Published in final edited form as:

Differentiation. 2013 ; 85(0): 140–149. doi:10.1016/j.diff.2013.02.006.

Sex steroid receptor expression and localization in benign prostatic hyperplasia varies with tissue compartment

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Abstract

Androgens and estrogens, acting via their respective receptors, are important in benign prostatic hyperplasia (BPH). The goal of this study was to quantitatively characterize the tissue distribution and staining intensity of androgen receptor (AR) and estrogen receptor-alpha (ER α), and assess cells expressing both AR and ER α , in human BPH compared to normal prostate. A tissue microarray composed of normal prostate and BPH tissue was used and multiplexed immunohistochemistry was performed to detect AR and ER α . We used a multispectral imaging platform for automated scanning, tissue and cell segmentation and marker quantification. BPH specimens had an increased number of epithelial and stromal cells and increased percentage of epithelium. In both stroma and epithelium, the mean nuclear area was decreased in BPH relative to normal prostate. AR expression and staining intensity in epithelial and stromal cells was significantly increased in BPH compared to normal prostate. ER α expression was increased in BPH epithelium. However, stromal ER α expression and staining intensity was decreased in BPH compared to normal prostate. Double positive (AR & ER α) epithelial cells were more prevalent in BPH, and fewer double negative (AR & ER α) stromal and epithelial negative cells were observed in BPH. These data underscore the importance of tissue layer localization and expression of steroid hormone receptors in the prostate. Understanding the tissue-specific hormone action of androgens and estrogens will lead to a better understanding of mechanisms of pathogenesis in the prostate and may lead to better treatment for BPH.

Keywords

Benign Prostatic Hyperplasia; Androgen Receptor; Estrogen Receptor alpha

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Introduction

With increasing age, a growing proportion of men will develop enlarged prostates with histologic evidence of benign prostatic hyperplasia (BPH) (Berry et al., 1984). Millions of American men suffer with associated lower urinary tract symptoms (LUTS); the result is billions of dollars in annual healthcare costs (Parsons, 2010; Roehrborn, 2011). While much remains to be learned about the basic biology of BPH, it is a heterogeneous disease and the histologic variability among BPH patients makes personalized therapies a possibility.

Androgens acting via the androgen receptor (AR) are important in BPH, and a mainstay of contemporary medical management for BPH is the use of 5-alpha reductase inhibitors (5ARI) that inhibit the metabolism of testosterone to the more potent AR ligand, dihydrotestosterone. These drugs prevent the progression of LUTS, reduce the risk of BPH complications such as urinary retention, and decrease the need for surgical treatment in some patients (McConnell et al., 2003). However, 5ARI are not effective for all patients, and a concern with these drugs is undesirable side effects such as gynecomastia and sexual dysfunction. Unfortunately, *de novo* erectile dysfunction with 5ARI therapy is typically persistent for the up to 20% of men who experience that disturbing side effect (Irwig, 2012).

In addition to androgens, estrogens are also important in prostate development, and have been implicated in BPH (Prins and Korach, 2008). *In vivo* models of BPH suggest that androgens and estrogens may act in synergy to induce prostate growth (Coffey and Walsh, 1990; Kumar et al., 2012). Estrogens mediate their effects via estrogen receptors; the subtype estrogen receptor alpha (ER α) is necessary for induction of prostate proliferation with estradiol and is considered a key mediator of prostatic epithelial proliferation (Risbridger et al., 2001). Selective estrogen receptor modulators (SERMs) are a promising new treatment strategy for targeting estrogen pathways implicated in BPH. A recent report indicates that SERMs in combination with 5ARI may be particularly promising for decreasing prostate cell proliferation in BPH (Kumar et al., 2012). Therefore, a better understanding of the localization and relative quantification of ER α expression may be helpful in selecting compounds to target ER α signaling in the prostate.

