Seminars article

Predicting therapy response and resistance in metastatic prostate cancer with circulating tumor DNA

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Abstract

The treatment of metastatic castration-resistant prostate cancer (mCRPC) is empirical, with progress to a more precision medicine approach hampered by lack of predictive biomarkers. This is due in large part to the historical difficulty of molecularly profiling a bone-predominant metastatic disease. Focus has turned to minimally invasive sources of tumor material to better understand the molecular drivers of therapy resistance. Circulating cell-free tumor DNA (ctDNA) is highly abundant in the bloodstream of mCRPC patients and appears to provide an accurate snapshot of real-time tumor genomics. Already, the analysis of androgen receptor gene alterations in the ctDNA of mCRPC patient cohorts has suggested significant potential for guiding the use of androgen receptor-directed therapy. Furthermore, the monitoring of patient ctDNA burden in the wake of systemic therapy may offer a powerful surrogate for tracking tumor responses and emerging resistant subclones. This seminar covers recent advances in mCRPC patient ctDNA profiling, emerging associations of distinct molecular subtypes with clinical outcomes, and the potential for ctDNA to augment precision oncology. © 2017 Elsevier Inc. All rights reserved.

Introduction

Metastatic prostate cancer is initially reliant on circulating androgens activating endogenous androgen receptor (AR) protein. Although androgen-deprivation therapy (ADT) elicits a strong response in most patients, the duration of response is highly variable and all patients eventually progress to metastatic castration-resistant prostate cancer (mCRPC) [1]. Continued targeting of the AR signaling axis with abiraterone or enzalutamide has changed clinical practice and improved the overall survival of mCRPC patients compared with a relatively recent era where the taxane-based chemotherapy docetaxel was the only life-extending option.

Significant challenges persist, including primary resistance to abiraterone or enzalutamide in 20% to 40% of chemotherapy-naïve patients, and inevitable acquired resistance to all mCRPC therapies [2–4]. Abiraterone is an androgen synthesis inhibitor, whereas enzalutamide is an AR ligand binding domain (LBD) antagonist. However, although these drugs act on different aspects of AR signaling, cross-resistance is common and is likely to be further compounded by the introduction of additional analogous agents currently in clinical development [5,6]. Furthermore, recent trial data suggests that abiraterone or docetaxel may soon be widely used prior to mCRPC development, in conjunction with up-front ADT [7–10].

With an increasing number of effective treatments for metastatic prostate cancer, and a shifting consensus on when during disease progression each is best applied, there is an urgent need for practical molecular biomarkers that allow improved sequencing of therapies and selection of agents for distinct molecular subtypes. Whole-exome sequencing studies of metastatic tumor tissue have given insights into the complexity and distribution of genomic aberrations [11–15]. This data offers exciting opportunities for precision medicine, including the use of PARP (poly ADP ribose polymerase) inhibitors in DNA damage repair defective mCRPC [16–18]. Unfortunately, metastatic tissue

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biopsies are impractical to perform routinely in clinical practice owing to the bone-predominant metastatic spread, and because the majority of men relapsing with mCRPC have low volume disease. Repeated sampling to obtain real-time molecular information as patients develop treatment-resistant disease is even more unrealistic. Focus has therefore turned to the development of minimally invasive biomarkers from peripheral blood, with cumulating data prioritizing circulating cell-free tumor DNA (ctDNA) as a powerful source of clinically relevant somatic information.

Plasma ctDNA detection and prognostic implications

Cell-free DNA (cfDNA) is shed into the bloodstream by apoptosing cells, both tumor and nonmalignant. The tumor-derived proportion of cfDNA (known as the ctDNA fraction) varies significantly between patients and is challenging to predict before genomic profiling, in sharp contrast to a metastatic tissue biopsy where tumor cellularity can be accurately estimated by a pathologist. However, ctDNA fraction associates approximately with patient overall tumor burden; correlating with clinical metrics of metastatic disease volume such as serum alkaline phosphatase or lactate dehydrogenase [19,20]. In low tumor volume settings such as minimal residual disease after local therapy, ctDNA is typically very rare among nonmalignant cfDNA. Conversely, over 75% of progressing mCRPC patients have ctDNA fractions above 2%, with approximately half of advanced patients having fractions above 30% [19–21]. The half-life of cfDNA in the bloodstream is measured in mere minutes, so it is thought to be representative of contemporary proliferating tumor cell populations.

