Metabolic imaging to differentiate aggressive versus indolent prostate cancer

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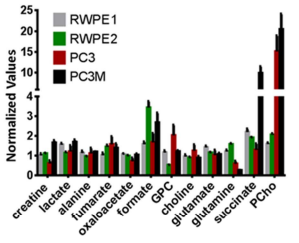
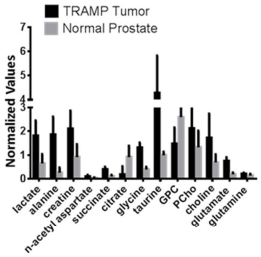
Purpose: Many prostate cancers (PCa) detected by screening are indolent (will not leave the prostate) however, 90% of patients will receive immediate treatment such as surgery or radiation therapy. There is a pressing need in the clinic for determining the aggressiveness of PCa that allows the whole prostate to be examined and is directly correlated to the metastatic behavior of the tumor. We are using a multi-prong approach to understand the metabolic changes that occur in the progression of PCa. (1) We are metabolic profiling tumor tissue and normal prostate tissue. (2) We are metabolic profiling both the intracellular and secretion/consumption of metabolites of four human prostate cancer cell lines with different aggressiveness profiles. (3) We are following the progression of PCa with hyperpolarized agents both in xenograft animal models of PCa and in a transgenic PCa animal model (TRAMP). We are hyperpolarizing metabolic agents to interrogate metabolic pathways such as: hyperpolarized choline (choline uptake/metabolism), bicarbonate/carbon dioxide (pH imaging) and acetate (TCA cycle/fatty acid metabolism). Hyperpolarization allows for over >10,000 fold sensitivity enhancement using conventional MRI and MRS. The polarization (signal enhancement) can be retained on the metabolites of the hyperpolarized molecule.[1-3]

Methods: We have analyzed PCa from palpable TRAMP animals.[4] We have analyzed the metabolites extracted from cells (intracellular metabolites) and secreted/consumed metabolites from four different prostate cancer cells lines with different aggressive profiles: RWPE-1 (non-tumorigenic, considered benign), RWPE-2 (non-metastatic), PC3 (aggressive, castration resistant), most aggressive cell line PC3M (castration resistant).[5, 6] Tumor tissue, normal prostate from control mice, and cell pellets were homogenized and water soluble metabolites were extracted with water and methanol. All samples were dissolved in phosphate buffered (pH 7.6) deuterium oxide with DSS-d_6 (Sigma 613150). 1D ^{1}H proton spectroscopy was performed with water suppression on a 500 MHz Bruker Avance III HD NMR equipped with a Prodigy BBO cryoprobe and analyzed with TopSpin or MestReNova. The concentrations of metabolites between the types of tissue were normalized based on the wet mass of tissue or cell count in each NMR sample (Figure 1). Student t-tests were determined using GraphPad Prism 6. We have recently published a new method of generating multiple hyperpolarized imaging agents concurrently called chemical reaction-induced multi-molecular polarization (CRIMP).[7] In the initial iteration of the CRIMP technique, we react polarized 1,2-^{13}C-pyruvate with hydrogen peroxide to generate hyperpolarized 1-^{13}C acetate and ^{13}C-carbon dioxide. We are currently utilizing the CRIMP technique and other existing hyperpolarized metabolic imaging agents to determine the aggressiveness of PCa in animal models.

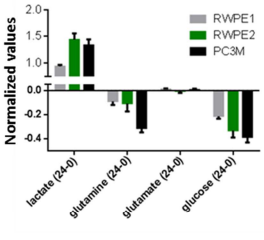
Figure 1



Intracellular Metabolites: Statistically significant changes seen between PC3 and PC3M cell pellets (n = 4): succinate (P = 0.002), GPC (P = 0.0003), choline (P = 0.0006), glutamate (P = 0.03), and glutamine (P < 0.001).



Tissue Metabolic Profiles: Metabolic differences between TRAMP palpable tumor tissue (n = 5) and normal mouse prostate tissue (n =4). Some of the major metabolic differences were lactate (P = 0.02), succinate (P = 0.002), glycine (P < 0.001), GPC (P = 0.04), and glutamate (P = 0.003).



Excreted/Consumed Metabolites: Average difference of metabolite in the media in a 24 hour growth. Statistically significant difference was seen only in glutamine consumption (P < 0.002).

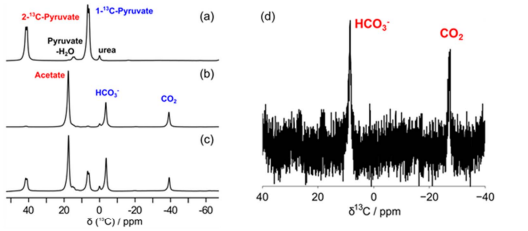


Figure 2 Hyperpolarized MRS (a) Hyperpolarized ^{13}C magnetic resonance spectrum of 1,2-^{13}C$_2$-pyruvate. (b) CRIMP reaction with H_2O_2 (full conversion). (c) Reaction with H_2O_2 (partial conversion). (d) *In vivo* spectrum of hyperpolarized bicarbonate and carbon dioxide (200 µl tail vein injection).

Results: Based on our metabolic profiling PCa tissue and cell culture, we observe significant differences in uptake of glutamine between RWPE2 and PC3M cells; in the amount of intracellular glutamine in PC3M versus PC3 cells; differences in phosphocholine (PCho) and glycerophosphocholine (GPC) between PC3 and PC3M; in the amount GPC and PCho in TRAMP tumor tissue versus normal prostate tissue; differences in intracellular succinate levels between PC3 and PC3M; and differences in succinate levels in TRAMP versus normal prostate tissue. In addition, in our hands we see no significant difference in the glycolytic rate (production of lactate) between the indolent RWPE2 and aggressive PC3M cell lines in culture or in the intracellular lactate values (Figure1).

Using DNP polarization, we are designing new methods for interrogating metabolic pathways. Using our CRIMP technique, we can fully transfer the high polarization levels of pyruvate in the irreversible reaction to 1-^{13}C acetate and $^{13}CO_2$ without substantial signal loses (Figure 2). Because hyperpolarized carbon dioxide nearly instantly equilibrated with bicarbonate in the aqueous environment, the pH of the media can be simply calculated from the signal intensity ratio $^{13}CO_2$ and $H^{13}CO_3^-$ using the Henderson Hasselbalch equation.

Discussion: As an alternative to hyperpolarized pyruvate for analysis of the aggressiveness of prostate cancer, compounds that more comprehensively interrogate glutaminolysis, choline metabolism, and the Krebs cycle in combination may provide better metabolic imaging information. Using the CRIMP technique and single component hyperpolarization of ^{15}N-choline, we are currently imaging PCa animal models.

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