An Update on Biochemical and Genomic Markers for Prostate Cancer

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Abstract:

Purpose: Detecting prostate cancer, developing therapeutic plans after negative biopsies, and prognosis-based patient counseling can be challenging for many urologists dealing with prostate cancer-specific antigens. New Biomarkers advances made improvement for prediction of responses to therapeutic option and can tell us about survival and recurrence. In this review, we have assessed current and upcoming biomarkers that are opening a new era in diagnosing the disease.

Materials and Methods: We conducted a comprehensive literature review of studies describing prostate cancer biomarkers. Two independent investigators searched PubMed, Embase, Web of Science, and Cochrane Databases to identify biomarkers in prostate cancer conducted a literature review.

Results: Recently, combining prostate cancer-specific biomarkers into a single test has gained increasing attention, especially since the introduction of genomic and molecular tools. The development of the Prostate Health Index (PHI), SelectMDx, and Confirm MDx have shown promising results for prostate cancer detection, in addition to risk stratification and biopsy avoidance.

Conclusion: Despite major improvements and innovations in prostate cancer biomarkers, application in current clinical practice is limited. However, these biomarkers have an important role in determining risk, preventing unnecessary prostate biopsies, and predicting prognoses. Additional confirmatory studies will be needed to fully understand the impact of prostate cancer-specific biomarkers.

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Introduction

Prostate cancer is one of the most common cancer and the fifths leading cause of cancer-related mortality in men.(1) This is compounded by the fact that prostate cancer incidence is increasing, especially in countries with higher socioeconomic development. However, global mortality rates have only marginally improved.(2) There is a considerable debate concerning the role that current prostate-specific cancer biomarkers have in decreasing mortality rates. Issues include over-diagnosis and biopsies with negative results or indolent cancers that can cause complications. This has led many to suspect that biomarkers have only a small effect on patient survival.(3) Finding new and better genomic and biochemical markers to detect those at high risk of prostate adenocarcinoma is therefore essential. It is also important to differentiate benign and aggressive tumors and biomarkers that contribute to decision making after biopsy are required. Finally, more biomarkers that are accurate will allow a better discussion with patients concerning prognosis, enabling medical practitioners to develop the most effective therapeutic plans as showed in Image 1 and 2. Therefore, the purpose of this review is to provide an up-to-date assessment of new and upcoming prostate-specific cancer biomarkers to help clinicians and patients come to the best possible treatment decisions.

Biomarkers that aid in reducing unnecessary biopsies

Prostate-specific antigen (PSA) is a conventional biomarker commonly used for the detection of prostate cancer, although it has limited specificity. PSA is a member of the kallikreins, a regulatory family of 15 serine proteases that are involved in the development of many malignant, inflammatory, and degenerative diseases. They all are expressed by prostatic tissue but PSA (hKLK3) is solely secreted by prostate epithelial cells.(4) Both complexed and free PSA are found circulating in the blood of patients. PSA starts as a zymogen (preproPSA or [−7] proPSA) that is cleaved by hK2 to make a proPSA. Subtraction of the proPSA leads to the active form of PSA. Partial cleavage of proPSA can produce other proPSAs that are particularly elevated in prostate cancer, such as [−2] proPSA, [−4] proPSA, and [−5] proPSA. Among the different PSA isoforms

that have shown a role in prostate cancer detection, [-2] proPSA (p2PSA) has received the most attention as it has been found to be possess greater precision than other isoforms. (5) For applications in clinical settings, several studies have shown that a set of four serum kallikrein biomarkers used together can increase prostate cancer diagnostic accuracy in comparison to using only PSA. (4) This test is known as the 4Kscore test and includes tPSA, fPSA, iPSA, and hK2. Image 1 provides an overview of the biomarkers recommended for prostate cancer diagnosis and determining therapeutic approaches.

Mixed Biomarkers

The 4Kscore panel (OPKO Lab, Nashville, TN, USA) in combination with an assessment of clinical features, such as age and digital rectal examination (DRE), and total PSA levels, has been reported to be more accurate for diagnosing Gleason 7 or more severe prostate cancers.(6) Recent studies have also shown that the 4Kscore panel helps physicians reclassify severity after an initial biopsy, although it did not add any predictive value for men diagnosed with prostate cancer during later surveillance biopsies.(7) recently, a report showed that combination of 4Kscore panel and MRI may decrease redundant prostate biopsies furthermore.(8)

Prostate Health Index (PHI)

