

Title: NDUFAF4-Driven Metabolic Vulnerabilities Guide Precision PARP Inhibition in Prostate Cancer

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Background: PARP inhibitors (PARPi) have transformed treatment of BRCA1/2-mutant prostate cancer (PCa), yet most patients lack BRCA1/2 alterations, and primary and acquired PARPi resistance remain major obstacles. Current biomarkers focus on homologous recombination repair (HRR) deficiency and do not capture metabolic or redox states that may determine PARPi response. How mitochondrial dysfunction and iron-dependent oxidative stress shape PARPi sensitivity and resistance in PCa is poorly understood.

Methods: We combined genome-wide CRISPR-Cas9 loss-of-function screens in HR-proficient PCa cell lines with mechanistic biochemical assays, genetic perturbations, and pharmacologic interventions. NDUFAF4 function was interrogated using CRISPR knockouts and partial-loss models with readouts including complex I activity, mitochondrial respiration, ROS, oxidative DNA damage, replication dynamics, and lipid peroxidation. We evaluated therapeutic combinations (PARPi with complex I inhibition or iron-export modulation) in cell lines, xenografts, and patient-derived xenografts (PDXs).

Results: Genome-wide CRISPR screens identified NDUFAF4, a mitochondrial complex I assembly factor at 6q16 (deep deletion ~6%, shallow deletion ~30% in metastatic PCa), as an HR-independent determinant of PARPi response. NDUFAF4 deficiency impaired complex I activity and mitochondrial respiration, triggering a surge in mitochondrial reactive oxygen species (ROS) that induced a dual vulnerability through oxidative DNA base damage (8-oxo-dG) and iron-dependent lipid peroxidation. This oxidative stress enhanced PARP1 trapping, replication-associated ssDNA gaps, and PARPi-induced cytotoxicity in vitro and in vivo.

Deep NDUFAF4 loss conferred marked sensitivity to PARPi monotherapy. In contrast, shallow deletions preserved partial complex I function but created a metabolically primed state; in these models, PARPi plus the clinically used complex I modulator metformin selectively augmented ROS, lipid peroxidation, DNA damage, and tumor growth suppression. Transcriptomic analyses further revealed NRF2-driven upregulation of the iron exporter SLC40A1 (ferroportin) as a recurrent adaptive response to complex I dysfunction and chronic PARPi exposure in both cell lines and PDX models. This iron-efflux program reduced intracellular Fe²⁺, limited ferroptosis, and promoted PARPi resistance. Genetic or pharmacologic SLC40A1 inhibition with PTG-300 increased labile iron and lipid peroxidation and restored PARPi sensitivity in NDUFAF4-deficient and PARPi-resistant models.

Conclusions: We define a mitochondrial complex I-iron redox axis as a key regulator of PARPi response in PCa. NDUFAF4 loss creates an ROS- and ferroptosis-linked vulnerability that can be exploited by PARPi monotherapy (deep loss) or PARPi plus metformin (shallow loss), whereas NRF2/SLC40A1-mediated iron efflux represents an adaptive resistance mechanism targetable with SLC40A1 inhibition. These findings support biomarker-guided therapeutic strategies incorporating mitochondrial and iron-targeted interventions to expand and restore PARPi efficacy beyond canonical BRCA1/2-mutant disease.

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