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INVITED REVIEW

Prostate Cancer

DNA alterations in the tumor genome and their associations with clinical outcome in prostate cancer

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Although most prostate cancer (PCa) cases are not life-threatening, approximately 293 000 men worldwide die annually due to PCa. These lethal cases are thought to be caused by coordinated genomic alterations that accumulate over time. Recent genome-wide analyses of DNA from subjects with PCa have revealed most, if not all, genetic changes in both germline and PCa tumor genomes. In this article, I first review the major, somatically acquired genomic characteristics of various subtypes of PCa. I then recap key findings on the relationships between genomic alterations and clinical parameters, such as biochemical recurrence or clinical relapse, metastasis and cancer-specific mortality. Finally, I outline the need for, and challenges with, validation of recent findings in prospective studies for clinical utility. It is clearer now than ever before that the landscape of somatically acquired aberrations in PCa is highlighted by DNA copy number alterations (CNAs) and *TMPRSS2-ERG* fusion derived from complex rearrangements, numerous single nucleotide variations or mutations, tremendous heterogeneity, and continuously punctuated evolution. Genome-wide CNAs, *PTEN* loss, *MYC* gain in primary tumors, and *TP53* loss/mutation and *AR* amplification/mutation in advanced metastatic PCa have consistently been associated with worse cancer prognosis. With this recently gained knowledge, it is now an opportune time to develop DNA-based tests that provide more accurate patient stratification for prediction of clinical outcome, which will ultimately lead to more personalized cancer care than is possible at present.

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INTRODUCTION

Globally, PCa is the most common cancer among men, with an estimated 1 442 000 new cases in 2013.[1,2] While most PCa patients have an indolent form of the disease that may not require treatment, about 10%–20% of men affected with PCa have an aggressive form of the disease that may progress to metastases and death, thus requiring more intensive therapies. The inability to reliably distinguish between these two forms of PCa early in the course of the disease has resulted in overtreatment of many and undertreatment of some cases.[3–5] This is because all patients with PCa are treated similarly in most cases even though the underlying genetic causes of their PCa tumors are likely different.

With high-resolution microarrays and next-generation sequencing (NGS), significant advances have recently been made in genetic dissections of both germline and prostate tumor genome, which is characterized by diverse somatic mutations and pathway alterations[6–11] derived from complex DNA rearrangements.[12–14] Each tumor genome harbors genetic aberrations affecting numerous genes as the result of successive clonal expansion and punctuated evolution.[13,15–20] While most of these affected genes may be neutral "passengers," aberrations of multiple "drivers" cause increased cell proliferation and PCa progression.[21–23] Moreover, chromothripsis and chromoplexy with catastrophic and chained genomic rearrangements,[12,13,24,25] respectively, suggest that multiple cancer genes are often altered coordinately. These massive and coordinated genetic alterations affect the expression of many genes, which define the fate of PCa.

Delineating the genomic and molecular characteristics of various types of PCa at different stages can improve our ability to understand the mechanisms that drive reemergence as aggressive PCa from indolent tumors and progression of malignant tumors to advanced stages. Analyzing the relationships between genomic alterations and clinical parameters may lead to better molecular markers for more accurate patient stratification and personalized cancer care. Due to a broad coverage of topics by other articles in this special issue, this review primarily focuses on somatically acquired aberrations of genomic DNA in the tumor genome and highlights their potentials for use as molecular biomarkers for prognosis and prediction of clinical outcome.

LANDSCAPE OF GENOMIC ALTERATIONS IN CLINICALLY LOCALIZED PROSTATE CANCER

Detailed genomic alterations in PCa have been the subject of extensive reviews recently for their biological and therapeutic implications.[26–32] This work aims to briefly highlight the major somatic alterations with potential to distinguish aggressive from nonaggressive PCa.

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DNA copy number alterations

Studies on genome-wide analyses of PCa have revealed that DNA copy number alterations (CNAs) are a major component of the landscape alterations in the tumor genome. The most frequently detected CNAs in primary tumors include amplifications of genomic regions containing oncogenic *MYC* (8q24.21, 10%–40%), and deletions of DNA sequences harboring tumor suppressor genes such as *NKX3-1* (8p21.2, 40%–70%), *PTEN* (10q23.31, 10%–40%), *CDKN1B* (12p13.1, 20%–30%), *RB1* (13q14.2, 30%–50%), and *TP53* (17p13.1, 20%–30%). In addition, CNAs of many other genes with various biological functions have also been reported in many cohorts.[7,33–36] It is notable that the frequencies of these CNAs vary among different tumor samples, depending on cohort composition, tumor grade, degree of tumor cell heterogeneity, and pathological stage, thus reflecting the complex roles of these CNAs in PCa. However, the major CNA regions across the whole genome among different cohorts are remarkably similar (**Figure 1**), suggesting a core CNA commonality of most PCa.

