Clinical variability and molecular heterogeneity in prostate cancer

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Prostate cancer is a clinically heterogeneous disease, with some men having indolent disease that can safely be observed, while others have aggressive, lethal disease. Over the past decade, researchers have begun to unravel some of the genomic heterogeneity that contributes to these varying clinical phenotypes. Distinct molecular sub-classes of prostate cancer have been identified, and the uniqueness of these sub-classes has been leveraged to predict clinical outcomes, design novel biomarkers for prostate cancer diagnosis, and develop novel therapeutics. Recent work has also elucidated the temporal and spatial heterogeneity of prostate cancer, helping us understand disease pathogenesis, response to therapy, and progression. New genomic techniques have provided us with a window into the remarkable clinical and genomic heterogeneity of prostate cancer, and this new perspective will increasingly impact patient care.

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INTRODUCTION

Understanding the clinical heterogeneity seen in prostate cancer (PCa), where some men have indolent disease that may never need treatment while others have lethal disease, is one of the greatest challenges faced by urologists and oncologists. Prostate cancer is fundamentally a genetic disease, driven by genomic instability causing the activation of oncogenes and the inactivation of tumor suppressors. Recent work has begun to unravel some of the genomic heterogeneity in prostate cancer and to define distinct molecular sub-classes of the disease. Leveraging this genomic heterogeneity may fundamentally change how we diagnose and manage prostate cancer in the decades to come. This review will not only examine the heterogeneity of prostate cancer at multiple levels, including clinical and pathological, but also primarily focus on the molecular heterogeneity of prostate cancer. We will discuss the implications of this heterogeneity for patient care across disease states, ranging from low-risk clinically localized prostate cancer to lethal castration-resistant disease.

PROSTATE CANCER PATHOLOGICAL HETEROGENEITY

The histologic spectrum of prostate cancer ranges from its precursor lesion, high-grade prostatic intra-epithelial neoplasia (HgPIN), to de-differentiated cancer. HgPIN is characterized by cellular proliferation within pre-existing glands with cytopathological changes mimicking cancer.¹ The presence of HgPIN is certainly associated with the presence of prostate cancer, but the definitive development of PCa from HgPIN is much less clear. Historically, it was believed that adenocarcinoma would develop in most men with HgPIN within 10 years,¹ and therefore men found to have HgPIN on biopsy generally underwent closer surveillance than men with no evidence of disease, with repeat biopsy typically recommended. However, more recent data suggest a lower risk of HgPIN progression, particularly with the standardization of biopsies consisting of more cores, limiting undersampling.² Importantly, HgPIN harboring specific molecular alterations (such as ERG overexpression) may be more associated with subsequent cancer detection, implying considerable heterogeneity in behavior even within HgPIN sub-classes.³

Historically, perhaps, the most powerful correlate to prostate cancer behavior is the histologic Gleason scoring system. Gleason grading was devised in the 1960s and 70s by Donald F. Gleason and the Veterans Administration Cooperative Urological Research Group, and uses the pattern of carcinoma cells in Hematoxylin and Eosin stained sections to generate a histologic score.⁴ Gleason grade, combined with other clinical factors such as prostate-specific antigen (PSA), age, clinical stage, MRI findings, number of positive cores, and percentage of each core that contains cancer has been incorporated into models which attempt to predict the behavior of prostate cancer. The prognostic ability of these models has led to the stratification of treatment according to risk category. In the absence of other adverse features, it is widely believed that men with isolated Gleason 6 disease may have a low risk of disease progression, and therefore do not require treatment when they are closely monitored.⁵ In contrast, men with localized disease with a Gleason score of 7 or above are usually recommended for local treatment (either radiation or surgery), while men with particularly high-risk clinical or pathologic features may benefit from multimodality therapy.⁶

Despite local treatment with surgery or radiation, some patients’ cancers will progress again in a variable fashion, with a mix of local recurrence, in a nodal basin, or as a distant metastasis to bone or
other sites. In terms of metastatic disease, it is well known that localized prostate cancer nearly universally responds to androgen deprivation therapy (ADT), while over time, almost all tumors will develop resistance to ADT and become castrate-resistant prostate cancer (CRPC). Some of these cancers will then acquire neuroendocrine features, which is associated with a poor response to treatment and prognosis.

