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Lysosomal Localization of Secretory Prostatic Acid Phosphatase in Human Hyperplastic Prostate Epithelium

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Summary. The ultrastructural localization of secretory prostatic acid phosphatase (PAP) in human benign prostate tissue was accomplished using the immunogold technique on ultrathin Lowicryl sections. Polyclonal antibodies directed against secretory PAP (MW 50 kD) and the lysosomal enzymes α-glucosidase and β-galactosidase as well as an antiserum dircted against prostatic antigen (PA) were used. PAP was found in secretory vacuoles of columnar secretory epithelial cells. In addition, double labeling experiments revealed that secretory PAP was also localized in electron-dense organelles of columnar epithelial cells containing α-glucosidase and β-galactosidase. PA was exclusively found in secretory vacuoles of columnar secretory epithelial cells. The results demonstrate the presence of secretory PAP within functional lysosomes and secretory vacuoles of the prostatic columnar epithelial cells and the absence of such PAP-containing lysosomes in the basal cells of the prostatic acini.

Key words: Prostatic acid phosphatase, Lysosomes, Prostatic antigen, Immunoelectron microscopy.

Introduction

Antibodies directed against prostatic acid phosphatase (PAP) and prostatic antigen (PA) are used to identify the prostatic origin of malignant tumors [5, 10]. Prostatic acid phosphatases show a considerable heterogeneity in molecular weight and isoelectric point, but none of these isoenzymes is fully restricted to the prostate [14, 15, 18]. Lysosomal and secretory isoenzymes of PAP can be discriminated on the basis of molecular weights and antigenicity [9, 14]. The secretory moiety of PAP is found in relatively large quantities in prostatic tissues as compared to other tissues [8, 18]. This explains its usefulness in the diagnosis of prostatic cancer and therefore this isoenzyme is sometimes being referred to as prostate specific acid phosphatase [11].

Secretory glands of the prostate consist of columnar secretory epithelial cells and basal cells [4]. Apart from their separate localization these two epithelial cell types have distinct morphological features. Thus, basal cells lack secretory vacuoles and a proportion of these cells contain myofibrils [16]. The differentiation grade of a prostatic carcinoma might be a reflection of the normal maturation pathway of basal cells to secretory epithelial cells. Very recently the exclusive presence of PAP and PA in secretory epithelial cells was demonstrated [16, 17]. The presence of these PAP and PA containing organelles might be indicative of the degree of the differentiation of prostatic carcinoma. In this study we investigated the ultrastructural localization of secretory PAP and of PA in prostatic secretory and basal cells. Since data on the ultrastructural localization of PAP were conflicting (Table 1) we aimed to further clarify the localization of secretory PAP in prostatic epithelia.

Materials and Methods

Transurethrally resected prostatic tissue was obtained from three patients with BPH, minced into 1 mm^3 cubes and immediately fixed in one of three different fixatives:

a) 1% v/v glutaraldehyde in 0.1 M phosphate buffer pH 7.2 at 0–4 °C.

b) 1% v/v acroleine plus 0.4% v/v glutaraldehyde in 0.1 M phosphate buffer pH 7.2 at 0–4 °C (Fix I).

c) 1.5% v/v paraformaldehyde plus 0.1% v/v glutaraldehyde in 0.1 M phosphate buffer pH 7.2 at 0–4 °C (Fix II).

