

Emerging Biomarkers for the Diagnosis and Prognosis of Prostate Cancer

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BACKGROUND: Early detection of prostate cancer (CaP), the most prevalent cancer and the second-leading cause of death in men, has proved difficult, and current detection methods are inadequate. Prostate-specific antigen (PSA) testing is a significant advance for early diagnosis of patients with CaP.

CONTENT: PSA is produced almost exclusively in the prostate, and abnormalities of this organ are frequently associated with increased serum concentrations. Because of PSA's lack of specificity for CaP, however, many patients undergo unnecessary biopsies or treatments for benign or latent tumors, respectively. Thus, a more specific method of CaP detection is required to augment or replace screening with PSA. The focus recently has been on creating cost-effective assays for circulating protein biomarkers in the blood, but because of the heterogeneity of CaP, it has become clear that this effort will be a formidable challenge. Each marker will require proper validation to ensure clinical utility. Although much work has been done on variations of the PSA test (i.e., velocity, density, free vs bound, proisoforms) with limited usefulness, there are many emerging markers at various stages of development that show some promise for CaP diagnosis. These markers include kallikrein-related peptidase 2 (KLK2), early prostate cancer antigen (EPCA), PCA3, hepsin, prostate stem cell antigen, and α -methylacyl-CoA racemase (AMACR). We review biomarkers under investigation for the early diagnosis and management of prostate cancer.

SUMMARY: It is hoped that the use of panels of markers can improve CaP diagnosis and prognosis and help predict the therapeutic response in CaP patients.

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Prostate cancer (CaP)⁵ is the most prevalent malignancy in men and is the second-leading cause of cancer deaths in North America. One in 6 men have a lifetime risk of a CaP diagnosis and a 3.4% chance of death due to CaP (1). Most diagnoses are currently being made in patients who have early stages of the disease and no symptoms. Because of this stage migration, the classic approaches for prognosis, such as the Partin tables and the Kattan nomograms, are no longer as effective as in the past. The focus has now moved from early detection to determining the clinical significance of these early-stage tumors. One objective is to find ways of distinguishing clinically relevant tumors that have the ability to metastasize. Currently, 30% of tumors removed by radical prostatectomy are deemed clinically insignificant and would not have required such invasive treatment. Most diagnosed cases have a latent, nonaggressive form of CaP; thus, it is important that these patients not be overtreated. Control of CaP could be achieved through early detection and selection of the appropriate treatment; however, we have yet to reach this level of diagnostic sophistication.

The biomarker currently used for CaP diagnosis is prostate-specific antigen (PSA). It is considered both the best tumor marker available for any cancer and a marker with many shortcomings. PSA was originally used for monitoring CaP patients and was subsequently implemented for screening. The discovery of PSA and its introduction into the clinic in the early 1990s has had a profound impact on the early diagnosis of CaP and has produced an increase in the documented incidence of CaP (2). PSA is currently used as a marker for diagnosis, but PSA values are now being recognized as representing the relative degree of risk for CaP. The upper limit of the reference interval set at 4 $\mu\text{g/L}$ fails to detect a large number of cancers, and

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⁵ Nonstandard abbreviations: CaP, prostate cancer; PSA, prostate-specific antigen; BPH, benign prostatic hyperplasia; fPSA, free PSA; KLK2, kallikrein-related peptidase 2; PSMA, prostate-specific membrane antigen; PCA3, prostate cancer antigen 3; AUC, area under the ROC curve; EPCA, early prostate cancer antigen; AMACR, α -methylacyl-CoA racemase; uPA, urokinase plasminogen activator; uPAR, uPA receptor; IGF, insulinlike growth factor; IGFBP, IGF-binding protein; PIN, prostatic intraepithelial neoplasia; TGF- β_1 , transforming growth factor β_1 ; PSP94, prostate secretory protein 94; CRISP-3, cysteine-rich secretory protein 3; ANXA3, annexin A3; PSCA, prostate stem cell antigen; IL-6, interleukin-6.

