

Evaluating the metabolic profile of prostate cancer cells using an MR compatible bio-reactor

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INTRODUCTION In Europe and the US, prostate cancer is the second most common cancer diagnosis and the third most common cause of cancer-related deaths in men. Although serum Prostate Specific Antigen is the most widely used biomarker for prostate cancer detection, its use is associated with false-positive and false-negative test results¹, causing great anxiety amongst patients. Thus, there is an urgent need for the identification of additional prostate cancer biomarkers. We utilized NMR spectroscopy to identify differences in the metabolic profile of two representative prostate cancer cell lines; LNCaP (poorly metastatic) and LNCaP-LN3 (highly metastatic) and evaluate the changes in metabolites with and without the addition of a pyruvate dehydrogenase kinase inhibitor, dichloroacetate (DCA)²

METHODS: Human LNCaP and LNCaP-LN3 prostate cancer cells and rat P22 sarcoma cells (used as a control) were cultured and then monitored in a customized bioreactor system, adapted for a 5mm broadband 9.4T NMR Probe (Bruker) at 310K, using previously published methods. Cells were treated with 50mmol DCA for 24hrs and their metabolism observed. Spectral acquisition parameters and the bioreactor system were optimized using rat P22 sarcoma cells with a combination of 1H and 31P spectra. Cell metabolism was monitored for a total of 12hrs using 1H spectra using the Bruker zgpgw5 water suppression sequence (NUC1=1H; NS=64; DS=2; SWH=11160Hz; AQ=0.2s; D1=2s; Total time for one spectral acquisition = 148s). 20mmol Trimethylsilyl Propanoic Acid was used as a chemical shift and concentration reference located in a coaxial tube within the 5mm NMR tube.

RESULTS: Zymography assays (n=4), showed an absence of the Lactate Dehydrogenase-B (LDH-B) subunit in both the LNCaP-LN3 and rat p22 cells, whereas LNCaP cells expressed both LDH-A and LDH-B subunits. In total, 6 metabolites were confirmed with ¹H-¹H DQF-COSY and ¹H-¹³C HSQC spectra: lactate, fatty acids, alanine, glutamine, choline and creatine against the Spectral Database for organic compounds (SDBS). Median lactate concentration measured at 1hr (Fig 1) showed highest levels in the LNCaP-LN3 cells at 19500 μ mol/10⁸ cells, which was greater than rat P22 by 12000 μ mol/10⁸ cells (p=0.006). As expected from zymography results, LNCaP cells had the lowest median lactate concentration of 864 μ mol/10⁸ cells (p=0.001). Median fatty acid concentration at 1hr in the rat P22 and LNCaP-LN3 cell lines was similar at ~4000 μ mol/10⁸ cells but greater than LNCaP cells at 820 μ mol/10⁸ cells (p values = 0.006 and <0.001) respectively. LNCaP-LN3 had a 8.5 and 0.8 greater Lac/Cho ratio than LNCaP within 1hr and 12hr (p=0.001). LNCaP-LN3 had the largest increase in creatine over 12hrs at 0.25 μ mol/10⁸ cells/s, double the rate of the LNCaP cells. Following DCA treatment there was a reduction in this gradient for all 3 cell lines. Choline production rate was drastically reduced following DCA treatment for LNCaP-LN3 cells (Fig 2). Following DCA treatment, median Cho/Cr ratios measured at 1hr in LNCaP and LNCaP-LN3 decreased by 0.26 (p<0.001).

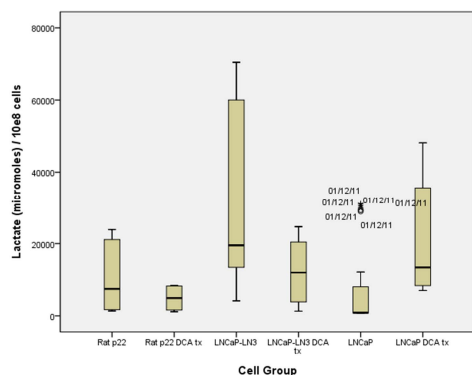


Figure 2: A Box-whisker plot showing lactate concentration (μ mol/10⁸ cells) by cell group within t=1hr shows the highest lactate levels for LNCaP-LN3 cells

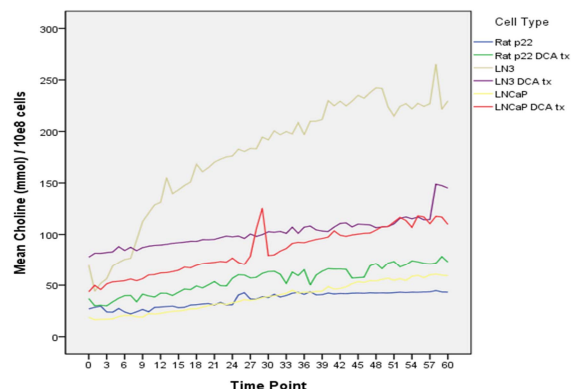


Figure 1: A time-series plot of mean choline concentration showing the LNCaP-LN3 cells produce choline more rapidly than the other cell lines

CONCLUSIONS: Lactate production is particularly pronounced in the metastatic prostate cancer cell line compared with the non-metastatic and the sarcoma cell lines despite the latter having the same LDH isoenzyme profile as the metastatic cell line. This suggests that lactate may be important for prostate cancer cell progression. In addition, the different metabolites of the representative prostate cancer cells measured could be related to their metastatic potential. DCA therapy alters the metabolism of prostate cancer cells.

REFERENCES: 1. EVANS, C. A. et al, 2009. Prostate cancer proteomics: The urgent need for clinically validated biomarkers. *PROTEOMICS – Clinical Applications*, 3, 197-212. 2. MICHELAKIS, E. et al, 2008 Dichloroacetate (DCA) as a potential metabolic-targeting therapy for cancer. *Br J Cancer*, 99, 989-94.