**DEFINING MOLECULAR SUB-CLASSES OF PCA**

Over the past 10 years, our understanding of PCA genomics has changed dramatically. The availability of next-generation sequencing technologies has allowed researchers to classify prostate cancers by their multiple levels of molecular signatures, exposing genomic, transcriptomic, and epigenetic heterogeneity. Distinct molecular sub-classes have emerged, with the potential to transform the disease from a poorly understood, heterogeneous disease with a highly variable clinical course to a collection of more homogeneous molecular sub-classes.

**ETS family members**

In 2005, a series of landmark papers discovered fusions of the 5′ untranslated region (UTR) of the TMPRSS2 gene with the ETS family transcription factor family members. This discovery provided the framework for organizing prostate cancers into those with ETS rearrangements and those without those re-arrangements. The most common ETS family re-arrangement is TMPRSS2:ERG which has now been identified in approximately half of prostate cancers and accounts for 90% of ETS family fusions. Fusions of other ETS family members, including ETV1, ETV4, ETV5, and FLII have been identified. These re-arrangements result in overexpression of the ETS family transcription factors which confer a neoplastic phenotype.

The original report of the fusion products, which has subsequently been validated in other cohorts, found that fusions between ETV1 and ERG appear to be largely mutually exclusive. Several other 5′ partners have also subsequently been identified, most notably a fusion product involving the ETS family member ELK4 to SLC45A3 in 5%–10% of prostate cancers.

ETV fusion seems to be an early event in carcinogenesis and has been detected in HgPIN. ERG re-arrangements when detected in HgPIN have also been detected in the adjoining prostate cancer and appear to precede other mutations. ERG re-arranged cancer is rarely identified distant from cancer foci in prostatectomy specimens, suggesting that ERG is important for the transition from HgPIN to cancer. Indeed, ERG re-arrangements in biopsy specimens with HgPIN have been shown to be predictive of the development of prostate cancer (53% vs 35%).

Multiple studies have demonstrated that ETS-positive cancers have distinct features at a number of levels. These show a distinct gene expression signature from ETS-negative cancers. In addition, ETS fusion-positive tumors have distinct copy number aberrations and a distinct pattern of genomic re-arrangements involving chains of balanced re-arrangements, a phenomenon described as “chromoplexy.” Mice engineered to overexpress ERG or ETV1 under androgen regulation develop neoplastic prostate lesions similar to PIN, and ERG overexpression accelerates prostate cancer pathogenesis when combined with deletions in PTEN.

Clinically, there is some evidence to support that ETS-rearranged cancers being more aggressive than cancers without these re-arrangements. This observation is largely derived from two studies from active surveillance cohorts of men diagnosed with prostate cancer on transurethral resection of the prostate (TURP). In both studies, men with TMPRSS2-ERG rearranged cancers had an increased risk of prostate cancer death. In addition, ERG-rearranged tumors have been demonstrated to have an increased risk of progression while on active surveillance. Importantly, it has been demonstrated that a urine test from TMPRSS2:ERG in conjunction with PCA3 is able to stratify patients undergoing active surveillance to determine their risk of having higher Gleason scores or a larger tumor volume. Analysis of prostatectomy specimens, however, has yielded conflicting results regarding the relative aggressiveness of ETS-rearranged cancers. ETS re-arrangements also appear to be more common in peripheral zone tumors than transition zone tumors.

