Hyperpolarized ^{13}C Biomarkers of Androgen Independent Prostate Cancer

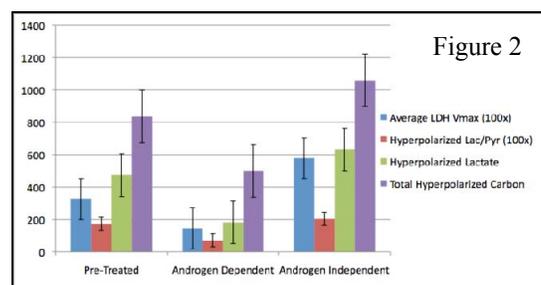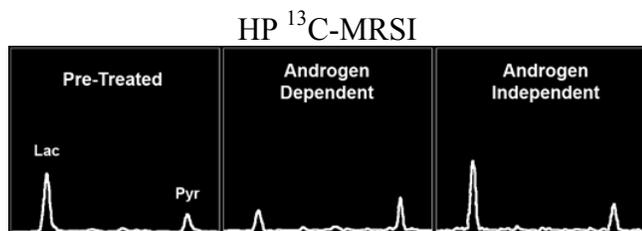**P. Vafaei[1], R. Bok[1], L. DeLosSantos[2], V. Zhang[2], P. Guan[2], D. Vigneron[2], and J. Kurhanewicz[2]**

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Introduction: Hormone therapy (also called androgen deprivation therapy) affects the cellular bioenergetics of the prostate and is a major component of prostate cancer treatment. Recurrent prostate cancer, following prostatectomy or radiation therapy, is commonly treated with androgen deprivation. Ultimately, this fails and the cancer progresses to an androgen independent state. However, there are currently no accurate biomarkers that clearly identify this transition. The aim of this study is to determine the metabolic and biologic phenotype of Androgen Independent Prostate Cancer (AIPC) in the Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) model. Differences in the percentage of poorly differentiated cells, Ki67 staining, and lactate dehydrogenase activity, prior to and post hormone therapy (surgical castration), were investigated in conjunction with hyperpolarized ^{13}C magnetic resonance spectroscopy (HP ^{13}C-MRSI) of tumors *in vivo*.

Methods: Three categories of mice and their prostates/tumors were investigated: pre-treated (not castrated), treated (androgen-dependent), and treated (androgen-independent). The pre-treated group in this study was not followed through castration, for tissue acquisition purposes, and consisted of a mix of disease states. The TRAMP mice were dissected and their tumors were histologically analyzed for grade and cell proliferation. Immediately adjacent tumor tissue was used to conduct lactate dehydrogenase (LDH) activity assays. The activity was measured through a previously described NADH-linked spectrophotometric method (1), in which the decrease in NADH absorbance was observed after the addition of varying pyruvate concentrations. Tumor androgen responsive status

Figure 1

HP ^{13}C-MRSI

Figure 2

was assessed through both morphometric and histological analysis. Spectroscopy was acquired with a dual-tuned mouse coil in a GE 3T scanner as previously described (2). The spectral data acquired was then optimally aligned with the traced tumor region of interest. The peak area-to-noise ratio of HP lactate and total hyperpolarized carbon (THC = lactate+pyruvate+alanine) were calculated. Spectral data was correlated to the LDH maximum velocity (V_{max}) values, calculated using the Lineweaver–Burk plot.

Results: Representative HP ^{13}C-spectra in untreated animals (pre-treated), those with no proliferation/growth after castration (androgen dependent), and those with proliferation/growth after castration (androgen independent) are shown in Figure 1. Qualitatively, we observed significantly lower hyperpolarized lactate and THC levels in androgen dependent versus androgen independent cancer. Specifically, the androgen independent phenotype had a significantly higher HP lactate/noise (632 ± 180 vs 182 ± 115, $p<0.0004$), THC (1060 ± 281 vs 501 ± 326, $p<0.004$), HP lactate/pyruvate (2.04 ± 0.71 vs 0.713 ± 0.27, $p<0.002$), and LDH activity (5.79 ± 2.76 vs 1.45 ± 0.30 nM-NADH/min/μg-protein/ml, $p<0.003$) relative to the androgen dependent phenotype. Figure 2 is a bar plot quantitatively summarizing the differences between androgen dependent and independent disease.

Discussion: AIPC is defined as continued tumor progression in the face of castrate-levels of androgen. In this study we show that in the TRAMP model, it is also characterized by significantly elevated LDH activity that correlates with elevated HP ^{13}C-lactate levels *in vivo*. Additionally THC, a measure of HP pyruvate uptake, was also significantly elevated in androgen independent disease. These studies suggest that these HP biomarkers could be helpful in delineating disease status for improved therapeutic selection.

References:

(1) Vassault, A., *Methods of Enzymatic Analysis*, *3*, 1983.
(2) Albers et al., *Cancer Research, 68(20):8607-15, 2008.*