Benign Prostatic Hyperplasia:

An Ultrastructural Study

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Abstract

Benign prostatic hyperplasia (BPH) is a prevalent disease but its molecular mechanism remains unknown. Using human tissue samples from 16 patients diagnosed with BPH, we performed an ultrastructural study to clarify the mechanism and the role of glandular cells in this pathology. We have made a description of all the changes that suffers the prostatic epithelium. We have shown that the glandular architecture presents many non-physiological forms such as papillae and papillary fronds. Basal cells present a prominent nucleolus, cytoplasmic projections through the basal membrane, with caveolae along them. On the other hand, we have found in luminal cell abundant lysosomal-like granules, lipofuscin and myelinoid bodies in the cytoplasm. Another notable finding is the presence of mast cells surrounding the pathologic glands very close to the basal membrane. Some ultrastructural findings have been reported in induced prostatic hyperplasia in the dog.
Barcelona 16 de July de 2014

To whom it may concern,

By this letter, I grant authorization to present this work as a conference paper to Joan Gil Ortells.

If further details are needed, please do not hesitate to contact me.

Sincerely,

[Signature]

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Introduction

Benign prostatic hyperplasia (BPH) is a very common disease, related to aging, and represents significant burdens for patients and health-care systems in many countries. In men over 60 years of age, 50% have been diagnosed with BPH; and by 85 years of age, 90% of men have symptoms of BPH (1). Despite the prevalence of BPH, its pathogenesis still remains poorly understood.

The human prostate is a muscular and gland male organ. The epithelium of the main prostatic glands contains two distinct cell types: luminal cells and basal cells. Luminal or secretory cells produce an alkaline liquid which function is to neutralize acid vaginal content, contribute to spermatozoid nutrition and condition semen to be less dense (2). On the other hand, basal cells play a role as a progenitor stem cell of the luminal cell lineage (3). The stromal compartment is a complex arrangement of stromal cells and extracellular matrix components. The principal sources of extracellular matrix are fibroblasts, smooth muscle cells, which synthesize the structural and regulatory components; and telocytes which seem to have a role as a stromal stem cell. There are also vasculature, nerves and immune components (4,5).

BPH is a noncancerous enlargement of the prostate gland that can interfere with the normal flow of urine. The specific mechanisms that regulate this enlargement leading to the BPH phenotype are essentially unknown. However, it is becoming clearer that many complex alterations occur that involve chronic inflammatory and wound repair processes. BPH is characterized by a progressive, but discontinuous, hyperplasia of both glandular epithelial and stromal cells leading to expansion of the prostate gland and clinical symptoms (4).

Previous ultrastructural studies with samples from dogs with hormonal induced BPH indicate a central role for basal cells in the early phases of induction of prostatic hyperplasia in the dog. Furthermore the luminal cells also present prominent changes such as irregular microvilli and abundant secretory granules(6,7).

Working hypothesis

Previous ultrastructural studies using dog as animal model suggests that the prostatic acini is where BPH phenotype manifests with more ultrastructural changes (6). Our propose is to keep in this line of investigation but now using samples from human patients with BPH.
Objectives

To determine if the results in human BPH correlate with the animal model and support the dog model as a good model of human BPH.

Material and Methods

This study has been based in 16 human tissue samples obtained by radical prostatectomy (9 samples) or adenomectomy (7 samples). The ages of male patients are comprised between 59 and 80 years, with an average age of 65.17 (±6.04 Standard Deviation). All subjects were diagnosed of BPH, 8 of them had prostate cancer as well, and 1 had prostatic intraepithelial neoplasia concomitant to the BPH. The samples analyzed under the electron microscope were from BPH areas.

Pieces were immediately immersed in 2.5% cacodylate-buffered glutaraldehyde and diced in 1-mm³ cubes. Tissue was postfixed in 2% osmium tetroxide, dehydrated in graded ethanol solutions, and embedded in epoxy resin. Ultrathin sections were prepared with diamond knives, stained with uranyl acetate and lead citrate, and examined under a Philips-FEI-CM100 electron microscope.

Electron microscope images were taken using iTEM Olympus Soft Imaging Solutions GmbH (version 5.2; Münster, Germany) Software.

Results

Glandular architecture is extremely altered in human benign prostatic hyperplasia (BPH). The physiological structure is preserved in few glands that have the normal acinar pattern, with a regular luminal cell layer and a more or less continuous basal layer (Fig. 1A). The vast majority of glands in BPH display pathological shapes with different patterns.

