Targeting Transporters to improve Prostate Cancer Therapeutics

A role for Monocarboxylate Transporters as a Metabolic Target to Improve Prostate Cancer Therapeutics

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Introduction

- Metabolic reprogramming in cancer cells is required in order for cells to survive and proliferate in the unstable microenvironment. One strategy is to) induce adjacent fibroblast to switch to anaerobic glycolysis with lactate "fuel". Such "lactate shuttle" requires monocarboxylate transporters (MCT) 1 and 4, acting as a bi-directional transporter and exporter respectively.
- Both MCT1 and MCT4 are overexpressed in a number of malignancies, including prostate cancer (PCa), associating with higher tumour grade, stage, recurrence and overall poor prognosis, thus presenting an attractive target for therapy. It is particularly relevant in PCa, as the development of resistance to hormonal therapy leads to the progression to metastatic castrate-resistant PCa, for which no curative therapies currently exist.
- We have employed Enzalutamide (MDV, generated 'in-house' from parental drug –sensitive LNCaP cells, as models of castrate –resistant PCa, to assess the role of MCT1 and MCT4 transporters in their metabolism and survival.

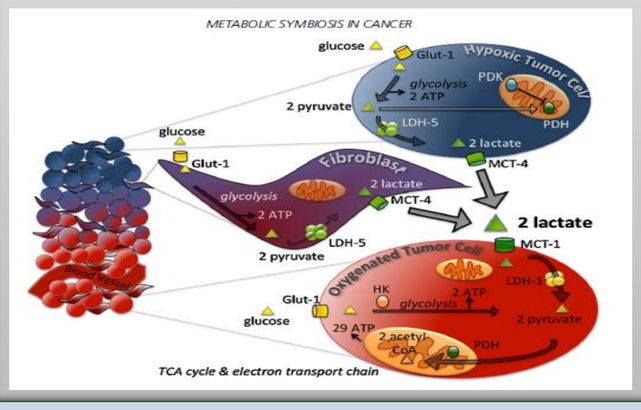


Figure 1 **: Epithelial tumour cell promotes "Reverse Warburg" effect in adjacent fibroblast**

Aims:

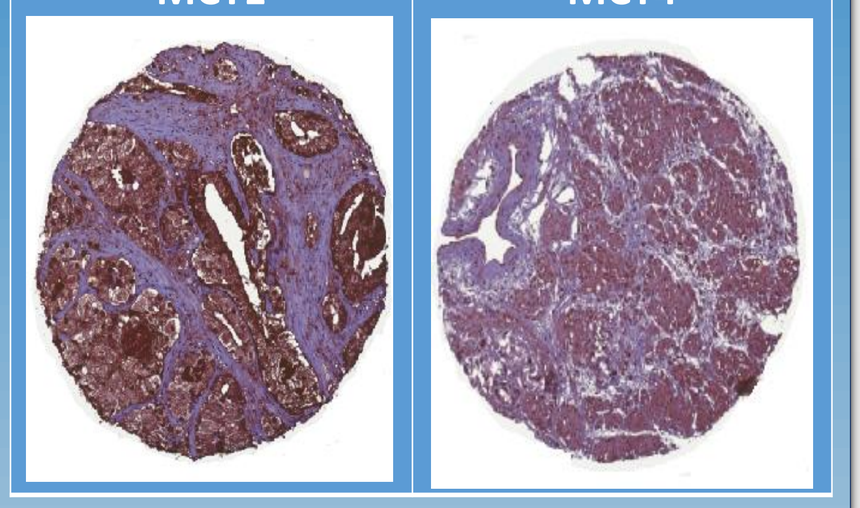
- To evaluate MCT1 and MCT4 expression in patient prostate cancer tissue sample, LNCaP and MDV cell lines microarrays
- To investigate the role of "metabolic symbiosis" in contributing to prostate cancer progression
- To assess the impact of MCT1 inhibition in drug-resistant cell line models and the effect that MCT1 inhibitor drug has on tumour-stromal interaction

