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Evidence for Molecular Differences in Prostate Cancer between African American and Caucasian Men

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Abstract

Purpose—To compare the frequency of *ERG* rearrangement, *PTEN* deletion, *SPINK1* overexpression, and *SPOP* mutation in prostate cancer in African American and Caucasian men.

Experimental design—Dominant tumor nodules from radical prostatectomy specimens of 105 African American men (AAM) were compared to 113 dominant nodules from Caucasian men (CaM). Clinical and pathologic characteristics of the two groups were similar. *SPINK1* overexpression was evaluated by immunohistochemistry, *ERG* rearrangement and *PTEN* deletion by FISH, and *SPOP* mutation by Sanger sequencing.

Results—*ERG* rearrangement was identified in 48/113 tumors (42.5%) in CaM and 29/105 tumors (27.6%) in AAM (p=0.024). *PTEN* deletion was seen in 19/96 tumors (19.8%) in CaM and 7/101 tumors (6.9%) in AAM (p=0.011). *SPINK1* overexpression was present in 9/110 tumors (8.2%) in CaM and 25/105 tumors (23.4%) in AAM (p=0.002). *SPOP* mutation was identified in 8/78 (10.3%) tumors in CaM and 4/88 (4.5%) tumors in AAM (p=0.230). When adjusted for age, BMI, Gleason score, and pathologic stage, *ERG* rearrangement and *SPINK1* overexpression remain significantly different (p=0.018 and p=0.008, respectively), and differences in *PTEN* deletion and *SPOP* mutation approach significance (p=0.061 and p=0.087, respectively).

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Conflicts of Interest: A patent has been issued to Weill Medical College of Cornell University on *SPOP* mutations in prostate cancer; C.E.B and M.A.R are listed as co-inventors.

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Conclusions—Significant molecular differences exist between prostate cancers in AAM and CaM. *SPINK1* overexpression, an alteration associated with more aggressive prostate cancers, was more frequent in AAM, while *ERG* rearrangement and *PTEN* deletion were less frequent in this cohort. Further investigation is warranted to determine if these molecular differences explain some of the disparity in incidence and mortality between these two ethnic groups.

Keywords

African American; prostate cancer; race; pathology; molecular

Introduction

Prostate cancer is known to exhibit differences among racial/ethnic groups

African American men (AAM) have a higher incidence and mortality from prostate cancer than that observed in Caucasian men (CaM) as well as other ethnicities (1). Many factors have been postulated to contribute to incidence and/or mortality differences, such as access to care, attitudes toward care, socioeconomic and educational disparities, differences in type and aggressiveness of treatment, and dietary fat intake (2). Some studies have shown that when these factors are controlled for, there is no difference in mortality, but the incidence of prostate cancer in AAM has consistently been shown to be higher (3).

Biochemical recurrence has also been demonstrated to be higher in locally advanced disease in AAM, although a difference in biochemical recurrence was not detected between AAM and CaM with organ-confined prostate cancer after radical prostatectomy (4). PSA levels have also been demonstrated to be higher in AAM than CaM with locally advanced prostate cancer (4). Genetic differences in prostate cancer between AAM and CaM are postulated to contribute to these disparities.

Genetic differences in prostate cancer

Differences in genes involved in the androgen signaling pathways have been observed between AAM and CaM, favoring increased androgen activity in AAM (5–7). Also, increased testosterone levels in AAM as compared to CaM have been shown in some studies (8,9). Several more recent studies have demonstrated differences in gene methylation and aggressive biomarker expression in prostate cancers between CaM and AAM (10–13), strongly suggesting that genetic differences do exist and at least partially contribute to differences observed in clinical outcomes between these two populations.

More is now known about specific molecular aberrations in prostate cancer, with several new discoveries over the past decade. These include recurrent gene fusions involving androgen regulated genes (*i.e.* *TMPRSS2*) and *ETS* family genes (14), *PTEN* genomic deletion (15–18), overexpression of *SPINK1* (a low molecular weight trypsin inhibitor) (19,20), and, more recently, non-synonymous somatic mutations of *SPOP* (21). Several previous studies have examined the prevalence of *ERG* rearrangement in AAM (22–24), all of which found a lower frequency of *ERG* rearrangement and/or *ERG* overexpression in AAM. Similarly, another study found increased *ERG* gene expression in prostate cancers among CaM relative to AAM when gene expression profiling was performed (24). To our

knowledge, our study is the first to compare the prevalence of *PTEN* deletion, *SPINK1* overexpression, and *SPOP* mutation between AAM and CaM. Furthermore, our study reports on all four of these molecular aberrations in AAM and CaM who were treated at a single academic medical center and demonstrated similar pre- and post-operative clinicopathologic features.

Materials and Methods

Case selection

All parts of this retrospective study were carried out following Institutional Review Board approval. Archival formalin-fixed, paraffin-embedded (FFPE) radical prostatectomy (RP) specimens from 105 consecutive self-identified AAM who underwent RP between 2001 and 2011 were retrieved. Archival FFPE specimens from an existing tissue microarray cohort of 113 representative self-identified CaM who underwent RP from 2007 to 2009 were included as controls. Although year of surgery was more variable in the AAM cohort, the remaining clinical and pathologic characteristics of the two groups were similar (Table 1). All patients were treated at our institution, a tertiary care academic medical center, and all patients had pre-existing health insurance, suggesting equal access to care. Furthermore, there was no significant difference in the type of primary insurance between the two groups (private versus government-sponsored) with 81/105 AAM (77%) and 81/113 CaM (72%) having only private insurance ($p=0.36$). No patients received hormonal or radiation therapy prior to surgery.

Biochemical recurrence information was available for the majority of men; however, these rates were not adjusted for post-RP treatment, as post-RP treatment was administered at the discretion of the treating physicians. Biochemical recurrence was defined as a post-operative PSA value of >0.2 ng/mL on two separate occasions. The median follow-up time in the CaM cohort was 44 months, and the median follow-up time in the AAM cohort was 41 months. There were 3 CaM and 24 AAM who were lost to follow-up.

