Review

Genomic Profiling Defines Subtypes of Prostate Cancer with the Potential for Therapeutic Stratification

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Abstract

The remarkable variation in prostate cancer clinical behavior represents an opportunity to identify and understand molecular features that can be used to stratify patients into clinical subgroups for more precise outcome prediction and treatment selection. Significant progress has been made in recent years in establishing the composition of genomic and epigenetic alterations in localized and advanced prostate cancers using array-based technologies and next-generation sequencing approaches. The results of these efforts shed new light on our understanding of this disease and point to subclasses of prostate cancer that exhibit distinct vulnerabilities to therapeutics. The goal of this review is to categorize the genomic data and, where available, corresponding expression, functional, or related therapeutic information, from recent large-scale and in-depth studies that show a new appreciation for the molecular complexity of this disease. We focus on how these results inform our growing understanding of the mechanisms that promote genetic instability, as well as routes by which specific genes and biologic pathways may serve as biomarkers or potential targets for new therapies. We summarize data that indicate the presence of genetic subgroups of prostate cancers and show the high level of intra- and intertumoral heterogeneity, as well as updated information on disseminated and circulating tumor cells. The integrated analysis of all types of genetic alterations that culminate in altering critical biologic pathways may serve as the impetus for developing new therapeutics, repurposing agents used currently for treating other malignancies, and stratifying early and advanced prostate cancers for appropriate interventions. *Clin Cancer Res; 19(15); 4058–66. ©2013 AACR.*

Introduction

Prostate cancer is the second most commonly diagnosed cancer among men in the United States, with more than 240,000 cases reported annually. These carcinomas exhibit a remarkable diversity in behavior ranging from decades of indolence to rapid growth, dissemination, and lethality. Although pathologic grading provides a powerful indicator of disease behavior, clinical outcomes of tumors with the same histologic patterns can vary substantially. While significant morbidity results from the overtreatment of indolent tumors, delayed diagnosis and undertreatment of aggressive malignancies contribute to an excess of 30,000 deaths per year from metastatic prostate cancers. A better understanding of the genetic and molecular characteristics defining indolent and lethal prostate cancers is key for improved patient stratification and selection of optimal therapies.

This review focuses on the field of prostate cancer genomics, highlighting chromosomal alterations that may drive cancer behavior and serve as biomarkers to guide future therapeutic directions. Genomic studies have recently strengthened our understanding of prostate cancer by clarifying (i) the frequency, types, and mutation characteristics in prostate cancer relative to other cancers; (ii) the progression of genomic alterations during disease evolution; and (iii) tumor heterogeneity and clonality. Collectively, these studies indicate that integrated analyses of genetic aberrations, changes in gene expression, and resulting contributions to biologic functions are necessary to understand the key features underlying prostate cancer behavior.

The Mutational Landscape of Prostate Cancer

Prostate cancer is characterized by extraordinary genomic complexity (1, 2), including somatic copy number alterations, point mutations, and structural rearrangements. Advanced prostate cancer may be aneuploid or have large regions of copy-neutral LOH (cnLOH; ref. 3). Recent advances that collectively involve detailed analyses of hundreds of primary and metastatic prostate cancers now provide a clearer picture of genomic aberrations that accompany indolent and aggressive disease.

Somatic copy number alterations

Somatic copy number alterations (SCNA) are genetic gains or losses that arise during cancer development. They

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are evident in nearly 90% of primary prostate tumors, with deletions typically outnumbering amplifications. These SCNAs tend to be focal ($\leq$1–5 Mb), affecting only a small portion of the genome (4, 5). Metastatic prostate tumors, however, display dozens to hundreds of aberrations, which can affect a large portion of the genome. This difference suggests increased genomic instability as the disease progresses. A recent detailed comparison of SCNAs among cancer types determined that prostate cancer displayed more SCNAs (averaging 46 per sample) than most of the other 26 cancer types (4). Frequent deletions are seen on chromosomes 6q, 8p, 10q, and 13q and include genes such as *NKX3-1*, *PTEN*, *BRCA2*, and *RB1*. Castration-resistant metastatic tumors show frequent amplification of chromosomes X, 7, 8q, and 9q, which include the androgen receptor (*AR*) and *MYC* oncogenes. Table 1 summarizes the most frequent SCNAs in different stages of prostate cancer development.

Clinically, detection of prostate SCNA from alternative tissue sources is of great current interest, as the success rate for prostate biopsy is only 60% to 70% even with computed tomographic (CT) guidance. Circulating and disseminated tumor cells (CTC and DTC) in the blood and bone marrow present an opportunity for repeated testing. The difficulties lie in their rare numbers and complicated techniques for isolation. Nevertheless, new methods promise new results. Genomic profiling of DTCs from patients with advanced disease showed a large number of SCNAs, mostly concordant with corresponding metastases and previous tumors (Table 1; refs. 6, 7), although DTCs from men with localized disease generally have fewer SCNAs, which may not correspond well with the primary tumor SCNAs.

Structural rearrangements

Double-stranded breaks can occur when DNA unwinds during replication or transcription. Improper repair of these breaks can result in intra- and interchromosome rearrangement. Almost 50% of all primary prostate tumors have *TMPRSS2:ERG* rearrangement, which places the growth-promoting activity of the *ERG* oncogene under the control of the regulatory elements of androgen-responsive *TMPRSS2* (8). Rearrangements can also result in new fusion proteins that are constitutively active or have altered function or cellular localization, as in the example of *ESRP1:CRAF* rearrangement (3). Several other rearrangements have been described for prostate cancer, including other *ETS* family rearrangements (9, 10) and *RAF* kinase gene fusions (11) as reviewed previously (12).

Although *ERG* rearrangement does not affect the overall frequency of SCNAs, it is associated with deletions of 10q, 17p, and 3p14 (5). These tumors have a distinct expression signature (8, 13). Tumors without *ERG* rearrangement are significantly enriched for 6q deletion, 7q gain, and 16q deletion (5).

