Steroid hormone metabolism mediated racial disparity in men with benign prostatic hyperplasia

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INTRODUCTION

Racial disparity in prostate cancer has been well established, with African American (AA) men having higher rates of diagnoses and death from the disease compared to Caucasian American (CA) men. AA men also have a high incidence of benign prostatic hyperplasia (BPH), a disease associated with lower urinary tract symptoms (LUTS) that affect >210 million men worldwide. Furthermore, AA men with BPH have an increased incidence of non-surgical treatment failure, larger prostates at time of surgery, and surgery occurring at a younger age. Estrogens have been shown to induce lower urinary tract dysfunction in multiple animal models suggesting a seminal role in BPH. Hormone differences, especially increased estrogen exposure, between AA and CA males have been identified however little is known if there is an association with BPH in these two populations.

OBJECTIVE

In this study, we examine the prostate expression and localization changes in estrogen receptors (ERα, ERβ) as well as steroid metabolism genes in AA and CA with or without BPH.

METHODS

- *Formalin-fixed, paraffin-embedded (FFPE) prostate tissues were obtained from 58 men (see **Table 1**)*
- *FFPE sections were H&E stained to identify regions of interest*
- *Using multispectral quantitative multiplex IHC, we examined the steroid hormone related protein expression of six proteins related to estrogen receptor signaling (see **Table 2**)*
- *Using InForm® software, we spectrally unmixed each fluorophore and quantified optical density*
- *Cell and tissue segmentation was also performed to examine protein localization*

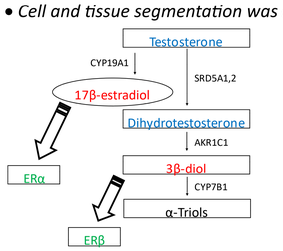

Figure 1. Steroid hormone metabolism pathway. Estrogens (red) are metabolized from androgen (blue) precursors and bind estrogen receptors (green). 17β-estradiol is the high affinity ligand for ERα. 3β-diol is the high affinity ligand for ERβ. 3β-diol is metabolized by AKR1C1 from dihydrotestosterone (DHT); CYP7B1 metabolizes 3β-diol for degradation and excretion.

Table 1. Distribution of prostate tissue.

	CA	AA
Normal	10	11
BPH	15	22

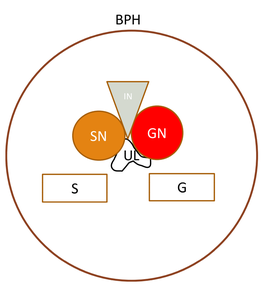

Table 2. Multispectral multiplex IHC protein targets.

Protein Target	OPAL Stains	Color
ERα	520	
COX2	540	
ERβ	570	
AKR1C1	620	
CYP7B1	650	
E-cadherin	690	
Nucleus	DAPI	

Figure 2. Regions of interest in human BPH samples. Upon H&E staining, regions of interest were identified within each BPH sample for multispectral, multiplex IHC analysis. UL—urethral lumen; SN—stromal nodule; GN—glandular nodule; IN—internodule; S—BPH adjacent normal stroma; G—BPH adjacent normal gland

RESULTS

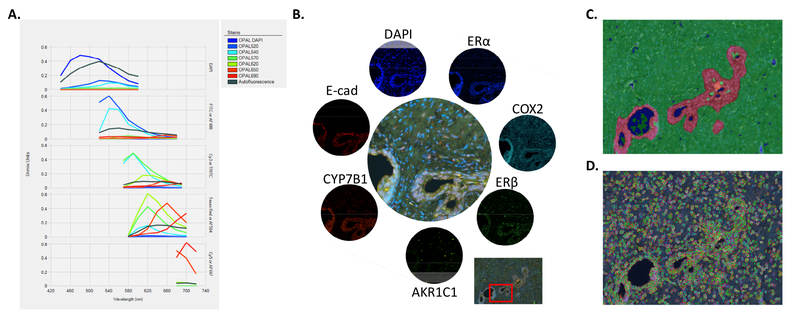

Figure 3. Image processing of multispectral, multiplex IHC. A. Spectral library of Opal stains account for autofluorescence inherent to tissues. **B.** Spectral unmixing using the spectral library allows for quantitation of each protein. Area represented in red inset. **C.** Machine learning algorithms using E-cadherin as a marker to segment epithelium (pink) from stroma (green). **D.** Using the DAPI nuclear stain, cell segmentation identifies nucleus, cytoplasm, and membrane.

