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Racial Variations in Prostate Cancer Molecular Subtypes and Androgen Receptor Signaling Reflect Anatomic Tumor Location

Farzana A. Faisal^{a,*}, Debasish Sundi^a, Jeffrey J. Tosoian^a, Voleak Choeurng^b, Mohammed Alshalalfa^b, Ashley E. Ross^a, Eric Klein^c, Robert Den^d, Adam Dicker^d, Nicholas Erho^b, Elai Davicioni^b, Tamara L. Lotan^a, and Edward M. Schaeffer^a

^a Brady Urological Institute, Johns Hopkins University, Baltimore, MD, USA

^b GenomeDx Biosciences, Vancouver, BC, Canada

^c Glickman Urological and Kidney Institute, Cleveland Clinic, Cleveland, OH, USA

^d Department of Radiation Oncology, Thomas Jefferson University, Philadelphia, PA, USA

Abstract

Prostate cancer (PCa) subtypes based on *ETS* gene expression have been described. Recent studies suggest there are racial differences in tumor location, with PCa located anteriorly more often among African-American (AA) compared to Caucasian-American (CA) men. In this retrospective analysis of a multi-institutional cohort treated by radical prostatectomy (179 CA, 121 AA), we evaluated associations among molecular subtype, race, anatomic tumor location, and androgen receptor (AR) signaling. Subtype (m-ERG⁺, m-ETS⁺, m-SPINK1⁺, or triple-negative) was determined using distribution-based outlier analysis. AR signaling was investigated using gene expression profiling of canonical AR targets. m-ERG⁺ was more common in CA than AA men (47% vs 22%, $p < 0.001$). AA men were more likely to be m-SPINK1⁺ (13% vs 7%; $p = 0.069$) and triple-negative (50% vs 37%; $p = 0.043$). Racial differences in molecular subtypes did not

* Corresponding author. Brady Urological Institute, Johns Hopkins University, CRBII-532, 1550 Orleans Street, Baltimore, MD 21231, USA. Tel: +1 410 5028180; Fax: +1 410 6148096. ffaisal1@jhmi.edu (F.A. Faisal).

Author contributions: Farzana A. Faisal had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Faisal, Choeurng, Alshalalfa, Ross, Schaeffer.

Acquisition of data: Faisal, Sundi, Ross, Schaeffer.

Analysis and interpretation of data: Ross, Alshalalfa, Davicioni, Choeurng, Schaeffer.

Drafting of the manuscript: Faisal, Ross, Alshalalfa, Davicioni, Schaeffer.

Critical revision of the manuscript for important intellectual content: Faisal, Tosoian, Ross, Alshalalfa, Davicioni, Klein, Den, Dicker, Lotan, Erho, Schaeffer.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.eururo.2015.09.031>.

persist when tumors were analyzed by location, suggesting a biologically important relationship between tumor location and subtype. Accordingly, anterior tumor location was associated with higher Decipher scores and lower global AR signaling.

Patient summary—This study demonstrates associations among patient race, prostate cancer molecular subtypes, and tumor location. Location-specific differences in androgen regulation may further underlie these relationships.

Keywords

Prostatic neoplasm; Race; African-American; Molecular subtype; TMPRSS2-ERG fusion; ETS gene fusion; SPINK1

Recent studies suggest that African-American (AA) men exhibit more aggressive prostate cancer (PCa) and a propensity for anteriorly localized tumors [1]. Many PCa-associated genes are differentially expressed between AA and Caucasian-American (CA) men [2,3], and prior genome-wide studies have identified common molecular subtypes based on gene fusions [4].

Fusions involving *ETS* transcription factors (*ERG*, *ETV1*, *ETV4*, *ETV5*) and other genes (*TMPRSS2*, *SLC45A3*, *NDRG1*) are the most common genomic rearrangements in prostate-specific antigen (PSA)-screened cohorts, occurring in 40–50% of CA men, and *ERG* rearrangement is more common in CA than AA men [5]. In ETS-negative PCa, other alterations (*SPINK1* overexpression, *SPOP* mutation, and *CHD1* deletion) have been identified. While varying molecular subtypes confer important prognostic value, little is known about molecular heterogeneity in relation to other clinical findings such as race and tumor location.

