New Strategies in Prostate Cancer: Translating Genomics into the Clinic

Himisha Beltran1,2 and Mark A. Rubin1,3

Abstract

With the rapidly developing use of next-generation sequencing technologies, there has been a surge in our knowledge of the genomic landscape of prostate cancer and a movement toward developing a molecular subclassification system for the disease. With this new understanding comes great clinical potential, both for the development of biomarkers as well as new therapeutic targets. Herein, we highlight the potential clinical use of recent discoveries and how they fit into our current paradigm. We describe the challenges that lie ahead as we move from genomic sequencing toward routine clinical practice and adopt precision cancer care for patients with prostate cancer. Clin Cancer Res; 19(3); 517–23. ©2012 AACR.

Disclosure of Potential Conflicts of Interest

M.A. Rubin is a coinventor of the patent on the detection of gene fusions in prostate cancer, filed by The University of Michigan and the Brigham and Women’s Hospital. The diagnostic field of use for ETS gene fusions has been licensed to Hologic Gen-Probe. No potential conflicts of interest were disclosed by the other author.

CME Staff Planners’ Disclosures

The members of the planning committee have no real or apparent conflict of interest to disclose.

Learning Objectives

On completion of this activity, the participant should be able to identify the common genomic alterations seen in prostate cancer and understand some of the advantages and challenges in implementing genomic studies into routine clinical practice.

Acknowledgment of Financial or Other Support

This activity does not receive commercial support.

Background

Although prostate cancer is associated with a longer natural history than most other tumor types and most of the 230,000 men diagnosed with prostate cancer every year in the United States do not die from the disease, it remains the second leading cause of cancer death in men, accounting for 32,000 deaths annually (1). Despite an improved understanding of prostate tumor biology, we have not yet delineated why certain tumors behave more aggressively than others. Furthermore, all patients with prostate cancer are treated similarly once metastases have developed, and patients vary widely in their response to therapies. One key step toward unraveling this clinical heterogeneity seen among patients with prostate cancer is through deciphering molecular heterogeneity within their prostate tumors. Recent advances in cancer genome sequencing have provided critical new insights into the molecular classification of several solid tumors, including colon (2), glioblastoma (3), and breast (4) cancers, and emerging data from prostate cancer sequencing have nominated potential initiating and driving genetic alterations (5–8). Understanding the molecular alterations driving prostate cancer can aid in the design of more effective targeting strategies both for disease prevention and for the treatment of systemic disease.

The Genomic Landscape of Primary Prostate Cancer and Clinical Implications

Gene fusions

In a landmark study in 2005, Tomlins and colleagues first reported that gene rearrangements involving the Ets family of transcription factors (most commonly ERG) fused with an androgen-regulated 5’ gene partner (most commonly TMPRSS2) occur in approximately 50% of all prostate cancers (9). These gene fusions occur early during the disease pathogenesis (present in high-grade prostate intraepithelial neoplasia; refs. 10, 11) and are
prostate-cancer specific (12), and therefore not present in benign prostate or any other tumor type. This has important diagnostic implications, and the TMPRSS2–ERG fusion is now being prospectively evaluated as a diagnostic urinary test to complement prostate-specific antigen (PSA) screening (13, 14). Testing for ERG gene fusion is also indicated in cases in which the diagnosis of prostate cancer is not clear, particularly in evaluating metastases of unknown primaries, in which its presence can confidently secure the diagnosis (50% sensitivity, 100% specificity). The FISH ERG break-apart assay is gold standard for detecting ERG rearrangement (9). Immunohistochemistry (IHC) to detect ERG overexpression can also be used with a sensitivity of 95.7% and specificity of 96.5% for primary prostate cancer (15). Notably, in cases of advanced prostate cancer that become dedifferentiated and lose androgen receptor (AR) expression, the ERG protein may be absent even in the setting of ERG gene rearrangement (16, 17), and FISH should be used in these cases to confirm the diagnosis. In addition to TMPRSS2–ERG, a number of other Ets family gene rearrangements have been identified in prostate cancer, including TMPRSS2–ETV1, TMPRSS2–ETV4, TMPRSS2–ETV5, SLC45A3–ERG, and others (9, 10, 18). When present, Ets gene fusions are mutually exclusive of one another. Therefore, by incorporating probes that include each of the identified Ets rearrangements, future next-generation sequencing (NGS) diagnostic assays will have an even higher sensitivity in detecting or confirming prostate cancer.

