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Biomarkers for Prostate Cancer

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ABSTRACT

The detection of prostate cancer using a blood test has by many standards changed the face of the disease. Despite this tremendous success, there are limitations attributed to the use of prostate specific antigen (PSA) as a means to screen and detect prostate cancer. PSA, as its name implies, is not specific for prostate cancer and as such is often found elevated in other prostatic diseases/symptoms associated with the aging male. Clearly, more specific marker(s) that could identify which individuals actually have prostate cancer and differentiate them from those without the disease would be of tremendous value. The search for more accurate and clinically useful biomarkers of prostate cancer has been extensive. This has focused on individual markers, as well as groups of markers. Included among these are PSA isoforms, pathological indicators and stains, nucleic acids and others. This article highlights the discovery of PSA as a first blood-based biomarker for prostate cancer detection, as well as other molecular biomarkers and their potential application in detection of the disease. J. Cell. Biochem. 108: 3–9, 2009.

KEY WORDS: PROSTATE CANCER; BIOMARKERS; PSA

In 2009, the American Cancer Society estimates that there will be 192,280 new cases and 27,360 related deaths [ACS, 2009] for prostate cancer. As recently as 20 years ago, more than half of the men representing with prostate cancer had metastatic disease on initial observations. Today, this is a rarity. Prostate cancer is perhaps the only cancer type in which biomarkers have changed the course of the disease. Prostate cancer is often suspected during a routine check-up where an elevated level of prostate specific antigen (PSA) in the blood test is detected, and/or an abnormality noted on digital rectal examination (DRE), which typically accompanies the blood test. In 1986, PSA testing was introduced after a reported increase of prostate cancer incidence in the United States [Ferro et al., 1987]. The PSA test was originally approved by the US Food and Drug Administration (FDA) to aid in the care of patients who already had been diagnosed with prostate cancer. Since then, the PSA test has been used for prostate cancer detection, and in 1994, the FDA approved the use of PSA as the first blood test for prostate cancer screening [Constantinou and Feneley, 2006]. It is estimated that over 25 million PSA tests are performed in the US every year [Constantinou and Feneley, 2006].

In 1990, an upper threshold of normal PSA level was established at 4.0 ng/ml [Cooner et al., 1990]. Utilizing this cut-off along with DRE increased the likelihood of prostate cancer detection. Nevertheless, the use of this cut-point has continuously been evaluated. There is a growing concern that a number of men with prostate cancer actually have PSA values lower than 4.0 ng/ml. Similarly, a PSA level above 4.0 ng/ml does not always indicate the presence of prostate cancer but can be associated with other prostate conditions such as benign prostatic hyperplasia (BPH), inflammation and prostatitis. Without any means to discriminate which men with "elevated" PSA levels have prostate cancer, a large number of apparently unnecessary biopsies have been performed. A study by Potter and Partin reported that in 1998, 15% of 15 million men screened by the PSA test had PSA levels higher than the established cut-off and therefore underwent biopsies [Potter and Partin, 1999].

A study performed by Thompson et al. [2004] on 2,950 men enrolled in the Prostate Cancer Prevention Trial (PCPT) revealed that 26.9% of men with PSA levels between 3.1 and 4.0 ng/ml had prostate cancer. Furthermore, 17% of men with PSA levels between 1.1 and 2.0 ng/ml had the disease. These studies have raised the question if there should be any cut-off used. Most contemporary studies have suggested that a PSA level of 2.5 ng/ml should be the upper threshold for the normal range [Catalona et al., 1997; Punglia et al., 2003; Thompson et al., 2004, 2005].

In over two decades since its introduction, the major limitation of PSA has been its specificity in detecting men with prostate cancer.

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More than 75% of men who undergo biopsies as a result of their PSA levels being in the range of 4.0–10.0 ng/ml turn out to be cancer-free [Barry, 2001]. Another challenge for PSA is its inability to differentiate prostate cancer from other prostatic symptoms such as BPH and prostatitis. Several concepts have been proposed to improve the use of PSA as a tumor marker. Our group has recently reviewed the use of age-adjusted PSA, analyses of PSA isoforms (free and bound/complex PSA), PSA kinetics (doubling time and velocity), as well as PSA density in improving the specificity of PSA [Leman and Getzenberg, 2007]. Of these concepts, the PSA velocity and free/total PSA ratio seem to be the most promising. The free/total PSA ratio appears to be able to be applied in conjunction with total PSA level to provide additional indication of the existence of clinically pertinent prostate cancer. In addition, PSA doubling time may serve as an indicator of mortality after radical prostatectomy [Carter et al., 1992]. Both concepts seem to be promising in terms of improving the standard PSA test and are in the process of being applied clinically.