**SPINK1**

Using the same Cancer Outlier Profile Analysis (COPA) used to define ETS gene re-arrangements, Tomlins et al. identified a second sub-class of prostate cancers, which overexpress Serine peptidase inhibitor, Kazal type 1 (SPINK1). SPINK1 outlier expression has been identified in ~10% of prostate cancers, and appears to be mutually exclusive from ERG re-arrangements. Interestingly, patients harboring these tumors were found to have a shorter time to biochemical recurrence than patients who do not overexpress SPINK1. SPINK1 outlier status, independent of Gleason score, lymph node status, surgical margin status, seminal vesicle invasion, extracapsular extension, and preoperative PSA, has been shown to be a significant predictor of clinical recurrence. SPINK1 is an extracellular secreted protein and therefore is amenable to both therapeutic targeting and noninvasive diagnosis. Indeed, studies using antibodies against SPINK1 in mouse prostate cancer xenografts have identified SPINK1 as a likely target in patients harboring SPINK1+/ETS+ tumors.

**SPOP/CHD1**

Recurrent mutations in the SPOP gene are found in 5%–15% of tumors, making it the most common point mutation in prostate cancer. SPOP encodes the substrate-binding sub-unit of a Cullin-based E3 ubiquitin ligase, and mutations affect conserved residues in the structurally defined substrate-binding cleft. SPOP mutation appears to occur exclusively in tumors without ERG re-arrangement and constitute a unique sub-class of prostate cancer. SPOP mutations have been identified in HgPIN adjacent to adenocarcinoma, and likely represent early events in the natural history of prostate cancer. SPOP mutant tumors have been found to have recurrent somatic deletions at 5q21 at the CHD1 locus. CHD1 is an ATP-dependent chromatin-remodeling enzyme, and the genomic locus is deleted in ~5%–10% of prostate cancers. A recent study found no association between SPOP mutation and clinical or pathological parameters. However, others have reported that mutations and decreased expression of the SPOP gene are associated with worse progression-free survival. Functionally, SPOP mutation has been shown to modulate carcinogenesis by preventing the degradation of oncogenic factors including ERG and the androgen receptor. Importantly, our group recently demonstrated that SPOP mutation alters DNA double-strand break (DSB) repair, is associated with genomic instability, and sensitizes to DNA-damaging agents such as PARP inhibitors.

**HETEROGENEITY BETWEEN PROSTATE CANCER CLONES**

**Primary prostate cancer**

Primary prostate cancer has relatively few genomic aberrations compared to other cancers. Recent work found the mutation rate to be ~0.9 per Mb, which is similar to that observed in acute myeloid leukemia and...
Metastatic prostate cancer

Metastatic prostate cancer arises from rare subclones that attain metastatic potential. This “seed and soil” hypothesis, where rare cells acquire metastatic potential has been supported in a number of studies. Interestsingly, a recent study of longitudinally collected primary and metastatic samples identified one metastasis comprising three distinct clones derived from two separate waves of metastasis from the prostate.

The most commonly mutated gene in metastatic PCA is the androgen receptor (AR). AR mutations occur almost exclusively in mCRPC, and are virtually never seen in localized disease. After ERG and AR, the most common alterations involve the inactivation of PTEN in approximately 40% of cancers resulting in activation of the PI3K pathway. PTEN copy number loss is consistently associated with aggressive disease features, cancer progression, and the development of castration-resistant disease. PTEN alterations are enriched in the ERG-rearranged subclass of PCA, and in this class, in particular, PTEN loss may be associated with more aggressive disease. Interestingly, PTEN deletion appears to occur after ERG rearrangement in these tumors, further complicating intratumoral heterogeneity and the search for the “lethal” subclone that leads to CRPC. Other common genetic alterations include p53 mutation (40%), RB loss (28%), aberrations in BRCA1, BRCA2, and ATM (19%), and MYC amplification also commonly occur in metastatic lesions.

The heterogeneity in mutation status between patients has already begun to be exploited for therapeutic benefit. Our institution recently published the results of whole exome sequencing in 154 tumor-normal pairs from 97 patients with metastatic prostate cancer, 94% of whom were found to have alterations which were potentially actionable, similar to the 89% of actionable mutations previously published. Five percent of these patients actually received therapy based on these results.

