Urine TMPRSS2: ERG Fusion Transcript as a Biomarker for Prostate Cancer: Literature Review

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Abstract

Prostate cancer (PCa) is one of the most common male malignancies. Serum prostate-specific antigen (PSA) is one of the most valuable biomarkers in tumor biology and remains the standard marker in detecting and monitoring PCa. However, the high number of serum PSA false positive and false negative results make the identification of novel biomarkers extremely welcome to improve our diagnostic accuracy in detecting PCa and distinguishing the aggressive from the indolent ones. In this study, we analyzed the current role of urinary gene fusion transcripts involving v-ets erythroblastosis virus E26 oncogene homolog, commonly known as ERG, and the androgen-regulated gene transmembrane protease, serine 2 (TMPRSS2), as a biomarker for PCa. Used as a single marker, urinary TMPRSS2:ERG has low sensitivity but high specificity. However, its combination with the other urinary marker PCa antigen 3 (PCA3) has been reported to provide high specificity and sensitivity. Finally, a commercially available assay combining serum PSA with urinary PCA3 and TMPRSS2:ERG provides a 90% specificity and 80% sensitivity in diagnosing PCa. Urinary TMPRSS2:ERG also seems to be indicative of PCa aggressiveness upon biopsy. Should these findings be confirmed in larger studies, urinary TMPRSS2:ERG might become a valuable test not only for diagnosing PCa but also for distinguishing the aggressive tumors from the indolent ones.

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Introduction

Prostate cancer (PCa) is one of the most common male malignancies worldwide.[1] This finding could also be due to the extensive use of serum prostate-specific antigen (PSA) testing, which is currently the only widely used serum biomarker for PCa.[2] In the past 20 years, PSA testing has allowed clinicians to screen, diagnose earlier, and monitor PCa patients,[3] ultimately leading to 2 apparently opposing facts: an increased cancer-specific mortality,[4] and the diagnosis also of indolent tumors. This last fact, namely overdiagnosis/overtreatment of tumors not carrying a significant lethal potential, involves the drawbacks of unnecessary morbidity,[5] and unnecessary health care costs.[6]

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Another disadvantage of PSA testing is its low specificity (ranging from 25% to 40%). Increased serum PSA levels might be detected in several nonneoplastic diseases such as prostatitis and benign prostatic hyperplasia (BPH), thus representing false positive results (negative prostate biopsy in patients with PSA > 4 ng/mL); conversely, patients with normal (< 4 ng/mL) PSA levels might harbor PCa (false negative results).[7] This led to the recent recommendation from the main US and European PCa guidelines against using PSA-based mass PCa screening.[6,8] Because of these limitations of PSA testing, great efforts have been made to search for additional more specific biological markers (biomarkers), with a focus on PSA isoforms[9] as well as the development of novel biomarkers.

A biomarker is defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[10] Beyond its high diagnostic accuracy, an ideal biomarker in the clinical practice of PCa should be measurable with a simple, inexpensive, and repeatable test carried out in an easily available body fluid such as serum, urine, or prostatic fluid.[11]

Because of the facility of collection and the fact that prostate cells are directly discharged into the urethra through prostatic ducts after applying pressure to the prostate, as is performed with a digital rectal

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examination (DRE), urine has become the optimal substrate for noninvasive biomarker testing.[12]

Current technologies have widened the spectrum of putative biomarkers, by allowing the more and more careful analysis of DNA, mRNA, proteins, metabolites, or processes such as apoptosis, angiogenesis, or proliferation[13] using new-generation methods such as DNA microarrays or quantitative and semiquantitative polymerase chain reaction (PCR)-based techniques.[14,15]

Such technologies have allowed the identification of novel PCa biomarkers in tissue and, most importantly, in noninvasive samples.[11,16,17] Nevertheless, no biomarker has so far replaced the routine use of PSA as a screening and follow-up method of PCa, because none have been through all 5 phases of testing (ie, preclinical exploratory studies, clinical assay and validation, retrospective longitudinal studies, prospective screening studies, and randomized controlled trials; reviewed in Huang et al[18]), which seal its official use in clinical practice.