To date, there is still a considerable amount of both clinical and basic science research effort devoted to define the precise factors that will maximize the sensitivity and specificity of PSA testing. With the ongoing research efforts to optimize the utilization of PSA, there have been an increasing number of efforts to identify novel biomarkers for prostate cancer. Advancements in both genomic and proteomic technologies have resulted in biomarker discovery as a focus in prostate cancer research. Many of these biomarkers are currently being tested as either detection or prognostic markers for prostate cancer. The primary goal of biomarker discovery is to develop a highly specific marker (or a panel of biomarkers) that can reduce the false positive tests and unnecessary biopsies for prostate cancer, as well as to produce markers that will provide additional value to the clinical and pathological factors of the disease. In this review, we will highlight the discovery of molecular markers for prostate cancer in recent years, as well as their potential application in detection of the disease.

BIOMARKERS FOR PROSTATE CANCER DETECTION

Although the utilization of PSA as a serum marker in clinical practice has improved the care of men with prostate cancer, the most documented limitation of PSA is its lack of specificity. Clearly, there is a need to identify and characterize additional biomarkers for prostate cancer detection in order to more precisely determine who should undergo a prostate biopsy. Advances in biotechnology have allowed many researchers to identify additional prostate cancer biomarkers to improve the diagnosis and treatment of this disease. Emerging technologies such as genomics and proteomics have been used extensively to characterize novel biomarkers for prostate cancer. In this section, we will examine several molecular biomarkers and their potential application for prostate cancer detection.

ALPHA-METHYLACYL COENZYME A RACEMACE (AMACR)

A number of genes that appear to be involved in prostate cancer have been reported using genomic analyses. AMACR was identified via subtractive hybridization and microarray studies [Dhanasekaran et al., 2001; Luo et al., 2001; Welsh et al., 2001]. This protein is an enzyme that is involved in peroximal beta oxidation of branched fatty acids [Ferdinandusse et al., 2000; Kotti et al., 2000]. AMACR has been shown to be upregulated in prostate cancer tissues by approximately ninefold in comparison to normal tissues [Luo et al., 2002]. A study by Rubin et al. [2002] using tissue microarrays containing 342 samples with different stages of prostate cancer demonstrated that tissue AMACR protein expression is 97% sensitive and 100% specific in detecting prostate cancer. Using serum samples from 109 patients, the same group further demonstrate that AMACR is more sensitive and specific than PSA (sensitivity and specificity of 78% and 81% vs. 46% and 50%, respectively) in differentiating prostate cancer from the control subjects [Sreekumar et al., 2004]. Additional studies have also shown that AMACR mRNAs were detected in urine samples following prostatic massage or biopsies. When this transcript was normalized to PSA mRNA, this combination resulted in the differentiation of low from high risk prostate cancer patients [Zielie et al., 2004]. Immunohistochemical analysis using samples from 204 men treated by radical prostatectomy and 188 men followed expectantly showed that AMACR tissue expression was lower in patients with poorer outcome, independent of the clinical variables [Rubin et al., 2005]. Among those with low AMACR expression and high Gleason score, the risk of prostate cancer death was 18-fold higher. This study suggests that AMACR can be used as a marker to identify aggressive prostate cancer, and is currently being used clinically.

HEPSIN

Hepsin is a transmembrane serine protease that is found to be over-expressed in prostate cancer when compared to normal and benign hyperplastic prostate tissues [Chen et al., 2003]. Analysis of tissue microarrays from over 700 clinically stratified prostate cancer specimens demonstrates that hepsin expression correlates significantly with measures of clinical outcome [Dhanasekaran et al., 2001]. Other studies using 90 matched prostate tissue samples from the tumor and non-tumor sections of the same tissue samples show that hepsin was over-expressed in 90% of the samples [Stephan et al., 2004]. In addition, 53% of the samples show 10-fold higher expression of hepsin in the tumor section.

