We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Biomarkers for Diagnosis and Prognosis of Prostate Cancer

Meghan A. Rice and Tanya Stoyanova

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.79726>

Abstract

Since its discovery, elevated prostate-specific antigen (PSA) has been the measurement to indicate possibility of prostate cancer, as well as biochemical recurrence following treatment. Although PSA has led to decrease in prostate cancer-related mortalities, PSA is a nonspecific prostate cancer biomarker reflective of other prostate-related conditions such as benign prostatic hyperplasia (BPH), resulting in a high false-positive rate. This has led to overtreatment of men with clinically insignificant disease. While most prostate cancer patients have slowly progressive disease and should be treated conservatively, roughly 10% of patients will progress to have metastatic disease, of which the majority of prostate cancer deaths can be attributed. Stratifying these patients based on prognosis so that they may benefit from aggressive treatment is critical to their survival. Biomarkers for prostate cancer diagnosis and subsequent prognostic screening have significantly advanced this field. Here, we review some of the current blood, tissue, and urine biomarker tools used to measure an array of molecules including DNA, RNA, protein, or even epigenetic modifications. Utilizing the technologies described here, as well as looking to the future, correct early identification of prostate cancer with powerful prognostic value is much closer than ever before.

Keywords: prostate cancer, biomarker, early detection, prognosis, risk stratification

1. Introduction

Prostate cancer remains the most commonly diagnosed cancer in men in the United States with over 200,000 new cases detected annually [1]. Gleason grade of prostate cancer, developed by Dr. Donald Gleason in the 1960s, remains the most prognostic indicator of prostate cancer to date. Gleason grade ranges from 1 (normal) to 5 (most abnormal) and is assigned based on the histology of prostate tissues from biopsies. The Gleason score ranges from 2

to 10 and is the sum of the two most common Gleason grades. However, assessing Gleason grade requires invasive tissue biopsies. Less than one-third of men tested for prostate cancer through biopsy are diagnosed with cancer by histological analysis. Meanwhile a negative biopsy does little to reassure patients and clinicians of negative cancer status. This leads to a large number of patients undergoing painful initial biopsy procedures that may ultimately be repeated due to uncertainty of diagnosis [2]. Prostate cancer biopsies are a painful and invasive procedure, with the chance of complications including bleeding and infection [3–5].

Of those patients with positive diagnoses, roughly 10% will progress to metastatic prostate cancer, resulting in about 30,000 deaths annually in the United States. It is obvious that these patients should receive aggressive treatment at the earliest sign of disease. However there is concern as to over-diagnosis and over-treatment of indolent prostate cancer [6], resulting in some cases of high risk prostate cancer being treated conservatively with active surveillance, or first step intervention with radiation or radical prostatectomy. It is imperative to the respective disparate patient populations to receive the most accurate, timely, prognostic diagnosis.

The national cancer institute (NCI) dictionary of cancer terms defines biomarker as a “biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or a condition or disease.” Advancements in the field of biomarker discovery have shaped the way medicine is performed and patients are diagnosed [7]. Biomarkers are used throughout the scope of clinical progression from early detection and diagnosis through clinical endpoint determinations.

Ideal biomarkers should have high sensitivity and specificity. That is, the power to correctly identify a high proportion of true cases, or those that will experience an event - in this case, developing prostate cancer. A biomarker should also have high specificity, correctly identifying patients who are truly negative for harboring prostate cancer. Typically, a balance is met between specificity and sensitivity. These results are presented as receiver operating characteristic (ROC) curves which are a visual means to describe the statistical ability of a model to correctly classify cases from non-cases [8]. Complete random distribution creates an “area under the curve” or AUC value of 0.50, graphed as a straight slope line, while the value from a perfect prediction model would be 1.0. A reliable prediction model therefore should have an AUC value nearing 1.0.

Here we will discuss the scope of biomarkers currently used for prostate cancer diagnosis, as well as prognosis to aid in disease monitoring and treatment oriented decision-making. Prostate cancer biomarkers are currently among three categories: blood, tissue or urine based biomarkers.

2. Blood-based biomarkers

An ideal biomarker screen is non-invasive. Blood collection is considered a minimally invasive technique with quick turnaround time that is also indicative of real-time alterations and disease states in the body, while robust or stable enough for findings to be reproducible across clinics. For prostate cancer as with many other diseases, the use of blood to monitor disease progression was the first and hallmark analysis performed to detect the disease.

In recent years, the phrase “liquid biopsy” has been coined, expanding a simple blood draw into an extensive cancer screen, testing for circulating tumor cells or circulating tumor free DNA in the blood. These tests have shown great promise in detecting cancer at early stages across a wide array of malignancies including prostate cancer [9].

Today, we continue to use blood based screens as means to detect prostate cancer, inform patients and clinicians on necessity of treatment, as well as to plan and monitor treatment response.

2.1. Prostate-specific antigen

The discovery of prostate and prostatic fluid associated antigens, most notably prostate-specific antigen (PSA) occurred in 1970 by Richard Ablin [10]. PSA is a glycoprotein produced by human kallikrein-3 (hK3), a member of a class of highly homologous serine proteases, the tissue kallikreins. PSA is normally produced by the epithelial cells of the prostate gland and secreted into the lumen to aid in liquefaction of semen ejaculate. However, other pathological conditions in the prostate, such as benign prostatic hyperplasia (BPH) or prostatitis can elevate the serum PSA levels, resulting in a “false positive” PSA test. PSA concentration in blood has been heavily explored for detection of prostate cancer, as well as treatment response and progression free survival monitoring thereof.

The number of diagnosed prostate cancer cases surged with the implementation of PSA screening tests, peaking at around the time of its approval by the United States Food and Drug Administration (FDA) in 1994 for prostate cancer detection. While PSA is prostate specific, it is not, however, specific to cancer, being additionally increased in the aforementioned benign prostate conditions. Since its discovery, PSA has been extensively studied in randomized clinical trials as a screening test for prostate cancer. Despite this discovery, prostate cancer remains the most commonly diagnosed non-cutaneous cancer in men in the United States, with solid tumor-associated deaths only second to lung cancer [1]. In addition to poor cancer specificity, PSA also has low sensitivity. Reported in the prostate cancer prevention trial (PCPT) 15% of men with PSA 0–4 ng/ml have prostate cancer, 15% of those are high Gleason score [11, 12].

Ultimately, implementation of PSA as a screening tool led to an over-diagnosis and subsequent over-treatment of low-risk disease. Perspective studies indicated up to 10% of patients who received curative therapy by either radical prostatectomy or radiation were over-treated [13]. In 2012 The United States Preventative Services Task Force (USPSTF) recommended against PSA based screening for prostate cancer [14]. This recommendation has recently been amended to suggest PSA may be used specifically in men 55–69 on a case-by-case basis with informed patient consent regarding potential harms of screening. In all, the usefulness of PSA will continue to persist especially in disease monitoring, but recent advances lose faith in PSA alone as a diagnostic tool. In-depth analysis of PSA has revealed several molecular variations and functions of PSA which may prove to be more specific to cancerous tissues.

2.1.1. Free vs. bound PSA

PSA is typically observed complexed to protease inhibitors such as alpha 1-antichymotrypsin (ACT) or alpha 2-macroglobulin, known as bound PSA [15, 16]. Discovered in the 1990s, a

higher ratio of free PSA, that is, not bound to protease inhibitors and considered inactive, is associated with increased likelihood of BPH rather than cancer [15, 17–19]. Specific assays have also been developed to measure bound PSA (complexed-PSA), which is usually PSA-ACT and is elevated in cancer [20]. A percentage free PSA (free PSA/total PSA) can be calculated, and is typically lower in men with prostate cancer [21], where early studies linked <25% free PSA to detection of prostate cancer with a sensitivity of 95% [22]. However, follow-up analyses have had less promising results likely due to the relative instability of free or uncomplexed PSA compared to bound, making this an unreliable clinical parameter for patient diagnosis [23, 24].

