Available online at www.sciencedirect.com
ScienceDirect
Cancer Letters 249 (2007) 5–13
CANCER Letters
www.elsevier.com/locate/canlet

Mini-review

Molecular markers for prostate cancer

Mark A. Reynolds [a,*], Kumar Kastury [a], Jack Groskopf [a], Jack A. Schalken [b], Harry Rittenhouse [a]

[a] *Gen-Probe Incorporated, 10210 Genetic Center Drive, San Diego, CA 92121, USA*
[b] *Radboud University Medical Center Nijmegen, 6525 GA Nijmegen, The Netherlands*

Abstract

Serum PSA testing has been used for over 20 years as an aid in the diagnosis and management of prostate cancer. Although highly sensitive, it suffers from a lack of specificity, showing elevated serum levels in a variety of other conditions including prostatitis, benign prostate hyperplasia, and non-cancerous neoplasia. During this period, numerous serum protein analytes have been investigated as alternative and/or supplemental tests for PSA, however in general these analytes have likewise suffered from a lack of specificity, often showing serum elevations in other clinical presentations. More recently, molecular assays targeting prostate disease at the DNA or RNA level have been investigated for potential diagnostic and prognostic utility. With the aid of modern genomics technologies, a variety of molecular biomarkers have been discovered that show potential for specific correlation with prostate cancer. Much of this discovery has been retrospective, using microdissected tissue from prostatectomy. The goal of current research is to apply genomic assays to noninvasive specimens such as blood and urine. Progress in this area is the subject of this review.

Keywords: Prostate cancer; Molecular test; Epigenetic markers; Gene expression markers; Genetic polymorphisms; Chromosomal translocations; Non-coding RNA

1. Introduction

The detection and management of prostate cancer using serum PSA has increased dramatically since its introduction over 15 years ago and has been the subject of numerous reviews [1–4]. The frequent use of PSA has led to a downward stage migration with fewer men diagnosed with distant disease. The PSA levels for men have also steadily declined as smaller tumors are detected today. These changes in the epidemiology of prostate cancer have led to current serious limitations of PSA for cancer detection. PSA lacks diagnostic specificity considering that more than half of the men with PSA over 4.0 ng/ml are negative on initial biopsy [5]. The accumulation of men with chronically elevated PSA for non-cancer reasons, for example enlarged prostates, has resulted in a population of over 10 million men who are especially difficult to monitor for biopsy decisions using PSA. The ''PSA dilemma'' population of men (those with elevated PSA who are negative on initial biopsy) is frequently

* Corresponding author. Tel.: +1 858 731 5956; fax: +1 858 731 5900.
E-mail address: markr@gen-probe.com (M.A. Reynolds).

0304-3835/$ - see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2006.12.029

biopsied multiple times as they age to assess the possible development of clinically significant cancers. Furthermore, for those men who are diagnosed and undergo curative surgical treatment, about 20–30% will clinically relapse, revealing that for many men cancer was not likely detected early enough.

The tissue specificity of PSA is responsible for its utility as a serum marker. Studies have shown that quantitation of PSA isoforms together with free PSA may provide incremental improvements in the detection and clinical management of prostate cancer [6]. However, serum proteins appear to have intrinsic limitations being distal from the primary tumor, with detection complicated by variable clearance kinetics and turnover of normal tissue. The specific identification and detection of marker proteins in serum is further complicated by a background of thousands of other circulating proteins in concentrations spanning 8–9 logs of dynamic range [7].

In this review, we will look at the use of molecular markers to directly detect cancer cells in biological specimens such as urine, illustrated schematically in Fig. 1. It has been known since 1869 that cancer cells break away from the primary tumor and are present in bodily fluids [8]. More recently it has been recognized that even in early cancer development, these cells are shed and can be detected in biological fluids such as blood [9]. For prostate cancer both blood and urine are now viewed as informative specimens for diagnostic assays [10]. Molecular tests for cancer cell-associated genes and other genetic markers, being a direct indicator of the presence of the cancer cell in the biological specimen, are predicted to provide new improvements in diagnostic specificity. Furthermore, through prospective clinical validation, such tests are predicted to aid urologists in the prognosis and monitoring of prostate cancer, as well as in the assessment of treatment options.

