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Mini-review

Molecular alterations in prostate cancer

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Abstract

Prostate tumors display a range of clinical phenotypes, from indolent to aggressively metastatic. Numerous gene expression profiling studies have been conducted toward the potential molecular staging of these pathologies, however the identification of genetic markers that predict aggressive disease has not yet been demonstrated in the clinical setting. A recent survey of the literature has shown that molecular alterations in prostate carcinomas can occur through a variety of different mechanisms, ranging from upstream epigenetic changes and genetic polymorphisms to downstream modulations through alternative splicing and other post-translational processes, some of which could involve noncoding RNAs. A summary of these results and recommendations for future work are the subject of this review.

Keywords: Prostate cancer; Gene expression markers; Genetic polymorphisms; Gene fusions; Epigenetic modulation; Methylation markers; Alternative splicing; Non-coding RNA; MicroRNA

1. Introduction

Prostate cancer is a highly heterogeneous disease, both in terms of its pathology and clinical presentation. It is the second leading cause of cancer-related deaths in Western males, yet because prostate tumors can take decades to progress to clinically significant disease, they are difficult to diagnose and treat. Latent prostate cancer is common, with many older men showing localized prostate tumors upon autopsy that died from other causes [1]. In contrast, once metastasis has been detected by imaging, the vast majority of patients will likely die from prostate cancer. Over the past two decades, serum PSA screening has provided a valuable tool for prostate cancer surveillance, however the age-adjusted incidence of mortality has remained relatively constant, therefore new tools are needed to guide prognosis and treatment.

Research into the genetic origins of prostate cancer has accelerated dramatically in recent years, aided by the availability of new high-throughput microarray and sequencing technologies. Numerous gene expression studies have been conducted to characterize prostate cancer initiation and progression, and many of them have shown correlation with clinical outcome. However, molecular associations with prostate cancer phenotypes continue to be fragmentary, and in some cases are poorly substantiated by follow-up investigations [2]. Early studies that profiled cancerous *versus* benign tissues have now been supplemented by micro-dissection

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procedures, revealing differences in gene expression patterns across individual tumor foci isolated from the same specimen [3]. Guided by such observations, investigators are now directing their attention to underlying molecular features that could be associated with gene expression alterations, toward a more comprehensive understanding of prostate cancer initiation and progression. These include genetic polymorphisms and epigenetic modifications, alternative splicing, and post-translational regulation involving non-coding RNA. This review surveys studies that have been conducted in each of these areas.

2. Gene expression profiling in prostate cancer

Shortly following the introduction of gene expression microarray technologies, a number of early profiling studies were reported using prostate cancer tissues and tumor-derived cell lines [4–8]. Such early studies successfully identified genes in key regulatory pathways that are widely associated with cancer phenotypes, for example genes involved in cell cycle regulation, DNA replication, and DNA repair [9]. Singh et al. was the first group to show a correlation between gene expression signatures and clinical outcome following prostatectomy [10]. They derived a 5-gene algorithm that predicted cancer recurrence with 90% accuracy, however the sample size was relatively small (52 tumor specimens profiled with 21 patients evaluable with respect to clinical outcomes).

Despite the promising results cited above, a comprehensive review of published gene expression profiling studies in the area of prostate cancer showed variable results between laboratories, potentially due to tissue heterogeneities and differences in analytical performance of the microarray platforms that were used [2]. Investigators resorted to gene-specific assays to validate candidate genes, including quantitative RT-PCR and immuno-histochemistry. Employing such techniques, for example, Bubendorf et al. demonstrated that insulin-like growth factor-binding protein 2 (IGFBP2) exhibited high expression in 100% of hormone-refractory clinical tumors, in 36% of primary tumors, and in none of the benign prostate hyperplasia specimens [4]. Similar albeit less significant expression patterns were observed for the 27-kd heat-shock protein (HSP27). By applying these procedures together with proprietary microarrays produced using cDNA library subtraction, Corixa Corporation identified a gene designated P504S, which was later found to code for the protein alpha-methyl CoA racemase (AMACR) [5]. Rubin et al. subsequently demonstrated that AMACR is a highly specific tissue biomarker for prostate cancer [11]. Indeed, antibodies targeting AMACR are now widely used by pathologists to help detect prostate cancer morphologies in prostate needle biopsies [12]. Verification of candidate gene up regulation by immunohistochemistry has been extended to a variety of proteins in the hope of identifying biomarkers of prostate cancer disease phenotypes. These included *STK11, STK15, PTTG1, STK15, MYBL2, PIM1, IGFBP-5, DAN1, FAT, RAG5A,* and *HEVIN* [9,13]. Attempts to associate such markers with pathological features of prostate cancer are continuing to progress with the aid of tissue microarrays for histological validation. For example, Lapointe et al. [14] found that MUC1 was associated with "aggressive" pathological features and a high risk of recurrence following prostatectomy, whereas ASGP1 was associated with a decreased risk of recurrence. Although such results have been encouraging, no molecular or histological markers have yet been found to sufficiently predict aggressive disease phenotypes for clinical staging.

A main drawback to the experiments described above is their inability to discriminate tumor heterogeneity within and between tissue specimens. To address this challenge, tissue micro-dissection techniques coupled with amplification procedures were employed to expand the extremely small amounts of extracted RNA. With this approach, True et al. identified an 86-gene panel capable of distinguishing low-grade from high-grade carcinomas that had been classified according to the Gleason grading system [15]. This was the first published study showing that gene expression signatures could be used for pathological staging of prostate cancer. In another study, Petrovics et al. [16] demonstrated that ETS-related gene (ERG), AMACR, and DD3 (later designated PCA3) are highly expressed in prostate tumor micro-dissections, and that over-expression of one or more of these genes occurred in over 98% of these tumors (55/56). Nonetheless, not every tumor micro-dissection exhibited over-expression of all three genes, and surprisingly this research team later reported that ERG expression decreased with increasing Gleason stage [17].

Nanni et al. employed a different strategy to reduce the complexity of prostate tissue heterogeneity, whereby epithelial cell cultures were derived

from finely dissected tissues explanted from patients undergoing radical prostatectomy [18]. The resulting primary cultures were characterized phenotypically by immunohistochemistry and then used for gene expression profiling using Affymetrix Human U133A GeneChip microarrays. Using this approach, these investigators were able to identify 89 common genes that were differentially expressed between cancer and non-cancer specimens, a subset of which were further analyzed by qRT-PCR. Transcripts found to be generally up regulated in tumors included AKT1, IGFBP5, IGFBP3, KRAS2, and hTERT. However, qRT-PCR analysis revealed overlapping expression patterns for the majority of these transcripts, with down regulation evident in a subset of the tumor-derived cultures.

