

Therapy-induced stress signaling induces mitochondrially mediated survival during androgen receptor blockade in prostate cancer

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Background: The standard-of-care treatments for men with metastatic prostate cancer are androgen receptor pathway inhibitors (ARPIs), such as abiraterone acetate and enzalutamide, which effectively block androgen receptor (AR) signaling. These therapies substantially extend overall survival, yet acquired resistance to ARPIs remains almost inevitable. Although many patients initially experience durable and profound responses that may last for years, resistance ultimately develops as cancer cells regain proliferative capacity. Targeting the persister cell population that withstands therapy may therefore offer an opportunity to prevent disease progression and improve clinical outcomes. We hypothesized that, despite underlying genomic heterogeneity, enzalutamide-resistant (EnzaR) prostate cancer cells converge on conserved stress-adaptive signaling and metabolic programs that enable survival. These convergent adaptations may, in turn, expose therapeutically actionable vulnerabilities that can be exploited to eliminate persister cells.

Methods: To characterize the molecular features underlying survival following prolonged enzalutamide exposure, we established a panel of enzalutamide-resistant (EnzaR) prostate cancer models, including LNCaP EnzaR, CS2 EnzaR, and LN95 EnzaR cells generated through chronic exposure to escalating enzalutamide concentrations over approximately 6-9 months, as well as MDA-PCa-2b and LAPC4 EnzaR lines and an *in vivo*-derived LNCaP-XER xenograft model. We integrated genomic (whole-exome sequencing and array comparative genomic hybridization), transcriptomic (RNA-seq), and phosphoproteomic (reverse-phase protein array) profiling across models and compared these findings with clinical circulating tumor cell RNA-seq data from the PROPHECY cohort and immunohistochemical analyses of metastatic biopsies. Mitochondrial function was evaluated using Seahorse extracellular flux analysis, intact- and permeabilized-cell respirometry, and JC-1 membrane potential assays. Functional validation employed pharmacologic p38 inhibition with SB203580, siRNA-mediated ATP5IF1 knockdown, and treatment with the mitochondria-targeted cation d-TPP *in vitro* and in xenograft models.

Results: EnzaR cells exhibited marked genomic and transcriptomic heterogeneity but converged phospho-proteomically on activation of p38/MAPK stress signaling. p38 activation coincided with a reduced pERK:p-p38 ratio, p21 upregulation, and a metabolic quiescence phenotype with suppressed oxygen consumption. EnzaR cells displayed reduced mitochondrial membrane potential (ψ) and evidence of non-canonical, reverse-mode ATP synthase activity that hydrolyzes ATP to sustain the proton gradient, linked to downregulation of the ATP synthase inhibitory factor ATP5IF1. Consistently, RNA-Seq analysis of CTCs from a subset of PROPHECY pre- vs post-ARPI patients also revealed a downregulation of ATP5IF1 with androgen pathway modulation resistance. p38 inhibition restored ATP5IF1 levels, while ATP5IF1 knockdown promoted enzalutamide resistance, establishing a functional p38-ATP5IF1 survival axis. The resulting mitochondrial depolarization selectively sensitized EnzaR cells to the mitochondria-targeted cation d-TPP, which when combined with enzalutamide significantly reduce xenograft growth without added toxicity.

Conclusions: AR pathway inhibition triggers a convergent, pro-survival p38/ATP5IF1-mitochondrial stress program to permit cellular persistence in prostate cancer, despite substantial underlying genomic heterogeneity. This adaptive program creates sensitivity to mitochondria-targeted cationic compounds, offering a novel combination strategy to eliminate persister cells and delay or prevent the emergence of subsequent acquired ARPI resistance.

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