The Human Genome Sequencing Project stimulated the development of a variety of new technologies to study prostate disease at the molecular level. These include methods for cloning and enrichment of disease-associated genes and the development of microarray technologies for profiling their expression. Rapid sequencing methods were developed to

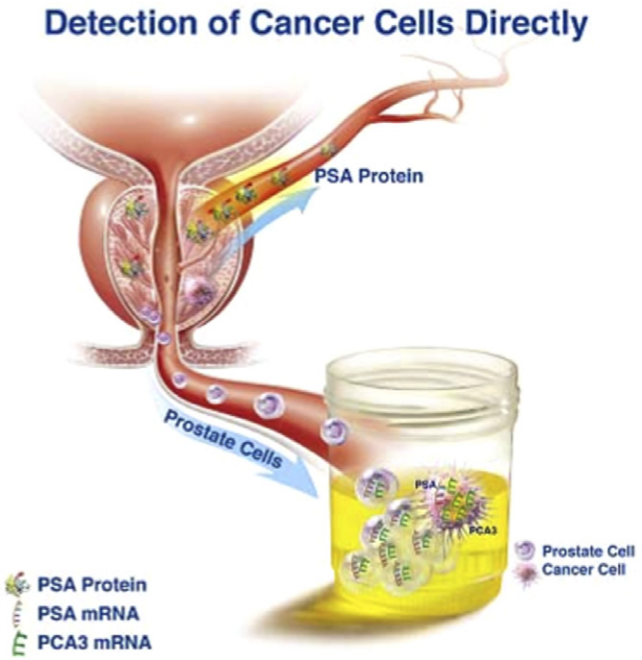

Fig. 1. Diagram representing independent mechanisms for PSA serum protein detection as a surrogate marker for prostate cancer (Top), and for the direct detection of prostate cancer cells shed into urine using the molecular markers PCA3 mRNA and PSA mRNA (Bottom). In the latter case, cells are expressed into the urine using a digital rectal exam (DRE) and the first void urine specimen is then collected. PSA mRNA copies are used for normalization, providing a PCA3 Score that represents the ratio between PCA3 and PSA mRNA copy numbers.

identify gene polymorphisms that could potentially be associated with an increased risk for prostate cancer and/or disease progression. Currently, compendia of gene expression data are being analyzed to identify common features of genomic instability that could potentially be associated with prostate cancer phenotypes. Finally, non-coding RNAs (including micro-RNAs, or miRNAs) have been associated with other types of cancer and could potentially be useful biomarkers for prostate cancer.

2. Epigenetic markers

Hypermethylation of CpG islands, located within promoter regions of tumor suppressor genes, is an important mechanism for gene inactivation and has been described in almost every tumor type [11]. Such epigenetic changes may result in the disruption of key regulatory pathways leading to cancer transformation and progression [12].

The most common method of identifying hypermethylation regions involves chemical conversion of methylated cytosines to uridine residues followed by amplification by PCR and comparison of uridine/cytosine (unmethylated) ratios through a variety of means, including mini-sequencing and hybridization with mutation-sensitive oligonucleotide probes.

In prostate cancer, a number of studies have demonstrated that detection of aberrant hypermethylation has potential for disease detection and prognosis. The majority of these studies restricted their analysis to prostatic tumor tissue, pelvic lymph nodes, noncancerous prostate tissue, and prostate cell lines. Disease association involves the comparison of methylation percentages in targeted promoter regions between diseased and non-diseased specimens. Typically, such studies have implicated a number of different genes, and the objective has been to identify limited subsets of hypermethylated (or in some cases hypomethylated) genes that collectively provide adequate diagnostic or prognostic utility. For example, a methylation index, defined as the ratio between hypermethylated genes to total genes analyzed, was shown to correlate with poor prognosis, although no individual gene provided a sufficient correlation [13].

To date, the hypermethylation of the gene glutathione *S*-transferase pi (GTSP1) has been found to be the most common epigenetic alteration in prostate cancer, being present in up to 90% of cases [13–17]. The search for synergistic methylation markers has met with some success. A study of 118 prostate carcinomas, 38 paired high-grade prostatic intraepithelial neoplasia (HG-PIN), and 30 benign prostatic hyperplasia (BPH) demonstrated that GSTP1 measured in combination with a second gene adenomatous polyposis coli (APC) provided 98.3% sensitivity for prostate carcinoma, with 100% specificity [18]. In a related study of 170 prostate carcinoma and 69 BPH specimens, GSTP1 and APC measured in combination with a third gene multidrug resistance 1 (MDR1) provided diagnostic sensitivity of 75.9% with a specificity of 84.1% [19]. In another related study, combination of GSTP1 and APC with another gene prostaglandin-endoperoxide synthase 2 (PTGS2) discriminated between prostate cancer tissues and BPH tissues with sensitivity ranging from 71.1% to 96.2% and specificity ranging from 92.9% to 100% [20].