Pathologic evaluation and tissue microarray construction

Slides of the FFPE tissue from all RP specimens were reviewed by study pathologists to confirm the pathologic characteristics (TNM stage, Gleason score, margin status). The dominant tumor nodule, defined as the tumor with highest pathologic tumor stage, was selected from each case for construction of tissue microarrays (TMAs). TMAs were constructed using 0.6 mm cores from the FFPE blocks, with each sample represented in triplicate.

Fluorescence *in situ* hybridization (FISH) analysis of *ERG* rearrangement and *PTEN* deletion

Five μm -thick tissue sections from the TMA blocks were used for FISH analysis. For detection of *ERG* rearrangement, a dual-color break-apart interphase FISH assay was performed as previously described (14,25). Briefly, *ERG* rearrangement status was assessed using centromeric (BAC clone RP11-24A11 labeled red) and telomeric (BAC clone RP11-372O17 labeled green) probes (Figure 1). If $>20\%$ of tumor cells were found to have

translocation or deletion, the tumor was considered to have an *ERG* rearrangement. For detection of *PTEN* deletion, a gene specific probe (BAC clone CTD-2047N14) and a reference probe located at 10q25.2 (RP11-431P18) were used (Figure 1). Deletion of *PTEN* was defined as fewer than two copies of the gene specific probe in the presence of two reference signals in >20% of the tumor nuclei. For detection of both *ERG* rearrangement and *PTEN* deletion, at least 200 tumor nuclei per case were evaluated using a fluorescence microscope (Olympus BX51; Olympus Optical, Tokyo, Japan).

Immunohistochemical analysis of *ERG* and *SPINK1* overexpression

Immunohistochemical staining was applied using a commercially available antibody for *SPINK1* (clone 4D4, 1:100 dilution, Abnova) and *ERG* (clone EPR 3864, 1:100 dilution, Epitomics) on the Discovery XT biomarker platform (Ventana Medical Systems, Inc.) (Figure 1). Semi-quantitative evaluation of cytoplasmic *SPINK1* expression and nuclear *ERG* expression were separately performed. Staining of 5% of tumor cells was considered positive for each case.

SPOP mutation analysis

Using tissues cores from either fresh frozen material or archival FFPE blocks, samples of the same tumor nodule used for TMA construction were evaluated for *SPOP* mutations. DNA from fresh frozen material was extracted using phenol-chloroform and purified by ethanol precipitation method as previously described (26). DNA from archival FFPE material was extracted using the Qiagen Biorobot Universal system. High resolution melt analysis (HRM) followed by direct Sanger sequencing of putative *SPOP* somatic mutations was performed by standard methods following PCR amplification using specific primers. Sequences of the primers used for amplifying and sequencing *SPOP* have been recently described (21).

Statistical analysis

Chi-square or Fisher's exact tests were used to evaluate association between categorical variables. Wilcoxon Rank Sum Test was performed to compare continuous variables (*e.g.* age) between groups. For all statistical tests, a *p*-value <0.05 was considered statistically significant.

Results

ERG rearrangement, *PTEN* deletion, *SPINK1* overexpression, and *SPOP* mutation in prostate cancer differ in African American vs. Caucasian men

ERG rearrangement was identified in 48/113 tumors (42.5%) of CaM. In AAM, however, *ERG* rearrangement was found in 29/105 tumors (27.6%; *p*=0.024). There was no significant difference in the mechanism of gene fusion between the two cohorts (translocation vs. translocation with deletion). Of note, *ERG* rearrangement by FISH and protein overexpression by immunohistochemistry were concordant in all cases. Hemizygous deletion of *PTEN* was seen in 19/96 tumors (19.8%) in CaM but only 7/101 tumors (6.9%) in African American men (*p*=0.011). *SPINK1* overexpression was present in 9/110 tumors (8.2%) from CaM in contrast to 25/105 tumors (23.8%) from AAM (*p*=0.002). *SPOP* mutations were present in 8/78 (10.3%) prostate cancers in CaM in contrast to 4/88 (4.5%)

prostate cancers in AAM; however, this difference was not statistically significant ($p=0.230$). In CaM, *SPOP* mutations involved the F133 (6 cases), F102 (1 case), and K129 (1 case) residues. *SPOP* mutations in AAM involved the F133 (2 cases), F102 (1 case), and Y87 (1 case) residues.

When adjusted for age, BMI, Gleason score, and pathologic stage, *ERG* rearrangement and *SPINK1* overexpression remained significantly different between the two cohort ($p=0.018$ and $p=0.008$, respectively), and differences in *PTEN* deletion and *SPOP* mutation status approached statistical significance ($p=0.061$ and $p=0.087$, respectively). Table 2 summarizes the frequency of all of these molecular findings in the two cohorts.

Association of molecular abnormalities with clinical and pathologic characteristics

When considering both ethnic groups combined, prostate cancers harboring *PTEN* deletions were found to be significantly associated with higher average age ($p=0.001$), higher Gleason score ($p<0.001$), higher pathological stage ($p=0.003$), and increased rate of biochemical recurrence ($p=0.024$). All other clinicopathologic parameters were statistically similar with respect to each molecular abnormality. Table 3 summarizes these findings.

Among CaM alone, prostate cancers harboring *PTEN* deletions were found to be significantly associated with higher average age ($p=0.002$), higher Gleason score ($p=0.009$), higher pathological stage ($p=0.006$), and increased rate of biochemical recurrence ($p=0.034$) (Supplemental Table S1). *SPINK1* overexpression was associated with a lower Gleason score in the CaM cohort ($p=0.016$) (Supplemental Table S1). In the AAM cohort, all clinical and pathologic parameters were statistically similar with respect to each molecular abnormality (Supplemental Table S2).

Since the calendar years in which the AAM and CaM cases were accrued were disparate (2001-2011 and 2007-2009, respectively), statistical comparisons of the clinicopathological characteristics of patients accrued before 2007, from 2007-2009, and after 2009 were performed. No significant differences were observed with respect to pre-operative PSA, Gleason score, pathological stage, or the frequency of each of the molecular abnormalities ($p>0.05$ for all; data not shown).