The Prostate Cancer Prevention Trial has concluded that there is no PSA concentration that rules out cancer (3). Measurement of total PSA has been shown to be useful as a prognostic tool, with high preoperative values being associated with advanced disease and a poor clinical outcome. The controversy surrounding the use of this marker is currently being debated, because it is unclear whether PSA screening has led to a decline in mortality due to CaP. In 2008 and 2009, 2 major randomized prospective clinical trials, the European Randomized Study of Screening for Prostate Cancer, and the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, will report on whether PSA screening reduces mortality. The relationship of PSA to tumor grade is also not clear. The tissue PSA concentration has been shown to decrease with increasing Gleason sum (4), albeit concentrations in the serum increase because of disruption of the basement membrane surrounding the prostate epithelial cells and in the overall prostate tissue architecture. PSA is not specific for CaP and can serve as a marker for benign prostatic hyperplasia (BPH) and growth in prostate volume. Key statistics for the PSA test have been shown to be inadequate, with positive predictive values of 37%, patients in the gray zone of 4–10 $\mu\text{g/L}$ having a 25% chance of harboring occult CaP (5), and 15% of men with PSA concentrations of $<4 \mu\text{g/L}$ displaying CaP (6). The inadequacies of PSA as a marker have created a need for novel markers of CaP to prevent overtreatment of indolent tumors. In addition to diagnostic markers, prognostic, predictive, and therapeutic markers are needed to act as surrogate endpoints in forecasting disease severity, choosing treatments, and monitoring responses to therapies, respectively. Guidelines for biomarker development have been established to aid in the validation of candidates (7, 8). This review focuses on upcoming biomarker candidates that show promise for the early detection and management of CaP (see Table 1).

PSA-Derived Forms

It has become clear that the operating characteristics of PSA need to be improved. One approach has been to measure PSA derivatives, including the rate of PSA change over time (PSA velocity), the ratio of PSA concentration to prostate volume (PSA density), and age-specific PSA intervals. In addition, improvements in measuring PSA and PSA-related proteins have allowed the measurement of percent free PSA (fPSA), which is the ratio of free to total PSA. One recent study in particular has shown the value of percent fPSA as a late-stage predictor of CaP (9). Other forms include complexed PSA, which is a measure of how much PSA in serum is bound to α_2 -macroglobulin,

α_1 -protease inhibitor, or α_1 -antichymotrypsin, as well as different cleavage isoforms of PSA, such as [–2]proPSA and bPSA. Several reviews on PSA have been written, and the derived forms are not discussed in this review (10).

Human Kallikrein-Related Peptidase 2

Human kallikrein-related peptidase 2 (KLK2, previously known as hK2) is a secreted serine protease from the same gene family as PSA. Data for CaP tissues have shown that KLK2 increases during CaP progression and therefore may have use as a CaP biomarker. Studies of serum have shown improvements in CaP diagnosis when KLK2 is used combination with total PSA (11) and fPSA (12), specifically with respect to extracapsular extension and tumor volume (13). KLK2 also provided improved independent prognostic information compared with PSA regarding the risk of biochemical recurrence in men with PSA values of $\leq 10 \mu\text{g/L}$ (14). Additional validation studies are required to elucidate the full prognostic potential of KLK2.

Prostate-Specific Membrane Antigen

Prostate-specific membrane antigen (PSMA) is a membrane glycoprotein that is produced in high concentrations in epithelial cells of healthy individuals and CaP patients. The relative production of PSMA was found to be increased in epithelial cells of CaP tissue. Cytogen has developed a commercial imaging test for PSMA (ProstaScint) that uses an ^{111}In -conjugated 7E11 antibody to PSMA in a radioimmunosciintigraphy assay (15). Finally, PSMA has been studied as a target for therapy through the use of antibodies conjugated to radioisotopes or toxins or by activating dendritic cells against PSMA (16). The use of PSMA has not yet been adopted into clinical practice, and its role as a diagnostic and therapeutic tool is still evolving.

Other Tissue Kallikreins

Until recently, *KLK3*⁶ (kallikrein-related peptidase 3; previously known as *PSA*), *KLK2* (kallikrein-related peptidase 2), and *KLK1* (kallikrein 1; also known as pancreatic/renal kallikrein 1) were the only genes

⁶ Human genes: *KLK3*, kallikrein-related peptidase 3; *KLK2*, kallikrein-related peptidase 2; *KLK1*, kallikrein 1; *ERG*, v-ets erythroblastosis virus E26 oncogene homolog (avian); *ETV1*, ets variant 1; *TMPS2*, transmembrane protease, serine 2; *EZH2*, enhancer of zeste homolog 2 (Drosophila); *GSTP1*, glutathione S-transferase pi 1.

