BIOMARKERS OF PROSTATE CANCER: A REVIEW

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ABSTRACT: Prostate cancer, an enigma and challenge is the most frequently diagnosed cancer in males in the United States, accounting for an estimated 186,320 new cases in 2008. Most diagnoses are currently being made in patients who have early stages of the disease and no symptoms. The focus has now moved from early detection to determining the clinical significance of these early-stage tumors. The identification of specific prostate cancer biomarkers can have a significant effect on the prognosis, diagnosis and treatment options for patients. Prostate-specific antigen (PSA) and digital rectal exams remain the hallmark assessments for screening individuals for prostate cancer. Genomic- and proteomic-based studies have led to the identification of a large number of candidate biomarkers, as well as signature patterns of multiple markers for prostate cancer diagnosis, disease progression and prediction of survival. While these candidates include the usual suspects, including oncogenes, proliferation markers and cytoskeletal proteins, there are many additional unexpected molecules involved in processes such as transcriptional repression and fatty acid metabolism. These markers can be measured in serum, tissue or urine samples. Future research should focus on validation of already existing biomarkers and the discovery of new markers to identify men with aggressive prostate cancer.

Key words: *Prostate cancer, biomarkers.*

INTRODUCTION

Prostate cancer is the most frequently diagnosed cancer in males in the United States, accounting for an estimated 186,320 new cases in 2008. (Jones *et al.*, 2008). The lifetime risk of developing prostate cancer is 1 in 6, whereas the lifetime risk of death due to metastatic prostate cancer is 1 in 30 (Thompson *et al.*, 1984). Most diagnoses are currently being made in patients who have early stages of the disease and no symptoms. The focus has now moved from early detection to determining the clinical significance of these early-stage tumors (McDavid *et al.*, 2004).

The identification of specific cancer biomarkers can have a significant effect on the prognosis, diagnosis and treatment options for patients (Anonymous, 2004). One of the recommendations of the 1995 American Urological Association Guideline Panel is that watchful waiting (surveillance or no treatment) should be offered to any man with locally confined prostate cancer (Middleton *et al.*, 1995). In parallel with this is the equipoise recognized by the ongoing prostate, lung, colorectal, and ovarian (PLCO) cancer screening trial of the National Cancer Institute that is currently following a large number of men with and without screening for prostate cancer to determine whether early detection impacts survival (Gohagan *et al.*, 1994).

In addition to both the academic and clinical struggles related to decision-making for patients with newly diagnosed disease, there has been a constant impetus for the improvement of existing biomarkers used for early detection. Before the identification of prostate-specific antigen (PSA) in the 1970s by Albin (Albin 1998), physicians had only digital rectal examination (DRE) to identify early-stage prostate cancer. Using DRE methodology, even in the context of an early-detection effort, fully two thirds of prostate cancers were extraprostatic at diagnosis (Thompson *et al.*, 1984). Currently, more than 95% of all prostate cancers are clinically locally confined and the likelihood is 97% that a prostate cancer in a man undergoing serial PSA determinations is clinically confined (Smith *et al.*, 1994). Prostate-specific antigen (PSA) and digital rectal exams remain the hallmark assessments for screening individuals for prostate cancer. The combined use of these diagnostic techniques has changed the clinical course of the disease by allowing for an earlier detection of tumors (Catalona *et al.*, 1991).

Genomic- and proteomic-based studies have led to the identification of a large number of candidate

biomarkers, as well as signature patterns of multiple markers for prostate cancer diagnosis, disease progression and prediction of survival. While these candidates include the usual suspects, including oncogenes, proliferation markers and cytoskeletal proteins, there are many additional unexpected molecules such as those involved in processes such as transcriptional repression and fatty acid metabolism. Patterns of expression serving as useful biomarkers is a new and, as yet, clinically untested concept which promises to permit a consideration of the complex milieu of cancer. Exciting as these developments are, clinical application will have to await careful validation of these candidates by independent biochemical approaches over large and diverse samples (Kumar-Sinha *et al.*, 2003).

