Noninvasive Detection of TMPRSS2:ERG Fusion Transcripts in the Urine of Men with Prostate Cancer

Bharathi Laxman*,2, Scott A. Tomlins*–2, Rohit Mehra*–1, David S. Morris1, Lei Wang*, Beth E. Helgeson*, Rajal B. Shah*–1,1–8, Mark A. Rubin*–8, John T. Wei1,1–8 and Arul M. Chinnaiyan*–1,1–8

*Department of Pathology, University of Michigan Medical School, Ann Arbor, MI 48109, USA; †Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI 48109, USA; ‡Department of Urology, University of Michigan Medical School, Ann Arbor, MI 48109, USA; §Michigan Urology Center, University of Michigan Medical School, Ann Arbor, MI 48109, USA; †Department of Pathology, Harvard Medical School, Boston, MA, USA; ‡Brigham and Women’s Hospital, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

Abstract
We recently reported the identification of recurrent gene fusions in the majority of prostate cancers involving the 5’ untranslated region of the androgen-regulated gene TMPRSS2 and the ETS family members ERG, ETV1, and ETV4. Here we report the noninvasive detection of these gene fusions in the urine of patients with clinically localized prostate cancer. By quantitative polymerase chain reaction, we assessed the expression of ERG and TMPRSS2:ERG transcripts in urine samples obtained after prostatic massage from 19 patients (11 prebiopsy and 8 pre–radical prostatectomy) with prostate cancer. We observed a strong concordance between ERG overexpression and TMPRSS2:ERG expression, with 8 of 19 (42%) patients having detectable TMPRSS2:ERG transcripts in their urine. Importantly, by fluorescence in situ hybridization, we confirmed the presence or the absence of TMPRSS2:ERG gene fusions in matched prostate cancer tissue samples from three of three patients with fusion transcripts in their urine and from two of two patients without fusion transcripts in their urine. These results demonstrate that TMPRSS2:ERG gene fusions can be detected in the urine of patients with prostate cancer and support larger studies on prospective cohorts for noninvasive detection of prostate cancer.

Keywords: Gene fusions, prostate cancer, noninvasive detection, urine, quantitative PCR.

Introduction
Chromosomal rearrangements play causal roles in numerous human malignancies and have been exploited diagnostically and therapeutically [1,2]. Using a novel bioinformatics strategy to nominate candidate oncogenes, we identified recurrent gene fusions involving the 5’ untranslated region of the androgen-regulated gene TMPRSS2 to members of the ETS gene family (ERG, ETV1, or ETV4) in the majority of prostate cancers [3,4]. Subsequently, multiple studies have confirmed the presence of TMPRSS2:ETS gene fusions, particularly TMPRSS2:ERG, in 40% to 80% of prostate cancers [5–8]. In addition to likely playing a central role in the pathogenesis of prostate cancers, these studies highlight the potential of TMPRSS2:ETS gene fusions to serve as a specific biomarker of prostate cancer.

In an effort to develop a noninvasive method to detect TMPRSS2:ERG gene rearrangements, we explored the possibility of identifying this fusion in urine samples obtained from patients with prostate cancer using quantitative polymerase chain reaction (qPCR). Here we show that RNA isolated from sedimentoed urine and subjected to qPCR revealed the presence of TMPRSS2:ERG fusions in 8 of 19 (42%) patients with prostate cancer. We validated the specificity of this assay by confirming the presence or the absence of TMPRSS2:ERG gene rearrangements in matched tissue samples from a subset of our cohort. The results demonstrate the feasibility of the noninvasive detection of TMPRSS2:ETS gene fusions from the urine of patients with prostate cancer.

Materials and Methods

Urine Collection, RNA Isolation, and Amplification
This study was approved by the Institutional Review Board (IRB) of the University of Michigan Medical School (Ann Arbor, MA, USA). All patients provided informed consent. Samples were obtained from 19 previously untreated men with clinically localized prostate cancer, of whom 11 were prebiopsy and 8 pre–radical prostatectomy. RNA was isolated from urine samples obtained after prostatic massage (Urine Collection, RNA Isolation, and Amplification).

Address all correspondence to: Arul M. Chinnaiyan, MD, PhD, Department of Pathology, University of Michigan Medical School, 1400 East Medical Center Drive 5316 CCGC, Ann Arbor, MI 48109-0602. E-mail: arulkimich.edu

This work was supported, in part, by the National Institutes of Health Early Detection Research Network (U01 CA111275–01 to A.M.C. and J.T.W.), the Prostate SPORE (P50CA69568 to K.J.A.M.C. and R.B.S.), the Prostate Cancer Foundation (to A.M.C.), and the Department of Defense (PC040517 to R.M.). S.A.T. is supported by a Rackham Predoctoral Fellowship and is a fellow of the Medical Scientist Training Program. A.M.C. is supported by a Clinical Translational Research Award from the Burroughs Welcome Foundation.

Co–first authors.
Received 3 October 2006; Revised 3 October 2006; Accepted 3 October 2006.