With genomic deletions outnumbering gains, the majority of the deletions affecting tumor suppressor genes involves the loss of only one copy. It is speculated that either these genes are haploinsufficient or these hemizygous deletions are eventually complemented by additional genetic and/or epigenetic alterations in another copy of the gene during cancer progression. For example, *NKX3-1* and *LPL* were reported to be affected by both deletion and methylation.[37,38] The combination of a hemizygous germline frameshift in one copy and an acquired somatic deletion of the other copy was found to inactivate *BRCA2*.[19,39] The combination of a somatic point mutation in one copy and a hemizygous deletion of another was reported to abolish *PTEN* and *TP53*.[39,40]

Homozygous deletions causing loss of both copies of the sequence have been observed in many genes. The most common homozygously deleted genes are *PTEN* (~15%) and *CHD1* (~10%) in PCa.[41] Others include *BNIP3L* (8p21.1), *LRP1B* (2q22.1), *RB1* (13q14.2), *USP10* (16q24.1), *HTR3A* (11q23.2), *RYPB* (3p13), *MAP3K7* (6q15), *TP53* (17p13.1), *CDKN1B* (12p13.1), and miR-15a/miR-16-1 (13q14.2) with much lower frequencies.[41,42] While most gains are hemizygous, amplifications with more than two extra copies involved both chromosomes, primarily on 8q24 including *MYC*, are also reported in PCa.[43]

Distinct CNA profiles across the tumor genome have been observed among patients of different races. Castro *et al.* reported that CNAs at six regions in clinically localized tumors from 20 African American (AA) men resembled those in metastatic cancers from Caucasian American (CA) men.[44] Rose *et al.* identified four CNAs with significantly higher frequencies in AA than in CA men.[45] The frequencies of deletions between *TMPRSS2* and *ERG* that create a fusion of these two genes, as well as *PTEN* loss, are found to be significantly lower in AA, Chinese, Japanese, and Korean PCa populations than in CA.[46–52]

Single nucleotide variations (SNVs)

Before NGS, targeted sequencing identified many point mutations (SNVs in somatic tissues) in selected genes thought to be biologically important for cancer development.[6,7] It has been reported that PCa harbors the fewest point mutations (~0.33–1.4 per Mb) among the major human cancers.[6,9] However, relatively high frequencies of SNVs at *TP53* (12%), *PTEN* (7%), *SPOP* (7%), *KRAS* (4%), *FOXA1* (3%), *KMT2C* (3%), *EGFR* (3%), and *CTNNB1* (3%) have been documented (**Figure 2**). Systematic analyses of SNVs across the tumor genome using exome and whole genome NGS have revealed a comprehensive mutation landscape of clinically localized PCa.[9,11–14,53] While the majority of these mutations is observed in a relatively small proportion of tumors, many genes, including *SPOP*, *FOXA1*, *TP53*, *PTEN*, *CDKN1B*, *MED12*, *THSD7B*, *SCN11A*, *NIPA2*, *PIK3CA*, ZNF595, *C14orf49*, *CDC27*, *MLL3*, *KDM6A*, and *KIF5A*, are considered to be significantly mutated. *SPOP* and *TP53* are the most frequently mutated genes with 10%–15% across different cohorts in primary PCa.[9,11]

The mutation landscape and biological implications of mutated genes in PCa have been reviewed extensively.[27,28,30–32,54–57] Although drugs have been developed to target specific pathways of some mutations, such as the PI3K/Akt/mTOR signaling pathway in tumors with *PTEN* and *PIK3CA* mutations, these significantly mutated genes have not been systematically investigated for their clinical utility in prognosis and predication of cancer progression or lethal PCa. This is partially due to the lower frequencies of mutated genes (in comparison to CNAs), small cohorts used in PCa genomic studies, and tremendous heterogeneity of PCa. Notably, there were only two overlapping genes identified as being significantly mutated in two independent cohorts of primary tumors analyzed by exome sequencing.[9,11] In addition, the detected frequency of gene mutations depends on the depth used in NGS and the sampled tissue location because of intra- and inter-tumor heterogeneity.[16,18,19,53,58]

Genomic subtypes of primary tumors

Since the discovery of *TMPRSS2-ERG* fusion transcripts caused by genomic DNA translocation[59] and deletion,[35,60] many studies have been carried out to investigate recurrent gene fusions and their roles in PCa (see reviews).[61,62] The fusion between *TMPRSS2* and E26 transformation-specific (ETS) family genes, observed in ~50% of PCa patients, is the most commonly acquired genetic alteration defining a distinct subtype of PCa. Moreover, there is a significant correlation between *ERG* rearrangements and *PTEN* loss. For example, Carver *et al.* observed a reduction or absence of *PTEN* expression in14 of 15 tumors with *TMPRSS2-ERG* fusion among 40 cases ($P = 0.007$).[21] King *et al.* found that 14 of 17 tumors with *PTEN* deletion also harbored *ERG* rearrangements in 121 cases ($P = 0.002$).[22] Analyzing specimens from 281 patients, Han *et al.* demonstrated a significant association between *PTEN* deletions and *ERG* rearrangements ($P = 0.0008$).[63] Reid *et al.* observed a significant association between *PTEN* loss and *ERG*/*ETV1* rearrangements among 308 patients ($P < 0.001$).[64] Bismar *et al.* reported that 71% of homozygous and 44% of hemizygous *PTEN* deletions were concurrent with *ERG* rearrangements among 220 patients.[65] Krohn *et al.* showed that *PTEN* deletion was significantly associated with *ERG* fusion among 2177 tumors ($P = 0.0001$).[66] Recently, Qi *et al.* also reported a significant correlation between *PTEN* deletion and *ERG* rearrangements in a cohort of 176 Chinese PCa patients ($P = 0.0008$).[67] These findings support the notion that a synergistic cooperation between *PTEN* deletion and *ERG* rearrangement drives the development and progression of this subtype of PCa.[21,22,68,69]

