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REVIEW

Diagnostic associations of gene expression signatures in prostate cancer tissue

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Purpose of review
Over the past several years, multiple biomarkers designed to improve prostate cancer risk stratification have become commercially available, while others are still being developed. In this review, we focus on the evidence supporting recently reported biomarkers, with a focus on gene expression signatures.

Recent findings
Many recently developed biomarkers are able to improve upon traditional risk assessment at nearly all stages of disease. Prominent examples are reviewed in this article. ConfirmMDx uses gene methylation patterns to improve detection of clinically significant cancer following negative biopsy. Both the Prolaris and Oncotype DX Genomic Prostate Score tests can improve risk stratification following biopsy, especially among men who are eligible for active surveillance. Prolaris and the Decipher genomic classifier have been associated with risk of adverse outcome following prostatectomy, while Oncotype DX is being studied in this setting. Finally, recent reports of the association of androgen receptor-V7 in circulating tumor cells with resistance to enzalutamide and abiraterone raise the possibility of extending the use of genetic biomarkers to advanced disease.

Summary
With the development of multiple genetic expression panels in prostate cancer, careful study and validation of these tests and integration into clinical practice will be critical to realizing the potential of these tools.

Keywords
biomarker, gene expression profile, prostate cancer, risk stratification

INTRODUCTION

As treatment options for prostate cancer increase, accurate risk assessment of prostate cancer is critical for treatment guidance at all stages of disease. While clinical staging, prostate-specific antigen (PSA) level, Gleason score, and extent of biopsy involvement continue to be important in risk assessment for patients with prostate cancer; these parameters lack the precision required to guide decision-making among treatment options. For example, under the typical risk group approach endorsed by the American Urological Association and National Comprehensive Cancer Network guidelines, among others, a patient with low-volume, low-PSA, Gleason 3+4 cancer with a small component of pattern 4 is lumped into the same risk category as men with high-volume Gleason 4+3 disease, leading to a non-optimized menu of treatment options offered to these patients [1,2].

This scenario is further complicated by the fact that even expert urologic pathologists often disagree over the presence of very small volumes of Gleason pattern 4 [3]. Likewise, in the advanced disease setting, men may appear to have clinically similar disease and yet some will respond to a chosen therapy while others will continue to progress rapidly. Recently, the incorporation of genomic data with clinical risk assessment has shown the potential to provide valuable information about risk in nearly all disease states, from mRNA-based gene expression profile signatures for patients with localized prostate cancer to specific gene alterations that may predict therapy response in castrate-resistant prostate cancer (CRPC). This review will focus on new and emerging gene expression signatures that

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KEY POINTS

- Gene expression signatures have been associated with the risk of prostate cancer and prostate cancer outcomes in multiple settings, and offer independent prognostic information above and beyond traditional risk stratification.
- For appropriate clinical application, careful attention must be paid to the populations and clinical scenarios in which these tests have been validated.
- Gene expression signatures and other prostate cancer biomarkers have potential to better identify men with clinically significant disease, which could decrease the burden associated with overdiagnosis and treatment of indolent prostate cancer.
- The true impact these tests will have on the management and outcomes of men with prostate cancer is not yet known and must be the subject of further study.

will help clinicians provide patients with a more personalized risk stratification and assessment of their cancer [4].

PRINCIPLES OF BIOMARKER DEVELOPMENT AND VALIDATION

Many genomic pathways alterations have been documented in prostate cancer, including somatic mutations, chromosomal abnormalities, copy number variations, and epigenetic changes [5]. Recognizing this heterogeneity both at the molecular level and in clinical terms is a critical first step in developing potential biomarkers for prostate cancer. Genetic biomarkers must go through thorough preclinical evaluation, appropriate validation, and careful implementation of these tests into clinical practice before they will be useful for patients.

Several authors have provided guidelines for the development of potential biomarkers into clinically meaningful prognostic tests [6,7▪]. Although discovery methods can vary, the characteristics of the test population and outcome of interest should be well defined. The way the test population was assembled (i.e., random sampling, matched cohort, sequential enrollment versus select cases) must be specified. Biospecimens from this population must be processed, accessioned, and stored the same way, every time, regardless of anticipated outcome. Statistical analysis must be performed on a dataset that is locked once outcomes and marker values are known but before any analysis has been started.

Once a promising biomarker is identified, it must be validated in a separate cohort that is independent of the discovery cohort. Once again, the way in which this validated population is identified is important, as the characteristics of this population will affect the generalizability of the test. The window of opportunity to evaluate the utility of these markers may occur early in their development. Once a test becomes readily available and widely used, our ability to evaluate its effectiveness is greatly diminished. There is, perhaps, no better example of this than the implementation of PSA level as a screening test [8].

In this review, we discuss both commercially available, validated genetic biomarkers and some that are still in development. It is important to note that although rigorous discovery and validation methods provide a critical foundation for these tools, appropriate clinical application and ongoing evaluation of the role of these tests in decision-making will ultimately determine how effective they are in clinical practice.

