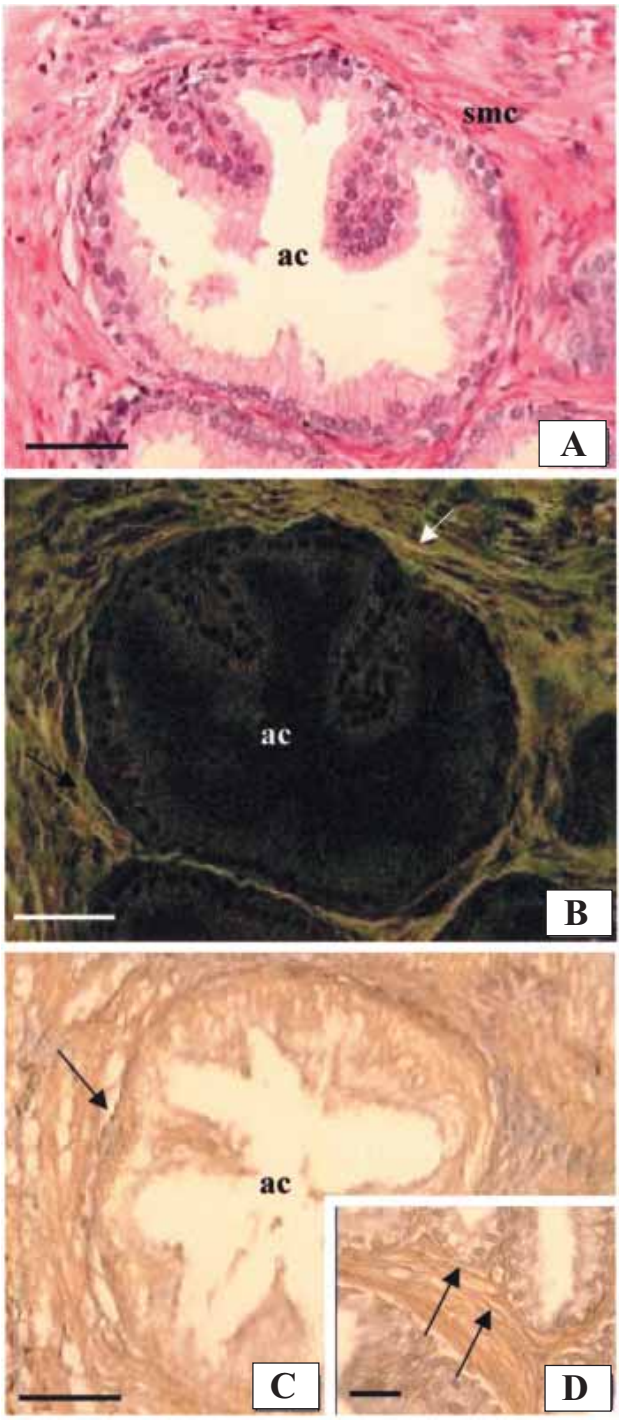

Figure 1. Normal (control) human prostate. (**A**) HE staining, (**B**) HE plus fluorescence green filter, (**C-D**) Weigert´s resorcin-fuchsin staining. The prostatic acinus (**ac**) is surrounded by a concentric fibrous extracellular matrix and smooth muscle cells (**smc**). The elastic fibers are thin and inconspicuous (**arrows**). Bars = 20 µm.

biochemical alterations in stromal cells and in the surrouding extracellular matrix [13,28,32]. However, genetic damage will only increase invasiveness if the extracellular matrix microenvironment is permissive and/or dismantled by the invading cells.

During tumor progression, novel interactions are established between the carcinoma cells and the surrounding stroma. Ronnov-Jessen *et al.* [29] postulated that without the dynamic responses of the stroma, the tumor establishment would be hampered. Bosman *et al.* [2] also suggested the existence of an association between certain fibroblast lineages, but not smooth muscle cells, and the anaplastic growth of colon carcinoma. In the prostate, the detailed mechanisms for these interactions are unknown. In particular, it is unclear whether neoplastic cells can reprogram smooth muscle cells to assume more synthetic or degenerated phenotypes [31], or whether they can induce a local proliferation of fibroblasts [13]. Smooth muscle cells play an active role in modifying collagen fibers in neoplasias [32] and in the stromal reorganization following androgen deprivation [37].