We examined a multi-institutional cohort to validate associations between molecular subtypes and race. In a single-institution subset with detailed tumor annotations, we tested for systematic differences in subtypes and androgen signaling with respect to race and tumor location. We then performed gene expression profiling to identify molecular differences between anterior and posterior tumors.

A total of 300 radical prostatectomy (RP) specimens with complete clinicopathologic data were selected by a matching algorithm applied to 579 RP patients pooled from multiple institutions, including Johns Hopkins Hospital (JHH; $n = 134$, 67 AA, 67 CA), Thomas Jefferson University ($n = 48$, 16 AA, 32 CA), Cleveland Clinic ($n = 44$: 14 AA, 30 CA), and Memorial Sloan Kettering Cancer Center ($n = 72$: 24 AA, 48 CA) [6]. CA men were matched to AA men within the same institution on the basis of Cancer of the Prostate Risk Assessment score (CAPRA-S) [7] using a two-point caliper.

Formalin-fixed, paraffin-embedded tissue blocks were sampled from dominant tumor nodules (largest tumor with the highest Gleason score) following histopathologic re-review. Methods for tissue acquisition and analysis are described in the Supplementary material. From punch biopsies, RNA was isolated, amplified, and profiled with the ~1.4-M probe-set Decipher platform. The 300-specimen cohort was used to assess association between subtype and race. In the subset of 109 patients from JHH (59 AA and 50 CA men with very

low risk), RP specimens were annotated by genitourinary pathologists for dominant tumor volume and location [1]; this subset was used to assess correlation between subtype, race, and tumor location.

Molecular subtype classification was determined using distribution-based outlier analysis on validated human exon 1.0 ST arrays to categorize patients into one of four molecular subtypes: m-ERG⁺; m-ETS⁺ (m-ETV1⁺, m-ETV4⁺, m-ETV5⁺, or m-FLI1⁺); m-SPINK1⁺; or triple negative (m-ERG⁻ m-ETS⁻ m-SPINK1⁻). In addition, we characterized androgen receptor (AR) signaling using canonical AR targets (AR, KLK2, KLK3, STEAP1, STEAP2, NKX3-1, RAB3B, FKBP5, PDE9A, PPAP2A, ACSL3, TMPRSS2).

Within the matched cohort ($n = 300$; 121 AA, 179 CA) there were no significant racial differences in baseline characteristics or pathologic outcomes (Supplementary Table 1). The m-ERG⁺ subtype was more common among CA than among AA men (47% vs 22%; $p = 0.001$, Supplementary Table 2). AA men were more likely to have the m-SPINK1⁺ (13% vs 7%; $p = 0.069$) or triple-negative (50% vs 37%; $p = 0.043$, Fig. 1A) subtype.

Since the anatomic origin of larger advanced tumors is difficult to determine, we mapped tumor location and volume in men with very low risk disease in the JHH subset [2]. In AA men, the m-ERG⁺ subtype was less frequent (20% vs 46%; $p = 0.007$) but the m-ETS⁺ subtype was more frequent (14% vs 2%; $p = 0.037$) among AA compared to CA men (Supplementary Table 3). Anterior tumors were less likely to be m-ERG⁺ (13% vs 42%; $p = 0.002$) and more likely to be triple negative (76% vs 46%; $p = 0.004$) compared to posterior tumors (Supplementary Table 3). Among anterior tumors, m-ERG⁺ subtype (14% in AA, 10% in CA; $p = 1.000$) and triple-negative status (72% in AA, 91% in CA; $p = 0.399$) were independent of race (Supplementary Table 4).