In addition to tissue analysis, methylation marker analyses have been extended to serum toward the development of noninvasive diagnostic and prognostic tests. In this case, the assumption is that circulating DNA originating from prostate carcinoma can be detected and discriminated against a background of DNA originating from other tissues. Such analysis requires high sensitivity and high analytical precision for robust performance. In one study, hypermethylated GSTP1 was detected in the serum of 12% of men with clinically localized prostate cancer and in 28% of men with metastatic prostate cancer [21].

3. Expression markers

The application of microarray technologies for profiling prostate cancer gene expression has been reviewed extensively [22–25]. As in the case of epigenetic markers, the vast majority of these studies have been conducted retrospectively in tissue, more recently using laser capture microdissected tissue. Numerous studies have utilized both cDNA and oligonucleotide microarrays to identify genes that are expressed differentially in prostate cancer compared to benign prostate, BPH, and HG-PIN tissues. In the former case, cDNA clones are either selected randomly or enriched through subtractive cloning techniques and then deposited at high density onto glass microscope slides to permit the profiling of as many as 80,000 expressed sequence tags in a single experiment. In the latter case, oligonucleotide sequences are designed using bioinformatic approaches to provide a comprehensive representation of the human

transcriptome. Recently, synthetic oligonucleotide microarrays have also been designed to discriminate aberrantly spliced mRNAs, as can be generated through cancer transformation.

Prostate-specific expression markers such as PSA and PSMA have been commonly identified at the mRNA level using microarrays. Other genes that have been found to be commonly over-expressed in prostate cancer include Hepsin, α-methylacyl-coenzyme A racemase (AMACR), telomerase, the serine protease TMPRSS2, δ-catenin, and a prostate-specific non-coding RNA called PCA3 (formerly called DD3) [26]. A comprehensive PubMed search of microarray studies comparing prostate cancer tissues with normal tissues revealed a number of discrepancies as well as commonalities [27]. It was noted that differences in results might have been attributed to variations in tissue selection and microdissection as well as differences in the microarrays themselves. Indeed, considerable effort in recent years has been directed toward standardization of microarray platforms [28].

One advantage of the cDNA approach is that it permits focused analysis of selected genes. In one such study, a custom microarray was produced using clones randomly selected from cDNA libraries prepared from the LNCaP prostate cancer cell line [29]. This strategy identified many genes that had been previously shown to represent markers of prostate cancer, as well as several new genes including HOXB13. In a related study, a PCR-based subtraction method was used to produce cDNA clones that resulted in the identification of several novel genes markers, designated P704P, P712P, and P775P [30]. Their differential expression by prostate carcinomas compared to normal prostate and BPH was verified subsequently by quantitative RT-PCR. This approach also identified a novel gene designated P504S (later shown to be AMACR), which was subsequently shown to be a useful histochemical marker for prostate needle biopsy [31]. In fact, AMACR represents an early example of prostate cancer markers discovered through molecular technologies that is currently being used in routine clinical pathology.

Although a number of common gene expression markers such as those described above have been identified using microarray techniques, complementary analytical approaches such as quantitative PCR are generally conducted to provide further validation of these prospective markers. For example, matched prostatectomy tissues from 106 patients were analyzed by quantitative RT-PCR for four housekeeping genes (GAPDH, HPRT, PGD, TBP) and nine prostate-related genes (AibZIP, D-GPCR, EZH2, PCA3, PDEF, prostein, PSA, PSCA, TRPM8) [32]. This study confirmed the predictive utility of PCA3, with three additional genes (EZH2, prostein, and TRPM8) providing synergistic utility. In a related study, paired laser microdissected benign and malignant prostate cells from 114 prostatectomies were analyzed by quantitative RT-PCR for ETS-related gene (ERG), AMACR, and DD3 (also known as PCA3) [33]. Overexpression of at least one of these genes was observed in nearly all (>98%) of the specimens.

The prospective validation of gene expression markers is just emerging in clinically relevant specimens such as urine and blood. For example, PCA3 has been studied extensively in our laboratories as a noninvasive diagnostic marker in urine [34]. Molecular tests were developed that assay PCA3 directly in urine collected following a digital rectal exam. To our knowledge this is the first noninvasive diagnostic test targeting human mRNA.