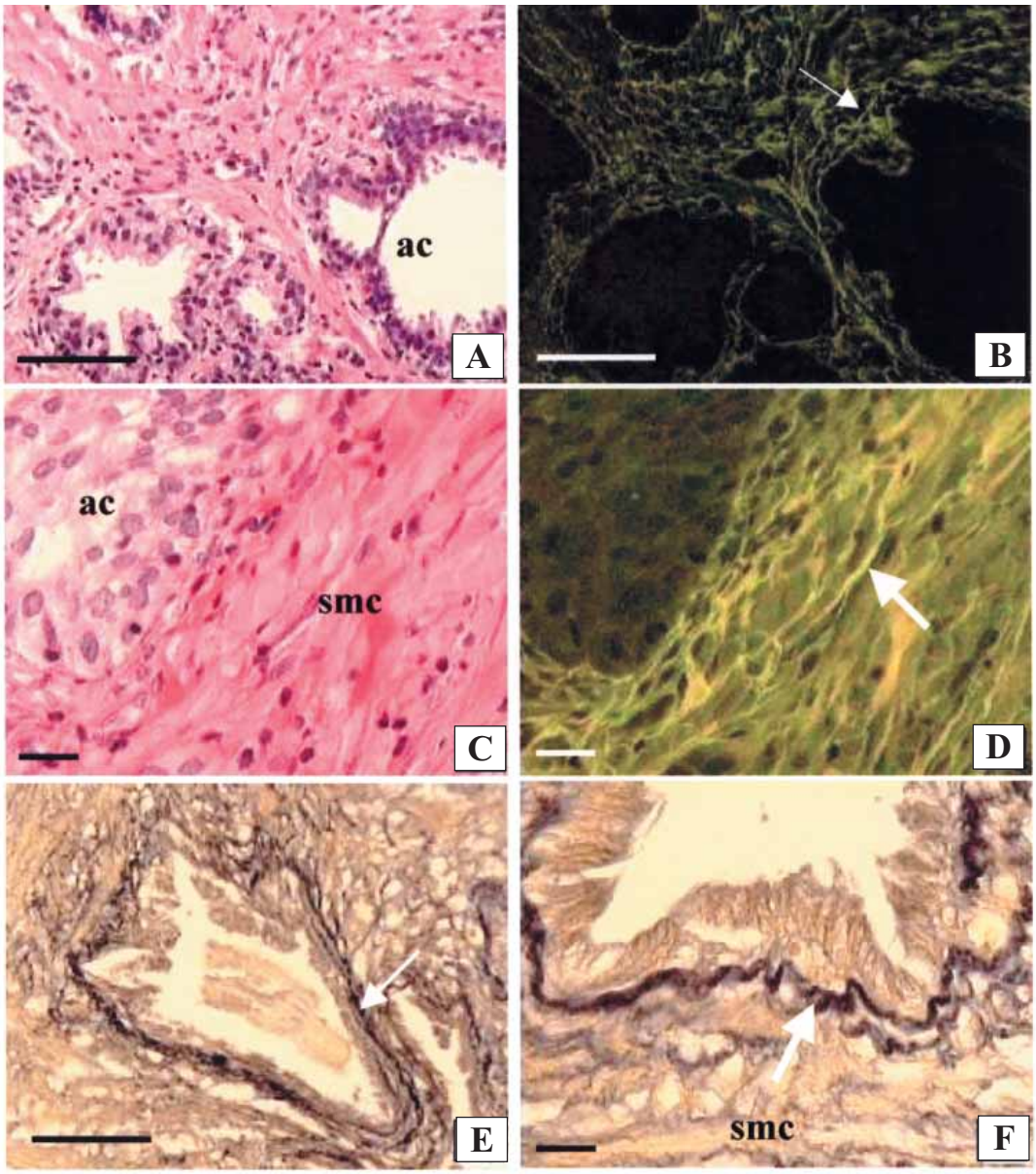

Figure 2. Human benign prostatic hyperplasia. (**A,C**) HE staining, (**B,D**) HE plus fluorescence green filter, (**E,F)** Weigert´s resorcin-fuchsin staining. Note the increase in elastic fibers (**arrows**) at the base of the epithelium, their variable thickness and their arrangement in a tridimensional network. Prostatic acinus (**ac**), smooth muscle cell (**smc**). Bars = 20 μm.

Iozzo [17] proposed that if the extracellular matrix of the stroma acts as a barrier of biomechanical antagonistic forces, agonistic permissive forces can eventually lead to neovascularization and, consequently, to more aggressive tumor phenotypes. In this case angiogenesis would play an important role [30].

Our results suggest that tumor aggressiveness and anaplasia can also be evaluated by examining the fibrillar components of the extracellular matrix. The most aggressive and anaplastic tumor samples studied here had lost the original pattern of elastic fiber distribution and showed a scaffold of thin, branching pre-elastic fibers scattered amongst the invading epithelial cells. A similar reorganization occurs in the hepatic stroma during regeneration [21].These alterations in the elastic system fibers paralleled simultaneous rearrangements of collagen fibers [32].