EARLY PROSTATE CANCER ANTIGEN (EPCA)

Utilizing a focused proteomic approach, a series of novel prostate cancer associated biomarkers has been identified. One of the hallmarks of the cancer cell is alterations in the shape, size, and morphometry of the nucleus. Since nuclear changes are one of the key features the pathologist uses to identify cancer cells, the goal was to find something at the molecular level that would equal what the pathologist was seeing under the microscope. One such change in nuclear matrix proteins is termed EPCA. EPCA has been demonstrated to be expressed in prostate cancer tissues. Immunohistochemical analyses reveal that EPCA is expressed throughout the prostate and represents a ''field effect'' associated with prostate cancer [Dhir et al., 2004; Uetsuki et al., 2005]. Using tissues from negative biopsies, subsequent biopsies and prostatectomy speci-

mens, sensitivity of the EPCA immunohistochemical analysis is 84% with a specificity of 85% [Dhir et al., 2004]. The expression of EPCA in the ''negative biopsies'' of men can help reveal if prostate cancer is localized or non-confined disease. Furthermore, this test could also serve as an adjunct to the current diagnostic approach to patients who undergo prostate needle biopsies and could identify men with prostate cancer as much as 5 or more years earlier than the current diagnostic approach.

The EPCA immunohistochemical analysis was further validated by a separate group [Uetsuki et al., 2005]. In this study, EPCA staining was positive in 94% of prostate cancer tissues and it was negative in bladder cancer tissues. There was no correlation of EPCA staining intensity with Gleason scores or stage [Uetsuki et al., 2005]. In non-cancerous tissues adjacent to major cancer foci, EPCA staining was positive in 86% of prostate cancer [Uetsuki et al., 2005]. These studies suggest that EPCA may reflect alterations in the nuclear structure that occur in the earlier stage of prostate cancer. Recently, EPCA expression was evaluated by immunohistochemistry on benign biopsies from 98 patients [Hansel et al., 2007]. Biopsies were obtained from 4 groups that included 39 patients with first time negative biopsy, 24 patients with persistently negative biopsies, 8 patients with initially negative biopsies who were subsequently diagnosed with prostate carcinoma and negative biopsies obtained from 27 cases where other concurrent biopsies contained prostate carcinoma. A higher proportion of EPCA expression was found in initial negative prostate biopsy of patients who were diagnosed with prostate carcinoma on subsequent follow-up biopsies. A relatively high proportion of EPCA positivity (59%) was found in the group with first time negative biopsies and a potential 41% rate of false-negative EPCA staining was found in benign biopsies from cases with documented prostate carcinoma on concurrent cores. Although this study supports the previous findings that EPCA may be used as a tissue diagnostic marker, additional studies on the antigenic properties of EPCA in archival material are still required to further delineate the usefulness of EPCA immunostaining on biopsy material.

EARLY PROSTATE CANCER ANTIGEN-2 (EPCA-2)

Although unrelated to EPCA with the exception of its name and the technique used to identify it, EPCA-2 is a more recently studied marker associated with prostate cancer. Unlike EPCA, EPCA-2 is not associated with a ''field effect'' and appears only in the prostate cancer tissue. Antibodies have been raised which recognize three distinct epitopes of this protein: EPCA-2.19, 2.22, and 2.4. The antibodies against each of the three epitopes of the EPCA-2 protein have been utilized to develop immunoassays, and these assays were used to examine serum samples from control population as well as men with prostate cancer, prostatitis, and BPH.

The initial studies have demonstrated that an assay which detects one of the EPCA-2 epitopes, EPCA-2.22 in the serum can serve as a highly sensitive and specific test for prostate cancer. This study consisted of groups of men that had normal PSA levels, as well as a group of men had elevated PSA levels, but repeated negative biopsies indicating that they did not have prostate cancer within their prostates. Also included were sets of men with BPH, as well as those with prostatitis. Finally, samples obtained from individuals with a large number of other types of conditions were also examined to determine whether EPCA-2 was indeed found principally in prostate cancer versus other cancer types or disease states. Utilizing a prospectively defined cut-off, the EPCA-2 assay demonstrated that the marker is highly specific for prostate cancer. In this study, the specificity across all of the populations was 97%. Despite this high level of specificity, EPCA-2 detected 94% of the prostate cancers. Furthermore, there was differentiation between the prostate cancers that at the time of surgery were contained within the prostate from the cancers that had escaped the prostate at surgery (non-organ confined). The ROC analysis revealed an area under the curve of 0.89 indicating that EPCA-2 is highly accurate at differentiating between organ-confined and non-organ confined disease [Leman et al., 2007]. Optimization was able to be performed to lower the background of the assay resulting in a cut-off in the range of 1 ng/ml.