Another major subtype of PCa is characterized by *CHD1* deletions and/or *SPOP* mutations in the tumor genome.[8,9,41,70–73] Searching for homozygous deletions among a total of 244 primary tumors, Liu *et al.* discovered that *CHD1* was second only to *PTEN* as the most frequent (~10%) homozygously deleted gene in PCa.[41] More importantly, none of the 21 subjects with *CHD1* homozygous deletions harbored a deletion from the 3' end of *TMPRSS2* to the 5' end of *ERG*. Barbieri *et al.* found that somatic deletions at 5q21 including *CHD1* significantly associated with *SPOP* mutation in a cohort with 112 tumors.[9] Interestingly, all of the *SPOP* mutations were exclusively observed in the tumors without *TMPRSS2-ERG* fusion. Analyzing the relationship between *CHD1* and ETS status in 13 studies with a total of

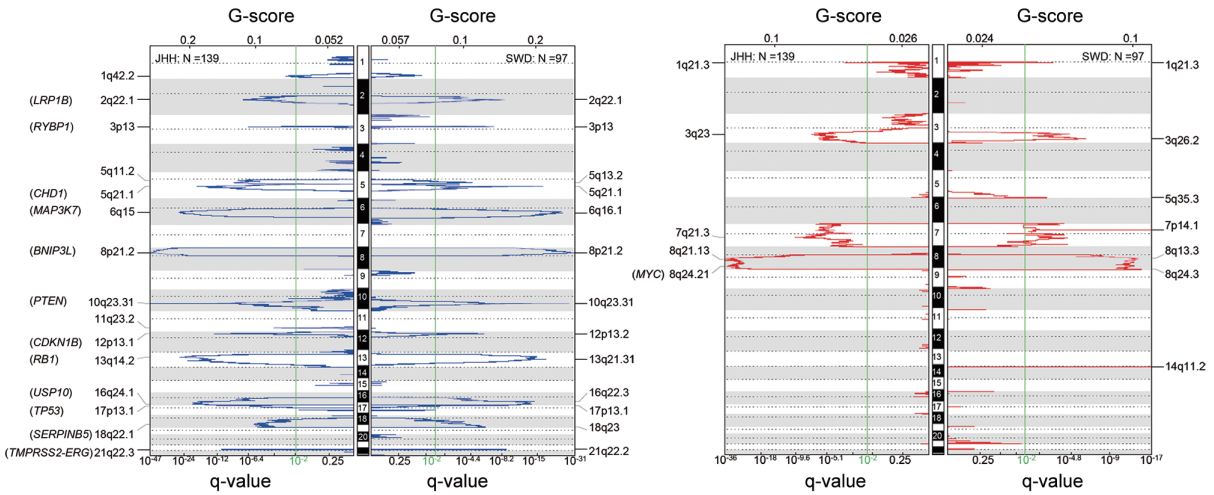

Figure 1: Significant genomic deletions (blue) and amplifications (red) in the tumor genomes from two independent cohorts of patients with clinically localized PCa. JHH: Johns Hopkins cohort, SWD: Swedish cohort.

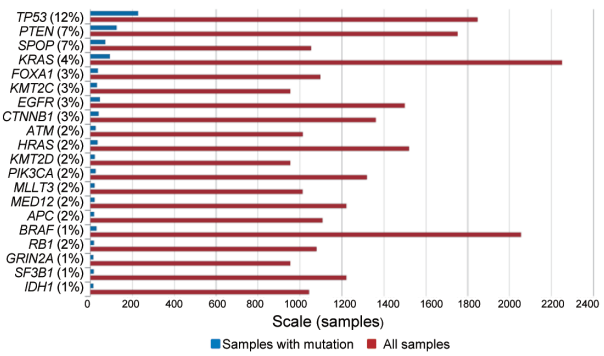

Figure 2: Top 20 genes mutated in prostate carcinoma as January 31, 2016 (http://www.sanger.ac.uk/genetics/CGP/cosmic).

945 cancers, Grasso *et al.* demonstrated a significant negative correlation between loss of *CHD1* and ETS rearrangements ($P < 0.0001$).[8] Using fluorescence *in situ* hybridization (FISH) to analyze the status of *CHD1* and *ERG* in 2093 tumors, Burkhardt *et al.* confirmed the significant negative association between *CHD1* deletion and *ERG* fusion ($P < 0.0001$).[71] Investigating *SPOP* mutations among 720 PCa samples from six international cohorts with Caucasian, AA, and Asian subjects, Blattner *et al.* further validated the inverse correlation between *ERG* rearrangements and *SPOP* mutations or *CHD1* deletion.[70]

In addition to *SPOP* mutation, tumors with deletions of *CHD1* are also enriched with deletions of *LRP1B* (2q22), *PDE4D* (5q11), *MAP3K7* (6q15), *FOXO3*, and *PRDM1* (6q21) and gain of *COL1A2* (7q21).[9,41] Loss of *CHD1* has been reported to be associated with a larger number of homozygous deletions at other locations in the tumor genome[41] and an increased number of CNAs.[13] Using whole genome NGS, Baca *et al.* found that tumors with *CHD1* deletions harbored significantly more intrachromosomal DNA rearrangements in the regions with low GC content. In contrast, tumors with ETS fusions contained more interchromosomal rearrangements in locations with active transcriptional hubs.[13] Burkhardt *et al.* demonstrated that *in vitro* inactivation of CHD1 impaired androgen receptor (AR)-dependent transcription required for translocation, thereby preventing *ERG* rearrangements.[71]