3. Somatic mutations

Gene expression heterogeneity in prostate cancer is perhaps not very surprising considering the numerous types of chromosomal abnormalities that have been described, including chromosomal insertions, deletions, amplifications, and translocations [19–25]. Genomic instability is an underlying characteristic of prostate carcinoma transformation. Nearly all solid tumors are genetically unstable, and it is now widely accepted that cancer results from the accumulation of genetic polymorphisms that directly control cell proliferation or cell death [26]. Alterations in chromosome number and chromosomal translocations have been observed for decades by classical cytogenetic methods.

Recent advances in molecular cytogenetics have elucidated more subtle genetic features, including gene amplifications and deletions toward the identification of genetic markers (e.g., oncogenes, tumor suppressor genes, and genes involved in structural morphology, adherence, etc.). These include fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH), which are capable of identifying insertions on the order of 0.1–10 megabases. CGH analyses have collectively shown that the most common chromosomal alterations in prostate cancer include losses at 1p, 6q, 8p, 10q, 13q, 16q, and 18q, and gains at 1q, 2p, 7, 8q, 18q, and Xq [23]. FISH analyses have provided more fine resolution of target genes, for example amplifications of AR (at Xq12), MYC (8q24), and EIF3S3 (8q23) have been identified in hormone-refractory prostate cancers [23]. Common chromosomal deletions have also led to the identification of tumor suppressor genes that could be potential biomarkers or therapeutic targets for prostate cancer. These include NKX3.1 (from 8p21), PTEN (10q23), P27/Kip1 (12p13), and KLF5 (13q21) [24]. Loss of the Kruppel-like transcription factor KLF6 located at 10p15 has been identified as a candidate tumor suppressor gene in prostate cancer [27], and was shown by PCR analysis to be deleted in 17 of 22 tumor specimens (77%) [28]. Such studies suggest that CGH analysis could be used together with gene expression analysis to further our understanding of molecular changes in clinical subtypes (see Section 7).

More recently, microarray-based CGH (or aCGH) has provided high-resolution quantitative detection of copy number aberrations in tumor micro-dissections with whole genome scanning [29]. This provides the ability to profile larger numbers of specimens in order to identify common features. For example, an aCGH profiling study of 64 tumors from men at intermediate to high risk for recurrence following radical prostatectomy identified approximately 40 candidate markers associated with metastatic potential [30]. Deletion at 8q23 was highly correlated with advanced disease and likelihood of recurrence, whereas amplification at 11q13.1 was predictive of recurrence regardless of tumor stage and grade.

Liu et al. conducted high-resolution genome-wide aCGH analysis of 22 matched frozen prostatectomy micro-dissections, identifying 355 recurrent deletions and 223 recurrent gains [31]. Overall, they found gains were more likely to occur within genes than deletions (74% *versus* 49%, respectively), however for the most frequent copy number alterations both gains (85%) and deletions (57%) were observed. Such fine resolution demonstrations of prostate cancer genetic instability highlight the potential for gene expression heterogeneity, as summarized in the previous section.

In a landmark study, Tomlins et al. [32] used a bioinformatics approach to discover oncogenic chromosomal aberrations in prostate cancer by correlating outlier gene expression signatures. This approach, termed Cancer Outlier Profiler Analysis (COPA), searches for highly correlative genes that are strongly over-expressed in only a subset of cancers. The authors hypothesized that such expression patterns could be caused by gene fusions resulting from chromosomal translocations (separate chromosomes) or deletions (same chromosome). They discovered that protein coding regions of ERG1

and ETV1, two members of the oncogenic ETS transcription family, were fused with the regulated promoter region of androgen-responsive gene TMPRSS2. They further demonstrated in a prostate cancer cell line harboring the TMPRSS2:ERG gene fusion that ERG expression is up regulated by androgens, suggesting a potential mechanism for prostate carcinoma transformation. By applying FISH analysis to prostatectomy-derived tissue microarrays, they subsequently demonstrated that the TMPRSS2:ERG gene fusion is present in about 50% of primary prostate cancers, and about 41% of hormone-na lymph node metastases [33]. Applying this approach, Tomlins et al. later reported additional recurrent ETS gene fusions, including TMPRSS2:ETV4, [34] and 5′-fusions of other genes with ETV1 [35]. A large number of investigators have confirmed the presence of these recurrent gene fusions, providing further evidence that they could potentially be early markers of prostate cancer [36–40]. Wang et al. [41] showed that certain isoforms of TMPRSS2:ERG fusions are associated with aggressive forms of prostate cancer and could potentially be used as prognostic markers. Such findings demonstrate the importance of taking genetic polymorphisms into account when studying gene expression patterns.

Localized genetic polymorphisms are also important to associate with gene expression information. Base replacements, insertions and deletions of one to several bases could potentially alter the stability of the expressed transcript and/or the integrity and function of its associated protein product(s). Furthermore, these polymorphisms could potentially modulate splicing and other post-translational regulation processes to be discussed later in this review. Hereditary polymorphisms were addressed in an earlier review by this author [42].

Somatic mutations that accumulate during the course of prostate carcinoma transformation are of keen interest toward targeted drug therapies, particularly those that occur within oncogenes, tumor suppressor genes, and stability genes. Mutation-targeted drugs could potentially be used to target proteins that are different in the cancer cell compared to normal tissues. This approach is already showing promise in human breast, colorectal, and lung cancers, for example [43]. A recent genome-wide scan of somatic mutations in breast and colorectal cancer specimens revealed a handful of genes that were mutated at a high frequency, and a total of 280 genes that were commonly mutated at a lower frequency [44]. By generating structural models from X-ray crystallography or nuclear magnetic resonance spectroscopy of their encoded proteins, the authors were able to show clustering of mutations around active sites of proteins or near protein-protein interfaces. In addition to altered protein function, such mutations could potentially also modulate gene expression patterns. Therefore, it is important to follow-up gene expression profiling with sequence analysis.