As a component of our validation of this marker, an initial evaluation of an assay that detects a distinct epitope of the same protein: EPCA-2.19 was performed. In this study, similar groups of serum samples as were previously studied for EPCA-2.22 were utilized. These samples were from men with PSA values $<$ and $>$2.5 ng/ml who had negative biopsies, men with BPH, men with organ-confined and non-organ confined prostate cancer, as well as control populations. At a cut-off of 0.5 ng/ml and above, EPCA-2.19 has a specificity of 94% and a sensitivity of 91% in separating the control men from those with prostate cancer. This cut-off was similar to that obtained from the EPCA-2.22 epitope. Receiver Operator Curve analyses of the EPCA-2.19 assay demonstrate an area under the curve of 0.982 [Leman et al., 2009]. While the data regarding serum-based EPCA-2 appear to be encouraging, the assay needs to be validated in larger multi-center studies. It also needs to be converted into an assay format that is more appropriate for clinical settings.

PROTEOMIC PATTERNS

A number of years ago, the ''holy grail'' of cancer biomarkers was to develop protein signatures of the disease. Utilizing proteomic patterns that could serve as fingerprints that would not only identify the presence of cancers but also to subclassify patients into groups that may have different prognoses or potential responses to therapy. The application of sensitive tools in the study of molecular biology is becoming essential for the discovery of novel tumor markers. Identifying unique genetic expression in cancer states compared with normal tissues will provide not only insight into the molecular etiology of disease but also novel methods for detection. In addition to identifying genes that are either on or off, identification of post-translational events that are unique to disease states will undoubtedly become invaluable in the discovery of cancer biomarkers. The identification of biomarkers from human serum and body fluids has been assessed by a variety of proteomic technologies. Much of the approach to develop such fingerprints came from the use of mass spectrometric (MS) analysis of blood and/or urine samples. The early MS studies utilized large machines with relatively low throughput. In order to increase the ability of this approach to analyze larger numbers of samples more rapidly, an approach known as SELDI was developed. A large number of these

instruments were placed in centers around the world. SELDI separates relatively small fragments of proteins (peptides) and uses these patterns to distinguish those with cancer from those with benign diseases [Malik et al., 2007]. Although in principal this approach is sound, it suffers from several significant drawbacks. These include the lack of ability to define the individual protein components and therefore the inability to produce more typical antibody-based tests. Secondly, while at single institutions, the instruments performed in a reasonably reproducible fashion, the design of the instrument was such that no two instruments performed with sufficiently high levels of concordance. After extensive investigation, it was concluded that SELDI-based approaches to analyzing serum samples are not a reliable tool with which to diagnose prostate cancer, at least at this time [McLerran et al., 2008].

In order to address these limitations, MS approaches have been developed that reveal the identities of the components. Prior to applying these approaches a significant amount of attention has been spent on standardizing protocols by which tissue, blood, and urine samples are collected. The apparently subtle differences in collection techniques have been shown to result in somewhat major and therefore confounding differences in proteomic patterns [Diaz et al., 2008]. Much advance has been made towards this end and the more recent findings have been demonstrative of the ability to distinguish diseased and normal populations. These include the use of 2D-DIGE as a means to identify serum markers for the differentiation of more aggressive prostate cancer [Byrne et al., 2009]. Similar studies have been performed in urine samples to develop a ''urine proteome'' for the identification of prostate cancer [M'Koma et al., 2007]. The idea of a fingerprint for the detection and classification of prostate cancer is still one that holds much promise but as of yet, needs further investigation and validation.

ENDOGLIN

Endoglin is a transmembrane glycoprotein that is otherwise known as CD105. While the expression of this protein is not prostate restrictive or even prostate cancer selective, it has been shown by a number of groups to be expressed in by vascular endothelial cells and therefore found to be elevated in cancerous states. Investigators have examined the ability to detect endoglin in the plasma as well as the urine of men with prostate cancer. When examining the plasma levels of the protein, the investigators demonstrated that with approximately 3 years of follow-up, preoperative endoglin levels correlate with an increased risk for biochemical progression of prostate cancer [Svatek et al., 2008]. Similarly, when endoglin is used in combination with several other blood-based biomarkers, they are able to provide further discrimination regarding the risk for biochemical recurrence [Svatek et al., 2009]. Endoglin is also expressed in the urine of men with prostate cancer in comparison to those that are biopsy negative after each group received a DRE. These urinary levels correlate with tumor volume and appear to be more accurate than PSA in the discrimination of biopsy outcome [Fujita et al., 2009]. These same investigators also examined the serum levels of endoglin and demonstrated that while they were not predictive of a diagnosis of prostate cancer they were able to distinguish between organ-confined and non-organ confined disease. These initial studies have been intriguing and certainly will need to be validated in multi-institutional trials.

