

Hyperpolarized [1-¹³C] Pyruvate Metabolism in a Human Prostate Tissue Culture Bioreactor

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Introduction: Published hyperpolarized ¹³C MRSI studies in the Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) model have demonstrated the clinical potential of hyperpolarized [1-¹³C] pyruvate uptake and metabolism for detecting intra-glandular and metastatic prostate cancer (1), providing a measure of pathologic grade (1) and early response to therapy (2). Although transgenic mouse models, like the TRAMP model, mimic several key aspects of human prostate cancer pathology and metabolism well (1), there are a number of important differences between these animal models and human disease. Human prostate tissue slice cultures (TSCs) can provide a more realistic experimental model for the biological characterization of the normal and malignant prostate and to carry out metabolic studies in a controlled environment (3). Combining the dramatically increased sensitivity provided by hyperpolarized ¹³C-labeled substrates with NMR-compatible bioreactors enables kinetic monitoring of metabolism on a second timescale without background signals from the cell culture media. In this study we demonstrate the first application of hyperpolarized ¹³C spectroscopy to 3D human prostate tissue cultures in a NMR-compatible bioreactor to monitor changes in [1-¹³C] pyruvate metabolism in prostate cancer.

Methods: Fresh tissue cores (8 mm diameter) from radical prostatectomy specimens were embedded in agarose and rapidly sectioned (30 - 250 μm sections) while immersed in chilled physiologic fluid. Tissue slices were then transferred to either an NMR-compatible bioreactor or processed for pre-culture histopathological analysis. [1-¹³C] pyruvate was hyperpolarized using the Hypersense (Oxford Instruments) as described previously (1) and 1mL of 10 mM pyruvate was injected into a custom designed 10mm flow system at 5 rpm. The bioreactor is a completely contained 3D culture system with a continuous flow of 35°C media (containing a custom DMEM based hormone defined formulation, 10% FBS, and Penn/Strep). ¹³C NMR spectra were acquired in intervals of 3 sec using a 13° pulse for 300 secs on a narrow-bore 11.7T Varian INOVA (125MHz ¹³C, Varian Instruments) equipped with a 10mm triple tune direct detect broadband probe. Prior to and after injection of the hyperpolarized compounds, a 4 hour time course of ³¹P spectra (202MHz ³¹P) were acquired with a 60° pulse, nt=2048, and at=1s to assess the β-NTP resonance as a function of time and infer cell health. ³¹P spectra were acquired from one benign sample for 32 hours. After perfusion in the bioreactor, samples were processed for pathology and LDH enzyme activity. Two pathologists used a five-point scale (1= excellent, 5 = poor) to quantify the quality of the pathology of the TSC's after the bioreactor study. Hyperpolarized metabolic data was processed using ACD 1-D NMR processor (ACD labs, Ontario, CA), and lactate (182 ppm) and pyruvate (171 ppm) peaks areas were integrated over time and normalized to tissue mass. The area under the metabolic time course curve (AUC), time to maximum hyperpolarized lactate, maximum hyperpolarized lactate peak, and LDH activity were statistically compared between benign and malignant tissue cultures using a Student T-test.

Results: NMR-compatible prostate TSC bioreactor studies were performed on 3 cancer TSCs (Gleason 3+3, 3+4 and 4+4), and 3 benign TSC's. The malignant and benign ³¹P TSC spectra (Figure 1A and B respectively) were identical to what has been previously published for *in vivo* ³¹P spectra from the human prostate (5), and the ³¹P spectra remained constant over a 32 hour time period (Figure 1C). Additionally, pathology at the end of the bioreactor study demonstrated preservation of *in vivo* tissue structure (Figure 1), with pathologist's giving an average pathologic score of good (3.3 ± 0.2). This tissue culture platform provided a unique opportunity to

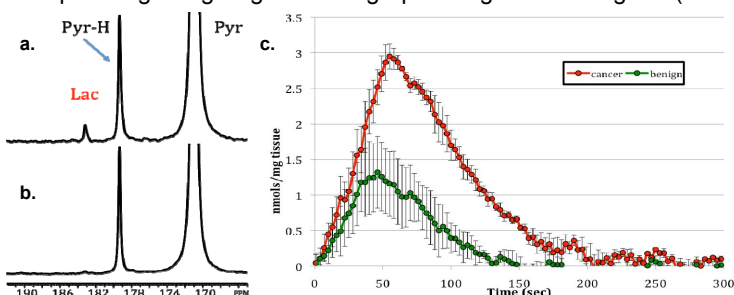


Figure 2. Hyperpolarized ¹³C spectra at 78 secs post-injection of [1-¹³C] pyruvate for both a gleason 4+3 prostat cancer (a) and benign (b) TSCs. An average time course of both cancer (N=3) and benign (N=3) TSCs (c) demonstrates the separation of hyperpolarized lactate signal in time. Error bars are the s.e.m. at each point.

Discussion: These studies demonstrate the feasibility of maintaining both the pathologic and metabolic integrity of benign and malignant human tissue cultures in the NMR compatible bioreactor for 32 hours. Benign tissues showed very low levels of hyperpolarized [1-¹³C] lactate consistent with the major utilization of pyruvate being citrate production (4), while cancer showed high lactate, similar to the prior observation of increased lactate concentrations in human biopsy samples (5) and increased LDH activity. Moreover, there was minimal overlap of the labeled hyperpolarized lactate signal in individual cancer and benign tissues, suggesting that hyperpolarized lactate will be an accurate biomarker of prostate cancer in patients.

References: 1. Albers, MJ. Cancer Research 2008; 68(20):8607-15 2. Chen, A. ISMRM 16th Scientific Meeting. 05/2008. 3. Albers MJ. ISMRM Fourteenth Scientific Meeting. (1270) : 264, 05/2006. 4. Costello LC. Prostate 1991; 18:25-46. 5. Tessem MB. Magnetic Resonance in Medicine 2008; 68(3):510-6. **Acknowledgements:** NIH R21 EB005363, NIH R01 EB007588, Special thanks to Paniz Vafaei and Rosalie Nollay

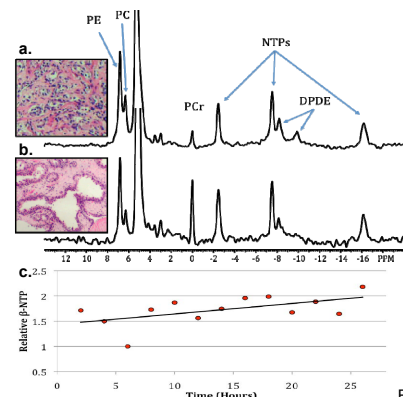


Figure 1. Representative ³¹P spectra of Gleason 4+3 (a) and benign (b) TSCs after 32 hours of perfusion. Time course of β-NTP (c) demonstrates stability of the tissue slices in time.