ERG rearrangements and *SPOP* mutations are mutually exclusive, as are *PTEN* deletions and *SPINK1* overexpression

ERG rearrangements and *SPOP* mutations were not seen together in any of the 178 tumors evaluable for both molecular alterations ($p=0.009$). *PTEN* deletion and *SPINK1* overexpression were also mutually exclusive in the 195 tumors evaluable for both events ($p=0.009$). Furthermore, *ERG* rearrangement and *SPINK1* overexpression were mutually exclusive in all but one of the 215 cases ($p<0.001$). No association was noted between *ERG* rearrangement and *PTEN* deletion, *SPOP* mutation and *PTEN* deletion, or *SPOP* mutation and *SPINK1* overexpression ($p>0.05$). These findings are graphically depicted in Supplemental Figure S1.

Discussion

We investigated molecular differences in prostate cancer between two clinicopathologically similar cohorts of AAM and CaM treated at our institution. Our findings are concordant with recent studies showing that there is a significantly lower prevalence of *ERG* gene rearrangements in prostate cancers of AAM when compared to CaM (22–24,27). To our knowledge, this is the first study to evaluate ethnic differences in the prevalence of hemizygous *PTEN* deletions, *SPINK1* overexpression, and *SPOP* mutation status in prostate cancer. These findings contribute to our understanding of biological differences in prostate cancer between AAM and CaM, building essential groundwork for the development of personalized cancer treatment regimens.

The discovery of recurrent gene rearrangements in prostate cancer involving androgen regulated genes (e.g. *TMPRSS2*) and *ETS* family genes (14) as well as more recent data from whole genome sequenced localized prostate cancers (26,28) has increased our understanding of the disease at the molecular level, identifying potentially diagnostic, prognostic, and therapeutic markers. Results from unscreened, population-based cohorts (e.g. Swedish Watchful Waiting Cohort) have suggested that untreated prostate cancer with *ERG* rearrangement runs a more aggressive clinical course than those without *ERG* rearrangement (29). In the setting of surgical or other interventions following diagnosis, the data are insufficient to make any reasonable conclusions. Yoshimoto M, *et al* later demonstrated that absence of *ERG* rearrangement and *PTEN* loss in prostate cancer is associated with a favorable outcome (30). Conversely, duplication of *ERG* rearrangement with interstitial deletion of sequences 5' to *ERG* identified cases of fatal human prostate cancer in patients that had been conservatively managed (15). Regarding distinct molecular characteristics of prostate cancer among different ethnic/racial groups, two recent studies have assessed the difference in prevalence of *ERG* rearrangements between AAM and CaM (22–24). In the study by Magi-Galluzzi *et al*, *ERG* rearrangements were present in 50% of CaM versus 31% of AAM ($p=0.07$) and in the study by Rosen *et al.*, *ERG* rearrangements were present in 41.9% of CaM versus 23.9% of AAM ($p<0.0001$). These findings are in concordance with our current study showing that *ERG* rearrangements are less frequent in prostate cancers in AAM (42.5% in CaM vs. 27.6% in AAM, $p=0.024$). When adjusted for age, BMI, Gleason score, and pathologic stage, the difference remained significant ($p=0.018$). Similar to the study by Magi-Galluzzi *et al*, *ERG* rearrangement in our study did not correlate with other clinicopathologic parameters aside from ethnicity (22).

Regardless of *ERG* rearrangement's correlation with clinicopathologic features, ethnic differences in the prevalence of *ERG* rearrangements may have diagnostic implications, with urine-based screening tests currently under investigation (31,32). A recent review by Truong M *et al.*, highlights *ERG* rearrangement transcripts as one of the more promising RNA markers for cancer detection in urine samples; a urine-based test which uses a combination of *ERG* rearrangement transcripts and prostate cancer antigen-3 (PCA3) has already been marketed for clinical use (33,34). Considering that our study and previous ones (22–24,27) have shown a decreased prevalence of *ERG* rearrangements or *ERG* expression in prostate cancer of AAM, such a urine-based diagnostic test will be less sensitive in this population and may not be as useful of a screening tool as it may be for prostate cancer in CaM.

PTEN, which encodes a phosphoinositide 3-phosphatase that negatively regulates the PI3K and mTOR signaling pathways, is a well-known tumor suppressor gene in many tumor types; in prostate cancer, mutations in *PTEN* have been found to be associated with higher Gleason score, a higher rate of metastasis, androgen independence, and an overall worse prognosis (15–18). In a more recent, large, nested case control study, decreased *PTEN* expression was shown to be associated with an increased risk of biochemical recurrence, independent of other clinicopathologic factors(35). Loss of *PTEN* results in elevated downstream activity in the PI3K and mTOR pathways, which have known therapeutic targets. Although therapeutic approaches to develop inhibitors targeting the PI3K-AKT pathway have failed in both pre-clinical and clinical trials for prostate cancer, there are newer AKT pathway inhibitors that show promise, such as AZD5363 (36). Specifically in prostate cancer cell lines, another recent study has shown that loss of *PTEN* and elevated AKT/mTOR activity are associated with sensitivity to ridaforolimus, a particular mTOR inhibitor under investigation (37). Although population-based mutational analyses on *PTEN* have been performed, there are few studies which have investigated ethnic differences in the prevalence of hemizygous loss of *PTEN* in specific tumors. One study by Winter JL *et al.*, showed no significant racial differences in the expression of *PTEN* between invasive breast cancers in African American women and those in non-African American women (38). Similar to the racial disparities observed in prostate cancer, breast cancers in African American women are known to have a worse prognosis when compared to those in non-African Americans (39), but *PTEN* does not appear to play a major role in this disparity (38).