While it is widely reported that stromal and epithelial cells express AR in BPH, many studies have detected little or no ER α expression (Alonso-Magdalena et al., 2009; Brolin et al., 1992; Ehara et al., 1995; Hetzl et al., 2012; Royuela et al., 2001; Schulze and Claus, 1990; Tsurusaki et al., 2003). To our knowledge, no studies have quantified tissue specific expression or colocalization of AR and ER in the same cell. Furthermore, traditional analyses of histologic and immunohistochemical markers are subjective and vulnerable to inter- and intra-observer variation and error.

A better understanding of the tissue distribution of cells expressing AR, ER α or both hormone receptors could provide insight into how drugs that affect the activity of these receptors could be used to target abnormal prostate growth in BPH. Furthermore, understanding the tissue specific hormone receptor status of a patient might be used to personalize hormonal therapies for BPH. Utilizing the an automated image analysis platform and a previously validated prostate tissue microarray (TMA) (Huang et al., 2012), we evaluated and quantified the localization and expression of AR and ER α in BPH compared to normal prostate.

Materials and Methods

Tissue microarray construction

The prostate TMA used in this study has been previously described (Warren et al., 2009). We recently validated quantification of spatially overlapping biomarkers with this TMA using chromogenic multiplexed immunohistochemistry (IHC) (Huang et al., 2012). Benign normal prostate was obtained from prostatectomy specimens from patients who were not treated with hormonal therapies (N = 104 duplicate cores from 52 patient specimens); the zone of the prostate as defined by McNeal (McNeal, 1988) was not determined at the time of tissue harvest, but specimens included both transition and peripheral zone tissue. BPH tissue was transition zone from patients with LUTS who underwent transurethral resection of the prostate (TURP; N = 48 duplicate cores from 24 patient specimens). All BPH patients had a history of LUTS; clinical indications for TURP included history of urinary retention and failure of medical therapy. Cores (0.6 mm in diameter) were placed on the recipient microarray block 0.2 mm apart vertically and horizontally using a Manual Tissue Arrayer (Beecher Instruments, Sun Prairie, WI, Model MTA-1). All tissue cores were evaluated by a genitourinary pathologist (WH) and scored for the presence of atrophy, defined as thinning of glandular epithelium, with or without diminished gland size.

Multiplexed IHC

The staining protocol with Vectra platform was performed as previously described (Huang et al., 2012). Antibodies to AR (1:50, Biocare Medical LLC, Concord, CA) were conjugated with Warp Red chromogen (Biocare), ER α (1:400, Lab Vision, ThermoFisher Scientific, Kalamazoo, MI) with Betazoid DAB chromogen (Biocare), Smooth muscle alpha-actin (ACTA2, 1:600, AbCam, Cambridge, MA) with Vina Green chromogen (Biocare), and counterstaining was performed with hematoxylin (HT, Biocare).

Automatic Image Acquisition and Analysis

Multispectral images (8-bit) acquired by the Vectra platform (Perkin Elmer, Waltham, MA) (Huang et al., 2012) were processed by Nuance 30.0 software (Perkin Elmer, Waltham, MA) to build unique spectral curves for each of the four chromogens, and then unmix the signals of multispectral images. To segment epithelium vs. stroma, InForm™ 2.1 software (Perkin Elmer, Waltham, MA) 18% of the total images were trained by a single genitourinary pathologist (WH). The total number of epithelial and stromal cells, the ratio of epithelial and stromal cells, and the area of the tissues were compared between normal prostate and BPH cores. AR and ER α expression were quantified as the percentage of positive nuclei divided by the total number of nuclei in the tissue compartment (stroma and epithelium). Staining intensity as a measure of AR and ER α expression was quantified by the optical density of the respective chromogen per unit area in pixels. Cells positive for both AR and ER α were counted with colocalization analysis using Nuance software. Mean nuclear size was calculated by dividing the total area of the nuclear tissue compartment measured in pixels (epithelium and stroma) by the number of cells in the respective tissue compartment. The area was then converted from pixels into μm^2 . The percent nuclear area was expressed as the area composed of nuclei within a tissue compartment (epithelium and stroma), divided by the total area of the tissue compartment. The following cores were excluded from analysis: less than 5% epithelial component, significant tissue loss or folding, and images with more than 5% poorly segmented nuclei.