In all cases the material was fixed for 1–4 h. After the primary fixation tissue fixed in "a" was transferred in 0.1 M phosphate buffer pH 7.2 at 0–4 °C for 8 h. Tissues fixed in Fix I or Fix II were transferred and stored in a sucrose buffer of 1 M sucrose in 1.1 M phosphate buffer pH 7.2 with 1% v/v paraformaldehyde at 0–4 °C. The tissue fixed in "a)" was then postfixed in 1% w/v OsO_4 in 0.1 M phosphate buffer pH 7.2 for 12 h at 0–4 °C, rinsed in the same buffer, ethanol dehydrated and Epon embedded for routine transmission electron microscopy. Tissues fixed in Fix I and Fix II were dehydrated in graded ethanol steps while the temperature was

Table 1. Summary of recent data on the ultrastructural localization of prostatic acid phosphatase

Authors	Description of PAP	Localization
Song et al. (1985)	HPAP-2	Golgi, secretory vacuoles
Aumüller and Seitz (1985)	76 kD (lysosomal) 100 kD (secretory)	Lysosomal structures, secretory vacuoles Secretory vacuoles
Warhol and Longtine (1985)	100 kD? (secretory)	Lysosomes
This study	50 kD (secretory)	Lysosomes, secretory vacuoles

progressively lowered. Finally the material was infiltrated with Lowicryl K4M-alcohol mixtures and pure catalysed Lowicryl at −35 °C. Polymerisation took place under UV light at −40 °C for 24 h and at room temperature for an additional 48 h. From the Lowicryl embedded material 1 μ thick sections were cut with glass-knife and stained with toluidine blue to select appropriate areas for ultrathin sectioning. The ultrathin Lowicryl sections were collected on carbon coated Formvar filmed mesh 100 copper grids.

The immunological methods for visualization of the rabbit antibodies bound to the antigenic sites were those described by Geuze et al. [6]. Briefly, grids were incubated with 0.14% w/v glycine in 0.1 M phosphate buffer pH 7.2 for 1 h to quench unreacted aldehyde groups. Then, the grids were incubated for 1 h with a solution of 5% normal rabbit serum in PBS (pH 7.6). After a short washing step in 0.14% w/v glycine in 0.1 M phosphate buffer pH 7.2 the grids were transferred to drops of the appropriate dilution of the respective antisera. After incubation for 1 h at room temperature the grids were washed in 0.14% w/v glycine in 0.1 M phosphate buffer pH 7.2 and incubated with a 10 nm colloidal gold-labeled goat anti-rabbit antiserum (GAR-10 nm) (Janssen, Belgium). For double immunogold labeling protein A gold probes of 5 nm and 10 nm were used (Janssen, Belgium). In the first incubation cycle antibodies specific for α-glucosidase [13] or β-galactosidase [2] (both kindly provided by Dr. A. J. J. Reuser, Dept. of Cell Biology and Genetics, Rotterdam) were tagged with protein A-5 nm gold. An incubation with free protein A (0.1 mg/ml) was performed to block free Fc regions. In the second incubation cycle the anti-PAP antibody (Ortho Diagnostics, Raritan, N.J.) was visualized with protein A-10 nm gold. Control sections were incubated with GAR-10 nm or protein A-5 nm gold only. Background labeling was negligible. Finally the grids were washed in 0.1 M phosphate buffer pH 7.2 at room temperature followed by distilled water and stained with uranyl acetate and lead citrate. Transmission micrographs were made on a Philips EM 300 operating at 80 kV.

Immunoblotting of the rabbit anti-PAP antiserum and the rabbit anti-PA antiserum (Ortho Diagnostics, Raritan, N.J.) on a SDS gel electroforesis of a lysate derived from BPH tissue showed that (in the presence of 2-Mercaptoethanol) the anti-PAP and anti-PA antisera recognized a 50 kD and 34 kD protein respectively (Data not shown).

Results

Figure 1 shows Epon embedded columnar secretory cells with lysosomes and secretory vacuoles.