GENOMIC ALTERATIONS IN ADVANCED METASTATIC PROSTATE CANCER

The majority of primary PCa cases is not life-threatening. However, approximately 293 000 men worldwide die annually from metastases of advanced PCa. While the initial driver of cancer cell dissemination from the prostate to other organs in untreated subjects is unknown, androgen deprivation therapy (ADT) apparently leads to metastatic castration-resistant prostate cancer (mCRPC) in most cases. Although a new generation of ADT agents, including enzalutamide[74,75] and abiraterone acetate,[76,77] has been proven to improve survival of patients with mCRPC, it is thought that cancers in nearly all affected subjects may eventually develop resistance to these new drugs via genomic alterations.[39,40,77–81] Therefore, the genomic landscape of advanced PCa, in addition to those inherited from primary tumors, is also shaped, in part, by selective pressure applied to cancer cells from treatment. The landscape of advanced PCa, highlighted by increased alterations of *AR*, *TP53*, and *PTEN*[7,8,10,17,19,39] and circulating tumor cells/DNA (CTC/ctDNA),[82–89] has recently been reviewed for clinical and therapeutic implications.[26–28,31,90–95] This section briefly outlines the major genomic aberrations and focuses on the genes that currently have potential for outcome prediction.

Lethal metastases are originally derived from primary tumors in the prostate although various subclones and multiclonal seeding have been documented in both clinically localized and distant metastatic PCa.[16–20,40,53,58] Genome-wide comparative genomic hybridization (CGH) has demonstrated that advanced metastatic PCa harbors similar CNAs, to those seen in primary tumors, but at much higher frequencies.[7,8,20,36,96] Analyzing DNA copy number in lethal PCa, Liu *et al.* reported that multiple distant metastases from anatomically distinct sites and tumors in the prostate shared a core profile of CNAs in the same subjects while each also accumulated a variable number of separate subclonally sustained changes.[20] Exome sequencing mCRPC from 150 individuals, Robinson *et al.* found that more than 40% of tumors had genetic alterations, including 27% with homozygous deletion of, as well as a number of mutations in, *PTEN*.[39] Comparing all gene-coding sequences between tumors and matched normal tissues, Barbieri *et al.* and Grasso *et al.* identified 12 and 9 significantly mutated genes in clinically localized[9] and castration-resistant[8] PCa, respectively. Importantly, *PTEN* and *TP53* are the only genes significantly mutated in

both of these two distinct types of PCa. To track the origin of lethal PCa, Haffner *et al.* analyzed three anatomically distinct autopsy metastases of liver, perigastric lymph node, and lung and nine lesions in the prostate obtained 17 years prior via radical prostatectomy (RP) from a subject who died of PCa.[18] They discovered that the lethal clone was derived from a small, low-grade focus harboring *PTEN* deletion and *TP53* mutation, rather than from the bulk, higher-grade primary cancer or from a lymph node metastasis that did not have these gene alterations. Using ultra-deep sequencing, Hong *et al.* demonstrated that PCa metastases were enriched with *TP53* mutations that apparently drove subclonal expansion.[19] Moreover, Ferraldeschi *et al.* found that loss of *PTEN* was associated with a shorter time on abiraterone treatment and worse patient survival for those with mCRPC,[97] possibly via resistance to ADT.[98] These findings emphasize the broad roles of these alterations in cancer initiation, progression, and treatment resistance.

AR amplification and mutation characterize 30%–80% of advanced metastatic PCa in various cohorts. Using FISH to analyze *AR* in transurethral resection of the prostate (TURP) from 23 subjects treated with ADT, Visakorpi *et al.* reported that 7 (30%) had *AR* amplification while none showed AR amplification from the same subjects before therapy.[99] Correspondingly, Koivisto *et al.* found that 28% of therapy-resistant tumors, but none of the untreated primary tumors, contained *AR* amplification in tumor specimens obtained via biopsy and TURP from 54 subjects.[100] Using SNP array to analyze multiple distant metastases from anatomically distinct sites in each subject from a cohort of 14 lethal PCa cases, Liu *et al.* found only two individuals harboring a normal single copy of *AR* in all metastatic sites. Seven subjects showed gains of two to eight copies and five subjects showed *AR* amplification ranging from 9 to 40 copies.[20] In contrast, they observed no *AR* gain in primary tumors in a separate study of 228 untreated subjects from two independent cohorts.[101] Similarly, Friedlander *et al.* reported that 11 of 15 of the metastatic samples harbored *AR* amplification.[96] Using exome NGS to analyze 50 pre-treated mCRPCs obtained by rapid autopsy, Grasso *et al.* found 30 harboring *AR* amplification and/or protein-coding mutations.[8] Moreover, Beltran *et al.* reported that 44% of 25 mCRPC cases had *AR* alterations.[102] Robinson *et al.* observed 63% of subjects with *AR* amplification and/or missense mutations in a prospective study cohort of 150 treated mCRPC patients.[39] These findings suggest that amplification and mutation of *AR* are primarily resulted from AR-related therapies.