Paired-end whole-genome sequencing suggests that rearrangements are much more common and complex than previously appreciated and implies the importance of surrounding chromatin structure (12, 14). Sequencing of pri-

mary tumors from patients with "high-risk" prostate cancer showed a median of 90 rearrangements, often complex, per tumor genome. Moreover, breakpoints in *TMPRSS2:ERG* rearranged tumors were precise and located in accessible chromatin that was enriched in transcription factors associated with androgen-regulated transcription (14). In contrast, in tumors without *TMPRSS2:ERG* rearrangement, breakpoints were located in transcriptionally repressed chromatin.

Point mutations

Primary prostate cancer has a somatic mutation rate of 1×10^{-6} to 2×10^{-6}, similar to breast, renal, and ovarian cancers (15–17). Although several thousand mutations may exist in each prostate tumor genome, only ~20 per genome are likely to affect protein stability or function. However, mutation of the DNA mismatch repair enzyme *MSH6* is associated with a hypermutator phenotype (5, 17–19), resulting in 25-fold more mutations than normally seen in prostate cancer. Mutations of common tumor suppressor genes, including *TP53*, *PTEN*, *RB1*, and *PIK3CA*, have also been defined in prostate cancer (15, 18, 20, 21), as have activating mutations in the oncogenes *KRAS* and *BRAF*. Additional recurrent mutations are detected in factors that mediate AR function, chromatin modification, and transcription. These are detailed below.

A new molecular subtype of prostate cancer has been suggested as defined by *SPOP* mutations (refs. 15, 18; Fig. 1). Point mutations at evolutionarily conserved residues of the substrate-binding cleft of this E3-ubiquitin ligase subunit were identified in up to 13% of primary tumors. *SPOP* mutations were enriched in tumors with somatic deletions of 5q21 and 6q21, which encode genes including the chromatin-modifying enzyme *CHD1*, the tumor suppressor *PRDM1*, and the transcription factor *FOXO3*. However, these tumors did not display *ETS* rearrangement or mutations in *TP53*, *PTEN*, and *PIK3CA*. *SPOP* mutations have recently been shown to influence the stability of the SRC3/NCOA3 protein and alter AR signaling in prostate cancer cells (22).

Integrating Genetic Information to Identify Novel Therapeutic Targets

As the spectrum of genetic aberrations becomes increasingly more complex in prostate cancer, integrated analysis of genetic aberrations, epigenetics, transcriptional regulation, and expression profiles is necessary to understand the molecular pathways that contribute to tumorigenesis. Results from such integrated approaches are now poised to define key targets for future prostate cancer therapeutics.

Androgen signaling pathway

Because the growth of prostate cancer is largely dependent on androgens, therapies blocking the AR signaling pathway are effective for most patients. However, several mechanisms can restore AR signaling and promote the development of castration-resistant metastatic disease (CRPC). These mechanisms include *AR* amplification,

Table 1. Most common SCNAs in human prostate cancer

Cytoband	Event	Size, Mb	Genes of interest	Reported frequency in prostate cancer[a]		
				Primary cancer	Advanced cancer	DTCs or CTCs
Xp11.22-q13.1	Gain	18–67.8	**AR**		50% CRPC (15, 24, 25, 68)	45% AdvDTC (6, 7, 69)
1p12-q43	Gain	117			45%–65% CRPC (24)	50% (69)
1q32.1-q32.3	Gain	12.50	ELK4, PTPRC, **ELF3**, PTPN7, MDM4, RAB7L1, RASSF5, IL24, IL10, CAMK1G	(5)	(24, 70)	45% AdvDTC
3q26.1	Gain	43.80	GMPS, **PIK3CA**, MLF1, SKIL, CCNL1, ECT2	13%–39% (5, 71)	(24, 70)	20% (7)
6q14.3-15	Loss	13.67	CYB5R4, NT5E, SNX14, SYNCRIP, HTR1E, CGA, GJB7	40% (5, 18, 64, 72, 73)	55% (64, 70, 72, 25)	25% (6, 7)
7p22.3-q36.3	Gain	158.40		(5, 18)	25%–55% CRPC (68, 70, 72, 25)	40% (6, 7)
8p12-q24.3	Gain	97.64	**MYC**, **MAF**, EYA1, MSC, TRPA1, KCNB2	20%–30% (5, 18, 64, 71–73, 74)	64%–82% CRPC (15, 62, 68, 70, 72)	50%–65% (6, 7, 69)
8p23-p11	Loss	19.58	**NKX3-1**	53%–67% (5, 18, 71–73, 74)	67%–74% of CRPC (15, 68, 72)	36%–90% of AdvDTCs; 20–23% LocDTCs (6, 7, 69)
9q31.3	Gain	22.79	PTPN3, AKAP2, DAPK1, SYK	(5)	30% (70, 72)	25%–45% AdvDTC (6, 7, 69)
10p13	Loss	1.12	ITGA8, PTER, C1QL3, RSU1	18% (64, 70, 73)	(25)	(6)
10q11.21	Loss	0.58	RET, RasGEF1A, HNRNPF, ZNF239, ZNF485, ZNF32	(64, 73)		55% AdvDTC (6)
10q22-q24	Loss	24.91	CFLP1, KILLIN, **PTEN**, RNLS, LIPJ, LIPF, LIPK, LIPN, LIPM, ANKRD22, STAMBPL1, ACTA2	12%–25% (5, 18, 62, 64, 72–74)	36%–80% (15, 62, 68, 70, 25)	36% (69)
11p13-p12	Loss	4.72		(4, 6, 64, 70, 73)	(25)	45% AdvDTC (6)
12p13	Loss	1.46	BCL2L14, LRP6, MANSC1, LOH12CR1, DUSP16, CREBL2, GPR19, **CDKN1B**, **ETV6**	30% (5, 18)	30%–50% (4, 64, 70, 72)	
13q12.3-q14.2	Loss	2.63	HSPH1, B3GALTL, **BRCA2**, N4BP2L1, CG030, PDS5B, KL, STARD13, EXOSC8, FAM48A, CSNK1A1L, POSTN, TRPC4, UFM1, FREM2, KBTBD6, KBTBD7, MTRF1, NAA16, OR7E37P, C13ORF15, SPERT, SIAH2, **RB1**, **FOXO1**	11%–40% (5, 18, 62, 64, 71–73, 74)	35%–95% mets (15, 62, 64, 68, 70, 72, 25)	21%–44% of LocDTC; 36%–55% AdvDTC (6, 69)
15q25.1-q26.3	Loss	21.30		(64)	40% CRPC	20–25% (7)
16q11.2-q24.3	Loss	33.56	WWOX	33%–38% (5, 18, 64, 71, 73, 74)	57%–82% (72)	33% (7)
17p13.1	Loss	4.28	RPAIN, AIPL1, XAF, DLG4, PER1, **TP53**	20%–30% (5, 18, 64)		
17p13.3-p11.2	Loss	19.50		30% (64, 73)	51%–61% CRPC (24)	
17q21.31	Loss	0.15	DHX8, **ETV4**	20% (18)		
17q24.2-q25.3	Loss	8.90			12%–41% CRPC (24, 70)	
18q22.3	Loss	0.29	CBLN2, NETO1	20%–25% (5, 18, 71, 73)	40% (24, 72)	
21q22.3	Loss	0.25	**ERG**, NCRNA00114, ETS2, PSMG1, BRWD1, HMGN1, WRB, LCA5L, SH3BGR, C21orf88, B3GALT5, IGSF5, PCP4, DSCAM, C21orf130, BACE2, PLAC4, FAM3B, MX2, MX1, **TMPRSS2**	33%–50% (5, 8, 18, 73, 74)	33% (25)	50% (7)