Immunogold Labeling with Antibodies Specific for Prostatic Acid Phosphatase and Prostatic Antigen

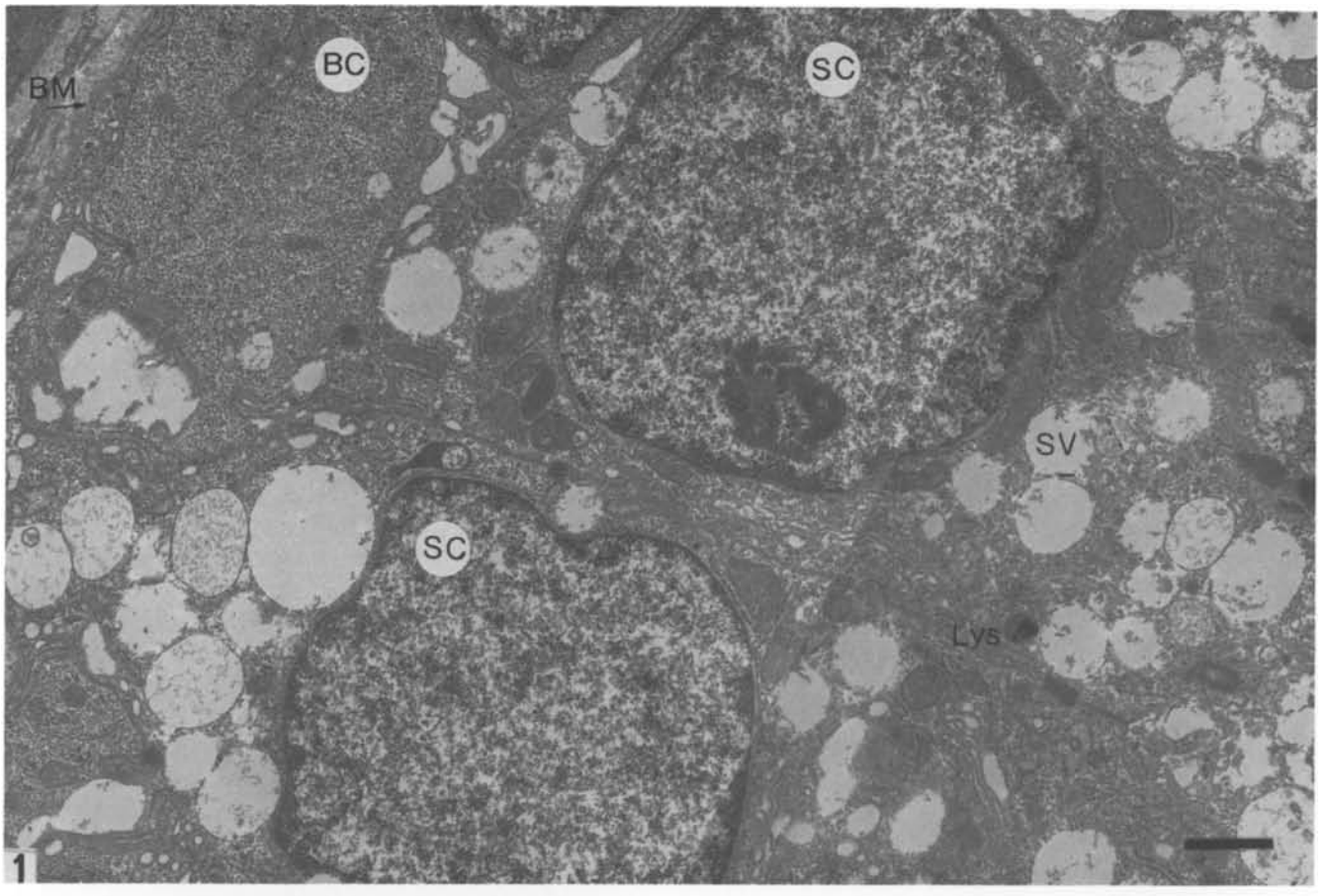
Immunogold labeling of prostatic secretory glandular epithelium with anti-PAP antibody resulted in the occurrence of gold particles over electron-dense spherical lysosome-like organelles (Fig. 2a), multivesicular bodies and secretory vacuoles (Fig. 2b) of secretory cells. Within the secretory vacuoles most gold particles were found at the periphery of the vacuoles, where electron-opaque material was present. At the margins of the glandular lumina gold particles were also present. A slight number of gold particles was found associated with the endoplasmatic reticulum of secretory cells. Labeling of the basal cells was not found, even in organelles resembling lysosomes. Generally fixation in Fix I resulted in a better preservation of morphology but immunolabeling was poor as compared to tissues fixed in Fix II.

