Identity fraud: lineage plasticity as a mechanism of anti-androgen resistance and target for therapy

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Background: Potent targeting of the androgen receptor (AR) in castration-resistant prostate cancer has altered the archetypal course of the disease, fueling the emergence of aggressive and incurable neuroendocrine prostate cancer (NEPC). These tumors can arise from non-neuroendocrine cells in response to AR pathway inhibitors (ARPIs), such as enzalutamide (ENZ), an observation consistent with lineage plasticity. What regulates this plasticity that allows cells to shed their dependence on the AR and re-emerge as "AR-indifferent" NEPC? Recent evidence suggests that evolution toward a NEPC phenotype is aligned with dynamic epigenetic reprogramming, but the molecular basis underlying this phenomenon remains poorly understood.

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 tumors were profiled by RNA-seq and ChIP-seq, and functionally assessed for stem cell-like properties. Our findings were validated across NEPC cell lines (NCI-H660), genetically engineered mouse models (PBCre4:*Pten*$^{f/f}$:*Rb1*$^{f/f}$), and patient tumors and organoids. CRISPR/Cas9-mediated genomic editing allowed us to assess the effect of knocking out reprogramming factors on therapy-induced neuroendocrine transdifferentiation.

Results: AR-indifferent ENZ-resistant tumors were enriched for a Polycomb/EZH2 signature; in particular, we identified EZH2 to be phosphorylated at threonine-350 (pEZH2-T350) by CDK1 in NEPC cell lines, mouse models, and patient tumors. Accordingly, RB1 loss was sufficient to enhance pEZH2-T350, which was required for prostate cancer cells to convert to a metastable stem-like state and, in turn, acquire neuroendocrine features under the pressure of ARPIs both *in vitro* and in patient-derived xenografts. This transdifferentiation to NEPC was associated with extensive reprogramming of the EZH2 cistrome, specifically at a core set of genes governing lineage identity. AR colocalized at the reprogrammed EZH2 binding sites via direct interaction. Treating NEPC-like cell lines with clinically relevant EZH2 inhibitors reversed the lineage switch and mitigated ENZ resistance.

Conclusions: This research establishes the centrality of epigenetic reprogramming in driving the insurgence of a neuroendocrine phenotype in response to ARPIs, and posits that epigenetic modulation may reverse or delay lineage transformation to extend the durability of clinically beneficial ARPIs.

Conflicts of Interest: None

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