Most of the glands consist of a solid homogeneous multilayer of cells; all cells have the same phenotype (Fig. 1B - 1D). Moreover, there is no evident difference between the basal and luminal cells. Sometimes, the cells usually grow forming papillary fronds devoid of connective tissue (similar to epitheliosis seen in breast gland hyperplasia) (Fig. 1B). In these glands, cell junctions are surrounding cells leaving little or no intercellular glandular space. They can form also papillae with a central axis or core made up of capillary blood vessels and connective tissue (Fig. 1C).
On the other hand, there are glands that have an intermediate state between physiological phenotype and this pathologic phenotype. In this pattern, it is possible to clearly distinguish between luminal and basal cells, but there is more than one layer of luminal cells (Fig. 1F).

However there are not only cases of hyperplastic glands in human BPH, there are also atrophied glands. These glands have lost the luminal cells and consist only of a layer of basal cells compressed by prostatic secretions (Fig. 1E).

**Basal Cells**

The basal cell layer is present in all glands observed although not always continuously. The nucleus usually has an oval shape with different degrees of irregularity and with prominent heterochromatin, corresponding to the hyperchromatic appearance by light microscopy (Fig. 2A – 2C). Although there are some nuclei that are divided into several lobes (Fig. 2B). Nucleoli can often be seen in different regions of the nucleus, either marginal, close to the nuclear membrane, or in a more central location; they have a well-developed nucleolonemama with a continuous reticular pattern (Fig. 2C – 2D). The
cytoplasm occupies only a small portion of the cell volume. It contains profiles of rough endoplasmic reticulum (RER) and mitochondria.

Another remarkable fact is the relationship of these cells with the basement membrane. In most cases, the basement membrane remains intact along the gland, but in most pathological situations, the basement membrane has many irregularities, in these cases basal cell cytoplasm appears to penetrate the basement membrane (Fig. 2C). The presence of caveolae in the basal aspect of most basal cells is also a prominent feature (Fig. 2E).

Luminal Cells

Luminal cells are present in all glands except in the extremely atrophic glands. These cells present the highest degree of diversity in human BPH. The glands that retain physiological architecture are few; most have more than one layer of luminal cells.
The cytoplasmic content is extremely irregular in its electrondensity. This is probably the result of the so-called dark/light cell phenomenon. One of the most remarkable findings of this study is the presence of abundant lysosomal-like granules in the cytoplasm. This seems to reflect or mimic increased lysosomal activity, and most of the granules are very similar to lipofuscin and myelinoid bodies (Fig. 3A). All cells have to greater or lesser extent these residual bodies, but in some of them the phenotype is more similar to the physiological presence of mitochondria in relation to abundant RER and protein secretion granules (Fig. 3B – 3D). The presence of lipid droplets was also quite common.

The presence and amount of microvilli is also one of the characteristics that differ between glands. While there are glands with few microvilli, there are many non-polarized cells, in part perhaps influenced by the section plane (Fig. 3C). However it is not uncommon to see all cells detached from the apical part of the gland. Secretion from these cells is also extremely variable. There are glands that have conventional merocrine protein secretion, while other glands appear to have apocrine secretion, with prominent tufts of apical cytoplasm protruding into the luminal space (Fig. 3C).
Nuclei have highly variable forms, but in all of them occupy most of the cell volume. In most cases, the nucleus presents with a hypochromatic (euchromatin-predominant) and rounded appearance (Fig. 3A), while in other glands, the nucleus appears to have a hyperchromatic (more abundant heterochromatin) content and an irregular morphology, similar to basal cell nuclei (Fig. 3B). Interestingly, nucleoli are found less often than in basal cells. In the few cases in which it can be seen, they are located in a marginal position, in the periphery of the nucleus, and present an irregular shape and a variability of heterochromatin content. The presence of nuclear projections and pockets is also remarkable. In one case, an intriguing finding was an intranuclear spherical body, with granular electron-dense texture, surrounded by a more electron-lucent, concentric membranous ring (Fig. 3D).

In this study we have observed several indications of cell stress; although in the context of all observed glands, the glands that present these indicators are few. The clearest indication can be seen in the mitochondria of both cell types. Swollen mitochondria were observed with cristae lost or displaced to the periphery. We have also seen mitochondria with electron-dense granules within luminal cells (Fig. 2D - 3B - 3D).
**Other cell types**

The presence of other cell types such as mast cells is also remarkable in human BPH. In this study we found mast cells in the prostatic stroma of all patients, and the most striking finding was its presence just a few micrometers away of non-physiological glands (Fig. 4A – 4B). This association between glands and mast cells has not been previously reported in a context of BPH. The presence of macrophages also appears to be common in human BPH. Macrophages can be seen in stroma and glandular cells. We have also identified several macrophages with endocytosis of protein gland secretion (Fig. 4C). In a few cases the presence of granulocytes in glands has been observed, therefore indicating superimposed inflammation (Fig. 4D).