Statistical analysis

Normal prostate and BPH were compared with a two-tailed Student's t-test for continuous variables and two-tailed Fisher's exact test for proportions. Correlation of AR and ER α

expression in tissue compartments was evaluated with simple linear regression. Type 1 error was defined as $\alpha < 0.05$ and statistical analysis was performed using GraphPad Prism (Graph Pad Software, Inc., La Jolla, CA).

Results

Patient Demographics, Atrophy, Tissue and Cell Segmentation

As shown in Table 1, BPH patients were older on average than normal prostate patients (69 vs. 61 years, $P = 0.0002$). The majority of patients in both groups were White/Non-Hispanic race (1 of 52 normal prostate patients self-identified as American Indian, Table 1). The prevalence of atrophy was similar among normal prostate (31%) and BPH patients (21%, $P = 0.4212$, Table 1).

Figure 1 shows unmixed images for each chromogen and multiplexed IHC image. As shown in Figure 2, Inform software was trained to segment stroma from epithelium, and automated cell quantification was performed for each tissue compartment (Figure 2A). Tissue segmentation analysis showed an increase in the average percent area of epithelial tissue in BPH samples ($P = 0.0409$; Figure 2B). There was no difference in the percent area of stromal tissue (relative to epithelium and other as shown in Figure 2A). Cell segmentation analysis (Figure 2A, right panel) revealed an increase in the number of epithelial cells ($P = 0.0018$; Figure 2B) and stromal cells ($P = 0.0014$; Figure 2C) in BPH compared to normal prostate. However, the proportions of epithelial cells and stromal cells were not significantly different in BPH compared to normal prostate (Figure 2B-C). In the epithelium and stroma, the mean nuclear size was decreased in BPH specimens relative to normal prostate (Table 2). There was no difference in the percentage of the tissue compartment that consisted of nucleus (% nuclear area, Table 2) when BPH was compared to normal prostate.

AR localization, intensity and tissue distribution

AR was localized to stromal and epithelial cell nuclei as we have described previously (Huang et al., 2012). As shown in Figure 3, overall, there was an increase in the percentage of AR positive cells ($P < 0.0001$, Figure 3A&B) as well as AR staining intensity in BPH ($P < 0.0001$, Figure 3A&B) compared to normal prostate. There was an increased percentage of AR positive epithelial ($P < 0.0001$, Figure 3A&C) and stromal ($P < 0.0001$, Figure 3A&D) cells and increased AR staining intensity in the epithelium ($P < 0.0001$, Figure 3A&C) and stroma ($P < 0.0001$, Figure 3A&D). Linear regression analysis revealed a distinct relationship among the percentage of AR positive cells in the stroma and epithelium in BPH compared to normal prostate (Figure 3E). The percentage of AR positive cells in the stroma and epithelium was positively correlated in normal prostate cores ($m = 0.04085 \pm 0.00752$, $r^2 = 0.3712$, $P < 0.0001$) and BPH cores ($m = 0.3086 \pm 0.08548$, $r^2 = 0.3720$, $P = 0.0016$; Figure 3E). However, the slope of the relationship was significantly greater in BPH relative to normal prostate ($P < 0.0001$, Figure 3E).

ER α localization, intensity and tissue distribution

Overall, there was no difference in ER α expression or staining intensity in BPH compared to normal prostate (Figure 4A&B). However, in BPH we observed an increased percentage of ER α positive epithelial cells ($P = 0.0454$, Figure 4A&C) that were predominantly basal cells, but no difference in ER α staining intensity (Figure 4A&C). In the stroma, there was a decrease in the percentage of ER α positive cells in BPH relative to normal prostate ($P = 0.0008$, Figure 4A&D) and a decrease in ER α staining intensity ($P = 0.0003$, Figure 4A&D). Linear regression analysis showed that the percentage of ER α positive cells in stroma vs. epithelia was positively correlated in normal prostate ($m = 1.579 \pm 0.4384$, $r^2 = 0.2059$, $P = 0.0007$) and BPH ($m = 0.2106 \pm 0.0556$, $r^2 = 0.3948$, $P = 0.0010$; Figure 4E).