Regardless of race, anterior tumors were associated with lower PSA density than posterior cancers (Wilcoxon test, $p = 0.07$; Supplementary Table 4). Anteriorly located dominant nodules were larger than posteriorly located dominant nodules (0.89 vs 0.32 cm², $p = 0.0004$). To assess whether these were secondary to tumor (epithelial) content, we assessed stromal signature expression (*MYLK*, *DESMIN*, *CNN1*, *FHL1*, *CAVI*) within each tumor nodule and found no significant association between stromal content and tumor location (Supplementary Fig. 1). On logistic regression analysis, AA race and ERG⁻ status were independent predictors of anterior tumors across multiple models (Supplementary Table 5).

The location-specific differences in AR molecular sub-types, tumor size, and PSA suggested that anterior tumors might have lower global AR signaling. Therefore, we examined expression of 12 AR genes among the 109 patients with very low risk and found that lower expression of AR genes was associated with ERG⁻ status and anterior tumors in the whole group and in the AA subset ($n = 59$; Fig. 2A). Evaluation of AR gene expression (Fig. 2B) revealed that tumors in the lowest AR signaling quartile were more likely to be anterior as compared to the highest quartile of AR signaling across all patients (46% vs 26%, $\chi^2 p = 0.018$). Anterior tumor location was associated with low AR signaling (univariable odds ratio [OR] 0.43; $p = 0.08$, Supplementary Table 6). A less significant trend was noted for the AA subset owing to the small number of cases (Fig. 2B). Additional work including more

cases might reveal stronger correlation. Characterizing the prognosis difference by tumor location revealed that the Decipher genomic classifier score (mean $\pm$ standard deviation) was significantly higher for anterior than for posterior tumors (0.31 ± 0.18 vs 0.22 ± 0.15 ; $p = 0.01$, Supplementary Fig. 2), suggesting tumor location is associated with a distinct genomic fingerprint.

In summary, AA and CA men have different *ERG*, *ETS*, and *SPINK1* PCa subtypes. Among low-risk cases, nearly 50% of tumors among AA men were anterior, compared to 20% among CA men. We also found that anterior tumors in both races had higher volume, lower PSA density, and higher genomic classifier scores. Accordingly, anterior tumors also had lower expression of canonical AR signaling genes.

Further research is warranted to explore upstream factors driving PCa subtypes and AR signaling, including factors in the prostatic stromal environment that may contribute to subtype differences by race and location. Limitations of our study include a focus on dominant tumor nodules, as it is possible that lower-grade or smaller nodules might also contribute to clinically important subtypes. Detection bias could have contributed to systematic differences found for anterior tumors, as anterior lesions are more difficult to capture using traditional biopsies. Finally, while these findings may be more applicable to lower-risk PCa, assessing the associations between race, subtype, and location in high-risk PCa may provide more insight into factors underlying the heterogeneity of anterior tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
(A) Molecular subtype by race in the full study cohort ($n = 300$; 121 AA, 179 CA). ERG⁺, $p < 0.001$; any ETS⁺ ERG⁻, $p = 0.177$; SPINK1⁺, $p = 0.069$; triple negative, $p = 0.043$ (Fisher's exact test). (B) Molecular subtype of dominant nodules by race and tumor location.

Fig. 2.

Androgen receptor (AR) signaling activity in patients with very low risk ($n = 109$). (A) Hierarchical clustering of the 109 patients (left) and AA patients (right) regarding expression of AR signaling genes collected from literature reveals that low AR activity is associated with ERG⁻ subtype and anterior tumor location. (B) AR signaling expression was averaged and grouped into quartiles. Among the 109 patients (left), anterior tumors are more frequent in the lowest compared to the highest AR signaling quartile (46% vs 26%; $p = 0.018$). In the AA subset (right, $n = 59$), patients with the lowest AR signaling are nearly twice as likely to have anterior tumors as patients with the highest AR signaling (53% vs 36%).