NOTE: SCNA regions are listed in chromosomal order. Well-characterized cancer genes are in bold. References are indicated for reported frequencies of SCNAs. In general, only SCNAs with a frequency >40% in at least one cancer category are listed. Size is based on reported results and indicates the broader region of overlap across studies. Actual size reported in individual samples may vary, especially for studies using recently developed technologies such as high-density single-nucleotide polymorphism comparative genomic hybridization (CGH) arrays and next-generation sequencing that permit a greater limit of resolution. In general, SCNAs are smaller in primary tumors than those observed in metastases and may only cover a portion of the region listed.

[a]Numbers in parentheses correspond with studies cited as references in this article.
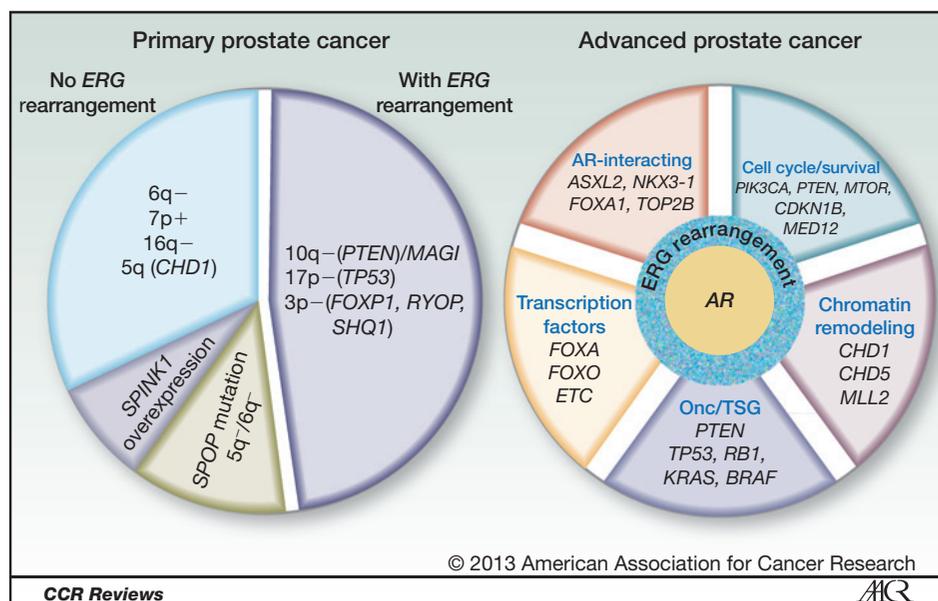
Figure 1. Relationships of common genomic alterations identified in localized and advanced in prostate cancer. Approximately 50% of both primary and advanced prostate cancers harbor *ERG* gene fusion or other *ETS* family gene rearrangement. Primary prostate cancers without *ERG* rearrangement can be subclassified on the basis of *SPINK1* overexpression (10), *SPOP* mutations, and select SCNAs, which are often mutually exclusive. The size of the pie chart pieces on the left represents approximate frequencies of each subgroup. Nearly all advanced-stage prostate cancers have amplification or mutation of *AR* or abnormalities of other AR pathway components. However, the genetic complexity associated with most advanced-stage prostate cancers precludes their classification into distinct subgroups based on genetic profiles. Among this complexity exist a number of commonly observed genetic aberrations, as shown. Metastatic tumors may display some or all of these aberrations. Therefore, the pie chart pieces on the right simply help define functional groups of various genes with mutations observed in advanced prostate cancer, without indication for frequencies or mutual exclusivity. See text for detail. Onc/TSG, oncogenes and tumor suppressor genes.

gain-of-function *AR* mutations, splice variants, and over-expression of *AR* or its coactivators. *AR* amplification is observed in metastases from approximately 50% of patients and can occur through focal amplification (23) or through gain of the entire X-chromosome, on which *AR* resides (5, 24). *AR* is also frequently mutated in advanced disease (5, 15, 25). The oncogenic H874Y *AR* mutation increases the binding affinity of AR for testosterone (26). Additional mutations in the ligand-binding domain (K580R, T877A, L701H, and V715M) permit inappropriate AR activation by other steroid hormones such as estrogens, progestin, and glucocorticoids (27). A new *AR* mutation, F876L, confers resistance to the potent AR antagonist, MDV3100 (28), attesting to the plasticity of the prostate cancer genome in responding to selective therapeutic pressures.

Beyond AR itself, other components of the AR signaling pathway are altered in up to half of primaries and nearly all metastases, indicating the critical nature of this pathway to prostate cancer at all developmental stages (5). The oncogenic transcriptional coactivator *NCOA2*, on 8q13.3, is amplified in 24% of metastases and 1.9% of primary tumors and correlates with elevated *NCOA2* transcripts. Overexpression of NCOA2 primes AR to respond to reduced androgen levels and boosts the total magnitude of AR transcriptional response. Mutations in the Ser/Thr-rich regulatory domain and the transcriptional activation domain of *NCOA2* are also frequent. Clinically, *NCOA2* may be an important AR pathway biomarker in primary prostate can-

cer as noncastrate patients who had *NCOA2* alterations showed significantly more recurrences (29).

