Ethnic Differences in Neuroendocrine Expression in Prostate Cancer Tissue

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Abstract. *Background/Aim: The role of neuroendocrine (NE) cells in prostate cancer biology remains unclear. We previously reported a large difference in NE expression in benign prostate tissue among men of different ethnicities; African-American men had significantly fewer NE cells compared to all other groups. This report describes NE expression in malignant prostate tissue. Patients and Methods: Paraffin-embedded tissue from 180 men who underwent radical prostatectomy at the University of Southern California between 1983 and 2003 was stained using standard immunohistochemistry technique for chromogranin A (ChrA), serotonin (Ser) and synaptophysin (Syn). There were 39 specimens from African-American patients, 39 Asian, 57 Hispanic and 45 non-Hispanic White. Staining intensity and the percentage of cells positive were determined by the automated cellular imaging system. Results were analyzed by univariate and multivariate general linear regression models. Results: There were significant differences in staining intensity for all markers between ethnic groups in univariate analysis. NE expression, judged by ChrA intensity, was highest in Hispanic patients, compared to non-Hispanic Whites and African-Americans. A similar pattern was observed for Syn and Ser. In multivariate analysis, controlling for age, Gleason score, PSA and stage, the differences in ChrA, Syn and Ser remained highly significant. Hispanic men had higher ChrA expression levels than African-Americans and non-Hispanic Whites (p=0.0077 and 0.0038, respectively); the p-values for the comparison were both <0.0001 for Ser. Both Hispanic and Asian patients had higher intensity Ser expression than African-American and Non-Hispanic Whites patients, with all p-values <0.018. Conclusion: As already shown in benign prostate tissue, we identified significant differences in NE expression among prostate cancer tissues from men of different ethnic backgrounds. The clinical impact of these differences in NE expression warrants exploration.*

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Prostate cancer (PC) is the most common cancer in men, affecting over 220,000 men in the United States each year, and causing approximately 23,000 deaths(1). The incidence of prostate cancer varies by geographic region and between ethnic groups within the same country; the lowest rates occur in Asian populations (100/100,000), whereas higher rates affect Caucasians in North America and Scandinavia, and the highest rates are seen in African-Americans (272/100,000). In addition to having the highest incidence, African-Americans present with more advanced stage and more aggressive grade, incurring the highest age-adjusted PC mortality (2). Although a variety of factors, such as genetic susceptibility, diet, socioeconomic status, and hormonal milieu, have been proposed as explanations for these observed differences, they do not account for the entire disparity (3-5).

Areas of neuroendocrine (NE) differentiation are found in almost all prostate adenocarcinomas, but can be found in normal prostate tissue as well (6). The role of NE cells in the development and progression of PC is controversial. NE cells are present in normal prostatic epithelium, where they represent a terminally differentiated population and characteristically do not express the androgen receptor (AR) or prostate-specific antigen (PSA) (7). Secretory granules of prostate NE cells contain bombesin, parathyroid hormone-related protein, calcitonin and neurotensin, which have been implicated in stimulation of cellular proliferation, differentiation, and angiogenesis (8). However, we have shown that exposure of PC cell lines to the paracrine effects of transdifferentiated NE cells results in growth inhibition and apoptosis (9). Some of the discrepancy in these findings could be explained if NE cells in benign prostate tissue represent a different population from that found in PC. Due to the lack of AR expression and low proliferative rate, NE cells represent a population which is resistant to standard therapy (7). In fact, some studies have identified NE expression in tissue or serum as a predictor of inferior

response to androgen deprivation therapy (10, 11). Elevated serum chromogranin A (ChrA) has also been shown to predict lack of response to docetaxel (12).

African-American men have a significantly higher incidence of PC than their white and Hispanic counterparts (248.5/100,000 compared to 156.7/100,000 and 138/100,000 respectively) and Asian men have much lower incidence (93.8/100,000). While recent studies show that outcomes do not differ for men of different races who receive curative therapy for early-stage PC (13), African-American men tend to present at younger ages and with higher Gleason scores (14). The biology behind the higher incidence and earlier, more aggressive presentation of prostate cancer among African-American men has not been explained. Our group has documented that significantly fewer NE cells are found in benign prostate tissue from African-American men compared to Asian, Caucasian, and Hispanic men (15). The current study was undertaken to determine whether differences in NE expression exist in malignant prostate tissue from men of different ethnic backgrounds, which could contribute to differences in clinical behavior. Understanding the influence of NE cells within PC is important, as this could yield novel therapeutic strategies via sub-classification, a trend which is rapidly revolutionizing the treatment of other solid tumors. In addition, NE cells may represent an opportunity for novel therapeutic targets.

