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Seminar article

Beyond PSA: Utility of novel tumor markers in the setting of elevated PSA☆

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Abstract

The introduction of prostate-specific antigen (PSA) for prostate cancer screening and detection has been used for over 20 years and has dramatically changed the face of prostate cancer. Although it is a highly sensitive serum test, its routine use has been the subject of continued controversy owing to its limited specificity. Due to this lack of specificity, many have proposed modifications of PSA in an attempt to bolster the performance of this analyte. The human genome project and high throughput gene expression profiling has recently yielded several promising molecular biomarkers for prostate cancer detection beyond PSA or PSA modifications. This review will first highlight several characteristics of an ideal biomarker, then focus on select emerging biomarkers for the detection of prostate cancer. Published by Elsevier Inc.

Keywords: Prostate cancer; Tumor markers; PSA

Introduction

The introduction and use of prostate-specific antigen (PSA) for prostate cancer screening and detection has changed the face of prostate cancer, in particular as it relates to the well-recognized stage migration of disease [1,2]. PSA is now the most widely used noninvasive screening tool in solid tumors, although its routine use has been the subject of continued controversy owing to its limited specificity [3]. More recent data (e.g., PCPT) have shed light on the substantial numbers of men who harbor prostate cancer within the so-called "normal range" of PSA (e.g., PSA $<$ 4 ng/ml) [4]. Furthermore, many of these cancers in the normal PSA range are found to be high grade and thus deemed clinically significant [5].

Due to the lack of specificity, many have proposed modifications of PSA in an attempt to bolster the performance of this analyte. These modifications include PSA density [6–9], age-specific PSA ranges [10–12], free to total PSA ratios [13–18], complexed PSA [19–22], transition zone PSA density [23,24], PSA velocity [25–28], and other PSA isoforms such as proPSA [29,30]. While these modifications have shown some promise in select patient cohorts, they have inherent limitations, prompting some to call for the end of the PSA era [31].

As a result, the search for improved biomarkers of detection has recently yielded several viable candidates beyond PSA or PSA modifications. Some of these biomarkers are cancer-specific and thus will have the potential to markedly improve the specificity of prostate cancer detection. This brief review will focus on select promising biomarkers for the detection of prostate cancer. Importantly, the review will not address markers of prognosis or response to therapy, although some of the documented markers may yield potential in these areas. This review is neither meant to be an exhaustive description of the individual biomarkers nor an inclusive list of all available biomarkers, as the list of biomarkers in development and discovery is far too lengthy to enumerate and describe here.

Biomarker characteristics

There are several characteristics of an ideal biomarker that would include a disease-specific, cost-effective, mini-

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mally invasive, reproducible assay with adequate sensitivity and specificity, ideally with correlation to disease outcome. With respect to this review, we will highlight key aspects of some of these characteristics. First, the biomarker target can represent different molecular forms. For instance, some targets represent genes with increased expression in prostate cancer and low or absent expression in normal tissue. Other molecular targets might be epigenetic changes that alter transcription of tumor suppressors or other genes involved in prostate cancer progression or carcinogenesis. These epigenetic changes would include DNA hypermethylation, histone modifications, chromatin remodeling, or micro-RNA regulation. Stable gene rearrangements are yet another target biomarker in development in prostate cancer, such as the now widely recognized fusion of androgen-regulated genes and those encoding transcription factors, e.g., the TMPRSS-2:ERG fusion protein and other related gene fusions. Last, there are certainly genomic markers of risk of prostate cancer diagnosis such as polymorphisms of chromosome 8q24, however, these are biomarkers of risk and not used in the same context as biomarkers of detection.

