Prostate Cancer Cells After Androgen Deprivation Therapy Demonstrate Unique Substrate Adaptability in the Setting of Metabolic Inhibition

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INTRODUCTION

- **Metabolic reprogramming is ubiquitous in tumorigenesis and progression**
- **Metabolic approaches to identifying tumor susceptibilities leads to improved eradication.**
- **Androgen deprivation therapy (ADT) is a primary approach to advanced hormone sensitive (HS) prostate cancer (PC), and recent data suggests it induces unique sensitivities to adjuvant therapy.**
- **Cellular oxidation-reduction (redox) is a sensitive measure of metabolism allowing for specific tumor differentiation and is often altered in malignancy due to changing metabolic demands.**
- **Optical Metabolite Imaging (OMI) is an real-time imaging technique that uses the fluorescent properties of NAD(P)H and FAD⁺ to quantify their levels and calculate a redox ratio.**
- **Our lab has discovered that ADT treated PC demonstrates an altered metabolite profile and reduced redox ratio, in a manner that is consistent with an attenuation in glycolysis.**

OBJECTIVES

- **We sought to determine the sensitivity of two HSPC cell lines following ADT to inhibition of individual key bio-energetic pathways through a concerted metabolomics approach.**

METHODS

- **HSPC cell lines LNCaP and VCaP were treated with androgen deficient media (CSS) for 4 and 8 days and compared to respective controls.**
- **To assess the importance of individual energy pathways in ADT cells, inhibitors of glycolysis, FA oxidation, and glutaminolysis were applied, followed by OMI to quantify changes in the redox ratio.**
- **Western blot analysis was performed for key regulatory enzymes and transporters in fatty acid (FA) and glutamine (Gln) metabolic pathways.**
- **Seahorse Mito Fuel Flex assay determined contributions of individual pathways to basal mitochondrial fuel oxidation.**

RESULTS

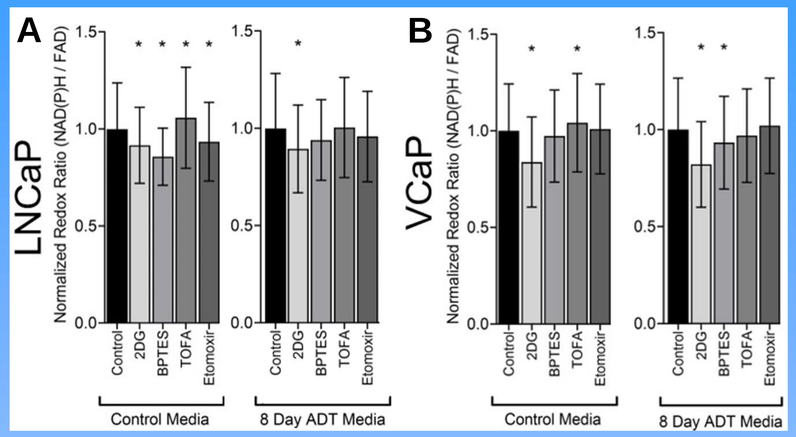

Figure 1: ADT induces resistance to key bio-energetic pathway inhibitors. (A) LNCaP and (B) VCaP cell lines were grown in control or CSS media for 8 days prior to OMI. After analysis of baseline redox ratio calculated by the ratio of NAD(P)H over FAD, application of inhibitors to separate plates was initiated. 2DG is taken up by cells, and trapped in a manner that inhibits glycolysis. Bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide (BPTES) is a selective inhibitor of Glutaminase 1 (GLS1) which converts Gln to glutamate so that it can enter the citric acid cycle. 5-(Tetradecyloxy)-2-furoic acid (TOFA) is an Acetyl-CoA carboxylase (ACC) inhibitor, a rate limiting step of FA synthesis. Etomoxir is an irreversible inhibitor of carnitine palmitoyltransferase-1 (CPT1) transporter which shuttles FA into the mitochondria for oxidation. ($p<0.05$ indicated by *).

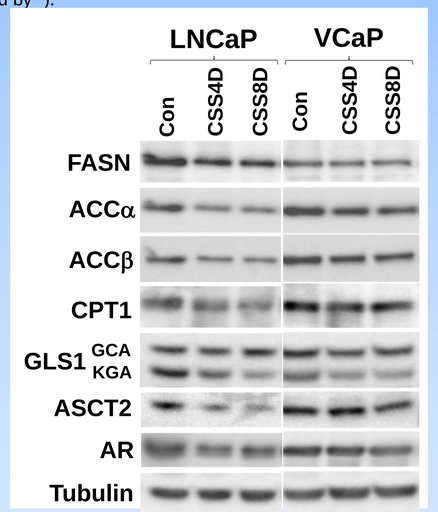

Figure 2: ADT depressed expression of key regulatory metabolic enzymes. LNCaP and VCaP cells were treated with 8 days of CSS media were compared to control cells grown in FBS and DMEM respectively. After collection, cells were lysed and subjected to Western Blot analysis.