CHARACTERISTICS OF BIOMARKERS

Biomarkers of carcinogenesis are quantifiable molecules involved in physiological or pathological events that occur between exposure to exogenous or endogenous carcinogens and the subsequent development of cancer (Kellof *et al.*, 1994). Biomarkers could be the consequence of a continuous process such as an increased cell mass or cell type, or of a discrete event, such as a genetic mutation (Srnivas *et al.*, 2001). Biomarkers of early carcinogenesis provide a means of diagnosing very early changes associated with cancer development before actual tumours or even polyps are present. In contrast to histological biomarkers, such as polyp formation or the presence of high-grade dysplasia, molecular biomarkers can often be assayed in surrogate tissue such as blood or faeces, which obviates the need for tissue biopsies. Alterations of molecular biomarkers are often detectable much earlier than histological changes, and assays of molecular biomarkers are frequently quantitative, reducing inter-observer variation.

SERUM MARKERS FOR PROSTATE CANCER

Prostate-Specific Antigen (PSA): The ''gold standard'' diagnostic marker for prostate cancer is prostate specific antigen (PSA) (McDavid *et al.*, 2004; Carter *et al.*, 2004). Although this analyte was first exploited as a tool to identify prostate cancer in tissue sections, where it is used to evaluate risk potential for the presence of malignancy (Christensson *et al.*, 1990). There is general agreement among clinicians that the PSA test has the highest predictive value for prostate cancer, that PSA screening can detect early-stage cancers possibly 10 years earlier than without the PSA test. However, all experts agree that PSA has limited specificity because benign disease, including prostatic enlargement and inflammation, can increase PSA levels.

PSA is not an ideal marker of aggressive cancer when the grade of disease is used as a surrogate marker for aggressiveness, because high-grade cancers actually produce less PSA than low-grade cancers when the levels are corrected for the cancer volume (Partin *et al.*, 1990). 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.

In an effort to make PSA more specific, so-called PSA derivatives, including PSA velocity, age-specific PSA, and PSA density have been realized. Although there has been considerable enthusiasm for these approaches, none have been shown in broad-based clinical trials to be useful in early detection. Age-specific cutoffs suffer from a lack of sensitivity, PSA density and transition zone density suffer from sampling bias, and both PSA velocity and the free/total ratio suffer from test variability (Christensson *et al.*, 1990).

Urokinase-type plasminogen activator receptor forms (uPAR): The plasminogen activation cascade has been reported to participate in degradation of the extracellular matrix during cancer progression

(Stephens *et al.*, 1999). Plasminogen is converted to the active form, plasmin, through the activation of the serine protease urokinase plasminogen activator (uPA) and binds to a specific receptor (uPAR) at the cell surface. Plasminogen enhances conversion of uPAR-bound pro-uPA to active uPA. uPA is inactivated by formation of stable complexes with various plasminogen activator inhibitors (PAI-1, PAI-2, or PAI-3/PCI), whereas plasmin, uPA, and several other proteases cleave and release uPAR from the cell surface. As a result, full-length intact uPAR and cleaved isoforms are liberated from the cell surface by several mechanisms (Stephens *et al.*, 1999).

Recently, specific in-house research immunoassays were developed by Piironen et al and facilitated quantification of the individual forms of uPAR, comprising the intact uPAR, and domains I–III (Piironen *et al.*, 2006). It was studied the ability of fPSA isoforms, uPAR fragments, and hK2 to improve prediction of biopsy outcome among 355 patients with an elevated PSA (Steuber *et al.*,

2007). As a result, uPAR fragments were significant univariate and multivariate predictors of Prostate Cancer. Increased tissue concentrations of uPAR in Prostate Cancer have been associated with osteoblastic metastases as well as with advanced Prostate Cancer progression (Miyake *et al.*, 1999). Shariat et al also recently reported that plasma levels of uPA and uPAR are higher in men with Prostate Cancer than in healthy controls and increase with disease progression (Shariat *et al.*, 2007). Although these results are exciting, an essential next step would be to quantify that adding data on urokinase levels to currently available clinical data allows more accurate diagnosis or prognosis. (Dhir *et al.*, 2004).

Early prostate cancer antigen (EPCA): 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 Prostate Cancer (Uetsuki *et al.*, 2005). Two unrelated nuclear matrix proteins were implicated to be associated with Prostate Cancer and, hence, called early prostate cancer antigen (EPCA) and EPCA-2. However, the structural composition of the corresponding target antigens detected by the EPCA or EPCA-2 antibodies still remains to be clarified.