PCA3 mRNA levels were quantified and normalized to the total amount of prostate RNA present using a prostate-specific ''housekeeping'' gene. The resultant ratio, or PCA3 Score, predicted biopsy outcome with sensitivity of 67–69% and specificity of 79–83% [34,35]. More recently, the PCA3 assay demonstrated good diagnostic accuracy for the ''PSA dilemma'' group of men with chronically elevated serum PSA and at least one previous negative biopsy [Leonard Marks et al., submitted]. The quantitative PCA3 Score correlated with the risk

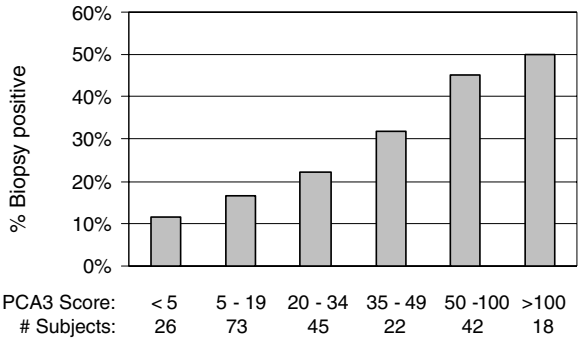

Fig. 2. Bar graph representing the percentages of positive biopsy results corresponding to patients with different PCA3 Score ranges. As shown, risk of a positive biopsy increases with increasing PCA3 Score.

of a positive biopsy in this population (Fig. 2). The PCA3 assay offers several advantages relative to other molecular methods: Specimen processing is relatively simple and utilizes whole urine (as opposed to urine sediments), the fraction of specimens yielding sufficient RNA for analysis is >95% and the assay is readily adaptable to a clinical laboratory setting using available instrumentation [34].

4. Genetic polymorphisms

Over the past two decades the genetic epidemiology of prostate cancer has become increasingly understood to be complex and difficult to classify [36]. Unlike other cancers such as breast cancer, where polymorphisms such as BRCA1 and BRCA2 have been found to have a relatively high penetrance, the vast majority of polymorphisms examined to date for prostate cancer have exhibited a relatively low penetrance, and frequently have not been validated in subsequent studies. Nonetheless, numerous studies have shown that immediate relatives of affected men have a 2- to 3-fold increased risk of developing prostate cancer [37], driving the expansion of polymorphism screens to increasingly larger populations. These studies are complicated by the high prevalence of prostate cancer in older men, and also by the considerably heterogeneous nature of the disease [38].

Despite the challenges just described, a number of loci have been shown to have a positive correlation with prostate cancer risk, including HPC1, HPC2, HPCX, HPC20, CAPB, PCAP, and an unnamed locus at 8p22–23 [39]. Many such genetic linkage studies have been based on a relatively small number of families (typically less than 100). In one study, results from several such association studies were reanalyzed as a combined dataset of 426 hereditary prostate cancer families [40]. This study led to the identification of a new susceptibility gene in the 17q22 region that comprises the BRCA1 gene. It was commented that the BRCA1 mutation had been shown in a previous study to correlate with a positive risk of prostate cancer. Another large-scale association study of >25,000 SNPs within 16,000 genes using 368 matched cases and controls provided evidence that genetic variants of ICAM5 are also associated with positive risk.

Many other such genetic association studies have been reported. From the standpoint of biomarker utility, more prospective studies are needed. Furthermore, it is apparent that multiple polymorphisms will need to be measured to obtain a test with adequate clinical utility.

5. Genetic instability and chromosomal translocations

Prostate cancer is a highly heterogeneous disease from the standpoint of chromosomal instability and cellular transformation. Numerous studies have attempted to apply in situ hybridization technologies to search for commonalities in prostate cancer phenotypes. Early clues to such commonalities came from gene expression profiling studies such as those described above. For example, using laser capture microdissection of tumor and adjacent benign prostate cells, it was shown that Ets Related Gene (ERG), a member of the Ets family of transcription factors, is over-expressed in a subset of prostate cancers and its level of expression correlated with cancer recurrence [33]. Using Cancer outlier profile analysis (COPA), the over-expression of ERG and ETV1 (another Ets family member) was found to be highly correlated with TMPRSS2, an androgen regulated type 2 transmembrane serine protease [41]. The expression patterns were shown to result from chromosomal translocations, presumed to be early events in prostate cancer transformation, where ERG or ETV1 genes are juxtaposed to the TMPRSSS2 gene. When such translocation events occur, the androgen-responsive promoter of TMPRSS2 regulates expression of the ERG and ETV1 oncogenes, providing a selective mechanism for cancer transformation. The occurrence of TMPRSS2:ERG or TMPRSS2:ETV1 translocations appears to be mutually exclusive and has been detected in over fifty percent of prostate cancers using tissue microarrays produced from prostatectomies [42]. These translocations have also been detected in biopsy tissue, suggesting a non-invasive means of measurement for diagnostic and prognostic applications [43]. Translocations between ERG and TMPRSS2 appear to be the most common (presumably because they are both located on chromosome 21, whereas ETV1 is on chromosome 7). TMPRSS2:ERG translocations represent submicroscopic, intronic deletions that cannot be detected with traditional karyotyping techniques. However, it has been possible to detect them directly using florescence in situ hybridization (FISH) applied to interphase nuclei of cancer cells [42], and also by using single