However, the slope of the linear relationship was significantly less in BPH relative to normal prostate ($P = 0.001$, Figure 3E).

Colocalization, intensity and tissue distribution

In BPH epithelium, cells that were double positive for ER α and AR were more prevalent compared to normal prostate ($P = 0.0018$, Figure 5A&B). There was a corresponding decrease in epithelial cells that were double negative for ER α and AR in BPH compared to normal prostate ($P < 0.0001$, Figure 5B). In the stroma, there was no significant difference in the proportion of double positive cells in BPH (Figure 5A&C). However, in BPH stroma, fewer cells were double negative for ER α and AR compared to normal prostate ($P = 0.0008$, Figure 5C).

Discussion

To our knowledge, this is the first study to objectively quantify relative tissue areas, cell counts, expression of AR and ER α , and colocalization of sex steroid receptors in glandular BPH. We found an increase in the ratio of epithelium to stroma and an increased number of stromal and epithelial cells in BPH specimens, but no differences in relative cell composition compared to normal prostate. The size of the nucleus was on average smaller in epithelial and stromal cells of BPH compared to normal prostate. We also found an increased percentage of AR positive cells and increased AR intensity in both epithelial and stromal cells in BPH compared to normal prostate. While overall, BPH and normal prostate had a similar percentage of ER α positive cells, there was increased expression of ER α in epithelial (primarily basal) cells of BPH tissues, but a decrease in BPH stromal ER α expression. An increased percentage of epithelial cells were double positive for AR and ER α in BPH. Taken together, the decreased prevalence of double negative cells in BPH epithelium and stroma, as well as the increased percentage of AR, epithelial ER α and double positive cells in BPH, suggest that there are more sex steroid hormone responsive cells in BPH compared to normal prostate.

Prior to the existence of automated software for quantification of biomarkers, studies of cell and tissue segmentation in BPH relied on manual or semi-quantitative methods. Therefore, few quantitative reports exist characterizing stromal and epithelial cells in BPH. The present results indicate an increase in the absolute number of stromal and epithelial cells per core in glandular BPH versus normal prostate. This relative increase in cell count is consistent with hyperplasia. Our finding of an increased percentage of epithelial tissue area is somewhat surprising, as many consider BPH a primarily stromal disease (Ho et al., 2008). With respect to tissue area, the present results are similar to reports of 16-17% epithelium in BPH specimens (Veltri et al., 2002).

While malignant nuclei have many characteristic features (Dey, 2010), nuclear morphometry in BPH has not been well characterized, and is rarely compared to normal prostate nuclei. Our finding of $32.50 \mu\text{m}^2$ as the mean epithelial nuclear size in BPH is consistent with other reports of $25.4 \mu\text{m}^2$ (Montironi et al., 1993) and $33.87 \mu\text{m}^2$ (Choi et al., 1999). Treatment of prostate adenocarcinoma with androgen deprivation therapy results in small, dense nuclei relative to the large nuclei observed in untreated prostate adenocarcinoma (Dey, 2010). This suggests that relative decreases in androgen exposure would result in decreased nuclear size. We found a decrease in the size of epithelial and stromal nuclei in BPH compared to normal prostate. While the finding of increased AR expression indicates that BPH cores have greater sensitivity to androgens, smaller nuclear size in BPH epithelia could represent decreased androgen exposure, which is known to occur in men as they age (Belanger et al., 1994).