Androgen receptor (AR) gene reactivation is a key transformational event in the development of hormone-refractory prostate cancer. Mechanisms of AR reactivation include gene amplification (see above), somatic mutations, and epigenetic modifications. Mutations in the AR gene have been studied extensively [45]. Studies have shown that somatic mutations of the AR gene are more common in metastatic specimens compared to primary specimens [46], suggesting a potential mechanism for the development of androgen-independent prostate cancer. One common mutation, T877A, is located in the ligand-binding domain and has been associated with adverse pharmacological effects from the AR inhibitor flutamide, where it acts as an agonist rather than an antagonist.

Mutations in the tumor suppressor gene p53 are also commonly observed in prostate cancer, with an overall frequency of about 20–30% [47,48]. They are more frequently detected in advanced prostate cancers and have also been associated with hormone-refractory disease. Mutations in the KLF6 gene have also been reported [28], in addition to its loss through genomic deletions described above. Mutations have also been reported in a number of other genes associated with prostate cancer, including checkpoint kinase 2 (CHEK2) [49,50], epidermal growth factor receptor (EGFR) [51], and plexin-B1 [52].

4. Epigenetic modulation

Epigenetic modulations allow transmission of cellular traits without changes in genomic sequence. They appear to be sensitive to a variety of environmental factors that have been associated with prostate cancer progression, such as diet and oxidative stress [53]. The most widely studied epigenetic alterations in cancer include histone modifications and DNA methylation [54]. Their potential application in the diagnosis, disease progression and treatment

of prostate cancer has been the subject of recent reviews [55–56].

Histone modifications regulate gene expression by altering transcription factor accessibility. These include acetylation and methylation of histone lysine and arginine residues. Histone acetylation facilitates transcriptional activation, whereas histone methylation can have activating or repressing effects depending on location within the histone tail.

Histone deacetylases (HDAC) are frequently up regulated in prostate cancer [57], with their highest levels found in specimens from patients with hormone-refractory disease [58]. Histone hypoacetylation is also highly associated with DNA methylation (discussed below). HDAC inhibitors are currently being investigated to treat a variety of cancers [59], and could potentially be useful drug targets for prostate cancer. Histone methyltransferases are also up regulated in prostate cancer, most notably EZH2 [60].

Genomic regions that are proximal to transcription start sites are often enriched in CpG dinucleotides, referred to as CpG islands. DNA methylation, a process involving the transmission of a methyl group to the C5 position of cytosine residues, represses transcription at these start sites in proportion to the density of modified residues. Gene silencing through DNA hypermethylation has been proposed as an early event in the genesis and propagation of oncogenic signal transduction pathways [61].

DNA methylation patterns can be detected through a variety of methods, most commonly using PCR following bisulfite treatment, which converts unmethylated cytosines to uracils. DNA hypermethylation profiles in primary and metastatic prostate cancer specimens have been summarized previously [55,56]. Many commonly hypermethylated genes are also frequently deleted or mutated in prostate cancer specimens (see above), demonstrating that gene expression heterogeneity can occur through multiple mechanisms. Hypermethylation of GSTP1 is one of the most frequently observed modifications, occurring in 72–100% of clinical specimens tested to date [62–64]. In addition, frequent hypermethylation has been reported in promoter regions of the MDR1 and APC genes [62,64]. Hypermethylation has been reported for a number of other prostate cancer markers, including NKX3.1 [65], a gene that has also been associated with chromosomal deletions (see above). More recently, global hypomethylation patterns in metastatic prostate cancer specimens have been described [66]. These and other observations indicate that DNA methylation is an important mechanism in prostate carcinoma transformation and should also be taken into consideration when correlating gene expression information.

5. Alternative splicing

Alternative splicing, the process by which exons of pre-mRNAs are spliced in different arrangements, plays a major role in the functional diversity of expressed gene transcripts. This came to be appreciated early this decade when expressed sequence tag (EST) databases such as Unigene predicted on the order of 120,000 human gene bins, whereas early drafts of human genomic sequences predicted only about 25,000–30,000 protein encoding genes. EST sequences can now be aligned to genomic sequences using programs that search for conserved splice site consensus sequences. Large databases have been created through these approaches to elucidate alternatively spliced gene transcripts. However, these databases are limited in that EST coverage is generally biased toward the 3′- and 5′-ends of these transcripts. Furthermore, Roy et al. [67] demonstrated that public EST databases are biased toward cancer-associated splice variants and are lacking in non-diseased splice variants. As reviewed previously, new technologies have been developed to expand our knowledge of alternative splicing as it relates to normal and disease physiology [68]. These include exon-specific microarrays that provide considerably more information than previous versions based on EST and cDNA content. For example, using such microarrays Pan et al. [69] profiled both expression and alternative splicing patterns across ten normal human tissues. They found that differential expression and alternative splicing could serve as independent predictors of tissue type, with similar numbers of unique genes identified between tissue pairs by either approach.

Examples of alternative splicing are illustrated in Fig. 1. These include exon skipping, alternative 3′ and 5′ splice sites usage, alternative 3′ splice sites, and intron retention. Spliceosome assembly and the resulting splicing reaction is a highly regulated process with a tight balance between binding initiators and repressors, involving five small nuclear RNAs and between 50 and 100 proteins [70,71]. Changes in the balance of these factors through pro-

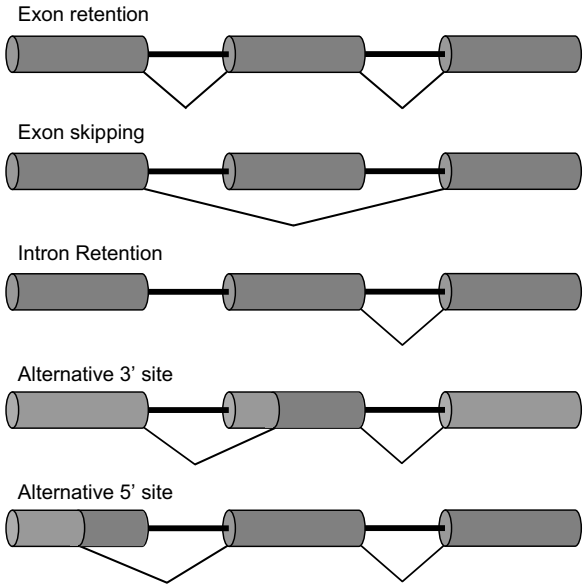

Fig. 1. Common alternative splicing mechanisms leading to, for example, alternative exon retention, exon skipping, intron retention, alternative 5′-initiation and 3′-polyadenylation sites, frame shift changes, etc.

cesses described above can lead to aberrant splicing events, as can mutations in the pre-mRNA sequence itself, resulting in loss of function, functional modification, or nonproductive products that are rapidly degraded. The study of aberrant splicing events is rapidly becoming appreciated as a potential source of future diagnostic markers and therapeutic targets for oncology [72–74]. A large number of splice variants have now been reported for many cancer-associated genes, some of which can be uniquely associated with cancer type [72].

