

## **EZH2 CONTROLS REACTIVATION OF A DORMANT STEM CELL TRANSCRIPTIONAL PROGRAM TO POTENTIATE NEUROENDOCRINE PROSTATE CANCER**

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**Background:** Potent targeting of the androgen receptor (AR) in castration-resistant prostate cancer (CRPC) has altered the archetypal course of the disease, fueling the emergence of highly aggressive and incurable neuroendocrine prostate cancer (NEPC). What regulates the plasticity that allows cells to shed their dependence on the AR and re-emerge as "AR-indifferent" NEPC, especially under the pressure of contemporary AR pathway inhibitors (ARPIs) such as enzalutamide (ENZ)? Recent data suggest that NEPC transdifferentiation is aligned with dynamic reprogramming of the epigenome by developmental regulators, like EZH2, making them attractive therapeutic targets.

**Methods:** ENZ-resistant (ENZR) cell lines established from LNCaP xenografts that recurred under the pressure of ENZ were transcriptionally profiled by RNA-seq, while epigenetic modifications were assessed by ChIP-seq. Public data was queried for EZH2 expression and phosphorylation status across patient specimens, which was confirmed by immunohistochemistry. Gain and loss of function studies were performed to pinpoint the requirement for EZH2 in reprogramming CRPC cells towards a more pluripotent (and neuroendocrine) state.

**Results:** Gene set enrichment analysis confirmed repression of canonical AR pathway genes in AR-indifferent/NEPC ENZR tumors, but enrichment for gene networks associated with embryonic stem cells and Polycomb/EZH2 activity. In particular, a phosphorylated form of EZH2 (EZH2-T350), uniquely expressed in AR-indifferent cell lines and patient tumors, was found to be indispensable for the emergence of a transient stem-like phenotype during the progression from an AR-driven state towards AR indifference and NEPC. This transition was concomitant with a redistribution of the EZH2 cistrome (genome-wide binding sites), particularly at loci associated with prostate stem cells and clinical progression to NEPC. AR colocalized at the reprogrammed EZH2 binding sites. Accordingly, treating AR-indifferent/NEPC cell lines with the EZH2 inhibitor GSK126 was sufficient to shift the AR cistrome back to a naive CRPC state and re-sensitize cells to ARPIs.

**Conclusions:** Our findings establish the centrality of epigenetic reprogramming in driving the insurgence of a clinically relevant NEPC phenotype. Drugging the epigenome via EZH2 inhibition to reverse the NEPC state and re-sensitize tumors to ARPIs has the potential to transform the treatment of prostate cancer.

**Conflict of Interest:** None to declare.

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