Table 1. List of candidate biomarkers for prostate cancer and their possible clinical utility.

Candidate CaP biomarker	Assessed clinical utility	References
KLK2	Diagnostic and prognostic predictor of extracapsular extension, tumor volume, and biochemical recurrence	(11–14)
PSMA	Imaging marker and target for therapy	(15, 16)
KLK11	Early predictor of CaP in serum	(17, 18)
PCA3	Urinary biomarker for detecting CaP	(19–23)
EPCA/EPCA-2	Immunohistochemical detection of CaP; serum marker to differentiate local from metastatic CaP	(24–27)
AMACR	Increased detection of autoantibodies in CaP; immunohistochemical detection as a prognostic factor for biochemical recurrence and death	(28–33)
uPA/uPAR	Increased tissue and serum concentrations predict biochemical recurrence and metastasis	(34–37)
IGF-1/IGFBP	IGF-1 slightly increased in CaP serum; IGFBP concentration inversely correlated to CaP progression	(38–40)
<i>TMPRSS2:ERG/ETV1</i>	Increased detection in urine of CaP and PIN patients vs BPH patients; gene fusion present in CaP tissue by FISH ^a	(41–44)
TGF- β_1	Increased immunohistochemical and serum concentrations with CaP progression and biochemical recurrence	(45–47)
EZH2	Gene expression in CaP tissue predicts progression	(48, 49)
GSTP1	Detection of gene promoter hypermethylation in urine to assess for biopsy	(50, 51)
PSP94	Predictor of Gleason sum, surgical margin status, and biochemical recurrence after local surgery	(52)
CRISP-3	Increased immunohistochemical staining in prostate tissues of men with high-grade PIN; independent predictor of CaP recurrence	(53, 54)
Chromogranin A	Monitoring of patients with androgen-independent late-stage CaP with neuroendocrine differentiation	(55, 56)
Progastrin-releasing peptide	Monitoring of patients with metastatic CaP with neuroendocrine and androgen-independent phenotype	(57, 58)
E-cadherin	Reduced immunohistochemical expression in CaP correlated with stage and reduced survival	(59, 60)
Annexin A3	Decreased production in CaP tissues by immunohistochemistry; prognostic risk marker	(61)
PSCA	Immunohistochemical marker associated with Gleason sum and stage; target for therapy	(62, 63)
Hepsin	Immunohistochemical detection in PIN and CaP compared with BPH	(64, 65)
IL-6	Elevated serum concentrations in late-stage CaP	(66–68)

^a FISH, fluorescence in situ hybridization.

identified in the human kallikrein locus on chromosome 19. The locus is now known to span 300 kb and to consist of 15 genes that share significant homology and sequence similarity at the DNA and protein levels. In addition to KLK2, other kallikreins have shown utility as biomarkers for CaP and other diseases (17). Eight kallikreins are produced at relatively high concentrations in prostate tissue: KLKs 2–4, 10–13, and 15. Of these kallikreins, KLK11 shows promise as a serum biomarker for CaP. The use of KLK11 in combination with total PSA and percent fPSA has shown some improved ability to predict CaP (18).

Prostate Cancer Antigen 3

Also known as DD3, prostate cancer antigen 3 (PCA3), a noncoding RNA produced almost exclusively in the prostate, has been shown to be highly overproduced in CaP tissues, including metastases, compared with BPH tissue (19). Several assays can measure PCA3 mRNA in urine sediment. The only commercially available test is APTIMA[®] (Gen-Probe), which uses transcription-mediated amplification (20). A PCA3 score is derived by normalizing the PCA3 mRNA concentration to the PSA concentration. A recent large multi-institutional

study of patients undergoing biopsy that included analysis of PCA3 in voided urine after prostatic massage, used a PCA3 score cutoff of 58, and obtained an area under the ROC curve (AUC) of 0.66, compared with an AUC of 0.57 for PSA (21). In a study of 233 patients who underwent repeat biopsy after a negative biopsy result, the PCA3 score had an AUC of 0.68 and a diagnostic sensitivity and specificity of 58% and 72%, respectively (22). This test has the potential to be useful for improving the diagnostic specificity of PSA. A combination of PCA3 and 3 other urinary biomarkers (GOLPH2, SPINK1, and *TMPRSS2:ERG* gene fusion) improved the diagnostic sensitivity and specificity over PCA3 alone (23).