Herein, we analyzed the current role of urinary transmembrane protease, serine 2 (TMPRSS2):v-ets erythroblastosis virus E26 oncogene homolog (ERG) as a biomarker for PCa.

Transmembrane Protease, Serine 2:ERG as a PCa Biomarker

On the basis of the compelling evidence that genomic rearrangements represent initial events in oncogenesis, gene fusion transcripts involving ERG (also known as TMPRSS2:ERG, or T2E), a member of the E 26 (ETS) family of oncogenes-transcription factors in prostate cells, have been identified as promising urinary novel biomarkers.[18] In 2005, using a new biostatistical method called cancer outlier profile analysis, Tomlins et al[19] identified strong outlier profiles for the gene members of the ETS family of oncogenes in 16 PCa patients (55%), in keeping with the results of a previous study in which ERG overexpression was found in 59 (72%) PCa cases.[20] Moreover, by performing combined assays, they detected fusion of these ETS family members with the 5′ untranslated region of the prostate-specific and androgen-regulated gene *TMPRSS2* in 19 cases (>90%) overexpressing ERG or ets variant gene (ETV) 1, implying that the overexpression is most probably caused by the fusion.[19] Of them, TMPRSS2 fusion with ERG is the main variant in approximately 50% (range, 40%-70%) of PCa patients, although other fusions involving the ETS family members ETV4 and ETV5 have been described as a rarer molecular event in PCa,[21,22] which might account for the aberrant androgen-regulated overexpression of ETS family members in some PCa because TMPRSS2 is androgen-dependent.[19] Because ERG proto-oncogene overexpression is the more commonly reported in PCa cells, TMPRSS2:ERG fusion is believed to play a main role in prostate tumorigenesis,[23] and has been recognized as the most frequent genetic anomaly recounted so far in human solid cancers.[24]

From bench to bedside, such androgen-regulated gene fusion between TMPRSS2 and ERG might try to accomplish the task of detecting and managing PCa, because these TMPRSS2:ERG gene fusions were further described in almost 50% of the PSA-screened Caucasian PCa patients and, with lower incidence, in the Asian population.[25,26] Such molecular markers are also seldom present in high-grade prostatic intraepithelial neoplasia (hg-PIN).[19]

A few years ago, Laxman et al detected the mRNA products of the TMPRSS2:ERG fusion gene in urine samples from PCa patients for the first time.[27] In a subsequent study, this urine test featured a quite low sensitivity of 37%, a specificity of 93%, and a positive predictive value of 94% in post-DRE urine samples from 78 men with PCa-positive biopsies and 30 men with PCa-negative biopsies using semiquantitative reverse transcription PCR (RT-PCR).[28]

In another study on a small cohort of patients, no TMPRSS2:ERG fusion transcripts were found in urine samples obtained from women, healthy young men, and post-radical prostatectomy (RRP) patients[17]; however, TMPRSS2:ERG fusion transcripts were found in 34.8% of the urine samples from PCa patients compared with 18.2% from men with negative biopsies (which the authors suggest were false negative).

In a recent editorial article, Tomlins raised concern regarding the reliability of this test, because of the well known multifocality and heterogeneity of PCa,[29] thus suggesting that a dual outcome (positive/negative) of TMPRSS2:ERG expression in urine PCa patients, as used in some studies,[24] has intrinsic limitations so that it does not overcome the need for an optimal cutoff to maximize the sensitivity and specificity balance.[29]

The TMPRSS2:ERG fusion has been also studied for its prognostic potential with controversial results. According to Leyten et al, the fact that cancer cells from high-risk PCa have a higher potential to infiltrate contiguous structures, resulting in their greater post-DRE release to the prostatic ducts, ultimately resulting in urine specimens bearing more TMPRSS2:ERG fusion mRNA, could account for the hypothesis that urine TMPRSS2:ERG might have prognostic value.[16]