The source of the biospecimen also merits consideration. There are various advantages and disadvantages to the traditional biospecimen source material, namely blood, urine, and tissue. While blood and urine are certainly more readily obtained for testing, they both require the biomarker of interest to gain access the source specimen. In other words, the biomarker needs to traverse the basement membrane to enter the vasculature or leak out into the urinary system. Additionally, in the event that the marker is not cancer-specific, the use of these specimen sources is open not only to contamination from other sources (e.g., normal prostate), which will certainly affect specificity, but also may have detection limit issues, which would affect sensitivity. While a tissue source certainly allows for a wealth of molecular analyses, both protein and RNA/DNA, the analyses would be hampered by the requirement for tissue acquisition, most probably from a biopsy, thus subjecting patients to invasive source sampling and also limiting the sample quantity.

Last, for the scope of this review, one has to consider the setting in which these biomarkers have been tested or will be tested in the future. As previously mentioned, the ideal biomarker would perform well both in detection and prognosis, however, comments here will be limited to diagnosis alone. Within the diagnostic arena, one must consider the performance of the proposed biomarker and whether it is based in a screening population vs. a referral population, a biomarker used as an adjunct to PSA vs. an independent biomarker, and the utility of the biomarker in the setting of a previous negative biopsy vs. at the initial biopsy. The overwhelming majority of available data in these new biomarkers have been produced from studies in the referral population and specifically as an adjunct to PSA. The remainder of this review will be framed around these specific studies.

Prostate cancer gene 3 (PCA3)

Prostate cancer gene 3 (PCA3) was first described by Bussemakers et al. in 1999 as a noncoding prostate-specific mRNA that was highly overexpressed in prostate cancer with reported low expression in normal tissue [32]. By Northern blot and polymerase chain reaction (PCR) analysis, the PCA3 transcript was expressed at a median 66-fold increase levels in prostate cancer compared with benign prostate tissue, and it was not expressed in the bladder, seminal vesicle, testis, or kidney [32]. Thus, although PCA3 is prostate-specific, it is not cancer-specific. To date, the functional mechanism by which PCA3 contributes to prostate cancer carcinogenesis or progression is unknown.

The methodology of PCA3 measurement presents some difficulty because PCA3 mRNA is not translated to a cognate protein, consequently, immunohistochemistry and enzyme-linked immunosorbent assay (ELISA) assays are impossible. Several assays have been developed that utilize various methods of PCA3 mRNA detection including nucleic acid sequence-based amplification (NASBA) [33–35], reverse transcriptase-polymerase chain reaction (RT-PCR) [36,37], and transcription mediated amplification (TMA) [38,39]. The source material for these investigations involved urine sediments from the first voided urine after digital rectal examination (DRE). The PCA3 mRNA is normalized to PSA mRNA to yield a composite PCA3 score. By varying the PCA3 score cut-point, investigators have reported significant improvements of PCA3 over PSA alone in the detection of prostate cancer. Representative data using the third generation TMA assay will be presented in this review. Complete details of this assay and methodology are presented elsewhere [38]. In brief, a digital rectal examination (DRE) is performed by applying firm pressure enough to depress the prostate surface approximately 1 cm from base to apex and from lateral to the median line for each lobe. Three strokes of each lobe are performed, then the first void of approximately 20–30 milliliters of urine is collected immediately after this “attentive” DRE. Urine is then kept on ice and processed within 4 hours by mixing with a stabilization buffer. Samples can then be stored either on cold packs overnight or stored at –70°C for up to 8 months before testing.

Deras and colleagues examined the performance of PCA3 in a multi-center prospective study of 570 men immediately before prostate biopsy [40]. Using a PCA3 cut-off of 35, the study yielded 54% sensitivity, 74% specificity, with AUC of 0.69 compared with the PSA AUC of 0.55. There were 2 other important and interesting results of this study. First, there was minimal difference in test performance in men undergoing initial biopsy vs. men with previous negative biopsies, suggesting that PCA3 is robust in both the initial and repeat biopsy cohort. Second, although PSA is directly correlated with prostate volume, thus contributing to the specificity concerns of PSA, the investigators found that PCA3 was not correlated with prostate vol-