Copyright © 2006 Neoplasia Press, Inc. All rights reserved 1522-8002/06/$25.00 DOI 10.1593/neo.06625
Hybridization (FISH) fusion transcripts in prostate cancer cells are as follows: (10), and (11) primers were as described. All primers and probe (MGB-labeled, threshold cycle) values greater than 38 cycles TMPRSS2: Taqman overexpression ex- Power SYBR Green Master Mix ETV1 Noninvasive Detection of TMPRSS2:ERG Gene Fusion Laxman et al. Neoplasia . Vol. 8, No. 10, 2006 with informed consent of the patients, urine samples were obtained following a digital rectal exam before ... amount of GAPDH. 886 Noninvasive Detection of TMPRSS2:ERG Gene Fusion Laxman et al. Neoplasia . Vol. 8, No. 10, 2006 overexpression gene rearrangement, we PSA ... (exons 6 and 7) (4), PSA ... TM-ERGa2_MGB-f: CGCGGCGAGGAAGCTTA TM-ERGa2_MGB-r: TCCGTAGGCACACTCAAACAC TM-ERGa2_MGB-probe: 5'-MGB-CAGTTGTGAGTGAGGACC-NFQ-3'.
microarray analysis [9]. Using this strategy, we assessed two cohorts containing a total of 19 men with prostate cancer. After a digital rectal exam, urine was collected from 11 men before the performance of needle biopsy, which revealed the presence of prostate cancer. We also assessed a cohort of eight patients with prostate cancer from whom urine was collected after a digital rectal exam but before radical prostatectomy. Cohort characteristics are presented in Table 1.

For each patient, we determined the expression of ERG relative to PSA, in addition to determining whether the sample expressed TMPRSS2:ERG fusion transcripts. To confirm the specificity of our TMPRSS2:ERG Taqman primer/probe assay, we assayed urine samples spiked with 1.6 million VCaP or LNCaP cells. We detected TMPRSS2:ERG fusion transcripts exclusively in VCaP cells, which we have previously shown to markedly overexpress ERG and to harbor TMPRSS2:ERG rearrangement [4]. By this same assay, 8 of 19 (42%) urine samples expressed TMPRSS2:ERG, including the seven samples with the highest expression of ERG (Table 1). These results are consistent with previous studies demonstrating an overall frequency of 40% to 80% for TMPRSS2:ERG fusions in prostate cancer tissue samples and demonstrating that ~95% of samples with ERG overexpression harbor TMPRSS2:ERG gene fusions [4–8]. We did not detect ETV1 overexpression in any sample.

As a confirmation of the specificity of our qPCR assay, we used FISH on matched tissue samples to determine the presence or the absence of the TMPRSS2:ERG gene rearrangement in the patient’s prostate cancer. We used a split-probe FISH assay, with probes located 5’ and 3’ to the ERG, where a TMPRSS2:ERG gene rearrangement is indicated by splitting of one pair of probes or by loss of the 5’ ERG probe, which is consistent with an intrachromosomal deletion between TMPRSS2 and ERG on chromosome 21q [4,5,8]. We expected that prostate cancer tissues from patients with high levels of ERG and TMPRSS2:ERG transcripts in their urine should be positive by FISH, whereas prostate cancer from patients with low levels of ERG and no detectable TMPRSS2:ERG transcripts in their urine should be negative by FISH. Thus, we assessed matched prostate tissue samples from three patients with detectable TMPRSS2:ERG in their urine and from two patients without detectable TMPRSS2:ERG in their urine. As expected, tissues from the three patients with high levels of ERG and detectable levels of TMPRSS2:ERG in their urine were positive for ERG rearrangement by FISH, whereas the two samples without TMPRSS2:ERG in their urine were negative for ERG rearrangement by FISH (Table 1). Hematoxylin and eosin–stained tissue sections and corresponding negative FISH assay from sample 5778, and a positive FISH assay from sample 5790 with deletion of the 5’ ERG probe are shown in Figure 2, A–D.

In summary, we have described the noninvasive detection of TMPRSS2:ERG fusion transcripts in the urine of patients with prostate cancer. We and others have recently described the presence of TMPRSS2:ETS gene fusions in the majority of prostate cancers and the utility of these gene fusions as a specific tissue biomarker of prostate cancer [3–8]. One limitation of the TMPRSS2:ERG Taqman assay we used for this study is that it only detects the TMPRSS2:ERGa isoform.
which is expressed in approximately 85% to 95% of fusion-positive prostate cancers [4,7]. Thus, additional assays will be needed to detect alternative isoforms expressed in the remaining 10% to 20% of positive cases. Isoform-specific assays may be particularly relevant, as particular isoforms have been associated with aggressive disease [7]. The presence of prostate cancer cells in the sedimented urine of prostate cancer suggests that other approaches to detect TMPRSS2:ETS gene rearrangements, such as urine-based FISH similar to the UroVysion system for detecting bladder cancer [12], may also be feasible. A FISH-based assay would also be able to identify TMPRSS2:ERG cases with intra-chromosomal deletion between TMPRSS2 and ERG, which has also been associated with aggressive disease in some cohorts [5,8]. In conclusion, the results reported herein support large-scale studies in prospective cohorts to determine the specificity and the sensitivity of urine-based assays for the detection of prostate cancer.

Acknowledgements
The authors thank Bo Han, Anjana Menon, and Alex Bond for technical assistance.

References