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Epigenetic reprogramming: A key mechanism driving therapeutic resistance

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Abstract

Prostate cancer initiation, development and progression is driven by androgen receptor (AR) signaling. Androgen deprivation therapy is the primary treatment for patients that present with locally advanced or metastatic disease. However, androgen deprivation therapy is not curative, and patients will progress to castrate-resistant disease (CRPC). Although most patient's progress to CRPC via restoration of AR signaling (CRPC-Ad), approximately a quarter of patients will progress via mechanisms independent of AR signaling. This highly lethal phenotype is termed aggressive variant prostate cancer (AVPC). Data from clinical and preclinical studies demonstrate that AVPC involves combinatorial loss-of-function mutations in key tumor suppressor genes, low to absent AR levels, and re-expression of reprogramming, stem, and neuroendocrine related gene signatures. Further, AVPC is shown to evolve from a CRPC-Ad phenotype. Overall, lineage plasticity underlying progression to AVPC is thought to be provoked by genome-wide chromatin remodeling. Here, we will discuss an emerging focus on key drivers of chromatin remodeling in AVPC, and how their identification could provide noninvasive biomarkers to predict or detect AVPC emergence, and therapeutic targets to prevent or reverse progression to AVPC.

Keywords: CRPC; aggressive variant; neuroendocrine; epigenetics; therapy; EZH2

Introduction

Targeted therapy is the foundation of personalized medicine [1]. In practice, however, efforts to employ targeted therapies have been curbed by the inevitable outcome of acquired resistance. Lineage plasticity as a mechanism of resistance has been observed in numerous cancer types including tumors of the prostate, brain, blood, and lung [2,3]. Epigenetic reprogramming enables a molecular and phenotypic evolution of the cancer cell that enables it to survive targeted therapy. The evolution of prostate cancer (PCa) in response to therapy provides an excellent example of this phenomenon.

Androgen receptor (AR) plays a critical role in the initiation, development and progression of PCa [4]. Patients that present with low-risk PCa—defined as localized disease with prostate specific antigen (PSA) readings of less than 10 and a Gleason score (GS) of 6—are unlikely to die from the disease and are managed via active surveillance to ensure the cancer does not progress [5]. Surgery or radiation are effective treatments for intermediate-risk PCa patients (localized disease, PSA of 8–10, GS of 7) and provides them the chance of a cure. There is no curative approach available for the treatment of high-risk PCa (advanced disease, PSA $>$ 20, GS $>$ 8) [5]. High-risk PCa is more likely to progress to metastatic PCa (mPCa) which is incurable.

Androgen deprivation therapy (ADT), which targets androgen synthesis and AR function, has been the cornerstone treatment for mPCa [6]. Conventionally, orchiectomies, luteinizing hormone-releasing hormone agonists, or luteinizing hormone-releasing hormone antagonists are used to achieve castrate levels of androgen. Second-generation ADT include the CYP17 inhibitor abiraterone and the AR antagonist, enzalutamide, are subsequently prescribed to patients that progress despite castrate levels of androgen. Although mPCa is initially responsive to ADT, nearly all

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patients progress to castration resistant prostate cancer (CRPC) within a median of a year due to acquired resistance to therapy. The STAMPEDE [7] and LATITUDE [8] trials have recently demonstrated that abiraterone and prednisone outperforms ADT alone or abiraterone and placebo in extending overall survival and progression-free survival in castration-sensitive patients with mPCa. These studies support the addition of abiraterone to conventional ADT as first-line treatment of mPCa. Although this is likely to change the standard of care for castration-sensitive mPCa, it remains to be seen whether moving abiraterone or enzalutamide as first-line treatment for androgen-sensitive mPCa will result in cure of disease, or a more rapid progression to CRPC.

