Genomewide analysis of AR and GR chromatin occupancy and associated mRNA expression in enzalutamide-treated GR+ prostate cancer (PC) cells +/- selective glucocorticoid receptor modulators (SGRM) treatment

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Background: Increased glucocorticoid receptor (GR) GR transcriptional activity in CRPC is postulated to generate a gene expression bypass mechanism that counteracts AR antagonism (e.g. enzalutamide) and impairs therapeutic efficacy. Therefore, inhibiting GR activity could extend the benefit of AR antagonism. Currently the only FDA-approved GR antagonist is mifepristone (mif). However, mif is a PR/GR mixed antagonist/agonist and can have AR agonist activity in AR ligand-binding domain mutations. Therefore, we investigated whether two novel, non-steroidal and highly GR modulators (SGRMs) could alter GR chromatin occupancy and subsequent gene expression in PC cell lines treated with enzalutamide.

Methods: Two PC cell lines (LAPC4 and CWR-22Rv1) were treated with combinations of vehicle, R1881 (R, 1nM), enzalutamide (E, 10μM), GR agonist dexamethasone (D, 100nM), SGRMs CORT-108297 (297, 1μM) or CORT-118335 (335, 1μM). Cells were treated for 3 days with vehicle/R/RE, followed by 1 hour D +/- SGRMs prior to anti-AR and GR ChIP-sequencing. To measure subsequent gene expression, mRNA was collected in parallel for sequencing (RNA-seq) following 2 or 6 hour D ± SGRM. Sequential pairwise differential steady-state mRNA expression analysis was performed comparing conditions. Ingenuity Pathway Analysis (IPA) Z-scores (a numerical score of activation/inactivation of an altered gene expression pathway) were identified.

Results: There were ~70-260x10^6 mapped RNA reads per condition for the RNA-seq. After differential expression gene (DEG) analysis, N= 385 qualified DEGs for LAPC and N=175 for CWR-Rv1 cells were identified as 1) altered 1.5 fold by R vs. Vehicle, 2) reversed by E, 3) then altered in the opposite direction by addition of D, and 4) then reversed back again with SGRM. There were 34 DEGs that were shared between lines, including several known AR/GR-regulated genes (e.g. *SGK1, KLK3,* and *FKB5)*. IPA cell function analysis revealed that cell proliferation and movement were significantly inactivated pathways following SGRM treatment. AR and GR ChIP-seq analysis in LAPC4 cells demonstrated the loss of ~1500 AR binding sites within 100kb of genomewide transcription start sites (TSSs) following enzalutamide treatment and the acquisition of ~700 GR sites following glucocorticoid treatment. Interestingly, subsequent SGRM treatment significantly altered GR chromatin association through both loss and gain of GR binding sites.

Conclusion: Genomewide analysis of PC cell lines revealed that SGRMs can reverse GR-mediated gene expression changes. Despite their divergent biology, LAPC4 and 22RV1 had 34 GR regulated/SGRM antagonized DEGs. Moreover, treatment with SGRMs resulted in gene expression changes consistent with reduced oncogenic activity. GR chromatin occupancy was both lost and gained following SGRM treatment, highlighting the complex nature of GR-mediated gene regulation. Further studies to identify the key downstream mechanisms of pro-oncogenic GR activity in PC are ongoing, with the goal of developing effective GR modulators for tumors acquiring GR-mediated oncogenic gene expression.

Conflict of Interest: SDK, RZS are go-inventors on a patent regarding the use of GR antagonists with AR modulation in CRPC that has been licensed to Corcept Therapeutics.

Funding Sources: PCF-Movember Challenge Award