The prognostic significance of the TMPRSS2–ERG gene fusion has also been studied by several groups, evaluating primary prostate tumors from different cohorts. In a population-based watchful waiting cohort, ERG fusion was associated with worse clinical outcomes (i.e., prostate-specific death; ref. 19). However, mixed results have been seen in several retrospective prostatectomy cohorts (20–24), mostly evaluating association with the development of biochemical recurrence. Larger prospective cohorts with survival outcomes are likely needed to clarify the prognostic significance of ERG fusion for patients undergoing intervention for primary localized disease. One possible explanation for the discrepancy seen in prior studies may be that the oncogene was removed early in the retrospective surgical series and therefore may not be reflected in outcomes, as opposed to watchful waiting cohorts in which the primary tumors are followed for their natural history of disease.

The TMPRSS2–ERG transcript does not encode for a chimeric protein but rather results in overexpression of a truncated ERG protein in the setting of active androgen signaling, which drives a unique transcriptional program linked to DNA damage, invasion, and metastasis (25). Genetically engineered mice overexpressing ERG develop precursor lesions (26), and when these features are combined with AR or PTEN loss, develop invasive carcinomas (27, 28), suggesting that ERG may also play a role in disease initiation. Because these fusions are prostate cancer specific, ERG is an attractive therapeutic target. In general, it is challenging to develop drugs that target transcription factors, but most recently, short peptidomimetics that bind to ERG and interfere with ERG binding to gene promoters have shown promise in preclinical prostate cancer models (29). ERG also interacts with and functionally cooperates with PARP1 (30), and the PARP inhibitor ABT888 is also being evaluated as another means by which to target ERG fusion–positive tumors (NCT01576172).

A lack of Ets gene fusion may also be clinically significant, as these prostate tumors may represent a distinct molecular subclass. Ets-negative prostate cancer has been linked to overexpression of the gene SPINK1 (serine peptidase inhibitor, Kalal type 1) in 10% of all prostate cancers (31), which is potentially targetable using an EGF receptor (EGFR) inhibitor (32), and/or point mutations involving the speckle-type POZ protein (SPOP) gene in 6% to 13% (6), as outlined below.

Another less common but clinically significant rearrangement seen in prostate cancer involves the RAF kinase gene. Present in less than 1% of all prostate cancers, preclinical studies suggests that these may be targetable using clinically available RAF/MEK inhibitors (33); thus, detection of RAF fusions may have direct clinical potential.

**PI3K/Akt/PTEN pathway alterations**

Alteration of genes leading to aberrant activation of the phosphoinositide 3-kinase (PI3K)/Akt pathway is frequent in prostate cancer, implicated in both primary (42%) and metastatic (100%) disease (8). Although activating point mutations involving PIK3CA or AKT1 are rare, loss-of-function mutations or deletions involving the tumor suppressor gene PTEN are frequent and especially apparent in advanced disease (with loss of heterozygosity of PTEN present in 20% to 60% of metastatic prostate cancer; refs. 7, 34, 35). Whole-genome sequencing studies have also recently identified complex rearrangements involving PTEN and the PTEN-interacting protein MAGI2 as another mechanism for PTEN inactivation (5).

The PI3K/Akt pathway stimulates various processes implicated in cancer, including growth, proliferation, survival, and metabolism (36). Introduction of PTEN loss in genetically engineered mice leads to precursor prostate cancer lesions, and the mice develop invasive carcinoma when these features are combined with other alterations (i.e., ERG or TP53; refs. 27, 28). Furthermore, the PI3K/Akt signaling pathway has been shown to inhibit AR signaling, and by reciprocal negative feedback, AR inhibition activates Akt signaling by inducing the Akt phosphatase PHLP (37). Targeted therapeutics designed to inhibit the PI3K/Akt pathway, as single agents and in combination with AR-targeted drugs, are currently under clinical investigation for patients with advanced prostate cancer.