Initial immunohistochemical studies using an antibody against EPCA revealed that biopsy specimens from men with Prostate Cancer expressed a more intense EPCA staining compared to specimens from men with no evidence of cancer (Dhir *et al.*, 2004). A field effect was also seen in non cancerous areas adjacent to tumor tissue and in 86% of Prostate Cancer tissue, and EPCA aided in identifying at-risk patients who have a negative biopsy result (Paul *et al.*, 2005). An enzyme-linked immunosorbent assay (ELISA) developed to measure blood levels of EPCA-2 using a novel anti-epitope antibody EPCA-2.22 was used to assess diagnostic cut-off points and the performance in discriminating Prostate Cancer patients from healthy controls (Leman *et al.*, 2007). Therefore, larger independently generated validation studies are urgently required to confirm whether the early promising data using EPCA-2 serum measurements could be replicated by others (Bradley *et al.*, 2005).

Prostate cancer-specific autoantibodies: The existence of autoantibodies against Prostate Cancer-specific antigens has been immunodetected in blood such as Huntington-interaction protein 1, prostasomes, and a-methyl-acyl-coenzymeA-racemase (AMACR) (Bradley *et al.*, 2005; Minelli *et al.*, 2005). Recently, Wang *et al* reported the use of a technique that combines phage display technology with protein microarrays to identify and characterize new autoantibody-binding peptides derived from Prostate Cancer tissue (Wang *et al.*, 2005).

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 Prostate Cancer. 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 developing Prostate Cancer (Chan *et al.*, 1998). Another prospective study found that the IGF-1 concentration increased slightly with Prostate Cancer risk but did not outperform PSA as a marker (Harman *et al.*, 2000); however, others have failed to reproduce these results and have found no association with Prostate Cancer progression. The main IGFBP produced by the prostate, IGFBP-2, has also been reported to be increased in Prostate Cancer, although the concentrations in localized tumors were inversely correlated with tumor size and Prostate Cancer 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 Prostate Cancer and healthy individuals have not shown any differences (Sharia *et al.*, 2002).

Human aspartyl (asparaginyl) ß-hydroxylase (HAAH): Hossein, *et al.*, have investigated the utility of human aspartyl (asparaginyl) ß-hydroxylase (HAAH) as a cancer molecular marker. HAAH has been detected by immunohistochemical staining (IHC) in a broad range of cancers including Prostate Cancer. It is highly specific having been detected by IHC in >99% of tumor specimens tested (n>1000) while absent in adjacent non-affected tissue, or in tissue samples from non-affected individuals. They have previously identified the presence of HAAH at detectable levels in the sera of cancer patients and have developed a sandwich ELISA for its detection. Preliminary results suggest that measurement of serum HAAH levels may enhance and potentially supplant PSA testing for the early detection of Prostate Cancer, and potentially reduce the number of prostate biopsies performed due to false positive screening results. (Ghanbari *et al.*, 2006).

Adiponectin: Serum levels of adiponectin were measured in patients with benign prostatic hyperplasia and prostate cancer of pT2 and pT3 stage. Adiponectin ELISA assay, immunohistochemistry, and selected metabolic and biochemical parameters measurement was

performed. Serum adiponectin levels are higher in locally advanced relative to organ-confined prostate cancer and may thus serve as an auxiliary marker providing further improvement for discrimination between pT2 and pT3 stages (Housa et al., 2008).

TISSUE MARKERS FOR PROSTATE CANCER

Human Tissue kallikrein-related peptidases: 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 identified in the human kallikrein locus on chromosome 19. In addition to KLK2, other kallikreins have shown utility as biomarkers for Prostate Cancer and other diseases (Diamandis *et al.*, 2002). 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 Prostate Cancer. The use of KLK11 in combination with total PSA and percent fPSA has shown some improved ability to predict Prostate Cancer (Stephan *et al.*, 2006).