The largest study so far, in a cohort of 1180 men treated with RRP with a median follow-up of 12.6 years, reported overexpression of ERG in 49% of tissue samples using immunohistochemistry, with a significant association with tumor stage, but not with Gleason score, metastases, biochemical recurrence, and cancer-related and overall mortality,[30] thus suggesting that TMPRSS2:ERG is not a strong predictor of outcome in surgically-treated PCa patients. Another study that assayed the urinary TMPRSS2:ERG fusion transcript in 37% of pre-biopsy PCa patients found no correlation was found with Gleason score assessed on biopsy, which can be a potential pitfall because of the usual occurrence of upgrading in RRP specimens.[28] Indeed, in a subsequent study that measured urine TMPRSS2:ERG transcript levels through a clinical-grade, transcription-mediated amplification assay, Tomlins et al showed a significant association with cancer volume and grade and upgrading of Gleason score at the time of RRP.[31]

There is a growing body of literature proposing that the presence of the TMPRSS2:ERG fusion is a possible prognosticator of PCa outcome. In a cohort of localized PCa patients treated by watchful waiting, TMPRSS2:ERG fusion was reported in association with Gleason score and cancer-specific death; the selection bias of this low-stage population possibly explains an incidence as low as 15% compared with the average 50% reported in most studies.[32] However, in this cohort TMPRSS2:ERG fusion was detected in only 15% of the PCa patients compared with the average 50% reported in most studies, potentially because of the selection bias of this low-stage population.[32] In keeping with such findings, Attard

et al found that a deletion-based fusion detected using a fluorescent in situ hybridization assay on tissue microarray specimens was associated with clinical stage, Gleason score, baseline PSA, and a poor cancer-specific survival,[33] as confirmed by other studies.[16]

Ongoing research topics are the exploitment of TMPRSS2:ERG gene fusion in patients with localized PCa and hg-PIN deserving active surveillance.[34]

Taken together, such studies highlight the limitations of TMPRSS2:ERG fusion assay as a urine biomarker of PCa for screening purposes; the main pitfalls are: (1) the lower incidence of the fusion in some cohorts of men (such as localized PCa patients) seem to provide lower screening sensitivity[32]; (2) a suitable, comprehensive cutoff has not been identified yet, and the issues of PCa heterogeneity and multifocality seem to trouble its assessment; (3) the test estimates the quantity of TMPRSS2:ERG fusion RNA in urine samples in relation to PSA mRNA, to measure the amount of prostate cells in the urine sediment; the assay is considered invalid when the PSA level is too little[35]; and (4) most studies describe a sensitivity that is too low to allow clinical value for the test alone, with a most recent systematic review and meta-analysis reporting a specificity of 86% and sensitivity of 45%.[36]

Although the high specificity of TMPRSS2:ERG urine assay could be exploited for risk stratification of patients with a negative biopsy, thus urging an urgent rebiopsy or magnetic resonance imaging, this assay is not yet verified in a clinical setting as a PCa biomarker to point out the urge for rebiopsy.

In Table 1 the most relevant studies dealing with the role of TMPRSS2:ERG as a PCa biomarker are summarized.

V-ets Erythroblastosis Virus E26 Oncogene Homolog in a Panel of PCa Biomarkers

In keeping with the heterogeneity of the disease and the apparent low sensitivity of TMPRSS2:ERG, the construction of multiplexed models on the basis of a combination of cancer-specific biomarkers would be more appropriate to increase the overall diagnostic accuracy of this assay in the clinical management of PCa.[35]

Biomarkers other than PSA have been developed recently. PCa antigen 3 (PCA3) is a prostate-specific noncoding mRNA that is raised in more than 95% of PCa, compared with benign prostate tissues,[11] thus being probed as a urine assay for PCa early detection[37] and also proved to be related to histologic grade and tumor volume in surgical specimens.[38] In the United States, Progensa PCA3 (Gen-Probe) is a commercially available assay used in the United States after the US Food and Drug Administration (FDA) approval in 2012 as a diagnostic test to help in risk stratification for patients with a negative prostate biopsy, to select those who will benefit from a repeat biopsy.