Early CaP Antigens

Changes in nuclear matrix proteins have been shown to be associated with carcinogenesis. Early prostate cancer antigen (EPCA) is a nuclear matrix protein that was initially detected by proteomic profiling of rat prostate tissue. It has since shown promise as a diagnostic marker for CaP. Immunohistochemical studies of CaP tissue biopsies with autoantibodies to EPCA showed increased staining relative to noncancerous samples (24). A field effect was also seen in noncancerous areas adjacent to tumor tissue and in 86% of CaP tissue; EPCA aided in identifying at-risk patients who have a negative biopsy result. A recently developed blood-based assay for EPCA showed a 92% diagnostic sensitivity and a 94% diagnostic specificity in a small cohort of 12 CaP and 34 healthy patients (25). Another study that measured the EPCA-2 protein in serum showed a 92% diagnostic specificity and a 94% sensitivity for identifying CaP and found that EPCA-2 was able to differentiate localized CaP from metastatic CaP with an AUC of 0.89 (26). Other, larger independent studies are awaited to confirm these promising data; however, methodologic deficiencies with such markers have been identified, casting doubt on their actual validity (27).

α -Methylacyl-CoA Racemase

α -Methylacyl-CoA racemase (AMACR) is an enzyme involved in the oxidative metabolism and synthesis of branched-chain fatty acids found in dairy products and red meat. Besides being strongly produced in CaP tissue, the enzyme is encoded by a gene located in a region (5p13.3) that contains polymorphisms associated with CaP. A metaanalysis of microarray data showed with high confidence that AMACR is up-regulated in CaP (28). A multi-institutional study of immunohistochemical staining of AMACR helped distinguish benign from cancerous prostate tissue with a 97% di-

agnostic sensitivity and a 92% specificity (29). In addition, decreased AMACR production has recently been shown to have prognostic value in predicting biochemical recurrence and death due to CaP (30). Circulating concentrations of AMACR mRNA in serum and urine have been measured by reverse transcription-PCR analysis (31). The concentration of the AMACR protein is low in serum, but it has been detected in urine by western blotting (32). Increased concentrations of autoantibodies to AMACR were able to distinguish CaP patients from healthy individuals in the PSA interval of 4–10 $\mu\text{g/L}$. This test showed a diagnostic sensitivity of 62% and a specificity of 72% (33). Additional studies are under way to fully elucidate the potential of AMACR as a biomarker for CaP.

Urokinase Plasminogen Activator and Receptor

The degradation of the extracellular matrix has been associated with cancer progression and the urokinase plasminogen-activation cascade has been shown to participate in this process. Plasminogen is converted to the active form, plasmin, through the activation of the serine protease urokinase plasminogen activator (uPA) and binding to the uPA receptor (uPAR). One study demonstrated increased concentrations in BPH and CaP compared with healthy individuals, albeit there was no statistically significant association with CaP (34). The detection of uPAR isoforms in combination with detection of PSA isoforms and KLK2 showed an improved ability in both univariate and multivariate models to predict biopsy outcome in patients with increased PSA concentrations (35). Increased tissue concentrations of uPAR in CaP have been associated with osteoblastic metastases as well as with advanced CaP progression (36). A recent study has shown increased serum concentrations of uPA and uPAR in CaP patients with bone metastasis (37). These studies reported preoperative plasma uPA to be a predictor of biochemical recurrence and metastatic disease, indicating the presence of distant disease at time of localized therapy. Large prospective studies are needed to elucidate the full prognostic potential of uPA and uPAR for preoperative models of disease progression and metastases.

Insulinlike Growth Factors and Binding Proteins:

Serum concentrations of insulinlike growth factors (IGFs) and their binding proteins (IGFBPs) have been found to be associated with CaP. The IGF family consists of 2 ligands (IGF-1, IGF-2), 2 receptors (IGFR-1, IGFR-2), and 6 binding proteins (IGFBPs 1–6). Increased IGF-1 and decreased IGFBP-3 concentrations have been correlated with an increased risk of develop-

ing CaP (38). Another prospective study found that the IGF-1 concentration increased slightly with CaP risk but did not outperform PSA as a marker (39); however, others have failed to reproduce these results and have found no association with CaP progression. The main IGFBP produced by the prostate, IGFBP-2, has also been reported to be increased in CaP, although the concentrations in localized tumors were inversely correlated with tumor size and CaP progression. The serum IGFBP-3 concentration has been reported to be inversely correlated with the presence of metastases to the bone, but patients with localized CaP and healthy individuals have not shown any differences (40).