To our knowledge, our current study is the first to compare the prevalence of deletions in *PTEN* in prostate cancer between AAM and CaM. We found that hemizygous deletions in *PTEN* were less frequently present in our cohort of AAM compared to that observed in CaM (6.9% vs. 19.8%, $p=0.011$). However, when adjusted for age, BMI, Gleason score, and pathologic stage, the difference in prevalence was less pronounced and only trended toward significance ($p=0.061$). Our findings suggest that *PTEN* deletions may not be critical contributors to the increased incidence or mortality of prostate cancer in AAM, but larger studies with more power are warranted to confirm or refute our finding.

As expected, and consistent with prior literature, *PTEN* deletions in our study were significantly associated with a higher average age of patients, higher Gleason score, higher pathological stage, and increased rate of biochemical recurrence, though our analysis of biochemical recurrence is only a crude estimate that does not adjust for post-RP therapy and is limited by relatively short follow up time. These associations were statistically significant in CaM when analyzed alone, while no significant clinicopathologic associations with *PTEN* deletion were identified in AAM alone, possibly attributable to the low number of *PTEN* deletions in our AAM cohort. Although alterations in the mTOR/AKT and PI3K may still be present in AAM and prove to be therapeutic targets, they may be less frequently due to *PTEN* deletion than in CaM.

SPINK1 has structural similarity to epidermal growth factor (EGF) and has been demonstrated to activate the epidermal growth factor receptor (EGFR) on the surface of prostate cancer cells, leading to cell growth (20). Using a model of SPINK1-positive

prostate cancer (22RV1 cells), Ateeq *et al.* showed that monoclonal antibodies to either SPINK1 or EGFR (cetuximab) could slow the growth of SPINK1-positive tumors by over 60% and 40%, respectively, suggesting that it may be a reasonable therapeutic target (40). Moreover, SPINK1 overexpression has identified an aggressive subtype of *ETS* negative prostate cancer, validated in different cohorts (19). SPINK1 and *ERG* rearrangements have been found to be mutually exclusive in other studies as well (41,42), similar to our current findings. A study by Leinonen *et al.* found SPINK1 overexpression to be present in 10% of prostate cancers and also found it to be associated with an aggressive form of the disease, although mutual exclusivity with *ERG* rearrangements were not observed in this particular study (43).

Our study is the first to show that SPINK1 overexpression in a particular tumor type correlates with African American ethnicity. In our study, prostate cancers from AAM showed SPINK1 overexpression in 23.8% of cases compared with 8.2% of prostate cancers in CaM ($p=0.002$), and this difference remained statistically significant after adjusting for age, BMI, Gleason score, and pathologic stage ($p=0.008$). In the context of the aforementioned literature, which has demonstrated that SPINK1 overexpression is associated with more aggressive prostate cancers, our study suggests that SPINK1 overexpression may be one molecular aberrancy that plays a role in the increased incidence and/or mortality observed in AAM with prostate cancer, although we emphasize that our study was not designed to demonstrate association of these molecular alterations with clinical outcomes. Furthermore, any targeted therapies to SPINK1 that could develop in the future, as proposed by Ateeq B *et al.* (40), potentially may benefit more AAM than CaM with prostate cancer.

More recently, whole genome and exome sequencing of prostate cancer has elucidated novel recurrent mutations in prostate cancer such as *SPOP*, *MED12*, and *FOXAI* (25,26). The most common non-synonymous somatic mutation involves *SPOP*, which encodes the substrate-binding subunit of a cullin-based E3 ubiquitin ligase (44,45). This recurrent mutation defines a new molecular subtype of *ETS*-negative prostate cancer (21). After having sequenced the *SPOP* gene in more than 300 primary prostate cancers and metastases, all *SPOP* mutations affected conserved residues in the structurally defined substrate binding cleft (21). Recent work in breast cancer has shown that *SPOP* directly interacts with a p160 steroid resistant coactivator, SRC-3, part of a family of proteins which are overexpressed in numerous human cancers; they are associated with poor clinical outcomes and resistance to therapy and are considered to be potential therapeutic targets (45,46). The interaction of *SPOP* and SRC-3 in breast cancer promotes cullin 3-dependent ubiquitination and proteolysis, thereby supporting *SPOP*'s role as a potential tumor suppressor (47).

In prostate cancer cell lines, *SPOP* mutants have been shown to be unable to interact with SRC-3 protein or promote its ubiquitination and subsequent degradation, suggesting that *SPOP* plays a critical tumor suppressor role in prostate cancer and supporting the potential of SRC-3 as a therapeutic target in prostate cancer (48). In our study, *SPOP* mutations were less frequently seen in prostate cancers from AAM than from CaM, although this difference did not reach statistical significance ($p=0.230$). When adjusted for age, BMI, Gleason score, and pathologic stage, however, this difference approaches statistical significance ($p=0.087$).

Although further work is needed in order to fully elucidate the biological and prognostic significance of *SPOP* mutations in prostate cancer *in vivo*, as well as the therapeutic potential of SRC-3, our findings suggest that *SPOP* mutations are less likely to play a significant role in prostate cancer in AAM compared to CaM.

Our study certainly is not devoid of limitations. First, our cohorts contained a relatively modest number of patients from a single institution; larger studies are needed to validate our findings. In addition, there were some cases for which there was missing data on the molecular alterations, highlighted in Supplemental Figure S1, which was attributable to missing or insufficient tissue on the TMAs (for *ERG* rearrangement, *SPINK1* overexpression, and *PTEN* deletion) or insufficient tissue for DNA extraction (for *SPOP* mutation analysis). Second, while two techniques were used to assess *ERG* rearrangement and were concordant, only one technique was used for the other molecular alterations, limiting our ability to confirm these molecular changes. Third, we also must emphasize that our study was not designed or powered to assess clinical outcomes of our patient cohorts, and, therefore, any conclusions as to whether the molecular alterations in these patients have prognostic value would be premature. Assessment of biochemical recurrence in our cohorts was performed only to show rough consistency with prior literature on *PTEN* and its association with worse outcomes(15–18). In addition, the median length of follow up time in both cohorts was relatively short (41 months and 44 months in AAM and CaM, respectively) with a large number of AAM lost to follow up (24 patients). Lastly, we did not obtain socioeconomic data on our patient population, which has been shown to contribute to prostate cancer outcomes(2). However, given that all patients in our study were treated at a single academic tertiary care facility, that all patients had pre-existing health insurance, and that >70% of patients in each cohort had private insurance coverage suggest that our patients had comparable access to oncologic care. The comparable (and mostly low) pathologic stages of the tumors between both cohorts at radical prostatectomy also suggest that screening and early diagnosis of prostate cancer occurred in both groups.