Defects in DNA repair are apparently the initial cause of genome-wide alterations and subsequent genomic instability, with higher frequencies in advanced metastatic PCa. Biallelic *BRCA2* inactivation is reported in more than 10% of mCRPC, with ~5% damaging germline mutations.[39,102] Correspondingly, subjects carrying *BRCA2* mutations have a significantly higher PCa-specific mortality rate and shorter median survival time.[103–106] In addition, aberrations of the mismatch repair genes *MSH2* and *MSH6*, which are associated with hypermutation, have been found at higher frequencies in advanced metastatic PCa cases[39,107] although changes in *MSH2* and *MSH6* are rarely observed in untreated primary tumors.[7,9,11] Pritchard *et al.* showed that 7 of 60 (12%) patients with advanced PCa had structural rearrangements in *MSH2* and *MSH6*, with all being hypermutated and microsatellite instable.[107] Robinson *et al*. reported that 2% of 150 patients with mCRPC harbored *MSH2* alterations.[39] Tracking the origin and driver of subclonal expansion in a longitudinal study, Hong *et al.* identified *MSH2* alterations in both the original clone of a primary tumor and the clone of a shoulder metastasis.[19] However, whether defects of these genes lead to the dissemination of a lethal clone is warranted for further investigation.

IMPLICATIONS OF DNA-BASED MARKERS FOR PROGNOSIS OF CLINICAL OUTCOME

With the unprecedented amounts of genomic data generated from the tumor genome as mentioned above, there is hope for identifying genomic markers that can distinguish aggressive from indolent PCa at the time of diagnosis, as well as genes that drive cancer progression. Because there is no consensus on the definition of aggressiveness, this section primarily focuses on DNA alterations associated with clinical outcome in PCa.

CNAs associated with biochemical recurrence (BCR)/clinical relapse of PCa

BCR/clinical relapse of PCa is the most common characteristic of clinical outcomes that have been investigated for association with CNAs.[7,34,66,108–130] The most frequently investigated candidate genes include *PTEN* on 10q23.31 and *MYC* on 8q24.21 (**Table 1**). Significant associations between *PTEN* loss and PCa recurrence have been reported in multiple cohorts.[66,109,123,127,128] In other cohorts, loss of *PTEN* alone by FISH is not significantly associated with BCR[111,130] whereas loss of PTEN by immunohistochemistry (IHC) is significantly associated with BCR.[111,115,131–136] The inconsistency of these observations is likely derived from variations in cohort composition, including homozygous versus hemizygous deletions, and the interval to PCa recurrence after treatments such as prostatectomy and radiotherapy.

MYC gain, either itself or together with other CNAs, is found to be associated with an earlier recurrence of PCa.[117,123,130] This is consistent with findings that patients with increased *MYC* expression have an earlier disease relapse.[123,133,137,138] In addition, the combined gain of *MYC* and loss of either *PTEN* or *NKX3.1* have been reported to be better prognostic predictors of PCa relapse after radiotherapy.[117,130] Similarly, concurrent loss of *PTEN* and loss of 16q or fusion of *TMPRSS2-ERG* are reported to contribute to independent prognostic information regarding BCR.[115,128]

Genome-wide CNAs have been consistently reported to be associated with PCa relapse.[7,66,113,116,120,124,129] Using an unsupervised hierarchical clustering of CNAs identified by the Agilent 244K CGH among 181 primary and 37 metastatic tumors, Taylor *et al*. classified PCa into 6 distinct groups, with some significantly associated with BCR.[7] Extending this study with a contemporary cohort containing 104 patients, Hieronymus *et al*. demonstrated that CNA burden, measured as the percentage of tumor genome affected, predicted PCa recurrence independent of histopathological parameters.[113] For example, patients with CNAs ≥1.34% had a 29%–38% risk of BCR within 5 years as opposed to a 13%–15% risk for those harboring CNAs <1.34%. Lalonde *et al*. reported that every 1% increase of CNAs in the tumor genome led to a 5%–8% decrease of 5-year BCR-free survival.[116] These findings are consistent with Strohmeyer *et al.*'s observation that the total number of deletions was significantly higher in patients with PCa relapse than in those without although no difference in total number of gains was observed between progressors and nonprogressors.[124] Selected multiple genomic CNAs across the tumor genome are also demonstrated to be significantly associated with PCa relapse.[116,120,129] Using a Genomic Evaluators of Metastatic Prostate Cancer (GEMCaP) score consisting of 39 CNAs, Paris *et al*. were able to increase the area under the receiver operating characteristic curve (AUC) up to 0.85 in prediction of PCa recurrence.[120] A 100-locus CNA signature has been claimed to be prognostic for disease recurrence in patients treated with radiotherapy and prostatectomy.[116] Moreover, it helps identify subjects (with an AUC of 0.68) who are most likely to fail initial treatment in 18 months with BCR, which has been considered a robust prognostic factor for PCa mortality after radiotherapy.[139]

