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Understanding cancer lineage plasticity: reversing therapeutic resistance in metastatic prostate cancer

“...an increasingly accepted mechanism of resistance in approximately a quarter of tumors involves lineage plasticity.”

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Prostate cancer (PCa) is among the most commonly diagnosed cancers in men throughout westernized countries and is a leading cause of cancer-related mortality [1]. Androgen-deprivation therapy (ADT) has been the backbone for PCa treatment by inhibiting androgen receptor (AR) signaling. However, resistance to this therapy is inevitable and men will progress to castrate-resistant PCa (CRPC). Although most patients will undergo resistant mechanisms involving the restoration of AR signaling and maintenance of an adenocarcinoma phenotype (CRPC-Ad), an increasingly accepted mechanism of resistance in approximately a quarter of tumors involves lineage plasticity. These tumors express from low to absent AR levels, often demonstrating neuroendocrine features (CRPC-NE) and are indifferent to AR signaling. Further, genomic analysis reveals that CRPC-NE evolves from CRPC-Ad [2], but currently the molecular mechanisms underlying CRPC-NE evolution are not completely understood. Both clinical and preclinical data are emerging to where it is now possible to begin understanding molecular mechanisms driving resistance to antiandrogen therapy via lineage plasticity.

Data from independent investigations have started to shed light on driver genetic events governing the progression of CRPC-Ad to CRPC-NE, and how molecular alterations contribute to therapy resistance via tumor evolution. An initial study investigating the status of *RB1*, *TP53* and *PTEN* tumor suppressor genes in prostate adenocarcinomas and small-cell PCa (SCPC) subtypes revealed that combined alterations in these three tumor suppressor genes was a common occurrence. A major observation by Tan *et al.*, was that deletion of *RB1* was a common event in AR negative SCPC, but not in high-grade AR positive adenocarcinomas, suggesting *RB1* deletion is critical for progression of SCPC [3]. Aparicio *et al.* also reported in independent studies, that combined alterations in *RB1*, *TP53* and/or *PTEN* genes were more frequent in CRPC-NE tumors when compared to CRPC-Ad tumors, demonstrating that indeed CRPC-NE tumors share molecular features with SCPC [4]. This was further validated by more recent studies in human samples that showed progression of CRPC-Ad to a CRPC-NE phenotype involved the acquisition of alterations in

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RBI and *TP53* [5,6]. The concept that partnering mutations are required for development of SCPC or CRPC-NE has been significantly supported by genetically engineered mouse models. This was initially demonstrated by the generation of the transgenic adenocarcinoma mouse prostate murine model which overexpresses an SV40 transgene specifically

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in the prostatic tissue of animals [7]. The resulting tumors are largely due to transgene-mediated attenuation of *Rb1* and *Tp53* expression/function. Later, a more direct mouse model was generated involving specific codeletion of *Rb1* and *Tp53* [8]. Both models result in progression to metastatic disease and resistance to castration via progression to a prostate neuroendocrine phenotype.

Apart from mutations occurring in key tumor suppressor genes, additional genes have been identified as potential key regulators of CRPC-NE and may offer novel molecular targets for therapeutic interventions. Overexpression and gene amplification of *MYCN* (N-MYC) and *AURKA* (Aurora-A) is a significant occurrence in human CRPC-NE [9,10]. In preclinical human cell lines and mouse models, it has now been well demonstrated that N-MYC is associated in the progression to CRPC-NE [9,11]. Interestingly, stabilization of N-MYC can be achieved via dimerization with Aurora-A, which can be targeted using allosteric Aurora-A inhibitors [12,13]. Targeting of Aurora-A with an allosteric inhibitor (alisertib) in preclinical neuroendocrine prostate cancer (NPEC) models demonstrated significant anti-tumor activity associated with degradation of N-MYC [11]. More recently, Phase II clinical data reported substantial single-agent activity with alisertib treatment of patients with CRPC-NE (Beltran, NCT01799278), though a Phase I/II trial of alisertib in combination with abiraterone and prednisone yielded low activity in patients with metastatic castrate-resistant prostate cancer (mCRPC) [14]. Selection of patients with CRPC-NE was performed by Beltran *et al.*, which may explain the reported differences in patient responses. Nevertheless, further evaluation of alisertib in patients with CRPC-NE as single-agent and combination approaches is warranted.