In an era where precision therapy of prostate cancer is rapidly changing, molecular characterization of both localized and metastatic prostate tumors will help stratify which men will benefit from active surveillance, surgery, targeted therapy, and hormonal and/or chemoradiation therapy. Already, recent studies have shown that ethnicity is an important factor in the progression of prostate cancers under active surveillance (12,49), suggesting perhaps that prostate cancers among different races should be managed differently. It has also been shown previously that prostate cancers with *ERG* rearrangement have a worse outcome under active surveillance(29), highlighting its potential importance in influencing therapeutic management. Furthermore, a study by Bismar *et al.* suggests that molecular aberrancies in *PTEN*, *ERG*, and *SPINK1* may be involved in the development of castration-resistant prostate cancer, emphasizing their clinical importance (42). Our current study highlights the significant differences that exist at the molecular level when prostate cancers from clinicopathologically similar AAM and CaM are compared. We have demonstrated that *ERG* gene rearrangement, *PTEN* deletion, and *SPOP* mutation have a lower prevalence in prostate tumors of AAM and likely play a lesser role in incidence or mortality differences. In contrast, *SPINK1* overexpression, a molecular aberrancy that has been found to be

associated with more aggressive prostate cancer (19,41,50), is more prevalent in AAM, suggesting that it plays a more important role in the disease within this ethnic group. As our study was not designed to assess clinical outcomes in association with these molecular alterations, larger studies with more detailed clinical outcome data will be needed in order to determine if any of these molecular differences at least partially explain the disparities in incidence and mortality between these two ethnic groups. In addition, future work on whole genome/exome sequencing of prostate cancer will help us to better characterize potential therapeutic targets in prostate tumors among different ethnic groups.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Ries, LAG.; Eisner, MP.; Kosary, CL.; Hankey, BF.; Miller, BA.; Clegg, L.; Mariotto, A.; Feuer, EJ.; Edwards, BK., editors. SEER Cancer Statistics Review, 1975-2002. National Cancer Institute; Bethesda, MD: http://seer.cancer.gov/csr/1975_2002/, based on November 2004 SEER data submission, posted to the SEER web site 2005
2. Freedland SJ, Isaacs WB. Explaining racial differences in prostate cancer in the United States: sociology or biology? *Prostate*. 2005; 62:243–52. [PubMed: 15389726]
3. Alexander G, Brawley O. Prostate cancer treatment outcome in blacks and whites: a summary of the literature. *Semin Urol Oncol*. 1998; 16:232–4. [PubMed: 9858331]
4. Powell IJ, Banerjee M, Novallo M, Sakr W, Grignon D, Wood DP, et al. Prostate cancer biochemical recurrence stage for stage is more frequent among African-American than white men with locally advanced but not organ-confined disease. *Urology*. 2000; 55:246–51. [PubMed: 10688088]
5. Platz EA, Rimm EB, Willett WC, Kantoff PW, Giovannucci E. Racial variation in prostate cancer incidence and in hormonal system markers among male health professionals. *J Natl Cancer Inst*. 2000; 92:2009–17. [PubMed: 11121463]
6. Bennett CL, Price DK, Kim S, Liu D, Jovanovic BD, Nathan D, et al. Racial variation in CAG repeat lengths within the androgen receptor gene among prostate cancer patients of lower socioeconomic status. *J Clin Oncol*. 2002; 20:3599–604. [PubMed: 12202660]
7. Gaston KE, Kim D, Singh S, Ford OH, Mohler JL. Racial differences in androgen receptor protein expression in men with clinically localized prostate cancer. *J Urol*. 2003; 170:990–3. [PubMed: 12913756]
8. Ross R, Bernstein L, Judd H, Hanisch R, Pike M, Henderson B. Serum testosterone levels in healthy young black and white men. *J Natl Cancer Inst*. 1986; 76:45–8. [PubMed: 3455741]
9. Gapstur SM, Gann PH, Kopp P, Colangelo L, Longcope C, Liu K. Serum androgen concentrations in young men: A longitudinal analysis of associations with age, obesity, and race: The CARDIA male hormone study serum androgen concentrations in young men. *Cancer Epidemiol Biomarkers Prev*. 2002; 1041–7. [PubMed: 12376505]
10. Kim HS, Moreira DM, Jayachandran J, Gerber L, Bañez LL, Vollmer RT, et al. Prostate biopsies from black men express higher levels of aggressive disease biomarkers than prostate biopsies from white men. *Prostate Cancer Prostatic Dis*. 2011; 14:262–5. [PubMed: 21519348]

