

## **Glucocorticoid receptor activation regulates both androgen receptor (AR)-dependent and independent cancer-related genes following AR blockade**

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**Background:** Despite the recent discovery of several potent AR-targeted therapies, progressive castration-resistant prostate cancer (CRPC) remains the second leading cause of male cancer deaths within the United States. While androgen-deprivation therapy (ADT) and AR antagonism continue to be the standard of care for progressive metastatic CRPC, identifying alternative molecular mechanisms that bypass AR activity is increasingly recognized as a crucial treatment approach. For example, our lab and others have shown that increased GR expression and activity can contribute to CRPC progression. This discovery has led to the development of selective GR modulators (SGRMs) as potential therapies for CRPC. Although GR activity clearly contributes to cell growth in AR-blocked PC cells, it is unclear whether GR solely recapitulates AR-driven cancer-related mechanisms or if GR additionally regulates critical gene expression pathways unique to GR (and different from AR) allowing a novel growth advantage for tumor cells.

**Methods:** LAPC4 and CWR-22Rv1 PC cell lines were treated with androgen, +/- enzalutamide, +/- dexamethasone, and +/- SGRMs in order to activate or inhibit GR function in both AR active and AR-blocked conditions. For transcriptome analysis we performed next generation sequencing (NGS) of RNA followed by gene expression pathway analysis using the Ingenuity Pathway Analysis platform. To determine GR and AR chromatin binding sites, we performed GR and AR ChIP followed by DNA NGS.

**Results:** Our recent transcriptomic analysis of AR-activated gene expression in LAPC4 PC cells compared to GR-activated gene expression (following AR antagonism) suggests that a subset of GR activated genes initiate proliferative pathways similar to AR. We then considered the total number of GR-regulated genes (N=7,541) and found that 44% of these genes are unique to GR and not regulated by AR (N=3,282). Analysis of these GR-unique transcriptomes uncovered Cell Signaling, Molecular Transport, and Metabolism functional pathways distinct from the proliferative pathways shared by AR and GR compensating genes. These pathways involve GR-mediated gene expression encoding proteins regulating lipid metabolism and associated autocrine and paracrine signals. These AR-distinct/GR-unique pathways suggest that GR-mediated cancer cell mechanisms allow cancers to evolve new mechanisms of progression. Furthermore, GR and AR ChIP-sequencing suggest that GR-activation following AR blockade results in GR chromatin association at both overlapping and unique sites compared to AR. Interestingly, SGRMs alter GR chromatin binding and gene expression within regulatory regions of both AR-bypass and GR-unique genes.

**Conclusions:** These data indicate that the increased GR activity observed in progressive CRPC following AR blockade results in both the compensation of AR-driven molecular mechanisms and novel GR-specific mechanisms of CRPC progression.

**Conflicts of interest:** SDC, RZS are co-inventors on a patent regarding the use of GR antagonists with AR modulation in CRPC that has been licensed to Corcept Therapeutics.

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