Human kallikrein 2 (hK2 or KLK2) is an abundant ''prostate-specific'' serine protease that shares 80% amino acid sequence identity with PSA. It has predominantly a prostate-restricted expression pattern regulated by both the action of androgens and a functional androgen receptor (AR). Extensive immunologic cross-reaction between hK2 and PSA and much lower abundance (about 10_2- fold) of hK2 than PSA may contribute to the reason very few data are reported from comparisons of tissue expression between hK2 and PSA. However, it is unclear whether this can be used to justify claims that hK2 is more closely linked to the biology of Prostate Cancer than PSA. Nevertheless, recent reports using referral and screening cohorts suggest that improved discrimination of patients with or without cancer is accomplished by combining measurements of tPSA, fPSA, and hK2 compared to that of tPSA alone (Scorilas *et al.*, 2003). Similarly, hK2 levels improve the ability to predict PCa stage (Haese et al., 2001) and risk of biochemical recurrence (BCR) (Steuber *et al.*, 2006) after radical prostatectomy (RP) both in referral and screening patient cohorts.

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 Prostate Cancer patients. The relative production of PSMA was found to be increased in epithelial cells of Prostate Cancer tissue. Cytogen has developed a commercial imaging test for PSMA (ProstaScint) that uses an 111In-conjugated 7E11 antibody to PSMA in a radioimmunoscintigraphy assay (Elgamal *et al.*, 2000).

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 (Mincheff *et al.*, 2006). The use of PSMA has not yet been adopted into clinical practice and its role as a diagnostic and therapeutic tool is still evolving (Diamandis *et al.*, 2002).

α-Methylacyl-CoA Racemase (AMACR): 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 (Wanders *et al.*, 2001). Besides being strongly produced in Prostate Cancer tissue. AMACR gene polymorphisms are found to cosegregate in prostate cancer families (Zheng *et al.*, 2002).

A metaanalysis of microarray data showed with high confidence that AMACR is up regulated in Prostate Cancer (Rhodes *et al.*, 2002). A multi-institutional study of immunohistochemical staining of AMACR helped distinguish benign from cancerous prostate tissue with a 97% diagnostic sensitivity and 92% specificity (Jiang *et al.*, 2004). In addition, decreased AMACR production has recently been shown to have prognostic value in predicting biochemical recurrence and death due to Prostate Cancer (Rubin *et al.*, 2005). Circulating concentrations of AMACR mRNA in serum and urine have been measured by reverse transcription–PCR analysis (Zehentner *et al.*, 2006). Increased concentrations of autoantibodies to AMACR were able to distinguish Prostate Cancer patients from healthy individuals in the PSA interval of 4–10 _g/L. This test showed a diagnostic sensitivity of 62% and a specificity of 72% (Sreekumar *et al.*, 2004).

Transforming growth factor-β1 and interleukin6: Transforming growth factor β-1 (TGF-β1) is a pleiotropic growth factor that regulates several cellular mechanisms such as proliferation, angiogenesis, immune response, and cellular differentiation. Increased concentrations of TGF- β 1 in Prostate Cancer tissue have been correlated with tumor grade and stage and with lymph node metastasis (Shariat *et al.*, 2001). TGF-β1 can also be detected in the circulation by using a commercialized quantitative sandwich enzyme immunoassay, which does not cross-react with TGF-bβ2 and TGF-β3. In vitro and in vivo studies have shown that human Prostate Cancer expresses

both interleukin 6 (IL-6) and its receptor (IL-6R), allowing for establishment of an autocrine/paracrine loop (Lee *et al.*, 2003). Elevated circulating levels of IL-6 and soluble IL-6R have been associated with features of aggressive Prostate Cancer (Tan *et al.*, 2005).

Hyperpolarized 13C lactate, pyruvate, and alanine: A study was done by Albers *et al,* to quantify, for the first time, differences in hyperpolarized [1-(13)C] pyruvate and its metabolic products between the various histologic grades of prostate cancer using the transgenic adenocarcinoma of mouse prostate (TRAMP) model. In summary, elevated hyperpolarized lactate and potentially THC and alanine are noninvasive biomarkers of prostate cancer presence and histologic grade that could be used in future three-dimensional (13) C spectroscopic imaging studies of prostate cancer patients (Albers *et al.,* 2008).