11. Grisanzio C, Werner L, Takeda D, Awoyemi BC, Pomerantz MM, Yamada H. Genetic and functional analyses implicate the NUDT11, HNF1B, and SLC22A3 genes in prostate cancer pathogenesis. *Proc Natl Acad Sci USA*. 2012; 109:11252–7. [PubMed: 22730461]
12. Iremashvili V, Soloway MS, Rosenberg DL, Manoharan M. Clinical and demographic characteristics associated with prostate cancer progression in patients on active surveillance. *J Urol Elsevier Inc*. 2012; 187:1594–9.
13. Kwabi-Addo B, Wang S, Chung W, Jelinek J, Patierno SR, Wang BD, et al. Identification of differentially methylated genes in normal prostate tissues from African American and Caucasian men. *Clin Cancer Res*. 2010; 16:3539–47. [PubMed: 20606036]
14. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science (80-)*. 2005; 310:644–8.
15. Shen MM, Abate-Shen C. Pten inactivation and the emergence of androgen-independent prostate cancer. *Cancer Res*. 2007; 67:6535–8. [PubMed: 17638861]
16. Pourmand G, Ziaee AA, Abedi AR, Mehraei A, Alavi HA, Ahmadi A, et al. Role of PTEN gene in progression of prostate cancer. *Urol J*. 2007; 4:95–100. [PubMed: 17701929]
17. Reid AHM, Attard G, Ambroisine L, Fisher G, Kovacs G, Brewer D, et al. Molecular characterisation of ERG, ETV1 and PTEN gene loci identifies patients at low and high risk of death from prostate cancer. *Br J Cancer*. 2010; 102:678–84. [PubMed: 20104229]
18. Li Y, Su J, DingZhang X. PTEN deletion and heme oxygenase-1 overexpression cooperate in prostate cancer progression and are associated with adverse clinical outcome. *J*. 2011; 224:90–100.
19. Tomlins SA, Rhodes DR, Yu J, Varambally S, Mehra R, Perner S, et al. The role of SPINK1 in ETS rearrangement-negative prostate cancers. *Cancer Cell*. 2008; 13:519–28. [PubMed: 18538735]
20. Paju A, Hotakainen K, Cao Y, Laurila T. Increased expression of tumor-associated trypsin inhibitor, TATI, in prostate cancer and in androgen-independent 22Rv1 cells. *Eur Urol*. 2007; 52:1670–9. [PubMed: 17306443]
21. Barbieri C, Baca S, Lawrence M. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. *Nat*. 2012; 44:685–9.
22. Magi-Galluzzi C, Tsusuki T, Elson P, Simmerman K, LaFargue C, Esgueva R, et al. TMPRSS2-ERG gene fusion prevalence and class are significantly different in prostate cancer of Caucasian, African-American and Japanese patients. *Prostate*. 2011; 71:489–97. [PubMed: 20878952]
23. Rosen P, Pfister D, Young D, Petrovics G, Chen Y, Cullen J, et al. Differences in frequency of ERG oncoprotein expression between index tumors of Caucasian and African American patients with prostate cancer. *Urology Elsevier Inc*. 2012; 80:749–53.
24. Powell IJ, Dyson G, Land S, Ruterbusch J, Bock CH, Lenk S, et al. Genes associated with prostate cancer are differentially expressed in african american and European american men. *Cancer Epidemiol Biomarkers Prev*. 2013; 22:891–7. [PubMed: 23515145]
25. Perner S, Demichelis F, Beroukhim R. TMPRSS2: ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer. *Cancer Res*. 2006; 66:8337–41. [PubMed: 16951139]
26. Berger MF, Lawrence MS, Demichelis F, Drier Y, Cibulskis K, Sivachenko AY, et al. The genomic complexity of primary human prostate cancer. *Nature*. 2011; 470:214–20. [PubMed: 21307934]
27. Mosquera JM, Mehra R, Regan MM, Perner S, Genega EM, Bueti G, et al. Prevalence of TMPRSS2-ERG fusion prostate cancer among men undergoing prostate biopsy in the United States. *Clin Cancer Res*. 2009; 15:4706–11. [PubMed: 19584163]
28. Baca S, Prandi D, Lawrence M. Punctuated evolution of prostate cancer genomes. *Cell*. 2013; 153:666–77. [PubMed: 23622249]
29. Demichelis F, Fall K, Perner S, Andr n O, Schmidt F, Setlur SR, et al. TMPRSS2:ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. *Oncogene*. 2007; 26:4596–9. [PubMed: 17237811]