nucleotide polymorphism (SNP) microarrays [44]. One particular TMPRSS2:ERG splice variant has been shown to be associated with an aggressiveness prostate cancer phenotype [45]. Biologically, over expression of ERG may promote cell proliferation and angiogenesis, since ERG is implicated in endothelial cell differentiation [46].

6. Noncoding RNA

The field of non-coding RNA is a relatively new area of molecular biology impacting a variety of regulatory processes between gene expression and protein synthesis [47]. Included in this category are micro RNAs (miRNAs) that have recently

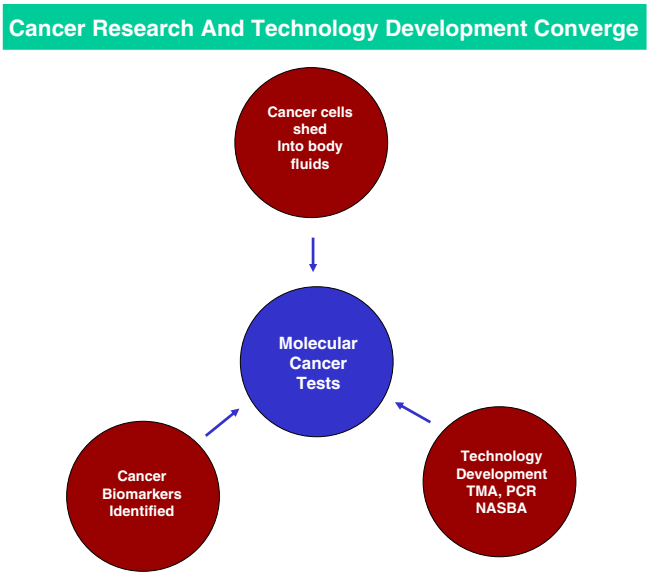

Fig. 3. Schematic illustrating the convergence of research and assay development leading to molecular tests for the direct detection of cancer cells in biological specimens.

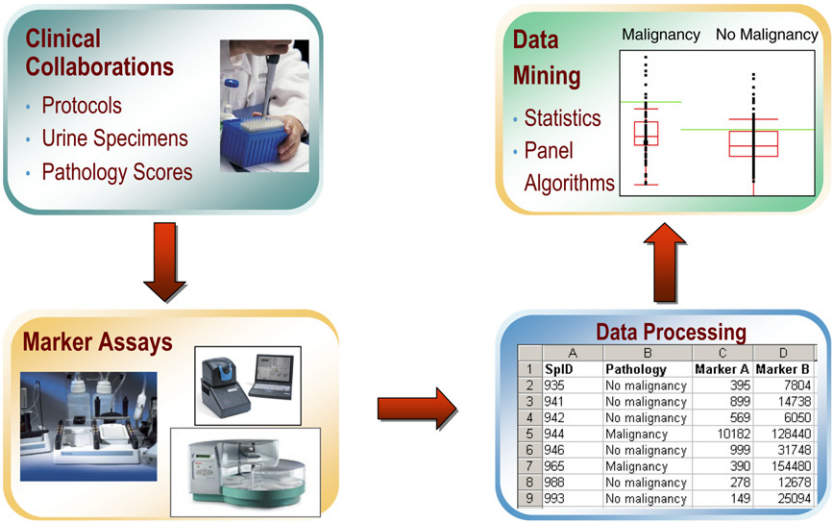

Fig. 4. Illustration of a systems approach for the preclinical evaluation of candidate biomarkers for diagnostic and/or prognostic clinical utility.

been shown to have potential as biomarkers of cancer transformation and progression [48,49]. For example, in the case of breast cancer, a panel of five miRNAs (mir-125b, mir-145, mir-21, and mir-155) were shown to clearly distinguish normal versus breast tumor tissue [50]. Although biomarker studies employing miRNA have not yet been reported in the area of prostate cancer, it is possible that this category of biomarkers could be shown to have diagnostic clinical utility in the future. It is worth noting that PCA3, mentioned in a previous section, is also a non-coding RNA [51], although its biological role in normal and diseased prostate cells remains to be elucidated.