NEW TOOLS FOR BETTER PREDICTION OF PROSTATE CANCER DETECTION

Although early detection of high-risk prostate cancer through PSA screening does clearly provide a prostate cancer survival benefit, it is also well established that using PSA alone leads to both unnecessary biopsy of men without cancer and underdiagnosis of men with significant cancer [9,10]. The recently developed serum tests, in particular the Prostate Health Index and 4 kallikrein panel, which improve on the predictive accuracy of PSA for detecting prostate cancer [11–15] are the subject of other articles in this issue. However, even with improvement in the accuracy of prebiopsy screening markers, a large number of men who undergo prostate biopsy will continue to have negative biopsies because of both false positive screening tests and sampling error from the biopsy itself.

Two other tests are available that can improve our ability to identify men at higher risk for cancer detection on follow-up biopsy: the prostate cancer antigen 3 (PCA-3) test and ConfirmMDx (MDx Health, Irvine, California, USA). The PCA-3 test, marketed as Progensa (Gen-Probe, San Diego, California, USA), measures mRNA levels of PCA-3 (a noncoding mRNA transcript) in the urine. PCA-3 mRNA levels in the urine are positively associated with the risk of cancer detection and are better able to predict both the presence of cancer and the presence of higher Gleason grade cancer on repeat biopsy than serum PSA alone [16]. This test is detailed in an accompanying manuscript in this issue.

Methylation of the *GSTP1*, *APC*, and *RASSF1* genes in tissue from negative biopsy specimens

has also been associated with risk of prostate cancer on future repeat biopsy [17,18]. Core-specific analysis of these methylation patterns has been developed into the ConfirmMDx test, which utilizes this methylation pattern to identify men at low risk for occult disease following negative biopsy. ConfirmMDx has been validated in both European and U S cohort, with an 88–90% negative predictive value on follow-up biopsy [19,20▪]. The PCA-3 and ConfirmMDx have not been compared head-to-head to each other or to the serum-based tests mentioned above, and the ability of these tests to reduce the number of follow-up biopsies depends on the tolerance of patients and physicians to the risk of occult cancer.

GENOMIC SIGNATURE TO BETTER RISK STRATIFY PATIENTS WHO ARE CANDIDATES FOR ACTIVE SURVEILLANCE

Once cancer is detected, discrimination between clinically indolent and clinically significant cases is of paramount importance. Identifying men at low risk for disease progression opens up the possibility of avoiding treatment while the disease is monitored carefully, a management strategy known as active surveillance [21]. As noted above, current clinical risk stratification paradigms may misclassify men with both indolent and clinically significant disease. Several genomic expression signatures have been developed to improve risk assessment in this area. Technology allowing the use of formalin-fixed, paraffin-embedded samples for RNA-based studies and the use of tissue archives with well documented follow-up has been critical to the development of these tests.

A cell-cycle progression (CCP) score marketed as the Prolaris test (Myriad Genetics, Salt Lake City, Utah, USA) is derived from a 31-gene subset of 126 preidentified cell-cycle-related genes. The genes chosen were representative of the mean expression of the panel as a whole in 96 radical prostatectomy specimens. A separate cohort of 336 patients who underwent radical prostatectomy was then used to derive the CCP score, which was correlated with biochemical recurrence and death [22,23]. The CCP score has been validated against a separate radical prostatectomy cohort of 413 men and shown to add predictive value to a commonly used post-operative risk model, the postsurgical Cancer of the Prostate Risk Assessment (CAPRA-S) score [24▪]. In a pilot study, the CCP score accurately predicted staging of active surveillance men detected by multi-parametric MRI-guided biopsy [25]. In addition, the CCP score generated from biopsy sample from 582 patients (three cohorts, treated with prostatectomy) was significantly associated with BCR and metastasis, suggesting that the CCP score is a valuable marker for disease outcome at diagnosis [26▪]. The CCP score has also been tested in transurethral resection of prostate specimens [20▪].

The Oncotype DX Genomic Prostate Score (GPS) test (Genomic Health, Redwood City, California, USA) is a 17-gene, RT-PCR-based panel designed to identify clinically significant disease in men with low to low-intermediate risk prostate cancer who are candidates for active surveillance. These genes were identified from 732 candidate genes via two separate studies: a prostatectomy study including 127 patients who experienced recurrence and a control set of 374 nonrecurrence patients and a biopsy study including 167 patients who underwent prostatectomy within 6 months of diagnostic prostate biopsy. This 17-gene signature includes 12 genes related to androgen receptor signaling, cellular and proliferation, and stromal responses in the tumor microenvironment that have been shown to correlate with tumor aggressiveness and five housekeeping genes. Finally, the 17-gene panel was validated in a separate cohort of 395 men with low and low-intermediate clinical risk characteristics to offer improved prediction of adverse pathologic features over clinical risk predication models alone [27▪,28]. These tests and others may expand our ability to offer men active surveillance as a management option while identifying others who are harboring more aggressive disease than it appears on biopsy [29].