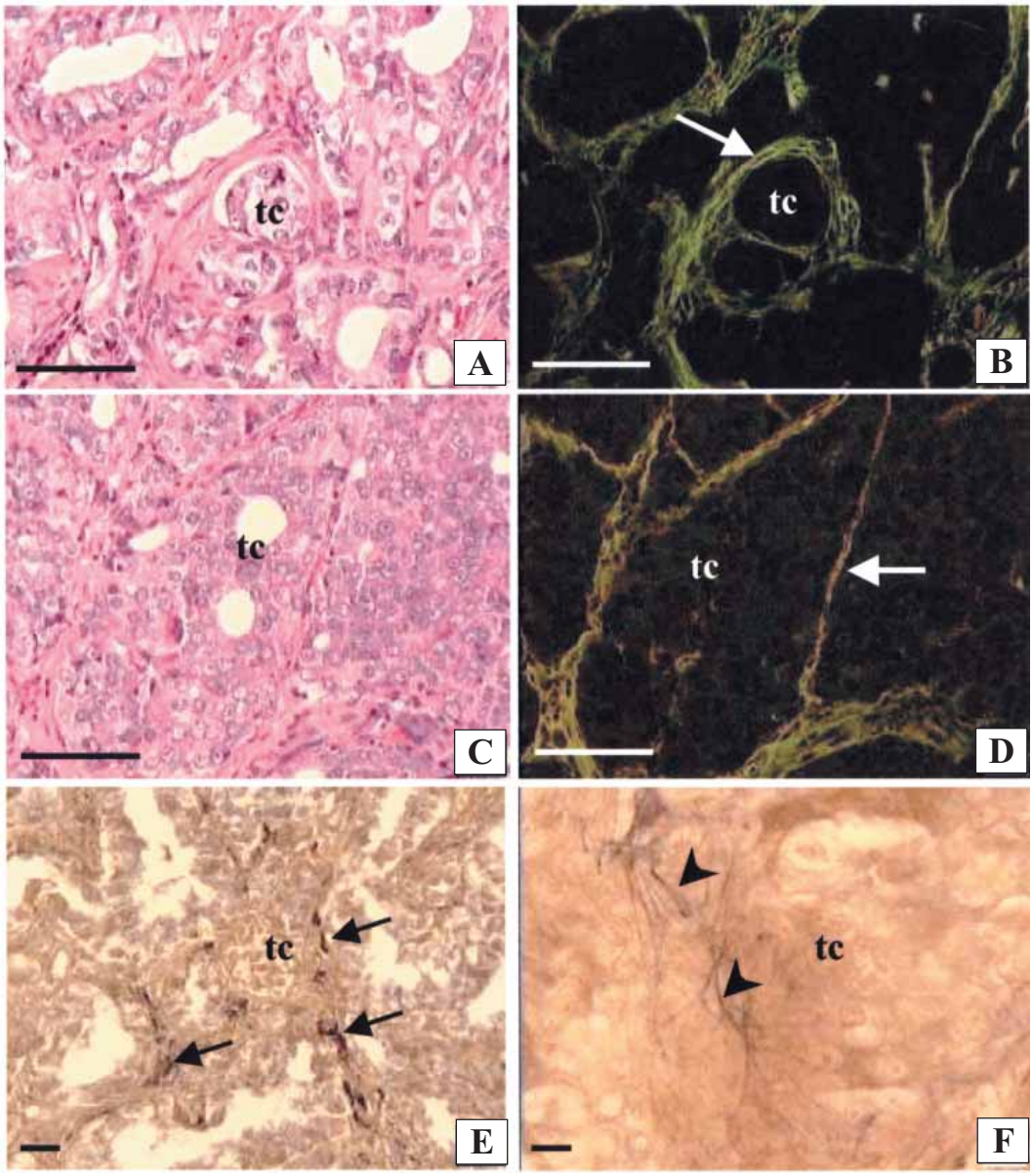

Figure 3. Human prostatic adenocarcinomas. (**A,C**) HE staining, (**B,D**) HE plus fluorescence green filter, (**E,F**) Weigert´s resorcin-fuchsin staining. In some areas, a well-defined pre-elastic (**arrowheads**) and elastic fiber network (**arrows**) delimits the base of transformed cells (low and medium Gleason´s scores). In other areas, the elastic fibers are disorganized and not well delimited between transformed epithelial cells (**tc**) and the surrounding stroma (higher Gleason´s scores). In some areas, elastin degradation and fragmentation can be seen (**E**). Bars = 20 µm.