2.1.2. Proenzyme PSA (proPSA)

Free PSA can be found in three different forms; proenzyme PSA (proPSA), benign PSA (BPSA) and intact PSA. proPSA is found increased in patients with prostate cancer [25, 26]. Several isoforms exist of proPSA based on varying truncations including [-2] and [-4] proPSA. [-2] proPSA or p2PSA has shown promise as a prostate cancer biomarker as it is not detected in BPH, and in trials increased the AUC from 0.52 for PSA or 0.53 for percentage free PSA to 0.73 [27]. [-2] proPSA has been used preferentially to total or free PSA for prostate cancer detection or biopsy [28–30].

2.1.3. Prostate-specific antigen density (PSAD) test

The PSAD test attempts to add specificity to PSA testing in prostate cancer by determining the amount of PSA produced in relation to size of the gland, as size has been highly correlated with prostate cancer prognosis [31, 32]. Prostate size can be measured with magnetic resonance imaging or transrectal ultrasound by a physician. High density indicates that a small volume prostate is responsible for making a large amount of PSA, and reflective of prostate cancer. In contrast, low density reflects an enlarged prostate, most likely due to BPH that is responsible for the PSA elevation.

2.1.4. PSA velocity

Another factor suggested to provide more accuracy to PSA in ability to predict prostate cancer lies in the rate at which increase is observed, referred to as PSA velocity. PSA testing is performed at routine intervals in men on active surveillance, and elderly men at risk of developing prostate cancer. Elevated PSA is considered in the range of 4.0–10.0 ng/ml, though prostate cancer may still be found in men below this range. PSA velocity factors the rate of PSA increase over time, such that an increase greater than 0.5 ng/ml per year may be indicative of prostate cancer [33].

2.2. The prostate health index

The Prostate Health Index (PHI) is an intuitive formula based upon utilization of several well characterized PSA forms—total PSA, free PSA, and [-2] proPSA or p2PSA, such that:

$$\left(\frac{p2PSA}{free\ PSA} \right) \times \sqrt{PSA} \quad (1)$$

The PHI's multifactorial approach has compounded the precision of each of the PSA measurements providing one patient score, shown to drastically increase the specificity for prostate cancer [28, 34]. PHI has been approved by the FDA for men with PSA in the 4.0–10.0 ng/ml range.

Several clinical trials have retrospectively performed direct comparison to PHI against other early detection biomarkers across blood or urine analysis. In a European cohort of men undergoing either an initial or repeat biopsy, comparing PHI to PSA or free PSA, PHI increased the AUC values to 0.70 compared to 0.65 or 0.53 respectively [35]. In one prospectively performed trial it was determined that 30.1% of patients who underwent a biopsy could have been spared the painful procedure based on PHI score [36]. PHI has additionally been compared against urine biomarkers (to be discussed further in this chapter), with PHI increasing AUC over PCA3 or TMPRSS2:ERG [35, 37]. While results were similar, PHI was the only one correlated with Gleason grade greater than 7 [38].

2.3. The four-Kallikrein panel and 4Kscore® test

The four-kallikrein panel, subsequently referred to as the 4Kscore test is a reflex, or follow-up blood test for men who have an abnormal PSA or digital rectal exam (DRE) result and are being considered for an initial or repeat prostate biopsy after a prior negative biopsy result. True to its name, the test is based upon inclusion of four-kallikreins, total PSA, intact PSA, free PSA in addition to human kallikrein-2 (hK2). The test is generated by OPKO Labs (Nashville, TN) and has been marketed as accurately identifying the risk of aggressive prostate cancer (Gleason >7) in a subsequent biopsy or radical prostatectomy, aiding in patient action plan-based decision making.

The first clinical report of the four-kallikrein panel was among 740 previously unscreened men who underwent biopsy for a PSA above 3.0 ng/ml in the European Randomized Study of Screening for Prostate Cancer [39]. Subsequent studies have been performed for at least 10 cohorts totaling over 15,000 subjects (reviewed in [40]), each of which observed an AUC between 0.80 and 0.90 for the four-kallikrein testing. Results from these studies consistently demonstrate the four-kallikrein panel effectively identified high-grade disease while reducing the number of unnecessary biopsies 49–57% among men being screened for the first time. The 4 k panel is the only test, aside from PSA that has been linked to long-term end-points including prostate cancer metastasis [17, 41]. Studies were initially performed in Europe, and limitations include only retrospective analysis, in primarily white populations with an alternative Gleason scale used. In translation to the United States to incorporate FDA guidelines, modifications of the test were implemented with positive results.

2.4. Stockholm-3

The Stockholm 3 model (S3M) is a combination of blood biomarkers initially including PSA, free PSA, intact PSA, hK2, MSMB, MIC1, genetic polymorphisms (SNPs) and other variabilities such as age, family history, previous prostate biopsies or exam [42, 43]. Later algorithm modification replaced intact PSA with HOXB13 [44]. The goal of the study was to increase the accuracy of high-risk prostate cancer diagnosis. S3M was tested in over 100,000 men, 50–69 years of age with no diagnosis of prostate cancer in Stockholm, Sweden [42, 43]. The performance

of S3M was compared to PSA alone. The use of S3M was found to decrease the number of biopsies by more than 50%, avoid negative biopsies and significantly improve the detection of high-risk prostate cancer [42, 43].

2.5. Prostate-specific membrane antigen

Prostate-specific membrane antigen (PSMA) is another glycoprotein with enzymatic function uniquely expressed in the prostate. As its name suggests, what makes it unique from PSA is that it is not a secreted protein, rather it is an integral membrane protein. Yet, like PSA, PSMA is also not specific to prostate cancer.

While PSMA has had little success as a serum based diagnostic marker, it is now being used as the target of an FDA approved radiographic scan (ProstaScint) in which an antibody against PSMA (7E11) is linked to a radiographic agent ¹¹¹indium. ProstaScint increased predictive value for metastatic prostate cancer, identifying positive lymph node metastases [45]. Several new PSMA specific tracers have been developed for use in PET and PET/CT scanning with performance characteristics that exceed those of ProstaScint and are likely to be approved soon for clinical use.

2.6. Prostatic acid phosphatase

Prostatic acid phosphatase (PAP), or prostatic specific acid phosphatase (PSAP) is a glycoprotein enzyme secreted by prostate cells like PSA. Discovered in the 1930s as a diagnostic biomarker [46], it was replaced with the discovery of PSA in the 1970s. However, PAP reemerged following the discovery that PAP was highly expressed in correlation with tumor staging, and is the target of the first prostate cancer immunotherapy, Sipuleucel-T, approved by the FDA in April, 2010 [47, 48], and which increased overall survival of men with metastatic prostate cancer in its first IMPACT trial [49].

2.7. AR-V7

Androgen receptor splice variant 7 (AR-V7) is a splice variant of androgen receptor (AR) that lacks the ligand binding domain leading to its constitutive transcriptional activity independent of androgens. Due to its androgen independent function, AR-V7 has been implicated in the resistance to second-generation anti-androgen therapies. AR-V7 can be detected in circulating tumor cells (CTCs) and its presence is correlated with resistance to second generation anti-androgens including enzalutamide and abiraterone [50, 51]. These results suggest the use of AR-V7 as a treatment selection biomarker.

3. Tissue-based biomarkers

Tissue based prognosticators are among the most diverse in functionality. Tissue based assays can be performed from as little tissue as a single core of a biopsy up to radical prostatectomy. Yet, these assays are the most invasive due to the nature of tissue extraction through surgical resection or biopsy. Patients may undergo one or multiple sets of biopsies in the course of disease detection and active surveillance. Biomarker screening may also be performed on

patients post radical prostatectomy to predict treatment response, recurrence free survival and likelihood of disease progression. Many of these tests are commercial panels available to analyze multiple mRNA signatures in the prostate, but recent advancements in protein and cancer epigenetics are expanding the possibilities of prostate cancer prognosis. Assays are monitored by The National Comprehensive Cancer Network (NCCN), an alliance of U.S. cancer centers directing clinical practice guidelines, as well as the Food and Drug Administration (FDA).