A number of alternatively spliced genes have been associated with prostate cancer including PSA, a highly studied serum biomarker [72]. New assays designed to detect PSA isoforms are being developed based on these discoveries [75]. All members of the kallikrein gene family, including PSA, have at least one splice variant, some of which could potentially be used as diagnostic markers [76]. These include KLK2 and KLK3 [77], KLK11 [72], and KLK15 [76], which have all been found to be up regulated in prostate cancer. Splice variant specific RT-PCR assays for KLK2 were shown to have some prognostic utility in lymph node specimens [78].

The ligand-binding specificity of fibroblast growth factor receptors is regulated primarily through alternative splicing [79], and certain isoforms of FGFR2 have been associated with hormone-refractory prostate cancer [80]. Other alternatively spliced genes that have been shown to be associated with increased prostate cancer risk include KLF6, a Kruppel-like zinc finger transcription factor [81], which was also found to be frequently mutated in prostate cancer specimens (see above), and the vascular epithelial growth factor receptor flt-4 (VEGFR3) [82].

Fourteen distinct splice variants have now been characterized for TMPRSS2:ERG gene fusions (mentioned above) that were previously found to be highly recurrent in prostate cancer [41,83], with certain isoforms showing correlation with aggressive disease.

6. Other post-transcriptional modulators

Recent estimates indicate that protein encoding genes comprise only about 2% of the human genome, however, tiling microarray experiments have shown that over 50% of the genome is transcribed to RNA [84]. The majority of these transcripts are termed non-coding RNA (ncRNA), the sub-categories of which have been reviewed recently [85]. Non-coding RNAs appear to play important regulatory roles in cellular processes, although many of the mechanisms are not yet understood. For example, Louro et al. [86] identified 39 intronic ncRNAs that are up regulated when hormone-responsive LNCaP cells were treated with synthetic androgen, demonstrating that ncRNAs could be involved in differentiation and maintenance of the prostate. Correspondingly, aberrant expression of ncRNAs could potentially lead to disease phenotypes.

PCA3 (formerly known as DD3) is a ncRNA that is highly up regulated in prostate carcinomas [87], and has recently been shown to be a useful marker in urine for the determination of prostate cancer in men with elevated serum PSA who have received at least one previous negative biopsy [88].

MicroRNAs (miRNAs) have been shown to inhibit mRNA translation, either through a cleavage mechanism (known as RNA interference, or RNAi) or through other mechanisms that are still being elucidated [89]. Regardless, miRNAs are known to bind directly to mRNA sequences, and therefore changes in miRNA expression can also have an impact on mRNA expression (and presumably vice versa). Mutations in mRNA sequences would also be predicted to have an impact on their regulation by miRNAs. The expanding role of miRNAs in cancer progression has been reviewed elsewhere [90–93].

In particular, the identification of microRNAs associated with chromosomal rearrangements in other solid tumors suggests that similar associations could be made in the area of prostate cancer [94]. Indeed, microRNA expression changes in prostate cancer have already been reported [95–98]. This continues to be an active area of research.

7. Discussion

As illustrated throughout this review, expression patterns for prostate-cancer-causing genes can be modulated through a variety of different mechanisms (Fig. 2). Furthermore, the original concept of an oncogene has taken on new meaning with the elucidation of somatic mutations and alternative splicing events that result in subtle or even gross differences in protein function. Despite these observations, a number of important oncogenic gene pathways have been elucidated over the past several decades. This suggests that cancer cells can become "addicted" to a finite number of gene pathways, and therefore the promise of more specific molecular assays and therapeutic modalities for prostate cancer appears to be emerging.

Table 1 summarizes promising molecular markers described in this review that are now being tested with biopsy cores or biofluids such as urine or semen in support of diagnostic utility. A molecular urine test for PCA3 has already been commercialized. This represents significant progress, however the field is expanding as new molecular markers become elucidated. Challenges for progressing such markers to commercialization can be considered in the context of the FDA's description of a "known valid biomarker."

"*Known* valid biomarker: *A biomarker that is measured in an analytical test system with well-established performance characteristics and for which there is widespread agreement in the medical or scientific community about the physiologic, toxicologic, pharmacologic, or clinical significance of the results* [99]."

From an assay standpoint, an important consideration is the ability to discriminate the biomarker signal against a background of non-target molecules and/or target molecules that are also expressed in normal tissues albeit at lower (or higher) levels. Robust assays require extensive optimization to achieve desired analytical sensitivity, specificity, and reproducibility. In addition, sample preparation is vitally important to the reproducibility of the test method. Taking these issues into consideration, it is important to understand the extent to which the candidate biomarker is uniquely expressed in the target tissue (or not), and/or the extent to which it is over (or under) expressed in the carcinoma to