Patients and Methods

From 1972 to 2008, 3804 men consented to participate in the institutional database, in the context of undergoing radical prostatectomy at the University of Southern California. Of these, 180 specimens from 1983-2003 were selected with the following ethnic distribution: 39 specimens from African-American patients, 39 Asian, 57 Hispanic, and 45 non-Hispanic white. Tissue specimens were stained using our standard immunohistochemistry technique (15) for ChrA, serotonin (Ser), synaptophysin (Syn) and AR. Briefly, deparaffinization was performed with xylene followed by rehydration in graded ethanol solutions. Slides were buffered with hydrogen peroxide and blocked with 20% fetal bovine serum, then incubated for 1 hour at room temperature (except for AR which was incubated overnight) with 1:1000 dilution of a monoclonal antibody against ChrA (Dako, Carpinteria, CA, USA), serotonin (Santa Cruz Biotech, Santa Cruz, CA, USA) and synaptophysin (Santa Cruz Biotech). The tissue was then incubated for 1 hour at 4˚C with second antibody consisting of a 1:1000 dilution of conjugated rabbit anti-mouse antibody (Dako). Slides were developed with diaminobenzidine tetrahydrochloride (DAB) solution (Dako), lightly counterstained with hematoxylin, and coverslipped. All of the slides were assessed using the Automated Cellular Imaging System II (ACIS II; Clarient, Aliso Viejo, CA, USA). After the slides were scanned, the pathologist (DH) manually selected 5 fields (×40) of interest. The ACIS II software, using wavelength-specific technology to detect color differences, was used to differentiate the DAB-positive, marker-positive cells from the hematoxylin-positive, marker-negative cells. The system provides objective and reproducible measures of percentage of cells staining and the intensity of staining (ACIS output expressed as an arbitrary score from 0-100) for the markers of interest. Results were analyzed by univariate and multivariate general linear regression models.

Table I. *Baseline and demographic characteristics of men whose radical prostatectomy specimens were included in this study.*

Characteristic	Number (%)	*p*-Value by ethnic group
African-American	39 (22%)	
Asian	39 (22%)	n/a
Hispanic	57 (32%)	
Non-Hispanic White	45 (25%)	
Age (years)		0.4805
<60	42 (23%)	
60-69	92 (51%)	
≥70	46 (26%)	
Stage		0.129
Organ-confined	117 (65%)	
T3a/T3b	49 (27%)	
Lymph node involvement	14 (8%)	
Gleason score*		0.097
≥6	88 (49%)	
7	62 (34%)	
8-10	29 (16%)	
Pre-operative PSA†		<0.0001
≤10	104 (58%)	
10-20	28 (16%)	
>20	22 (12%)	
Pre-op ADT		0.076
African-American	8 (20.5%)	
Asian	14 (36%)	
Hispanic	17 (30%)	
Non-Hispanic White	6 (13%)	

*1 Missing; †26 missing. ADT: Androgen-deprivation therapy.

Results

Baseline and demographic characteristics of the included population are summarized in Table I. All characteristics were similar among ethnic groups, except that pre-operative PSA was more commonly less than 10 ng/ml in African-Americans (79%) compared to Asians (60.5%), Hispanics (66%), and non-Hispanic Whites (64%). Forty-five men (25%) had received neoadjuvant androgen deprivation therapy; this was not different among the ethnic groups (p=0.076).

AR staining was present in more cells in PC tissue from African-Americans (mean 71.3% of cells, SE 5.3%) compared to non-Hispanic Whites (mean 48.4% of cells, SE 5.5, p=0.0099) but did not differ significantly compared to other ethnic groups (mean 63.7% of cells for Hispanics, 55.1% of cells for Asians). Mean AR intensity in African-American PC tissue was 71.4 (SE 1.6), compared to 69.3 for Asian, 69.8 for Hispanic, and 68.4 for non-Hispanic Whites (p=0.88).