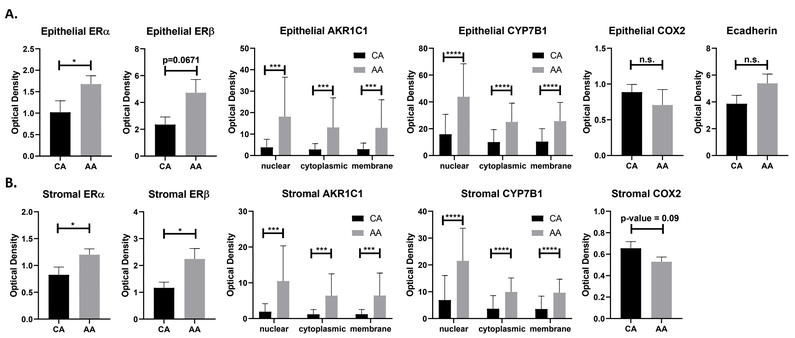

Figure 4. Racial differences in protein expression and localization. A. Expression of proteins in the epithelial compartment. **B.** Expression of proteins in the stromal compartment. ERα and ERβ are localized to the nucleus. COX2 is localized to the cytoplasm. E-cadherin is localized to the membrane. *, p < 0.05; ***, p < 0.001; ****, p < 0.0001

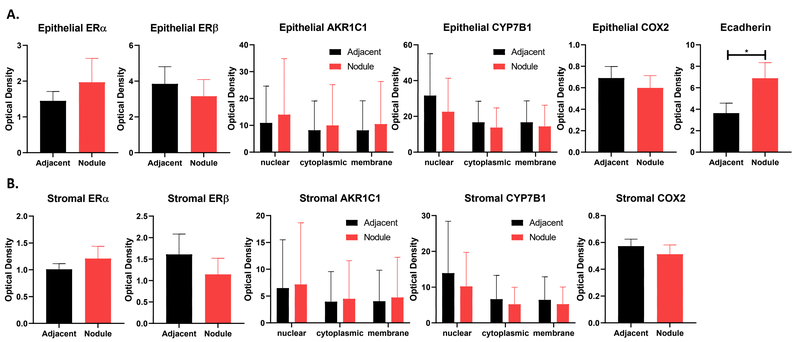

Figure 5. Disease differences in protein expression and localization. A. Expression of proteins in the epithelial compartment. **B.** Expression of proteins in the stromal compartment. ERα and ERβ are localized to the nucleus. COX2 is localized to the cytoplasm. E-cadherin is localized to the membrane. *, p < 0.05

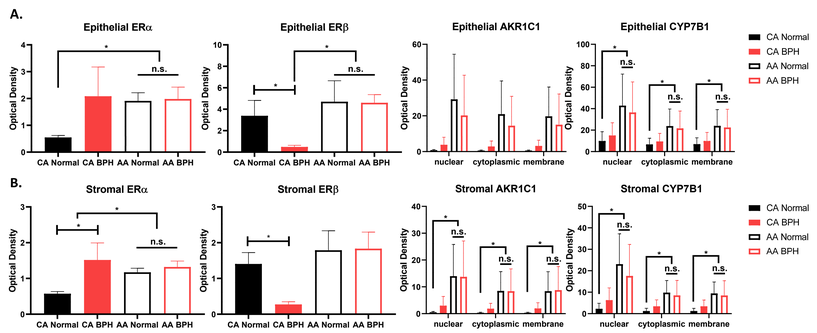

Figure 6. Racial and disease differences in protein expression and localization. A. Expression of estrogen pathway proteins in the epithelial compartment. **B.** Expression of estrogen pathway proteins in the stromal compartment. ERα and ERβ are localized to the nucleus. *, p < 0.05; solid bars = CA, unfilled bars = AA

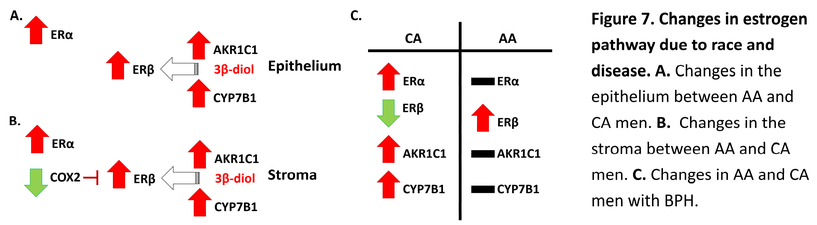

Figure 7. Changes in estrogen pathway due to race and disease. A. Changes in the epithelium between AA and CA men. **B.** Changes in the stroma between AA and CA men. **C.** Changes in AA and CA men with BPH.

CONCLUSIONS

- There is a racial difference in steroid metabolism enzymes affecting the expression of ERα and ERβ between normal prostate and BPH tissue.
- The dysregulation of steroid enzyme genes due to BPH in CA men is different to that found in AA men, complicating treatment strategies targeting the estrogen pathway.

FUTURE DIRECTIONS

- Examine patient matched (adjacent vs. nodule) expression in the context of race
- Examine the colocalization vs. coexpression of the ER pathway proteins
- Examine the importance of age/treatment/comorbidities on the expression of ER pathway proteins
- Examine the contribution of ER pathway proteins on collagen deposition

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