The Prostate Health Index (PHI) is an encouraging new test based on prostate-specific antigen (PSA) that aims to mathematically estimate the risk of prostate cancer using the formula (p2PSA/free PSA) × √PSA. (9)The PHI test has been used to prevent more than one third of avoidable biopsies and its failure rate for detecting prostate cancer is below 2%.(10) A recent meta-analysis of the diagnostic accuracy of the PHI and 4Kscore tests for detecting and predicting high-grade prostate cancer rates found PHI more sensitive but 4Kscore more specific. Specifically, pooled sensitivity was 0.93 for PHI and 0.87 for the 4Kscore panel, whereas the diagnostic accuracy of PHI was 0.82 and 0.81 for 4Kscore. Both the PHI and the 4Kscore tests had acceptable diagnostic accuracy rates for identifying overall and high-grade prostate cancer.(11) A recent

Meta-Analysis showed that combination of these 4Kscore and prostate cancer antigen 3 may be more predictive together than any of these test lonely.(12)

Prostate-specific membrane antigen (PSMA)

Prostate-specific membrane antigen (PSMA) is a glycoprotein found in the serum, urine, and tissues of patients with prostate cancer and is a well-established biomarker. PSMA is expressed by the epithelium of prostate tissues, although it is also secreted by the central nervous system and intestine. Recently, three splice variants of PSMA (PSM′) have been defined as a possible new biomarker for prostate cancer, although data are limited.(13) PSMA is also a promising molecular probe for positron emission tomography (PET) that offers better detection compared to conventional imaging methods, particularly at the very low PSA levels found during biochemical recurrence. The current imaging modalities used for detecting prostate cancer metastases have only modest accuracy. In addition to the possible diagnostic applications of PSMA, it may also have a therapeutic role involving the immune system that can delay disease progression.(14) For instance, a controlled *in vivo* study using a mouse model found that anti-PSMA monoclonal antibodies led to a decrease in tumor growth and prolonged survival rates.(15)

Prostate cancer antigen 3 (*PCA3*)

Prostate cancer antigen 3 (*PCA3*) is non-coding RNA (ncRNA) that can be measured by quantitative amplification using reverse transcription polymerase chaining reaction (RT-PCR]. The detection of *PCA3* in urine by RT-PCR can improve prostate cancer diagnoses. (16)More recently, a newer technique has been developed for *PCA3* detection that has improved sensitivity and quantitation. (16)The technique has been approved by the FDA for informing therapeutics decisions and is typically used after a negative prostate biopsy. However, appropriate thresholds have become a matter of concern as different cutoff levels result in variable sensitivity and specificity rates.(17) A meta-analysis by Cui *et al.* indicated that *PCA3* levels in the urine have high sensitivity and specificity,(18) although a more recent report has questioned the use of *PCA*3

and suggested it may not be useful for determining intermediate- or high-risk prostate cancers.(19) Zhikui Jiang et al report that score cutoff value of 20 as a best diagnostic efficacy.(20)

***TMPRSS2:ERG* gene fusions**

In normal prostate tissue, the *ERG* proto-oncogene is inactive, although limited expression has been reported in other tissue types. During prostate cancer, *ERG* is activated by gene fusion events, most frequently with *TMPRSS2* to create *TMPRSS2:ERG*.(21) The fusion of the *TMPRSS2* promoter region to the *ERG* oncogene is reported in half of all cases of prostate cancer and is a highly specific biomarker.(22) However, an important consideration is that the prevalence of *TMPRSS2:ERG* fusions is lower in men of African descent, indicating that alternate genomic biomarkers may be more suitable for this population.(23)

EXO106 score

Exosomes are small lipid membrane vesicles produced by most cells of the body that often contain nucleic acids.(24) Both exosomal *PCA3* and *TMPRSS2:ERG* transcript levels are higher in the first voided urine after prostate massage and may be useful for diagnosis.(25) However, a combination of *PCA3* and *ERG* transcript levels can also assessed without prostate massage by RT-PCR, termed the EXO106 score.(26) Although measuring exosomes remains challenging, it may become a standard method to assess the levels of important biomarkers, such as *PCA3* and *ERG* .