It is generally accepted that in the prostate, AR is found in the nuclei of luminal epithelial and stromal cells. Many studies have shown abundant AR expression in BPH epithelium and stroma (Alonso-Magdalena et al., 2009; Tsurusaki et al., 2003) although some have reported similar levels of AR intensity in BPH compared to normal prostate (Hetzl et al., 2012). However, estimates for AR expression in normal prostate vary widely depending upon the study. Utilizing a hotspot technique, which likely results in overestimation of protein expression, Qiu et al. reported 85.3% of epithelial cells were AR positive in benign prostate, which is a substantially greater estimate than our finding of 29.18% AR positive cells in normal prostate and 55.61% in BPH (Qiu et al., 2008). Consistent with our findings, Brolin et al. subjectively measured the relative numbers of positively stained cells, and reported a significant increase in the percentage of AR positive epithelial cells in BPH (78.1%) compared to normal prostate (32.5%) (Brolin et al., 1992). The importance of AR negative luminal epithelial cells in the prostate is unknown, but it is possible that molecular derangements that lead to abnormal epithelial growth in BPH result in increased AR expression in luminal epithelial cells.

Because prostate specific antigen (PSA) is a target gene of AR, increased AR activity is likely responsible for the increase in serum PSA that often accompanies BPH. Indeed, while the role of PSA in prostate cancer screening has been debated, it has emerged as a powerful biomarker for BPH (El Melegry et al., 2010). In retrospective studies, PSA predicts changes in LUTS, urinary flow rate, acute urinary retention and need for surgery in BPH patients (Roehrborn et al., 1999a; Roehrborn et al., 1999b). This underscores the importance of serum PSA as a customized therapeutic tool to target androgens in BPH therapy. While we found an increase in AR expression in both the stromal and epithelium of BPH, we were interested in how relative numbers of AR positive cells were related among the tissue compartments. In normal prostate, the percentage of AR positive stromal cells remained relatively low even as the relative number of AR positive epithelial cells varied widely. However, in BPH, there was a positive relationship among AR localization in epithelium and stroma: BPH tissues with high AR positivity in the stroma also had high AR positivity in the epithelium. This suggests a synergistic or positive-feedback relationship among AR positivity in BPH that is distinct from normal prostate, which is consistent with aberrant paracrine interactions among stroma and epithelia thought to be important in prostate growth.

Understanding the tissue distribution of ER α in BPH will be critical for the development of new treatment strategies targeting estrogen pathways. In the epithelium of BPH, we found 5.3% cells, compared with 2.2% ER α positive cells in normal prostate epithelia. Consistent with our findings, other investigators have reported that ER α positive epithelial cells were increased in BPH (10.1% relative to 0.4% in normal prostate epithelium) (Royuela et al., 2001). Further semiquantitative studies (Hetzl et al., 2012) demonstrated increased epithelial ER α staining in BPH (33-66%) vs. <33% in normal prostate). Interestingly, we found a distinct relationship among stromal and epithelial ER α expression in BPH compared to normal prostate. This association suggests that in BPH, as the number of ER α -positive epithelial cells increases, the number of ER α -positive stromal cells remains relatively constant, in contrast to normal prostate. Likely due to lower stromal and higher relative numbers of epithelial ER α -positive cells, the resulting slope of the relationship was significantly smaller in BPH compared to normal prostate. These data suggest that epithelial ER α is important in the proliferative events associated with BPH (Risbridger et al., 2001). Alternatively, a decreased number of stromal ER α -positive cells suggests that estrogen regulated stromal growth inhibitory factors may be diluted in BPH relative to normal prostate. Discerning the mechanism of estrogen hormone action may be particularly important for better understanding the mechanisms of hormone regulation in BPH pathogenesis and developing future therapies.