Although most CRPCs remain AR-dependent, via mechanisms by which CRPC tumors evolve to survive ADT include AR gain, AR alterations such as point mutations and splice variants, as well as bypass mechanisms, such as PI3K/AKT pathway alterations, that enable AR signaling to continue despite loss of androgen ligand [9]. In up to 25% of patients with CRPC [10,11], the acquisition of molecular alterations enable the tumor to function independently of AR signaling. This lethal subtype is termed aggressive variant PCa (AVPC), or neuroendocrine, anaplastic, or AR-indifferent PCa [12]. AVPCs represent a spectrum of PCas that display neuroendocrine features [13] and have a median OS of less than a year. An important unanswered question is, if a specific genetic/epigenetic landscape predisposes patients to resistance mechanisms driving progression to AVPC, the treatment schedule changes championed by the LATITUDE and STAMPEDE trials could result in these patients ungoing transformation years earlier than what would be expected compared to the current standard of care applied to patients. Therefore, it is critical that molecular mechanisms driving progression to APVC are identified and rapidly translated into biomarkers, and therapeutic strategies to intercept patients progressing to this lethal PCa phenotype.

Progression to AVPC is associated with lineage plasticity and involves the molecular evolution of the tumor cell involving histological transformation from an adenocarcinoma (CRPC-Ad) to neuroendocrine morphology [14]. AVPC is associated with a low mutation rate, with copy-number loss of tumor suppressor genes RB1 and TP53 being the most significant genetic features, observed in 70% and 66.7% of AVPC and 32% and 31.4% of CRPC-Ad patient samples, respectively [15]. AVPC is further associated with loss of known luminal (adenocarcinoma) markers and gain of neuroendocrine, stem, and reprogramming markers. This reprogramming of the tumor cell allows for lineage plasticity, which serves as a molecular switch from a CRPC-Ad to AVPC phenotype. Further, there is evidence that demonstrates that epigenetic reprogramming is an important mechanism in this adaptive response to therapy. With no current therapy to successfully treat AVPC, a better understanding of underlying mechanisms that drive this lethal phenotype is critical toward designing therapeutic strategies for these patients.

Molecular basis of therapeutic resistance

Next generation RNA sequencing efforts have demonstrated (1) AVPC evolves from CRPC-Ad, evidenced by retained TMPRSS2-ERG fusion expression and (2) AVPC and CRPC-Ad have distinct gene expression profiles [14]. CRPC-Ads and AVPCs also have distinct epigenomes. Levels of histone H3 lysine 4 mono-methylation, di-methylation, and tri-methylation (H3K4me1, H3K4me2, and H3K4me3) are significantly increased in CRPC-Ad compared to hormone-dependent PCa [16]. The epigenetic enzyme, enhancer of zeste homolog 2 (EZH2), which trimethylates histone H3 lysine 27 (H3K27), is also overexpressed in metastatic CRPC-Ad [17] and have therefore been extensively studied in the context of PCa. EZH2 transcript upregulation was observed in human AVPC samples compared to CRPC-Ad samples by Beltran et al. [14,15]. Subsequent data from Clermont et al. [18] also found that chromobox 8 (CBX8) and EZH2 were the most and second-most overexpressed epigenetic regulators in AVPC clinical samples and patient-derived xenograft models of AVPC, positioning these 2 components of the polycomb group as drivers of epigenetic remodeling leading to progression to AVPC. Using genome-wide DNA methylation analysis Beltran et al. [15] also demonstrated that AVPC has an epigenome that is distinct from CRPC-Ad, and plays a key role in this clonal evolution.

Although this work is still ongoing, some progress has been made in elucidating the molecular events that drive the evolution of CRPC-Ad to AVPC. Genomic-sequencing studies and preclinical modeling provide evidence that combined molecular alterations in retinoblastoma 1 (*RB1*), tumor protein 53 (*TP53*), and phosphatase and tensin homolog (*PTEN*) drive AVPC and occur more frequently in these tumors—thus differentiating AVPC from CRPC-Ad tumors [3]. In these independent studies, loss of *RB1* expression or function is overall the most common and significant feature of AVPC. RB1 is a known negative regulator of cell cycle progression via repression of the transcriptional activity of E2F class proteins [19]. More importantly, RB1 has also been implicated in chromatin remodeling [20]. RB1 is demonstrated to collaborate through dimerization with EZH2 to determine distribution of H3K27me3 sites [21]. Further, *RB1* deletion in fibroblasts promotes a pluripotent state by H3 acetylation and redistribution of H3K4me3 and H3K27me3 marks at multiple pluripotency genes [22]. This data indicates that in the absence of RB1 expression or function, EZH2 function can be significantly altered.

Recent studies have more explicitly linked the loss of RB1 and chromatin remodeling to AR-independent resistance mechanisms. Marked resistance to antiandrogen