ExoDx Prostate (IntelliScore)

The ExoDx prostateurine-based test (IntelliScore; Exosome Diagnostics, Inc., Waltham, MA, USA) is an application of EXO106 that aims to identify the presence of high-grade prostate cancer in men over 50 years of age and PSA levels between 2-20 mg/mL. The test detects RNA from three specific genes (*ERG*, *PCA3*, and *SPDEF*) in the urine and combines analysis with clinical findings (PSA levels, age, race, and family history) to diagnose the disease.(27)

Decipher

Decipher, developed by Genome Dx Biosciences (Vancouver, Canada) and The Mayo Clinic (Rochester, MN, USA), is a genomic panel of RNA biomarkers that assays the expression of 22 different genes. It is a validated genomic classifier used to predict metastasis after radical prostatectomy. Decipher can be used to predict metastasis and prostate cancer-specific mortality using an initial diagnostic biopsy in intermediate and high-risk patients after radiotherapy or radical prostatectomies.(28) A Systematic Review Proposed that Decipher genomic classifier is best for intermediate-risk PCa and after radical prostatectomy therapeutic plans.(29)

SelectMDx

The mRNA levels of *DLX1*, *HOXC6*, and *KLK3* have also been shown to be promising candidates for the detection of prostate cancer. By assessing the levels of these transcripts post-DRE using first-void urine, unnecessary biopsies can often be avoided. Higher levels of these mRNAs after biopsy can also have a predictive role in significant prostate cancer. The SelectMDx algorithm (MDxHealth, Irvine, CA, USA) combines RT-PCR of *HOXC6* and *DLX1* with clinical and para-clinical findings (PSA levels, PSAD, DRE, age, and family history).(30) Dijkstra *et al.* propose that applying SelectMDx in patients with PSA levels greater than 3 ng/mL can result in a reduction in therapeutic costs and an increase in quality-adjusted life years (QALYs).(31) Quintana discussed that SelectMDx is a vaoluable diagnostic tools in patients with a very low risk or patient with negative biopsy and patient withdoubtful mpMRI.(32)

Michigan Prostate Score (MiPS)

Prostate cancer antigen 3 (PCA3) assessment has also been recommended by the National Comprehensive Cancer Network (NCCN) and approved by the FDA to assess men at high risk of prostate cancer but with negative needle biopsy results.(33) This test, in combination with *TMPRSS2:ERG* fusion-gene transcript assays, has emerged as a potentially valuable novel biomarker. The Michigan Prostate Score (MiPS) is an

application of this methodology to diagnose prostate cancer that combines serum PSA levels with *TMPRSS2:ERG* and PCA3 genes levels in the urine.(34)

Oncotype DX and Prolaris

The Oncotype DX multi-gene RT-PCR (Genomic Health, Redwood City, CA, USA) has also shown promising results. The test uses quantitative RT-PCR to measure 12 specific cancer-related RNAs using prostate biopsy specimens.(35) Prolaris (Myriad Genetics, Salt Lake City, UT, USA) is a similar molecular-based test that assays 31 genes and is valuable for risk assessment in patients with prostate cancer.(36) However, a recent meta-analysis found insufficient evidence to show the effectiveness of the Prolaris test when determining prostate cancer clinical outcomes.(37) Moschovas and colleagues showed that Oncotype DX higher scores is related to high pathologic grade of the tumor after surgery.(38)

Other genes and single nucleotide polymorphisms (SNPs)

Several other genetic markers have been identified as mutated in prostate cancer, particularly in hereditary cancers, that may serve as biomarkers. These include the tumor suppressor genes breast cancer type 1 and 2 (*BRCA1* and *BRCA2*), the MDM2 promoter P1 region, the development gene *HOXB13*, and multiple mismatch repair genes, including several from the melanocyte-stimulating hormone (MSH) family.(39-41) However, the full roles of these genes in prostate cancer and whether they could be applied to diagnosis require further study.While single nucleotide polymorphisms (SNPs) have been identified in many genes and loci related to prostate cancer, the relationships are often weak. Therefore assessing effects multiplicatively may be a more valuable approach.(42)

Epigenetic alterations

While changes that lead to cancer are often due to genetic effects, epigenetic alterations that do not affect DNA sequence can also influence gene activity and expression. Hypermethylation, hypomethylation, and histone post-translational modifications have all been associated with prostate cancer and may serve as

potential biomarkers. For example, methylation of the glutathione S-transferase Pi 1 promoter region, the Ras association domain family protein 1 isoform A promoter region, retinoic acid receptor beta 2, adenomatous polyposis coli, and several other loci (including AOX1 and RARB), has been linked to prostate cancer.(43, 44)

ConfirmMDx

One potential application of epigenetics to diagnose prostate cancer is ConfirmMDx, developed by MDxHealth. This test uses post biopsy specimen analysis of hypermethylation in CpG islands of the promoter regions of *GSTP1*, *APC*, and *RASSF* genes.(45) However, there is still little evidence to support the effectiveness of the test.

