SPECTROSCOPY OF THE PROSTATE
MRS - Metabolite Profiling & Metabolism

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Metabolic changes that are monitored by proton Magnetic Resonance Spectroscopic Imaging (^{1}H MRSI) have been shown to significantly improve the ability of MRI to detect and assess the location, volume and aggressiveness of cancer within the prostate, as well as improve the assessment of extracapsular spread in patients (1). It provides a non-invasive method of detecting small molecular biomarkers, specifically the metabolites choline, creatine, citrate and polyamines, within the cytosol and extracellular spaces of the prostate and is always performed in conjunction with high-resolution anatomic imaging.

Proton (^{1}H) MRSI of the prostate is typically acquired using a combination of point resolved spectroscopy (PRESS) volume localization and 3-dimensional (D) chemical shift imaging (CSI) (2). Robust acquisition of prostate ^{1}H MRSI data has required the development of very accurate volume selection and efficient outer volume suppression techniques (3-5). The resonances for citrate, choline, creatine and polyamines occur at distinct frequencies or positions in the spectrum. The areas under these signals are related to the concentration of the respective metabolites, and changes in these concentrations can be used to identify cancer with high specificity (6). In spectra taken from regions of prostate cancer the citrate and polyamines are significantly reduced or absent, while choline is elevated relative to spectra taken from surrounding healthy peripheral zone tissue. The specificity of ^{1}H MRSI for detecting, localizing and characterizing the aggressiveness of prostate cancer relies on the unique metabolism of the prostate and the specific metabolic changes that occur with the evolution and progression of this disease and following therapy, as described in the following sections.

Biochemical Rationale for Metabolic Changes Prior to Therapy: The metabolic changes observed by ^{1}H MRSI take advantage of the well documented unique metabolism of healthy prostate epithelial cells. These cells have the specialized function of synthesizing and secreting large amounts of citrate that is dramatically reduced or lost in prostate cancer (7-10). The elevation of choline-containing metabolites [phosphocholine (PC), glycerophosphocholine (GPC) and free choline (Cho)] and the over and under-expression of key enzymes in the Kennedy cycle have been associated with the progression and therapeutic response of a variety of human cancers including prostate (1). The elevation of the *in vivo* “choline” resonances which appear as one peak at 1.5T has become the most specific marker of prostate cancer prior to therapy (1) and currently the only marker of residual/recurrent disease after therapy (11). Healthy prostate epithelial cells also contain very high concentrations of polyamines, particularly spermine (1, 12, 13). Polyamines are dramatically reduced in prostate cancer due to changes in the levels of expression of genes that regulate polyamine metabolism (1, 12). The fact that choline and citrate change in opposite directions has led to the (choline + creatine)/citrate (CC/C) emerging as one of the most widely used metabolic biomarkers for detecting prostate cancer (54), and has been used in numerous publications on prostate spectroscopy. It has also been demonstrated that when the CC/C ratio was ≥ 3 standard deviations above the normal value there was minimal overlap between spectroscopic voxels from regions of cancer and healthy peripheral zone tissues (54), and elevated choline has shown a correlation with cancer grade. For more information please see three recent reviews of prostate metabolism which go into detail on it’s clinical potential and why it is currently limited by 1.5T ^{1}H MRSI, as well as how both 3T ^{1}H MRSI and hyperpolarized ^{13}C MRSI has the potential to overcome these limitations (14-16).

Biochemical Rationale for Metabolic Changes After Therapy: The spectroscopic criteria used to identify residual/recurrent prostate cancer needs to be adjusted due to a time dependent loss of prostate metabolites following therapy. For example, prostatic citrate production and secretion have been shown to be regulated by hormones (17), and an early dramatic reduction of citrate and polyamines after initiation of complete hormonal blockade has been observed by ^{1}H MRSI (18). There was slower loss of choline and creatine with increasing duration of hormone deprivation therapy (18). This loss of prostatic metabolites correlates with the presence of tissue atrophy and is considered to be a indicator of effective therapy (18). Similar time dependent reductions in prostate metabolites also occurred after radiation therapy (19, 20).

Studies have also demonstrated the ability of MRI/^{1}H MRSI to discriminate residual or recurrent prostate cancer from residual benign tissue and atrophic/necrotic tissue after cryosurgery (21-23), hormone deprivation therapy (18, 24) and radiation therapy (19, 25). These studies have relied on elevated choline to creatine as a metabolic marker for prostate cancer since polyamines and citrate tend to disappear early after therapy in both residual healthy and malignant tissues. Two published MRI/^{1}H MRSI studies have demonstrated that three or more consecutive voxels having choline/creatine >1.5 resulted in the ability to predict the presence of cancer after radiation therapy with an accuracy of ≈ 80% {Coakley, 2004 #11424;Westphalen, 2010 #16526}. The detection of residual cancer at an early stage following treatment and the ability to monitor the time course of therapeutic response would allow earlier intervention with additional therapy and provide a more quantitative assessment of therapeutic efficacy.