Androgen signaling can also promote corecruitment of AR with topoisomerase IIβ (TOP2B) to DNA, resulting in TOP2B-mediated double-stranded breaks and rearrangements, including *TMPRSS2:ERG* (30). Furthermore, in response to genotoxic stress—as may be experienced by cells during radiation or other anticancer therapeutics—AR recruits the enzymes AID (activation-induced cytidine deaminase) and the LINE-1–encoded ORF2 endonuclease (31), which may also contribute to formation of rearrangements. Given that chromosomes form three-dimensional "transcriptional hubs" that simultaneously coordinate the chromatin structure and transcriptional activity of multiple genes (32), transcriptional hubs may facilitate rearrangements of genes that spatially colocalize when errors in DNA processing cause DNA breaks in response to androgen signaling (33). These new factors warrant further investigation.

Transcription factors and chromatin modifiers

Transcription factors and chromatin modifiers work cohesively to mediate sequence-specific chromatin modifications that regulate gene expression. They play important roles in embryonic stem cells and cellular differentiation and are altered in many cancer types, including prostate cancer. Given the potentially broad effects of alterations in chromatin structure and transcriptional regulation,

disruptions in these genes may have extensive effects. These global changes may contribute to the high degree of genetic instability that is characteristic of metastatic prostate cancer, perhaps through increasing accessibility of DNA to factors that induce DNA breaks or by altering three-dimensional chromatin structures and interactions. Most notable are recurrent aberrations in the chromodomain helicase DNA-binding (CHD) proteins and FOXA and FOXO transcription factor families. Nevertheless, their contribution to prostate cancer oncogenesis is not fully understood to date.

Alterations in *CHD* genes have been commonly detected in primary and metastatic prostate cancer and may distinguish a new subgroup of prostate cancers with increased aggressiveness. CHD proteins function in ATP-dependent chromatin remodeling. *CHD1* on 5q21 is disrupted by focal deletions or mutations in up to 17% of all prostate tumors (14, 15, 34). Intragenic breakpoints in *CHD1* yield truncated proteins (14). Knockdown of *CHD1* in prostate cancer cell lines has been associated with morphologic changes and increased cell invasiveness (34). CHD1 deficiency is strongly correlated with lack of *ETS* family gene rearrangements and may represent a novel subclass of aggressive prostate cancer (15, 18). Another CHD protein, CHD5, is a tumor suppressor whose expression is altered in several solid tumor types by focal deletions, mutations, or DNA methylation. *CHD5* mutations have been detected in multiple prostate tumors (25). Loss of *CHD5* correlates with increased proliferation and decreased apoptosis via the p53 pathway (35–37). Finally, the H3/K4-specific methyltransferase gene *MLL2* is also frequently mutated in prostate cancer, as has previously been seen in other cancer types (15, 38, 39).

The forkhead box protein A (FOXA) and O (FOXO) families belong to the larger group of highly conserved forkhead proteins, which are deregulated in several tumor types (40.) FOXO and FOXA members are transcription factors that bind to AR and regulate its association with androgen response elements (15, 41). However, their roles in prostate cancer appear to be diverse based on frequent amplification or activation of *FOXA1* but loss of *FOXO* in tumors. FOXA1 is required for prostate epithelial cell differentiation and promotes proliferation (42). Focal amplifications of *FOXA1* and mutations in the transactivation and DNA-binding forkhead domains have been reported in approximately 10% of prostate cancer cases (5, 15, 18, 25). Increased expression of *FOXA1* correlates with prostate-specific antigen (PSA) and Gleason score and is associated with biochemical recurrence and poor prognosis (43). FOXA1 likely functions by repressing AR signaling, thereby leading to dedifferentiated tumors that are more aggressive and have a higher risk for metastatic relapse. In prostate cancer cell lines, increased FOXA1 activity promotes proliferation, tumorigenesis, and xenograft growth (15).

FOXO proteins have tumor suppressor activity and control the transcription of genes involved in metabolism, stress response, cell-cycle arrest, cell death, and DNA damage repair. In prostate cancer, FOXO1 and FOXO3A are inhibited by AKT-mediated phosphorylation, resulting in their nuclear exclusion and ubiquitin-mediated degradation (44). Deletion of *FOXO1* on 13q14 has been observed in approximately one third of prostate cancer cell lines, primary tumors, and xenografts (45). Loss of *FOXO1* increases the basal activity of AR and sensitizes it to lower androgen levels or other nonandrogenic ligands (46). FOXO1 also inhibits the transcriptional activity of Runx2, a transcription factor that contributes to prostate cancer cell migration, invasion, and metastasis (47). Restoration of FOXO3A activity in cancer cell lines sensitizes them to radiation (48), suggesting that combination of radiation with therapies that increase FOXO3A activity might be beneficial.

Phosphoinositide 3-kinase and AKT signaling pathways

Phosphoinositide 3-kinase (PI3K) pathway is a critical regulator of cell survival and proliferation. In prostate cancer, aberrant activation of the PI3K pathway is associated with higher Gleason grades, earlier recurrence, and a higher risk of cancer-specific mortality (49). Up to 50% of primary prostate tumors and 100% of metastases have aberrant expression, SCNAs, or mutations in PI3K pathway members.

The major route for PI3K pathway activation is via the loss of PTEN's tumor suppressor function as a result of *PTEN* copy number loss, inversions, or mutation (5, 14, 25). Focal deletion of *PTEN* or aneuploidy of chromosome 10 is present in nearly half of primary prostate cancers and all metastatic tumors (5, 50), whereas inactivating mutations of *PTEN* account for 5% to 10% of primary cancers (18). In addition, loss-of-function mutations and rearrangements in a PTEN-associating protein, MAGI (membrane-associated guanylate kinase inverted) have been detected in prostate cancer (14). MAGI enhances PTEN's ability to suppress AKT activation. Besides PTEN, several other phosphatases can regulate AKT activity, including PHLPP and INPP4B. Deletion or loss of their expression is correlated with higher Gleason score and shorter time to biologic recurrence (51, 52).

