Imaging, Diagnosis, Prognosis

Clinical Cancer Research

Evaluation of the *ETS*-Related Gene mRNA in Urine for the Detection of Prostate Cancer

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Abstract

Purpose: Prevalent gene fusions in prostate cancer involve androgen-regulated promoters (primarily *TMPRSS2*) and *ETS* transcription factors (predominantly *ETS*-regulated gene (*ERG*)], which result in tumor selective overexpression of *ERG* in two thirds of patients. Because diverse genomic fusion events lead to *ERG* overexpression in prostate cancer, we reasoned that it may be more practical to capture such alterations using an assay targeting *ERG* sequences retained in such gene fusions. This study evaluates the potential of an assay quantitating *ERG* mRNA in post–digital rectal exam (DRE) urine for improving prostate cancer detection.

Experimental Design: Patients scheduled to undergo transrectal ultrasound-guided needle biopsy of the prostate were prospectively enrolled. On the day of biopsy, patients provided a urine sample immediately following a DRE. Urine *ERG* mRNA was measured and normalized to urine prostate-specific antigen (PSA) mRNA using the DTS 400 system. Demographic traits, clinical characteristics and biopsy results were analyzed for association with urine *ERG* score.

Results: The study was conducted on 237 patients. Prostate cancer was shown on biopsy in 40.9% of study subjects. A higher urine *ERG* score associated significantly with malignancy on biopsy ($P = 0.0145$), but not with clinical stage or Gleason score. Urine *ERG* score performed best in Caucasians and in men with a PSA of ≤4 ng/mL (area under the curve = 0.8).

Conclusions: A higher urine *ERG* score in post-DRE urine is associated with the diagnosis of prostate cancer on biopsy. Urine *ERG* score performed particularly well in men with a PSA of ≤4.0 ng/mL, a segment of the screening population in which further diagnostic markers are needed to determine in whom biopsy should be done. *Clin Cancer Res; 16(5); 1572–6. ©2010 AACR.*

The use of prostate-specific antigen (PSA) has revolutionized screening, management, and postintervention surveillance resulting in earlier diagnosis, downward stage migration, and expedited detection of recurrence after definitive therapy (1). However, the excellent sensitivity of PSA is tempered by its poor specificity. There is an extensive research effort in progress to find an early detection modality without the limitations of PSA. Ideally, it would be one that complements PSA in such a way as to help identify patients who are likely to have disease that warrants definitive intervention.

Comparative evaluations of gene expression alterations in benign and malignant prostate epithelial cells from other groups and from our laboratory have defined frequent overexpression of *PCA3*, *AMACR*, and *ETS*-regulated gene (*ERG*) in prostate tumor cells (2–6). Our study further highlighted a combined specificity of >98% for *PCA3*, *AMACR*, and *ERG* in discriminating microdissected prostate tumor cells from normal prostatic epithelial cells (6). The discovery of prevalent gene fusions involving regulatory sequences of the androgen receptor–regulated prostate-associated genes (predominantly *TMPRSS2*) and protein coding sequences of the nuclear transcription factors in the *ETS* gene family (*ERG*, *ETV1*, *ETV4*, *ETV5*, and *ELK4*; predominantly *ERG*) have defined the underlying genetic mechanism of frequent overexpression of the *ERG* family members in prostate tumors (5–8). Extensive evaluations of gene fusions involving *ERG* have highlighted exclusive prostate tumor cell association and the potential causal nature of *ERG* alterations in prostate cancer (8).

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Note: The opinions and assertions contained herein are the private views of the authors and are not to be considered as reflecting views of the U.S. Army or the Department of Defense.

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Translational Relevance

The use of urine biomarkers to assist in diagnosing and prognosticating prostate cancer is a promising new strategy. Frequent overexpression of *ETS*-regulated gene (*ERG*), as a result of cancer-specific gene fusion events, represents the most common oncogenic activation in prostate cancer. This study highlights a new utility of *ERG* mRNA measurements in post–digital rectal exam urine especially in a defined patient cohort [prostate-specific antigen (PSA) ≤4.0 ng/mL] in which serum PSA test is least specific and, therefore, further diagnostic markers are needed to determine in whom biopsy should be done. The impressive performance of urine *ERG* score in predicting the biopsy result for this cohort of patients indicates that this assay has a potential translational relevance in determining which patients with minimally elevated serum PSA may undergo prostate biopsy.