**Complex rearrangements**

A key discovery from sequencing the first 7 prostate cancer whole genomes was the identification of frequent and complex genomic rearrangements in prostate cancer, with a median of 90 rearrangements per genome (5).
There were distinct patterns of balanced breaking and rejoining of DNA, not previously observed in solid tumors, and many of these rearrangement breakpoints involved tumor suppressors (PTEN, MAGI2) or putative tumor suppressors (CHD1, ZNF407), or were adjacent to other cancer-regulated genes (TBK1, TP53, MAP2K2, CADM2). Emerging data also suggest that there may be subclasses that tend to have more of these types of rearrangements. This suggests a novel previously unrecognized mechanism for gene alteration that may play a key role in disease pathogenesis. The mechanism underlying development of these complex rearrangements is an area of active investigation and a first step toward elucidating how best to target these events.

**SPOP mutations**

In the first whole-exome sequencing study from a cohort of 112 patients with prostate cancer (6), the overall frequency of point mutations was low, with 0.9 to 1.5 mutations observed per megabase. Recurrent missense point mutations involving the SPOP gene were present in 6% to 13% of cases, now confirmed in multiple independent cohorts. This makes SPOP mutation the most common nonsynonymous point mutation in prostate cancer. Importantly, SPOP gene mutations occur mutually exclusive of Ets gene rearrangements and PTEN loss, supporting SPOP-mutated prostate cancer as a molecularly distinct subclass. SPOP encodes for the substrate-binding subunit of a Cullin-3–based ubiquitin ligase. Mutations were found exclusively in the substrate-binding cleft, and structural analyses suggest that these mutations inactivate SPOP function by disrupting SPOP–substrate interaction. Ongoing work is focused on elucidating the biologic consequences and clinical implications of the SPOP mutation in prostate cancer.

**The Genomic Landscape of Advanced Prostate Cancer and Clinical Implications**

Deciphering molecular events associated with prostate cancer progression, the development of castration resistance, and formation of metastases has been challenging. Recently, Grasso and colleagues published exome sequencing results of 50 cases of metastatic castration-resistant prostate cancers (CRPC) obtained at rapid autopsy (7). Notably, the mutation rate remained low (overall 2 mutations per megabase), but the number of copy number alterations was significantly higher in CRPC compared with primary prostate cancer. Multiple candidate driver mutations and copy number alterations were identified in CRPC, involving genes associated with AR signaling, DNA damage response, histone/chromatin modification, and the spindle checkpoint, and have provided clues toward mechanisms of resistance and insight for developing new therapeutic strategies.

**AR alterations**

Despite castrate levels of circulating testosterone, most CRPC tumors remain dependent on AR signaling (38). This can occur through various mechanisms (reviewed in the work of Knudsen and Scher; ref. 39), including alterations in the AR gene itself through acquisition of gene amplification, point mutations, or the development of splice variants. AR gene alterations are common in CRPC and have not been observed in any cases of hormone-naïve prostate cancer. Specifically, in the recent prostate cancer exome sequencing study from Barbieri and colleagues, none of the 112 clinically localized tumors showed AR gene alterations (6). In the study of Taylor and colleagues and Grasso and colleagues of a total of 87 CRPCs, alterations in AR were present in approximately 60% of CRPC (7, 8). Another NGS study with more than 900-fold coverage identified 44% AR gene alterations in 25 CRPC cases (40). Cofactors that physically interact with AR (i.e., FOXA1, MLL complex genes, ASXL1-3, UTX) are also often mutated in CRPC (7) and may be another newly identified mechanism of treatment resistance. Taken together, these results suggest that AR and AR cofactor mutations are largely a result of treatment and do not represent a predisposition. However, there may be other germline polymorphisms that predispose to such mutations that would help us to understand why only a subset of treated men develop these alterations.

**Aurora kinase and N-myc amplification**

Less commonly, CRPC tumors can lose AR expression in late stages of disease as another mechanism of resistance. The development of AR-independent anaplastic or neuroendocrine prostate cancer (NEPC) is associated with low PSA, frequent visceral metastases, and an aggressive clinical course (41). Co-amplification of genes encoding the cell-cycle kinase Aurora kinase A (AURKA) and the transcription factor N-myc (MYCN) occurs in 40% of NEPC (16) and can occur early before the development of NEPC (42). Aurora kinase A and N-myc functionally cooperate to induce transdifferentiation of prostate cancer cells to a neuroendocrine phenotype and are potentially targetable using an Aurora kinase inhibitor (16). A clinical trial evaluating the AURKA inhibitor alisertib for patients with NEPC is under way. AURKA and MYCN co-amplification are being explored as potential predictive biomarkers and may be used to select NEPC and patients with high-risk prostate cancer for early intervention with AURKA-targeted therapy.