PCA–3

Utilizing differential display and Northern blot analysis to compare normal and prostate cancer tissue, the DD3 prostate-specific gene was identified on chromosome 9q21-22 [Bussemakers et al., 1999]. Study of this gene has determined that it may function as non-coding RNA as it has been found to be alternatively spliced, contains a high density of stop codons, and lacks an open reading frame. Expression of the *DD3PCA-3* protein has been localized to prostatic tissue and has been found in 95% of prostate cancer and prostate metastasis specimens. A real-time quantitative reverse transcriptase PCR (QRT-PCR) assay for DD3 and showed 66-fold upregulation of this protein in cancerous tissues compared with normal control tissues [de Kok et al., 2002]. Furthermore, DD3 was detected from specimens containing as little as 10% cancer, indicating that this test was capable of finding cancer within a large background of normal cells. Utilizing this discovery, a test was developed to detect DD3 in urine specimens from men following prostate massage and biopsy [Hessels et al., 2003]. Using QRT-PCR to analyze the urine from these men undergoing biopsy for serum PSA greater than 3 ng/mL, the test demonstrated 67% sensitivity and 83% specificity for men diagnosed with cancer after confirmatory biopsy. As also reported, a negative predictive value of 90% supports the potential of the test as a modality to reduce the number of invasive diagnostic procedures such as TRUS biopsy. Several clinical studies and further assay development is currently under way for DD3/PCA-3.

This urine-based biomarker has been investigated extensively in the recent years. After its identification in the prostate cancer tissue, investigators have been able to demonstrate its detection using RT-PCR within the urine of men with prostate cancer. To enhance the sensitivity of such assays, urine samples have been collected after an ''attentive'' DRE which conceptually will loosen and shed the cells within the prostate and therefore enhance the chance of detection. Utilizing this approach, urine-based assays have been developed that can detect prostate cancer [Tinzl et al., 2004]. Several clinical studies have been performed which evaluate the utility of PCA-3 to serve as a prostate cancer biomarker. Some studies have focused on the utility of this biomarker in the setting of individuals that have undergone at least one biopsy of their prostate for the determination of prostate cancer [van Gils et al., 2008]. PCA-3 has been shown to have some ability to discriminate among those that would benefit from a second biopsy in that these individuals ended up with prostate cancer on their subsequent biopsy [Deras et al., 2008]. PCA-3 has also been reported to be associated with Gleason score of men with the disease, where higher levels of the message appear to correlate with Gleason score. PCA-3 levels are reported as a ratio of PCA-3 message over PSA message within the urine. The levels of PSA message are therefore utilized as a means to normalize these levels. In a recent report, it has interestingly been noted that urine levels of PCA-3 do not appear to be elevated with increasing prostate size but do correlate with tumor volume [Whitman et al., 2008]. While this provides support for the concept that enlarged prostates would not by themselves result in higher PCA-3 levels, it is somewhat surprising in that since PSA message is used as in the

normalization factor to evaluate PCA-3 levels, in men with enlarged prostates, one would expect to have an elevated level of PSA message within the urine as a result of the increase in cells within the prostate along with a breakdown of architecture would result in more PSA message being released into the urine an therefore an actual decline in urine-based PCA-3 message. The investigators actually did not note any change in PCA-3 levels and do not provide an explanation as to why this is the case [Whitman et al., 2008].

Recently, the PCA-3 diagnostic test has been standardized for whole urine, in an easy to use platform retaining the 69% sensitivity and 79% specificity (AUC-ROC 0.746) in men undergoing prostate biopsy [Groskopf et al., 2006]. However, the AUC-ROC was only 0.69 in a larger cohort of men [Deras et al., 2008]. The molecular assay has been further evaluated in a multicenter trial in Holland [van Gils et al., 2007] and in the United States [Sokoll et al., 2008] where the test has also performed similarly. Although these results are promising, further validation is necessary to understand fully the potential clinical utility of this test. The US FDA has not yet approved the PCA-3 diagnostic test, but several reference laboratories which run the test are available in the US.

