The Genomic Landscape of Prostate Cancer

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Prostate cancer is a common malignancy in men, with a markedly variable clinical course. Somatic alterations in DNA drive the growth of prostate cancers and may underlie the behavior of aggressive versus indolent tumors. The acceleration of genomic technologies over the last two decades has identified mutations that drive prostate cancer formation, progression, and therapeutic resistance. Here, we discuss exemplary somatic mutations in prostate cancer, and highlight mutated cellular pathways with biological and possible therapeutic importance. Examples include mutated genes involved in androgen signaling, cell cycle regulation, signal transduction, and development. Some genetic alterations may also predict the clinical course of disease or response to therapy, although the molecular heterogeneity of prostate tumors poses challenges to genomic biomarker identification. The widespread application of massively parallel sequencing technology to the analysis of prostate cancer genomes should continue to advance both discovery-oriented and diagnostic avenues.

Keywords: prostate cancer, genomic, genome sequencing

Prostate cancer is the second most common cancer in men worldwide and causes over 250,000 deaths each year (Jemal et al., 2011). However, many men with prostate cancer do not develop symptomatic disease. Overtreatment of indolent tumors may result in significant morbidity. A deeper understanding of the genomic differences between lethal and indolent prostate cancer, as well as elucidation of “druggable” effectors dysregulated by genetic alterations, should improve patient stratification and speed the development of targeted therapies.

With the advance of genome characterization technologies over the last two decades, the somatic alterations that may drive prostate tumors have come into sharper focus. In this mini-review, we survey the field of prostate cancer genomics, highlight recent findings, and discuss prospects for future research.

THE MUTATIONAL SPECTRUM OF PROSTATE CANCER

All categories of DNA sequence alterations contribute to prostate tumorigenesis, including point mutations, small insertions or deletions, copy number changes, and chromosomal rearrangements (Figure 1). An overview of each category of alteration, and its contribution to prostate cancer biology, is presented below.

SOMATIC COPY NUMBER ALTERATION

Most prostate cancers exhibit somatic copy number alterations (SCNAS), with genomic deletions outnumbering amplifications in early stages of disease (Visakorpi et al., 1995). Early studies relied on cytogenetics, fluorescence in situ hybridization and molecular genetic approaches to map candidate cancer genes to regions of SCNA (Brotman et al., 1999). In recent years, comparative genomic hybridization and high-density single nucleotide polymorphism arrays have allowed high-resolution genome-wide analysis of SCNAS. Statistical analyses of genome-wide copy number data have narrowed the boundaries of recurrent alterations considerably and have pinpointed novel cancer genes in these regions (Beroukhim et al., 2007; Taylor et al., 2010; Robbins et al., 2011).

The extent of SCNA is generally modest in pre-cancerous prostate intraepithelial neoplasia (PIN), but becomes increasingly prevalent along the spectrum from localized adenocarcinoma to metastatic disease (Zittlauberger et al., 2003). Particular recurrent SCNAS are enriched in advanced tumors. For example, tumors that fail androgen ablation therapy show frequent amplification of chromosomes 7, 8q and X (Visakorpi et al., 1995; Ales et al., 2006; Holcomb et al., 2009). Animal models of prostate cancer indicate that genes in these regions, such as the androgen receptor gene (X) and the MYC proto-oncogene (8q), contribute to cancer progression (discussed in detail below).

POINT MUTATIONS AND SMALL INSERTIONS–DELETIONS

Relative to structural alterations, recurrent point mutations are less common in primary prostate cancers (Kan et al., 2010). Primary tumors generally harbor one to two somatic variants per million base pairs – far fewer than known carcinogen-driven tumors such as lung cancer or melanoma, but comparable to breast, renal, or ovarian cancers (Greenman et al., 2007; Plesance et al., 2010a,b; Berger et al., 2011). While most of these mutations confer no proliferative advantage, a handful of recurrent oncogenic mutations have been defined.

