Expression of Estrogen Related Proteins in Hormone Refractory Prostate Cancer: Association With Tumor Progression

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Abbreviations and Acronyms

5AR = 5α-reductase

ADT = androgen deprivation therapy

AR = androgen receptor

BCAR1 = breast cancer anti-estrogen resistance 1

ERA = estrogen receptor α

ERB = estrogen receptor β

HRPC = hormone refractory prostate cancer

LHRH = luteinizing hormone-releasing hormone

pAR210 = Ser210 phosphorylated AR

PSA = prostate specific antigen

TMA = tissue microarray

TUR = transurethral resection

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Purpose: Despite increasing evidence that estrogen signaling has a key role in prostate cancer development and progression, few studies have focused on the estrogen pathway in the transition from hormone sensitive to hormone refractory tumors. We investigated the expression of proteins related to androgen and estrogen metabolism in paired prostate cancer samples collected before androgen deprivation therapy and after hormonal relapse.

Materials and Methods: The study included 55 patients treated for prostate cancer only with androgen deprivation therapy and in whom tissue was available before treatment induction and after recurrence. Immunohistochemistry was performed using tissue microarray with antibodies directed against androgen receptor, phosphorylated androgen receptor, estrogen receptor α, estrogen receptor β, 5α-reductase 1 and 2, aromatase, BCAR1 and the proliferation marker Ki67.

Results: Compared to hormone sensitive samples, tissues collected after hormonal relapse were characterized by increased expression of Ki67, androgen receptor, phosphorylated androgen receptor ($p < 0.001$) and BCAR ($p = 0.03$), and by lower staining for 5α-reductase 2 ($p = 0.002$), estrogen receptor β ($p = 0.016$) and aromatase ($p < 0.001$). Shorter time to hormonal relapse was associated with high expression of aromatase and BCAR1 on diagnostic biopsy, together with low staining for estrogen receptor α in stromal cells. Overall survival was significantly shorter when tissues collected after relapse showed a high proliferation index and low estrogen receptor α expression.

Conclusions: Results revealed dysregulation of proteins involved in androgen pathways, and in estrogen synthesis and signaling during the development of hormone refractory prostate cancer.

Key Words: prostate, prostatic neoplasms, androgen antagonists, estrogens, gene expression

Several pathways are involved in progression to androgen independence in cases of advanced prostate cancer treated with hormone deprivation, often associated with AR signaling. Genomic modifications of the AR gene have been described, including amplification or mutations.[1] More recently AR protein phosphorylation was also identified in association with HRPC.[2] In addition, tissue testosterone levels seem to be sufficient to activate even WT AR despite androgen deprivation, suggesting a role for enzymes involved in local androgen metabolism.[3] Conversion of testosterone to more active dihydrotestosterone is mediated through 5AR, which exists as 2 isoen-

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zymes, 5AR1 and 5AR2, expressed in normal and tumor prostate tissue.[4–6]

Despite increasing evidence that estrogen signaling has a major role in prostate cancer development and progression, few studies have focused on estrogen related genes in HRPC cases. The effects of estrogens on target tissues are mediated by ERA and ERB, which are receptors in the prostate.[7–9] The aromatase enzyme, which is responsible for local estrogen production, is also present in the human prostate.[10–12]

Although gene expression in prostate cancer has important variations among patients,[13] most previous reports comparing hormone sensitive and hormone refractory tumors tissues from different patients were used. Thus, there is a need for studies designed to compare gene expression before and after hormonal treatment in the same individuals to remove variability among patients. We analyzed the expression of proteins involved in androgen and estrogen metabolism in paired prostate samples from patients treated only with androgen deprivation.

MATERIALS AND METHODS

Patients and Tissues

We selected 55 patients from 323 treated at our institution exclusively with ADT for prostate cancer between 1988 and 2008. Patients were selected if they initially responded to exclusive ADT, ie decreased PSA without clinical or radiological progression, and had a pre-hormonal and post-hormonal relapse tissue sample suitable for analysis.

The hormonal therapy used was LHRH agonist, steroidal or nonsteroidal antiandrogen, or complete androgen blockage. No patient received chemotherapy, radiation therapy, prostatectomy or 5AR inhibitors. Hormonal relapse was defined as 2 consecutive PSA increases at a 1-week interval at least and serum testosterone below the castrate level (50 ng/dl).[14]

Prostate tissues at diagnosis were obtained from needle biopsy and tissues after hormonal relapse were collected by TUR, which was done in all cases due to decrease urinary tract symptoms associated with local tumor progression. Table 1 lists patient characteristics. Median age was 72.1 years (range 55.7 to 85.6) and median PSA at diagnosis was 37.0 ng/ml (range 1.8 to 900.0).

Table 1. *Patient characteristics*

	No. Pts (%)
TNM stage:	
T2	35 (63.6)
T3	16 (29.1)
T4	4 (7.2)
Metastasis	11 (20.0)
Gleason score at diagnosis:	
6	3 (5.5)
7	17 (30.9)
8	15 (27.3)
9	15 (27.3)
10	5 (9.1)
ADT:*	
LHRH agonist only	31 (56.4)
Steroidal antiandrogen only	2 (3.6)
Nonsteroidal antiandrogen only	1 (1.8)
Complete androgen blockade	4 (7.2)
Combined therapy	17 (30.9)

* No patient underwent bilateral orchiectomy only.

TMA Construction

The TMA block was prepared from the 55 formalin fixed, paraffin embedded tumor pairs. In each patient we transferred to the recipient block 3 cores from the tissue collected before hormonal treatment (each core from 1 biopsy) and 3 from the tissue collected after relapse. Biopsies were included in TMA as previously described.[15]

Immunohistochemistry

Immunostaining was done in tissue sections using antibodies to Ki67 (DakoCytomation, Glostrup, Denmark), AR, ERA (Novocastra®), pAR210 (Imgenex, San Diego, California), aromatase (Affinity BioReagents, Golden, Colorado), 5AR1, 5AR2 (Santa Cruz Biotechnology, Santa Cruz, California), ERB (GeneTex, Irvine, California) and BCAR1 (R & D Systems®). Negative controls were obtained using the irrelevant antibody, monoclonal Ig. Colonic samples were also included in the TMA to serve as negative controls. Samples from other tissues known to express each marker served as positive controls, including breast carcinoma for ERA and ERB.

Antibody Staining Scoring

Slides were analyzed by 2 pathologists (GF and MY) in blinded fashion. For markers with nuclear epithelial staining (AR, Ser210 AR, ERB, 5AR1 and Ki67) positive cells are expressed as a percent of total epithelial cells. ERA, a marker with stromal nuclear staining, was scored as the mean number of positive stromal cells per core. The cytoplasmic staining pattern of 5AR2 and BCAR expression in tumor cells was classified as 0—no stained cells, +—rare stained cells, ++—fewer than 50% positive cells and +++—greater than 50% positive cells. Cytoplasmic staining for aromatase expression in tumor epithelial cells was classified as 0—no stained cells, +—focally positive and ++—diffusely positive.

In cases of interobserver variability (different categories for categorical data or more than 10% variability for continuous data), slides were rescored by the 2 pathologists until consensus was achieved.

Statistical Analysis

Protein expression before ADT induction and after hormonal relapse was compared using the Friedman and Wilcoxon paired tests for categorical and continuous data, respectively. Survival analysis was done using the Kaplan-Meier method and curves were compared with the log rank test. The HR was calculated using Cox regression for multivariate analysis. Correlations between protein expression and cell proliferation were assessed using the Spearman correlation, Mann-Whitney or Kruskal-Wallis test.