Combating lineage plasticity to suppress therapeutic resistance in advanced prostate cancer

Alastair Davies[1,2], Shaghayegh Nouruzi[1,2], Daksh Thaper[1,2], Soojin Kim[1], Chiara Bostock[1], Sahil Kumar[1], Loredana Puca[3], Musaddeque Ahmed[4], Jennifer Bishop[1], Ladan Fazli[1], Haojie Huang[5], David Goodrich[6], Hansen He[4], Himisha Beltran[3], and Amina Zoubeidi[1,2]

[1]*Vancouver Prostate Centre, Vancouver, Canada*
[2]*University of British Columbia, Vancouver, Canada*
[3]*Weill Cornell Medicine, New York, USA*
[4]*Princess Margaret Cancer Centre, Toronto, Canada*
[5]*Mayo Clinic Cancer Center, Rochester, USA*
[6]*Roswell Park Cancer Institute, Buffalo, USA*

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. 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-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 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: Using a multicolor genetic lineage tracing approach, we demonstrate that prostate cancer cells convert to a stem cell-like state permissive of lineage plasticity and, in turn, transition to a neuroendocrine phenotype under the pressure of ARPIs. This plasticity was found to be driven by EZH2; in particular, we identified EZH2 to be phosphorylated at threonine-350 (pEZH2-T350) in NEPC cell lines, mouse models, and patient tumors. Notably, RB1 loss was sufficient to enhance pEZH2-T350 via CDK1 activation, which facilitated rapid NEPC transdifferentiation. This transition was associated with a marked redistribution of the EZH2 cistrome, specifically to a core set of genes governing lineage identity. AR colocalized at the reprogrammed EZH2 binding sites, and was found to be part of the same complex with EZH2. Treating AR-indifferent/NEPC 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 drugging the epigenome via EZH2 inhibition may reverse or delay lineage transformation to extend the durability of clinically beneficial ARPIs.

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