Between metastatic foci

Characterization of lethal prostate cancer by whole genome sequencing of 51 sites from 10 patients found metastasis-to-metastasis spread to be common, either through de novo monoclonal seeding of daughter metastases or transfer of multiple tumor clones between metastatic sites. Importantly, lesions affecting tumor suppressor genes usually occurred as single events while mutations in genes involved in AR signaling commonly derived from events in different metastases. By sequencing circulating tumor DNA, Carreira et al. examined the tumor clone dynamics of 106 sequential plasma samples, CRPC tumor biopsies, and precastration tumor cores in 16 ERG-positive patients. Multiple independent clones were differentially expressed in circulation in metastatic disease (Figure 2). Importantly, treatment...
with abiraterone and enzalutamide in men who subsequently progressed was correlated with the emergence of clones harboring mutations in AR that are activated by glucocorticoids.81

**CLINICAL IMPLICATIONS**

**Localized disease**

The high degree of heterogeneity in individuals has wide ranging implications for prostate cancer diagnosis and treatment. In terms of diagnosis, the genomic heterogeneity seen in localized prostate cancer challenges the idea of a “dominant lesion,” which is defined solely by size or histologic criteria, being largely responsible for a patient’s clinical course. Molecular heterogeneity seen in PCa may instead suggest that genomic features, rather than size or histology alone, will determine the biology of disease.85 This raises questions about the underlying assumptions of both standard template biopsy, as well as image-guided targeted biopsies. Template biopsy, commonly utilizing 12–14 cores, is based on probabilistic sampling of the prostate to detect larger lesions. Given the spatial heterogeneity of prostate cancer, sampling of one larger lesion does not necessarily provide insight into the other lesions that are present. Similarly, targeted biopsy assumes that the MRI-visible lesion is clinically the most relevant, which also may not be the case that ongoing studies are crucial to validate this idea. This type of heterogeneity is also problematic for personalized genomic testing, as prognostic information may be clouded by a lack of adequate sampling.

Similarly, tumor heterogeneity is particularly problematic, given the increasing acceptance of focal therapy for prostate cancer. As discussed above, prostate cancer is often multifocal, however even the surrounding normal tissue can harbor clonal mutations.87 Furthermore, the marked heterogeneity seen in localized disease re-inforces how little understood prostate cancer pathogenesis is and how difficult it is to predict who requires therapy. This is because even if we are able to determine which mutations confer worse prognosis, the ability to sample all clones continues to be problematic.

**Metastatic disease**

Multiple reports have demonstrated polyclonal sub-populations in metastatic foci, as well as heterogeneity within a single focus and between foci. The dynamics of resistant clones in response to therapy suggest that preexistent clonal populations are responsible for resistance to therapy and disease progression. This has important implications for how we treat metastatic disease, as using multiple concurrent agents may be preferable to single-agent therapy as these will target multiple populations, similar to the rationale for highly active antiretroviral therapy for human immunodeficiency virus treatment. Indeed, recent work has demonstrated that combination of docetaxel and ADT was superior to ADT alone, perhaps because of this reason.83

In addition, there is increasing evidence that local treatment with radiation or surgery of lymph node-positive or oligo-metastatic disease may be beneficial in prostate cancer.84–89 While the data to this point are limited to case series, ongoing clinical trials will help to further elucidate this question. One hypothesis for how local control may benefit patients is the clearance of foci harboring resistant or more aggressive clones even if some cancer cells remain.

**CONCLUSIONS**

The past decade has brought new insights into the genomic pathogenesis of prostate cancer. The discovery of sub-classes of prostate cancer categorized by ETS rearrangement, SPOP mutation, and SPINK1 overexpression has paved the way for novel insights into the diagnosis, prognosis, and treatment of the disease. The molecular characterization of prostate cancer has also allowed examination of the spatial and temporal heterogeneity of prostate cancer with previously unobtainable resolution. These findings have important implications for prostate cancer screening and diagnosis. They also provide insights into the treatment of disease, and will likely be the basis for future therapeutic approaches.

**AUTHOR CONTRIBUTIONS**

JS and CEB reviewed the relevant literature and wrote the manuscript. All authors read and approved the final manuscript.

**COMPETING INTEREST**

All authors declare no competing financial interests.

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