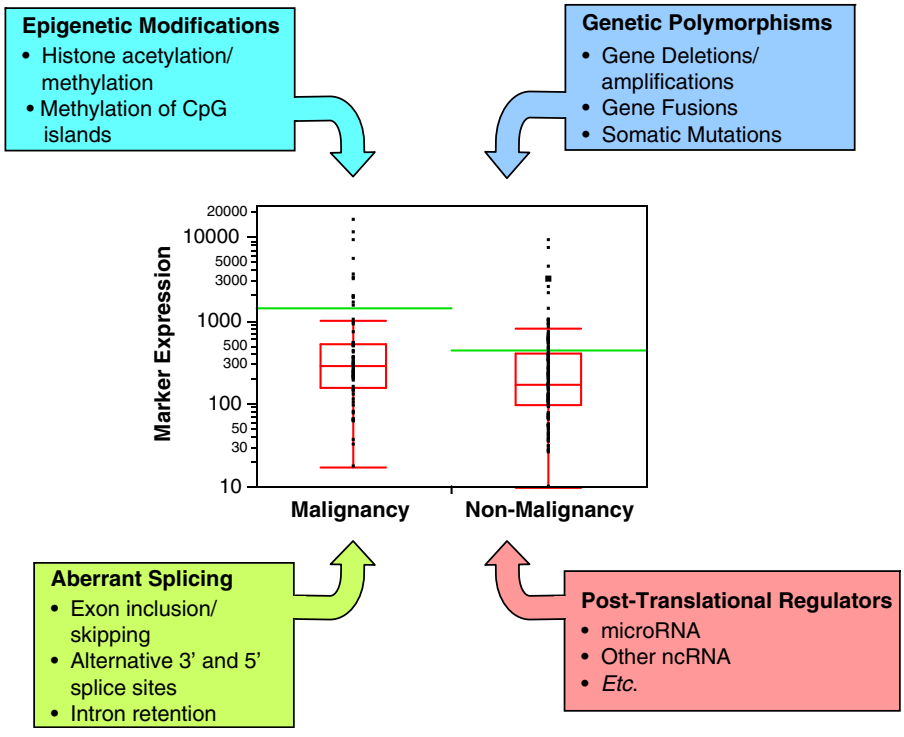

Fig. 2. Diagram illustrating potential molecular events leading to observable changes in gene expression.

Table 1
Examples of promising molecular markers in prostate cancer

Marker(s)	Disease association status	References
AMACR	Specific histochemical stain for biopsy cores	[10,11]
	Combination panel with PCA3 and ERG	[14]
PCA3	Molecular urine test for men with elevated serum PSA and previous negative biopsies	[88,102–104]
TMPRSS2:ERG	Marker of invasive prostate cancer pathology	[105]
	Potential diagnostic utility in urine sediments and combination with PCA3	[106,107]
GSTpi, APC, and EDNRB (DNA methylation marker panel)	Molecular urine test correlative with biopsy status	[108]

be detected. This becomes even more important when developing a test for the candidate biomarker in a biofluid such as urine or blood. PCA3 represents one example where a successful molecular urine test method has been developed and shown to have clinical utility in multiple clinical studies [42,88,102,103]. Its expression is relatively specific to the prostate, and it is over-expressed in prostate carcinomas by over 60-fold [104].

Once analytical performance has been achieved and demonstrated, the test method must then be investigated in multiple clinical studies in order to demonstrate reproducible clinical utility, as discussed in a previous review by this author [42]. The expense and time commitments required of such studies must be weighed against the anticipated probability of success; therefore it is important to understand the candidate biomarker from the standpoint of its underlying tumor biology.

The biological characterization of candidate biomarkers can also help drive medical acceptance, particularly as they become associated with carcinoma initiation and progression. This helps explain the growing interest in the TMPRSS2:Ets gene fusion markers discovered by Chinnaiyan and co-workers [32], which have been characterized as early (and potentially transforming) signals of prostate carcinoma. Moreover, certain splice variants of TMPRSS2:ERG gene fusions have been associated with aggressive disease [83].

Taking these issues into account, and considering the variety of mechanisms that can lead to gene expression differences, it should be appreciated that a detailed knowledge of epigenetic changes, genetic polymorphisms, somatic mutations and alternative splicing events will ultimately be needed in order to develop more specific diagnostic assays and therapeutic strategies. A number of molecular markers associated with prostate cancer have now been shown to arise through two or more of these mechanisms (see Table 2). Therefore, investigators are now resorting to orthogonal analytical approaches to elucidate the underlying molecular events leading to observed expression changes. For example, Lapointe and co-workers [14] originally used gene expression profiling to identify three distinct clinical subtypes of prostate cancer ranging from localized disease to metastatic disease. More recently, they applied array CGH analysis to identify recurrent gene deletion and amplification patterns within each subtype [100]. Subtype 2 tumors contained deletions in 8p21 (NKX3.1, as mentioned in a previous section) and 21q22 (resulting in TMPRSS2:ERG gene fusion), whereas subtype 3 tumors showed higher overall frequencies of both gains and deletions, including gains at 8q24 (MYC) and losses at 10q23 (PTEN). Such studies suggest that molecular staging of prostate cancer is feasible.

Table 2
Examples of genetic alterations associated with molecular markers of prostate cancer

Marker	Genetic alteration	References
Androgen receptor (AR)	Gene amplification (Xq12)	[23]
	Somatic mutations	[45,46]
KLF6	Gene deletion (10p15)	[27,28]
	Somatic mutations	[28]
	Alternative splicing	[81]
NKX3.1	Gene deletion (8p21)	[24]
	Hypermethylation	[65]
TMPRSS2:ERG	Gene fusion	[32,33,36–40]
	Alternative splicing	[41,83]
p53	Somatic mutations	[47,48]
	MicroRNA transactivation (miR-34a)	[101]

As stated above, some of the more commonly identified genes associated with prostate cancer have been shown to arise through multiple genetic events, including epigenetic changes, chromosomal alterations, and somatic mutations. Furthermore, characterization of alternative splicing could potentially provide more specific identification of prostate cancer phenotypes, since aberrantly spliced gene transcripts would not be expected from normal tissues. Based on these considerations, it is evident that further characterization of these complex molecular mechanisms will be needed in order to realize the vision of customized diagnosis and treatment for prostate cancer.