The present results revealed low levels of ER α expression in the stroma of BPH. Other studies reported ER α expression in stromal nuclei of the peripheral zone of BPH specimens, but no ER α expression in transition zone stroma, luminal epithelial cells or basal cells (Tsurusaki et al., 2003). Alonso et al. (Alonso-Magdalena et al., 2009) reported rare ER α positive stromal cells in three (of sixteen) BPH specimens; these studies did not observe ER α positive epithelial cells in BPH specimens. Schulze and Claus reported ER α expression in the epithelium but not the stroma, and no difference in semi-quantitative levels of ER α staining intensity among normal prostate, non-obstructive and obstructive prostate (Schulze and Claus, 1990). Finally, another study reported no ER α positive nuclei in the stroma of normal prostate or BPH (Brolin et al., 1992). In contrast, Ehara et al. found ER α positive stromal cells, but not glandular epithelial cells in normal prostate specimens; these authors reported ER α -positive cells in fibromyoadenomatous and myoadenomatous hyperplasia subtypes of BPH, but not adenomatous hyperplasia (Ehara et al., 1995). These conflicts in the literature support the use of an objective and quantitative analysis system to detect low levels of stromal ER α expression.

While AR and ER expression have been previously evaluated in BPH, few have quantified these biomarkers in a reproducible and highly precise manner. Our study is also unique because we evaluated colocalization of AR and ER α in the same cell. We found a higher percentage of double positive epithelial cells in BPH compared to normal prostate, which has not been reported previously. While ER α positive cells in BPH were typically basal, and AR positive cells were usually luminal epithelia, double positive cells were both luminal and basal epithelial cells in BPH specimens. The implication of increased colocalization of AR and ER α in epithelial cells in BPH remains unclear. A number of studies support the synergy of androgens and estrogens in promoting growth of the prostate (Bernoulli et al., 2008; Bernoulli et al., 2007; Kumar et al., 2012; Nicholson et al., 2012; Ricke et al., 2008; Yarkin et al., 2009). While the population of double positive cells is small, this suggests they have an important role in BPH etiology and progression. In epithelium and stroma, there were fewer double negative cells in BPH, suggesting that more cells in BPH would be susceptible to local and systemic androgens and estrogens.

A limitation of our study was that we do not conclusively determine the cell identity of AR, ER α and double positive cells. In normal prostate and BPH, luminal and basal epithelial cells can be easily distinguished based on histology, but localization of ER α -positive epithelial cells could be confirmed with specific basal cell marker. Furthermore, due to the diversity of stromal cells (smooth muscle cells, fibroblasts, endothelial cells, resident macrophages, inflammatory cells) it remains unclear which cells are most important in mediating paracrine interactions in the prostate. Because InForm software depends on the nucleus for cell-segmentation, any cell that lacks a nucleus, whether due to sectioning plane or low intensity staining, is not included in the analysis. This may be particularly important in the stroma, where the cell body may be quite large with respect to the nucleus. This limitation may result in an underestimation of cell numbers. Additionally, adjacent nuclei without intervening cytoplasm may be counted as one large nucleus by InForm, resulting in an underestimation of the number of cells, or overestimation of nuclear size. Cellular segmentation of nuclei can also vary depending on the orientation of the tissue core, which underscores the importance of using duplicate cores in the TMA. Technical issues with nuclear segmentation are important to recognize when using automated technologies for cell and marker quantification. Future improvements in cellular segmentation with quantitative pathology software would greatly improve the utility of these tools in BPH research.

Contemporary quantitative pathology tools applied to tissue microarrays allow for precise and objective quantitation of biomarkers in samples of many patients with BPH. The present investigation is the one of many that will be needed to identify factors involved BPH

etiology and progression and ultimately narrow the focus of basic science investigations to clinically useful targets. Understanding tissue specific hormone action and overall hormone responsiveness of a patient's BPH lesion could lead to personalized therapeutic interventions targeting sex steroid action. Furthermore, these findings suggest that BPH therapies targeting estrogen pathways, such as SERMs, could be important adjuncts to current anti-androgen strategies.

Acknowledgments

We thank Emily Ricke and Laura Hogan for manuscript editing assistance. We also thank the National Institutes of Health for financial support for these studies: DK093690, ES018764 (WAR). TMN is a trainee in the Medical Scientist Training Program at the University of Rochester funded by NIH T32 GM07356; TMN is also supported by F30DK093173 from the National Institutes of Health under Ruth L. Kirschstein National Service Award from the NIDDK. The project described was also supported by the Clinical and Translational Science Award (CTSA) program, through the NIH National Center for Advancing Translational Sciences (NCATS), grant UL1TR000427. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of General Medical Sciences or NIH.

