SOX2 is an AR Repressed Gene That Drives Castration-Resistant Prostate Cancer Progression and Mediates AR-Targeted Therapeutic Resistance

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Background: Prostate cancer is driven by androgen signaling mediated by the Androgen Receptor (AR) and can be treated by androgen deprivation therapy (ADT). Although initially effective, recurrence of disease following ADT is virtually inevitable, and the development to castration-resistant prostate cancer (CRPC) marks transition to the lethal form of the disease, for which current therapeutic modalities are only palliative. Work by our group and others has identified overexpression of *SOX2* [sex determining region Y-box 2], an essential transcription factor for maintaining the survival and pluripotency of undifferentiated embryonic stem cells, to be associated with aggressive prostate cancer. We have demonstrated that *SOX2* is an AR-repressed gene, and that the constitutive over-expression of SOX2 in a hormone-sensitive, SOX2-negative prostate cancer cell line is sufficient to generate a castration-resistant phenotype *in vitro* and *in vivo*. Further, our data demonstrate canonical SOX2 transcriptional co-factors OCT4 and NANOG are frequently not expressed in prostate cancer, implying that SOX2 has unique, non-stem cell gene targets and binding partners within prostate malignancies.

Methods: To elucidate novel SOX2 gene targets in CRPC, we performed SOX2 chromatin immunoprecipitation and sequencing (ChIP-Seq) in CWRR1 prostate cancer cells, normal prostate epithelial cells (PrECs) and human ES cells (WA01) for comparative analyses. To assess SOX2 activity in prostate cancer under androgen deprivation, we perfomed RNA-sequencing (RNA-Seq) in CWRR1 cells cultured in 10% charcoal-stripped serum, treated with Enzalutamide, a potent AR antagonist. The functional impact of SOX2 depletion was assessed by measuring proliferative capacity and cell survival of CWRR1 cells infected with virus containing CRISPR-Cas9 constructs silencing SOX2 expression. A tissue microarray (N = 499) was used to assess SOX2 protein expression in FFPE prostate cancer tumors collected following radical prostatectomy, evaluating associations with biochemical recurrence and metastatic disease.

Results: ChIP-Seq revealed SOX2 binding of prostate-specific, prostate-cancer specific, and both stem cell and non-stem cell gene targets in prostate cancer for SOX2. Functional analyses of these cells show significantly decreased proliferative capacity and survival in SOX2-silenced cells under treatment with Enzalutamide, as compared to cells infected with a non-silencing control. SOX2 protein expression in FFPE tissues not associated with time to biochemical recurrence, but was associated with lymph node metastases ($p = 0.006$).

Conclusions: Our data identify non-stem, and cancer-specific functions for SOX2 in the development of CRPC. In addition to its role in CRPC, our data suggest a role for SOX2 in mediating resistance to AR-targeted therapies in CRPC. The SOX2 target genes and their pathways identified herein represent potentially novel therapeutic targets to better manage advanced prostate cancer and CRPC.

Conflict Disclosures: All authors have no conflicts of interest to disclose regarding this work.

Funding Acknowledgment: This work was made possible by an NIH/NCI R01 award (CA178431-01, PI: Vander Griend).