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Differences in the frequency of ERG oncoprotein expression between index tumors of Caucasian American and African American prostate cancer patients

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Abstract

OBJECTIVE—To systematically evaluate ERG alterations in the multifocal tumor context by using whole-mount prostatectomy specimens of African American and Caucasian American patients matched for age, pathologic grade and stage. Oncogenic activation of the ETS-Related Gene (*ERG*) is the most common early genomic alteration in prostate cancer patients in Western countries. However, ERG alterations have not been systematically examined in African American patients with known higher risk of prostate cancer incidence and mortality.

METHODS—ERG oncoprotein expression was analyzed in 91 Caucasian American and 91 African American prostate cancer patients matched for age, Gleason score and pathologic stage. A unique aspect of this study was the evaluation of ERG in whole-mount prostatectomy sections, minimizing sampling bias and allowing the careful assessment of ERG in the multifocal tumor context of prostate cancer.

RESULTS—The frequency of ERG positive prostate tumors was significantly greater among Caucasian Americans vs. African Americans when assessed in all tumor foci (41.9% vs. 23.9%, $p < 0.0001$). Markedly higher frequency of the ERG oncoprotein expression was noted between the index tumors of Caucasian Americans (63.3%) and African Americans (28.6%). Of note, in African American patients the higher grade index tumors were predominantly ERG negative.

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CONCLUSIONS—ERG typing of prostate tumors establishes a major difference between the index tumors of Caucasian and African American patients. ERG negative index tumors may indicate less favorable outcome in African American patients. This study underscores that typing of prostate tumors for ERG may enhance our understanding of biological differences between the examined ethnic groups.

INTRODUCTION

Carcinoma of the prostate (CaP) displays health disparities reflected by differences in cancer incidence and mortality among different populations with a higher incidence in Western countries and a lower incidence in Asian countries.¹ African Americans (AA) with CaP are diagnosed with significantly worse clinical parameters, e.g., with a 2.5-fold greater chance of dying from the disease in comparison to Caucasian Americans (CA).² However, biological reasons for these differences remain poorly understood. Increased associations of a genetic variant of CYP3A4, germ line mutations of EphB2 tyrosine kinase and macrophage scavenger receptor 1 (MSR1), and single nucleotide polymorphism (SNPs) have been reported in AA CaP.³ Differences of serum levels of vitamin D, IGF1, IGFBP3, and LDL have also been reported between AA and CA CaP patients.⁴

Frequent genomic alterations in CaP involve fusions of regulatory sequences of the androgen receptor (AR) regulated genes (predominantly *TMPRSS2*) and protein coding sequences of ETS transcription factors (predominantly *ERG*).⁵ Recent evaluation of ERG oncoprotein from our group in whole-mounted CaP specimens demonstrated 65% frequency and very high concordance (96.5%) of ERG positive prostatic intraepithelial neoplasia (PIN) and prostatic adenocarcinoma.⁶ Presence of ERG oncoprotein also shows high correlation with *ERG* fusions.^{6–9} Although, differences in ERG alterations between AA and CA have been suggested by us and others^{10–12}, this study has systematically examined ERG alterations in the multifocal tumor context by using whole-mount prostatectomy specimens of AA and CA patients matched for age, pathologic grade and stage.

MATERIAL AND METHODS

Radical prostatectomy specimens were obtained from patients enrolled in the Center for Prostate Disease Research (CPDR) program and processed as whole-mounts between 1996 and 2010 under an Institutional Review Board-approved protocol from the Walter Reed National Military Medical Center. Archived specimens from the 1237 cases were examined with complete data on age at surgery [mean 59.5, (SD: 7.7), median 60.4 (range 39.2–80.1)], serum PSA at diagnosis in ng/mL [mean: 10.8 (SD:143.0), median 5.2 (range 0.4–5065)], self declared ethnicity [AA 327 (26.4%) and CA 876 (70.8%)], family history of CaP [none in 696 (65.5%), yes in 366 (34.5%)], pathological T stage [pT2 in 771 (62.3%), pT3 in 466 (37.7%)], and pathological Gleason sum [6 or less in 656 (53.0%), 7 in 451 (36.5%) and 8 to 10 in 130 (10.5%)]. From the 1237 radical prostatectomy specimens 91 CA and 91 AA CaP patients were matched for age, Gleason sum and pathologic stage (Table 1). The average and medium number of tumor foci among AA and CA patients were approximately two with no statistically significant difference (Table 2). One representative whole-mount cross section of each specimen was selected from each of the 182 patients. Pathology evaluation was performed for each tumor focus.

