Differentiating metabolic response to treatments in androgen -sensitive and -independent prostate cancer tissue slices

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Introduction

- **Prostate cancer (PCa)** is the second leading cause of malignancy in men.
- **One problem** is that determining the efficacy of prostate cancer (PCa) treatments like androgen-deprivation therapy (ADT) can take months.
- Recent studies suggest that metabolomics can offer **solutions (Figure 1)**.
  - Screening the metabolic response to therapies in PCa tissue biopsies can rapidly and proactively predict the efficacy of therapies.
  - Metformin and rapamycin are potential therapies for PCa and may affect mitochondrial function in PCa tissue.
- **Challenges** exist in current PCa metabolomics research.
  - PCa metabolic assay research is limited to cell cultures, which do not reflect the complex *in vivo* metabolic behavior of a 3D tumor block.
  - Metabolic profiling tissue slices can better reflect the complexity of tumors but is difficult and rarely accomplished.

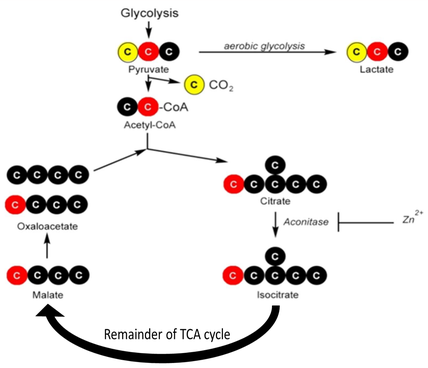

Figure 1. Normal prostate aconitase is inhibited to build-up citrate (energy for sperm). PCa tissue can lose this unique metabolic trait, leading to increased oxidative phosphorylation or the Warburg effect.

Aims

1. To **differentiate** the effects of MDV (ADT agent enzalutamide), metformin, and rapamycin on the metabolic responses in **androgen-sensitive (LNCaP)** and **androgen-independent (CSS90)** PCa tissue slices.
2. To improve characterization of PCa metabolism in a complex 3D tumor by establishing **novel methods of PCa tissue slicing and metabolic profiling**.

Materials and Methods

- Orthotopic PCa tumors in SCID mice were resected and prepared as 200µm tissue slices (Leica VT1200S at 1.00mm/s speed and 3.00mm amplitude).
- During slicing, tumors were supported by an agar block (30% w/v in H2O), submerged in chilled 1X Ca2+/Mg2+ free PBS, and surrounded by ice.
- PCa tissue medium was RPMI-1640 with 2mM L-Gln, 1% PenStrep, 1mM pyruvate, and 10% FBS for LNCaP or 9% charcoal-stripped FBS + 1% FBS for CSS90. 15µM DHT, 20µM MDV, 10µM metformin, and 5µM rapamycin were added in the respective treatment groups.
- PCa tissue **oxygen consumption rate (OCR)** and **extracellular acidification rate (ECAR)** were measured with the Seahorse XF Analyzer **(Figure 5).**

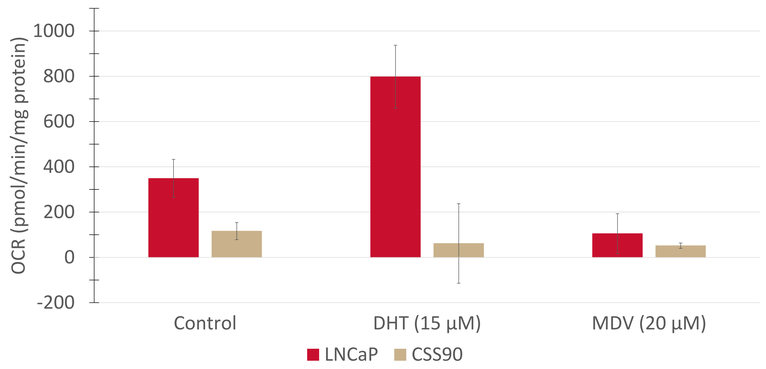

Figure 2. Seahorse XF OCR measurements of **(a.)** LNCaP and **(b.)** CSS90 PCa tumor slices. Control, DHT (androgen induction), and MDV (AR inhibition) treatment groups were incubated for 24 hours in medium.

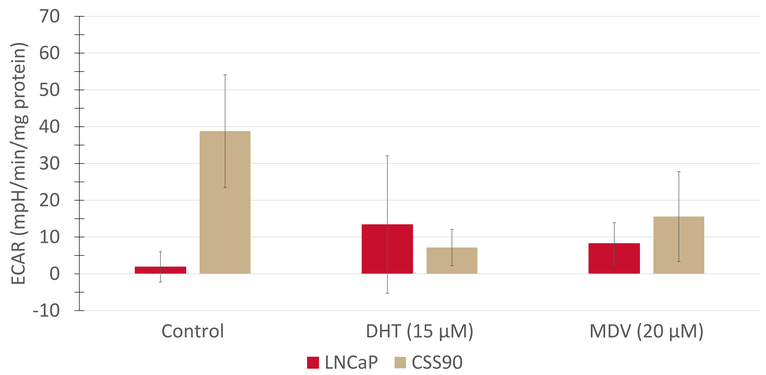

Figure 3. Seahorse XF ECAR measurements of **(a.)** LNCaP and **(b.)** CSS90 PCa tumor slices. Control, DHT (androgen induction), and MDV (AR inhibition) treatment groups were incubated for 24 hours in medium.