Tests with the anti-PA antibody yielded staining of secretory vacuoles and multivesicular bodies of the secretory cells (Fig. 2c). In contrast, the electron-dense organelles remained unlabeled. Some slight gold labeling of the rough endoplasmatic reticulum and the Golgi apparatus was present. No gold label was found in organelles of the basal cells.

In tissue from one patient, a few stromal macrophages in contact with the secretory glands were present. These

Fig. 1. Ultrastructural detail of prostatic gland. Columnar secretory cells (*SC*) with lysosomes (*Lys*) and secretory vacuoles (*SV*) are visible. Also present a basal cell (*BC*) and basal membrane (*BM*). (Epon, Bar = 1 μm) ▶

Fig. 2a–f. Cytoplasmic details of prostatic columnar secretory cells reacted with various antibodies, visualised with colloidal gold. Lowicryl embedding. a Gold (10 nm) marked lysosomes with anti-PAP. (Bar = 1 μm). b Gold (10 nm) marked (*arrow*) secretory vacuoles with anti-PAP. (Bar = 1 μm). c Gold (10 nm) marked secretory vacuoles with anti-PA. (Bar = 1 μm). d Gold (10 nm) marked lysosomes with anti-α-glucosidase. (Bar = 1 μm). e Gold (10 nm) marked multivesicular body (*arrow*) with anti-α-glucosidase. (Bar = 1 μm). f Lysosomes detail with 10 nm gold (*arrow*) with anti-PAP and 5 nm gold (*open arrow*) with anti-α-glucosidase. (Bar = 100 nm)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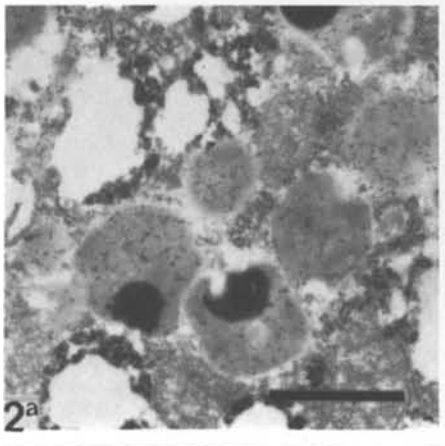