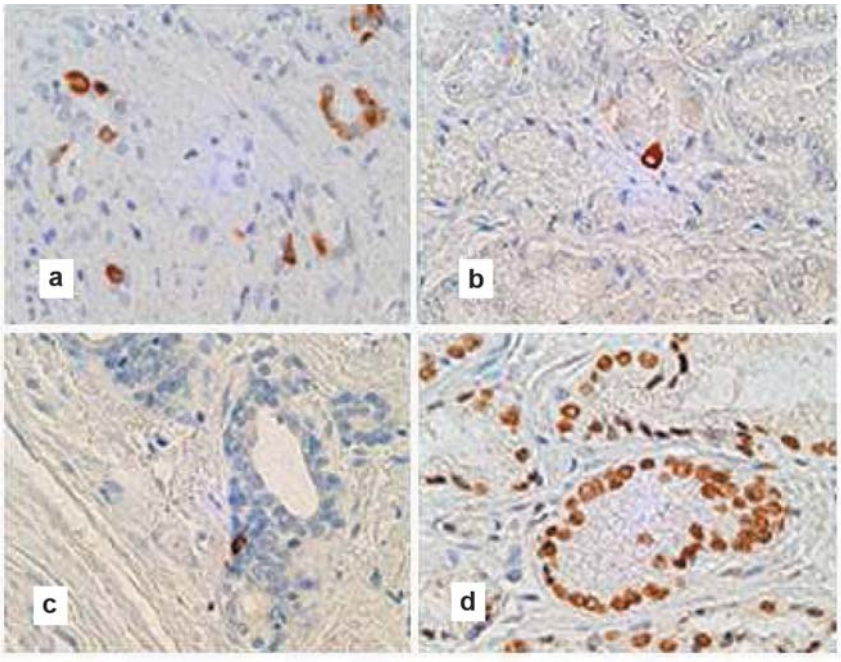

Figure 1. *Representative photographs showing immunohistochemical staining of neuroendocrine expression and androgen receptor in prostate cancer tissue in this study, representing immunoactivity for serotonin (a), synaptophysin (b), chromogranin A (c) and androgen receptor (d) (original magnification, ×600).*

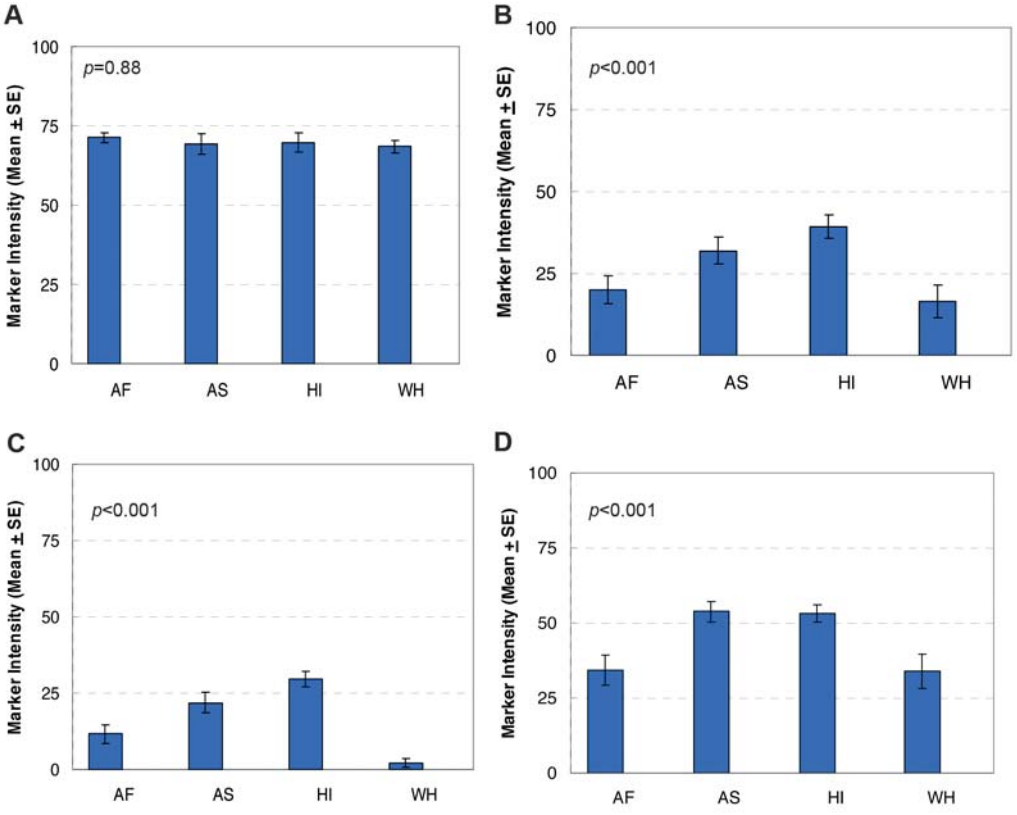

Figure 2. *Intensity of staining for androgen receptor (A), chromogranin A (B), serotonin (C), synaptophysin (D). AF, African-American; AS, Asian; HI, Hispanic; WH, non-Hispanic White, expressed as ACIS score (arbitrary units).*