30. Yoshimoto M, Joshua AM, Cunha IW, Coudry Ra, Fonseca FP, Ludkovski SR, et al. Absence of TMPRSS2:ERG fusions and PTEN losses in prostate cancer is associated with a favorable outcome. *Mod Pathol.* 2008; 21:1451–60. [PubMed: 18500259]
31. Young A, Palanisamy N, Siddiqui J, Wood DP, Wei JT, Chinnaiyan AM, et al. Correlation of urine TMPRSS2:ERG and PCA3 to ERG+ and total prostate cancer burden. *Am J Clin Pathol.* 2012; 138:685–96. [PubMed: 23086769]
32. Tomlins SA, Aubin SMJ, Siddiqui J, Lonigro RJ, Sefton-Miller L, Miick S, et al. Urine TMPRSS2:ERG fusion transcript stratifies prostate cancer risk in men with elevated serum PSA. *Sci Transl Med.* 2011; 3:94ra72.
33. Truong M, Yang B, Jarrard DF. Toward the detection of prostate cancer in urine: a critical analysis. *J Urol.* 2013; 189:422–9. [PubMed: 23017522]
34. Salami SS, Schmidt F, Laxman B, Regan MM, Rickman DS, Scherr D, et al. Combining urinary detection of TMPRSS2:ERG and PCA3 with serum PSA to predict diagnosis of prostate cancer. *Urol Oncol.* 2013; 31:566–71. [PubMed: 21600800]
35. Chaux A, Peskoe SB, Gonzalez-Roibon N, Schultz L, Albadine R, Hicks J, et al. Loss of PTEN expression is associated with increased risk of recurrence after prostatectomy for clinically localized prostate cancer. *Mod Pathol.* 2012; 25:1543–9. [PubMed: 22684219]
36. Lamoureux F, Zoubeidi A. Dual inhibition of autophagy and the AKT pathway in prostate cancer. *Autophagy.* 2013; 9
37. Squillace RM, Miller D, Wardwell SD, Wang F, Clackson T, Rivera VM. Synergistic activity of the mTOR inhibitor ridaforolimus and the antiandrogen bicalutamide in prostate cancer models. *Int J Oncol.* 2012; 41:425–32. [PubMed: 22614157]
38. Winter JL, Stackhouse BL, Russell GB, Kute TE. Measurement of PTEN expression using tissue microarrays to determine a race-specific prognostic marker in breast cancer. *Arch Pathol Lab Med.* 2007; 131:767–72. [PubMed: 17488163]
39. American Cancer Society. *Cancer Facts & Figures for African Americans 2013-2014.* Atlanta: American Cancer Society; 2013.
40. Ateeq B, Tomlins S. Therapeutic targeting of SPINK1-positive prostate cancer. *Sci Transl Med.* 2011; 3:1–18.
41. Lippolis G, Edsjö A, Stenman UH, Bjartell A. A high-density tissue microarray from patients with clinically localized prostate cancer reveals ERG and TATI exclusivity in tumor cells. *Prostate Cancer Prostatic Dis.* 2013; 16:145–50. [PubMed: 23459095]
42. Bismar T, Yoshimoto M, Duan Q. Interactions and relationships of PTEN, ERG, SPINK1 and AR in castration-resistant prostate cancer. 2012; 60:645–52.
43. Leinonen K, Tolonen T. Association of SPINK1 expression and TMPRSS2: ERG fusion with prognosis in endocrine-treated prostate cancer. *Clin Cancer.* 2010; 16:2845–51.
44. Zhuang M, Calabrese MF, Liu J, Waddell MB, Nourse A, Hammel M, et al. Structures of SPOP-substrate complexes: insights into molecular architectures of BTB-Cul3 ubiquitin ligases. *Mol Cell.* 2009; 36:39–50. [PubMed: 19818708]
45. Nagai Y, Kojima T, Muro Y, Hachiya T, Nishizawa Y, Wakabayashi T, et al. Identification of a novel nuclear speckle-type protein, SPOP. *FEBS Lett.* 1997; 418:23–6. [PubMed: 9414087]
46. Xu J, Wu RC, O'Malley BW. Normal and cancer-related functions of the p160 steroid receptor co-activator (SRC) family. *Nat Rev Cancer.* 2009; 9:615–30. [PubMed: 19701241]
47. Li C, Ao J, Fu J, Lee DF, Xu J, Lonard D, et al. Tumor-suppressor role for the SPOP ubiquitin ligase in signal-dependent proteolysis of the oncogenic co-activator SRC-3/AIB1. *Oncogene.* 2011; 30:4350–64. [PubMed: 21577200]
48. Geng C, He B, Xu L, Barbieri CE, Eedunuri VK, Chew SA, et al. Prostate cancer-associated mutations in speckle-type POZ protein (SPOP) regulate steroid receptor coactivator 3 protein turnover. *Proc Natl Acad Sci USA.* 2013; 110:6997–7002. [PubMed: 23559371]
49. Abern M, Bassett M, Tsivian M. Race is associated with discontinuation of active surveillance of low-risk prostate cancer: Results from the Duke Prostate Center. *Prostate Cancer Prostatic Dis.* 2012; 16:85–90. [PubMed: 23069729]
50. Bismar T, Yoshimoto M, Vollmer R. PTEN genomic deletion is an early event associated with ERG gene rearrangements in prostate cancer. *BJU Int.* 2011; 107:477–85. [PubMed: 20590547]

Translational Relevance

African Americans have a higher incidence of prostate cancer and higher mortality from the disease than that observed in Caucasians. Although socioeconomic factors may contribute to these differences, underlying genetic differences are believed to play a role as well. In the current study, we highlight significant differences in ERG gene rearrangement, PTEN deletion, SPINK1 overexpression, and SPOP mutation status in prostate cancer of African- American men compared to Caucasian men. Our findings suggest biologic differences between prostate cancers from these two ethnic groups, with ERG rearrangement, PTEN deletion, and SPOP mutation less frequent in African American men and SPINK1 overexpression more frequent. In view of forthcoming new molecular diagnostic modalities and targeted therapies for prostate cancer, molecular classification of this disease is germane; understanding ethnic differences in this disease will allow for optimizing screening methods and selecting appropriate treatment plans.

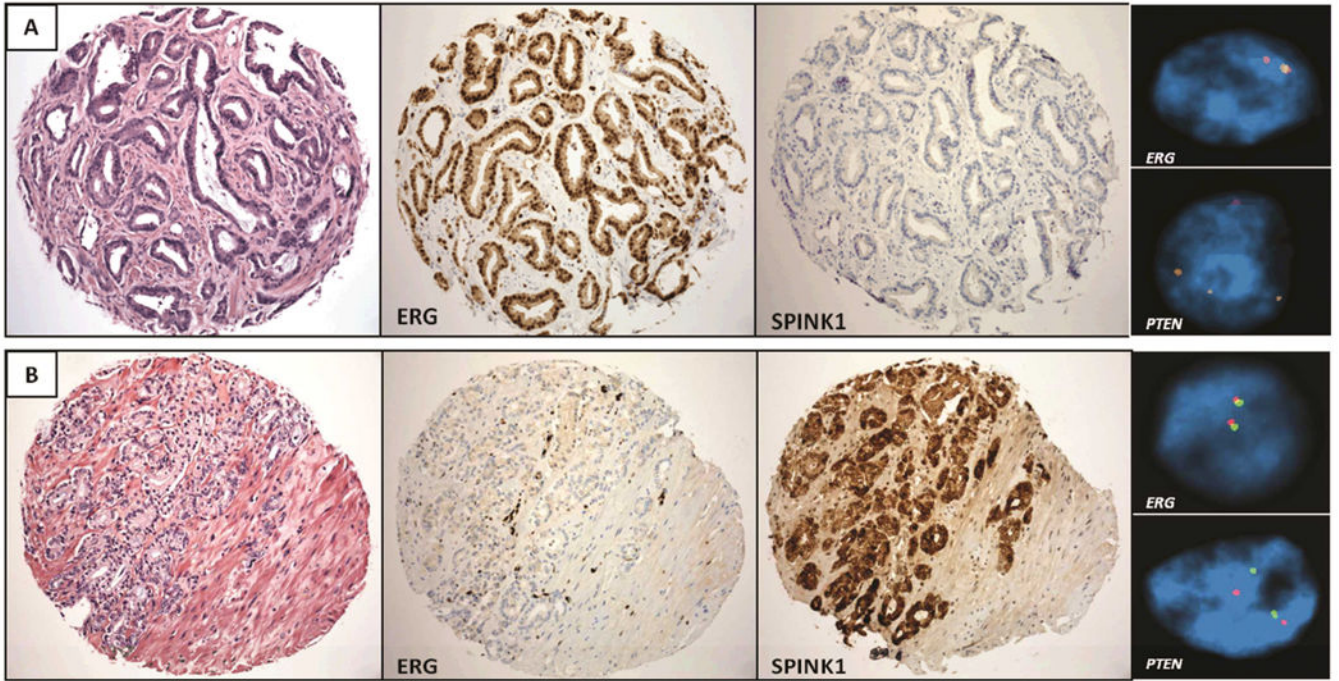


Figure 1. Immunohistochemical Staining for ERG/SPINK1 and FISH for *ERG* Rearrangement and *PTEN* Deletion