Table 1: Significant CNAs associated with BCR/clinical relapse of PCa

Gene or cytoband	*CNAs*	*Method*	*Tumor or patient*	*Tissue*	*Univariate*	*Multivariate*	*Reference*	*Year*
11q13.1	Gain	CGH	64	RP	**	*	119	2004
6q/7q/13q	Loss/gain/loss	CGH	51	RP	**/*/*	*	124	2004
6q15¶	Loss	CGH	55	RP	*		34	2007
8p22	Loss	FISH	156	RP	*	NS	126	2002
16q	Loss	FISH	3542	RP	***	**	115	2015
16q and *PTEN*	Loss	FISH	3373	RP	***	**	115	2015
CHD1	Loss	FISH	1713	RP	**		71	2013
CNAs†	Loss and gain	CGH	54	RP	*		120	2010
CNAs	Loss and gain	CGH	218‡	RP	*		7	2010
CNAs	Loss and gain	CGH	104	RP	**		129	2012
CNAs	Loss and gain	CGH	126	Bx	***		116	2014
CNAs	Loss and gain	CGH	168	RP	***	*	113	2014
CNAs	Loss and gain	CGH	104	RP	**	*	113	2014
EIF3S3	Gain	FISH	183	RP	*		122	2001
ERG	Gain	FISH	344	RP	**		125	2011
HER2/neu	Gain	FISH	113	RP	*		121	1997
MAP3K7	Loss	FISH	2289	RP	***		114	2013
MYC	Gain	FISH	50	RP	**		123	2006
MYC	Gain	CGH, FISH	126	RP	***	**	102	2012
NKX3-1	Loss	CGH, FISH	126	Bx, RP	**		117	2012
NKX3-1 and MYC	Loss and gain	CGH, FISH	126	Bx, RP	***		117	2012
PTEN	Loss	FISH	107	RP	***	*	127	2007
PTEN	Loss	FISH	2266	RP	***	*	66	2012
PTEN and *TMPRSS2-ERG*	Loss	FISH	125	RP	***	*	128	2008
PTEN	Loss	FISH	43 and 64§	RP	*		109	2012
PTEN and *MYC*	Loss and gain	CGH, FISH	126	Bx	***	***	130	2012

†39 BAC-based DNA markers, termed GEMCaP; ‡Include 181 primaries and 37 metastases; §Two different cohorts; *P≤0.05; **P≤0.01; ***P≤0.001; ¶Negative correlation. Bx: biopsy; RP: radical prostatectomy; NS: not significant; BCR: biochemical recurrence; CNAs: copy number alterations; CGH: comparative genomic hybridization; FISH: fluorescence *in situ* hybridization; PCa: prostate cancer; GEMCaP: genomic evaluators of metastatic prostate cancer

Furthermore, CNA signature has been demonstrated to provide prognostic information beyond RNA-based profiling for PCa relapse.[113,116] For example, Hieronymus *et al*. reported that CNAs outperformed an RNA-based cell cycle progression signature for BCR in Gleason 7 tumors while both of these signatures were independently associated with PCa relapse.[113] In addition, *CHD1* deletion is reported to be associated with BCR[71,140] while there is no correlation between *SPOP* mutation and BCR.[70] Systematic investigation on the relationship between *CHD1* deletion or *SPOP* mutation and clinical outcomes, such as PCa recurrence, metastasis, and cancer-specific death, are apparently needed although knockdown of CHD1/MAP3K7 has been reported to alter cell morphology, increase cell invasiveness, and promote aggressive PCa.[41,71,72,141]

CNAs associated with metastasis or mortality of PCa

Metastasis is a more definitive clinical outcome parameter than BCR for lethal PCa because most patients with distant metastasis eventually die from the disease. However, the relationship between CNAs and metastasis has only been reported in a few studies. Paris *et al*. identified 40 CNAs in primary tumors that appear to be associated with metastasis.[119] The percentage of CNAs in the tumor genome as a continuous variable is also reported to be significantly associated with metastasis in one (168 primary tumors) of two cohorts that Hieronymus *et al*. investigated.[113] *PTEN* copy number loss is not associated with metastasis in this cohort although PTEN protein loss by IHC is observed to be associated with decreased time to PCa metastasis in another cohort.[142] Conversely, using FISH, Krohn *et al*. found that *PTEN* hemizygous and homozygous deletions were significantly correlated with lymph node metastasis in a cohort with 1111 tumors.[66]

The association between CNAs and PCa-specific mortality has been investigated in a number of cohorts (**Table 2**).[64,67,101,123,143–149] Using FISH to study the relationship between CNAs on chromosome 8 and clinical outcome, three independent groups found that gains of *MYC* were significantly associated with systematic disease progression and earlier PCa-specific death.[123,146,147] Using the Affymetrix 6.0 SNP array to analyze normal and tumor DNA from 125 high-risk subjects, Liu *et al*. uncovered seven regions in the tumor genome that were significantly associated with lethal PCa.[101] These included gains at 8q24.21 (*MYC*), 1q21.3 (*ADAR*), and 8q21.13 (*TPD52*) and deletions at 18q21.33 (*SERPINB5*), 16q24.1 (*USP10*), 10q23.31 (*PTEN*), and 17p13.1 (*TP53*). Among these CNAs, gains of *MYC* conferred the greatest risk of dying from PCa with an OR of 4.75, consistent with previous findings.

