Metabolic Profiling of Post-Radiation Prostate Biopsy Tissues

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Introduction: Despite excellent success rates with radiotherapy for the treatment of prostate cancer, a significant number of patients will experience a rise in their serum prostate specific antigen (PSA) level suggesting tumor recurrence (1). A variety of salvage therapeutic options exist for these patients and the choice to pursue surveillance, single focal therapy (cryotherapy, brachytherapy or salvage surgery) or combination focal and systemic (androgen deprivation) therapy depends on clinical assessment of risk and location of tumor recurrence. The ability to determine the presence of residual prostate cancer is confounded by the long time to PSA nadir (typically ≥2years), and radiation induced effects on prostate tissue histology resulting in the inability to accurately identify residual proliferating from dying cancer (2). Proliferative markers such as Ki-67 have been shown to be helpful in distinguishing viable tumor cells from inactive tumor following radiotherapy, and have been shown to be predictive of treatment efficacy (2). The goal of this study is to obtain quantitative HR-MAS spectra from snap frozen TRUS guided biopsies in men with a rising PSA and a positive MRI/^{1}H-MRSI exam after radiation therapy and to correlate metabolite concentrations with pathology and Ki-67 immunohistochemistry of the same biopsy samples in order to identify a metabolic phenotype of proliferating residual cancer.

Methods: 18 benign and 15 cancer snap frozen TRUS guided biopsies were acquired from patients with a rising PSA and a positive MRI/^{1}H-MRSI exam after radiation therapy. Samples were weighed (5.73 ± 1.24 mg) and placed into custom designed 20 µl leak proof zirconium rotors containing 3.0 µl D2O + 0.75% TSP. 1H HR-MAS data were acquired at 11.7T, 1°C, and 2,250 Hz spin rate using a Varian INOVA spectrometer, equipped with a 4 mm gHX nanoprobe. A fully relaxed (TR=6s) water pre-saturated (2s) spectra were acquired (40,000 points, SW= 20,000 Hz, and 124 transients). Metabolite concentrations were quantified using HR-QUEST, a custom version of QUEST (3), and metabolite concentrations were calculated using the ERETIC method (4). Only peaks fits having Cramer-Rao Bounds of ≤ 10% were quantified. Following HR-MAS analysis, samples were frozen in OCT, sectioned, and stained (H&E) and Ki-67 immunohistochemistry was performed. Slides were reviewed by two prostate pathologists, who determined the Gleason grade, percentage of cancer benign glandular and stromal tissue within each sample, and the % of tissue that stained positive for Ki-67 (none, < 5%, = 5%-15%, >15%).

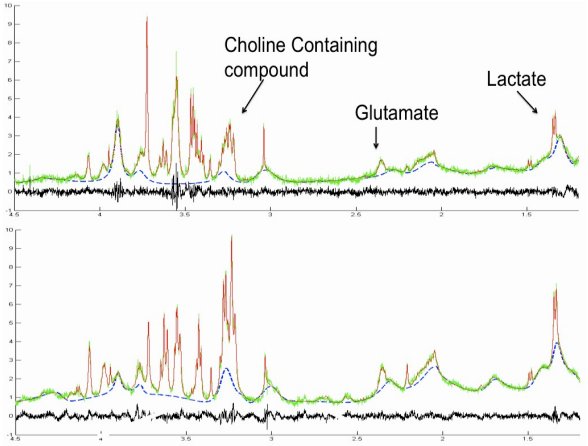

Figure 1: Representative ^{1}H HR-MAS spectra from benign (KI-67 negative) (top) and malignant (bottom, Gleason 4+4, Ki-67 – 25%) biopsy samples. The original spectrum is green, the HR-QUEST fitted spectrum is red, the estimated baseline is blue and the residual is black.

	Cases	KI67		
		Negative	<15% expression	≥15% expression
Treated normal	18	17	1	0
Treated Cancer	15	0	10	5

Table 1: Ki-67 expression for treated healthy vs treated cancer biopsy samples. p-value = 0.0006

Results: Figure 1 shows a representative ^{1}H HR-MAS spectra and the corresponding HR-QUEST fit data from benign and malignant biopsy samples after radiation therapy. As illustrated in the representative spectra, citrate and polyamine concentrations were typically reduced to the level of the noise in both benign and malignant tissues after radiation therapy.

Visually, both the choline containing metabolites and lactate appear elevated in residual cancer relative to treated benign tissues. Table 1 summarizes the Ki-67 expression results. Ki-67 expression was positive in only 1 of 18 treated healthy biopsy samples, while all 15 treated cancer biopsy samples stained positive for Ki-67, with the average cancer ki-67 staining being 13.5±11.8%. In cancer samples staining positive for Ki-67, Glycerophosphocholine (GPC) + Phosphocholine (PC), lactate and glutamate were found to significantly higher in cancer vs. benign biopsy tissues (Figure 2).

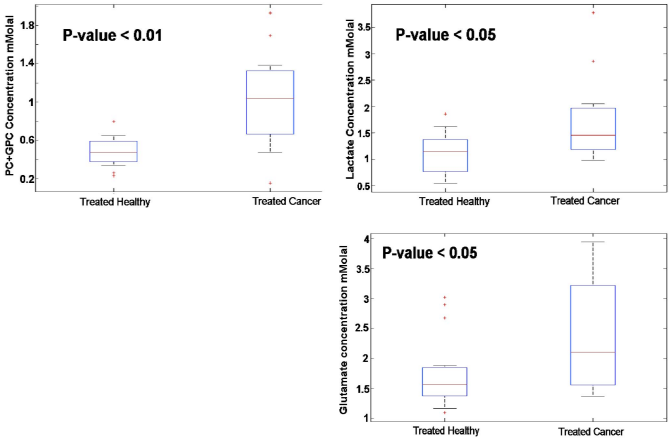

Figure 2: Significantly different metabolite concentrations (mmol/kg) between benign and malignant biopsy tissues after radiation therapy.

Discussion: In this study, we demonstrate, for the first time, that proliferating cancers after radiation therapy are associated with significantly higher levels of PC and GPC, lactate and glutamate concentrations relative to surrounding benign treated tissues. An elevation of PC and GPC in residual cancer is consistent with prior *in vivo* ^{1}H MRSI studies of patients with rising PSA after radiation therapy, in which elevated choline in multiple contiguous voxels correlated with biopsy proven residual cancer (5). While *in vivo* prostate lactate levels have not been typically monitored by ^{1}H MRSI studies of patients due to complications with overlapping lipid, fast ^{13}C MRSI studies after injection hyperpolarized [1-^{13}C] pyruvate could take advantage of elevated lactate in proliferating residual cancer after radiation therapy. Elevated hyperpolarized [1-^{13}C] lactate levels have been shown to correlate with prostate cancer grade (6), and with failed androgen deprivation therapy in a transgenic mouse model of prostate cancer (7). Significantly elevated Glutamate in residual cancer could also have significance for future hyperpolarized ^{13}C MRS studies, since the conversion of hyperpolarized glutamine to glutamate by michondrial glutaminase was successfully demonstrated in cultured human hepatoma cells (HepG2) (8).

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