Several investigators showed that useful diagnostic and even prognostic information can be obtained through the molecular genetic analysis of exfoliated prostatic epithelial cells found in the urine after digital rectal exam (DRE; refs. 2–4). Most of these studies evaluated the utility of *PCA3* and/or *TMPRSS2-ERG* fusion assays (2, 4–11). Because diverse genomic fusion events may lead to *ERG* overexpression, we reasoned that it may be more practical to capture such alterations using assays targeting *ERG* sequences that are retained in all gene fusions involving *ERG*. In support of this hypothesis, our recent study confirmed higher frequency of overall *ERG* expression targeting *ERG* sequences 3′ to fusion compared with the detection of a common *TMPRSS2-ERG* fusion (12). Our findings are also supported by a recent discovery of *SLC45A3-ERG* fusions in a subset of prostate cancer in addition to more frequent *TMPRSS2-ERG* fusions (13). This study focuses on the diagnostic utility of quantitative measurements of *ERG* mRNA in post-DRE urine of prostate cancer patients.

Materials and Methods

Patient cohort and urine specimens. This study was approved by the Institutional Review Boards at Walter Reed Army Medical Center and the Uniformed Services University of Health Sciences. Patients were accrued between September 2006 and March 2008. Patients were excluded if they reported use of medications that would affect serum PSA levels (e.g., 5α-reductase inhibitors, herbal supplements, or the use of testosterone).

Urine samples were collected immediately following a DRE involving three sweeps of the physician's finger over both lateral lobes of the prostate. Samples were obtained immediately before prostate biopsy. *ERG* and PSA mRNA were measured using a DTS® 400 system, as previously described (2, 3, 8). *ERG* mRNA was normalized to PSA mRNA in each sample. This score was then correlated to transrectal ultrasound-guided needle prostate biopsy result. A total of 12 cores were obtained from each patient. Three cores were taken from the base, middle, and apex of each lateral lobe with three additional biopsies taken from the more lateral base, middle, and apical regions. Urine samples were processed within 4 h of collection by mixing with an equal volume of detergent-based stabilization buffer (urine transport medium) to lyse cells and stabilize RNA. The urine transport medium was the same formulation that is found in the APTIMA® Urine Specimen Collection kit for Male and Female Urine Specimens (Gen-Probe Incorporated). Once processed, specimens were stored at −70C until they were tested.

All researchers collecting or processing specimens and collecting clinicopathologic data or doing *ERG* assays were blinded to results until a biostatistician provided the data.

Biomarker assay procedure. *ERG* and PSA mRNA levels were quantified using a urine assay. Target mRNA was captured onto magnetic microparticles coated with sequence-specific oligonucleotides. Transcription-mediated amplification of mRNA was followed by detection with chemiluminescent DNA probes. Transcript calibrators were then used to quantify *ERG* and PSA mRNA. The *ERG* score was calculated using the following equation:

$$ERGscore = 100,000 \times \frac{EGR_{c/mL}}{PSA_{c/mL}}.$$

The components for the *ERG* and PSA mRNA assays include analyte-specific (*ERG* and PSA) target capture, amplification, and probe reagents. The *ERG* target capture oligo sequence, primers, and probe targeted the 3′ untranslated region of *ERG* mRNA, the same region targeted previously in our quantitative reverse transcription-PCR assays (9). The PSA target capture oligo sequence, primers, and probe targeted the exon 2/3 splice junction in the PSA mRNA. All other reagent formulations are identical to the corresponding Gen-Probe PROGENSA PCA3® reagents. The *ERG* and PSA assay protocols are identical and the reagent addition volumes and incubation times and temperatures specified in Gen-Probe PROGENSA PCA3® reagents.

Statistical analysis. Demographic and biopsy characteristics of the study sample were examined. Frequencies were reported for categorical patient features, whereas measures of central tendency (mean and median) and dispersion (SD and range) were reported for continuous patient features. The Wilcoxon rank-sum test or the Kruskal-Wallis test were used to examine the association between urine *ERG* score and patient demographic and biopsy features. Logistic regression and receiver operating characteristic (ROC) analysis were used to examine the biopsy prediction value of urine *ERG* score. A cutoff value of continuous *ERG* score was identified by using the minimum *P* value approach according to Mazumdar and Glassman (14) in multivariate extension. The cutoff value of continuous *ERG* score was identified by using minimum *P* value

Table 1. Demographic, clinical characteristics, and biopsy results (n = 237)

Variable	
Biopsy results	
Negative	140 (59.1%)
Positive	97 (40.9%)
Race	
Caucasian and other	162 (68.4%)
African-American	75 (31.6%)
Mean age at biopsy (y)	64.5 ± 10.3
Mean PSA at biopsy (ng/mL)	6.3 ± 8.2
Clinical stage*	
T1	52 (59.1%)
T2	32 (36.4%)
T3-4	4 (4.5%)
Preoperative Gleason sum	
5-6	65 (67%)
7	16 (16.5%)
8-10	16 (16.5%)