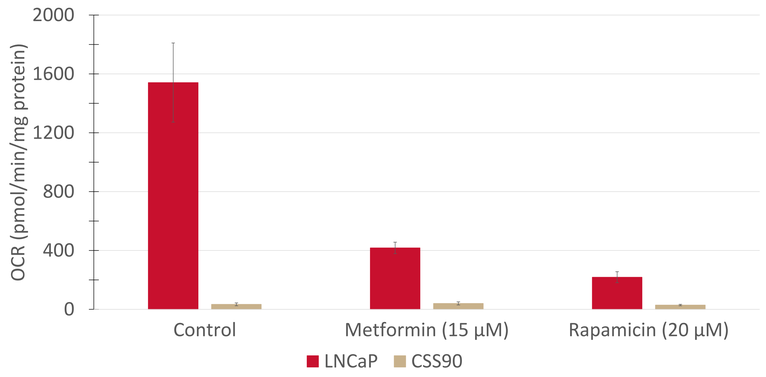

Figure 4. Seahorse XF OCR measurements of **(a.)** LNCaP and **(b.)** CSS90 PCa tumor slices. Control, metformin, and rapamycin treatment groups were incubated for 24 hours in medium.

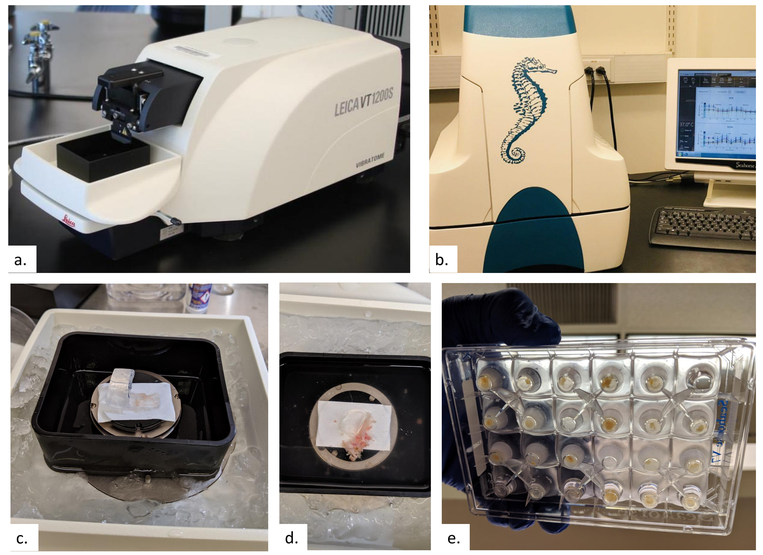

Figure 5. We developed a novel tissue slicing method for the soft PCa tumor with the **(a.)** Leica VT1200S microtome. **(c.)** Agar gel tissue "backboard" and high blade amplitude were critical in acquiring **(d.)** PCa tissue slices for **(b.)** the Seahorse XF analyzer **(e.)** Islet Capture Microplate samples.

Results

- Compared to control (no treatment), **MDV treatment** decreased **OCR** by 70.0% in LNCaP tissue and by 55.5% in CSS90 tissue **(Figure 2)**.
- Compared to control, **MDV treatment** increased **ECAR** by 338% in LNCaP tissue and decreased ECAR by 59.9% in CSS90 tissue **(Figure 3)**.
- Compared to control, **metformin treatment** decreased **OCR** by 72.9% in LNCaP tissue and increased OCR by 18.1% in CSS90 tissue **(Figure 4)**.
- Compared to control, **rapamycin treatment** decreased **OCR** by 85.8% in LNCaP tissue and by 14.3% in CSS90 tissue **(Figure 4)**.

Discussion

- The greater decreases of OCR in LNCaP tissue slices suggest that existing PCa ADTs (i.e., enzalutamide) and potential PCa therapies (i.e., metformin, rapamycin) **inhibit mitochondrial respiration in androgen-sensitive PCa tissue but not in androgen-independent PCa tissue**.
- OCR decrease and ECAR increase in MDV-treated LNCaP tissue support that MDV inhibits mitochondrial respiration in androgen-sensitive PCa tissue as pyruvate could be shunted towards aerobic glycolysis.
- OCR and ECAR decreases in MDV-treated CSS90 tissue support that MDV does not inhibit mitochondrial respiration in androgen-independent PCa tissue. Explanations like general MDV cytotoxicity are more likely.
- We established a **novel method of screening metabolic responses to treatments in PCa tissue slices**, which can be completed within two days.
- In future studies, the goal is to demonstrate that metabolic screening of human PCa tissue biopsies can **predict whether a treatment will be effective for a specific PCa patient at or before the start of treatment**.

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References

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