Establishing a real time metabolic assay in bladder cancer models to test response to standard-of-care therapy

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Summary

Bladder cancer (BC) is the 10th most common cancer globally and presents as a highly heterogenous disease that frequently reoccurs post-surgery. There are limited post-operative treatments, and discoveries for novel therapy approaches are required. Altered cellular metabolism underpins cancer progression and resistance to standard-of-care (SOC) therapies, however little is known about BC metabolic alterations and whether these changes can be exploited for additional therapies.

Objective:

We aim to characterise the complex bioenergetics of BC *in situ* and how metabolic dependencies may present novel therapeutic targets to improve patient outcomes.

Aims

1. To establish a real-time bioenergetics assay using patient-derived organoids (PDO) and tissue punches using the Seahorse XFe96 platform
2. Use this assay to define the metabolic pathways of *ex vivo* BC tissue and identify metabolic vulnerabilities.

Methodology

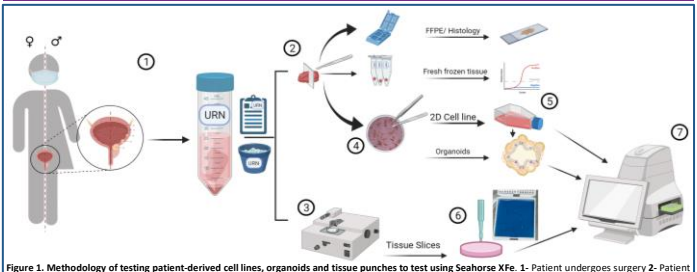

Figure 1. Methodology of testing patient-derived cell lines, organoids and tissue punches to test using Seahorse XFe. **1-** Patient undergoes surgery **2-** Patient tissue is dissected in the lab. Portion taken for histology, fresh frozen tissue, organoids and tissue slices. **3-** Section of tissue is embedded into agarose and sliced into 200µm sections. **4-** Tissue is manually minced then filtered to allow for organoids to re-assemble **5-** Cell lines and organoids develop over time and then can be used in Seahorse XFe 96 spheroid plate **6-** 1.0-1.2mm biopsy punches are taken from slices and then placed in Seahorse XFe 96 spheroid plate **7-** metabolic analysis of patient-derived cell lines, organoids and biopsy punches of tissue slices using Seahorse XFe 96 analyser. Created with BioRender.com

Bladder Cancer Biopsy Tissue Punches

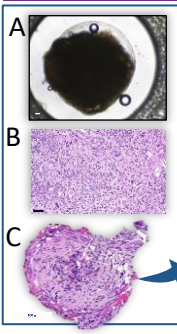

Figure 2. (A) Image of BC tissue biopsy punch in a 96 well Seahorse XFe spheroid plate post analysis Scale, 100µm. **(B)** Haematoxylin/ eosin (H&E) of parental bladder cancer tissue. Scale, 50µm **(C)** Corresponding H&E of parental bladder cancer tissue biopsy punch post Seahorse XFe analysis to observe architecture and integrity. Scale, 100µm.

1.0mm biopsy punch of 200µm tissue slice mimics the phenotype of original patient tissue histology including TME

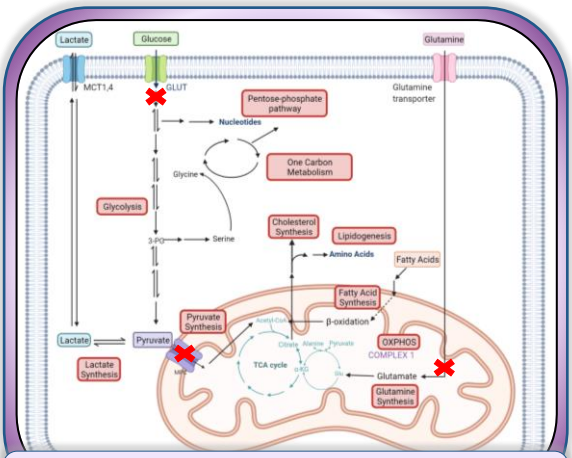

Figure 3. Metabolic pathways of cancer cell. Metabolic inhibitors act on major fuel entry points to determine fuel dependency. These inhibitors are used during Seahorse XFe96 analysis to identify dependency and flexibility (red crosses) in metabolic pathways to record measurements of oxygen consumption rate and extracellular respiration rate as an indicator of metabolic activity. Created with BioRender.com

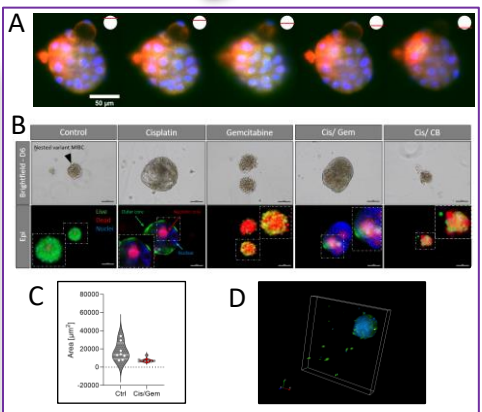

Figure 4. (A) Z-stacked image of Bladder cancer PDO stained with Hoechst (nuclear DNA), Pico green for total dsDNA (Nuclear and mtDNA), MitoTracer Orange CTMRos (accumulates according to mitochondrial activity) and LysoTracker Deep Red (acidic organelles in live cells) on the Incell 6500 **(B)** Organoid viability following 6-day treatment with cisplatin, gemcitabine and CB-839 (glutaminase inhibitor)(Cis; 10 µM)/gemcitabine (Gem; 10 nM)(Cis; 10 µM)/(CB-839; 20 µM) Scale, 100 µm. **(C)** Violin plot showing decreased viability of organoid area (µm²) following 6-day treatment with Cis/Gem. **(D)** 3D confocal Z-stacked image of bladder cancer PDO with Hoechst and LIVE/DEAD stain.

Conclusions

Our pilot study demonstrates it is feasible to generate BC tumour organoids from patient tissue that are representative of parental tumour metabolic fuel dependencies using the Seahorse XFe platform.

- Tissue punches model the patient tissue tumour microenvironment and histological features
- Assessing metabolic vulnerabilities of BC using PDOs may give an insight to the metabolic profile of BC
- Utilising these models, assessing SOC therapies in combination with metabolic inhibitors can potentially impact clinical management of BC patients

Ultimately, combining metabolic inhibitors with SOC therapies to provide a personalised medicine approach may improve treatment efficacy in patients with BC

References & Acknowledgements

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This research was supported by funding from a Princess Alexandra Research Foundation award, and the Medical Research Future Fund (MRFF) Rapid Applied Research Translation Program CPAC. The Translational Research Institute receives support from the Australian Government.

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2021 Embracing diversity in healthcare
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24–27 August 2021