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Sustainable healthcare: A future for all

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Establishing a real-time metabolic assay in prostate cancer models to test response to therapy

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Introduction

- Metastatic prostate cancer (PCa) is treated with androgen deprivation therapy and as disease progresses, is augmented by androgen receptor inhibitors (ARI) such as enzalutamide.
- Despite more advanced ARIs entering the clinic therapeutic resistance is a major issue[1]. Over 3000 Australian men die from CRPC each year[2]. Fresh approaches are needed to combat resistance and therapy failure.
- Continual AR inhibition gives rise to the emergence of more aggressive, incurable phenotypes of PCa[3,4]. Continuous AR suppression also drives major molecular reprograming in PCa cells, including tumour metabolism.
- However, little is known about PCa metabolism in 3D models due to lack of an established metabolic assay protocol but is important for translation.

Hypothesis

Understanding the differences in metabolism in the spectrum of disease progression may assist with improving detection or treatment stratification.

Aims

- To define the metabolic dependencies of the PCa molecular subtypes in 2D cell lines.
- To develop a protocol for the analysis of 3D spheroids using the Seahorse Extracellular Flux analyser and compare 2D metabolic dependencies to 3D.

Methods

PCa cell lines representative of different PCa disease phenotypes are cultured in 2D and 3D spheroid models. Table 1 depicts the chosen cell line models.

Table 1: *Cell line models of PCa subtypes.*

Stage / Subtype of PCa represented	Cell line
AR-positive, castration-sensitive, ATT-sensitive (ARPC)	LNCaP
AR-positive, enzalutamide sensitive, castration-resistant (CRPC)	V16D
AR-positive, enzalutamide resistant, castration-resistant (CRPC-ENZR)	MR49F, MR42D

Figure 1: Methodology for 2D and 3D bioenergetic analysis of PCa models.

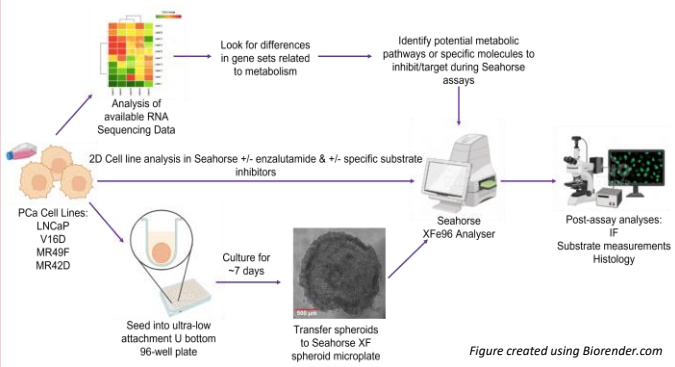

Figure 1: *Methodology for 2D and 3D analysis of PCa models. Established PCa cell lines (LNCaP, V16D, MR49F and MR42D) are cultured in vitro and directly analysed in the Seahorse XF analyser with and without 10 μM enzalutamide treatment and specific substrate inhibitors. Cell lines are also cultured as spheroids with and without enzalutamide treatment for subsequent analysis using the Seahorse XFe96 (3D).*

Optimisation of 3D spheroid metabolism platform

- Developing PCa cell lines into 3D spheroid models for metabolic analysis in the Seahorse XF analyser required optimisation of 3D culture methods and assay parameters.

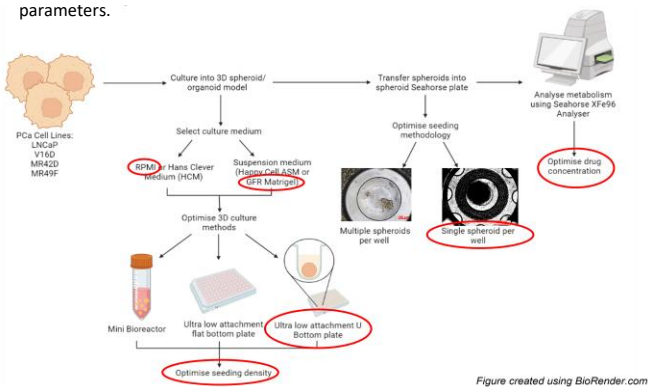

Figure 2: *Optimisation of 3D culture methods for analysis of PCa cell lines on the Seahorse XF analyser. Red circles indicate the optimal condition discovered for analysis using the Seahorse XF analyser. The optimised method involves culturing PCa cell lines in RPMI medium containing 5% growth factor reduced (GFR) Matrigel at a density of 5 X 10 ^{4} cells per well of an ultra-low attachment U bottom plate for approximately 7 days. A single, large spheroid is then transferred into each well of a spheroid seahorse plate and analysed in the Seahorse XF analyser with optimised drug concentrations.*

Mitochondrial ATP production changes with PCa progression & treatment

- Production of ATP from the glycolysis pathway and oxidative phosphorylation (OXPHOS) in the mitochondria is separately measured during a Seahorse experiment and converted to an ATP rate index to show where majority of ATP is produced (Figure 3).

