Validation of Hyperpolarized ^{13}C Lactate as a Prostate Cancer Biomarker Using a Human Prostate Tissue Slice Culture Bioreactor

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INTRODUCTION: The treatment of prostate cancer has been impeded by both the lack of clinically relevant disease models and metabolic markers that track tumor progression. Hyperpolarized (HP) ^{13}C MR spectroscopy has emerged as a new technology to investigate the metabolic shifts in prostate cancer [1], and the safety of ^{13}C pyruvate as a metabolic probe was confirmed in a recent clinical trial [2]. The present study provides the first validation of HP ^{13}C lactate as a prostate cancer biomarker in human tissues, critical for the interpretation of *in vivo* studies. A patient-derived prostate tissue slice culture (TSC) model that recapitulates the metabolic profile of prostate cancer *in vivo* was developed, applied to a perfused cell (bioreactor) platform, and investigated by ^{13}C MR. ^{13}C spectra following injection of hyperpolarized ^{13}C pyruvate demonstrated significantly increased pyruvate to lactate flux in malignant as compared to the normal prostate TSCs. This increased flux in the malignant prostate TSCs correlated with both increased expression of monocarboxylate transporters (MCT) and activity of lactate dehydrogenase (LDH), providing mechanistic evidence for HP ^{13}C lactate as a prostate cancer biomarker.

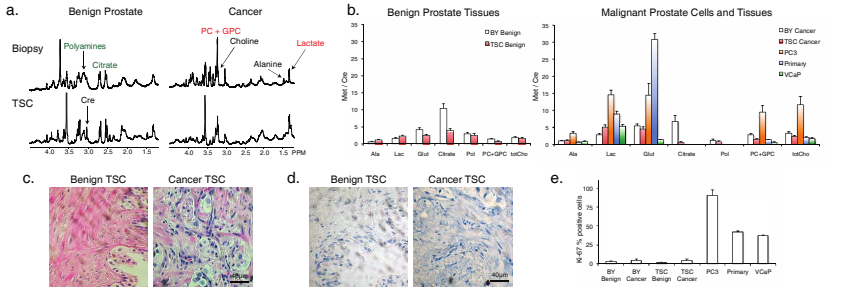

Figure 1. (a) ^{1}H HRMAS of TSCs and snap frozen biopsies. (b) Comparison of metabolism in TSCs, biopsies and both immortal (PC3 and VCaP) and primary prostate cancer cells. (c/d) histopathology of TSCs for H&E and Ki-67. (e) Relative Ki-67 showing low numbers of dividing cells.

METHODS: *Cell Culture and TSCs:* PC-3, VCaP and primary prostate cancer cells were grown to 80% confluency in Dulbecco's Modified Eagle's medium (DMEM) with high glucose and supplemented analogous to the PFMR-4A previously described, which included 10nM R1881 [3]. For TSCs, 8mm cores were taken from radical prostatectomy specimens and rapidly sectioned (300µm) and cultured in the same medium as cell studies on a rotator inside of a standard cell culture incubator [4]. Labeling studies utilized DMEM containing 5 mM [3-^{13}C] pyruvate (Isotec, Miamisburg, OH) and 1 mM unlabeled glucose for 2 hours. TRUS-guided prostate biopsies, acquired from untreated patients (mean age = 64 ± 10 years [range: 35–83 years]; median PSA = 5.6 ± 18.4 µg/L [range: 1.6 –115.9 µg/L]), were used for metabolic comparison [5]. *Bioreactor Studies:* Tissue slices were perfused in an MR-compatible bioreactor with a continuous flow of 37ºC media (perfused with the same medium as was used for cell culture) [6]. HP MR: Hyperpolarization was conducted by the DNP method using the HyperSense (Oxford Instruments, Oxford, UK) using [1-^{13}C]pyruvic acid and the trityl radical OX063 to an average polarization of 20% and 10mM concentration (pH =7.5). *Histopathology and Biochemical Assays:* Tissues were stained for hematoxylin & eosin (for structure) and KI-67 (for proliferation). Lactate dehydrogenase (LDH) activity was assayed using standard methods. qRT-PCR for the expression of the monocarboxylate transporters (MCT1 and 4) as well as LDHA utilized primers purchased from Applied Biosystems (Foster City, CA). *Data Analysis:* All NMR data was processed using ACD Labs NMR processor (version 9; ACD/Labs, Toronto, Canada) and jMRUI. Peak areas or volumes were integrated and used to derive the necessary concentrations. All statistics were calculated using JMP software (SAS Corp.).

