

Targeting fatty acid metabolism in *SPOP* mutated Prostate Cancer

Joanina K. Gicobi,¹ Susanna Gregory,² Elizabeth A. Bering,³ John A Copland ^{3rd}⁴, Taro Hitosugi,¹ Jacob J. Orme¹

¹Department of Oncology, ²Department of Biochemistry and Molecular Biology,

³Mayo Clinic Graduate School of Biomedical Sciences, Mayo Clinic, Rochester, Minnesota, USA, ⁴Department of Cancer Biology, Mayo Clinic, Jacksonville, FL, USA.

Background: About 300,000 men are diagnosed with prostate cancer (PCa) in the United States each year, with cases expected to double by 2040. Androgen receptor (AR) signaling is critical for normal prostate cells functions but upon cancer development, AR signaling becomes the driver of disease progression. Therapies targeting AR signaling have dramatically changed the clinical outcome of PCa in men around the world. While initially successful, prostate cancers eventually become resistant to treatment and develop into castration resistant prostate cancer (CRPC). Up to 15% of PCa patients harbor mutations in the Speckle type BTB/POZ protein (SPOP) and are more sensitive to Androgen Receptor Pathway Inhibitors (ARPIs) before and after CRPC status. SPOP is an adaptor protein of the Cullin3-based E3 ubiquitin ligase that facilitates both degradative and non-degradative polyubiquitination of cellular molecules. This study aims to determine the role of SPOP in prostate cancer metabolism to expand therapeutic options for patients with CRPC through targets that are efficacious as monotherapy or in combination with other therapies.

Methods: Live cell metabolic assays such as seahorse analysis, RNA sequencing and mass-spectrometry analysis, Biomolecular techniques such as CO-IP and western blotting and cell viability assay, and Genetic manipulation tools such as CRISPR, mutagenesis kits, siRNAs etc.

Results: Our preliminary data show that critical metabolic enzymes such as those involved in fatty acid metabolism have SPOP binding motifs and may be subject to SPOP-mediated ubiquitination. However, there is limited research on how SPOP regulates metabolic enzymes in PCa. We have found that rate-limiting enzymes in fatty acid synthesis such as Stearoyl-CoA desaturase 1 (SCD1) are upregulated upon SPOP inhibition and contain SPOP binding motifs, implying they are ubiquitinated in a SPOP-dependent manner. Co-immunoprecipitation shows that SPOP interacts with both SCD1 and fatty acid synthase (FASN). Inhibition of SCD1 induces cell death in AR-negative prostate cancer cell lines at very low concentrations and shows minimal synergy with SPOP inhibition. Interestingly, FASN inhibitor does not. AR-dependent and AR-V7 expressing cell lines are sensitive to both FASN and SCD1 inhibitors and show synergy with SPOP inhibitor.

Conclusions: SCD1i show promising results for application in AR- and AR+ prostate cancers. This implies that *SPOP* loss in patients with prostate cancer could sensitize them to novel metabolic inhibitors depending on whether the disease is AR-positive or negative.

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