Here we will discuss several of the most commonly used tests:

3.1. DNA

Deoxyribonucleic acid (DNA) is the genetic material of all living things. In comparison to other cancers, prostate cancer has little in the way of genetic mutations. Researchers have used the several well described genomic alterations to their advantage as prognosticators of disease.

3.1.1. Epigenetic testing

Current biopsy strategies sample areas of the prostate in a gridded fashion in attempt to have the most representative assessment of the prostate. Even with this strategy in place, less than 1% of the prostate is sampled. As less than one-third of biopsies return positive results for cancer, there is large concern over inconclusive biopsy results.

The field cancerization effect was first observed in the 1950s when it was noticed that tissues surrounding cancerous lesions contained markers associated with tumor development of oral squamous-cell carcinoma [52]. This phenomenon has since been observed in most solid tumors. Further understanding of the concept is explained in [53]. Today, field effect can translate to modifications in cellular morphology, epigenetics, genomic or mitochondrial DNA alterations, and changes in gene expression or protein levels (reviewed in [54]).

One such assay, ConfirmMDx, tests the epigenetic field effect by observing the molecular changes in methylations occurring in prostate cancer. DNA methylation is among the most common measures of epigenetic abnormality, and easiest to test. These alterations are not detectable in histological analyses, but visible with methylation specific PCR (MSP). Biologically these methylations may be responsible for silencing of key tumor-suppressive genes critical to preventing cancer development, and because of the cancer field effect, this test dramatically amplifies the tested area of the prostate. ConfirmMDx is recommended for men having undergone an initial negative biopsy.

Prostate cancer-associated epigenetic biomarkers used in this assay include glutathione S-transferase-Pi (GSTP1), APC and RASSF1. Methylation of GSTP1 is among the most common somatic alterations observed in prostate cancer with high specificity and sensitivity, and which correlates strongly with Gleason score, age, PSA and DRE [55–57].

3.1.2. PTEN loss and ERG rearrangements

Phosphatase and tensin homolog (PTEN) is a tumor suppressor commonly lost in many cancers. Loss of PTEN is one of few genomic alterations occurring in prostate cancer. PTEN deletion associates with poor outcome and is an established prognostic biomarker for prostate

cancer. Analysis of prostatic tissue by Immunohistochemistry (IHC) or Fluorescence in situ hybridization (FISH) demonstrated that PTEN loss is associated with prostate cancer biochemical recurrence, disease progression and metastasis [58–63].

TMPRSS2:ERG fusion is found in ~50% of prostate cancer [64, 65]. TMPRSS2:ERG is a result of gene rearrangement and fusion between androgen regulated transmembrane protease, serine 2 (TMPRSS2) and ERG transcriptional factor genes [64, 65]. This leads to significant overexpression of ERG reported to promote prostate cancer oncogenesis [66–70]. TMPRSS2-ERG rearrangements are accompanied by PTEN loss, which cooperates to promote prostate cancer progression [69, 70]. Moreover, loss of PTEN and presence of TMPRSS2:ERG fusion together predict prostate cancer biochemical recurrence [71] and Metamark further provides screening for loss of PTEN and ERG rearrangement in their PTEN/ERG screen.

3.2. mRNA

Messenger RNA or ribonucleic acid (mRNA) is genetic material carrying information between DNA and protein.

3.2.1. *The genomic prostate score*

Oncotype DX offers a diverse array of genomic health testing including breast, colon and prostate cancer. The Genomic Prostate Score (GPS) is a prostate specific array which aids in decision-making between initiating immediate treatment or active surveillance. The test measures expression from 12 genes in four prostate cancer associated biological pathways: androgen signaling (AZGP1, FAM13C, KLK2, SRD5A2), cellular organization (FLNC, GSN, GSTM2, TPM2), stromal response (BGN, COL1A1, SFRP4) and cellular proliferation (TPX2), as well as 5 reference genes (ARF1, ATF5E, CLTC, GPS1, PGK1) [72]. This assay has been validated prospectively as an independent predictor of tumor aggressiveness based on adverse pathology, and death associated with prostate cancer and metastasis [73, 74]. GPS is advised for patients with low-risk clinical prostate cancer (very low, low or intermediate NCCN risk). AZGP1 was further validated as a potential biomarker for significant disease [75]. Loss of AZGP1 assessed by RNA in situ hybridization and immunohistochemical analysis is associated with worse outcome and overall survival [75].

3.2.2. *Prolaris*

Prolaris is a prognostic genetic test developed by Myria Genetic Laboratories based on a 46-gene expression signature strongly tied to cell cycle progression genes. Uniquely paired with cellular proliferation and Gleason grading, The Prolaris Score is generated as a metric of an individual's prostate cancer aggressiveness. This score provides a relative risk among patients of the same risk group defined by the American Urological Association (AUA), and a 10-year prostate cancer specific mortality risk in men with localized disease [76].

3.2.3. *Decipher*

The Decipher Biopsy was generated from GenomeDX, based on whole genome technology. In men with localized prostate cancer undergoing biopsy or radical prostatectomy, this test divides patients into Low Risk or High Risk, aiding clinicians and patients in decision making

toward active surveillance or intensification of treatment with multi-modal therapies. Decipher Biopsy measures 22 RNA biomarkers to correlate the probability of clinical metastasis within 5 years following radical prostatectomy [77] and is predictive of lymph node metastasis [78]. This test can be performed on either biopsy or prostatectomy samples reproducibly [79].

3.3. Protein

3.3.1. ProMark

ProMark is the first protein based prognostic test for prostate cancer from Metamark Genetics Inc. Based on the understanding that mRNA levels may not be completely reflective of a diseased state, ProMark assays protein levels in intact, formalin fixed biopsy samples to infer prognostic information about the patients' condition at the time of biopsy. Based on a quantitative multiplex immunofluorescence (QMPI) platform in which tissues are fixed in formalin, samples are stained for eight protein markers for cancer and normal regions and quantified in situ [80]. Markers include SMAD4, PDSS2, HSPA9, FIS, pS6, and YBOX1 to designate regions of prostate cancer, as well as proteins found in tumor and benign tissues, DERL1 and CUL2. Selected by computational modeling, these combinations of protein markers reflect the morphology from tumor epithelium for reliable prognostication [80–82]. Cost to perform ProMark protein screening is additionally quite low compared to usual guideline-based care [83]. ProMark has been utilized in clinical studies to predict lethal outcome. The test is currently recommended for men with Gleason grade 3 + 3 or 3 + 4 prostate cancer as part of the NCCN Clinical Care Guidelines.

3.3.2. p63 and AMACR

p63 has been identified as a marker of basal cells in multiple epithelial tissues including normal prostate [84]. Significant downregulation or loss p63 is commonly observed in prostate cancer [84, 85]. Alpha-methylacyl coenzyme A racemase (AMACR) is commonly found overexpressed in prostate cancer and exhibits little to no expression in the normal prostate tissues [86–89]. A combination of high-molecular weight cytokeratins, AMACR and loss of p63 can be used to define normal prostate tissues, prostate intraepithelial neoplasia and prostate adenocarcinoma [90, 91].

4. Urine biomarkers

Urine analysis is a non-invasive screening technique for prostate cancer.

4.1. PCA3

Prostate cancer antigen 3 (PCA3) (also known as DD3) is a non-coding mRNA specifically expressed in human prostate tissues, and highly overexpressed in prostate cancer [92].