Materials and Methods

- Drug-resistive **LNCaP** and Enzalutamide **(MDV)**- Resistant cell lines (MDV Res) were employed as PCa models, with primary fibroblasts representing tumour stroma.
- Incucyte assay is employed to find Gi50 of LNCaP and MDV cell line when treated with AZD3965 drug for 7 days; this help deduce the exact concentration of MCT1 inhibitor (ADZ3965) that induce 50% apoptosis in both LNCaP and MDV Res which will be used in Seahorse Bioscience XF24 analyser.
- Seahorse Bioscience XF24 analyser is used to measure oxygen consumption rate (OCR) in different experimental cell models of sensitive and resistant prostate cancer. OCR is reflective of the rate of oxygen phosphorylation (OXPHOS); it quantify mitochondrial respiration.
- Immunohistochemistry staining of prostate cancer tissue microarrays was employed to evaluate the localisation of MCT1 and MCT4 protein expression in both cancerous and benign clinical tissue samples, including matched paired patient tissue samples (before and after relapse on conventional anti-androgen treatment).

Immunohistochemistry Results: MCT1 and MCT4 expression in patient tissue microarray

Figure 2 : Expression of MCT1 and MCT4 proteins in prostrate benign and cancerous tissue microarray detected by immunohistochemistry staining



- **MCT1 protein is mainly expressed in the cytoplasm of PCa, whereas MCT4 protein is mainly expressed in the stroma surrounding PCa**
- **Expression of MCT1 is more common in hormone naïve prostate cancer**
- **MCT1 overexpression associated with high tumour grade, stage, invasion and recurrence/relapse**
- **Both MCT1 and MCT4 transporters are significantly overexpressed in PCa compared to benign tissue as revealed by histological and quantitative evaluation**
- **MCT1 expression increased, while MCT4 expression decreased inpatients with later stage, castrate-resistant disease**

	Number with expression	Percentage with expression	Mean Cytoplasmic score
Total MCT1	101/135	74.81%	1.56
Hormone Naïve	61/75	81.33%	1.6
Hormone Sensitive	5/16	31.25%	0.625
Castrate resistant	35/44	79.55%	1.84
Total MCT4	35/131	26.72%	0.37
Hormone Naïve	26/73	35.62%	0.49
Hormone Sensitive	3/16	18.75%	0.19
Castrate resistant	6/42	14.29%	0.21

Figure 3 : Total number of patients with TMA that had a positive MCT1/MCT4 expression, hormone naïve, hormone sensitive and castrate resistant, and the mean score of MCT1/MCT4 protein expressed in the cytoplasm

