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

D. J. Joun1, M. Albers1, K. Keshari1, R. Bok1, C. Ward2, D. Peehl1, S. Ronin2, D. Vigneron2, and J. Kurhanewicz2

1Radiology, UCSF, San Francisco, California, United States, 2Radiology, UCSF, San Francisco, California, 3Urology, Stanford

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 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 13º 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 (200MHz 31P) were acquired with a 60º pulse, n=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 curve (AUC) of β-NTP (c) demonstrates stability of the tissue slices in time.

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 investigate the metabolism of hyperpolarized [1-13C] pyruvate in the human prostate prior to actual patient studies. As shown in Figure 2A prostate cancer TSCs demonstrated a significantly higher production of labeled hyperpolarized [1-13C] lactate than benign TSCs, as evidenced by a significantly larger peak hyperpolarized lactate (3.0 ± 0.1 versus 1.3 ± 0.5 nmol/mg, p=0.03) and hyperpolarized lactate AUC (262 ± 17 versus 80 ± 47, nmol/mg total, p=0.02, Figure 2C). The significant increase in hyperpolarized lactate signal is consistent with a significant increase in LDH activity in cancer (0.0027 ± 0.0003 versus 0.0018 ± 0.0003 nmol/mg/min of protein, p=0.05). There was no significant difference in the time to maximum hyperpolarized lactate (58 ± 5 sec versus 49 ± 3 sec, p=0.2) between benign and malignant TSCs.

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.