Several studies have demonstrated that ctDNA fraction in mCRPC patients correlates with poor prognosis [20,22]. This is consistent with the association between ctDNA fraction and clinical indices of tumor burden. Similarly, the depth of ctDNA decline in the wake of systemic therapy also appears to inform on tumor response. Patients sampled while responding to AR-directed therapy seem to have much lower ctDNA fractions than at baseline or clinical progression [20,23]. Most impressively, in the recent TOPARP-A trial of the PARP inhibitor olaparib in mCRPC, a greater than 50% decline in total plasma cfDNA yield (a loose surrogate for ctDNA fraction) after only 4 weeks of treatment was independently associated with longer progression-free survival [17,19].

The abundant plasma ctDNA in progressing mCRPC provides an opportunity for broad somatic genome profiling, although there still exists a tradeoff between cost, proportion of genome covered, and sensitivity to the minority of patients with ctDNA fractions below 1%. Relatively deep targeted sequencing (×1,000) across established cancer gene panels captures somatic mutations in most progressing mCRPC patients, although copy number calling is challenging in patients with ctDNA fractions

below 10% to 20% [19]. This is true even if using a panel that surveys changes in germline SNP heterozygosity accompanying chromosomal aneuploidy, as per commercial assays screening maternal plasma fetal cfDNA for common triploidies [24]. Mutant alleles in patients with ctDNA fractions between 0.1% and 1% can be detected with droplet digital polymerase chain reaction or bead-based polymerase chain reaction, but a priori knowledge of tumor genotype (e.g., from profiling of matched tissue) or the likely presence of hotspot mutations (e.g., within the AR LBD or the SPOP math domain) are required [25–27]. Ultra-deep sequencing ($>\times 10{,}000$) with incorporation of unique molecular identifiers can also detect mutations in the setting of very low ctDNA, but costs become prohibitively high if a large genomic territory is profiled [28]. Ultimately, given that patients with very low ctDNA fractions seem to have the best prognoses with current standard-of-care approaches [22], costly attempts to profile their tumor genotype must be balanced against the potential clinical utility of that information.

The somatic landscapes derived from plasma ctDNA profiling are highly consistent with the established molecular landscape from metastatic tissue analysis [19,20,23,29,30]. A recent study of 45 matched plasma cfDNA samples and metastatic tissue biopsies from a mixed cohort of mCRPC patients demonstrated concordance of above 90% for detection of mutations in key prostate cancer driver genes such as *AR*, *SPOP*, *FOXA1*, *PTEN*, *BRCA2*, and *RB1* [19]. Global copy number profiles were also highly correlated. However, there were several notable examples of clinically relevant alterations (e.g., AR amplification, PI3K, and WNT signaling pathway mutations) present in ctDNA but not detected in matched metastatic tissue. Supporting the notion that ctDNA is shed from multiple metastatic niches, this discordance was observed in 2 patients with bone-predominant disease where only a soft tissue metastasis was biopsied.

AR gene alterations and response to AR-directed therapy

Alterations to the *AR* gene promote resistance to the standard hormonal therapies used for metastatic prostate cancer. *AR* copy number gain leads to overexpression of the *AR* at the transcript and protein level, which have been shown to overwhelm AR-antagonists or drive hypersensitivity to castrate-levels of androgen [31]. Mutations within the AR LBD can alter ligand specificity, permitting agonism by adrenal androgens or AR-antagonists themselves [32].

The influence of *AR* copy number or LBD mutations on therapy response in the mCRPC setting is highly context dependent. Early ctDNA-based studies suggested a strong association between their detection and very poor prognosis in patients treated with AR-directed therapy [20,23,33,34]. However, initial results were difficult to generalize given