The combined approach of PCA3 and TMPRSS2:ERG gene fusion testing for PCa diagnosis is supported by the evidence of the different increase of each marker in prostatic diseases; in the recent in vitro study by Robert et al on 32 normal prostate tissues, 48 BPH, and 48 PCa,[39] PCA3 levels showed gradual increase from BPH to normal prostate tissue and PCa tissue (3 and 30 times, respectively) and the TMPRSS2:ERG gene fusion was detected in 4 (8.3%) of the BPH, 5 (15.6%) of the normal prostate tissue, and 24 (50%) of the PCa samples.[39] Although the authors showed that the use of both combined markers improved the sensitivity, because most false-negative results of the PCA3 test could be corrected using the TMPRSS2:ERG fusion assay, they also suggested the need to assess an optimal cutoff to withdraw all false positive results.[39]

Interestingly, in one study that detected the TMPRSS2:ERG and PCA3 in urine samples from a huge cohort of 246 men,[40] it was reported that a significant lower area under the receiver operating characteristic curve (AUC) for TMPRSS2:ERG (0.63) than for PCA3 (0.74), and the use of combined parameters did not result in a significant increase in diagnostic accuracy compared with single markers.[40]

However, through a series of reports over the past 7 years, the combined use of PCA3 and TMPRSS2:ERG gene fusion transcripts, also included in widely used PCa risk calculators, was shown to significantly increase the reliability at diagnosis of each single test,[16,28,31,37,41-43] and some authors claimed that they might be related to PCa aggressiveness.[31,38]

Table 1 Association Between Urine TMPRSS2:ERG Fusion and Clinicopathological Findings From Selected Studies

Study	Sample Size	Study Design	Significant Correlation	Limitations
DeMichelis et al[32]	111 Men with localized PCa	Population-based cohort study	PCa specific death	NA
Tomlins et al[31]	1312 Men	Cohort study	Clinically significant PCa at the time of biopsy and prostatectomy (tumor size, high Gleason score at the time of prostatectomy, upgrading of Gleason grade at the time of prostatectomy)	Most patients in the study (>85%) were Caucasian Men in this study were PSA-screened (and elected to undergo biopsy)
Salami et al[41]	45 Men	Cohort study	PCa found at biopsy The greatest discriminatory value in predicting PCa compared with PCA3 and PSA alone	Small sample size
Cornu et al[45]	291 Men	Cohort study	PCa at biopsy	Lack of a prospective design, thus the data are limited to odds ratios, which do not permit causality to be assessed
Leyten et al[16]	443 Men	Multicenter cohort study	Significant additional predictive value to the ERSPC risk calculator parameters to predict biopsy Gleason score and clinical tumor stage	Lack of correlation with Gleason score in radical prostatectomy specimens

Abbreviations: ERG = v-ets erythroblastosis virus E26 oncogene homolog; ERSPC = European Randomised Study of Screening for Prostate Cancer; PCa = prostate cancer; PCA3 = prostate cancer antigen 3; PSA = prostate-specific antigen; TMPRSS2 = transmembrane protease, serine 2.

In a recent review of the literature on the role of genetic analysis in PCa, the authors have pointed out PCA3 and TMPRSS2:ERG fusion transcripts as promising RNA diagnostic and predictive markers.[44]

The first report by Hessels et al[28] was based on a prospective study on 108 prebiopsy post-DRE urine samples, 78 (72%) of which were later diagnosed with PCa upon biopsy. Because of its high specificity (93%), the combination of PCA3 and TMPRSS2:ERG increased the test sensitivity by 11% (from 62% for PCA3 alone to 73% for both markers). Such results have been subsequently corroborated by Cornu et al.[45] The latter showed that a PCA3 and TMPRSS2:ERG combined assay raised the outcome of the multivariate PCPT (PCa Prevention Trial) risk calculator in predicting cancer on biopsy by increasing the AUC from 0.66 to 0.75, although their findings were biased by the lack of uniformity of assay threshold among the centers involved in the study.