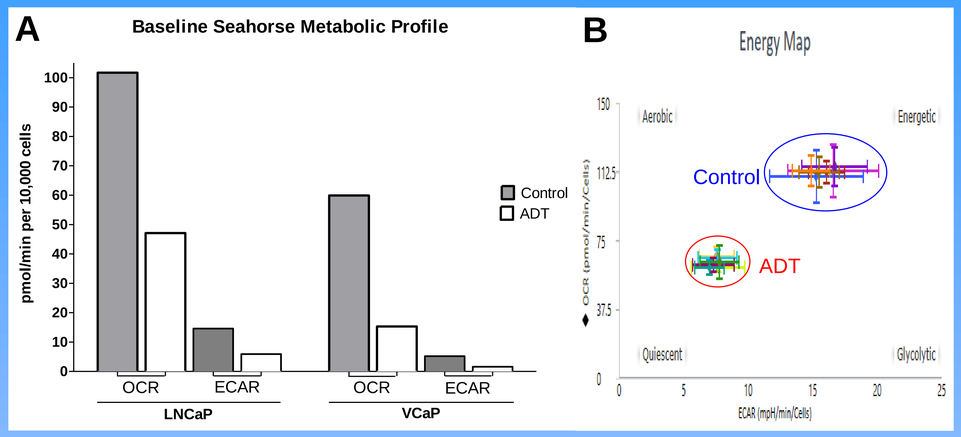

Figure 3: ADT treated cells demonstrate metabolic depression. LNCaP and VCaP cells were grown in FBS or CSS and DMEM or CSS media for 8 days prior to being plated on Seahorse plate coated with Cell-Tak. 10,000 and 30,000 cells or LNCaP and VCaP, were respectively split into the plate on the day prior to assay. (A) Baseline measurements in Oxygen Consumption Rate (OCR) and Extracellular Acidification Rate (ECAR) were measured via Seahorse XFe96 analyzer. Values were normalized via DNA Hoescht staining. (B) Energy map phenotype generated for control and 8 day ADT treatment LNCaP cells.

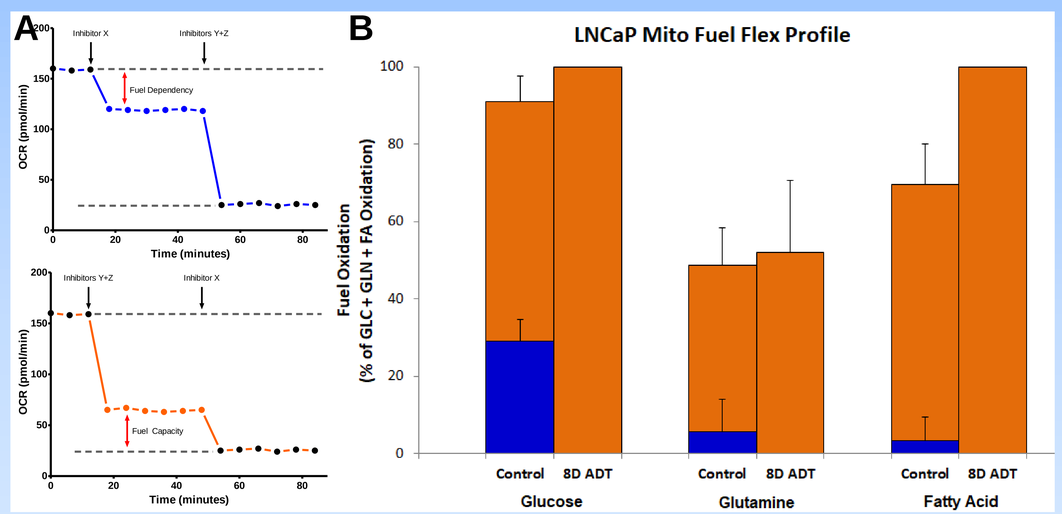

Figure 4: ADT treated cells display no dependence on single fuel source. (A) Description of calculation of substrate dependency and capacity measures as performed by WAVE XF Agilent analysis tool. (B) LNCaP Mito fuel flex flexibility assay was performed according to protocol provided by Agilent. 10,000 cells of control and 8D CSS treated cells were plated on day prior to analysis. OCR and ECAR values were normalized via DNA hoescht staining. Calculations performed using WAVE XF software.

SUMMARY AND CONCLUSIONS

- **ADT treatment confers resistance to inhibition of key bio-energetic pathways via monitoring real-time redox changes in PC cell lines**
- **Protein expression analysis revealed treatment with 4 and 8 days of ADT induces depression of critical metabolic regulatory enzymes.**
- **Via Seahorse technology, ADT treated cells phenotypically appear less metabolically active, appearing to have no dependence on any single pathway as a fuel source.**
- **In early ADT treatment, cells appear quiescent, may explain why metabolic inhibitors are not particularly effective against this stage of disease.**

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