Activation of the PI3K pathway can also occur following oncogenic activation or amplification of *PIK3CA*, *AKT1*, and *MTOR* (25). *PIK3CA* on 3q26.32 encodes the catalytic subunit of PI3K. Amplification of *PIK3CA* has been detected in 13% to 39% of primary tumors and up to 50% of CRPCs (53, 54). Activating mutations in *PIK3CA* and *MTOR* have also been detected in about 4% of primary prostate tumors (18, 25, 54).

Clonal Heterogeneity

The high level of genetic heterogeneity within and across prostate tumor foci likely contributes to a tumor's ability to develop therapeutic resistance. Pathologically distinct tumor foci have few commonly shared mutations, supporting the largely independent clonal origin of each neoplastic region (55). Even among different sites within a tumor focus, unique mutation profiles are observed. These results are concordant with the high degree of intratumoral heterogeneity that has been characterized in breast, kidney, and

myeloproliferative cancers (56–59). Meanwhile, the shared pattern of aberrations in patients' metastatic tumors supports a monoclonal origin of metastasis and may indicate aberrations that are important to the progression of prostate cancer (23, 25). However, metastases also accumulate SCNAs that are unique, particularly in lymph node, brain, and bone metastases (23). These may either be passenger events that do not influence disease progression or may result from tissue-specific selection pressures.

Given the high levels of heterogeneity, integrated analysis of biologic pathways that are altered in prostate cancer will be critical. Integrated analysis of genetic and transcription data has revealed new pathways in glioblastoma (60) and is promising to do so in prostate cancer. Recently, Taylor and colleagues found that three well-known pathways, PI3K, RAS/RAF, and RB1, are altered in 34% to 43% of primary tumors and 74% to 100% of metastases (5). Although RNA expression could not predict recurrence, they found that DNA copy-number profiling was significantly associated with outcomes. They also identified a separate group of patients whose tumors did not carry any major SCNA or

aneuploidy and who remained largely free of recurrence at 5 years. Thus, genomic analyses may also identify patients who are good candidates for active surveillance.

Conclusions and Future Studies

The use of genomic profiling to identify robust subtypes of prostate cancer lags far behind efforts in other cancer types, where the molecular subclassification has improved our clinical ability to predict patients' overall risk and response to treatments. Examples include *HER2* in breast cancer, *BRAF* in melanoma, and *KRAS* and *EGFR* in lung cancer. Thus, it is imperative that future studies correlate clinical data with molecular and genetic classification of cancer samples. With the high prevalence of aberrations in the AR and PI3K/AKT signaling pathways, additional treatments targeting these signaling programs are in order. Factors that regulate chromatin, epigenetics, and transcription have also emerged as highly significant and deserve further investigation (61). Ideally, targeted therapy based on key perturbed pathways, as illustrated in Fig. 2, can be tested in prospective clinical trials.

Figure 2. Considerations for targeted therapy based on key pathways perturbed in prostate cancer. Current standard of care involves active surveillance for low-risk localized prostate cancers; hormonal therapy, radical prostatectomy, or radiation therapy for higher-risk localized disease; and androgen pathway suppression for metastatic disease with chemotherapy and immunotherapy at the time of disease progression. This figure shows the potential for targeted therapy in molecularly defined subtypes of prostate cancer. Genomic alterations are classified on the basis of the class of molecular pathways affected (inner circle). Therapeutic agents (outer circle) targeting respective pathways are grouped with the genes (middle circle) commonly altered in these pathways, coordinated by color wherever possible. Selected agents in various phases of clinical trials are superscripted: [a]FDA-approved, [b]phase III clinical trials, [c]phase I/II clinical trials; preclinical development not marked. Although the antiandrogen therapy abiraterone, the microtubule inhibitor cabazitaxel, and the immunotherapy sipuleucel-T are already in clinical use, aberrations of *NCOA2* and *FOXA1* genes (white) are recent findings, the functional significance and therapeutic implications of which await further investigation. HDACi, histone deacetylase inhibitor.

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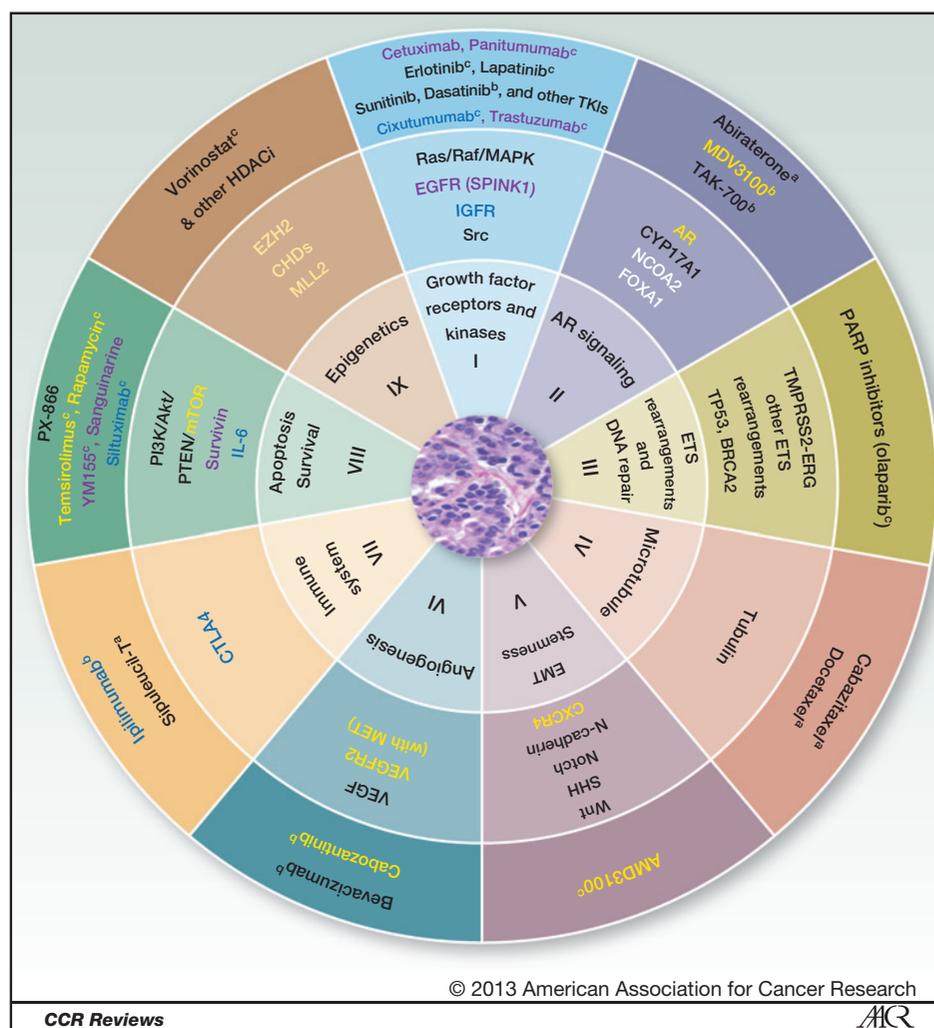
An underlying question is how SCNAs, particularly heterozygous deletions and low copy number amplifications, affect expression of the genes located within the affected region and cause indirect effects on other genes. Kim and colleagues show a modest correlation of copy number with gene expression; approximately 38% of amplified genes had concordant increases in expression (62). The area of cnLOH also warrants further attention, which can only be detected through next-generation sequencing approaches or by genomic arrays incorporating single-nucleotide polymorphism markers (63). Large cnLOH is typically associated with homozygous mutations of gene(s) residing in the respective sequence.