*Clinical stage data were not available for nine men.

approach according to Mazumdar and Glassman (14) in multivariate extension. All observed values of *ERG* score, except a proportion of top and bottom 10% of the extreme values in the data (15), were examined as candidates for the cutpoint ($C_1, C_2, C_3, \ldots$). To simplify the calculation, *ERG* score was rounded to integers. The i^{th} cutpoint of *ERG* score is chosen using its association with biopsy outcome (biopsy) and it is known that other variables such as age, race, and serum PSA affect this relationship. Then, the χ^2 due to the i^{th} cutpoint of *ERG* score was defined as:

$$-2[\log lik(biopsy \sim C_i,\ age,\ race,\ PSA) - \log lik(biopsy \sim age,\ race,\ PSA)],$$

in which the log likelihoods were obtained from corresponding multivariate logistic regression models. The value that best separates biopsy outcomes according to the maximum χ^2 statistic was chosen.

A P value of 0.05 was adopted as statistically significant. The SAS version 9.1 was used for all data analysis.

Results

A total of 269 men provided urine before undergoing prostate biopsy. Measurement of urine *ERG* was not done in 32 men due to insufficient urine volume or due to PSA mRNA concentration of <7,500 c/mL.

Prebiopsy characteristics of the 237 patients included in the final analysis is provided in Table 1. Notably, the study group included 31.6% African-American men. Ninety-seven (40.9%) men showed adenocarcinoma of the prostate on biopsy. The majority of the patients were clinical stage T1 and had low-grade disease (Gleason score 5-6) on biopsy (Table 1).

Higher urine *ERG* score associated significantly with a positive biopsy (P = 0.0145; Table 2). No significant association of urine *ERG* score with race or a family history, as defined as prostate cancer in a first-degree relative, was noted. Urine *ERG* score did not correlate with clinical stage or biopsy Gleason sum.

Using ROC analysis, an *ERG* score cutoff of 144 yielded a sensitivity of 31%, specificity of 84%, and AUC of 0.592. The number of positive cases for *ERG* in patients with positive and negative biopsies were 30 of 97 (30.9%) and 23 of 140 (16.4%), respectively. We evaluated the performance of the urine *ERG* score in relation to serum PSA cutoff of 4.0 ng/ mL, which is widely used in prostate cancer screening. *ERG* showed improved specificity, but decreased sensitivity when compared with serum PSA using a cutoff of 4.0 ng/mL. When evaluating all patients, adding urine *ERG* to serum PSA and other clinical parameters in a multivariate logistic regression model did not significantly improve performance in predicting positive biopsy (Table 3).

Although there was no difference in urine *ERG* score predicted by race alone (Table 2), urine *ERG* score performed better in predicting prostate cancer in Caucasians with an AUC of 0.678, as well as in patients with serum PSA ≤4 ng/mL with an AUC of 0.679 (Table 4). No significant differences in clinical stage, biopsy Gleason score, or mean serum PSA was appreciated between African-Americans and Caucasians.

The most significant finding of this study was the predictive ability (AUC of 0.802) of *ERG* score in Caucasians

Table 2. Association of urine *ERG* score with clinical characteristics and biopsy results

Variable	Urine *ERG* score		
	n	Median (range)	*P*
Race			0.183
Caucasian and other	162	31 (0-17,836)	
African-American	75	23 (0-2,331)	
Prostate cancer family history			0.089
No	188	25 (0-2,826)	
Yes	41	48 (0-17,836)	
Biopsy result			0.0145
Negative	140	19 (0-2,057)	
Positive	97	59 (0-17,836)	
Clinical T stage*			0.6398
T1	52	55 (0-721)	
T2	32	78 (0-17,836)	
Biopsy grade			0.4739
2-6	65	50 (0-17,836)	
7	16	70 (0-1,134)	
8-10	16	81 (0.2-2,826)	

NOTE: Due to very small sample size, four cases with clinical T3-4 stage were excluded from analyses (data not shown).

Table 3. Multivariate logistic regression model for predicting biopsy result by using urine *ERG* score with other clinical features

Variable	OR	95% CI of OR	*P*
Age (continuous)	1.004	0.977-1.033	0.7557
Serum PSA (continuous)	1.049	0.986-1.115	0.128
Race			
Caucasian and other	1		
African-American	0.873	0.494-1.543	0.6411
Urine ERG score (continuous)	1	1.000-1.001	0.3831

Abbreviations: OR, odds ration; 95% CI, 95% confidence interval.