The reported prevalence of mutations in several known cancer genes varies widely and depends on tumor purity, stage, histological grade, and exposure to treatments. For example, RB1, TP53, and PTEN are preferentially mutated in locally advanced...
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tance as well. Activating mutations in KRAS and BRAF occur in
~10% of Asian patients but are rare in Caucasian men, perhaps
defining different environmental etiology or biological behavior
of cancers in these populations (Watanabe et al., 1994; Komishi
et al., 1997; Cho et al., 2006).
Defects in DNA mismatch repair (MMR) machinery have been
reported in prostate cancers and may accelerate progression to
castration-independence (Dahiya et al., 1997; Chen et al., 2001).
Large-scale sequencing studies have recently identified a subset of
tumors with markedly elevated rates of point mutation (Taylor
et al., 2010; Kumar et al., 2011; unpublished data). It remains to be
determined whether the high levels of mutation in these tumors are
displayed by MMR deficiency, and whether hyper-mutated cancers
caused more clinically aggressive behavior.
STRUCTURAL REARRANGEMENTS
The discovery of ETS family gene fusions in roughly half of
prostate cancers heralded a novel class of alterations in epithelial
malignancies as a whole (Tomlins et al., 2005). The most common
and prototypical ETS fusion places the oncogenic ERG transcrip-
tion factor under control of the androgen-regulated TMPRSS2
gene, leading to high expression in the prostate epithelium. Subse-
quent research has identified a host of similar oncogenic fusions,
where a proto-oncogene is adjoined to a highly active promoter
(Tomlins et al., 2007; Kumar-Sinha et al., 2008; Palanisamy et al.,
2010). Since mutation or amplification of oncoproteins is less com-
mon in early-stage prostate cancer, genomic rearrangements may
comprise an important means of cancer gene dysregulation in
nascent tumors.
Complete sequencing of prostate cancer genomes has provided
further insight into chromosomal rearrangements in prostate can-
cer. Primary tumors may harbor an average of approximately
100 rearrangements, including translocations, deletions, inser-
tions, and inversions (Figure. 1, Berger et al., 2011). Some tumors
display “closed chains” of balanced rearrangements, in which
multiple DNA breaks occur throughout the genome and the result-
ing fragments are shuffled and rejoined to one another. These
rearrangements may arise when the affected genetic loci are phys-
ically proximal to each other, possibly due to co-regulation by
genomic rearrangement in open- or closed-chromatin compartments (Osborne et al., 2004; Berger
et al., 2011). Consistent with this hypothesis, androgen stimula-
tion can induce physical co-localization of TMPRSS2 and ERG
and permit fusion of these genes de novo via a topoisomerase
2B-mediated mechanism (Hallinan et al., 2010).
The diverse categories of genomic aberrations underscore the
need for comprehensive genomic analyses both to understand
tumor biology and to direct targeted therapies on a genotype-
specific basis (Roychowdhury et al., 2011).

Cellular Pathways Dysregulated by Recurrent
Prostate Cancer Genomic Alterations
Genomic alterations in prostate cancer can increasingly be con-
ceptualized in terms of the molecular processes and pathways on
which they impinge (Taylor et al., 2010). Mutations in prostate
cancer may affect signal transduction pathways that regulate
growth and proliferation, as well as genes involved in the nor-
mal development of the prostate. Below, we highlight several
themes and pathways that provide a framework for understanding
genomic alterations in prostate cancer.
Pik and Mapk signaling
The phosphoinositide 3-kinase (PI3K) pathway is a central medi-
or of cellular proliferation and growth that is aberrantly acti-
vated in prostate cancer. In response to pro-proliferative signals,
PI3K catalyzes the formation of phosphatidylinositol (3,4,5)-
triphosphate (PIP3), which recruits Akt to the plasma membrane.
Upon phospho-activation at the plasma membrane, Akt phospho-
rylates a wide array of substrates that promote proliferation and
cell survival.
Prostate tumors achieve activation of PI3K signaling most
frequently via inactivation of the tumor-suppressor gene PTEN
(Figure 2). PTEN encodes a lipid–protein phosphatase that
counteracts signaling by PI3K via dephosphorylation of PIP3. Loss of
heterozygosity at the PTEN locus is found in up to 70% of pri-
mary prostate cancers and inactivating mutations occur in 5–10%

