

Poster Title – Transcriptomics and Proteomics Analysis of Enzalutamide-Treated Castration-Resistant Prostate Cancer Cell Lines

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Abstract -

INTRODUCTION: Androgen deprivation therapy (ADT) is the standard treatment for metastatic prostate cancer. However, tumors often progress to metastatic castration-resistant prostate cancer (mCRPC). Enzalutamide (ENZ) and other treatments initially work well for mCRPC, but their effectiveness decreases due to the evolution of tumor resistance.

OBJECTIVE: Our study investigates how ENZ alters biological pathways in castration-resistant (CR) cell lines. We aim to compare whole-transcriptome and whole-proteome expression profiles of C4-2Bs (CR), following ENZ exposure.

METHODS: C4-2B cells were treated with ENZ, CAN, or DMSO (vehicle control) in 10% FBS for 24 h (RNA) and 36 h (protein). Total RNA and proteins were extracted, quantified, and analyzed by RNA sequencing or long-gradient shotgun proteomics. Data underwent quality control and PCA to verify reproducibility and treatment-specific clustering. Differentially expressed genes (DEGs) were identified using DESeq2 (R), defined as fold change $> |1.5|$ with FDR < 0.05 (low stringency) or Bonferroni-corrected $p < 0.05$ (high stringency). Over-Representation Analysis (ORA) of DEG and DEP lists was conducted using clusterProfiler (R) across KEGG and GO pathways. Late-stage ORA integration via WebGestalt combined DEG and DEP inputs to identify shared enriched pathways, calculating meta-p (combined enrichment significance) and meta-FDR (multiple-testing-corrected false discovery rate across datasets).

RESULTS: Transcriptomics identified 464 DEGs (FDR < 0.05) following ENZ treatment, with 193 remaining significant after Bonferroni correction. Downregulation of KLK3, NKX3.1, TMPRSS2, and DHCR24 confirmed AR inhibition. ORA (GO/KEGG) revealed enrichment in steroid hormone biosynthesis (hsa00140) and retinol metabolism (hsa00830), including hormone metabolic process (GO:0042445), response to steroid hormone (GO:0048545), and steroid metabolic process (GO:0008202). Proteomics identified 1,244 protein groups (PEAKS, $Q > 20$) and 93 DEPs by LFQ ANOVA, enriched in propanoate metabolism (hsa00640) and pyruvate metabolism (hsa00620), supported by small-molecule catabolic process (GO:0044282) and amino acid metabolic process (GO:0006520). Multiomics ORA highlighted fatty-acid metabolic (GO:0006631), lipid catabolic (GO:0016042), metabolism (hsa01100), and pentose/glucuronate interconversions (hsa00040) pathways. RT-qPCR in C4-2B cells validated CHRNA2, RDH16, PLA2G4E, UGT1A6, and AR-target suppression (KLK3, NKX3-1). GRIN3A was significantly suppressed by ENZ, whereas AKR1D1 did not show significance. In LNCaP cells, NKX3-1, CHRNA2, GRIN3A, and UGT1A6 showed similar responses when treated with ENZ, while PLA2G4E and AKR1D1 displayed divergent regulation.

CONCLUSION: ENZ treatment induces coordinated multi-omic remodeling of metabolic pathways while effectively suppressing AR signaling. Transcriptomic and proteomic analyses identified significant alterations in steroid hormone biosynthesis, retinol metabolism, fatty-acid metabolism, and small-molecule catabolic processes. RT-qPCR validation in C4-2B and LNCaP cells confirmed conserved regulation of CHRNA2, GRIN3A, and UGT1A6, supporting a shared antiandrogen-responsive program involving neuroactive ligand-receptor signaling and metabolic adaptation, whereas PLA2G4E and AKR1D1 exhibited divergent responses suggestive of metabolic rewiring during CRPC progression. Future

validation and ncRNA-sequencing studies will further define the regulatory networks linking AR inhibition to metabolic reprogramming and identify potential therapeutic targets associated with mCRPC progression.

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