PROSTATE BREAST OVER-EXPRESSED GENE 1 (PBOV1) OR UROC28

An et al. [2000] have cloned and characterized a novel gene *UROC28* found to be over-expressed in prostate, breast, and bladder carcinomas. This gene is on chromosome 6q23-24, and the expression product protein is measurable in serum. Early immunohistochemical studies have demonstrated differential expression and characterization between normal and cancerous tissues. This group has also identified and measured the protein UC28 in serum. Application of the antibody to a ProteinChip and SELDI analysis has provided encouraging results correctly distinguishing cancer specimens from normal and BPH specimens. This preliminary work remains under investigation.

ANNEXIN A3

Among the potential urine-based proteins that can be used as biomarkers for prostate cancer. Annexin A3 is part of a family of calcium and phospholipid binding proteins that has been shown to be altered in cancer [Wozny et al., 2007; Kollermann et al., 2008]. Recently, utilizing an immunoblot in which urine samples are blotted onto a membrane and probed for Annexin A3 utilizing an antibody, investigators were able to determine the potential clinical utility of urine-based Annexin A3 either as a stand-alone biomarker or together with PSA. In a blinded study that consisted of training and evaluation sets of 243 and 264 men respectively the investigators were able to show that the addition of Annexin A3, added to the ability of PSA to provide discrimination of these groups. Annexin A3 levels are inversely related to the presence of prostate cancer. While this marker appears to have potential utility, further studies are certainly warranted to validate this work.

NMP 48 (50.8 kDa)

Utilizing proteomic patterns based on SELDI-TOF mass spectroscopy, Hlavaty et al. [2003] discovered a unique 50.8-kDa protein. This protein was subsequently characterized as vitamin D binding protein using peptide mass fingerprinting. Utilizing preprocessed serum samples from men with prostate cancer, BPH, and no cancer, this group was able to identify correctly vitamin D binding protein in the samples from men with cancer. SELDI-TOF identified the protein in 50 of 52 (96%) cancer cases, 5 of 20 (25%) biopsy-confirmed benign cases, 3 of 10 (30%) BPH cases, and 2 of 50 (4%) normal controls. Validation studies are under way for this potential prostate cancer tumor marker.

METABOLITES

One of the characteristics of cancer cells are modifications in their metabolism. The cancer cell in general, uses a less efficient source of generating energy and therefore surviving. With the explosion of molecular biology and the large amounts of information that have been learned about cell-cycle regulation little attention has been placed upon the differences that exist within the metabolic process of the cancer cell. Utilizing a high throughput approach that utilizes chromatography combined with mass spectroscopy, a number of potentially important differences in metabolomic profiles have been observed when combing prostate cancer tissue to normal. Among these, one of the differences, sarcosine, has been demonstrated within the urine of men with prostate cancer [Sreekumar et al., 2009]. Although the average levels of urine-based sarcosine are indeed different between the men with prostate cancer an those without the disease, from the data provided, it would appear that a cut-off that separates these populations in a clinically robust fashion is unlikely. Despite this, there certainly is potential for the use of cancer metabolites to serve as markers of the disease and this is an area of active investigation.

CONCLUDING REMARKS

The discovery of PSA represents an important development in the early detection of prostate cancer. Serum PSA measurement along with DRE continue to be the most performed test recommended by most physicians for prostate cancer detection. Although the utilization of PSA as a serum marker in clinical practice has improved the care of men with prostate cancer, the most documented limitation of PSA is its lack of specificity. As described in this review article, biomarker discovery in recent years has become one of the major focuses in prostate cancer research. New technologies in both genomics and proteomics play a significant part in the area of biomarker discovery. Ongoing innovation efforts for new prostate cancer biomarkers will definitely contribute to diagnosis, prognosis, and prediction of the disease. Of these new markers, the ones that are able to detect cancer predict the outcome of disease and influence therapy options will have the most significant role in determining the future of prostate cancer oncology. With PSA being the currently "reliable" prostate cancer marker at this point, PSA test will more than likely be incorporated with any of the new biomarker (and/or a panel of biomarkers) to ultimately provide added sensitivity and specificity for detection of disease.

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