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Abbreviations

ACTA2	Smooth muscle alpha-actin
AR	Androgen Receptor
BPH	benign prostatic hyperplasia
DAB	d-amino benzene
ERα	Estrogen Receptor-alpha
LUTS	lower urinary tract symptoms
IHC	immunohistochemistry
HT	hematoxylin
PSA	prostate specific antigen
TMA	tissue microarray
TURP	transurethral resection of the prostate
5ARI	5-alpha reductase inhibitors

Highlights

- AR and ER α expression in BPH was evaluated with a tissue microarray.
- Protein expression was quantified with an automated IHC analysis suite.
- AR expression was increased in BPH relative to normal prostate.
- ER α expression was increased in BPH epithelium, but decreased in BPH stroma.
- Cells double positive (for AR and ER α) were more prevalent in BPH epithelium.

Figure 1.

Unmixed signals of four chromogens and composite multispectral images from multiplexed IHC of normal prostate (left panels) and BPH (right panels). Blue (HT), Vina green (turquoise, ACTA2), Warp red (red, AR), DAB (brown, ER α), bottom panels: composite multispectral image.

Figure 2.

Tissue compartment, cell counts and relative area in normal prostate and BPH. *A*, Normal prostate (top panels) vs. BPH [bottom panels; blue = hematoxylin (HT), turquoise = smooth muscle alpha-actin (ACTA2); E = epithelium, S = stroma]. Tissue architecture (left panel) is shown for a representative core of normal prostate and BPH. Tissue Segmentation (middle panel): InForm software was trained by a single pathologist to segment the image of the tissue core into epithelium (E, pink), stroma (S, green), and other (purple). Other tissue included image areas with no tissue, glandular lumen or secretions, and edge artifact. Cell Segmentation (right panel): Cells were automatically quantified with Nuance software in the epithelium (E, pink) and stroma (S, green). Multi-colored lines surround nuclei of individual cells. *B*, The percentage of epithelial tissue was increased in BPH relative to normal prostate (left bar graph, $*P = 0.0409$). There was an increase in the absolute number of epithelial cells in BPH cores compared to normal prostate (middle bar graph, $**P = 0.0018$). The percentage of epithelial cells was not significantly different in BPH vs. normal prostate (right bar graph). *C*, The percentage of stromal tissue was not significantly different in BPH relative to normal prostate (left bar graph). There was an increase in the absolute number of stromal cells in BPH cores compared to normal prostate (middle bar graph, $**P < 0.0014$). The percentage of stromal cells was not different in BPH from normal prostate (right bar graph).

Figure 3.

AR expression in normal prostate and BPH. *A*, Normal prostate epithelium composite image (blue = HT, red = AR) shows moderate AR expression in luminal epithelial cells (left upper panel). Luminal epithelial cells in BPH stain intensely for AR (right upper panel). Normal prostate epithelium shows scant AR positive stromal cells (left lower panel) while more AR positive stromal cells were observed in BPH cores (arrowheads, right lower panel). *B*, There was an increase in the overall percentage of AR positive cells in BPH relative to normal prostate ($***P < 0.0001$, left bar graph) and increased AR staining intensity ($***P < 0.0001$, right bar graph). *C*, The percentage of AR positive epithelial cells was increased in BPH relative to normal prostate (left bar graph, $***P < 0.0001$) and AR staining intensity in epithelial cells was increased in BPH (right bar graph, $***P < 0.0001$), indicating increased AR expression. *D*, The percentage of AR positive stromal cells in BPH was increased compared to normal prostate ($***P < 0.0001$) and stromal AR staining intensity was also greater than normal prostate ($***P < 0.0001$). *E*, Group differences in the relationship of AR positivity in stroma and epithelium were evaluated with simple linear regression. In normal prostate, AR expression was rarely seen in stroma, and positively correlated to epithelial AR expression ($P < 0.0001$). In BPH, AR positivity in stroma and epithelium were positively correlated ($P = 0.0016$). The slope of the relationship among epithelial and stromal AR positivity in BPH was significantly greater in BPH cores than normal prostate ($P < 0.0001$).