GOLPH2

GOLPH2 is coding the 73-kDa-type II Golgi membrane antigen GOLPH2/GP73. Upregulation of GOLPH2 mRNA has been recently reported in expression array analyses of prostate cancer. As GOLPH2 protein expression in prostate tissues is currently unknown. Importantly, GOLPH2 immunohistochemistry exhibited a lower level of intratumoral heterogeneity. This recent study clearly suggests GOLPH2 as an additional ancillary positive marker for tissue-based diagnosis of prostate cancer (Kristiansen *et al.,* 2008).

TRPM8

TRPM8 has clearly defined roles as a sensor for cooling in sensory neurones. It is expressed in prostate cancer cells and is associated with the pathophysiology of these cells, tumorigenic progression and metastasis,. TRPM8 is potentially a valuable diagnostic tissue marker and prognostic indicator for the progress of prostate cancer. Further studies are needed to compare TRPM8 expression with other established prostate cancer markers in terms of accuracy and probability. The limitation of using TRPM8 as a marker is that prostate tissues are needed for the examination of TRPM8 levels. The regulation of TRPM8 expression by androgens is important in terms of both fundamental knowledge and understanding the role of TRPM8 in prostate cancer. Finally, TRPM8 may be a potential target for pharmaceutical or genetic interventions for the treatment of prostate cancer and other cancers with over-expression of TRPM8. Further experiments might include screening for the specific and potent agonists for activation of the TRPM8 channel and exploring the strategy *in vivo* (Zhang *et al.,* 2006)

URINE MARKERS FOR PROSTATE CANCER

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 Prostate Cancer tissues, including metastases, compared with BPH tissue (Hessels *et al.,* 2003). Several assays can measure PCA3 mRNA in urine sediment. The only commercially available test is APTIMA_ (Gen-Probe), which uses transcription mediated amplification (Groskopf *et al.*, 2006).

A PCA3 score is derived by normalizing the PCA3 mRNA concentration to the PSA concentration. 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 (Laxman *et al.,* 2008).

CpG Island Hypermethylation: Hypermethylation of cytosine guanine (CpG) dinucleotide islands is considered to be an initial step in prostate cancer development. A number of different candidate genes have been evaluated, and the most consistently hypermethylated in prostate cancer patients is the glutathione S-transferase pi (GSTP1) gene. This gene was analyzed initially in tissue as a marker to distinguish benign from malignant tissue. Later, GSTP1 hypermethylation was studied in urine sediment as a non-interventional test for determining the need for prostate biopsies (Gonzalg *et al.*, 2004). Two recent studies looked at a panel of 10 candidate genes (APC, DAPK, ECDH1, GSTP1, MGMT, p14 [ARF], p16, RARβ2, RASSF1a, and TIMP3) (Roupret *et al.*, 2007). CpG island hypermethylation of multiple prostate cancer specific genes has become a promising molecular marker for prostate cancer diagnosis and detection. By applying qthese techniques to readily available clinical specimens such as urine or blood the current ability to diagnosis prostate cancer may be improved (Bastian *et al.,* 2004).

CONCLUSION: The PSA era has ushered in a significant rise in the number of men diagnosed with prostate cancer and resulted in a tremendous number of biopsies performed to rule out the disease. With detection rates of 25%–40%, and more than 200,000 incident cases of prostate cancer annually, the number of biopsies performed annually approaches 1 million. PSA testing has

survived as one of the few biomarkers accepted for cancer detection, despite its obvious limitations and poor specificity. A great deal of work has been done across the world in an attempt to improve upon the specificity of PSA by developing assays for different PSA isoforms. This effort has improved our understanding of the biochemistry behind PSA and prostate cancer but has produced no dramatic improvement in the accuracy of detection. Finally, more recent molecular inquiries suggest that even if the markers just discussed do not prove sufficiently useful, other more promising ones will surely come forth. There is no doubt that we are approaching a time when the use of proper biomarkers will help detect, monitor, and manage progression of the disease, as well as assist with therapeutic decisions. Discovery and validation of novel Prostate Cancer biomarkers remains a crucial goal for the future of patient care. To accomplish this, a large concerted effort is required to advance the field of Prostate Cancer biomarkers through systematic discovery, verification, and validation.

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