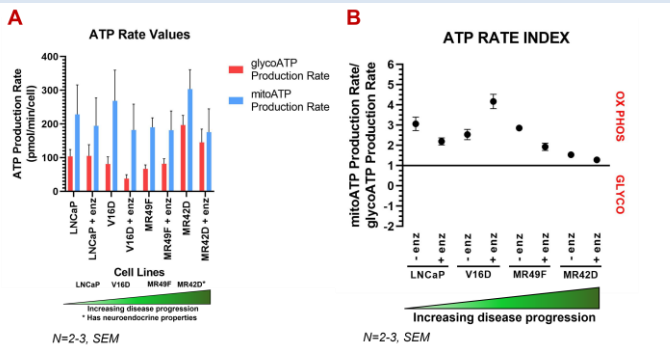

Figure 3: ***A)*** *Total ATP produced for each cell line +/- enzalutamide treatment. Red = ATP derived from glycolysis. Blue = ATP derived from mitochondrial respiration (OXPHOS).* ***B)*** *ATP rate index. Proportion of mitochondrial-derived ATP to glycolysis-derived ATP. Values above 1.0 indicate samples which produced more ATP from OXPHOS. Values below 1.0 indicate samples which produce more ATP from glycolysis.*

- Total ATP production is reduced with enzalutamide treatment for all cell line models excluding MR49F. This implies that ATT treatments induce metabolic remodelling. Understanding this remodelling could help rationalise metabolic interventions in ENZ-sensitive cell lines.
- Figure 3B shows that all models predominantly use OXPHOS for ATP production. We also see an increase in reliance on OXPHOS in models of earlier PCa (LNCaP & V16D) and a trend towards a more glycolytic phenotype in enzalutamide resistant models (MR49F & MR42D). This indicates a change in metabolic phenotype associated with disease progression which may reveal potential treatment targets.

Metabolic fuel dependency switches with PCa progression

- Fuel preferences and dependencies for each cell line were investigated using the Seahorse by treating with substrate specific inhibitors such as UK5099 (an inhibitor of pyruvate entry into the TCA cycle), CB839 (a glutaminase inhibitor), and etomoxir (fatty acid oxidation inhibitor) and comparing metabolic parameters, including basal respiration and spare respiratory capacity, with untreated (vehicle) cells (Figure 4).

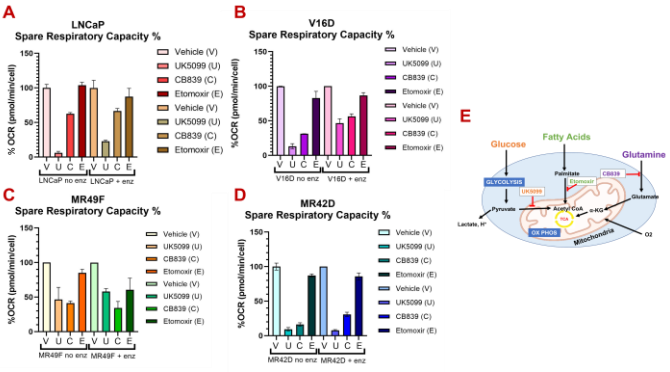

Figure 4: *Spare respiratory capacity of all cell lines with and without enzalutamide and substrate specific inhibitors. Spare respiratory capacity indicates the maximum respiration in a cell under stress. OCR = oxygen consumption rate, a measurement of mitochondrial respiration.* ***A)*** *Spare respiratory capacity of LNCaP cells.* ***B)*** *Spare respiratory capacity of V16D cells.* ***C)*** *Spare respiratory capacity of MR49F cells.* ***D)*** *Spare respiratory capacity of MR42D cells.* ***E)*** *Diagram showing target molecules of substrate specific inhibitors UK5099, CB839, & etomoxir.*

- ARI-sensitive cell lines (LNCaP & V16D) rely heavily on glucose as a fuel source for OXPHOS.
- There is a greater reliance on glutamine as a fuel source with increasing enzalutamide resistance.

Conclusions & Future Directions

- We have identified that metabolic dependencies develop during enzalutamide resistance and that metabolism differs between molecular subtypes of PCa.
- Future directions will use our optimised 3D seahorse method to validate these 2D differences. This will greatly advance our knowledge of PCa metabolism and therapy resistance and may reveal useful metabolic dependencies which can be exploited for imaging and disease staging.

References & Acknowledgements

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