FIGURE 1 | Genomic alterations in four high-risk prostate cancers. Cross plots depicting genomic rearrangements and copy number
alterations in four prostate tumors analyzed by whole-genome sequencing (unpublished data). Green and pink lines designate intrachromosomal and
interchromosomal rearrangements, respectively. Somatic copy number alterations are indicated in red (amplification) and blue (deletion) in the inner
rings. Gleason scores indicate the two most prevalent histologic grades in each tumor. Pathological stage is noted as well, where pT3 indicates locally
invase disease.
Somatic alterations in the PI3K pathway in prostate cancer.

Selected genes in the PI3K pathway are depicted, alongside the mechanisms by which they are altered in prostate cancer. Putative proto-oncogenes are boxed in red and tumor-suppressor genes in blue. PI3K signaling is frequently activated by deletion of PTEN. PHLPP1 encodes a phosphatase that dephosphorylates activated Akt, and is frequently co-deleted with PTEN in metastatic tumors (Chen et al., 2011). Genomic rearrangements disrupt MAGI2, which encodes a scaffolding protein that stabilizes PTEN (Gu et al., 2003; Berger et al., 2011). Recurrent deletions inactivate the FOND1A gene, which encodes a transcription factor substrate of Akt that mediates PI3K signaling. Although rare, oncogenic mutations in the receptor tyrosine kinase EGFR or AKT1 may activate the pathway upstream or downstream of PI3K (Kai et al., 2006; Boormans et al., 2008). The expression of most pathway members is dysregulated at the transcript level as well.

Amplification of PIK3CA, which encodes the catalytic subunit of PI3K, occurs in 13–39% of primary tumors and 50% of castration-resistant tumors (Edwards et al., 2003; Sun et al., 2009; Barbieri et al., 2012). Amplification of PIK3CA is enriched in advanced tumors and correlates with decreased cancer-specific survival (McMenamin et al., 1999; Sirac et al., 2009). PTEN loss in the mouse prostate collaborates with other tumor-promoting events such as loss of TP53 and overexpression of c-Myc or ERG (Chen et al., 2005; King et al., 2009; Kim et al., 2012).

Amplification of PIK3CA, which encodes the catalytic subunit of PI3K, occurs in 13–39% of primary tumors and 50% of castration-resistant tumors (Edwards et al., 2003; Sun et al., 2009; Agelli et al., 2011). Activating mutations have been observed in ~5% of primary tumors (Sun et al., 2009; Barbieri et al., 2012). PIK3CA activation and PTEN loss tend to be mutually exclusive, which suggests functional redundancy — although larger sample sizes are needed to assess this relationship robustly (Sun et al., 2009). Interestingly, PTEN loss and PIK3CA activation co-occur in endometrial cancer, suggesting that multiple lesions are required to activate the pathway, or that these events engage disparate oncogenic mechanisms (D’Ma et al., 2003). In support of the latter possibility, oncogenic Akt-independent signaling downstream of mutant PIK3CA has been observed in both primary tumors and cancer cell lines (Nauadevan et al., 2009).

The PI3K pathway may be activated by genomic alterations at additional pathway nodes and dysregulated expression of constituent genes (Figure 2; Dong et al., 2006; Cai et al., 2008; Taylor et al., 2010). Determining whether these lesions predict sensitivity or resistance to PI3K pathway inhibitors has become an active area of translational research.