7. Future considerations

Harnessing the potential of molecular markers for prostate cancer will not be a trivial exercise. The identification of such markers in tissue, the subject of the major portion of this review, represents only the first step in the process of developing a diagnostic assay. Further characterization of cancer cells that are shed into biological fluids is needed, as well as further development of robust assay platforms that are suitable for clinical laboratories. These components of assay development are illustrated in Fig. 3.

The preclinical validation of molecular markers is vitally important to any successful product development strategy. This can require the procurement and analysis of large numbers of patient specimens, preferably from multiple centers, obtained through well-defined collection protocols and archived in a manner that is amenable to high throughput analysis. This requires a systems approach, illustrated in Fig. 4, providing a seamless (preferably electronic) linkage between specimens, results, and data analysis.

The standardization of specimen collection and sample processing will be vitally important to the successful development of molecular tests for urine and blood, particularly for RNA-based tests where the samples must be collected in appropriate media in order to preserve the target nucleic acid. For example, although early results measuring PSA mRNA in blood were encouraging [52], there were considerable discrepancies in the confirmatory studies [53]. These discrepancies were attributed to a lack of standardized preanalytical and RT-PCR procedures.

Studies involving PCA3 as a molecular urine test for prostate cancer, described in a previous section, have shown very similar clinical diagnostic performance across four independent research groups. In each of these studies, the preanalytical sample handling followed a standardized protocol, whereas three different nucleic acid amplification platforms were used for detection. This illustrates the need for well-controlled specimen handling in biomarker experiments.

Finally, it seems there will be a paradigm shift in terms of prostate cancer diagnosis, treatment and monitoring based on the molecular signatures, as they become understood. For example, in the case of the TMPRSS2:ERG gene chromosomal translocations described above, there is already early evidence that specific splice variants could be more or less correlative with prostate cancer phenotypes (e.g. the aggressiveness of transformation, invasiveness, etc.) [54]. Furthermore, a recent microarray study using exon-specific target elements revealed that as many as 454 splice isoforms from as many as 200 genes are differentially regulated in prostate tumors compared to benign tissue [55]. Such studies suggest that it may eventually be necessary to measure multiple genes, including alternative splice isoforms, in order to provide accurate prognostic and monitoring solutions for individualized patient care.

References

[1] P.D. Maroni, E.D. Crawford, Screening for prostate cancer in 2006: PSA in the 21st century, N. C. Med. J. 67 (2006) 136–139.
[2] S.J. Freedland, A.W. Partin, Prostate-specific antigen: Update 2006, Urology 67 (2006) 458–460.
[3] J.A. Efstathiou, M.H. Chen, W.J. Catalona, et al., Prostate-specific antigen-based serial screening may decrease prostate cancer-specific mortality, Urology 68 (2006) 342–347.
[4] I.M. Thompson, D.P. Ankerst, C. Chi, et al., Assessing prostate cancer risk: Results from the prostate cancer prevention trial, J. Natl. Cancer Inst. 98 (2006) 529–534.
[5] T.A. Stamey, Preoperative serum prostate-specific antigen (PSA) below 10 microg/l predicts neither the presence of prostate cancer nor the rate of postoperative PSA failure, Clin. Chem. 47 (2001) 631–634.
[6] S.D. Mikolajczyk, Y. Song, J.R. Wong, R.S. Matson, H.G. Rittenhouse, Are multiple markers the future of prostate cancer diagnostics? Clin. Biochem. 37 (2004) 519–528.
[7] N.L. Anderson, N.G. Anderson, The human plasma proteome: history, character, and diagnostic prospects, Mol. Cell. Proteom. 1 (2002) 845–867.
[8] T.R. Ashworth, A case of cancer in which cells similar to those in the tumours where seen in the blood after death, Aust. Med. J. 14 (1869) 146.