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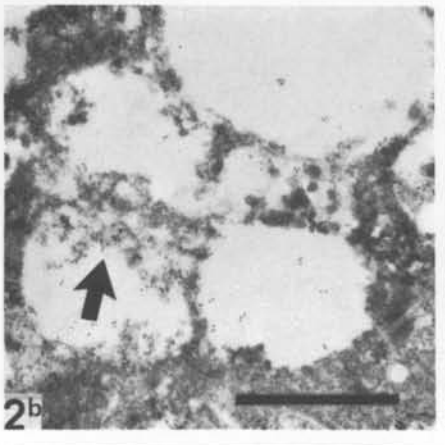
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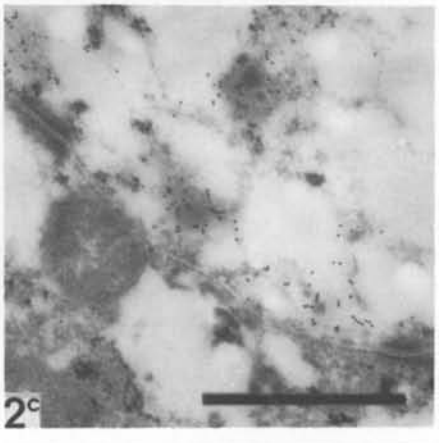
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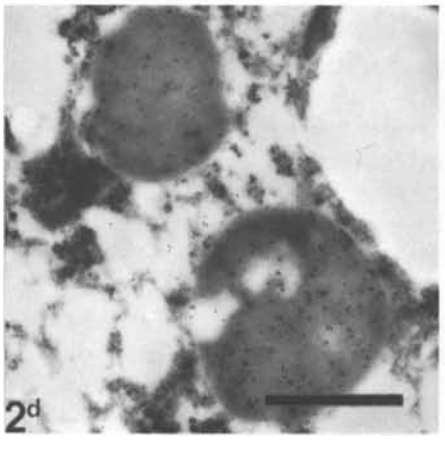
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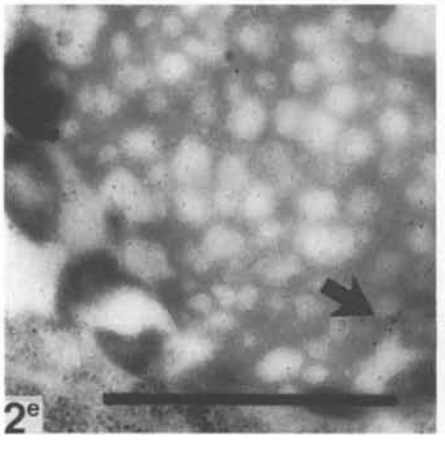
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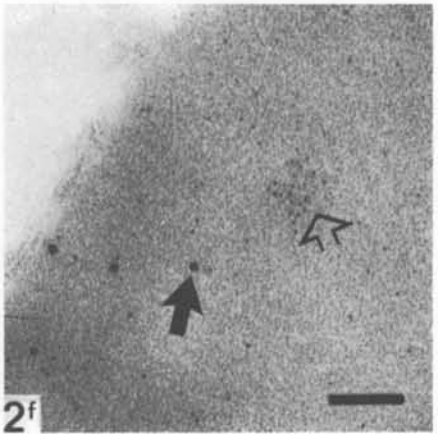
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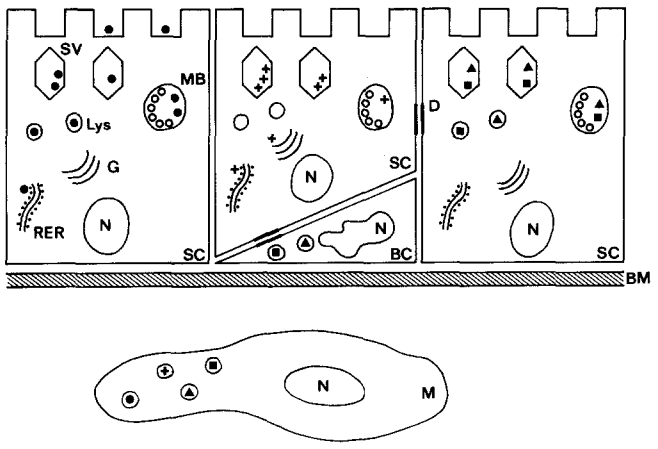

Fig. 3. Scheme of columnar secretory cells (*SC*), a basal cell (*BC*) and a stromal macrophage (*M*) summarizing the gold localization in different cell organelles reacted with various antibodies. Lysosome (*Lys*), secretory vacuoles (*SV*), multivesicular body (*MB*), nucleus (*N*), desmosome (*D*), rough endoplasmatic reticulum (*RER*), Golgi (*G*). Various antibodies: anti-PAP (•), anti-PA (+), anti-α-glucosidase (▲), anti-β-galactosidase (■)

macrophages did show labeling of organelles resembling lysosomes after incubation with antibodies directed against PAP and against PA.