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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] E.P. Gelmann, O.J. Semmes, Expression of genes and proteins specific for prostate cancer, J. Urol. 172 (2004) S23–S26, discussion S26–S27.
[3] C.S. Cooper, C. Campbell, S. Jhavar, Mechanisms of disease: Biomarkers and molecular targets from microarray gene expression studies in prostate cancer, Nat. Clin. Pract. Urol. 4 (2007) 677–687.
[4] L. Bubendorf, M. Kolmer, J. Kononen, et al., Hormone therapy failure in human prostate cancer: analysis by complementary DNA and tissue microarrays, J. Natl. Cancer Inst. 91 (1999) 1758–1764.
[5] J. Xu, J.A. Stolk, X. Zhang, et al., Identification of differentially expressed genes in human prostate cancer using subtraction and microarray, Cancer Res. 60 (2000) 1677–1682.
[6] D.T. Ross, U. Scherf, M.B. Eisen, et al., Systematic variation in gene expression patterns in human cancer cell lines, Nat. Genet. 24 (2000) 227–235.
[7] U. Scherf, D.T. Ross, M. Waltham, et al., A gene expression database for the molecular pharmacology of cancer, Nat. Genet. 24 (2000) 236–244.
[8] J. Luo, D.J. Duggan, Y. Chen, et al., Human prostate cancer and benign prostatic hyperplasia: molecular dissection by gene expression profiling, Cancer Res. 61 (2001) 4683–4688.
[9] E. LaTulippe, J. Satagopan, A. Smith, et al., Comprehensive gene expression analysis of prostate cancer reveals distinct transcriptional programs associated with metastatic disease, Cancer Res. 62 (2002) 4499–4506.
[10] D. Singh, P.G. Febbo, K. Ross, et al., Gene expression correlates of clinical prostate cancer behavior, Cancer Cell 1 (2002) 203–209.
[11] M.A. Rubin, M. Zhou, S.M. Dhanasekaran, et al., Alpha-methylacyl coenzyme A racemase as a tissue biomarker for prostate cancer, JAMA 287 (2002) 1662–1670.
[12] C. Magi-Galluzzi, J. Luo, W.B. Isaacs, J.L. Hicks, A.M. de Marzo, J.I. Epstein, Alpha-methylacyl-CoA racemase: a variably sensitive immunohistochemical marker for the diagnosis of small prostate cancer foci on needle biopsy, Am. J. Surg. Pathol. 27 (2003) 1128–1133.
[13] P.S. Nelson, Predicting prostate cancer behavior using transcript profiles, J. Urol. 172 (2004) S28–S32, discussion S33.
[14] J. Lapointe, C. Li, J.P. Higgins, et al., Gene expression profiling identifies clinically relevant subtypes of prostate cancer, Proc. Natl. Acad. Sci. USA 101 (2004) 811–816.
[15] L. True, I. Coleman, S. Hawley, et al., A molecular correlate to the Gleason grading system for prostate carcinoma, Proc. Natl. Acad. Sci. USA 103 (2006) 10991–10996.
[16] 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.
[17] S. Shaheduzzaman, G. Petrovics, V. Srikantan, B. Furusato, I.A. Sesterhenn, D.G. McLeod, S. Srivastava, in: ETS-Related Gene (ERG), a Frequent Proto-Oncogene Expression Alteration in Prostate Cancer With Potentials in Diagnosis and Prognosis, ASCO Prostate Cancer Symposium, February 24–26, San Francisco, CA, USA, 2006.
[18] S. Nanni, C. Priolo, A. Grasselli, et al., Epithelial-restricted gene profile of primary cultures from human prostate tumors: A molecular approach to predict clinical behavior of prostate cancer, Mol. Cancer Res. 4 (2006) 79–92.
[19] W.B. Isaacs, Molecular genetics of prostate cancer, Cancer Surv. 25 (1995) 357–379.
[20] P.A. Koivisto, Molecular cytogenetics of prostate cancer, Crit. Rev. Oncog. 7 (1996) 143–150.
[21] J.T. Dong, W.B. Isaacs, J.T. Isaacs, Molecular advances in prostate cancer, Curr. Opin. Oncol. 9 (1997) 101–107.
[22] R.S. Verma, M. Manikal, R.A. Conte, C.J. Godec, Chromosomal basis of adenocarcinoma of the prostate, Cancer Invest. 17 (1999) 441–447.
[23] N.N. Nupponen, T. Visakorpi, Molecular cytogenetics of prostate cancer, Microsc. Res. Tech. 51 (2000) 456–463.
[24] J.T. Dong, Chromosomal deletions and tumor suppressor genes in prostate cancer, Cancer Metastasis Rev. 20 (2001) 173–193.
[25] T. Visakorpi, The molecular genetics of prostate cancer, Urology 62 (2003) 3–10.
[26] C. Lengauer, K.W. Kinzler, B. Vogelstein, Genetic instabilities in human cancers, Nature 396 (1998) 643–649.
[27] G. Narla, K.E. Heath, H.L. Reeves, et al., KLF6 a candidate tumor suppressor gene mutated in prostate cancer, Science 294 (2001) 2563–2566.
[28] C. Chen, E.R. Hyytinen, X. Sun, et al., Deletion, mutation, and loss of expression of KLF6 in human prostate cancer, Am. J. Pathol. 162 (2003) 1349–1354.
[29] D. Pinkel, D.G. Albertson, Array comparative genomic hybridization and its applications in cancer, Nat. Genet. 37 (Suppl.) (2005) S11–S17.
[30] P.L. Paris, A. Andaya, J. Fridlyand, et al., Whole genome scanning identifies genotypes associated with recurrence and metastasis in prostate tumors, Hum. Mol. Genet. 13 (2004) 1303–1313.