Four μ m sections were dehydrated and blocked in 0.6% hydrogen peroxide in methanol for 20 min. and were processed for antigen retrieval in EDTA (pH 9.0) for 30 min in a microwave followed by 30 min of cooling in EDTA buffer. Sections were then blocked in 1% horse serum for 40 min and were incubated with the ERG-MAb mouse monoclonal antibody (9FY, now available from Biocare Medical Inc.) at a dilution of 1:1280 for 60 min

at room temperature. Sections were incubated with the biotinylated horse anti-mouse antibody at a dilution of 1:200 (Vector Laboratories, Burlingame, CA) for 30 min followed by treatment with the ABC Kit (Vector Laboratories, Burlingame, CA) for 30 min. The color was developed by VIP (Vector Laboratories, Burlingame, CA) treatment for 5 min. and the sections were counter stained by hematoxylin. ERG expression was reported as positive or negative in index tumors (largest tumor with highest grade within the prostate), as well as in the multi-focal tumor context. ERG protein expression was correlated with clinico-pathologic features.

ERG rearrangement status of chromosome 21q22.2 was analyzed by FISH break-apart assay.⁸ Biotin-14-dCTP labeled BAC clone RP11-24A11 (red avidin-rhodamine) and digoxigenin-dUTP-labeled BAC clone RP11-137J13 (green fluorescein-tagged anti-DIG antibody) was used in the assay. BAC clones were kindly provided by Dr. Settara C. Chandrasekharappa, National Human Genome Research Institute, and were prepared by the GenePro Diagnostics, Inc. (Rockville, Maryland). Tissues were assayed by hybridization and analyzed by fluorescence microscopy.

Categorical patient clinico-pathological features were compared across group of patients using frequencies and percentages. Continuously measured patient features were compared using measures of central tendency, including mean and median, as well as measures of dispersion, including standard deviation and range. Student test, Wilcoxon rank sum test or Chi square test were used to evaluate the difference of the distribution of clinic-pathological characteristics between AA and CA groups. The difference of ERG immunohistochemistry status (positive vs. negative) between AA and CA, as well as the association of ERG status with Gleason stratified by race/ethnicity, were examined by using Chi square test. Biochemical recurrence (BR), defined as two consecutive post-operative PSA values greater than 0.2 ng/mL measured at ≥ 8 weeks post-operatively, was correlated with ERG status. If BR status could not be assessed, only patients with at least 24 months of follow up were considered for analysis. For the assessment of differences in BR-free survival across ERG status, unadjusted Kaplan Meier (KM) survival analysis was conducted. The log rank test was used to test for differences in KM curves by ERG status; $p < 0.05$ was considered to be statistically significant. For all data analysis, the SAS version 9.3 was used.

RESULTS

Overall, a higher frequency of ERG oncoprotein expression was detected in prostate tumors of CA patients in comparison to AA patients (Table 2). Fifty-nine of 91 CA patients (65.9%) and 41 of 91 (42.9%) AA patients had at least one ERG positive tumor ($p = 0.0018$). Moreover, 80 of 191 (41.9%) individual tumor foci were ERG positive type of tumors in CA versus 49 of 205 in AA (23.9%; $P < 0.0001$). There was a high concordance of ERG positive or negative PINs with ERG positive or negative tumors in both CA (95.3%) and AA patients (95.5%).

A remarkable difference was noted in the frequency of ERG positive index tumors between CA (57/91; 63.3%) and AA (24/91; 28.6%) patients ($p < 0.0001$). Consistent with the higher frequency of ERG negative index tumor types in AA patients, the correlation of ERG status with pathologic grade revealed a significant association with higher grade tumors (Table 3A). Although not statistically significant, data indicated a trend of association between PSA recurrence and the frequency of ERG negative tumor types among AA patients ($p = 0.2201$) that was not found among CA patients ($p = 0.9057$). Evaluation of ERG rearrangement by fluorescence in situ hybridization (FISH) in 20 AA and 20 CA cases revealed 99% concordance with ERG protein frequencies noted for the ERG oncoprotein expression.

COMMENT

This study underscores a striking difference of ERG oncoprotein expression between prostate tumors of CA and AA patients by comprehensive evaluation of oncoprotein expression in multiple tumor foci of whole-mount prostatectomy specimens from patients matched for age and tumor pathologic features. The differences of ERG expression between AA and CA CaP are consistent with the data from our initial quantitative evaluations *ERG* mRNA in CaP¹⁰ as well as, from recent studies of *ERG* mRNA in post DRE urine.¹¹ Similar differences were also noted in studies of *TMPRSS2-ERG* fusion status or ERG protein expression in CaP^{6,12} including this study. Increased association of the *TMPRSS2-ERG* fusion with interstitial deletion (IDEL type) in AA CaP specimens noted in a previous report¹² was also observed in this study. Our study showed that the index tumor was most likely to express ERG if any of the tumor foci was ERG positive in CA patients (ratio of index/any = 0.96). In contrast, this ratio was much lower in AA CaP patients (ratio of index/any = 0.67). This finding suggests for a more frequent clonal expansion of ERG positive foci in CA than in AA patients as illustrated in Fig. 1. We postulate that the majority of index tumors in AA patients are utilizing distinct genetic mechanisms for progression. Of note, higher frequency of ERG negative tumor types of AA patients was reflected in preponderance of high-grade tumors, whereas, this difference was not apparent in tumors of CA cases (Table 3B).