[9] M.J. Duffy, Can molecular markers now be used for early diagnosis of malignancy? Clin. Chem. 41 (1995) 1410–1413.
[10] D. Hessels, H. Rittenhouse, J.A. Schalken, Molecular diagnostics in prostate cancer, EAU Update Series Series 3 (2005) 200.
[11] M. Esteller, CpG island hypermethylation and tumor suppressor genes: a booming present, a brighter future, Oncogene 21 (2002) 5427–5440.
[12] S.B. Baylin, W.Y. Chen, Aberrant gene silencing in tumor progression: Implications for control of cancer, Cold Spring Harb. Symp. Quant. Biol. 70 (2005) 427–433.
[13] R. Maruyama, S. Toyooka, K.O. Toyooka, et al., Aberrant promoter methylation profile of prostate cancers and its relationship to clinicopathological features, Clin. Cancer Res. 8 (2002) 514–519.
[14] C. Jeronimo, R. Henrique, P.F. Campos, et al., Endothelin B receptor gene hypermethylation in prostate adenocarcinoma, J. Clin. Pathol. 56 (2003) 52–55.
[15] K. Woodson, J. Gillespie, J. Hanson, et al., Heterogeneous gene methylation patterns among pre-invasive and cancerous lesions of the prostate: a histopathologic study of whole mount prostate specimens, Prostate 60 (2004) 25–31.
[16] Y. Tokumaru, S.V. Harden, D.I. Sun, K. Yamashita, J.I. Epstein, D. Sidransky, Optimal use of a panel of methylation markers with GSTP1 hypermethylation in the diagnosis of prostate adenocarcinoma, Clin. Cancer Res. 10 (2004) 5518–5522.
[17] R. Singal, L. Ferdinand, I.M. Reis, J.J. Schlesselman, Methylation of multiple genes in prostate cancer and the relationship with clinicopathological features of disease, Onco. Rep. 12 (2004) 631–637.
[18] C. Jeronimo, R. Henrique, M.O. Hoque, et al., A quantitative promoter methylation profile of prostate cancer, Clin. Cancer Res. 10 (2004) 8472–8478.
[19] H. Enokida, H. Shiina, S. Urakami, et al., Multigene methylation analysis for detection and staging of prostate cancer, Clin. Cancer Res. 11 (2005) 6582–6588.
[20] P.J. Bastian, J. Ellinger, A. Wellmann, et al., Diagnostic and prognostic information in prostate cancer with the help of a small set of hypermethylated gene loci, Clin. Cancer Res. 11 (2005) 4097–4106.
[21] P.J. Bastian, G.S. Palapattu, X. Lin, et al., Preoperative serum DNA GSTP1 CpG island hypermethylation and the risk of early prostate-specific antigen recurrence following radical prostatectomy, Clin. Cancer Res. 11 (2005) 4037–4043.
[22] C. Kumar-Sinha, D.R. Rhodes, J. Yu, A.M. Chinnaiyan, Prostate cancer biomarkers: a current perspective, Expert Rev. Mol. Diagn. 3 (2003) 459–470.
[23] K. Huppi, G.V. Chandramouli, Molecular profiling of prostate cancer, Curr. Urol. Rep. 5 (2004) 45–51.
[24] P.S. Nelson, Predicting prostate cancer behavior using transcript profiles, J. Urol. 172 (2004) S28–S32, discussion S33.
[25] L. Kopper, J. Timar, Genomics of prostate cancer: Is there anything to "translate"?, Pathol Oncol. Res. 11 (2005) 197–203.
[26] D. Hessels, G.W. Verhaegh, J.A. Schalken, J.A. Witjes, Applicability of biomarkers in the early diagnosis of prostate cancer, Expert Rev. Mol. Diagn. 4 (2004) 513–526.
[27] E.P. Gelmann, O.J. Semmes, Expression of genes and proteins specific for prostate cancer, J. Urol. 172 (2004) S23–S26, discussion S26-7.
[28] F.E. Ahmed, Microarray RNA transcriptional profiling: Part I. platforms, experimental design and standardization, Expert Rev. Mol. Diagn. 6 (2006) 535–550.
[29] S. Edwards, C. Campbell, P. Flohr, et al., Expression analysis onto microarrays of randomly selected cDNA clones highlights HOXB13 as a marker of human prostate cancer, Br. J. Cancer 92 (2005) 376–381.
[30] J.A. Stolk, Y. Jiang, C.H. Day, et al., and P775P: A genomic cluster of prostate-specific genes, Prostate 60 (2004) 214–226.
[31] Z. Jiang, B.A. Woda, Diagnostic utility of alpha-methylacyl CoA racemase (P504S) on prostate needle biopsy, Adv. Anat. Pathol. 11 (2004) 316–321.
[32] U. Schmidt, S. Fuessel, R. Koch, et al., Quantitative multigene expression profiling of primary prostate cancer, Prostate 66 (2006) 1521–1534.
[33] G. Petrovics, A. Liu, S. Shaheduzzaman, et al., Frequent overexpression of ETS-related gene-1 (ERG1) in prostate cancer transcriptome, Oncogene 24 (2005) 3847–3852.
[34] J. Groskopf, S.M. Aubin, I.L. Deras, et al., Aptima PCA3 molecular urine test: Development of a method to aid in the diagnosis of prostate cancer, Clin. Chem. 52 (2006) 1089–1095.
[35] D. Hessels, J.M. Klein Gunnewiek, I. van Oort, et al., DD3(PCA3)-based molecular urine analysis for the diagnosis of prostate cancer, Eur. Urol. 44 (2003) 8–15, discussion 15-6.
[36] D.J. Schaid, The complex genetic epidemiology of prostate cancer, Hum. Mol. Genet. 13 (Spec. No.1) (2004) R103–R121.
[37] D.J. Schaid, J.C. Guenther, G.B. Christensen, et al., Comparison of microsatellites versus single-nucleotide polymorphisms in a genome linkage screen for prostate cancer-susceptibility loci, Am. J. Hum. Genet. 75 (2004) 948–965.
[38] S.L. Slager, K.E. Zarfas, W.M. Brown, et al., Genome-wide linkage scan for prostate cancer aggressiveness loci using families from the university of Michigan prostate cancer genetics project, Prostate 66 (2006) 173–179.
[39] A.W. Hsing, A.P. Chokkalingam, Prostate cancer epidemiology, Front. Biosci. 11 (2006) 1388–1413.
[40] E.M. Gillanders, J. Xu, B.L. Chang, et al., Combined genome-wide scan for prostate cancer susceptibility genes, J. Natl. Cancer Inst. 96 (2004) 1240–1247.
[41] S.A. Tomlins, R. Mehra, D.R. Rhodes, et al., TMPRSS2:ETV4 gene fusions define a third molecular subtype of prostate cancer, Cancer Res. 66 (2006) 3396–3400.
[42] S. Perner, F. Demichelis, R. Beroukhim, et al., TMPRSS2:ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer, Cancer Res. 66 (2006) 8337–8341.
[43] M.J. Soller, M. Isaksson, P. Elfving, W. Soller, R. Lundgren, I. Panagopoulos, Confirmation of the high frequency of the TMPRSS2/ERG fusion gene in prostate cancer, Genes Chromosomes Cancer 45 (2006) 717–719.
[44] M. Yoshimoto, A.M. Joshua, S. Chilton-Macneill, et al., Three-color FISH analysis of TMPRSS2/ERG fusions in prostate cancer indicates that genomic microdeletion of chromosome 21 is associated with rearrangement, Neoplasia 8 (2006) 465–469.
[45] J. Wang, Y. Cai, C. Ren, M. Ittmann, Expression of variant TMPRSS2/ERG fusion messenger RNAs is associated with aggressive prostate cancer, Cancer Res. 66 (2006) 8347–8351.
[46] F. McLaughlin, V.J. Ludbrook, J. Cox, I. von Carlowitz, S. Brown, A.M. Randi, Combined genomic and antisense