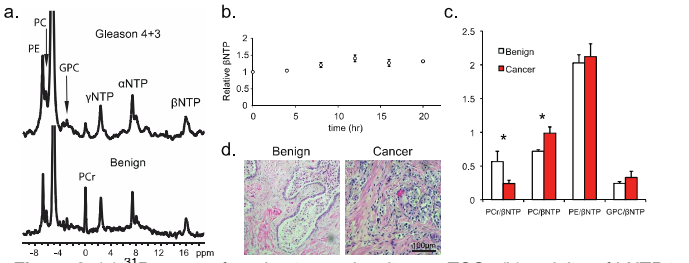

Figure 2. (a) ^{31}P spectra from benign and malignant TSCs (b) stability of bNTP in time showing steady state (c) quantification of ratios, demonstrating reproduction of *in vivo* ^{31}P (d) histology post-bioreactor showing preservation of tissue.

RESULTS AND DISCUSSION: Representative ^{1}H HRMAS data in **Figure 1a** demonstrate the similarities between TSCs and snap frozen biopsies. The distribution of metabolites, when compared to immortal (PC3 and VCaP) and primary prostate cancer cells is dramatically different (**Fig. 1b**), highlighting the need to study primary tissue cultures. Of note, TSCs recapitulated the steady state metabolic reprogramming of prostate biochemistry, showing increases in lactate and glutamate with decreases in citrate and polyamines. Histology confirms the preservation of architecture (**Fig. 1c**) and Ki-67 staining demonstrates the low levels of proliferation (**Fig. 1d** and quantified in **Fig 1e**). When incubated with [3-^{13}C] pyruvate, cancer TSCs labeled 10 fold more [3-^{13}C] lactate than benign TSCs (P=0.07), with an increased fractional enrichment. When TSCs were cultured in an MR-compatible bioreactor, ^{31}P MR spectra (**Fig. 2a**) demonstrate similar spectra to that of in vivo ^{31}P. These cultures are stable in time (**Fig. 2b**) and have ratios similar to the literature (**Fig. 2c**). When HP [1-^{13}C] pyruvate is injected, cancer TSCs produce significantly higher HP lactate (**Fig. 3**), which correlates to the LDH activity as well as expression of monocarboxylate transporters.

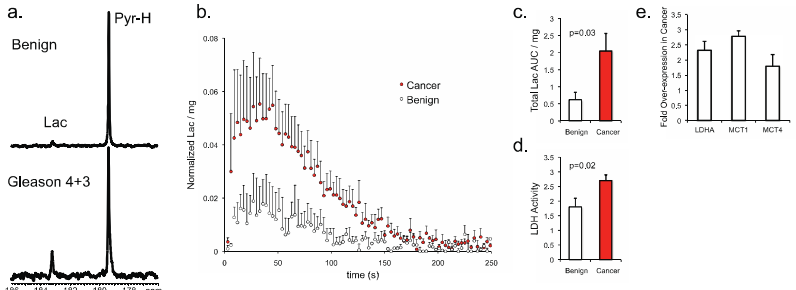

Figure 3. (a) HP ^{13}C at 90 secs post-injection (b) average time course of HP Lac (c) AUC of Lactate (d) significant increase in LDH activity (e) mRNA expression increase in MCT1, 4 and LDHA relative to benign TSCs

CONCLUSIONS: In this study we have characterized the metabolism of patient-derived prostate tissue slices in cultures, a novel model for study of prostate cancer. In contrast to immortal and primary prostate cancer cells in culture, the prostate TSCs exhibit structure, function and metabolism that recapitulate the *in vivo* situation. Utilizing this model, we interrogated the glucose reprogramming in human prostate cancer tissues using HP ^{13}C pyruvate MR, and provided mechanistic evidence for HP ^{13}C lactate as a prostate cancer biomarker. More broadly, this clinically relevant *ex vivo* metabolic model system in combination with HP MR can facilitate the identification of clinically translatable biomarkers of prostate cancer presence and aggressiveness, which are important for treatment selection. Moreover, this approach addresses one of the most pressing issues in prostate cancer research, the development of treatments and companion metabolic biomarkers of response that can be easily translated into the clinic. In future studies this research will be extended to smaller cultures (i.e. single human biopsies), multiple hyperpolarized probes and the use of novel therapeutics.

REFERENCES: [1] Albers *Cancer Res* 2008 [2] Nelson *ISMRM* 2012 [3] Levin MRM 2010 [4] [5] Tessem *MRM* 2008 [6] Keshari *MRM* 2010 **ACKNOWLEDGEMENTS:** NIH K99 EB014328, P41 EB013598 and DOD PC093725