Significant association between *PTEN* deletion and PCa-specific death has been documented by four independent groups.[64,101,143,148] In a cohort of 59 patients with hormone refractory PCa, Sircar *et al*. found that *PTEN* was deleted in 77% of the cases, including 25% homozygous, 34% hemizygous, and 18% mixture deletions, and correlated with cancer-specific mortality.[148] In a TURP cohort with 643 patients, Cuzick *et al*. observed significant associations of both deletion and amplification of *PTEN* with PCa-specific death.[143] Further analysis indicated that patients with homozygous deletions were at greater risk of dying from PCa. This is consistent with the finding that loss of PTEN expression is significantly associated with an increased risk of PCa-specific mortality.[134,150] In addition, Reid *et al*. reported

Table 2: Significant CNAs associated with mortality of PCa

Gene or cytoband	*CNAs*	*Method*	*Tumor or patient*	*Tissue*	*Univariate*	*Multivariate*	*Reference*	*Year*
8pter-p23	Loss	PCR	45	RP	**		149	2000
8q	Gain	CGH, FISH	61	Bx	**		146	2006
ERG	Fusion	FISH	244	TURP	*		67	2014
ERG/ETV1	Fusion	FISH	322	TURP	***	NS	64	2010
MYC	Loss	CGH	125	RP	**	**	101	2013
MYC	Gain	FISH	60	Bx	**		145	2007
MYC	Gain	FISH	144	RP	*		147	1999
MYC	Gain	FISH	50	RP	**		123	2006
PTEN	Loss	FISH	643	TURP	***	NS	143	2013
PTEN	Loss	CGH	125	RP	*	**	101	2013
PTEN	Loss	FISH	322	TURP	***	NS	64	2010
PTEN	Loss	FISH	59	TURP	*		148	2009
PTEN and *ERG/ETV1* fusion	Loss and negative	FISH	322	TURP	***	***	64	2010
PTEN and *MYC*	Loss and gain	CGH	125	RP		***	101	2013
Telomere length	Shorter and variable	FISH	596	RP	*		144	2013

*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$. Bx: biopsy; TURP: transurethral resection of the prostate; RP: radical prostatectomy; NS: not significant; CNAs: copy number alterations; CGH: comparative genomic hybridization; FISH: fluorescence *in situ* hybridization; PCa: prostate cancer

that patients harboring *PTEN* deletions in the absence of *ERG*/*ETV* rearrangements carried a significantly higher risk of dying from PCa in both univariate and multivariate analyses.[64] Moreover, using a model that incorporated both genetic markers and clinicopathologic variables in a multivariate logistic regression analysis, Liu *et al*. showed that the CNAs of *PTEN* and *MYC* conferred additional independent prognostic information.[101] Compared to patients without either *PTEN* or *MYC* alterations, patients with both alterations had a 53-fold higher risk for dying from the disease. This study is the first to demonstrate a stronger joint effect of *PTEN* and *MYC* on PCa-specific mortality. An important aspect of this study is the further confirmation of the joint effect of *PTEN* loss and *MYC* gain in 333 tumors from three additional distinct patient cohorts.[101]

Association between ETS fusions and clinical outcome of PCa

The relationship between *TMPRSS2-ERG* fusion and clinical outcome has been investigated extensively. However, conflicting, even opposite, results are reported. For example, using FISH and qPCR to analyze TURP samples from 111 patients in a watchful waiting cohort, Demichelis *et al*. identified 15% harboring *ERG* rearrangements associated with PCa-specific deaths.[151] Analyzing TURP specimens from 445 conservatively managed patients, Attard *et al*. found that subjects harboring a duplication of the fusion together with interstitial deletion of *ERG* exhibited extremely poor survival after 8 years.[152] Similarly, Qi *et al*. demonstrated that *ERG* rearrangements were associated with cancer-related death in 224 Chinese patients using TURP samples.[67] These data are consistent with the findings that higher ERG expression, usually caused by fusion, is significantly associated with BCR, distant metastasis or PCa-specific mortality.[153–157]

Conversely, using FISH to analyze samples from 521 cases treated by RP, Gopalan *et al*. demonstrated that *TMPRSS2-ERG* rearrangement was not associated with BCR, metastasis or mortality.[112] Toubaji *et al*. found that *TMPRSS2-ERG* fusion was not associated with PCa relapse in a cohort containing 172 patients with and 172 patients without recurrence after prostatectomy.[125] FitzGerald *et al*. observed no correlation between *ERG* rearrangements and cancer-specific death in a population of 214 tumors analyzed by FISH.[158] In addition, Fleischmann *et al*. reported that *TMPRSS2-ERG* fusion was correlated with favorable BCR-free survival in a cohort of 119 surgically treated tumors.[159] Similarly, Saramaki *et al*. demonstrated that *ERG* fusion was associated with longer progression-free survival in 150 patients treated by prostatectomy.[160] These results are consistent with the findings that ERG overexpression either is not associated with cancer relapse or mortality or is correlated with better BCR-free survival.[161–166]

The conflicting and opposite findings described above may be derived from a number of factors, including cohort composition, techniques used to detect the fusion, and other confounding alterations in the tumor samples. Because *TMPRSS2-ERG* fusion is significantly associated with *PTEN* loss that correlates with worse clinical outcomes as reviewed in previous sections, prognostic values of the fusion are likely confounded by *PTEN* status in various cohorts.[140] Indeed, Reid *et al*. found that *ERG* rearrangement alone or *PTEN* loss alone was not correlated with patient survival in multivariate analyses. However, patients with *PTEN* loss in the absence of the fusion exhibited a significantly poorer cancer-specific survival rate than those harboring *PTEN* loss and *ERG* rearrangement.[64]