CONCLUSION

The findings presented in this study have a potential to stratify CaP by the status of cancer gene alterations, such as ERG, towards the understanding of widely-observed differences of disease aggressiveness noted between AA and CA patients.^{13–14} The emerging ethnic differences of ERG alteration are intriguing. In a limited number of studies, much lower frequencies of ERG positivity have been reported in prostate cancer of Asian patients (~20%).^{12,15} Issues related to sampling biases, tumor biology or treatment effects have not been ruled out for the apparent differences noted between in ERG frequencies in prostate cancers from Western and Asian countries. In this regard, this report has taken into consideration these issues when establishing the differences of ERG frequencies between CA and AA patients. Since *ERG* oncogenic alterations are being extensively evaluated as a biomarker and therapeutic target in CaP, ERG typing of prostate cancers has potential to unravel biological differences of prostate cancer in different ethnic groups.

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Figure 1.

Representative images of whole-mount sections analyzed by ERG immunohistochemistry and by FISH in a CA and AA patient. **A**, on the left, an ERG positive index tumor and an ERG negative secondary tumor of a CA patient is outlined and representative view fields are enlarged. **B**, right panel represents an ERG negative index tumor and a secondary ERG positive tumor in an AA patient. **C**, *ERG* rearrangement by translocation in a CA patient and **D**, *ERG* rearrangement by deletion in a section from an AA are shown by FISH assay.

Table 1

Clinical-pathologic characteristics of matched AA and CA cases.

Variable	Caucasian	African American	P value
N	91	91	
Age at surgery (yr)			0.8518
<i>Mean (SD)</i>	59.4 (8.3)	59.6 (8.6)	
<i>Median (range)</i>	61.2 (40.2–74.4)	61.1 (40.2–73.5)	
Diagnostic PSA (ng/mL)			0.4448
<i>Mean (SD)</i>	7.0 (4.5)	6.7 (5.5)	
<i>Median (range)</i>	6.0 (1.1–23.4)	5.6 (0.6–41.2)	
Pathological T stage-N (%)			1.0000
T2A, B, C	42 (46.2)	42 (46.2)	
T3A, B, C	49 (53.8)	49 (53.8)	
Gleason sum-N (%)			0.6387
6	38 (41.7)	36 (39.6)	
3+4	33 (36.3)	38 (41.8)	
4+3	5 (5.5)	2 (2.2)	
8 or above	15 (16.5)	15 (16.5)	

Table 2

Association of ERG IHC status with AA and CA ethnicity

ERG IHC	Caucasian	African American	P value
Number of Patients	91	91	
<i>Patients with Index tumor[#] ERG +</i>	57 (63.3)	24 (28.6)	<.0001
<i>Patients with at least one tumor ERG+</i>	60 (65.9)	39 (42.9)	0.0018
Number of tumor foci	191	205	
<i>ERG +</i>	80 (41.9)	49 (23.9)	0.0001

[#] Index tumors were absent in one CA and seven AA cases.

Table 3A

Association of index tumor ERG IHC status with race/ethnicity stratified by Gleason sum. In the Gleason score 4+3 category there were two patients in the AA and five patients in the CA groups. Therefore, we have combined the 4+3 with 8–10.

	Overall	Caucasian	African American	P value
Gleason 3+3				0.0037
<i>Index tumor ERG –</i>	33 (48.5)	11 (31.4)	22 (66.7)	
<i>Index tumor ERG +</i>	35 (51.5)	24 (68.6)	11 (33.3)	
Gleason 3+4				0.0385
<i>Index tumor ERG –</i>	35 (50.7)	15 (39.5)	20 (64.5)	
<i>Index tumor ERG +</i>	34 (49.3)	23 (60.5)	11 (35.5)	
Gleason 4+3/8–10				0.0036*
<i>Index tumor ERG –</i>	25 (67.6)	7 (41.2)	18 (90.0)	
<i>Index tumor ERG +</i>	12 (32.4)	10 (58.8)	2 (10.0)	

* Fisher Exact p-value

Table 3B

Association of index tumor ERG IHC status with Gleason sum (GI) stratified by race.

Index ERG	Caucasian (n=90)		P value
	GI 3+3, 3+4 (* <25%)	GI 3+4 (* 25%), 4+3, 8–10	
Negative	20 (33.3)	13 (43.3)	0.3534
Positive	40 (66.7)	17 (56.7)	

Index ERG	African American (n=84)		P value
	GI 3+3, 3+4 (* <25%)	GI 3+4 (* 25%), 4+3, 8–10	
Negative	33 (63.5)	27 (84.4)	0.0394
Positive	19 (36.5)	5 (15.6)	

* worst tumor grade percentage