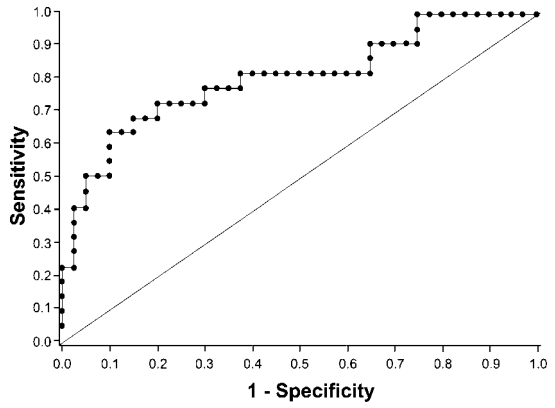

Fig. 1. ROC curve for *ERG* Score prediction of positive biopsy in Caucasians with a PSA of ≤4.0 ng/mL (AUC = 0.802).

with serum PSA ≤4 ng/mL (n = 62; Fig. 1). *ERG* score cutoff of 150 yielded a sensitivity of 50%, specificity of 95% and accuracy of 79% (Table 5). The AUC of *ERG* score among African-American s with serum PSA ≤4 ng/mL was 0.660 (data not shown).

Discussion

Frequent overexpression of *ERG*, as a result of cancer-specific gene fusion events, represents the most common oncogenic activation in prostate cancer. This study highlights the potential use of *ERG* mRNA measurements in post-DRE urine especially in a defined patient cohort (PSA, ≤4.0 ng/mL) in which serum PSA test is least specific.

Recent studies have evaluated the value of prostate cancer–associated gene expression detection in post-DRE urine. Laxman et al. (11) reported the detection of *TMPRSS2-ERG* fusion transcripts using qPCR in 8 of 19 (42%) patients who showed prostate cancer on biopsy. Hessels et al. (10) evaluated *TMPRSS2-ERG* fusion transcripts in post-DRE urine of 108 patients undergoing prostate biopsy, 78 of which showed prostate cancer. This study revealed 37% sensitivity and 93% specificity of *TMPRSS2-ERG*, and when combined with *PCA3*, sensitivity increased to 73%. Laxman et al. (9) investigated the use of a panel of seven markers: *PCA3*, *ERG*, *AMACR*, *TMPRSS2-ERG* fusion, *SPINK1*, *TFF3*, and *GOLPH2*. In this analysis, *PCA3*, *GOLPH2*, and *TMPRSS2-ERG* fusion showed a significant association with prostate cancer in either biopsy or prostatectomy specimens. In contrast to the present study, *ERG* expression was not significantly associated with prostate cancer on biopsy or prostatectomy. The investigators constructed a predictive model that included only those biomarkers that showed a significant association with malignancy on univariate analysis. This resulted in an improved AUC of 0.758.

Our study highlights a potential new utility of measuring *ERG* in post-DRE urine of a defined patient cohort. Despite a consensus that prostate cancer is being overdiagnosed due in large part to PSA screening, recent years have seen a move toward biopsying younger men for lower PSAs and decreased velocities than had been previously recommended. Thompson et al. (16) reported that 15.2% of patients with a PSA of <4.0 ng/mL and a normal DRE were diagnosed with prostate cancer. Of these patients, 14.9% showed a Gleason score of >7. Carter et al. (17) reported that in men with a pretreatment PSA of <4.0 ng/mL, a PSA velocity of >0.35 ng/mL/y was associated with a significantly worsened cancer-specific survival than those with a PSA

Table 4. Performance of urine *ERG* assay (n = 237)

	ERG score biopsy performance stratified by race		Urine *ERG* performance stratified by serum PSA*	
	Caucasian and other	African-American	≤4 ng/mL	>4 ng/mL
Subjects (n)	162	75	86	150
ROC AUC	0.678	0.425	0.679	0.544
Prostate cancer positive	68 (42%)	29 (40%)	29 (34%)	68 (45%)

*Serum PSA data were not available for one patient.

Table 5. Performance of urine *ERG* assay among Caucasian men with serum a PSA of ≤4.0 ng/mL (*n* = 62)

No. of subjects		62	
ROC AUC		0.8	
Cap positive		22 (35%)	
Cutoff	150	100	60
Sensitivity	50%	55%	68%
Specificity	95%	85%	75%
Accuracy	79%	74%	72%

velocity below this cutoff. Thus, PSA alone is not sufficient in determining which patients have more aggressive disease. The impressive performance of urine *ERG* score in predicting biopsy result for Caucasian patients with a PSA value of ≤4.0 ng/mL may indicate that this assay has a potential in determining which patients should undergo prostate biopsy in this subgroup. The reason for differences in the performance of *ERG* assays between Caucasian American and African-American patients are not clear at this stage, although we have previously noted the decreased *ERG* expression in prostate tumor cells of African-American patients (6). This observation warrants future investigations. Taken together, novel observation on diagnostic potential of *ERG* score in a defined patient cohort warrants further investigations as a urologist faced with a younger patient whose PSA is below 4.0 ng/mL and a marginal PSA velocity may be able to use a urine *ERG* score to determine whether the patient should be biopsied or observed.