The Progenesa PCA3 assay is an FDA approved urine based molecular test to aid in repeat biopsy decisions from Hologic [93]. Following DRE, a simple “first-catch” urine test captures prostate epithelial cells released into the urine. PCA3 mRNA levels are quantified in proportion to PSA. Also included in the NCCN's Clinical Practice guidelines for prostate cancer early

detection, this test's specificity lies in PCA3 which is highly upregulated in prostate cancer cells and not affected by instances of benign prostatic hyperplasia (BPH), prostatitis or other conditions as is the case for PSA. PCA3 testing is currently FDA approved for men previously having a negative biopsy with a persistently elevated PSA to help identify men who need a repeat biopsy. PCA3 is calibrated to identify men at low risk for a positive biopsy such that $PCA3 < 25$ indicates that it is safe to forgo the biopsy. Increase in score was directly correlated with likelihood of positive repeat biopsies, and predictive of 4-year biopsy outcome [94–96]. PCA3 has subsequently been explored and proven to positively predict detection of prostate cancer in initial biopsies with high specificity and may aid in initial biopsy decision making [97, 98].

4.2. SelectMDX

Utilizing first catch post-DRE urine, SelectMDx tests for mRNA levels of genes DLX1 and HOXC6. Analysis for this test incorporates multifactorial data from PSA density and prior biopsy data to increase significance of this liquid biopsy. This test has shown promise over PCA3 in two prospective clinical trials in identification of patients with high-grade prostate cancer (AUC of 0.90 in first cohort and 0.86 in validation cohort) [99]. SelectMDx was recently added to the European Association of Urology's list for added decision making before a repeat biopsy.

4.3. TMPRSS2:ERG

As ERG rearrangements occur at the genomic level, prostate cancer associated gene fusions such as TMPRSS2:ERG rearrangements are also detectable in patient urine [71, 86–89]. Urine TMPRSS2:ERG was found to associate with Gleason score and tumor size in a large multicenter study with 1312 men [100]. This strategy utilizes transcription mediated amplification (TMA) assay to quantify TMPRSS2-ERG mRNA normalized to PSA mRNA. Additionally, it was demonstrated that the combination of urine TMPRSS2:ERG with urine PCA3, improves the performance of serum PSA for predicting prostate cancer risk [100–102].

4.4. Mi-prostate score (MiPS)

The Michigan Prostate Score (MiPS) combines serum PSA levels with urine analysis for TMPRSS2:ERG and PCA3 mRNA as a predictive model for a positive prostate cancer biopsy. This compounded analysis of three independent prostate markers are closely correlated with presence of prostate cancer in an initial or repeat biopsy and provides a more accurate predictive model of biopsy detected prostate cancer [101].

4.5. Extracellular vesicles

Exosomes are small extracellular vesicles secreted from cells ranging in size from 30 to 120 nm. A portion of the parent cell cytoplasm is contained inside each exosome for the biological function of cell-to-cell communication. For the purpose of clinical diagnostics, this mechanism can be manipulated to measure exosomal genetic material released into blood, urine or other biological fluids. RNA expression from tumor cells is promising as they are highly representative of cell of origin. As exosomes are secreted freely into the urine, exosomal based testing does

not require biopsies to detect oncogenic signatures [103, 104]. For instance, PCA3 and ERG mRNAs can be detected in exosomes and be predictive for high grade prostate cancer [105].

4.6. ExoDX prostate (IntelliScore)

Exosomal analysis of PCA3 and ERG RNA copy number from prostate cancer patient urine was determined to positively predict presence of high-grade prostate cancer [105, 106]. This test, now marketed as ExoDX *Prostate(IntelliScore)* is considered a liquid biopsy, combining urine with PSA screening from blood sample. Clinically, *Prostate(IntelliScore)* correctly predicts the occurrence of Gleason scores above 7, and has been recommended for men over 50 with PSA levels in the 2–10 ng/ml range. Further evaluation of this biomarker assay is currently underway.

New technologies are expanding to increase the capture and analysis for extracellular vesicles as experimental material. Exosome Diagnostics, who market the *Prostate(IntelliScore)* additionally provide isolation kits for exosomal RNA. Alternatively, devices such as the Exosome Total Isolation Chip (ExoTIC), have been generated specific for the high-yield isolation of extracellular vesicles from biofluids (blood, urine, and lavage), even allowing for separation among vesicles based on size. This work has initially been applied to protein and microRNA analysis, increasing the scope of assayable markers for prostate cancer [107].

5. Conclusions

Assays and technologies have vastly improved prediction strategies for recurrent prostate cancer and metastatic disease. Collectively, the biomarkers and assays presented within this chapter represent great advances in the diagnosis and prognostic assessment of patients with prostate cancer and aid in decision-making for subsequent treatment strategies (reviewed in **Table 1**). However, even with this extensive armamentarium there is still improvement to be made in risk-stratification to accurately identify patients with cancer, and among them, those at risk of developing high grade disease. As biomarkers become available it is increasingly important to understand how these tests are helpful to know when and on which patients these tests should be utilized. Guidelines for care are consistently monitored by urological associations globally. While recommendations vary based on country and among individual institutions and providers, more than ever, patient led decision making is at the forefront of screening. This was evidenced by USPSTF's recent removal of PSA screening for healthy men as routine procedure, instead recommending individualized decision-making by physician counseling of patients as to the potential risks of inaccurate diagnosis leading to over-treatment.

Human nature understandably dictates a need for testing to be as minimally invasive as possible to eliminate painful procedures, and increase patient compliance and willingness to participate in early disease screening. The development of non-invasive screening methods such as blood and urine assays to limit prostate biopsy aids in reducing painful, and in the case of prostate cancer, often unnecessary procedures. This is even further amplified when taken into account the number of men who have to undergo the biopsy procedure repeatedly in the course of diagnosis and disease progression. The future of cancer screening, and hopefully diagnosis will come

Test	Specimen	Invasive	Implication	Biomarkers tested	Genetic Material Tested	FDA Status	Sources
PSA	Blood	Non-invasive	Treatment response and progression free survival	PSA	Protein	Approved	[10-33]
Prostate Health Index (PHI)	Blood	Non-invasive	Risk-assessment and repeat biopsy decision	total PSA, free PSA, [-2]proPSA	Protein	Approved	[28,34-38]
4Kscore	Blood	Non-invasive	Risk-assessment	total PSA, intact PSA, free PSA, hK2	Protein	total PSA, free PSA- Approved; 4kscore- CLIA	[17,39-41]
Stockholm-3	Blood	Non-invasive	Risk-assessment	PSA, free PSA, hK2, MSMB, MIC1, HOXB13, clinical variables, genetic markers and prostate examination	Protein/DNA	None	[42-44]
AR-V7	Blood	Non-invasive	Therapy resistance	AR-V7	RNA	CLIA	[50,51]
ConfirmMDx	Tissue	Invasive	Risk-assessment and repeat biopsy decision	Methylated GSTP1, APC and RASSF1	DNA	CLIA	[55-57]
PTEN/TMPRSS2:ERG	Tissue	Invasive	Risk-assessment and treatment planning	PTEN and ERG	DNA	CLIA	[58-71]
Oncotype DX Genomic Prostate Score	Tissue	Invasive	Risk-assessment and treatment planning	AZGP1, FAM13C, KLK2, SRD5A2, FLNC, GSN, GSTM2, TPM2, BGN, COL1A1, SFRP4, TPX2	RNA	CLIA	[72-75]
Prolaris	Tissue	Invasive	Risk-assessment and treatment planning	46 RNA biomarkers, Gleason score	RNA	CLIA	[76]
Decipher	Tissue	Invasive	Treatment planning	22 RNA biomarkers	RNA	CLIA	[77-79]
ProMark	Tissue	Invasive	Risk-assessment and treatment planning	SMAD4, PDSS2, HSPA9, FUS, pS6, YBOX1, DERL1, CUL2	Protein	CLIA	[80-84]
ProgenSA	DRE	Non-invasive	Risk-assessment and repeat biopsy decision	PCA3 and PSA	RNA/Protein	Approved	[92-98]
SelectMDx	DRE	Non-invasive	Repeat biopsy decision	DLX1, HOXC6 and PSA	RNA/Protein	Exempt	[99]
TMPRSS2:ERG	Urine	Non-invasive	Risk-assessment	TMPRSS2:ERG	RNA	CLIA	[71][86-89][100-102]
MiPS	Urine/Blood	Non-invasive	Risk-assessment	TMPRSS2:ERG, PCA3, PSA	RNA/Protein	CLIA	[101]
ExoDX Prostate (Intelliscore)	Urine/Blood	Non-invasive	Risk-assessment, active surveillance monitoring	ERG, PCA3, PSA	Exosomal RNA	Exempt	[105-107]