analysis reveals that the transcription factor erg is implicated in endothelial cell differentiation, Blood 98 (2001) 3332–3339.
[47] J.S. Mattick, I.V. Makunin, Non-coding RNA, Hum. Mol. Genet. 15 (Spec No 1) (2006) R17–R29.
[48] G.A. Calin, C.M. Croce, MicroRNA-cancer connection: the beginning of a new tale, Cancer Res. 66 (2006) 7390–7394.
[49] R.W. Carthew, Gene regulation by microRNAs, Curr. Opin. Genet. Dev. 16 (2006) 203–208.
[50] M.V. Iorio, M. Ferracin, C.G. Liu, et al., MicroRNA gene expression deregulation in human breast cancer, Cancer Res. 65 (2005) 7065–7070.
[51] M.J. Bussemakers, A. van Bokhoven, G.W. Verhaegh, et al., highly overexpressed in prostate cancer, Cancer Res. 59 (1999) 5975–5979.
[52] A.E. Katz, C.A. Olsson, A.J. Raffo, et al., Molecular staging of prostate cancer with the use of an enhanced reverse transcriptase-PCR assay, Urology 43 (1994) 765–775.
[53] L.G. Gomella, G.V. Raj, J.G. Moreno, Reverse transcriptase polymerase chain reaction for prostate specific antigen in the management of prostate cancer, J. Urol. 158 (1997) 326–337.
[54] C.M. Ahlers, W.D. Figg, ETS-TMPRSS2 fusion gene products in prostate cancer, Cancer Biol. Ther. 5 (2006) 254–255.
[55] C. Zhang, H.R. Li, J.B. Fan, et al., Profiling alternatively spliced mRNA isoforms for prostate cancer classification, BMC Bioinformat. 7 (2006) 202.