Conclusion

The use of prostate cancer–specific biomarkers in urine to assist in diagnosing and prognosticating prostate cancer is a promising new strategy. This study shows the performance of urine *ERG* score that approximates serum PSA in predicting prostate biopsy results. Importantly, urine *ERG* score performed particularly well (AUC = 0.8) in Caucasian men with a PSA of ≤4.0 ng/mL, a segment of the screening population in which further diagnostic markers are needed to determine in whom biopsy should be done. Further studies examining the long-term prognostic significance of these markers will show their full potential in augmenting the appropriate diagnosis and treatment of prostate cancer. Recent studies have reported higher specificity and moderate sensitivity of urine *PCA3* (3, 18) and *TMPRSS2-ERG* fusion assays (9–11). The urine *ERG* assay described in this study may complement other urine biomarker assays in enhancing sensitivity for predicting positive biopsy in patients with minimally elevated serum PSA of ≤4 ng/mL.

Disclosure of Potential Conflicts of Interest

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References

1. Jang TL, Han M, Roehl KA, Hawkins SA, Catalona WJ. More favorable tumor features and progression-free survival rates in a longitudinal prostate cancer screening study: PSA era and threshold-specific effects. Urology 2006;67:343–8.
2. Deras IL, Aubin SM, Blase A, et al. PCA3: a molecular urine assay for predicting prostate biopsy outcome. J Urol 2008;179:1587–92.
3. Marks LS, Fradet Y, Deras IL, et al. PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. Urology 2007;69:532–5.
4. Whitman EJ, Groskopf J, Ali A, et al. PCA3 score before radical prostatectomy predicts extracapsular extension and tumor volume. J Urol 2008;180:1975–8.
5. Reynolds MA. Molecular alterations in prostate cancer. Cancer Lett 2008;271:13–24.
6. Petrovics G, Liu A, Shaheduzzaman S, et al. Frequent overexpression of ETS-related gene-1 (ERG1) in prostate cancer transcriptome. Oncogene 2005;24:3847–52.
7. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644–8.
8. Kumar-Sinha C, Tomlins SA, Chinnaiyan AM. Recurrent gene fusions in prostate cancer. Nat Rev Cancer 2008;8:497–511.
9. Laxman B, Morris DS, Yu J, et al. A first-generation multiplex biomarker analysis of urine for the early detection of prostate cancer. Cancer Res 2008;68:645–9.
10. Hessels D, Smit FP, Verhaegh GW, Witjes JA, Cornel EB, Schalken JA. Detection of TMPRSS2-ERG fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer. Clin Cancer Res 2007;13:5103–8.
11. Laxman B, Tomlins SA, Mehra R, et al. Noninvasive detection of TMPRSS2:ERG fusion transcripts in the urine of men with prostate cancer. Neoplasia 2006;8:885–8.
12. Hu Y, Dobi A, Sreenath T, et al. Delineation of TMPRSS2-ERG splice variants in prostate cancer. Clin Cancer Res 2008;14:4719–25.
13. Tomlins SA, Laxman B, Dhanasekaran SM, et al. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Nature 2007;448:595–9.
14. Mazumdar M, Glassman JR. Tutorial in biostatistics categorizing a prognostic variable: review of methods, code for easy implementation and applications to decision-making about cancer treatments. Stat Med 2000;19:113–32.
15. Miller R, Siegmund D. Maximally selected chi square statistics. Biometrics 1982;38:1011–6.
16. Thompson IM, Pauler DK, Goodman PJ, et al. Prevalence of prostate cancer among men with a prostate-specific antigen level < or = 4.0 ng per milliliter. N Engl J Med 2004;350:2239–46.
17. Carter HB, Ferrucci L, Kettermann A, et al. Detection of life-threatening prostate cancer with prostate-specific antigen velocity during a window of curability. J Natl Cancer Inst 2006;98:1521–7.
18. Groskopf J, Aubin SM, Deras IL, et al. APTIMA PCA3 molecular urine test: development of a method to aid in the diagnosis of prostate cancer. Clin Chem 2006;52:1089–95.