Table 1. Screening assays for prostate cancer, classified by specimen and genetic material tested, invasiveness of the assay, clinical uses, biomarkers tested, and status of FDA approval. Certain tests have been proven exempt from FDA regulations, and these are also specified. The Clinical Laboratory Improvement Amendments (CLIA) certifies clinical laboratory developed tests to perform additional testing.

from less invasive procedures. One such advance may be the implementation of imaging strategies such as multi-parametric magnetic resonance imaging (mpMRI) to locate and diagnose prostate cancer. Offered prior to biopsy, patients with negative results are spared the biopsy, while those with cancerous lesions can undergo a targeted biopsy aided by mpMRI, minimizing

complications, and obtaining accurate biopsies with ample cancer tissue to aid in treatment plan determination. This technique has tested more accurate than standard of care transrectal ultrasound (TRUS) biopsy in predicting presence of aggressive prostate cancer [108]. The role of imaging in prostate cancer diagnosis is still evolving and these technologies stand to introduce new avenues to the field of prostate cancer diagnosis and even treatment which may lead to better patient risk-stratification with increased survival rates of aggressive prostate cancer.

Acknowledgements

This chapter is written in dedication to Gerald Robert Howley.

The authors thank James D. Brooks for helpful comments and review.

T.S. is supported by the National Institute of Health/National Cancer Institute and Stanford McCormick-Gabilan Faculty Award.

Conflict of interest

The authors declare no conflicts.

Author details

Meghan A. Rice and Tanya Stoyanova*

*Address all correspondence to: stanya@stanford.edu

Department of Radiology, Canary Center for Cancer Early Detection, Stanford University, Palo Alto, CA, USA

References

- [1] Siegel RL, Miller KD, Jemal A. Cancer statistics. CA: A Cancer Journal for Clinicians. 2018;**68**(1):7-30
- [2] Campos-Fernandes J-L, Bastien L, Nicolaiew N, Robert G, Terry S, Vacherot F, et al. Prostate cancer detection rate in patients with repeated extended 21-sample needle biopsy. European Urology. 2009 Mar;**55**(3):600-606
- [3] Nam RK, Saskin R, Lee Y, Liu Y, Law C, Klotz LH, et al. Increasing hospital admission rates for urological complications after transrectal ultrasound guided prostate biopsy. The Journal of Urology. 2013;**189**(1 Suppl):S12-S17; discussion S17-8
- [4] Loeb S, Vellekoop A, Ahmed HU, Catto J, Emberton M, Nam R, et al. Systematic review of complications of prostate biopsy. European Urology. 2013;**64**:876-892

- [5] Wade J, Rosario DJ, Macefield RC, Avery KNL, Salter CE, Goodwin ML, et al. Psychological impact of prostate biopsy: Physical symptoms, anxiety, and depression. *Journal of Clinical Oncology*. 2013;**31**(33):4235-4241
- [6] Andriole GL, Crawford ED, Grubb RL, Buys SS, Chia D, Church TR, et al. Prostate cancer screening in the randomized prostate, lung, colorectal, and ovarian cancer screening trial: Mortality results after 13 years of follow-up. *Journal of the National Cancer Institute*. 2012;**104**(2):125-132
- [7] Strimbu K, Tavel JA. What are biomarkers? *Current Opinion in HIV and AIDS*. 2011;**5**(6):463-466
- [8] Hanley A, Mcneil J. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology*. 1982;**143**:29-36
- [9] Albitar M, Ma W, Lund L, Albitar F, Diep K, Fritsche HA, et al. Predicting prostate biopsy results using a panel of plasma and urine biomarkers combined in a scoring system. *Journal of Cancer*. 2016;**7**(3):297-303
- [10] Ablin RJ, Soanes A, Bronson P, Witebsky E. Precipitating antigens of the normal human prostate. *Journal of Reproduction and Fertility*. 1970;**22**(December 1969):573-574
- [11] Lucia MS, Darke AK, Goodman PJ, La Rosa FG, Parnes HL, Ford LG, et al. Pathologic characteristics of cancers detected in the prostate cancer prevention trial: Implications for prostate cancer detection and chemoprevention. *Cancer Prevention Research*. 2008;**1**:167-173
- [12] Thompson IM, Pauler DK, Goodman PJ, Tangen CM, Lucia MS, Parnes HL, et al. Prevalence of prostate cancer among men with a prostate-specific antigen level ≤ 4.0 ng per milliliter. *The New England Journal of Medicine*. 2004;**350**(22):2239-2246
- [13] Miller DC, Gruber SB, Hollenbeck BK, Montie JE, Wei JT. Incidence of initial local therapy among men with lower-risk prostate cancer in the United States. *Journal of the National Cancer Institute*. 2006;**98**(16):1134-1141
- [14] Moyer VA. Screening for prostate cancer: U.S. preventive services task force recommendation statement. *Annals of Internal Medicine*. 2012;**157**:120-134
- [15] Lilja H, Christensson A, Dahlen U, Matikainen MT, Nilsson O, Pettersson K, et al. Prostate-specific antigen in serum occurs predominantly in complex with $\alpha 1$ -antichymotrypsin. *Clinical Chemistry*. 1991;**37**(9):1618-1625
- [16] Rannikko S, Tuhkanen K, Alfthan O. A complex between prostate-specific antigen and $\alpha 1$ -antichymotrypsin is the major form of prostate-specific antigen in serum of patients with prostatic cancer: Assay of the complex improves clinical sensitivity for cancer. *Cancer Research*. 1991;**51**(1):222-226
- [17] Steuber T, Nurmikko P, Haese A, Pettersson K, Graefen M, Hammerer P, et al. Discrimination of benign from malignant prostatic disease by selective measurements of single chain, intact free prostate specific antigen. *The Journal of Urology*. 2002;**168**(5):1917-1922
- [18] Finne P, Finne R, Bangma C, Hugosson J, Hakama M, Auvinen A, et al. Algorithms based on prostate-specific antigen (PSA), free PSA, digital rectal examination and prostate