Additional meta-analyses of existing genetic information may help identify aberrations that work synergistically to promote tumorigenesis. In a limited example involving five metastatic tumors, all 19q13.32 losses occurred in the presence of 1p22.1 loss, whereas 17q21.31 loss occurred with 18q22.3 loss, and 21q22.3 loss with 16q23.1 loss (64). Results such as these point to common regulation such as through colocalization in three-dimensional space.

An important question that must be addressed centers on the molecular heterogeneity within and between primary prostate cancer foci and discrete metastasis. Developing approaches to assess distinct clones will have important implications for anticipating response and resistance to targeted therapeutics. Furthermore, sampling of multiple metastatic sites for genomic analyses poses technical and safety challenges. Enumeration of CTCs and DTCs has been shown to predict risk of relapse and quantifies patients' treatment responses (65, 66). Building on these assessments of CTC numbers, technologic advances now allow for the direct molecular profiling of these populations on a single-cell basis. Results such as these could provide a view of the heterogeneity of

a patient's tumor burden and has the advantage of resampling over the course of disease. Direct sequencing of circulating cell-free DNA offers another avenue for identifying and monitoring genomic alterations that could influence therapy selection (67).

In closing, rapidly expanding technologies and declining costs for genomic analysis are providing insights into the genetic underpinnings of prostate cancer at a rate faster than ever before. As additional studies are undertaken and new gene candidates emerge, putative driver events will be evaluated as therapeutic targets. With more novel therapies tested and approved, determining the best approach to handle genetic heterogeneity among patients will be a top research priority.

Disclosure of Potential Conflicts of Interest

P. Nelson is a consultant/advisory board member for Johnson & Johnson and Astellas. No potential conflicts of interest were disclosed by the other authors.

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Conception and design: J.R. Schoenborn, M. Fang
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.R. Schoenborn, P. Nelson, M. Fang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.R. Schoenborn, M. Fang
Writing, review, and/or revision of the manuscript: J.R. Schoenborn, P. Nelson, M. Fang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.R. Schoenborn, M. Fang
Study supervision: M. Fang

References

1. Barbieri CE, Demichelis F, Rubin MA. Molecular genetics of prostate cancer: emerging appreciation of genetic complexity. Histopathology 2012;60:187–98.
2. Beltran H, Yelensky R, Frampton GM, Park K, Downing SR, Macdonald TY, et al. Targeted next-generation sequencing of advanced prostate cancer identifies potential therapeutic targets and disease heterogeneity. Eur Urol 2013;63:920–6.
3. Gonzalgo ML, Isaacs WB. Molecular pathways to prostate cancer. J Urol 2003;170:2444–52.
4. Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. The landscape of somatic copy-number alteration across human cancers. Nature 2010;463:899–905.
5. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010;18:11–22.
6. Holcomb IN, Grove DI, Kinnunen M, Friedman CL, Gallaher IS, Morgan TM, et al. Genomic alterations indicate tumor origin and varied metastatic potential of disseminated cells from prostate cancer patients. Cancer Res 2008;68:5599–608.
7. Weckermann D, Polzer B, Ragg T, Blana A, Schlimok G, Arnholdt H, et al. Perioperative activation of disseminated tumor cells in bone marrow of patients with prostate cancer. J Clin Oncol 2009;27: 1549–56.
8. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644–8.
9. Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BE, Cao X, Morris DS, et al. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. Nature 2007; 448:595–9.
10. Tomlins SA, Rhodes DR, Yu J, Varambally S, Mehra R, Perner S, et al. The role of SPINK1 in ETS rearrangement-negative prostate cancers. Cancer Cell 2008;13:519–28.
11. Palanisamy N, Ateeq B, Kalyana-Sundaram S, Pflueger D, Ramnarayanan K, Shankar S, et al. Rearrangements of the RAF kinase pathway in prostate cancer, gastric cancer and melanoma. Nat Med 2010;16: 793–8.
12. Rubin MA, Maher CA, Chinnaiyan AM. Common gene rearrangements in prostate cancer. J Clin Oncol 2011;29:3659–68.
13. Setlur SR, Mertz KD, Hoshida Y, Demichelis F, Lupien M, Perner S, et al. Estrogen-dependent signaling in a molecularly distinct subclass of aggressive prostate cancer. J Natl Cancer Inst 2008;100: 815–25.
14. Berger MF, Lawrence MS, Demichelis F, Drier Y, Cibulskis K, Sivachenko AY, et al. The genomic complexity of primary human prostate cancer. Nature 2011;470:214–20.

15. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, et al. The mutational landscape of lethal castration-resistant prostate cancer. Nature 2012;487:239–43.

16. Kan Z, Jaiswal BS, Stinson J, Janakiraman V, Bhatt D, Stern HM, et al. Diverse somatic mutation patterns and pathway alterations in human cancers. Nature 2010;466:869–73.