FUTURE PERSPECTIVES

Although many studies have been performed to explore the utility of DNA alterations for prognosis of clinical outcome via association, survival, and receiver operating characteristic analyses as reviewed above, none has been systematically validated in a Clinical Laboratory Improvement Amendments (CLIA) lab. In contrast, many new biomarkers, including RNA-based genomic predictors, have been validated for commercial use.[167,168] Clinical application of genomic DNA-based prognostic markers has lagged expression-based molecular markers even though they hold some advantages such as robustness to degradation and remaining constant despite physiological and environmental fluctuations.

The clinical validity and utility of DNA-based alterations associated with PCa outcome need to be fully assessed by systematic investigations with proper design to address a number of confounding factors in prospective studies. These include (1) intra- and inter-tumor (focal) heterogeneity,[16,18,19,53,58] (2) field effects of genomic alterations,[16] (3) choice and availability of tissues (e.g., biopsy, TURP, RP, CTC/ctDNA, etc.) and sampling bias, (4) composition of patient cohort (e.g., untreated patients with primary tumors at active surveillance [AS] or RP, patients with advanced PCa treated various drugs), (5) stratifications of age, family history, race, other genomic

changes, and environmental factors, and (6) technology for detecting alterations and standardization of experimental protocols.

Taking heterogeneity, for example, Cooper *et al*. identified mutations at high levels in morphologically normal tissues distant from the cancer site and multiple genetically distinct clones in a single tumor mass using NGS with an average depth of 10 000 ×.[16] They also documented a number of somatic mutations in the prostate that were not detected in tumor or blood samples from the same patient. Investigating tumor origin, Lindberg *et al*. observed no apparent common somatic mutations in different tumor foci of the same prostate among three of four patients.[58] Similarly, Boutros *et al.* reported multiclonal tumor foci with no shared CNAs and very few shared SNVs in the prostate of the same individuals.[53] These findings on heterogeneity complicate unbiased sampling for validating the clinical utilities of genomic biomarkers. Therefore, extensive retrospective and prospective investigations are warranted to track and validate driver alterations using longitudinal studies and clonal analyses,[16–20,40,53] to assess their associations with clinical outcomes, and to assess their potential utilities for personalized prognosis.

Genome-wide CNAs, *PTEN* loss, *MYC* gain in primary tumors, and *TP53* loss/mutation and *AR* amplification/mutation in advanced metastatic PCa have been consistently observed to be associated with poorer clinical outcome. Further investigations are necessary to validate their utilities as genomic markers for distinguishing aggressive PCa from indolent tumors, especially in AS patients, because RP was the major source of DNA used for discovery of the associations with BCR, metastasis, and mortality. Due to the complexity of genomic alterations, it is unlikely that a common set of genomic markers can be used for diagnosis of aggressive PCa, prognosis of outcome after surgery, and monitoring/prediction of adjuvant therapy. Therefore, a distinct set of genomic markers is needed for a particular subtype (e.g., *ERG*$^+$ *vs CHD*$^-$) of PCa at a specific stage (e.g., AS *vs* RP *vs* mCRPC) of the disease in an appropriate subpopulation (e.g., Caucasian *vs* African *vs* Asian men). While genome-wide analysis is feasible for RP patients due to ample amount of available tissues, targeted genetic analysis seems more practical using tissue samples obtained from biopsies and CTC/ctDNA samples for AS and mCRPC patients, respectively, until whole genome analysis becomes robust and cost effective.

In addition to association, AUC by receiver operating characteristic analysis and positive and negative predictive values of these genomic markers should be evaluated for prognostic accuracy and their utilities in clinical settings. Although new technological tools, such as NGS and high-throughput genotyping, are widely adapted by clinical labs, use of these tools for genomic prognosis/prediction of clinical outcome currently adds significant costs. Therefore, inexpensive/robust methods and simple/effective algorithms must be developed for common clinical practice until higher-cost technology with more complex analyses is proven necessary for added clinical benefits.

Furthermore, analytical validity of DNA-based genomic predictors in a given method must be assessed. This includes precision and reproducibility, accuracy and trueness, sensitivity and limit of detection, specificity, and interference. To maximize clinical benefit, various sets of cutoff criteria and algorithms for clinical prediction and analytical validity using genomic DNA profiling at different stages must be developed to minimize the risks of underestimating cancer progression, and vice versa.

CONCLUSIONS

Unprecedented amounts of data from recent genome-wide analyses of various tumor genomes now enable a better understanding of the relationships between specific genomic alterations and clinical characteristics. As a result, new DNA-based prognostic markers have been discovered with the potential for distinguishing aggressive PCa from indolent tumors and predicting clinical outcome. Validation of the utility of these genomic markers in prospective studies with proper experimental design is warranted before they can be used clinically as predictors of cancer outcomes. While DNA-based markers hold advantages over and complement expression-based markers, their real contributions to clinical practice and their net benefits to PCa patients must be the subjects of long-term prospective investigations in clinical settings.

COMPETING INTERESTS

The author has no competing interests to declare.

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