Examples of immunohistochemical staining results are shown in Figure 1. The absolute numbers of NE cells detected was low, with only 10 patients having more than 5% of cells stain for ChrA. Staining for Syn detected the largest number of NE cells, ranging from a mean of 6% of cells staining in non-Hispanic whites to 9.6% of cells in Asians. The small number of cells limited statistical power to detect differences among ethnic groups. There was a trend toward significance only for ChrA, in that African-American samples had fewer cells stain positively than the other ethnic groups (p=0.076).

The staining intensity for each of the three NE markers (ChrA, Ser, Syn) was significantly different among ethnic groups in univariate analysis. ChrA expression was most intense among Hispanic patients, compared to African-American and non-Hispanic Whites. Both Hispanic and Asian patients had higher intensity of Ser expression than African-Americans and non-Hispanic Whites. These findings are depicted in Figure 2. All differences remained significant after controlling for age, stage, Gleason score, and pre-operative PSA level in multivariate analysis. Hispanic men had more intense ChrA expression than African-American and non-Hispanic White men (p=0.0077 and 0.0038 respectively); the p-values for the comparison were both <0.0001 for Ser. Both Hispanic and Asian patients had higher intensity Ser expression than African-American and non-Hispanic White patients, with all p-values <0.018.

Discussion

This study documents that differences in NE expression exist in PC specimens from men of different ethnic groups, complementing our earlier work in which we found significant differences in NE expression in benign prostate tissue (15). NE cells are thought to be involved in carcinogenesis within the prostate (6, 8), although some data suggest they may actually exert a protective effect. There is a decrease in NE expression within the peripheral zone of the prostate, the region most susceptible to cancer development, as men age, which is when PC incidence increases (16). Decreased NE expression has also been reported around high-grade prostatic intraepithelial neoplasia, a PC precursor (17). *In vitro*, proliferation of PC cells was inhibited during exposure to NE-conditioned medium or in co-culture with NE cells (9).

The main biologic processes driving differentiation into NE phenotype (expression of neurosecretory granules, morphology) *in vivo* is not understood; the cytokine and hormonal milieu have been implicated. Since inhibition of AR signaling can induce NE differentiation in prostate cancer cells (18), we hypothesize that African-American men may have less NE expression due to the higher levels of testosterone seen in the second and third decades in this population (19). While the low absolute numbers of NE cells precluded statistically robust comparison, there was a trend toward fewer cells expressing ChrA in samples from African-American men, similar to the findings in benign prostate tissue. The intensity of staining, however, was markedly different among ethnic groups for all three NE markers. The overall pattern was of more NE staining in the PC tissue of Hispanic and Asian patients compared to African-American and non-Hispanic Whites. Given that androgen deprivation increases NE expression, it is important to note that there was no significant difference between use of neoadjuvant therapy among men of different ethnicities, although slightly more Asian and Hispanic patients had been treated, which could account for some of the higher NE staining seen in these groups.

The association between NE expression and PC outcomes is controversial; while our group and others have documented that higher NE expression is associated with a significantly higher rate of recurrence after prostatectomy and lower overall survival in D1 PC patients (20, 21), some series have not found a significant relationship (22, 23). We did not correlate NE expression with outcome in this study, since there are many confounding factors and the study was not designed to control for them. However, we hypothesize that NE cells influence the behavior of PC and note that higher levels of NE expression are seen in the groups with lower incidence and/or less aggressive behavior of PC.

The difference in number of cells staining for AR between tissue samples from African-American men and non-Hispanic White men has previously been reported (24). Given that AR expression has been correlated with more aggressive behavior of PC (25-27), this finding may explain some of the differences in outcomes seen in African-Americans with prostate cancer. The higher AR expression in the group with lower NE expression fits with the inverse relationship between AR signaling and NE differentiation noted above. NE expression may thus be one of the mechanisms by which AR signaling influences PC behavior.

Conclusion

As already shown in benign prostate tissue, we identified significant differences in NE expression among malignant prostate tissue from men of different ethnic backgrounds. Further work is needed to elucidate the clinical implications of differential NE expression.

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