***TMPRSS2:ERG* and *TMPRSS2:ETV1* Gene Fusion**

Gene rearrangements have been implicated in cancers, hematologic malignancies in particular. One such rearrangement involves the transcription-factor genes *ERG* [v-ets erythroblastosis virus E26 oncogene homolog (avian)] (21q22.2) and *ETV1* (ets variant 1) (7p21.1) and the gene encoding the membrane-anchored serine protease *TMPRSS2* [*TMPRSS2* (transmembrane protease, serine 2), located at 21q22.3]. This rearrangement was shown to occur in 80% of CaPs by cancer outlier profile analysis (41). The fusion products of these genes have been observed in 42% of CaP patients, in 20% of patients with prostatic intraepithelial neoplasia (PIN), and rarely in BPH (42). A prospective study that followed 252 men with stage T1a/b CaP for 9 years showed that the *TMPRSS2:ERG* fusion was associated more than the *TMPRSS2:ETV1* fusion with Gleason sums >7, metastatic disease, and death due to CaP (43). An isoform of the *TMPRSS2:ERG* fusion has been shown by fluorescence in situ hybridization analysis to be present in 80%–95% of CaP tissues, and this isoform could be a potential target for therapy. In addition, overproduction of SPINK1, a serine protease shown to promote tumor invasion in patients negative for *ERG* and *ETV1* rearrangements, has recently been associated with an adverse prognosis (44).

Transforming Growth Factor β_1

Transforming growth factor β_1 (TGF- β_1) is a widely acting growth factor involved in a variety of molecular processes, such as cellular differentiation, immune response, angiogenesis, and proliferation. Studies with model systems of CaP have shown a role for TGF- β_1 in CaP progression. Increased concentrations of TGF- β_1 in CaP tissue have been correlated with tumor grade and stage and with lymph node metastasis (45). An ELISA used to measure preoperative plasma concentrations of TGF- β_1 has shown TGF- β_1 to be increased in CaP patients (46) and correlated with extracapsular

extension, seminal vesicle invasion, metastasis, and biochemical recurrence (47). Thus, TGF- β_1 could prove useful as a prognostic marker for CaP.

Enhancer of Zeste Homolog 2

EZH2 [enhancer of zeste homolog 2 (*Drosophila*)] encodes a protein in the polycomb family of proteins involved with regulation of gene expression. Gene expression profiling of CaP tissues from autopsies of men who died from metastatic CaP has shown *EZH2* to be produced more in metastatic CaP than in localized CaP and BPH (48). In addition, this marker was found to outperform the preoperative PSA concentration and the Gleason score for determining CaP progression. The use of this marker in combination with E-cadherin was also shown to predict CaP recurrence after localized therapy (49). Development of a serum assay would aid in the validation of this candidate biomarker for identifying patients at risk of developing metastatic disease.

Glutathione S-Transferase π Hypermethylation

Hypermethylation of tumor suppressor genes at their promoter regions at cytosine/guanine (CpG) nucleotide islands have been implicated in CaP. Glutathione S-transferase π is an enzyme that protects DNA from free-radical damage. Reduced expression of the *GSTP1* gene (glutathione S-transferase pi 1) due to hypermethylation of the promoter has been shown consistently in CaP and has been measured in urine sediment to determine the need for biopsy (50). This assay has been improved through the application of prostatic massage before urine collection (51). Panels of genes, including *GSTP1*, have been studied in a similar manner.

Prostate Secretory Protein 94 and Binding Protein

Prostate secretory protein 94 (PSP94), also known as β -microseminoprotein, is a highly abundant protein in semen that plays a role in the regulation of cell proliferation and apoptosis. Bound forms of PSP94 exist as a complex with PSP94-binding protein. Serum concentrations of the ratio of PSP94 to free PSP94 and serum concentrations of PSP94-binding protein in CaP patients after local surgery were associated with Gleason sum, biochemical recurrence, and surgical margin status (52). Large prospective studies are still required to validate this candidate biomarker.

