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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 goals of this study were to quantitatively characterize the tissue distribution and staining intensity of androgen receptor (AR) and estrogen receptor-alpha (ERα), and assess cells expressing both AR and ERα, 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 ERα. 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. ERα expression was increased in BPH epithelium. However, stromal ERα expression and staining intensity was decreased in BPH compared to normal prostate. Double positive (AR and ERα) epithelial cells were more prevalent in BPH, and fewer double negative (AR and ERα) 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.

1. 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 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

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; SERMs, selective estrogen receptor modulators; TMA, tissue microarray; TURP, transurethral resection of the prostate; 5ARI, 5-alpha reductase inhibitors.

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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 (ERα) 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 ERα expression may be helpful in selecting compounds to target ERα 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 ERα 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, ERα 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 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 ERα in BPH compared to normal prostate.

2. Materials and methods

2.1. 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 (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.

2.2. 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) chromogen (Biocare), ERα (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).

2.3. 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 percentage of epithelial and stromal cells, and the area of the tissues were compared between normal prostate and BPH cores. AR and ERα 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 ERα expression was quantified by the optical density of the respective chromogen per unit area in pixels. Cells positive for both AR and ERα were counted with colocalization analysis using Inform™ 2.1 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.

2.4. 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 ERα 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).

3. Results

3.1. 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).

Fig. 1 shows unmixed images for each chromogen and multiplexed IHC image. As shown in Fig. 2, Inform software was trained to segment stroma from epithelium, and automated cell quantification was performed for each tissue compartment (Fig. 2A). Tissue segmentation analysis Figure 2A, middle panel showed an increase in the average percent area of epithelial tissue in BPH samples ($P=0.0409$; Fig. 2B). There was no difference in the percent area of stromal tissue Fig 2C (relative to epithelium and