Figure 4.

ERα expression in normal prostate and BPH. *A*, Normal prostate epithelium composite image (blue = HT, brown = ERα) shows scant ERα expression in epithelial cells (arrow, left upper panel). Basal epithelial cells in BPH stained for ERα (arrow, right upper panel). Normal prostate epithelium shows moderate ERα positive stromal cells (arrowheads, left lower panel) while few ERα positive stromal cells were observed in BPH cores (arrowhead, right lower panel). *B*, There was no significant difference overall in the percentage of ERα positive cells in BPH relative to normal prostate (left bar graph) and no differences in ERα staining intensity. *C*, The percentage of ERα positive epithelial cells was increased in BPH relative to normal prostate (left bar graph, $P = 0.0454$) and no difference in epithelial ERα staining intensity (right panel). *D*, The percentage of ERα positive stromal cells in BPH was decreased compared to normal prostate ($P = 0.0008$) and stromal ERα staining intensity was decreased compared to normal prostate ($P = 0.0003$). *E*, Group differences in the relationship of AR positivity in stroma and epithelium were evaluated with simple linear regression. In normal prostate, ERα positivity in stromal and epithelial cells was positively correlated ($P = 0.0007$). In BPH, ERα positivity in stroma and epithelia were positively correlated ($P = 0.0010$). The slope of the relationship among epithelial and stromal ERα positivity in BPH was significantly less than normal prostate ($P = 0.001$).

Figure 5.

Colocalization of AR and ER α in normal prostate and BPH. *A*, Normal prostate epithelium composite image (blue = HT, red = AR, brown = ER α) shows rare double positive (yellow) epithelial cells while BPH epithelia commonly had double positive cells (right upper panel). Rare double positive stromal cells were observed in normal prostate epithelium (left lower panel) and (right lower panel). *B*, There were significantly more double AR and ER α positive cells in BPH epithelium compared to normal prostate ($**P = 0.0018$) and fewer double negative cells were observed in BPH ($***P < 0.0001$). *C*, The percentage of double positive stromal cells was not significantly different in BPH relative to normal prostate. There was a decreased proportion of double negative cells in BPH ($***P = 0.0008$).

Table 1

Prostate tissue patient demographics.

	Normal prostate (n = 52)	BPH (n = 24)
Mean age $\pm$ SEM (range)	61 $\pm$ 1.2 (37 – 74)	69 $\pm$ 1.6 [*] (59 – 86)
Percentage race White/Non-Hispanic	98%	100%
Atrophy present in 1 core (%)	16 (31%)	5 (21%) ^a

^{*} P = 0.0002, compared to normal prostate with two tailed t-test.

^a P = 0.4212 with two-sided Fisher's exact test. To determine atrophy, cores were evaluated by a blinded pathologist, based on thinning of glandular epithelium, with or without diminished size of glands.

Table 2

Nuclear size and percent area in BPH compared to normal prostate.

	Normal prostate (n = 52)	BPH (n = 24)
Epithelium: mean nuclear size (μm^2)	41.28 $\pm$ 0.6905	32.50 $\pm$ 0.9231 ***
Epithelium: % nuclear area	12.34 $\pm$ 0.4790	12.81 $\pm$ 0.4766
Stroma: mean nuclear size (μm^2)	26.54 $\pm$ 0.3826	24.85 $\pm$ 0.4553 *
Stroma: % nuclear area	28.87 $\pm$ 0.5601	26.13 $\pm$ 0.7421

Values are means $\pm$ SEM, compared with two-tailed t-test (***
 $P < 0.0001$,*
 $P = 0.0105$ compared to normal prostate).