- volume reduce false-positive PSA results in prostate cancer screening. *International Journal of Cancer*. 2004;**111**(2):310-315
- [19] Mikolajczyk SD, Rittenhouse HG. Tumor-associated forms of prostate specific antigen improve the discrimination of prostate cancer from benign disease. *Rinsho Byori*. 2004;**52**(3):223-230
- [20] Lilja H. Biology of prostate-specific antigen. *Urology*. 2003;**62**:27-33
- [21] Christensson A, Björk T, Nilsson O, Dahlén U, Matikainen MT, Cockett AT, et al. Serum prostate specific antigen complexed to alpha 1-antichymotrypsin as an indicator of prostate cancer. *The Journal of Urology*. 1993;**150**(1):100-105
- [22] Catalona WJ, Smith DS. Cancer recurrence and survival rates after anatomic radical retropubic prostatectomy for prostate cancer: Intermediate-term results. *The Journal of Urology*. 1998;**160**(6 Pt 2):2428-2434
- [23] Djavan B, Remzi M, Zlotta AR, Ravery V, Hammerer P, Reissigl A, et al. Complexed prostate-specific antigen, complexed prostate-specific antigen density of total and transition zone, complexed/total prostate-specific antigen ratio, free-to-total prostate-specific antigen ratio, density of total and transition zone prostate-sp. *Urology*. 2002;**60**(4 Suppl 1):4-9
- [24] Khan MA, Sokoll LJ, Chan DW, Mangold LA, Mohr P, Mikolajczyk SD, et al. Clinical utility of proPSA and "benign" PSA when percent free PSA is less than 15%. *Urology*. 2004;**64**(6):1160-1164
- [25] Mikolajczyk SD, Grauer LS, Millar LS, Hill TM, Kumar A, Rittenhouse HG, et al. A precursor form of PSA (pPSA) is a component of the free PSA in prostate cancer serum. *Urology*. 1997;**50**(5):710-714
- [26] Mikolajczyk SD, Millar LS, Wang TJ, Rittenhouse HG, Marks LS, Song W, et al. A precursor form of prostate-specific antigen is more highly elevated in prostate cancer compared with benign transition zone prostate tissue. *Cancer Research*. 2000;**60**(3):756-759
- [27] Sokoll LJ, Wang Y, Feng Z, Kagan J, Partin AW, Sanda MG, et al. [-2]proenzyme prostate specific antigen for prostate cancer detection: A national cancer institute early detection research network validation study. *The Journal of Urology*. 2008;**180**(2):539-543; discussion 543
- [28] Loeb S, Catalona WJ. The prostate health index: A new test for the detection of prostate cancer. *Therapeutic Advances in Urology*. 2014;**6**(2):74-77
- [29] Catalona WJ, Bartsch G, Rittenhouse HG, Evans CL, Linton HJ, Amirkhan A, et al. Serum pro prostate specific antigen improves cancer detection compared to free and complexed prostate specific antigen in men with prostate specific antigen 2 to 4 ng/ml. *The Journal of Urology*. 2003;**170**(6 I):2181-2185
- [30] Sokoll LJ, Sanda MG, Feng Z, Kagan J, Mizrahi IA, Broyles DL, et al. A prospective, multicenter, national cancer institute early detection research network study of [-2]proPSA: Improving prostate cancer detection and correlating with cancer aggressiveness. *Cancer Epidemiology, Biomarkers & Prevention*. 2010;**19**(5):1193-1200

- [31] Benson MC, Seong Whang I, Pantuck A, Ring K, Kaplan SA, Olsson CA, et al. Prostate specific antigen density: A means of distinguishing benign prostatic hypertrophy and prostate cancer. *The Journal of Urology*. 1992;**147**(3):815-816
- [32] Benson MC, McMahon DJ, Cooner WH, Olsson CA. An algorithm for prostate cancer detection in a patient population using prostate-specific antigen and prostate-specific antigen density. *World Journal of Urology*. 1993;**11**(4):206-213
- [33] Vickers AJ, Brewster SF. PSA velocity and doubling time in diagnosis and prognosis of prostate cancer. *British Journal of Medical and Surgical Urology*. 2012;**5**:162-168
- [34] Catalona WJ, Partin AW, Sanda MG, Wei JT, Klee GG, Bangma CH, et al. A multicenter study of [-2]pro-prostate specific antigen combined with prostate specific antigen and free prostate specific antigen for prostate cancer detection in the 2.0 to 10.0 ng/ml prostate specific antigen range. *The Journal of Urology*. 2011;**185**(5):1650-1655
- [35] Scattoni V, Lazzeri M, Lughezzani G, De Luca S, Passera R, Bollito E, et al. Head-to-head comparison of prostate health index and urinary PCA3 for predicting cancer at initial or repeat biopsy. *The Journal of Urology*. 2013 Aug 1;**190**(2):496-501
- [36] Loeb S, Sanda MG, Broyles DL, Shin SS, Bangma CH, Wei JT, et al. The prostate health index selectively identifies clinically significant prostate cancer. *The Journal of Urology*. 2015;**193**(4):1163-1169
- [37] Ferro M, Bruzzese D, Perdonà S, Marino A, Mazzearella C, Perruolo G, et al. Prostate health index (phi) and prostate cancer antigen 3 (PCA3) significantly improve prostate cancer detection at initial biopsy in a total PSA range of 2-10 ng/ml. *PLoS One*. 2013;**8**(7):1-7
- [38] Stephan C, Vincendeau S, Houlgatte A, Cammann H, Jung K, Semjonow A. Multicenter evaluation of [-2]proprostate-specific antigen and the prostate health index for detecting prostate cancer. *Clinical Chemistry [Internet]*. 2013 Jan 1;**59**(1):306-314. Available from: <http://clinchem.aaccjnl.org/content/59/1/306.abstract>
- [39] Vickers AJ, Till C, Tangen CM, Lilja H, Thompson IM. An empirical evaluation of guidelines on prostate-specific antigen velocity in prostate cancer detection. *Journal of the National Cancer Institute*. 2011;**103**(6):462-469
- [40] Punnen S, Pavan N, Parekh DJ. Finding the wolf in sheep's clothing: The 4Kscore is a novel blood test that can accurately identify the risk of aggressive prostate cancer. *Revista de Urología*. 2015;**17**(1):3-13
- [41] Stattin P, Vickers AJ, Sjöberg DD, Johansson R, Granfors T, Johansson M, et al. Improving the specificity of screening for lethal prostate cancer using prostate-specific antigen and a panel of Kallikrein markers: A nested case-control study. *European Urology*. 2015 Aug;**68**(2):207-213
- [42] Eklund M, Nordström T, Aly M, Adolfsson J, Wiklund P, Brandberg Y, et al. The Stockholm-3 (STHLM3) model can improve prostate cancer diagnostics in men aged 50-69 yr compared with current prostate cancer testing. *European Urology Focus*. 2016;**229**:1-4
- [43] Grönberg H, Adolfsson J, Aly M, Nordström T, Wiklund P, Brandberg Y, et al. Prostate cancer screening in men aged 50-69 years (STHLM3): A prospective population-based diagnostic study. *The Lancet Oncology*. 2015;**16**(16):1667-1676

- [44] Ström P, Nordström T, Grönberg H, Eklund M. The Stockholm-3 model for prostate cancer detection: Algorithm update, biomarker contribution, and reflex test potential. *European Urology*. 2018;**76**:1-7
- [45] Rieter WJ, Keane TE, Ahlman MA, Ellis CT, Spicer KM, Gordon LL. Diagnostic performance of In-111 capromab pendetide SPECT/CT in localized and metastatic prostate cancer. *Clinical Nuclear Medicine*. 2011;**36**(10):872-878
- [46] Gutman AB, Gutman EB. An “acid” phosphatase occurring in the serum of patients with metastasizing carcinoma of the prostate gland. *The Journal of Clinical Investigation*. 1938;**17**(4):473-478
- [47] Huber ML, Haynes L, Parker C, Iversen P. Interdisciplinary critique of Sipuleucel-T as immunotherapy in castration-resistant prostate cancer. *Journal of the National Cancer Institute*. 2012;**104**:273-279
- [48] Gerritsen WR. The evolving role of immunotherapy in prostate cancer. *Annals of Oncology*. 2012;**23**(Suppl 8):viii22-vvii27
- [49] Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *The New England Journal of Medicine*. 2010;**363**(5):411-422
- [50] Antonarakis ES, Lu C, Wang H, Lubner B, Nakazawa M, Roeser JC, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *The New England Journal of Medicine*. 2014;**371**(11):1028-1038
- [51] Antonarakis ES, Lu C, Lubner B, Wang H, Chen Y, Zhu Y, et al. Clinical significance of androgen receptor splice variant-7 mRNA detection in circulating tumor cells of men with metastatic castration-resistant prostate cancer treated with first- and second-line abiraterone and enzalutamide. *Journal of Clinical Oncology*. 2017;**35**(19):2149-2156
- [52] Slaughter DP, Southwick HW, Smejkal W. “Field cancerization” in oral stratified squamous epithelium. Clinical implications of multicentric origin. *Cancer*. 1953;**6**(5):963-968
- [53] Braakhuis BJM, Tabor MP, Kummer JA, Leemans CR, Brakenhoff RH. A genetic explanation of slaughter’s concept of field cancerization. *Cancer Research*. 2003;**63**(8):1727-1730
- [54] Trujillo KA, Jones AC, Griffith JK, Bisoffi M. Markers of field cancerization: Proposed clinical applications in prostate biopsies. *Prostate Cancer*. 2012 May 14;**2012**:302894
- [55] Lee WH, Morton RA, Epstein JI, Brooks JD, Campbell PA, Bova GS, et al. Cytidine methylation of regulatory sequences near the pi-class glutathione S-transferase gene accompanies human prostatic carcinogenesis. *Proceedings of the National Academy of Sciences*. 1994;**91**(24):11733-11737
- [56] Zhou M, Tokumaru Y, Sidransky D, Epstein JI. Quantitative GSTP1 methylation levels correlate with Gleason grade and tumor volume in prostate needle biopsies. *The Journal of Urology*. 2004;**171**(6, Part 1):2195-2198
- [57] Van Neste L, Herman JG, Otto G, Bigley JW, Epstein JI, Van Criekinge W. The epigenetic promise for prostate cancer diagnosis. *The Prostate*. 2012;**72**:1248-1261