**Stroma**

The prostate stroma had a physiological aspect, except for the capillaries near the glands. These have a high number of filopodia (Fig. 5). This usually indicates an increase in pinocytosis and increased metabolic activity of the endothelial cell. Elastic fibers and collagen present a physiological aspect, as well smooth muscle cells.
**Discussion**

One of the most surprising findings of this study is that the nucleolus is more prevalent in basal than in luminal cells. This supports the idea that in human BPH the more active cells are the basal cells that are involved in the proliferation and differentiation of luminal cells. The presence of multiple nucleoli is a feature of many aggressive tumour cells, and it has been interpreted as the result of nuclear disruption or a defect in nucleolar assembly, as well as the response to increased transcriptional and translational demands. The nucleolus is involved also in the stress sensing mechanism of both normal and pathologic cells (8,9). But, it would be also a product of the section, the basal cells have smaller nucleus so they have more possibilities to be cut in a section that includes the nucleolus. The presence of caveolae in the basal membrane is notable finding in humans but it has been reported also in dogs with hormonal induced BPH, although in this model their presence was more important. Caveolae are specialized lipid raft microdomains 50-100 nm flask-shaped vesicular invaginations of the plasma membrane, which serve as a scaffold for signalling molecules related to cell adhesion, growth and survival. Caveolae are composed of cholesterol, sphingolipids, and structural proteins termed caveolins (6). Caveolin-1 is overexpresssed in prostate cancer and has been demonstrated to be involved in prostate cancer angiogenesis, growth and metastasis (10).

Furthermore, another important finding is the presence of heterogeneous secretory granules in the cytoplasm of the luminal cells, that are different from mucus or zymogen vesicles. Moreover, their appearance remembers to lysosomes and there are other granules similar to lipofuscin and myelinoid bodies, residues of lysosomal digestion. These findings would support the notion that in these cells there is an increased lysosomal activity and autophagy state. However the content of these lysosomal-like granules remains unknown. A possible component may be the prostatic acid phosphatase (PAP) that is a glycoprotein secreted by luminal cells. PAP has been used as a biomarker of prostate cancer. Although its role remains unclear, its function may be associated with the liquefaction process of semen (11). These heterogeneous granules have not been reported in other prostate pathologies. In the normal prostate the cytoplasm is reported as full of electron-lucent vacuoles and lipofuscin bodies are more frequent with aging. In prostatic intraepithelial neoplasia (PIN) the cellular content corresponds to secretory vacuoles, lysosomes and lipid droplets: this gives the cells a mixture of clear and dark appearance. Finally, cells are described as dark cells in prostatic adenocarcinoma because of high concentration of cytoplasmic organelles (12,13). So, taking into account all of this, we can conclude that BPH has some
features in common with aged non-hyperplastic epithelium, PIN and prostate cancer. This would question if the hormonal induced BPH in dogs is a good model of human BPH because of the absence of lysosomal-like granules and the presence of abundant secretory vesicles (6). In any case, further studies are needed to clarify the composition and function of these granules.

The most important quality that defines an epithelial glandular cell is its polarization. However we could see that in some cases, luminal cells have lost it, acquiring a phenotype that resembles them to mesenchymal cells. Some authors explain this by the epithelial–mesenchymal transition phenomenon (14).

The presence of inflammation in BPH can be explained as a consequence of the hyperplastic process. We hypothesize that when the gland is compressed by a process of atrophy, the epithelial barrier is broken and that attract inflammatory cells such as neutrophils. In conclusion, the inflammatory response would be a secondary phenomenon to hyperplasia. Other authors postulate that inflammation may contribute to the development of BPH. They think that the inflammatory injury may contribute to cytokine production by inflammatory cells driving local growth factor production and angiogenesis in the prostatic tissue (15). Maybe the truth is middle way between these two hypotheses, one can explain the beginning of BPH inflammation and the other the progression.

Finally, we found many mast cells surrounding hyperplastic glands, very close to them. There is no information about the role of mast cells in human BPH, but there are some authors that postulate that mast cells increased presence in prostate cancer could play an important role in its evolution (16). But nowadays there is a lot of controversy because some authors postulate that its presence is associated with good prognosis (17) and others argued for the opposite (18). New studies are needed to understand the role of mast cells in prostate, that seems to be quite important.