[31] W. Liu, B. Chang, J. Sauvageot, et al., Comprehensive assessment of DNA copy number alterations in human prostate cancers using affymetrix 100 K SNP mapping array, Genes Chromosomes Cancer 45 (2006) 1018–1032.
[32] S.A. Tomlins, D.R. Rhodes, S. Perner, et al., Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer, Science 310 (2005) 644–648.
[33] 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.
[34] 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.
[35] S.A. Tomlins, B. Laxman, S.M. Dhanasekaran, et al., Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer, Nature 448 (2007) 595–599.
[36] 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.
[37] 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.
[38] K. Iljin, M. Wolf, H. Edgren, et al., TMPRSS2 fusions with oncogenic ETS factors in prostate cancer involve unbalanced genomic rearrangements and are associated with HDAC1 and epigenetic reprogramming, Cancer Res. 66 (2006) 10242–10246.
[39] K.G. Hermans, R. van Marion, H. van Dekken, G. Jenster, W.M. van Weerden, J. Trapman, TMPRSS2:ERG fusion by translocation or interstitial deletion is highly relevant in androgen-dependent prostate cancer, but is bypassed in late-stage androgen receptor-negative prostate cancer, Cancer Res. 66 (2006) 10658–10663.
[40] S. Perner, J.M. Mosquera, F. Demichelis, et al., TMPRSS2-ERG fusion prostate cancer: an early molecular event associated with invasion, Am. J. Surg. Pathol. 31 (2007) 882–888.
[41] 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.
[42] M.A. Reynolds, K. Kastury, J. Groskopf, J.A. Schalken, H. Rittenhouse, Molecular markers for prostate cancer, Cancer Lett. 249 (2007) 5–13.
[43] N. Papadopoulos, K.W. Kinzler, B. Vogelstein, The role of companion diagnostics in the development and use of mutation-targeted cancer therapies, Nat. Biotechnol. 24 (2006) 985–995.
[44] L.D. Wood, D.W. Parsons, S. Jones, et al., The genomic landscapes of human breast and colorectal cancers, Science 318 (2007) 1108–1113.
[45] B.J. Feldman, D. Feldman, The development of androgen-independent prostate cancer, Nat. Rev. Cancer 1 (2001) 34–45.
[46] J.P. Elo, T. Visakorpi, Molecular genetics of prostate cancer, Ann. Med. 33 (2001) 130–141.
[47] D.J. Grignon, R. Caplan, F.H. Sarkar, et al., p53 status and prognosis of locally advanced prostatic adenocarcinoma: a study based on RTOG 8610, J. Natl. Cancer Inst. 89 (1997) 158–165.
[48] D.I. Quinn, S.M. Henshall, D.R. Head, et al., Prognostic significance of p53 nuclear accumulation in localized prostate cancer treated with radical prostatectomy, Cancer Res. 60 (2000) 1585–1594.
[49] L. Zheng, F. Wang, C. Qian, et al., Unique substitution of CHEK2 and TP53 mutations implicated in primary prostate tumors and cancer cell lines, Hum. Mutat. 27 (2006) 1062–1063.
[50] X. Wu, X. Dong, W. Liu, J. Chen, Characterization of CHEK2 mutations in prostate cancer, Hum. Mutat. 27 (2006) 742–747.
[51] D.A. Douglas, H. Zhong, J.Y. Ro, et al., Novel mutations of epidermal growth factor receptor in localized prostate cancer, Front. Biosci. 11 (2006) 2518–2525.
[52] O.G. Wong, T. Nitkunan, I. Oinuma, et al., Plexin-B1 mutations in prostate cancer, Proc. Natl. Acad. Sci. USA 104 (2007) 19040–19045.
[53] S.L. Thompson, G. Konfortova, R.I. Gregory, W. Reik, W. Dean, R. Feil, Environmental effects on genomic imprinting in mammals, Toxicol. Lett. 120 (2001) 143–150.
[54] P.A. Jones, S.B. Baylin, The epigenomics of cancer, Cell 128 (2007) 683–692.
[55] L.C. Li, P.R. Carroll, R. Dahiya, Epigenetic changes in prostate cancer: implication for diagnosis and treatment, J. Natl. Cancer Inst. 97 (2005) 103–115.
[56] J.R. Dobosy, J.L. Roberts, V.X. Fu, D.F. Jarrard, The expanding role of epigenetics in the development, diagnosis and treatment of prostate cancer and benign prostatic hyperplasia, J. Urol. 177 (2007) 822–831.
[57] S.K. Patra, A. Patra, R. Dahiya, Histone deacetylase and DNA methyltransferase in human prostate cancer, Biochem. Biophys. Res. Commun. 287 (2001) 705–713.
[58] K. Halkidou, L. Gaughan, S. Cook, H.Y. Leung, D.E. Neal, C.N. Robson, Upregulation and nuclear recruitment of HDAC1 in hormone refractory prostate cancer, Prostate 59 (2004) 177–189.
[59] C.B. Yoo, P.A. Jones, Epigenetic therapy of cancer: past, present and future, Nat. Rev. Drug Discov. 5 (2006) 37–50.
[60] S. Varambally, S.M. Dhanasekaran, M. Zhou, et al., The polycomb group protein EZH2 is involved in progression of prostate cancer, Nature 419 (2002) 624–629.
[61] S.B. Baylin, J.E. Ohm, Epigenetic gene silencing in cancer – a mechanism for early oncogenic pathway addiction?, Nat Rev. Cancer 6 (2006) 107–116.
[62] S. Yegnasubramanian, J. Kowalski, M.L. Gonzalgo, et al., Hypermethylation of CpG islands in primary and metastatic human prostate cancer, Cancer Res. 64 (2004) 1975–1986.
[63] D. Lodygin, A. Epanchintsev, A. Menssen, J. Diebold, H. Hermeking, Functional epigenomics identifies genes frequently silenced in prostate cancer, Cancer Res. 65 (2005) 4218–4227.
[64] 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.
[65] E. Asatiani, W.X. Huang, A. Wang, et al., Cancer Res. 65 (2005) 1164–1173.