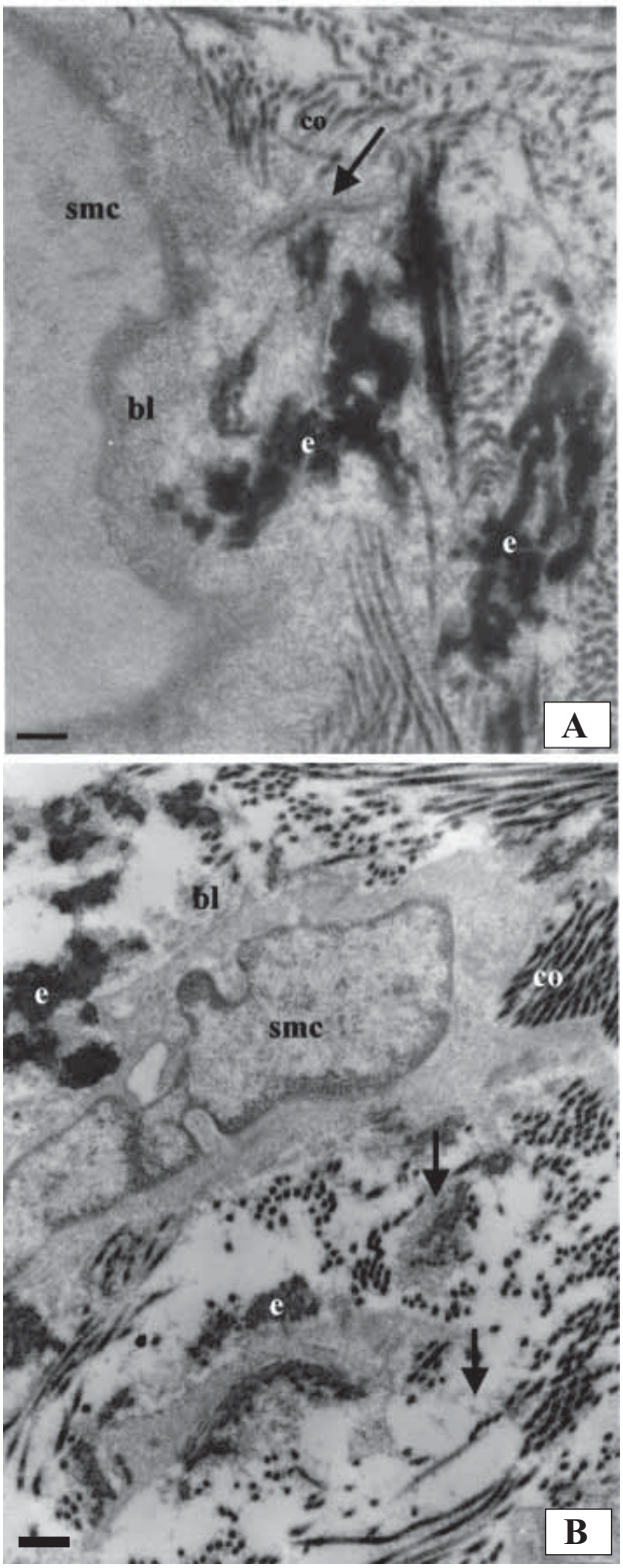

Figure 4. Ultrastructural aspects of the prostatic stroma. Elastic fibers were observed in association with smooth muscle cells in BPH (**A**) and adenocarcinoma (**B**). **SMC** - smooth muscle cell, **bl** - basal lamina, **co** - collagen fibrils, **e** - elastin, **arrows** - microfibrils. Bar = 2 μm.

The substitution of mature elastic fibers in normal tissue by the three dimensional network of pre-elastic fibers seen in invaded stroma most likely involves the degradation of elastic fibers, possibly under the influence of invading neoplastic cells. Accordingly, degraded elastic fibers were usually found close to tumor cells and in areas with a large number of vessels. It remains to be determined which cells produce the new pre-elastic fibers.

Zhang *et al.* [41] recently studied the distribution of elastic fibers in diseased prostate and found elastic fibers in the transition zone, an area usually affected with BPH. Since these authors used van Gieson staining and elastin immunohistochemistry, they were unable to detect the three-dimensional network of pre-elastic fibers described here because elastin is a minor component of these fibers.

The elastic system in BPH was similar to that of normal tissue. The apparent increase in the number of elastic fibers was apparently secondary to the generalized increase in stromal volume, especially in smooth muscle cells. The fibers were wavy and shorter, although this could be an artifact of the plane of sectioning. Elastic fibers and smooth muscle cells may act in concert to preserve the deformability of the prostate in BPH.

The modifications observed here demonstrate that prostate disorders involve extensive remodeling of the elastic system which varies according to the disorder (BPH or adenocarcinoma) when compared to unaffected areas of the prostate.

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