17. Kumar A, White TA, MacKenzie AP, Clegg N, Lee C, Dumpit RF, et al. Exome sequencing identifies a spectrum of mutation frequencies in advanced and lethal prostate cancers. Proc Natl Acad Sci U S A 2011; 108:17087–92.

18. Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat JP, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nat Genet 2012; 44:685–9.

19. Kumar A, Shendure J, Nelson PS. Genome interrupted: sequencing of prostate cancer reveals the importance of chromosomal rearrangements. Genome Med 2011;3:23.

20. Eastham JA, Stapleton AM, Gousse AE, Timme TL, Yang G, Slawin KM, et al. Association of p53 mutations with metastatic prostate cancer. Clin Cancer Res 1995;1:1111–8.

21. Cairns P, Okami K, Halachmi S, Halachmi N, Esteller M, Herman JG, et al. Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. Cancer Res 1997;57:4997–5000.

22. Geng C, He B, Xu L, Barbieri CE, Eedunuri VK, Chew SA, et al. Prostate cancer-associated mutations in speckle-type POZ protein (SPOP) regulate steroid receptor coactivator 3 protein turnover. Proc Natl Acad Sci U S A 2013;110:6997–7002.

23. Liu W, Laitinen S, Khan S, Vihinen M, Kowalski J, Yu G, et al. Copy number analysis indicates monoclonal origin of lethal metastatic prostate cancer. Nat Med 2009;15:559–65.

24. Holcomb IN, Young JM, Coleman IM, Salari K, Grove DI, Hsu L, et al. Comparative analyses of chromosome alterations in soft-tissue metastases within and across patients with castration-resistant prostate cancer. Cancer Res 2009;69:7793–802.

25. Robbins CM, Tembe WA, Baker A, Sinari S, Moses TY, Beckstrom-Sternberg S, et al. Copy number and targeted mutational analysis reveals novel somatic events in metastatic prostate tumors. Genome Res 2011;21:47–55.

26. Askew EB, Gampe RT Jr, Stanley TB, Faggart JL, Wilson EM. Modulation of androgen receptor activation function 2 by testosterone and dihydrotestosterone. J Biol Chem 2007;282:25801–16.

27. Shah S, Hess-Wilson JK, Webb S, Daly H, Godoy-Tundidor S, Kim J, et al. 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene stimulates androgen independence in prostate cancer cells through combinatorial activation of mutant androgen receptor and mitogen-activated protein kinase pathways. Mol Cancer Res 2008;6:1507–20.

28. Balbas MD, Evans MJ, Hosfield DJ, Wongvipat J, Arora VK, Watson PA, et al. Overcoming mutation-based resistance to antiandrogens with rational drug design. eLife 2013;2:e00499.

29. Agoulnik IU, Vaid A, Nakka M, Alvarado M, Bingman WE III, Erdem H, et al. Androgens modulate expression of transcription intermediary factor 2, an androgen receptor coactivator whose expression level correlates with early biochemical recurrence in prostate cancer. Cancer Res 2006;66:10594–602.

30. Haffner MC, Aryee MJ, Toubaji A, Esopi DM, Albadine R, Gurel B, et al. Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. Nat Genet 2010;42:668–75.

31. Lin C, Yang L, Tanasa B, Hutt K, Ju BG, Ohgi K, et al. Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer. Cell 2009;139:1069–83.

32. Rickman DS, Soong TD, Moss B, Mosquera JM, Dlabal J, Terry S, et al. Oncogene-mediated alterations in chromatin conformation. Proc Natl Acad Sci U S A 2012;109:9083–8.

33. Mani RS, Tomlins SA, Callahan K, Ghosh A, Nyati MK, Varambally S, et al. Induced chromosomal proximity and gene fusions in prostate cancer. Science 2009;326:1230.

34. Huang S, Gulzar ZG, Salari K, Lapointe J, Brooks JD, Pollack JR. Recurrent deletion of CHD1 in prostate cancer with relevance to cell invasiveness. Oncogene 2012;31:4164–70.

35. Zhao R, Yan Q, Lv J, Huang H, Zheng W, Zhang B, et al. CHD5, a tumor suppressor that is epigenetically silenced in lung cancer. Lung Cancer 2012;76:324–31.

36. Wu X, Zhu Z, Li W, Fu X, Su D, Fu L, et al. Chromodomain helicase DNA binding protein 5 plays a tumor suppressor role in human breast cancer. Breast Cancer Res 2012;14:R73.

37. Bagchi A, Papazoglu C, Wu Y, Capurso D, Brodt M, Francis D, et al. CHD5 is a tumor suppressor at human 1p36. Cell 2007;128:459–75.

38. Jones DT, Jager N, Kool M, Zichner T, Hutter B, Sultan M, et al. Dissecting the genomic complexity underlying medulloblastoma. Nature 2012;488:100–5.

39. Morin RD, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, et al. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. Nature 2011;476:298–303.

40. Yang JY, Hung MC. Deciphering the role of forkhead transcription factors in cancer therapy. Curr Drug Targets 2011;12:1284–90.

41. Sahu B, Laakso M, Ovaska K, Mirtti T, Lundin J, Rannikko A, et al. Dual role of FoxA1 in androgen receptor binding to chromatin, androgen signalling and prostate cancer. EMBO J 2011;30:3962–76.

42. Gao N, Ishii K, Mirosevich J, Kuwajima S, Oppenheimer SR, Roberts RL, et al. Forkhead box A1 regulates prostate ductal morphogenesis and promotes epithelial cell maturation. Development 2005;132:3431–43.

43. Imamura Y, Sakamoto S, Endo T, Utsumi T, Fuse M, Suyama T, et al. FOXA1 promotes tumor progression in prostate cancer via the insulin-like growth factor binding protein 3 pathway. PLoS One 2012;7:e42456.

44. Katoh M, Igarashi M, Fukuda H, Nakagama H, Katoh M. Cancer genetics and genomics of human FOX family genes. Cancer Lett 2013;328:198–206.

45. Dong XY, Chen C, Sun X, Guo P, Vessella RL, Wang RX, et al. FOXO1A is a candidate for the 13q14 tumor suppressor gene inhibiting androgen receptor signaling in prostate cancer. Cancer Res 2006;66:6998–7006.

