Valine Catabolism Reprogramming Promotes Therapeutic Resistance in Advanced Prostate Cancer

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Background

Prostate Cancer (PCa) is one of the most diagnosed cancers in men[1]. The objective of current targeted therapies against PCa is to inhibit the androgen receptor (AR) signalling axis which drives tumour growth and disease progression[2]. Unfortunately many patients will be subject to the onset of castrate-resistant prostate caner (CRPC), which is characterised by restoration of the AR despite castrate levels of androgens. These changes are accompanied by fundamental alterations to metabolic pathways to meet the heightened metabolic demand of the tumour[3]. Further exploration and investigation into these energetically dysregulated pathways is needed to aid in the discovery of novel therapeutic strategies.

Objectives

Branched chain amino acid catabolism (BCAA) is highly upregulated in clinical metastatic prostate cancer samples but little is understood about how this supports tumour survival and treatment resistance. Our preliminary research has demonstrated that the enzymes responsible for catabolising the BCAA - valine are highly elevated in response to anti-androgen therapy *in vitro* (Figure 1). The objective of this study is to identify valine catabolism's role in treatment resistance by:

- Assessing the metabolic dependency of PCa cells on valine by extracellular depletion of individual BCAAs from culture medium.
- Characterising the effects of valine catabolism suppression in PCa models representing multiple stages of disease progression.

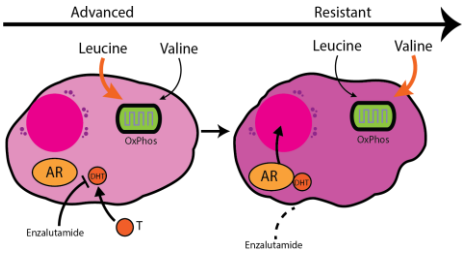

Figure 1. Proposed Model of Enhanced Valine Uptake and Catabolism following Prostate Cancer Cell Exposure to Androgen Targeted Therapy (Enzalutamide).

Methods

Lipid uptake was measured in order to characterise the metabolic response of PCa cells following BCAA starvation. Enhanced lipid uptake is a well-reported mechanism which is upregulated throughout PCa disease progression[4]. This was measured following 24 hours of LNCaP cell culture in individual BCAA-depleted medium and quantified by high-content imaging following the treatment of cells with the fluorescent palmitate analogue, C16-BODIPY. The competitive palmitate analogue 2-bromopalmitate (2-BP) was included as a positive control in order to reduce C16-BODIPY signal by competitive uptake via fatty acid transport.

HIBCH was inhibited using siRNA gene silencing. Cell growth was then monitored using the IncuCyte live cell imaging system before cell death was quantified using Hoechst and propidium iodide staining at end-point. Changes in mitochondrial morphology were investigated by staining LNCaP PCa cells with the MitoTracker Green FM stain. Measurements of oxygen consumption rate was quantified using the Seahorse XFe96 Analyzer.

Results

Measurements of C16 fatty-acid uptake following the depletion of branched chain amino acids demonstrated that exogenous starvation of valine selectively stimulated uptake via fatty acid transport in LNCaP PCa cells. Reversal of valine-dependant uptake was observed following co-treatment with the competitive palmate analogue 2-BP, suggesting this mechanism was transporter mediated (Figure 2).

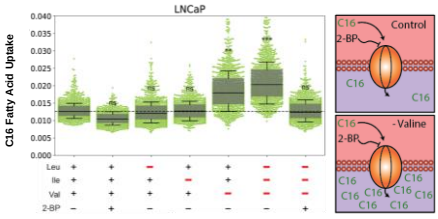

Figure 2. LNCaP PCa Cells Compensate for Extracellular Loss of Valine but not other BCAAs by Long Chain (C16) Fatty Acid Uptake. This can be reversed by the competitive palmitate analogue 2-BP, suggesting rescue occurs through fatty acid transport.

Inhibition of valine catabolism via HIBCH-targeted siRNA reduced cell growth and stimulated cell death in multiple models of PCa progression (LNCaP and PC3). Furthermore, inhibition of HIBCH induced mitochondrial dysfunction in the form of morphologic fragmentation and a notable reduction in oxygen consumption rate observed in 49F enzalutamide-resistant PCa Cells (Figure 3).

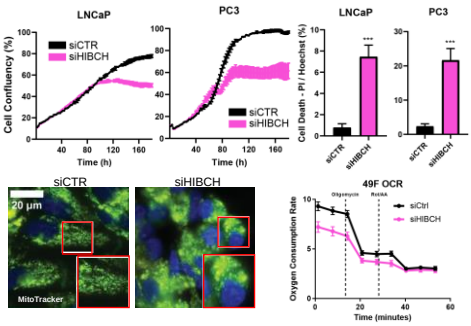

Figure 3. siRNA suppression of HIBCH reduces cell growth and increases cell death in LNCaP (AR+) and PC3 (AR-) PCa cells. Furthermore, suppression induces mitochondrial fragmentation in mitochondria labelled with MitoTracker Green and reduces the oxygen consumption rate of enzalutamide resistant PCa cells.

Discussion

This study has reported a unique metabolic dependency regarding the uptake and catabolism of valine within PCa cells. Here we described valine-dependent effects to PCa lipid metabolism and provided evidence suggesting that this response is transporter-mediated. Silencing of valine catabolism through siRNA mediated suppression of HIBCH has revealed a potential target with pre-clinical potential. Future work utilising drug-inducible models of HIBCH suppression is currently underway with *in vivo* animal-based work to follow.

References

1. AIHW. Cancer data in Australia. Australian Institute of Health and Welfare. 2019.
2. Lonergan PE, Tindall DJ. Androgen receptor signaling in prostate cancer development and progression. J Carcinog. 2011; 10:20.
3. Eidelman E, Twum-Ampofo J, Ansari J, Siddiqui MM. The Metabolic Phenotype of Prostate Cancer. Front Oncol. 2017;7:131.
4. Tousignant KD, Rockstroh A, Taherian Fard A, et al. Lipid Uptake Is an Androgen-Enhanced Lipid Supply Pathway Associated with Prostate Cancer Disease Progression and Bone Metastasis. Mol Cancer Res 2019; 17: 1166–79.