Hyperpolarized ^{13}C Metabolic Imaging of Prostate Cancer: While the current commercially available clinical MRI/^{1}H MRSI prostate exam relies on changes in choline, citrate, and polyamine metabolism, lactate and alanine have largely been ignored due to the difficulty of suppressing the large signals from periprostatic lipids which overlap lactate and alanine (26). Significantly higher concentrations of lactate and alanine have been found in prostate cancer biopsies compared to healthy biopsy tissue. High levels of lactate in cancer is consistent with prior studies and has been associated with increased glycolysis and cell membrane biosynthesis (27, 28). ^{18}F-2-deoxy-2-fluoro-D-glucose (FDG) positron emission tomography (PET) studies have shown high rates of glucose uptake in several human cancers and that the glucose uptake correlates directly with the aggressiveness of the disease and inversely with the patient's prognosis (29, 30). The high glucose uptake leads to increased lactate production in most tumors even though some of them have sufficient oxygen, a condition know as the Warburg Effect (31) or aerobic glycolysis (27). The increased glycolysis provides the parasitic cancer cells with an energy source that is independent of its oxygen supply, a carbon source for the biosynthesis of cell membranes that begins with lipogenesis (28), and an acid source that likely enables the cells to invade neighboring tissue (27). New hyperpolarized ^{13}C spectroscopic imaging techniques (32-34) and advances in lipid suppression and spectral edited ^{1}H spectroscopic imaging (5, 35) provide the opportunity to observe changes in lactate and alanine in clinical MRI/^{1}H MRSI exams.

^{13}C labeled substrates have recently been polarized using dynamic nuclear polarization (DNP) techniques to obtain tens of thousands fold enhancement of the ^{13}C NMR signals from the substrate as well as its metabolic products (32, 36, 37). Preliminary DNP studies in rats, rat xenograft tumors, and a transgenic mouse model of prostate cancer have demonstrated greater than 50,000-fold enhancements in the polarization of [1-^{13}C]pyruvate and its metabolic products, lactate and alanine, providing sufficient MR signal for high spatial and temporal resolution spectroscopic imaging of the metabolites (33, 34, 38). Pyruvate is ideal for these studies because the signal from C-1 carbon relaxes very slowly as a result of its long T_1 and it is at the entry point to several important energy and biosynthesis pathways. In particular, it is converted to lactate in glycolysis, to alanine for protein synthesis and/or lipogenesis, and to acetyl-CoA

and oxaloacetate to support the citric acid cycle and biosynthesis of membrane lipids. Several studies involving pre-clinical murine models of human prostate cancer have suggested that that hyperpolarized [1-^{13}C]lactate levels measured after the injection of hyperpolarized [1-^{13}C]pyruvate provide a non-invasive way to detect primary and metastatic disease and characterize the aggressiveness (histological stage) of prostate cancer (33, 39). Moreover, the presence of elevated hyperpolarized [1-^{13}C]lactate after injection of hyperpolarized [1-^{13}C]pyruvate was recently demonstrated in living human prostate cancer tissues in a NMR compatible tissue culture bioreactor(40). Additionally, after androgen deprivation therapy in the TRAMP model, significant decreases in [1-^{13}C]pyruvate uptake, [1-^{13}C]pyruvate to [1-^{13}C]lactate flux, and in perfusion based on hyperpolarized ^{13}C urea were observed and correlated with therapeutic response (41, 42).

Although pyruvate has shown clear value for metabolic imaging in initial pre-clinical prostate cancer studies, recent studies have shown the potential of other substrates including lactate(43), [2-^{13}C]fructose(44), ^{13}C sodium bicarbonate(45), and ^{13}C urea(42) to detect and characterize prostate cancer prior to and after therapy. A new method for the simultaneous polarization of [1-^{13}C]pyruvate, ^{13}C sodium bicarbonate, [1,4-^{13}C]fumarate, and ^{13}C urea with high levels of solution polarization and T_1 values similar to those recorded for the individual hyperpolairzed probes. This combination of agents was successfully injected into the TRAMP model providing the potential for measuring pH, metabolism, necrosis and perfusion in a single MR acquisition (46)**.** There is a clear path to the clinical translation of hyperpolarized ^{13}C metabolic imaging. Specifically, hyperpolarized [1-^{13}C]pyruvate has IND approval for initial use in prostate cancer patients, and the phase 1 clinical trial is underway with very encouraging initial results.

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