**Defects in DNA repair**

Genomic aberrations involving DNA defect repair genes have been reported in both CRPC and high-risk localized disease, including mutations or deletions involving BRCA2 and ATM present in up to 20% of cases (40). This may have important treatment implications, as tumors with defects in homologous recombination (such as those with BRCA or ATM mutations) are sensitive to inhibition of PARP1 (involved in base excision DNA repair). An ongoing trial, the TO-PARP study (NCT01682772), is investigating the role of the PARP inhibitor olaparib for patients with CRPC and evaluating these alterations and others prospectively as potential predictive biomarkers.
Recurrent homozygous deletions involving the chromodomain helicase DNA-binding protein gene, \textit{CHD1}, are also common in prostate cancer (present in 5% to 10%; refs. 6, 7, 43). \textit{CHD1} encodes a chromatin-remodeling enzyme and has been implicated to have a causal role in the prevention of somatic deletion events. Therefore, loss of \textit{CHD1} may be involved in the large number of copy number alterations seen in prostate cancer and

Figure 1. Molecular subclassification of prostate cancer. Disease initiation occurs through activation of androgen signaling, complex rearrangements, or other proposed mechanisms, leading to prostate tumor–wide clinical heterogeneity and distinct molecular subtypes. Disease progression and resistance to therapy lead to acquisition of new and potentially overlapping molecular alterations.

Figure 2. Proposed model of precision medicine for prostate cancer. Patients newly diagnosed with prostate cancer will have their prostate biopsy sequenced using NGS technologies. On the basis of molecular subtyping, patients with low-risk disease will remain on surveillance and those with high-risk tumors will undergo local therapy with or without the addition of targeted therapy identified through sequencing. At the time of disease progression, metastatic lesions will also be rebiopsied for comprehensive sequencing. Hormonal therapies will be initiated as the standard of care, with or without targeted therapy identified through sequencing. When patients progress on therapy, rebiopsy and sequencing will lead to use of targeted therapy based on the molecular profile of the resistant tumor.
with disease progression. Notably, CHD1 deletions are mutually exclusive of TMPRSS2–ERG fusions, thus representing another distinct molecular subclass of prostate cancer.

On the Horizon

Molecular subclassification of prostate cancer and precision cancer care

We propose taking a significant step toward transitioning prostate cancer from a poorly understood, clinically heterogeneous disease into a collection of homogeneous subtypes identifiable by distinct molecular criteria (Fig. 1). We should consider prostate cancer to be, like acute myelogenous leukemia or breast cancer, a collection of cancers that may best be defined by characteristic molecular alterations. We believe that in the future of targeted therapies and precision cancer care, we will start treating prostate cancers as “ERG rearranged,” “SPOP mutated,” “AR activated,” or “AURKA/MYCN amplified” (Fig. 2).

With the decreasing cost and increasing efficiency of genome sequencing, sequencing a cancer genome in a patient will soon be clinically feasible and can be incorporated into the standard of care. Numerous commercial high-throughput NGS assays are being developed, such as those available through Foundation Medicine. Currently focused on detecting predefined actionable mutations, these developers are pushing technology to sequence formalin-fixed, paraffin-embedded tissue with a lower DNA requirement and at higher depth and will likely move toward whole-genome sequencing in the near future. Noninvasive methods for sequencing tissue from alternative sources (circulating tumor cells, plasma DNA, exosomes, urine) are also being explored for diagnostic and predictive biomarker detection.

A number of ongoing and planned therapeutic trials are exploring novel biomarkers and response to targeted therapies for patients with prostate cancer. These include both biomarker-driven trial design (e.g., TO-PARP study) and correlative endpoint trials (e.g., alisertib), as well as studies focused on biomarker discovery. The Stand Up 2 Cancer/ AACR (a Program of the Entertainment Industry Foundation) and Prostate Cancer Foundation have recently funded a multicenter next-generation clinical trial in prostate cancer that will incorporate whole-exome sequencing of metastatic CRPC tumors.

Challenges

With our improved understanding of the genomic landscape of prostate cancer, we are also left with a number of questions and challenges.