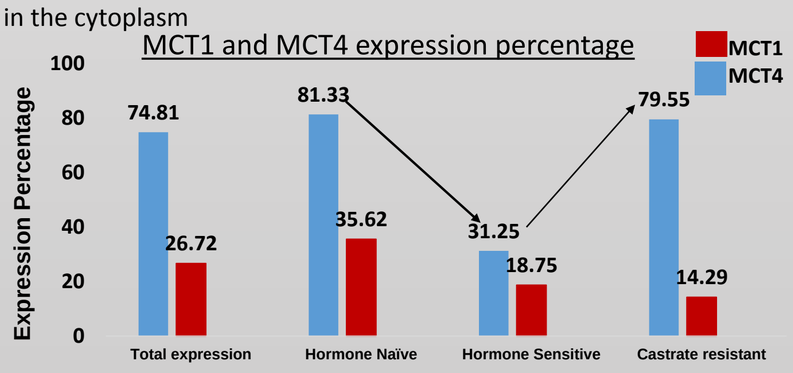
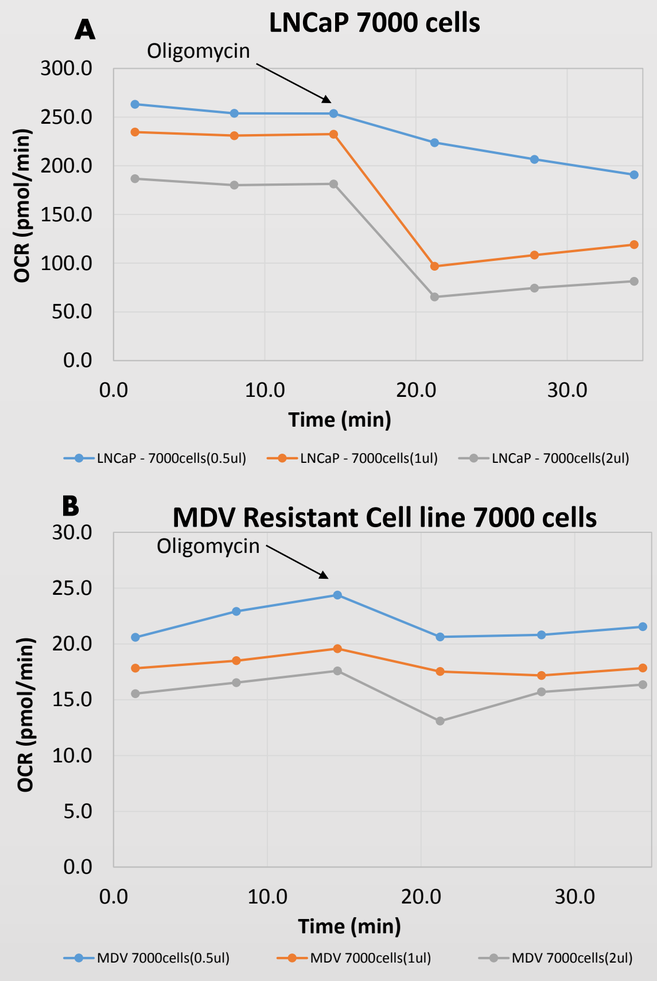


Figure 4 : MCT1 and MCT4 expression percentage on the entire sample and in subgroups of hormone naïve, hormone sensitive and castrate resistant disease

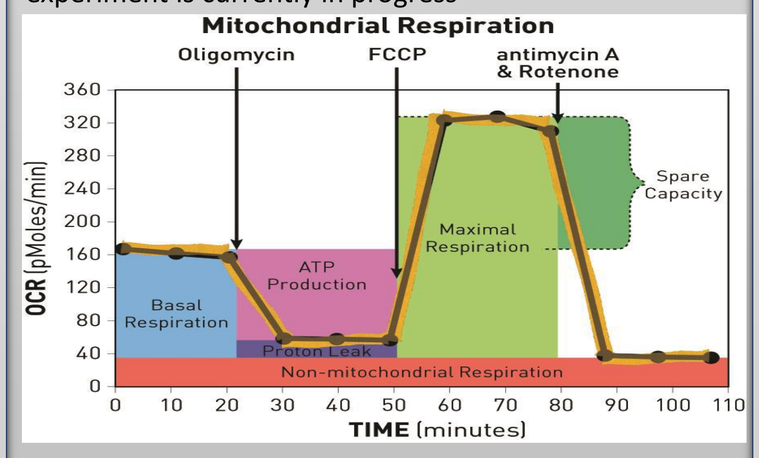
Seahorse Bioscience XF24 analyser: Optimisation with Oligomycin results

Figure 5: Measuring oxygen consumption rate over time after adding oligomycin in 7000 cells of LNCaP and MDV resistant cell line. **(A)** Oxygen consumption rate measured over time after treating 7000 LNCaP cells with 0.5ul, 1ul and 2ul of oligomycin. **(B)** Oxygen consumption rate measured over time after treating 7000 MDV Resistant cell line with 0.5ul, 1ul and 2ul of oligomycin



- **After adding 1ul of oligomycin in LNCaP cells, we observed a rapid decrease in oxygen consumption rate compared to MDV Resistant cell line. However, MDV Resistant cell line is derived from LNCaP cells (parent cell), therefore 1ul of oligomycin will be used in both cell lines during Seahorse XF Cell Mitochondria Stress analyser experiment.**