46. Liu P, Li S, Gan L, Kao TP, Huang H. A transcription-independent function of FOXO1 in inhibition of androgen-independent activation of the androgen receptor in prostate cancer cells. Cancer Res 2008;68:10290–9.

47. Renault VM, Thekkat PU, Hoang KL, White JL, Brady CA, Kenzelmann Broz D, et al. The pro-longevity gene FoxO3 is a direct target of the p53 tumor suppressor. Oncogene 2011;30:3207–21.

48. Chen MF, Fang FM, Lu CH, Lu MS, Chen WC, Lee KD, et al. Significance of nuclear accumulation of Foxo3a in esophageal squamous cell carcinoma. Int J Radiat Oncol Biol Phys 2008;71:1220–9.

49. Majumder PK, Sellers WR. Akt-regulated pathways in prostate cancer. Oncogene 2005;24:7465–74.

50. Pourmand G, Ziaee AA, Abedi AR, Mehrsai A, Alavi HA, Ahmadi A, et al. Role of PTEN gene in progression of prostate cancer. Urol J 2007;4:95–100.

51. Hodgson MC, Shao LJ, Frolov A, Li R, Peterson LE, Ayala G, et al. Decreased expression and androgen regulation of the tumor suppressor gene INPP4B in prostate cancer. Cancer Res 2011;71:572–82.

52. Chen M, Pratt CP, Zeeman ME, Schultz N, Taylor BS, O'Neill A, et al. Identification of PHLPP1 as a tumor suppressor reveals the role of feedback activation in PTEN-mutant prostate cancer progression. Cancer Cell 2011;20:173–86.

53. Edwards J, Krishna NS, Witton CJ, Bartlett JM. Gene amplifications associated with the development of hormone-resistant prostate cancer. Clin Cancer Res 2003;9:5271–81.

54. Sun X, Huang J, Homma T, Kita D, Klocker H, Schafer G, et al. Genetic alterations in the PI3K pathway in prostate cancer. Anticancer Res 2009;29:1739–43.

55. Lindberg J, Klevebring D, Liu W, Neiman M, Xu J, Wiklund P, et al. Exome sequencing of prostate cancer supports the hypothesis of independent tumour origins. Eur Urol 2013;63:347–53.

56. Navin N, Kendall J, Troge J, Andrews P, Rodgers L, McIndoo J, et al. Tumour evolution inferred by single-cell sequencing. Nature 2011;472:90–4.

57. Hou Y, Song L, Zhu P, Zhang B, Tao Y, Xu X, et al. Single-cell exome sequencing and monoclonal evolution of a JAK2-negative myeloproliferative neoplasm. Cell 2012;148:873–85.

58. Xu X, Hou Y, Yin X, Bao L, Tang A, Song L, et al. Single-cell exome sequencing reveals single-nucleotide mutation characteristics of a kidney tumor. Cell 2012;148:886–95.

59. Gerlinger M, Rowan AJ, Horswell S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med 2012;366:883–92.

60. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Cell 2010;17:98–110.

61. Kim JH, Dhanasekaran SM, Prensner JR, Cao X, Robinson D, Kalyana-Sundaram S, et al. Deep sequencing reveals distinct patterns of DNA methylation in prostate cancer. Genome Res 2011;21:1028–41.

62. Kim JH, Dhanasekaran SM, Mehra R, Tomlins SA, Gu W, Yu J, et al. Integrative analysis of genomic aberrations associated with prostate cancer progression. Cancer Res 2007;67:8229–39.

63. Fang M, Toher J, Morgan M, Davison J, Tannenbaum S, Claffey K. Genomic differences between estrogen receptor (ER)-positive and ER-negative human breast carcinoma identified by single nucleotide polymorphism array comparative genome hybridization analysis. Cancer 2011;117:2024–34.

64. Demichelis F, Setlur SR, Beroukhim R, Perner S, Korbel JO, Lafargue CJ, et al. Distinct genomic aberrations associated with ERG rearranged prostate cancer. Genes Chromosomes Cancer 2009;48:366–80.

65. Doyen J, Alix-Panabieres C, Hofman P, Parks SK, Chamorey E, Naman H, et al. Circulating tumor cells in prostate cancer: a potential surrogate marker of survival. Crit Rev Oncol Hematol 2012;81:241–56.

66. Panteleakou Z, Lembessis P, Sourla A, Pissimissis N, Polyzos A, Deliveliotis C, et al. Detection of circulating tumor cells in prostate cancer patients: methodological pitfalls and clinical relevance. Mol Med 2009;15:101–14.

67. Dawson SJ, Tsui DW, Murtaza M, Biggs H, Rueda OM, Chin SF, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013;368:1199–209.

68. Friedlander TW, Roy R, Tomlins SA, Ngo VT, Kobayashi Y, Azameera A, et al. Common structural and epigenetic changes in the genome of castration-resistant prostate cancer. Cancer Res 2012;72:616–25.

69. Magbanua MJ, Sosa EV, Scott JH, Simko J, Collins C, Pinkel D, et al. Isolation and genomic analysis of circulating tumor cells from castration resistant metastatic prostate cancer. BMC Cancer 2012;12:78.

70. Sun J, Liu W, Adams TS, Sun J, Li X, Turner AR, et al. DNA copy number alterations in prostate cancers: a combined analysis of published CGH studies. Prostate 2007;67:692–700.

71. Cheng I, Levin AM, Tai YC, Plummer S, Chen GK, Neslund-Dudas C, et al. Copy number alterations in prostate tumors and disease aggressiveness. Genes Chromosomes Cancer 2012;51:66–76.

72. Lapointe J, Li C, Giacomini CP, Salari K, Huang S, Wang P, et al. Genomic profiling reveals alternative genetic pathways of prostate tumorigenesis. Cancer Res 2007;67:8504–10.

73. Ishkanian AS, Mallof CA, Ho J, Meng A, Albert M, Syed A, et al. High-resolution array CGH identifies novel regions of genomic alteration in intermediate-risk prostate cancer. Prostate 2009;69:1091–100.

74. Boyd LK, Mao X, Xue L, Lin D, Chaplin T, Kudahetti SC, et al. High-resolution genome-wide copy-number analysis suggests a monoclonal origin of multifocal prostate cancer. Genes Chromosomes Cancer 2012;51:579–89.

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