GENE EXPRESSION SIGNATURE TO PREDICT CANCER PROGRESSION

There is currently a great deal of debate over which men benefit most from adjuvant treatment following localized treatment for prostate cancer. Several genetic panels offer improved risk assessment following treatment over pathologic parameters and PSA kinetics alone. The Decipher genomic classifier (GenomeDx Biosciences, Vancouver, British Columbia, Canada) is designed to predict early metastasis and disease-specific mortality after radical prostatectomy using a signature of 22 gene at the mRNA level. The gene signature was developed from a discovery set of 545 prostatectomy specimens with 192 metastatic patients and 353 nonmetastatic patients [30▪]. The genomic classifier was then validated in a separate set of 256 postradical prostatectomy patients, 73 of whom had documented metastases, to predict the occurrence of metastasis following radical prostatectomy [31]. In a separate analysis of the same patient group, both genomic classifier score and CAPRA-S were independently

associated with cancer-specific mortality and a combination of genomic classifier score and CAPRA-S showed the highest net benefit using decision curve analysis [31,32]. The CCP (Prolaris) score has also been validated in this space, showing an association with adverse outcome following prostatectomy [24▪]. In addition, at the time of this review, positive performance of the GPS score (Oncotype Dx) in predicting cancer recurrence after radical prostatectomy using a prospective cohort is due to be reported [33].

In contrast to the RNA-based test described above, the Genomic Evaluators of Metastatic Prostate Cancer is a DNA-based test that uses copy number alteration in a set of 36 loci. The panel of loci used was originally discovered in a set of 64 men at high risk of recurrence, 32 of whom had recurred [34]. This has been validated to predict biochemical recurrence in men at high risk of recurrence better than clinical risk stratification alone and is the only marker to have been validated in a cohort of African-American prostatectomy patients, although it is not yet commercially available [34–36]. Extracellular microRNA offers significant promise as another source of prostate cancer biomarkers which could be assayed noninvasively and repeatedly. One study found that a panel of three circulating microRNAs added independent predictive value to a standard clinicopathological risk assessment [37].

GENE EXPRESSION SIGNATURE IN ADVANCE METASTATIC CANCER CASTRATE-RESISTANT PROSTATE CANCER

There are currently no validated gene signatures to predict progression to castrate-resistant cancer or to evaluate response to therapy. Translation of basic science studies, such as mutations associated with resistance to 2nd generation androgen receptor signaling inhibitors, enzalutamide, and abiraterone, will be of critical importance in addressing these challenges [38,39]. One difficulty in using gene expression patterns in metastatic cancer is the availability of biopsy tissue. In this setting, circulating tumor cells (CTCs) may be of value in generating new biomarkers that can be obtained via a readily available peripheral blood draw. It is notable that CTC show 70% of the mutations that were present at the primary tumor and thus can provide useful prognostic information without invasive diagnostic procedures, and their distinct patterns of chromosome copy number alterations have been demonstrated to be prognostic of disease outcomes [40,41]. A constitutively active variant of androgen receptor (AR-V7) has been implicated in progression to CRPC. Detection of AR-V7 in CTC of CRPC patients initially treated with either enzalutamide or abiraterone has recently been associated with worse PSA response rate, lower clinical and radiographic progression-free survival, and worse overall survival [39]. Future validation studies evaluating AR-V7 in CTC will be important in developing a biomarker for predicting treatment response in advance metastatic prostate cancer.

CLINICAL IMPACT

Improved prognostic value does not translate automatically to better clinical decision-making. After validating the prognostic or diagnostic value a biomarker, the next step is integration into clinical practice to empower clinicians and their patients with more information about their cancer. Choosing the right assays throughout the spectrum of disease, from initial diagnosis to metastatic cancer, may help reduce uncertainty in deciding treatment options and improve results. Adopting these new tissue-based biomarkers into clinical practice will require a combination of accessibility, affordable cost, simplicity in interpretation of results, and improved understanding of their relationship to long-term clinical outcome. To reduce overtreatment of indolent disease while recognizing lethal cancer, urologists may need to integrate these new biomarkers into the current risk assessment of their patients.

For instance, urologists who are using CCP score in their risk assessment indicated that the test is leading them to shift patients to a more conservative approach, hence, could be valuable reducing overtreatment of low-risk disease and [42▪]. Recent report indicated that genomic classifier is also useful in the clinic when used as a part of the risk stratification in recommending adjuvant radiation to patients with high-risk pathologic features (43% of patients shifted to observation based on information of genomic classifier after radical prostatectomy) [43▪].

CONCLUSION

Current models of risk predication at all stages of prostate cancer are limited in their ability to predict true aggressiveness. Genomic gene expression profiling is being adopted in the clinic in an attempt to improve risk stratification. Ongoing evaluation of these tests and integration of genomic profiling into risk assessment models will be critical to realize the potential benefits of these tools.

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Conflicts of interest

Dr M.R.C. has previously received consulting fees from Myriad Genetics, Genomic Health, and GenomeDx. All three companies maintain research relationships with the Urology Department at UCSF.

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