Epigenetic reprogramming of AR function underlies lineage infidelity in advanced prostate cancer

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Background: Treatment of castration-resistant prostate cancer (CRPC) with potent androgen receptor (AR) pathway inhibitors (ARPIs), such as enzalutamide (ENZ), inevitably leads to tumour recurrence, with a sizable proportion undergoing lineage switching to a neuroendocrine state. These tumours, referred to as treatment-induced neuroendocrine prostate cancer (tNEPC), are characterized by loss of canonical AR signaling and expression of neuroendocrine lineage markers. Intriguingly, the AR remains expressed in a subset of these patients, hinting that an alternative AR-regulated transcriptional program may exist.

Methods: We developed an *in vivo* model of acquired ENZ resistance to (a) identify reprogramming factors that facilitate lineage plasticity, and (b) determine how to best capitalize on therapeutic strategies aimed at blocking or reversing lineage transformation. Cell lines derived from ENZ-resistant tumours were profiled by RNA-seq, ChIP-seq, and ATAC-seq, and functionally assessed for stem cell-associated properties. Our findings were validated across NEPC cell lines (NCI-H660), genetically engineered mouse models (PBCre4:*Pten*$_{f/f}$:*Rb1*$_{f/f}$), and patient tumours and organoids. CRISPR/Cas9-mediated genomic editing allowed us to assess the effect of knocking out reprogramming factors on therapy-induced neuroendocrine transdifferentiation.

Results: Utilizing a naturally occurring model of ENZ-resistance that captures the clinical progression of prostate cancer, we discovered that the AR flows between different binding profiles (cistromes) as CRPC tumours relapse as tNEPC. This was due, in part, to changes in chromatin accessibility. Investigating the mechanism, we found that the epigenetic modifier EZH2 co-occupies the reprogrammed AR binding sites in tNEPC. While EZH2 is often associated with establishing transcriptional repression, we found it functions with AR to transcriptionally activate stem cell and neuronal genes – granting privileges associated with both fates. This non-canonical activating function of EZH2 was mediated by threonine-350 phosphorylation (pEZH2-T350). Targeting EZH2 in AR-positive tNEPC reversed the lineage switch back to an adenocarcinoma state, while suppression of AR facilitated terminal neuroendocrine differentiation.

Conclusions: Our studies offer insight into how changes in chromatin architecture guide an alternative AR transcriptional program that enforces a plastic state of "lineage infidelity" during the evolution of tNEPC. Cooperation with EZH2 provides opportunity to reprogram AR function by drugging the epigenome, thus extending the durability of clinically beneficial ARPIs.

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