Panel A shows a prostatic adenocarcinoma which demonstrates positive *ERG* immunostaining and a corresponding *ERG* rearrangement by FISH. The tumor is negative for SPINK1 overexpression and shows a hemizygous deletion of *PTEN*.

Panel B shows a prostatic adenocarcinoma which demonstrates negative *ERG* immunostaining and no *ERG* rearrangement by FISH. The tumor shows SPINK1 overexpression and no deletion of *PTEN* by FISH.

Table 1
Clinical and Pathologic Characteristics of 218 Men with Prostate Cancer Treated by Radical Prostatectomy

	Caucasian	African American	<i>p</i> -value
Number of men	113	105	
Age at Surgery			0.148
Mean ± SD	61.2 ± 7.1	59.6 ± 7.6	
Range	45.6-75.5	37.0-73.1	
Body Mass Index (BMI)			0.924
Mean ± SD	26.9 ± 3.2	27.0 ± 5.4	
Range	22.0-38.0	19-68.0	
Gleason Score [no.(%)]			0.601
6	18 (16)	19 (18)	
7(3+4)	63 (56)	57 (54)	
7(4+3)	18 (16)	21 (20)	
8 and 9	14 (12)	8 (8.0)	
Pathological Stage [no. (%)]			0.160
T2	80 (71)	85 (81)	
T3a	25 (22)	13 (12)	
T3b	8 (7.1)	7 (6.6)	
Margin Positivity [no./total (%)]	18/113 (16)	13/105 (12)	0.561
Biochemical Recurrence [no./total (%)]	14/110 (12)	12/81 (15)	0.665

Table 2
Prevalence of Molecular Aberrations in Prostate Cancer of AAM versus CaM

	African American	Caucasian	p-value	Adjusted p-value [*]
<i>ERG</i> rearrangement	27.6% (29/105)	42.5% (48/113)	0.024	0.018
<i>PTEN</i> deletion	6.9% (7/101)	19.8% (19/96)	0.011	0.061
<i>SPINK1</i> overexpression	23.8% (25/105)	8.2% (9/110)	0.002	0.008
<i>SPOP</i> mutation	4.5% (4/88)	10.3% (8/78)	0.230	0.087

* Adjusted for age, BMI, Gleason score, and pathologic stage.

Table 3
Association of Clinical and Pathologic Characteristics with Molecular Abnormalities (combined AAM and CaM cohorts)

	ERG rearrangement			PTEN deletion			SPINK1 overexpression			SPOP mutation		
	Positive	Negative	P-value	Deleted	Wild type	P-value	Positive	Negative	P-value	Mutated	Wild type	P-value
Age at Surgery (Mean ± SD)	59.9 ± 7.0	60.7 ± 7.6	0.298	64.6 ± 7.4	59.5 ± 7.3	0.001	57.6 ± 8.2	60.9 ± 7.1	0.067	60.8 ± 7.2	60.3 ± 7.4	0.812
BMI (Mean ± SD)	26.9 ± 3.2	27.0 ± 4.8	0.622	26.4 ± 2.3	27.1 ± 4.6	0.628	26.0 ± 3.3	27.1 ± 4.5	0.190	27.0 ± 4.7	26.5 ± 3.8	0.899
Gleason Score [no./total (%)]			0.285			<0.001			0.163			0.305
6	13/37 (35)	24/37 (65)		1/35 (3)	34/35 (97)		9/36 (25)	27/36 (75)		0/29 (0)	29/29 (100)	
7(3+4)	48/120 (40)	72/120 (60)		12/109 (11)	97/109 (89)		20/118 (17)	98/118 (83)		7/96 (7)	89/96 (93)	
7(4+3)	9/39 (23)	30/39 (77)		5/35 (14)	30/35 (66)		4/39 (10)	35/39 (90)		4/34 (12)	30/34 (88)	
8 and 9	7/22 (32)	15/22 (68)		8/18 (44)	10/18 (56)		1/22 (5)	21/22 (95)		1/19 (5)	18/19 (95)	
Pathological Stage [no./total (%)]			0.139			0.003			1.000			0.259
T2	59/165 (35.8)	106/165 (64.2)		13/149 (8.7)	136/149 (91.3)		26/162 (16.0)	136/162 (84.0)		11/123 (8.9)	112/123 (91.1)	
T3a	16/38 (42.1)	22/38 (57.9)		8/33 (24.2)	25/33 (75.8)		6/38 (15.8)	32/38 (84.2)		0/28 (0)	28/28 (100)	
T3b	2/15 (13.3)	13/15 (86.7)		5/15 (33.3)	10/15 (66.7)		2/15 (13.3)	13/15 (86.7)		1/14 (7.1)	13/14 (92.9)	
Margin Positivity [no./total (%)]	8/218 (3.7)	23/218 (10.5)	0.311	2/197 (1.0)	25/197 (12.7)	0.541	4/215 (1.9)	27/215 (12.6)	0.793	1/166 (0.6)	26/166 (15.7)	0.440
Biochemical Recurrence [no./total (%)]	7/191 (3.6)	19/191 (9.9)	0.383	7/171 (4.0)	16/171 (9.3)	0.024	5/188 (2.6)	21/188 (11.1)	0.562	2/141 (1.4)	21/141 (14.9)	0.743