Distinguishing drivers

As sequencing technology evolves, we are discovering novel alterations at a rapid pace. It is a significant challenge to functionally characterize new mutations and to distinguish driving mutations (those essential for cancer growth and survival) versus those that arise as passengers, due to therapy or other events leading to genomic instability. Incorporating the role of epigenomic and tumor microenvironment changes also must be taken into consideration. Using computational methodologies to identify candidate alterations as functionally significant, such as those recently reported by Nijhawan and colleagues (44), may help provide initial clues in distinguishing driver versus passenger mutations. Furthermore, recent cataloging of the molecular and pharmacologic profiles of available cell lines, publically available through the Cancer Cell Line Encyclopedia (45), can help in the design of preclinical studies to functionally characterize nominated driving lesions and explore downstream consequences of mutations. Correlation of genotypes with both tumor biology and clinical phenotypes is essential as we proceed toward development of novel biomarkers and therapeutic strategies that have a direct impact on patient care.

Understanding resistance to targeted therapies

As targeted therapies are developed, tumor cells develop methods to evade therapy through acquisition of drug-resistance mutations and by activation of bypass pathways. To effectively implement snapshot sequencing technologies into routine cancer care, one must understand the context of the findings. We should learn from each patient and go back to the laboratory to better understand the basis of response and resistance to new targeted therapies. Even uncommon genomic alterations may be clinically significant and can directly affect an individual’s response to therapy and clinical outcome, as shown recently with underlying everolimus sensitivity of TSC1-mutated bladder cancer (46) and also possibly with RAF-rearranged prostate cancer.

Accounting for disease heterogeneity

It is well recognized that primary prostate cancer is multifocal. Both morphologic and molecular analyses have shown that by the time of cancer diagnosis, more than 80% of prostates harbor multiple separate cancer foci (47), and these foci may each have a distinct molecular profile (48). Within one focus, there tends to be homogeneity. For instance, TMPRSS2–ERG fusions, when present, are distributed among all nuclei within a discrete tumor lesion (49). Therefore, challenges in biomarker development will be assessing and prioritizing tumor foci with clinically significant alterations that may play a biologic role in disease progression. Evaluating for concordance of molecular alterations between primary tumors and metastases also provides clues toward identifying early events. For instance, several genes were identified as altered in both primary tumors and CRPC in the recent exome sequencing studies, including those that regulate AR (FOXA1, CDKN1B, MLL2, ASL20; refs. 6–8), and these may be important for disease initiation, as opposed to the AR amplifications and mutations implicated in treatment resistance.

Another challenge lies in determining molecular concordance between different sites of tumor metastases, as this
has important biologic and therapeutic implications (50). Biopsies are not routinely conducted in advanced disease, and if conducted, are usually limited to single biopsies of one site. But is this biopsy representative of the overall tumor burden? Will choosing therapies based on the mutational profile of this single site lead to ineffective therapy for the patient? Studies focused on systematic evaluation of disease heterogeneity within prostate cancer are planned and one hopes they will help answer many of these unanswered questions.

The future

Characterizing the genomic landscape of prostate cancer and defining a molecular subclassification system have enormous potential to translate into clinically relevant biomarkers and new targeted therapeutic approaches. However, many challenges lie ahead, including distinguishing drivers from passengers and understanding disease heterogeneity and treatment resistance. As we exploit rapidly developing genome sequencing technologies, basic scientists and clinicians must come together to understand what drives prostate cancer and design effective biomarker validation trials to rationally and effectively move the field toward precision cancer care for patients with prostate cancer. In the end, a well-designed targeted therapy study that incorporates biopsies and NGS of metastatic tumors before and during treatment will provide important insights that can allow investigators to learn from each clinical trial. Means of determining resistance, even for rare cases, will help drive the field forward and represent a more precise application of cancer care.

References

Clinical Cancer Research

New Strategies in Prostate Cancer: Translating Genomics into the Clinic

Himisha Beltran and Mark A. Rubin


Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-12-1452

This article cites 46 articles, 15 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/19/3/517.full.html#ref-list-1

This article has been cited by 1 HighWire-hosted articles. Access the articles at:
/content/19/3/517.full.html#related-urls

Sign up to receive free email-alerts related to this article or journal.
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.