Biomarkers for predicting prognosis

In addition to detecting prostate cancer, understanding severity and developing therapeutic plans are key issues for medical practitioners and are especially important for recently diagnosed patients. Several biomarkers have been proposed for predicting prostate cancer prognosis [Image 1]. Recently, immunohistochemical analysis of the fork-head box protein A1 (FOXA1 or HNF-3a) transcription factor using post-prostatectomy tissue from *ERG* negative patients has found that high *FOXA1* expression may be a useful prognostic.(46) In addition, aberrant androgen biosynthesis is often associated with prostate cancer and may have prognostic implications, although the underlying mechanisms are not well established.(47) For example, the synthesis of androgens in the prostate of patients with castration-resistant prostate cancer (CRPC) depends on the enzymatic activity of HSD3B1. Polymorphisms in this protein have shown some prognostic roles during CRPC.(48) Recent studies have also shown that estrogen receptors α/β and aromatase in the androgen synthesis and catalysis cascade can predict the outcome of prostate cancer.(49) Current evidence suggests that only the minority of prostate stem cells are androgen-independent and can cause CRPC, although many genes have been found to be more highly expressed in such cases, including *CCNB2*,

DLGAP5, *CENPF*, *CENPE*, *MKI67*, *PTTG1*, *CDC20*, *PLK1*, *HMMR*, and *CCNB1*. These likely have a prognostic role in the heterogeneous response to androgen-deprivation therapies used for prostate cancer.(50) Several other genes have been implicated in the variation inherent to prostate cancer prognoses. For example, Valla *et al.* showed that the RNA component of telomerase (TERC) is overexpressed in prostatic cancer and regulated by MYC. TERC therefore may serve as a potential new biomarker.(51) The phase II SWOG S0925 androgen deprivation combination study also suggested that circulating micro-RNAS (miRNAs) may have a prognostic role, including miR-141, miR-200a, miR-19a, and miR-375.(52, 53) The involvement of miRNAs is supported by a further study that found a high combined miRNA score in the miR-17-92 cluster was prognostic for shorter biochemical recurrence in patients with prostate cancer.(54) Additionally, the differential expression of several genes has been linked to metastasis occurrence, including *CD4*, *PCNA*, and baculoviral IAP repeats.(55) Finally, mutations in many DNA repair genes have also emerged as potential prognostics and patients with metastatic CRPC have a greater frequency of mutations in BRCA2, BRCA1, PALB2, CHEK2, and ATM.(56, 57) although based on racial variation, some of these genes role are matter of concern.(58)

PORTOS

A recent analysis of the 24 gene Post-Operative Radiation Therapy Outcomes Score (PORTOS) has shown that it is valuable in predicting metastases in patients with prostate cancer. The authors of the study suggest that adjuvant radiotherapy should be routinely practiced for men with high PORTOS scores.(59)

ProMark

ProMark (Metamark, Waltham, MA, USA) is a protein-based test that quantitatively examines multiplexed proteomics from prostate tissue. The panel utilizes 12 protein markers that can serve to predict prostate cancer aggressiveness.(60)

DNA-ploidy

Aberrant DNA has also been shown to be a prognostic factor in patients with prostate cancer. This includes the presence of deletions that amplify the risk of biochemical recurrence in diploid, tetraploid, and aneuploid tissues. This has led to the development of a diagnostic nomogram that uses an assessment of ploidy and deletions to determine prostate cancer prognosis.(61) Ersvaer et all suggested that the DNA ploidy beside automatically estimated stroma fraction is a useful test for prognosis assessment.(62)

Tumor circulating cells

The biomarker potential of circulating tumor cells (CTCs) has expanded significantly over recent years, especially since the development of tests that detect *PSA* mRNA during prostate cancer. However, CellSearch (Menarini-Silicon Biosystems, Castel Maggiore, Italy) is currently the only FDA-approved test for identifying circulating tumor cells. The system uses antibodies specific to EpCAM and cytokeratins 8, 18, and 19 (positive) with CD45 (negative) to detect tumor cells.(63) However, a recent study has developed a new system that applies analysis of 14 genes, including epithelial markers, stem cell markers, and epithelial-to-mesenchymal-transition markers, to perform *in vivo* CTC isolation. This allows downstream RNA analysis and may be of use for molecular diagnostics.(64) There has also been developments in improving CRPC survival, including better detection and therapeutic approaches.(65) The CTCs found in CRPC feature alternate active androgen receptors, a consequence of differential splicing that can occur in the human androgen receptor gene. These unique variants may offer a promising biomarker for predicting prognosis during CRPC. For example, AR-V7 mRNA expression is higher in CTCs isolated from patients with metastatic CRPC that have aggressive tumors. These patients also have poor treatment outcome after androgen deprivation therapy.(66, 67) However, tests based on CTCs are limited in clinical practice due to methodological limitations. Such as separation methods and purify that.