The mitogen-activated protein kinase (MAPK) pathway also plays a role in prostate cancer pathogenesis, especially in advanced and castration-resistant tumors. MAPK pathway activation is associated with higher tumor stage and grade and recurrent disease (Guo et al., 1999). In the setting of castration resistance, PI3K and MAPK signaling are often coordinately dysregulated (Gao et al., 2006; Kinkade et al., 2008). Evidence for collaboration between...
these pathways continues to emerge. For instance, PTEN-induced senescence may be overcome by up-regulation of MAPK signaling induced by overexpression of HER2 (Ahmad et al., 2011). Up-regulation of RAS family members, RAF1 and BRAF, or down-regulation of SPRY1 or SPRY2 genes, are common and enriched in prostate cancer metastases (Kwabi-Addo et al., 2004; McKie et al., 2005; Taylor et al., 2010). In some cases, expression of RAS, RAF1, and BRAF is activated by oncogenic fusions with highly expressed promoters (Palaisamy et al., 2010; Wang et al., 2011). Repression of the RAS-GAP gene DARZIP by EZH2 may activate MAPK signaling and drive progression and metastasis (Min et al., 2010). Defining the relevant mechanisms of pathway activation in greater detail will likely inform strategies for targeting castration-resistant tumors.

**CELL CYCLE REGULATORY GENES**

Several cell cycle regulatory genes are disrupted in prostate cancer. Inactivation of cell cycle inhibitors appears to be required to avoid senescence induced by oncogenic signaling and possibly to bypass androgen-regulation of growth in metastatic or castration-resistant tumors. Two critical cell cycle regulatory genes, TP53 and RB1, are commonly deleted or mutated in metastatic tumors (Bookstein et al., 1993; Heidenberg et al., 1995; Tricoli et al., 1996; Hyytinen et al., 1999). p53 activates expression of the p21WAF1 cyclin-dependent kinase inhibitor, and the Rb protein regulates transition from the G1 to S cell cycle phase. RB1 inactivation is common in castration-resistant tumors (Holcomb et al., 2009; Sharma et al., 2010). Likewise, inactivation of p53 is necessary to bypass cellular senescence mechanisms that are activated upon loss of PTEN (Chen et al., 2005).

Another key cell cycle regulator, CDKN1B, encodes the p27Kip1 cyclin-dependent kinase inhibitor, and resides within the 12p13 chromosomal region that is frequently deleted. Low p27Kip1 expression correlates with poor pathological prognostic markers (Vis et al., 2000; Dreher et al., 2004). Amplification of SKP2, which encodes a ubiquitin ligase that targets p27Kip1 for proteasomal degradation, may also serve to inactivate p27Kip1 (Taylor et al., 2010; Robbins et al., 2011). Duplication of CDKN1B promotes prostate cancer coordinately with hemizygous deletion of chromosome 8p appears to disrupt terminal differentiation and foster the mutational progression of the prostate gland and develop PIN-like lesions with age (Bhattachar and Visakorpi, 1999). In addition, NEK3-1-deficient mice exhibit defective branching morphogenesis of the prostate gland and develop PIN-like lesions with age (Bhattachar and Visakorpi, 1999). In addition, NEK3-1 appears to protect the differentiated prostate epithelium from oxidative DNA damage (Ouyang et al., 2005; Bowen and Gelmann, 2010). Therefore, loss of NEK3-1 may both disrupt terminal differentiation and foster the mutational inactivation of collaborating cancer genes such as PTEN (Kim et al., 2002).

The Wnt pathway regulates embryological development, and its contribution to prostate cancer is becoming increasingly recognized (Yardy and Brewster, 2005). Key pathway genes including APC, AXIN1, and the β-catenin gene CTNNB1 may be mutated at low frequency (Vooler et al., 1996; Chesselet et al., 2006; Yardy et al., 2009). APC undergoes LOH in roughly 20% of primary cancers and promoter CpG methylation in up to 90% (Brewster et al., 1994; Phillips et al., 1994; Yegnasubramanian et al., 2004). β-Catenin may promote proliferation through co-activation of AR-mediated transcription (Tusca et al., 2000; Cronauer et al., 2005). Additional mutations in Wnt pathway genes were recently documented in the progression to castration-resistant disease (Kumar et al., 2011). More pairs of pre- and post-relapse samples should be analyzed to clarify the importance of this pathway in refractory disease.

**GENOMIC HETEROGENEITY OF PROSTATE CANCER**

Prostate cancer is a clinically and genetically heterogeneous disease. Independent cancerous foci with distinct morphological features often coexist in a single prostate. The course of disease also varies widely: some cancers remain indolent for decades while others rapidly progress to lethality. Distinct molecular features appear to underlie the clinical and histological differences. Identifying genomic determinants of aggressive disease might improve experimental modeling and stratification of patients with intermediate-risk prostate cancer.

