Quantification of Soluble Metabolites in the Prostate: Applying LCModel to HRMAS NMR Data from Normal and Cancerous Prostate Tissue

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Introduction: Detection of pre-metastatic prostate cancer enables curative intervention to be applied to an otherwise lethal and common disease. An automated, quantitative and objective method for correlating lesion properties with patient prognosis would be valuable in assessing such patients. The combination of MRI with MRSI has yielded encouraging results over MRI alone, and higher field clinical magnets further enhance metabolite determination. In order to elucidate the differences in patterns of metabolites in normal versus cancer tissues, we have studied prostate tissue samples *ex vivo* at high field strength (11.74T). The non-destructive nature of high resolution magic angle spinning (HR-MAS) NMR spectroscopy has allowed histologic analysis of the same tissue sample. In this study, we describe the method required to adapt Provencher's LCModel software for quantifying prostate metabolite levels from HR-MAS NMR spectra and report upon differences that were observed between healthy and cancerous prostate tissue.

Methods: Five to fifteen milligrams of prostate tissue from surgical prostectomies were placed in a zirconium rotor with 3 microliters of D2O with TSP, spun at 2250 Hz at the magic angle (57.4º), and data was acquired using a presat pulse sequence on a 500 MHz NMR instrument (Varian, Palo Alto, CA) equipped with a magic angle spinning nanoprobe. To analyze this data, a library of 18 of the most common soluble metabolites in the prostate (alanine, choline, citrate, creatine, ethanolamine, glycerol phosphorylcholine, glucose, glutamate, glutamine, lactate, myo-inositol, phosphorylcholine, phophoethanolamine, putrascene, scyllo-inositol, spermadine, spermine, and taurine) was created. Each of these chemicals was prepared at pH 7.2, 5-20mM, 50% D2O, with quantitative TSP in a phosphate buffered solution (GPC, PC and spermine were not buffered) and analyzed in the inverse probe of the same 500 MHz NMR spectrometer. This library formed the basis set for Provencher's LCModel algorithm (1). Additionally the 2.2 ppm acetone peak, as well as lipid peaks at 1.31, 1.60, 2.05 and 2.27 ppm were simulated as single lorenzian-gausian peaks and added as members of the basis set. Sixteen cancer and eleven healthy samples were analyzed with LCModel (runtime approximately 5 minutes per spectrum on a SunBlade 2000 workstation). Several LCModel parameter modifications were needed to fit this prostate data set: a software version to accommodate a 6000 point window of analysis; a change in the primary spectral referencing from choline, creatine and NAA to creatine and lactate; a greater freedom in upfield translation of the data; and a preprocessing step in order to determine the zero order phase of the data. The LCModel concentration data that were output from each spectrum were scaled with the level of creatine, and the two groups were compared with a two-tailed student's t test.

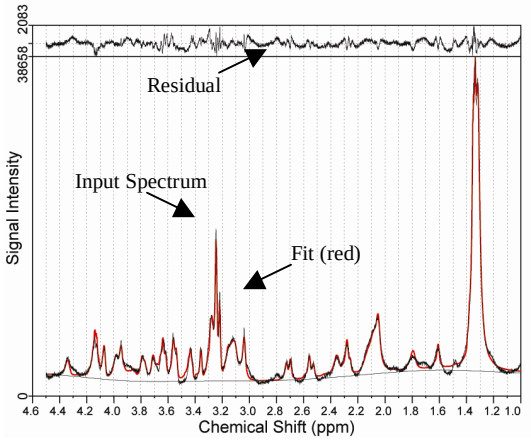

Figure 1: LCModel fit of HR-MAS NMR prostate caner spectra.

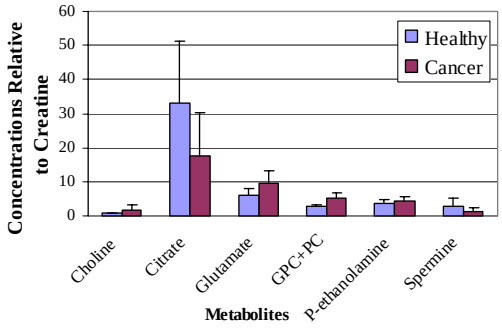

Figure 2: Five metabolites show significant changes in prostate cancer. A 3.97 ppm peak potentially corresponding to phosphoethanolamine also shows a change.

Discussion: Five metabolites show significant differences between cancer and normal tissue: the sum glycerophosphorlcholine (GPC) + phosphorylcholine (PC), glutamate and choline all rise with cancerous transformation, while citrate and spermine fall (p values 0.03, 0.001, 0.01, 0.02, 0.03 respectively). While choline's 3.19 ppm peak can be determined from the 3.24 ppm GPC/PC region, in this analysis GPC and PC could not be clearly distinguished. Citrate and spermine are clearly detectable at this resolution. Glutamate can be distinguished from glutamine as their respective peaks at 2.34 ppm and 2.44 ppm do not overlap. A peak at 3.97 ppm that corresponds to phosphoethanolamine also shows a significant decrease upon cancerous transformation (p=0.03). These results correspond with results published elsewhere (2).

Conclusions: The LCModel software package can be adapted to estimate the chemical composition of prostate tissue HR-MAS NMR data. The results of our analysis show significant differences between normal and cancerous tissue and may allow the determination of tissue type based on their metabolic profile. Performing *in vivo* studies is complex in terms of logistics, in addition to being time consuming and expensive. *Ex vivo* analysis of tissue characteristics are therefore critical for focusing future *in vivo* studies on the most appropriate metabolic markers.

References: (1) Provencher SW. Magn Reson Med 1993; 30(6):672-92.
(2) Swanson MG, Vigneron DB, *et al*. Magn Reson Med. 2003 Nov; 50(5):944-54.

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