Summary

Despite major developments in biomarkers, it is clear that additional work and more focused clinical trial design is required to develop effective diagnostic tests for prostate cancer that have prognostic capacity. It is therefore additional large, multi-center clinical studies are needed to provide more vigorous evidence that will aid the development of prostate cancer biomarkers and to further validate these findings

Abbreviations

- **PC:** Prostate cancer
- **PSA:** Prostate-specific antigen
- **PHI:** Prostate Health Index
- **4K:** Four-kallikrein
- **PCA3:** Prostate Cancer Antigen 3
- **AS:** Active surveillance
- **mRNA:** messenger RNAs
- **MiPS:** Mi prostate score
- **mpMRI:** Multiparametric magnetic resonance imaging of the prostate
- **miRNAs:** micro RNAs

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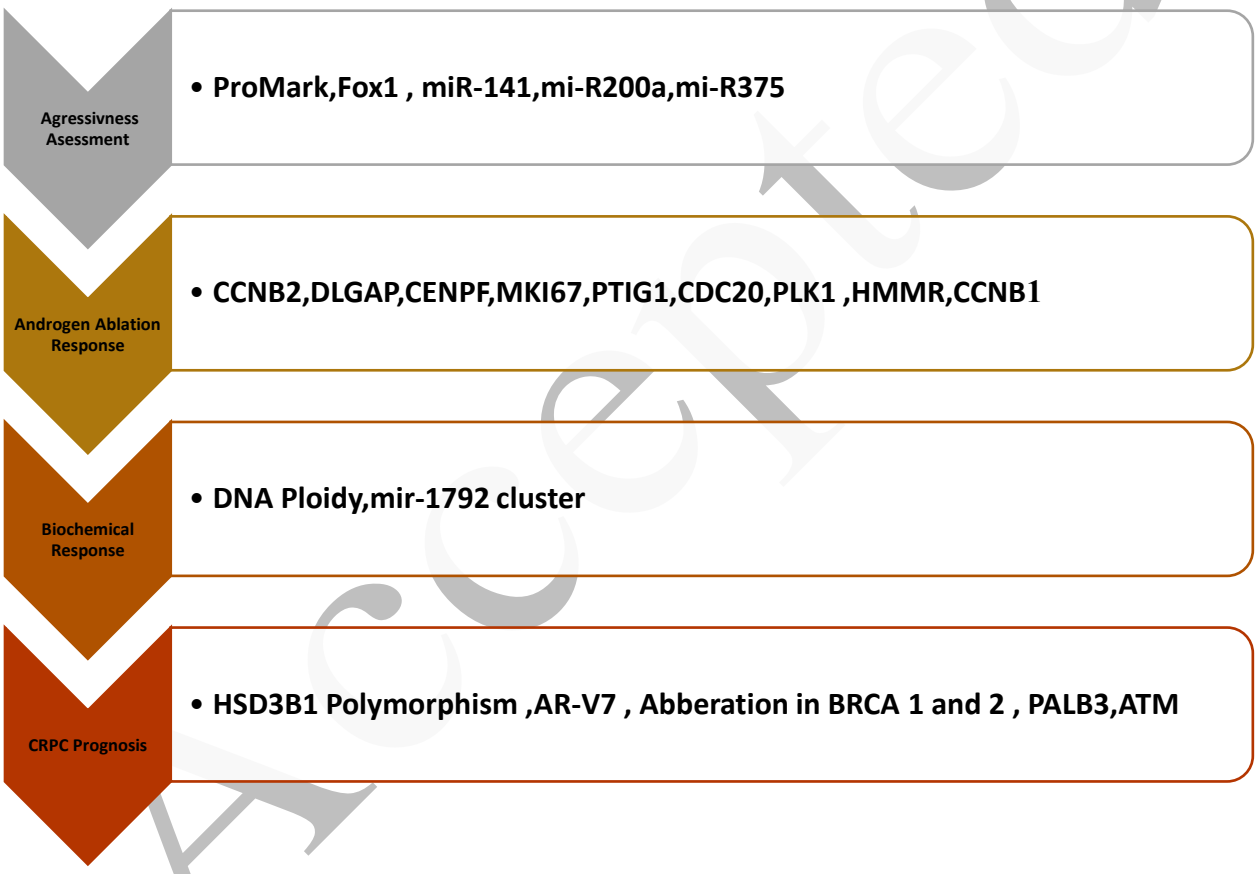

Image 1: Different biomarkers and their roles in prostate cancer prognosis

Image 2: New emerging prostate biomarkers and their roles in screening, treatment, and establishing prognoses.