Recently, the role of *RBI* toward cellular reprogramming and governing of pluripotency networks was reported [15]. This investigation utilized induced pluripotent stem-cell (iPSC) reprogramming as a cellular model to demonstrate that *RBI* regulates reprogramming of fibroblasts to iPSCs. It was found that *RBI* globally represses pluripotency networks, which

renders cells to be more amendable to reprogramming via deregulation of factors like *SOX2*. This intriguing finding is consistent to two more recent publications. Mu *et al.*, by engineering knockdown of *RBI* and *TP53* in a human PCa cell line that overexpresses AR (LNCaP-AR), a model of CRPC-Ad, demonstrated that only dual knockdown of both genes caused marked resistance to antiandrogen treatment. Also, cells lacking *RBI* and *TP53* showed lineage plasticity, with dramatic loss in expression of luminal epithelial cell markers and increased expression of basal epithelial and neuroendocrine cell markers [16]. In correlation to the study by Kareta *et al.*, *SOX2* expression was induced by silencing of *RBI* and *TP53* in LNCaP-AR cells, and interestingly, expression of basal and neuroendocrine markers was dependent on *SOX2* expression. In addition, using a mouse model of prostate adenocarcinoma (AR expressing) driven by the deletion of *Pten*, co-deletion of *Rb1* resulted in aggressive metastatic disease with heterogeneous histology expressing both markers of adenocarcinoma and neuroendocrine disease, where variable AR expression was observed [17]. Animals displayed transient response to castration, with all progressing to castrate-resistant disease. Few animals contained *Tp53* mutations, suggesting an important role for loss of *Tp53* toward castration resistance. Gene expression analysis revealed that loss of *Rb1* shifted tumors to a phenotype with significant overlap of genes observed in human neuroendocrine PCa. Most relevant were notable changes in neuroendocrine lineage genes, and genes related to stemness and epigenetic reprogramming, including *Sox2* and *Ezh2*. Excitingly, Ku *et al.* demonstrated that *Ezh2* inhibition increased levels of AR and restored sensitivity to antiandrogen therapy. Further, the importance of *Ezh2* as a key mediator of PCa lineage switching was recently shown in an independent study. Using the same prostate adenocarcinoma mouse model driven by loss of *Pten*, the concurrent overexpression of *MycN* initiated progression to neuroendocrine PCa with reduced overall AR expression [11]. Like the study presented by Ku *et al.*, overexpression of *MycN* resulted in increased expression of *Ezh2* and chromatin redistribution. Further, *NMyc* expression sensitizes cells to *Ezh2* inhibitors. These independent studies suggest mechanistic importance of *Ezh2* mediating progression to CRPC-NE and use of *Ezh2* inhibitors in combination with other therapies, including antiandrogen therapy to treat patients who have progressed to CRPC-NE.

Clinical data comparing human CRPC-Ad with CRPC-NE tumors also highlight significant deregulation of stemness/epigenetic regulators in

CRPC-NE [9,18]. Specifically, data from Clermont *et al.* demonstrated significant increased expression of numerous chromatin modifiers in NPEC samples including *DNMT3A*, *DNMT3B* and *DNMT1* involved in DNA methylation, and histone modifying enzymes including *EZH2*, *CBX8* and *DOT1L* [18]. By overlapping gene transcripts across multiple independent pre-clinical and clinical NPEC samples, the development of a 185-gene list referred to as ‘neuroendocrine-associated repression signature’ (NEARS) demonstrated significant enrichment of polycomb (involving *EZH2*) targeted genes [18]. NEARS selected patients with rapid development of metastatic disease, further validating polycomb action in development of lethal PCa. The development of gene signatures, like NEARS, from noninvasive sources including cell-free DNA/RNA or circulating tumor cells (CTCs) is of high importance for clinical use. These signatures may enable earlier detection of CRPC-Ad switching to CRPC-NE. Recent evaluation of CTCs from CRPC-NE patients compared with patients with CRPC-Ad revealed that CTCs from CRPC-NE patients carry unique characteristics [19]. More recently, exome sequencing comparing a patients metastatic tumor sample and CTCs was reported. Excitingly, this longitudinal analysis demonstrated the development of resistance to ADT via tumor evolution involving acquisition of neuroendocrine features involving a nonsense mutation of the *RBI* gene [20]. Such analysis increases confidence in the use of CTCs to track tumor evolution and selection of treatment options for patients progressing with ADT.

Moving forward, significant advances have been made in PCa modeling including genetically

modified mouse models, patient-derived xenografts, mouse and human 3D organoid models and availability of patient samples. These combined with genomic and proteomic technology will allow for a more rapid gain of knowledge into understanding the genetic and epigenetic interplay which drive lineage plasticity and therapy resistance. Overall, CRPC-NE tumors are significantly more epigenetically deregulated compared with CRPC-Ad tumors. To date, a large focus has been directed towards *Ezh2* action, and clinically relevant inhibitors of *Ezh2* have proven effective in reversing ADT resistance in pre-clinical studies [17]. Our data aligned with independent studies, position *Ezh2* as a primary epigenetic target to initialize clinical trials in CRPC-NE patients. In addition, alternate epigenetic/chromatin modifiers including the Switch/Sucrose Non-Fermentable (SWI/SNF) [21,22] complex and Bromo- and Extra-Terminal domains [ELLIS LAB, UNPUBLISHED DATA] need to be further tested in models of CRPC-NE. This continued growth in knowledge will ultimately allow for the implementation of targeted/epigenetic therapies to inhibit or reverse lineage plasticity and generate sustainable clinical responses in patients with CRPC-NE.

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