Modulation Of Chromatin Bivalency Facilitates Prostate Cancer Lineage Plasticity

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Prostate cancer lineage plasticity, characterized by histologic transformation of prostate adenocarcinoma (PRAD) to neuroendocrine prostate cancer (NEPC), is an emerging mechanism of treatment resistance. NEPC accounts for up to 15% of treatment-resistant prostate cancers and is associated with poor prognosis, highlighting an unmet need for new therapies. NEPC lineage reprogramming is primarily driven by epigenetic dysregulation including differential activity of histone and DNA methyltransferases. EZH2, the catalytic component of the Polycomb repressive complex 2 (Polycomb), is overexpressed in most treatment-resistant prostate cancers and is implicated as a driver of disease progression. In this study, we define the differential, lineage-specific action of Polycomb in both PRAD and NEPC subtypes to better understand its role in modulating differentiation and lineage plasticity, and to identify novel targetable drivers of NEPC.

Epigenetic H3K27me3 CUT&Tag profiling of treatment-resistant PRAD (n=9) and NEPC (n=9) rapid autopsy clinical samples revealed that Polycomb targets, including NE-lineage transcription factors, are de-repressed in NEPC. Mechanistically, Polycomb modulates H3K4/K27me3 bivalent genes, leading to the upregulation of NEPC-associated transcriptional drivers (e.g., *ASCL1*) and neuronal gene programs which facilitate forward differentiation following EZH2 targeting in NEPC patient-derived organoid/xenograft models. Notably, we identified prospero-homeobox 1 (PROX1) as an understudied cell-fate determining transcription factor that is epigenetically de-repression by differential Polycomb activity. Integrative H3K27ac CUT&RUN and Capture Hi-C analyses revealed potential enhancers of *PROX1* in NEPC that may be regulated by ASCL1.

We sought to functionally characterize the role of PROX1 in NEPC. An unbiased CRISPR screen in two NEPC patient-derived organoid models demonstrated high cellular dependency for *PROX1*; knockout of *PROX1* impeded tumor growth in NEPC models, while overexpression of *PROX1* in PRAD promoted tumor growth and spontaneous metastases. Transcriptomic and cistromic analyses across models of CRPC and NEPC pointed to PROX1 regulation of neuroendocrine-lineage transcriptional programs. Immunoprecipitation followed by mass spectrometry identified three novel phosphorylated sites in the DNA-binding domain of PROX1 that are critical for its stability and function. *In silico* analyses of these phosphorylation sites predicted CHEK1 and CDK2 asmpotential upstream kinases which could be exploited for therapeutic targeting of PROX1.

Our findings provide insights into the potential role for bivalent promoters in Polycomb-mediated lineage reprogramming, which may facilitate forward differentiation in NEPC upon EZH2 inhibition. Further investigation of de-repressed candidates defines the role of PROX1 as a driver of NEPC and a potential therapeutic target.

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