The diagnostic accuracy of the PCA3 and TMPRSS2:ERG transcripts assay has been studied also in combination with other markers. Laxman et al assayed the combination of 4 biomarkers, comprised of PCA3 and TMPRSS2:ERG, along with Golgi phosphoprotein 2 (GOLPH2) and serine protease inhibitor Kazal-type 1 (SPINK1), each one being an independent predictor of PCa, using a quantitative multiplexed RT-PCR analysis model; as a result, sensitivity and specificity for PCa detection in rebiopsies were up to 66% and 76%, respectively.[46] Subsequently, Cao et al reported a high diagnostic accuracy combined panel of PCA3, TMPRSS2:ERG, annexin A3, and sarcosine for PCa diagnosis (AUC 0.856 vs. PCA3: 0.739, TMPRSS2:ERG: 0.732, annexin A3: 0.728, and Sarcosine: 0.665).[47]

In a more recent study, Salami et al[41] supported the highest sensitivity (93%) of PCA3 and highest specificity (87%) of TMPRSS2:ERG fusion transcript for detection of PCa in a cohort of urine samples from 48 patients, 15 of whom were diagnosed with PCa. Multivariable algorithms including PCA3, urine TMPRSS2:ERG, and serum PSA, and PCA3 + TMPRSS2:ERG + PSA + DRE were used to diagnose PCa with high accuracy.[41,48]

Furthermore, in a recent study, Leyten et al assessed the independent additional predictive value of urinary TMPRSS2:ERG to PCA3 and the ERSPC (European Randomised Study of Screening for Prostate Cancer) risk calculator parameters (serum PSA level, DRE abnormal/normal, transrectal ultrasound abnormal/normal, and prostate volume) in a cohort of 443 men comprised of 44% PCa patients. Their results showed a stepwise increase in AUC from 0.799 (the ERSPC risk calculator alone) to 0.833 (ERSPC + PCA3) to 0.842 (ERSPC + PCA3 + TMPRSS2); interestingly, TMPRSS2:ERG showed prognostic value comparable with PCA3.[16] For the prediction of clinically significant PCa, TMPRSS2:ERG was more specific than PCA3 alone; the latter, along with TMPRSS2:ERG, raised its sensitivity to 88% not compromising its specificity.[16] Despite all such evidence for the 2 RNA-based biomarkers, PCA3 and TMPRSS2:ERG,[49] the available studies did not address the antagonistic effect of PCA3 and ERG score in a multivariable model. Moreover, all the cited studies did not integrate testosterone serum levels or genetic analyses, although both have been shown to affect the detection of PCa as well as its biological behavior.[50]

Having said this, there is already a commercially available diagnostic assay in the United States and Canada for PCa, with outcome as a percentage of risk, on the basis of blood PSA levels and urinary PCA3 and T2E test results, with a specificity and sensitivity as high as 90% and 80%, respectively,[41] thus outperforming traditional population-based nomograms.[18]

Conclusions

Serum PSA remains one of the most useful biomarkers in cancer biology and is here to stay. However, because of its high number of false positive and false negative results, it requires experienced interpretation. Therefore, the identification of novel biomarkers that can be used alone or in combination with PSA is extremely welcome to improve our diagnostic accuracy in detecting PCa and distinguishing the aggressive from the indolent ones.

Because PCa cells discarded into the urethra after a DRE, urine represents a noninvasive, easy to obtain milieu to gain information on PCa-related markers. Nevertheless, so far only few markers have been validated in large cohort studies. Of them, only PCA3 and TMPRSS2:ERG fusion transcripts have been shown to be promising RNA markers for detecting PCa and predicting its aggressiveness. PCA3 has been recently introduced in clinical practice upon FDA approval, and combined assay of both has been marketed for clinical use having been shown to perform better than the single marker in detecting PCa.

A major challenge in PCa management is the risk stratification of patients eligible for delayed treatment because of localized disease. Even though further evidence is needed, there is ground for believing that urinary ERG might have a predictive value as well.

Some potential limitations of using urine, including dilution, variability of collection methods, confounding effects of other urinary components, and biomarker degradation, nevertheless do not impair the future exploitment of the diagnostic and predictive potentials of such novel assays.

Disclosure

The authors have stated that they have no conflicts of interest.

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