In summary, our results show the striking heterogeneity of the BPH glands in humans and suggest the importance of the parenchyma in its pathogenesis. Our observations suggest that there is a link between inflammatory cells and specifically mast cells and BPH.
Conclusions

Certainly, the basal cells seem to play a predominant role in hyperplasia. The presence of caveolae is a common finding but it is more prevalent in the animal model. It could be explained because of the hormonal treatment of the dogs that increased the processing and internalization of hormonal signalling. The prominent presence of nucleoli in human basal cells supports that hyperplasia originates from an increase of luminal cells due to the basal cell proliferation.

The appearance of luminal cells is different between humans and dogs. The human luminal cells present a peculiar phenotype with many cell layers, although papillae have been found in both samples. But the cytoplasm component of human luminal cells is unique in BPH, it differs from the other prostatic pathologies and from the animal model.

Finally, we think that the model of induced canine prostatic hyperplasia can provide a very reliable knowledge about the role of the basal cells in human BPH. But there are pathological processes are superimposed in the human prostate, such as inflammation, that are more difficult to reproduce in the canine model.

Thus, the animal model is comparable to human BPH only partially. On the other hand, there is little information on the normal, young male prostate and even less information on the rare cases of aging but not hyperplastic prostate. Therefore, it is important to search for these samples to be able to compare all the changes.

The role of basal cells and some of inflammatory components, particularly mast cells, deserves further study.
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Human Prostatic Hyperplasia: an Ultrastructural Study

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Background
Benign Prostatic Hyperplasia (BPH) is a very prevalent disease affecting more than 90% of men over 85 years. Despite the prevalence of BPH, its pathogenesis still remains poorly understood.

The prostate is a muscular and a secretory gland organ. The secretory epithelium contains two distinct cell types: luminal cells and basal cells. Luminal or secretory cells produce an alkaline liquid which function is to neutralize acid vaginal content. On the other hand, basal cells play a role as a progenitor stem cell of the luminal cell lineage. The stromal compartment is a complex arrangement of stromal cells (smooth muscle and fibroblasts) and extracellular matrix components. BPH is a noncancerous enlargement of the prostate gland characterized by a progressive, but discontinuous, hyperplasia of both glandular epithelial and stromal cells leading to expansion of the gland and clinical symptoms. Previous ultrastructural studies with samples from dogs with hormonal induced BPH indicate a central role for basal cells in the early phases of induction of prostatic hyperplasia. Furthermore the luminal cells also present prominent changes such as irregular microvilli and abundant secretory granules.

Material and Methods
This study has been based in 16 human samples from BPH areas. They were processed for EM and examined under a Philips CM100 electron microscope.

Results

Glandular Architecture
Glandular architecture is altered in BPH. The physiological structure is preserved in few glands (a) and there are glands with more than one layer of luminal cells (f). Most of the glands consist of a solid homogeneous multilayer of cells with the same phenotype. Sometimes gland form papillary fronds devoid of connective tissue(b); and sometimes they forms proper papillae with a central axis made up of capillary blood vessels and connective tissue (c). Some glands are composed by luminal cells that have lost their polarization acquiring mesenchymal-like phenotype (d). However there are also atrophied glands which consist in a single layer of basal cells compressed by prostatic secretions (e).

Luminal Cells
Luminal cells are very diverse. They present abundant heterogeneous lysosomal-like granules (a) and lipofuscin and myelinoid bodies. The amount of microvilli is very variable between glands (b). In one case, an intriguing finding was an intranuclear spherical body, with granular electron-dense texture, surrounded by a more electron-lucent, concentric membranous ring (c).

Basal Cells
The basal cell layer is present in all glands although not always continuously. Basal cells have little cytoplasm (a-c), prevalent nucleolus (d) and cytoplasm projections to the basal membrane (c). The nuclei usually present oval form, but can exhibit very variable forms (b-d). Another remarkable finding is the presence of caveolae along the basal membrane (e).

Conclusions
Certainly, the prominent presence of nucleoli in human basal cells supports that hyperplasia originates due to the basal cell proliferation. The presence of caveolae is a common finding in previous studies using dogs with hormonal induced BPH. The human luminal cells present a peculiar phenotype, the cytoplasm component of human luminal cells is unique in BPH, it differs from the other prostatic pathologies and from animal models. Finally, we think that the model of induced canine prostatic hyperplasia can provide a very reliable knowledge about the role of the basal cells in human BPH.

Thus new studies are needed to understand the role of mast cells in BPH, that seems to be quite important. In summary, our results show the striking heterogeneity of the BPH glands in humans and suggest the importance of the parenchyma in its pathogenesis. Our observations suggest that there is a link between inflammatory cells and specifically mast cells and BPH.

Bibliography