Cysteine-Rich Secretory Protein 3

Cysteine-rich secretory protein 3 (CRISP-3), a secreted protein produced in the male reproductive tract, is in-

involved in sperm maturation. Large amounts have been detected in seminal plasma; in addition, staining of prostate tissue has shown increased CRISP-3 staining in high-grade PIN and several CaP samples (53). The association of CRISP-3 with CaP was evaluated in conjunction with β -microseminoprotein in tissues from radical prostatectomy patients and was shown to be an independent predictor of CaP recurrence (54). CRISP-3 is an emerging tissue marker for CaP prognosis.

Markers for Neuroendocrine Differentiation

Chromogranin A, a peptide produced by the neuroendocrine cells in the prostate, is currently used for CaP diagnosis and assessing prognosis for CaP tumors that show neuroendocrine differentiation. Increased chromogranin A concentrations in serum have been correlated with androgen-independent CaP progression and a poor prognosis (55) and have been shown to precede PSA increases and to improve diagnostic specificity when combined with fPSA (56).

Progastrin-releasing peptide is a growth factor released in the neuroendocrine type of CaP. Increased concentrations have been detected in metastatic CaP and have been associated with its progression (57). The concentration of progastrin-releasing peptide has also been shown to be predictive of the androgen-independent phenotype (58). Thus, both chromogranin A and progastrin-releasing peptide may be used to monitor patients with late-stage hormone-refractory CaP that displays neuroendocrine differentiation.

E-cadherin

Cell–cell adhesion plays an important role in non-pathologic tissue architecture and carcinogenesis. E-cadherin is a cell-adhesion molecule produced in epithelial cells, and E-cadherin production by these cells has been shown to predict CaP prognosis. An immunohistochemical study showed reduced E-cadherin production in 50% of CaP tumors, whereas nonpathologic prostate tissue showed uniform production (59). E-cadherin production was further studied and correlated with grade, tumor stage, and survival. Lower E-cadherin production detected immunohistochemically was associated with a shorter survival time for CaP patients (60).

Annexin A3

Annexin A3 (ANXA3) is a calcium-binding protein and a member of the annexin family of proteins. ANXA3 has been shown to be involved with activation of the immune response, as well as with membrane

trafficking and lymphocyte migration. ANXA3 was recently studied with immunohistochemistry approaches as a promising tissue marker for CaP prognosis and found to show lower production in CaP than in BPH, PIN, and healthy tissues (61). ANXA3 was able to stratify a large group of intermediate-risk patients into high- and low-risk subgroups.

Prostate Stem Cell Antigen

Prostate stem cell antigen (PSCA) is a membrane glycoprotein with a fairly specific production in the prostate. PSCA was detected in CaP tissues by immunohistochemistry, and PSCA RNA was found in blood samples. Increased PSCA production was correlated with an increased risk of CaP, a higher Gleason score, a higher stage, and the presence of metastasis (62). PSCA has also been investigated as a target for therapy (63); however, larger validation studies are required to confirm this marker's clinical utility.

Hepsin

Hepsin, a membrane serine protease first identified in human liver from cDNA libraries, is produced at high concentrations in prostate tissue. Expression profiling studies of mRNA have shown overexpression of the hepsin gene in 90% of CaP tumors (64). In one study, immunohistochemical staining showed hepsin to be highly produced in PIN lesions of the prostate and to be preferentially produced in CaP compared with BPH (65). Further studies with serum and urine samples are required to fully elucidate hepsin's diagnostic potential.

Interleukin-6 Ligand and Receptor

Interleukin-6 (IL-6), a cytokine secreted by a variety of cell types, is involved in the immune and acute-phase responses. Increased concentrations of IL-6 and its receptor have been demonstrated in metastatic and androgen-independent CaP (66) and have been suggested as candidate markers of CaP progression (67). Studies of IL-6 in combination with TGF- β_1 for CaP diagnosis have also shown promising results (68).

Circulating Tumor-Associated DNA

Dissemination of tumor cells is a prerequisite for metastasis; thus, early detection of these cells in the circulation can be useful for assessing the prognosis of CaP patients. Tumor cells have been detected with reverse transcription–PCR, which has proved to be analytically sensitive and to be useful for increasing the diagnostic accuracy of staging and prediction of disease recurrence with markers specific to the prostate (69).