- [58] Lotan TL, Wei W, Ludkovski O, Morais CL, Guedes LB, Jamaspishvili T, et al. Analytic validation of a clinical-grade PTEN immunohistochemistry assay in prostate cancer by comparison with PTEN FISH. *Modern Pathology*. 2016;**29**(8):904-914
- [59] Lotan TL, Heumann A, Dwertmann Rico S, Hicks J, Lecksell K, Koop C, et al. PTEN loss detection in prostate cancer: Comparison of PTEN immunohistochemistry and PTEN FISH in a large retrospective prostatectomy cohort. *Oncotarget*. 2017;**2**(2):180-188
- [60] Lotan TL, Wei W, Morais CL, Hawley ST, Fazli L, Hurtado-Coll A, et al. PTEN loss as determined by clinical-grade immunohistochemistry assay is associated with worse recurrence-free survival in prostate cancer. *European Urology Focus*. 2016;**2**(2):180-188
- [61] Lotan TL, Carvalho FL, Peskoe SB, Hicks JL, Good J, Fedor HL, et al. PTEN loss is associated with upgrading of prostate cancer from biopsy to radical prostatectomy. *Modern Pathology*. 2015;**28**(1):128-137
- [62] Lotan TL, Gurel B, Sutcliffe S, Esopi D, Liu W, Xu J, et al. PTEN protein loss by immunostaining: Analytic validation and prognostic indicator for a high risk surgical cohort of prostate cancer patients. *Clinical Cancer Research*. 2011;**17**(20):6563-6573
- [63] Chaux A, Peskoe SB, Gonzalez-Roibon N, Schultz L, Albadine R, Hicks J, et al. Loss of PTEN expression is associated with increased risk of recurrence after prostatectomy for clinically localized prostate cancer. *Modern Pathology*. 2012;**25**(11):1-7
- [64] Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun X-W, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science*. 2005;**310**(5748):644-648
- [65] Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate Cancer. *Cell*. 2015;**163**(4):1011-1025
- [66] Tomlins SA, Bjartell A, Chinnaiyan AM, Jenster G, Nam RK, Rubin MA, et al. ETS gene fusions in prostate cancer: From discovery to daily clinical practice. *European Urology*. 2009;**56**:275-286
- [67] Zong Y, Xin L, Goldstein AS, Lawson DA, Teitell MA, Witte ON. ETS family transcription factors collaborate with alternative signaling pathways to induce carcinoma from adult murine prostate cells. *Proceedings of the National Academy of Sciences*. 2009;**106**(30):12465-12470
- [68] Rostad K, Hellwinkel OJC, Haukaas SA, Halvorsen OJ, Øyan AM, Haese A, et al. TMPRSS2:ERG fusion transcripts in urine from prostate cancer patients correlate with a less favorable prognosis. *APMIS*. 2009;**117**(8):575-582
- [69] King JC, Xu J, Wongvipat J, Hieronymus H, Carver BS, Leung DH, et al. Cooperativity of TMPRSS2-ERG with PI3-kinase pathway activation in prostate oncogenesis. *Nature Genetics*. 2009;**41**(5):524-526
- [70] Carver BS, Tran J, Gopalan A, Chen Z, Shaikh S, Carracedo A, et al. Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. *Nature Genetics*. 2009;**41**(5):619-624

- [71] Yoshimoto M, Joshua AM, Cunha IW, Coudry RA, Fonseca FP, Ludkovski O, et al. Absence of TMPRSS2:ERG fusions and PTEN losses in prostate cancer is associated with a favorable outcome. *Modern Pathology*. 2008;**21**(12):1451-1460
- [72] Klein EA, Cooperberg MR, Magi-Galluzzi C, Simko JP, Falzarano SM, Maddala T, et al. A 17-gene assay to predict prostate cancer aggressiveness in the context of Gleason grade heterogeneity, tumor multifocality, and biopsy undersampling. *European Urology*. 2014;**66**(3):550-560
- [73] Cullen J, Rosner IL, Brand TC, Zhang N, Tsiatis AC, Moncur J, et al. A biopsy-based 17-gene genomic prostate score predicts recurrence after radical prostatectomy and adverse surgical pathology in a racially diverse population of men with clinically low- and intermediate-risk prostate cancer. *European Urology*. 2015;**68**(1):123-131
- [74] Knezevic D, Goddard AD, Natraj N, Cherbavaz DB, Clark-Langone KM, Snable J, et al. Analytical validation of the oncotype DX prostate cancer assay—A clinical RT-PCR assay optimized for prostate needle biopsies. *BMC Genomics*. 2013;**14**(1):1-12
- [75] Brooks JD, Wei W, Pollack JR, West RB, Shin JH, Sunwoo JB, et al. Loss of expression of AZGP1 is associated with worse clinical outcomes in a multi-institutional radical prostatectomy cohort. *The Prostate*. 2016;**76**(15):1409-1419
- [76] Cuzick J, Stone S, Fisher G, Yang ZH, North BV, Berney DM, et al. Validation of an RNA cell cycle progression score for predicting death from prostate cancer in a conservatively managed needle biopsy cohort. *British Journal of Cancer*. 2015;**113**(3):382-389
- [77] Klein EA, Haddad Z, Yousefi K, Lam LLC, Wang Q, Choeurng V, et al. Decipher genomic classifier measured on prostate biopsy predicts metastasis risk. *Urology*. 2016;**90**:148-152
- [78] Lee HJ, Yousefi K, Haddad Z, Abdollah F, Lam LLC, Shin H, et al. Evaluation of a genomic classifier in radical prostatectomy patients with lymph node metastasis. *Research and Reports in Urology*. 2016;**8**:77-84
- [79] Knudsen BS, Kim HL, Erho N, Shin H, Alshalalfa M, Lam LLC, et al. Application of a clinical whole-transcriptome assay for staging and prognosis of prostate cancer diagnosed in needle core biopsy specimens. *The Journal of Molecular Diagnostics*. 2016;**18**(3):395-406
- [80] Blume-Jensen P, Berman DM, Rimm DL, Shipitsin M, Putzi M, Nifong TP, et al. Biology of human tumors development and clinical validation of an in situ biopsy-based multimarker assay for risk stratification in prostate cancer. *Clinical Cancer Research*. 2015;**21**(11):2591-2600
- [81] Shipitsin M, Small C, Choudhury S, Giladi E, Friedlander S, Nardone J, et al. Identification of proteomic biomarkers predicting prostate cancer aggressiveness and lethality despite biopsy-sampling error. *British Journal of Cancer*. 2014;**111**(6):1201-1212
- [82] Shipitsin M, Small C, Giladi E, Siddiqui S, Choudhury S, Hussain S, et al. Automated quantitative multiplex immunofluorescence in situ imaging identifies phospho-S6 and phospho-PRAS40 as predictive protein biomarkers for prostate cancer lethality. *Proteome Science*. 2014;**12**(1):1-13