Simultaneous Demonstration of Prostatic Acid Phosphatase and the Lysosomal Enzyme α-Glucosidase or β-Galactosidase

Prostatic tissue fragments were stained for the presence of α-glucosidase and β-galactosidase using GAR-10 nm gold particles for visualization. Both enzymes were found in electron-dense spherical particles localized in secretory as well as basal cells of the prostatic glands (Fig. 2d). Also labeling of the secretory vacuoles and multivesicular bodies of secretory cells was observed (Fig. 2e).

The localization of PAP within lysosomes was further substantiated by performing double labeling for PAP and α-glucosidase or PAP and β-galactosidase. Both combinations were demonstrated in the electron-dense spherical organelles of the secretory epithelial cells (Fig. 2f). The results are schematically summarized in Fig. 3.

Discussion

Prostatic acid phosphatases comprise a heterogeneous group of enzymes and as many as 25 isoenzymes can be distinguished on the basis of isoelectric point [14, 15]. The group of prostatic acid phosphatases can basically be divided into the lysosomal and the secretory form of PAP. The secretory type of PAP is considered to be present in much higher concentration in prostatic tissues than in other cell types. In contrast lysosomal acid phosphatases can be found in many different organs and cell types. This paper demonstrates the localization of secretory PAP within both functional-active lysosomes and secretory vacuoles of the columnar secretory epithelial cells of the prostate. The functional status of the secretory PAP containing organelles is proven by the simultaneous demonstration of PAP and the lysosomal enzymes α-glucosidase and β-galactosidase (Fig. 2f). These observations contrast with those in previous papers locating secretory PAP either within secretory vacuoles or within lysosomes of the prostatic columnar epithelial cells (Table 1). The use of an immunoperoxidase method on frozen material by Song et al. [16] may have precluded the positive identification of PAP within lysosomes due to the presence of endogeneous peroxidase activity. Aumüller and Seitz [1] similarly identified a 100 kD secretory PAP within secretory vacuoles but not within lysosomes. Although these latter authors used epoxy resin the absence of immunoreactivity of lysosomes cannot be ascribed to the insensitivity of their method. With the antiserum used in our study a more intense staining of lysosomes was obtained as compared to the staining reaction on secretory vacuoles (Fig. 2a, b). Since the secretory form of PAP also consists of a heterogeneic group of (iso)enzymes [12, 14] it is likely that the antiserum of Aumüller and Seitz is directed against an enzyme different from the one detected by our antiserum. This possibility is further strengthened by the observation that the anti-secretory PAP antiserum of Aumüller and Seitz strongly cross-reacts with lysosomes of neutrophils, whereas the antiserum used in this study does not show such a cross-reactivity (data not shown). Finally, Warhol and Longtine reported secretory PAP to be present in lysosomes of secretory epithelial cells of the prostate, but these authors did not state its presence in secretory vacuoles [17].

The presence of secretory PAP both within lysosomes and in secretory vacuoles suggests that lysosomes fuse with the secretory vacuoles and empty out their contents in these secretory vacuoles. The finding that the lysosomal enzymes α-glucosidase and β-galactosidase are also both present in secretory vacuoles points to such a pathway. The presence of PA within multivesicular bodies also indicates the transition of lysosomal enzymes into secretory vacuoles as according to Blok multivesicular bodies can be considered to be the end product of crinophagic processes [3]. The only lysosomal localization of PA was found in macrophages, surrounding hyperplastic secretory glands. Via this macrophage-mediated pathway PA can be transported outside the prostatic glands and might eventually reach the blood circulation where it may be detectable serologically, in the absence of malignancy [7].

The presence of α-glucosidase and β-galactosidase containing organelles in the basal cells of prostatic glandular acini indicates the existence of functional lysosomes in these cells. Despite the presence of these organelles secretory PAP is not detected in this cell type. Apparently secretory PAP can be used as a differentiation marker for prostatic epithelial cells, just like PA. Since prostatic carcinoma may contain smaller or larger areas without immu-

noreactivity for PAP or PA [5], we intend to investigate the ultrastructural features of PAP and PA positive and negative prostatic carcinoma cell populations.

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