[66] W.G. Nelson, S. Yegnasubramanian, A.T. Agoston, et al., Abnormal DNA methylation, epigenetics, and prostate cancer, Front. Biosci. 12 (2007) 4254–4266.
[67] M. Roy, Q. Xu, C. Lee, Evidence that public database records for many cancer-associated genes reflect a splice form found in tumors and lack normal splice forms, Nucleic Acids Res. 33 (2005) 5026–5033.
[68] B.J. Blencowe BJ, Alternative splicing: new insights from global analyses, Cell 126 (2006) 37–47.
[69] Q. Pan, O. Shai, C. Misquitta, et al., Revealing global regulatory features of mammalian alternative splicing using a quantitative microarray platform, Mol. Cell 16 (2004) 929–941.
[70] B.R. Graveley, Alternative splicing: increasing diversity in the proteomic world, Trends Genet. 17 (2001) 100–107.
[71] T. Maniatis, R. Reed, An extensive network of coupling among gene expression machines, Nature 416 (2002) 499–506.
[72] B.M. Brinkman, Splice variants as cancer biomarkers, Clin. Biochem. 37 (2004) 584–594.
[73] E. Buratti, M. Baralle, F.E. Baralle, Defective splicing, disease and therapy: searching for master checkpoints in exon definition, Nucleic Acids Res. 34 (2006) 3494–3510.
[74] M.J. Pajares, T. Ezponda, R. Catena, A. Calvo, R. Pio, L.M. Montuenga, Alternative splicing: an emerging topic in molecular and clinical oncology, Lancet Oncol. 8 (2007) 349–357.
[75] K. Jung, J. Reiche, A. Boehme, et al., Analysis of subforms of free prostate-specific antigen in serum by two-dimensional gel electrophoresis: potential to improve diagnosis of prostate cancer, Clin. Chem. 50 (2004) 2292–2301.
[76] I.P. Michael, L. Kurlender, N. Memari, et al., Intron retention: a common splicing event within the human kallikrein gene family, Clin. Chem. 51 (2005) 506–515.
[77] A. David, N. Mabjeesh, I. Azar, et al., Unusual alternative splicing within the human kallikrein genes KLK2 and KLK3 gives rise to novel prostate-specific proteins, J. Biol. Chem. 277 (2002) 18084–18090.
[78] S.F. Shariat, M.W. Kattan, S. Erdamar, et al., occult prostate cancer metastases in lymph nodes using a splice variant-specific RT-PCR assay for human glandular kallikrein, J. Clin. Oncol. 21 (2003) 1223–1231.
[79] B.K. Yeh, M. Igarashi, A.V. Eliseenkova, et al., Structural basis by which alternative splicing confers specificity in fibroblast growth factor receptors, Proc. Natl. Acad. Sci. USA 100 (2003) 2266–2271.
[80] R.P. Carstens, J.V. Eaton, H.R. Krigman, P.J. Walther, M.A. Garcia-Blanco, Alternative splicing of fibroblast growth factor receptor 2 (FGF-R2) in human prostate cancer, Oncogene 15 (1997) 3059–3065.
[81] G. Narla, A. Difeo, H.L. Reeves, et al., A germline DNA polymorphism enhances alternative splicing of the KLF6 tumor suppressor gene and is associated with increased prostate cancer risk, Cancer Res. 65 (2005) 1213–1222.
[82] M.E. Stearns, M. Wang, Y. Hu, G. Kim, F.U. Garcia, Expression of a flt-4 (VEGFR3) splicing variant in primary human prostate tumors. VEGF D and flt-4t(Delta773-1081) overexpression is diagnostic for sentinel lymph node metastasis, Lab. Invest. 84 (2004) 785–795.
[83] J. Clark, S. Merson, S. Jhavar, et al., Diversity of TMPRSS2-ERG fusion transcripts in the human prostate, Oncogene 26 (2007) 2667–2673.
[84] P. Kapranov, A.T. Willingham, T.R. Gingeras, Genome-wide transcription and the implications for genomic organization, Nat. Rev. Genet. 8 (2007) 413–423.
[85] J.S. Mattick, I.V. Makunin, Non-coding RNA, Hum. Mol. Genet. 15 Spec. No. 1 (2006) R17–R29.
[86] R. Louro, H.I. Nakaya, P.P. Amaral, et al., Androgen responsive intronic non-coding RNAs, BMC Biol. 5 (2007) 4.
[87] M.J. Bussemakers, A. van Bokhoven, G.W. Verhaegh, et al., DD3: a new prostate-specific gene, highly overexpressed in prostate cancer, Cancer Res. 59 (1999) 5975–5979.
[88] L.S. Marks, Y. Fradet, I.L. Deras, et al., PCA3 molecular urine assay for prostate cancer in men undergoing repeat biopsy, Urology 69 (2007) 532–535.
[89] R.W. Carthew, Gene regulation by microRNAs, Curr. Opin. Genet. Dev. 16 (2006) 203–208.
[90] G.A. Calin, C.M. Croce, MicroRNA-cancer connection: the beginning of a new tale, Cancer Res. 66 (2006) 7390–7394.
[91] G.A. Calin, C.M. Croce, MicroRNA signatures in human cancers, Nat. Rev. Cancer 6 (2006) 857–866.
[92] R. Garzon, M. Fabbri, A. Cimmino, G.A. Calin, C.M. Croce, MicroRNA expression and function in cancer, Trends Mol. Med. 12 (2006) 580–587.
[93] B. Zhang, X. Pan, G.P. Cobb, T.A. Anderson, MicroRNAs as oncogenes and tumor suppressors, Dev. Biol. 302 (2007) 1–12.
[94] G.A. Calin, C.M. Croce, Chromosomal rearrangements and microRNAs: a new cancer link with clinical implications, J. Clin. Invest. 117 (2007) 2059–2066.
[95] J. Lu, G. Getz, E.A. Miska, et al., MicroRNA expression profiles classify human cancers, Nature 435 (2005) 834–838.
[96] S. Volinia, G.A. Calin, C.G. Liu, et al., A microRNA expression signature of human solid tumors defines cancer gene targets, Proc. Natl. Acad. Sci. USA 103 (2006) 2257–2261.
[97] M.D. Mattie, C.C. Benz, J. Bowers, et al., Optimized high-throughput microRNA expression profiling provides novel biomarker assessment of clinical prostate and breast cancer biopsies, Mol. Cancer 5 (2006) 24.
[98] K.P. Porkka, M.J. Pfeiffer, K.K. Waltering, R.L. Vessella, T.L. Tammela, T. Visakorpi, MicroRNA expression profiling in prostate cancer, Cancer Res. 67 (2007) 6130–6135.
[99] FDA draft guidance document, "Guidance for Industry – Pharmacogenomic Data Submissions", March 2005.
[100] J. Lapointe, C. Li, C.P. Giacomini, et al., Genomic profiling reveals alternative genetic pathways of prostate tumorigenesis, Cancer Res. 67 (2007) 8504–8510.
[101] T.C. Chang, E.A. Wentzel, O.A. Kent, et al., Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis, Mol. Cell 26 (2007) 745–752.
[102] J. Groskopf, S.M.J. 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.
[103] L.J. Sokoll, W. Ellis, P. Lange, et al., A multicenter evaluationand of the PCA3 molecular urine test: pre-analytical effects, analytical performance, and diagnostic accuracy, Clin. Chem. Acta 389 (2008) 1–6.
[104] D. Hessels, J.K. Gunnewiek, I. van Oort, et al., DD3(PCA3) based molecular urine analysis for the diagnosis of prostate cancer, Eur. Urol. 44 (2003) 8–15.

[105] S. Perner, M.-M. Mosquera, F. Demichelis, et al., TMPRSS2-ERG fusion prostate cancer: an early molecular event associated with invasion, Am. J. Surg. Pathol. 31 (2007) 882–888.

[106] B. Laxman, S.A. Tomlins, R. Mehra, et al., Noninvasive detection of TMPRSS2:ERG fusion transcripts in the urine of men with prostate cancer, Neoplasia 8 (2006) 885–888.

[107] D. Hessels, F.P. Smit, G.W. Verhaegh, et al., Detection of TMPRSS2-ERG fusion transcripts and prostate cancer antigen 3 in urinary sediments may improve diagnosis of prostate cancer, Clin. Cancer Res. 13 (2007) 5103–5108.

[108] C.G. Rogers, M.L. Gonzalgo, G. Yan, et al., High concordance of gene methylation in post-digital rectal examinations and post-biopsy urine samples for prostate cancer detection, J. Urol. 176 (2006) 2280–2284.