- [83] Roth JA, Ramsey SD, Carlson JJ. Cost-effectiveness of a biopsy-based 8-protein prostate cancer prognostic assay to optimize treatment decision making in Gleason 3 + 3 and 3 + 4 early stage prostate cancer. *The Oncologist*. 2015;**20**(12):1355-1364
- [84] Signoretti S, Waltregny D, Dilks J, Isaac B, Lin D, Garraway L, et al. p63 is a prostate basal cell marker and is required for prostate development. *The American Journal of Pathology*. 2000;**157**(6):1769-1775
- [85] Dhillon PK, Barry M, Stampfer MJ, Perner S, Fiorentino M, Fornari A, et al. Aberrant cytoplasmic expression of p63 and prostate cancer mortality. *Cancer Epidemiology, Biomarkers & Prevention*. 2009;**18**(2):595-600
- [86] Rubin MA, Zhou M, Dhanasekaran SM, Varambally S, Barrette TR, Sanda MG, et al. α -Methylacyl coenzyme a racemase as a tissue biomarker for prostate cancer. *Journal of the American Medical Association*. 2002;**287**(13):1662
- [87] Luo J, Zha S, Gage WR, T a D, Hicks JL, Bennett CJ, et al. Alpha-methylacyl-CoA race-mase: A new molecular marker for prostate cancer. *Cancer Research*. 2002;**62**:2220-2226
- [88] Zhou M, Chinnaiyan AM, Kleer CG, Lucas PC, Rubin MA. Alpha-methylacyl-CoA race-mase: A novel tumor marker over-expressed in several human cancers and their precursor lesions. *The American Journal of Surgical Pathology*. 2002;**26**(7):926-931
- [89] Wu CL, Yang XJ, Tretiakova M, Patton KT, Halpern EF, Woda BA, et al. Analysis of α -methylacyl-CoA racemase (P504S) expression in high-grade prostatic intraepithelial neoplasia. *Human Pathology*. 2004;**35**(8):1008-1013
- [90] Shah RB, Kunju LP, Shen R, LeBlanc M, Zhou M, Rubin MA. Usefulness of basal cell cocktail (34 β E12 + p63) in the diagnosis of atypical prostate glandular proliferations. *American Journal of Clinical Pathology*. 2004;**122**(4):517-523
- [91] Sung M-T, Jiang Z, Montironi R, MacLennan GT, Mazzucchelli R, Cheng L. Alpha-methylacyl-CoA racemase (P504S)/34 β E12/p63 triple cocktail stain in prostatic adenocarcinoma after hormonal therapy. *Human Pathology*. 2007;**38**(2):332-341
- [92] Bussemakers MJG, Van Bokhoven A, Verhaegh GW, Smit FP, Karthaus HFM, Schalken JA, et al. DD3: A new prostate-specific gene, highly overexpressed in prostate cancer. *Cancer Research*. 1999;**59**(23):5975-5979
- [93] Gittelman MC, Hertzman B, Bailen J, Williams T, Koziol I, Henderson RJ, et al. PCA3 molecular urine test as a predictor of repeat prostate biopsy outcome in men with previous negative biopsies: A prospective multicenter clinical study. *The Journal of Urology*. 2013;**190**(1):64-69
- [94] Aubin SMJ, Reid J, Sarno MJ, Blase A, Aussie J, Rittenhouse H, et al. PCA3 molecular urine test for predicting repeat prostate biopsy outcome in populations at risk: Validation in the placebo arm of the Dutasteride REDUCE trial. *The Journal of Urology*. 2018 Apr 15;**184**(5):1947-1952
- [95] Marks LS, Fradet Y, Lim Deras I, Blase A, Mathis J, Aubin SMJ, et al. PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy. *Urology*. 2007;**69**(3):532-535

- [96] Haese A, de la Taille A, van Poppel H, Marberger M, Stenzl A, Mulders PFA, et al. Clinical utility of the PCA3 urine assay in European men scheduled for repeat biopsy. *European Urology*. 2008;**54**(5):1081-1088
- [97] De La Taille A, Irani J, Graefen M, Chun F, De Reijke T, Kil P, et al. Clinical evaluation of the PCA3 assay in guiding initial biopsy decisions. *The Journal of Urology*. 2011;**185**(6):2119-2125
- [98] Chevli KK, Duff M, Walter P, Yu C, Capuder B, Elshafei A, et al. Urinary PCA3 as a predictor of prostate cancer in a cohort of 3,073 men undergoing initial prostate biopsy. *The Journal of Urology*. 2014;**191**(6):1743-1748
- [99] Van Neste L, Hendriks RJ, Dijkstra S, Trooskens G, Cornel EB, Jannink SA, et al. Detection of high-grade prostate cancer using a urinary molecular biomarker-based risk score. *European Urology*. 2016;**70**(5):740-748
- [100] Tomlins SA, Aubin SMJ, Siddiqui J, Lonigro RJ, Sefton-Miller L, Miick S, et al. Urine TMPRSS2:ERG fusion transcript stratifies prostate cancer risk in men with elevated serum PSA. *Science Translational Medicine*. 2011;**3**(94):1-24
- [101] Tomlins SA, Day JR, Lonigro RJ, Hovelson DH, Siddiqui J, Kunju LP, et al. Urine TMPRSS2:ERG plus PCA3 for individualized prostate cancer risk assessment. *European Urology*. 2016;**70**(1):45-53
- [102] Leyten GHJM, Hessels D, Jannink SA, Smit FP, de Jong H, Cornel EB, et al. Prospective multicentre evaluation of PCA3 and TMPRSS2-ERG gene fusions as diagnostic and prognostic urinary biomarkers for prostate cancer. *European Urology*. 2014;**65**(3):534-542
- [103] Mitchell PJ, Welton J, Staffurth J, Court J, Mason MD, Tabi Z, et al. Can urinary exosomes act as treatment response markers in prostate cancer? *Journal of Translational Medicine*. 2009;**7**:1-13
- [104] Nilsson J, Skog J, Nordstrand A, Baranov V, Mincheva-Nilsson L, Breakefield XO, et al. Prostate cancer-derived urine exosomes: A novel approach to biomarkers for prostate cancer. *British Journal of Cancer*. 2009;**100**(10):1603-1607
- [105] Donovan MJ, Noerholm M, Bentink S, Belzer S, Skog J, O'Neill V, et al. A molecular signature of PCA3 and ERG exosomal RNA from non-DRE urine is predictive of initial prostate biopsy result. *Prostate Cancer and Prostatic Diseases*. 2015;**18**(4):370-375
- [106] McKiernan J, Donovan MJ, O'Neill V, Bentink S, Noerholm M, Belzer S, et al. A novel urine exosome gene expression assay to predict high-grade prostate cancer at initial biopsy. *JAMA Oncology*. 2016;**2**(7):882-889
- [107] Liu F, Vermesh O, Mani V, Ge TJ, Madsen SJ, Sabour A, et al. The exosome total isolation chip. *ACS Nano*. 2017;**11**(11):10712-10723
- [108] Ahmed HU, El-Shater Bosaily A, Brown LC, Gabe R, Kaplan R, Parmar MK, et al. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): A paired validating confirmatory study. *Lancet*. 2017;**389**(10071):815-822