Autoantibodies

The immune system is known to elicit an autoantibody response to some antigens overproduced by tumors. Humoral responses to huntingtin-interaction protein 1, prostasomes, and AMACR have been reported (33). Through the use of phage display and protein microarrays in a new approach termed “cancer immunomics,” Wang et al. (70) were able to identify autoantibodies to peptides derived from CaP tissue. They were able to generate a 22-phage peptide array that was able to distinguish 68 CaP serum samples from 60 controls with 88.2% diagnostic specificity, 81.6% sensitivity, and an AUC of 0.93, which was superior to that for PSA (0.80). Studies are under way to further validate this detection tool in a larger cohort. A recent study by the same group applied a similar approach and then carried out a biological-network analysis to determine deregulated pathways in CaP progression (71). One concern is that the needle biopsies themselves may be eliciting an autoimmune response.

Nomograms

Nomograms are multivariable tools that combine clinical features such as tumor grade/stage and biomarkers to provide physicians with standardized patient care. They use evidence-based approaches for arriving at decisions regarding treatment at each stage of disease management. The value of a nomogram is derived from its performance characteristics and user-friendliness. Numerous nomograms have been developed for CaP, including a TGF- β_1 and IL-6 standard nomogram for biochemical recurrence (72) as well as nomograms for predicting the outcome of biopsy (73). A review by Karakiewicz and Hutterer summarizes nomograms that have been developed for CaP (74).

Multivariable Tests/Artificial Neural Networks

Combinations of biomarkers have been used widely to improve disease prediction for many different disease states. Heterogeneity exists among individuals, and disease states will therefore differ among these individuals in their biology. Thus, the use of multivariable tests will most likely be more applicable for population screening than a single marker. Recently, Parekh et al. (75) used a 54-protein biomarker panel that included adipokines, metalloproteinases, adhesion molecules, and growth factors. They used age-matched controls and measured prediagnostic serum concentrations of patients who later received CaP diagnoses. These investigators' results did not prove that the marker panel was able to outperform the risk factors from the Prostate Cancer Prevention Trial calculator. Artificial neu-

ral networks have been used to model complex relationships between variables and to identify data patterns. Stephan et al. have used the artificial neural network approach to assess various combinations of kallikrein biomarkers for their clinical utility in CaP diagnosis (76).

Proteomic Patterns

High-throughput proteomic analysis of biological fluids, tissues, and cell lines has recently become a popular approach for the identification of novel biomarkers. In particular, SELDI-TOF mass spectrometry has frequently been applied to profile biological samples. With respect to CaP, Adam et al. (77) used a decision-tree algorithm to identify a peak fingerprint capable of distinguishing CaP patients from healthy individuals with a diagnostic sensitivity and specificity of 83% and 97%, respectively. Petricoin et al. (78) used 266 serum samples from CaP patients and control individuals to achieve a 95% diagnostic sensitivity and a 78% specificity. Qu et al. (79) used a boosted decision-tree algorithm to analyze their SELDI-TOF data and were able to achieve a 97% diagnostic sensitivity and a 97% specificity. Other studies have also used proteomic profiling for CaP diagnosis, further demonstrating the usefulness of the approach (80). The use of proteomic-pattern fingerprinting has come under scrutiny, however, and the National Cancer Institute and the Early Detection Research Network have conducted a multi-institutional study to objectively validate this approach, the results of which were recently published (81). Although stage 1 of the validation confirmed the analytical reproducibility of the approach, stage 2 was unable to determine if it could predict CaP in a case-control series across institutions. The cause of this failure has been attributed to preanalytical, analytical, and bioinformatics biases, as previously described in the literature (82).

In summary, the introduction of PSA testing revolutionized how CaP is diagnosed and managed; however, controversy exists regarding both the utility of PSA screening for reducing CaP mortality and the risks associated with CaP overdiagnosis. Thus, novel markers are required to improve on the specificity of PSA testing. Evidence is pointing to the use of multiple markers to fully characterize the heterogeneity of prostate tumor phenotypes across the male population. The use of multiple markers in combination with clinical and demographic data will aid in predicting patients who are at risk for developing CaP and for assessing their prognoses. Novel technology platforms being used in the discovery of novel CaP markers will aid in the search for new markers; however, the use of appropriate study designs and clinical-data analyses are

key factors to obtain results that are unbiased and reproducible.

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