Figure 6 : Seahorse XF Cell Mitochondria Stress analyser experiment is currently in progress



- **In this experiment we hope will to measure oxygen consumption rate (OCR) which quantify mitochondrial respiration and extracellular acidification rate (ECAR) which is reflective of glycolysis.**

Incucyte cell count proliferation assay results: measures the proliferation rate of LNCaP cells after treated with ADZ3965 drug and MDV Res after being treated with ADZ3965 and enzalutamide

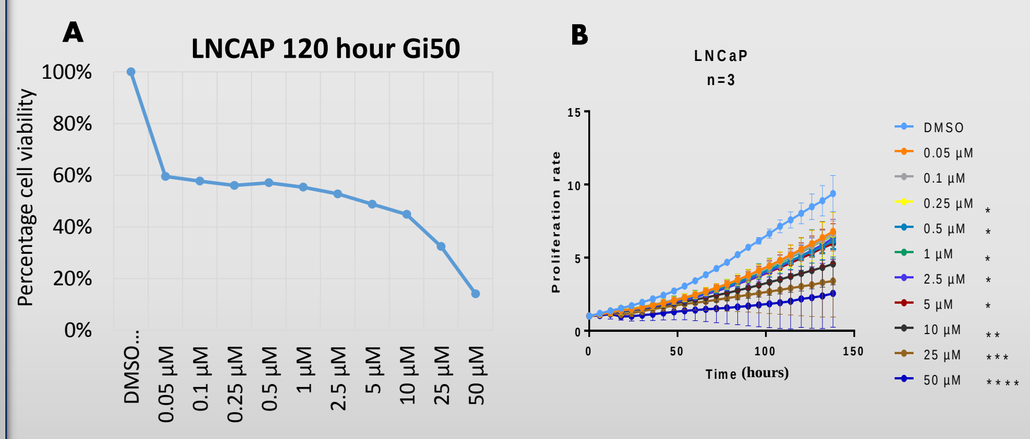


Figure 5 : Measuring the proliferation rate and cell viability of both LNCAP and MDV Res after adding MCT1 inhibitor (AZD965) and cells that are treated with DMSO (10uM) are classified as controls. **(A)** Measuring cell viability at 120 hours after treating LNCaP cells with different concentrations of AZD3965 to denote the exact Gi50. **(B)** Proliferation rate of LNCaP cells after AZD3695 drug treatment at different time. **(C)** Measuring cell viability at 120 hours after treating MDV Resistant cell line with different concentrations of AZD3965 to denote the exact Gi50. **(D)** Proliferation rate of MDV resistant cell line after AZD3695 drug treatment at different time.

- **5µM concentration of AZD3695 is the best concentration of MCT1 inhibitor as it induced approximately 50% cell apoptosis in both LNCaP and MDV Resistant cell line**
- **Proliferation rate in both LNCaP and MDV resistant cell line decreased dramatically when 50uM of MCT1 inhibitor (AZD3695) was added compared to the control (DMSO). This show that MCT1 transporter in particular is essential for metabolic balance and cell viability, and may be present as an attractive therapeutic target for overcoming drug resistance.**

Conclusions/Future work:

- MCT1 is important in cell metabolism and is linked to proliferative potential
- Therefore, using an MCT1 inhibitor ADZ3965 (AstraZeneca) alone or in combination with Enzalutamide in MDV Res cells could potentially compromise cell survival and delay therapeutic resistance.
- Carry a Seahorse XF Cell Mitochondria Stress analyser experiment in conditions that mimicking tumour microenvironment, LNCaP resistant cell line undergo significant changes in their own metabolism and drive stromal cells to switch to a "Reverse Warburg" state

Reference

1) Nakajima E.C. and Van Houten B. (2013), Molecular Carcinogenesis **52**:329-337