Prostate cancer may arise in multiple foci from independent precursor cells that are driven to neoplastic transformation by carcinogenic exposures or genetic predisposition (Androusi and Cheng, 2010). The presence of genomic lesions can vary between foci, including TMPRSS2-ERG fusion, MYC amplification, and TP53 mutation (Mirchandani et al., 1985; Jenkins et al., 1997; Mehra et al., 2007). Multiple distinct clones can be identified in a single biopsy (Ruiz et al., 2011), but most metastatic prostate cancers appear to originate from a single clone within a primary
tumor (Qian et al., 1995; Holcomb et al., 2009; Liu et al., 2009). Among other lesions, subclonal TP53 mutations may define cells in the primary tumor with metastatic potential (Michaudani et al., 1995; Navone et al., 1999). Intratumoral heterogeneity complicates efforts to define prognostic mutations or expression signatures from primary tumors, because the subclone within a primary tumor that gives rise to metastatic disease must be adequately sampled (Shoner et al., 2010).

Despite the challenges posed by tumor heterogeneity, expression signatures have been delineated that have prognostic and therapeutic implications. Some genetic alterations appear to have prognostic value as well. The TMPRSS2-ERG fusion, MYC amplification, and PTEN or TP53 deletion predict cancer-specific death in at least some patient cohorts (Sato et al., 1999; Demichelis et al., 2007; Sirac et al., 2004, in some cases, a mutational signature may underlie expression-based sub-classifications (Lapointe et al., 2004, 2007).

PROSTATE CANCER IN THE ERA OF GENOMICS-DRIVEN MEDICINE

High-throughput genomic profiling has advanced the understanding, prognostication, and treatment of several tumor types. For example, identification of mutations in BAP1 in uveal melanoma (Harbour et al., 2010) or IDH1 in glioblastoma and acute myeloid leukemia (Parsons et al., 2008; Mardis et al., 2009) demonstrated the power of genome sequencing to pinpoint novel cancer-driving mutations. Risk-predictive transcriptional signatures have been proposed that delineate histologically indistinguishable tumor types (Qian et al., 1995; Holcomb et al., 2009; Liu et al., 2009). As a result of these efforts to define prognostic mutations or expression signatures for discovery of novel cancer genes and biomarkers as well, since genomic characterization of large cohorts of aggressive tumors has not recently become feasible.

Indeed, whole-exome sequencing of 100 primary prostate tumors–normal pairs revealed that the ubiquitin ligase complex subunit gene SPOP is among the most frequently mutated genes in primary tumors, though its role in cancer was heretofore unrecognized (Barbieri et al., 2012). This study also identified novel recurrent mutations in the fork-head transcription factor gene FOXA1 and mediator complex gene MED12. Experimental study will be required to determine whether these mutations engage known molecular pathways relevant to prostate cancer or reflect novel mechanisms of oncogenesis.

Several hurdles must be overcome for prostate cancer genomics to impact the clinical management of this disease. For instance, biopsies produce scarce material for clinical genotyping and may not fully capture the relevant molecular heterogeneity within a tumor. Expression signatures have not yet demonstrated sufficient prognostic value to merit widespread use. In addition, recurrent genetic lesions identified thus far are largely not considered “druggable.”

These challenges can likely be surmounted by new approaches. For example, genomic characterization may identify opportunities to leverage synthetic lethality by inhibiting targets that are essential in the setting of a particular mutation, such as poly(ADP-ribose) polymerase in ETS-fusion positive prostate cancer (Brenner et al., 2011). The analysis of multiple samples from a primary tumor and perhaps from circulating tumor cells may allow aggressive tumor subclones to be identified. Ultimately, new paradigms for clinical trials may be required that incorporate cancer genomic information. In spite of these challenges, genomic profiling is likely to play a significant role in the clinical management of prostate cancer and ultimately in the clinical management of this malignancy.

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