

Identification and Analysis of metabolic biomarkers for predicting prostate cancer grades using ^1H HR-MAS Spectroscopy of biopsy tissues

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Introduction: Statistics indicate that less than 1 percent of men diagnosed with prostate cancers develop clinically significant disease. However, there is no cure for prostate cancer once it becomes metastatic and the average survival of patients with metastatic disease is less than two years. Currently there is no reliable way of predicting which cancers will be indolent versus those that will metastasize and result in death (1). Clinically, patients having a dominate Gleason 4 pathologic pattern are considered to have a higher risk of developing metastatic disease and are treated more aggressively. Prior HR-MAS studies of human biopsy and surgical samples have demonstrated significant differences in the metabolic patterns between healthy and cancer tissue (2, 3). The aim of this study was to determine using HR-MAS spectroscopy of snap frozen biopsies whether there are metabolic differences between low and high pathologic grade prostate cancers.

Methods: 26 TRUS guided biopsies were acquired from 22 patients, snap frozen on dry ice, and stored at -80°C until use. Samples were weighed (5.11 ± 1.03 mg) and placed into custom designed 20 μl leak proof zirconium rotors containing 3.0 μl D₂O + 0.75% TSP. 1D spectra were acquired on these samples using high resolution magic angle spinning (HR-MAS) spectroscopy. The concentrations were quantified using HR-QUEST, a custom version of QUEST (4) adapted for analysis of short-echo time HR-MAS spectra containing 40,000 points. Basis set spectra of 19 metabolites were collected in solution and incorporated into the HR-QUEST fitting routine.

Peaks from known macromolecules and unidentified compounds were also included as part of the basis set. HR-QUEST estimated the background signal using an HLSVD algorithm and iterated between fitting the metabolites and modeling the background 6 times (4). The concentrations of 13 metabolites were calculated relative to the peak area of the ERETIC signal (5). Following HR-MAS analysis, samples were frozen in OCT, sectioned, and stained (H&E). Slides were reviewed by two pathologists, who determined the Gleason grade and percentage of cancer tissue within the sample.

Results: For analysis, the cancer biopsy samples were separated into two groups based on their Gleason scores – samples with a Gleason score of 3+3 or less were categorized into as low grade cancer whereas samples with Gleason scores of $\geq 3+4$ were categorized as high grade cancer. Figure 1 shows a representative histology, ^1H HR-MAS spectra and HR-QUEST spectral fit from a low-grade biopsy tissue sample (left) and a high-grade biopsy tissue sample (right). Figure 2 summarizes the metabolites whose concentrations differed the most between the low grade and high grade prostate cancer tissues. Glycerophosphocholine (GPC), phosphoethanolamine (PE), free choline, glutamate and alanine were significantly higher in high grade cancer vs. low grade cancer tissues. PC and lactate followed a similar trend, increasing with cancer grade but, were not statistically different. Citrate, creatine, polyamines (putrescence, spermine and spermidine) were not significant between low and high grade prostate cancer.

Discussion and Conclusion: Previous studies have shown that Choline and ethanolamine-containing phospholipid metabolites including GPC, PC, and PE and GPE were significantly increased in prostate cancer relative to benign prostate tissues (3). In this study, we demonstrate that GPC, free choline and PE increased significantly in higher pathologic grade tumors. Lactate and alanine have also been found to be significantly elevated in prostate cancer as compared to benign prostate tissues (2). Additionally, ^{13}C MRSI studies after injection of hyperpolarized [^{13}C] pyruvate in a transgenic mouse model of prostate cancer (TRAMP model) demonstrated a significant pathologic grade-dependent increase in the production of hyperpolarized lactate and alanine (7). In this study there was a significant grade-depend increase in alanine but the increase in lactate was not found to be significant. Lactate could not be determined in several (2 samples) of the biopsy samples due to the presence of large lipid peaks and the lack of significance could be due to the small sample size. Glutamate was also observed to be significantly elevated in high grade cancer. This has significance for future hyperpolarized ^{13}C MRS studies, since the conversion of hyperpolarized glutamine to glutamate by mitochondrial glutaminase was successfully demonstrated in cultured human hepatoma cells (HepG2) (8). While it has been previously shown that citrate and polyamines concentrations significantly decreased in prostate cancer, there wasn't a grade dependent difference in the decrease of these metabolites. This may well be due to the fact that the cancer samples contained a variable mixture of benign glandular and stromal tissues, and

the stromal tissues contain low concentrations of citrate and polyamines. Additional studies involving increased numbers of cancer biopsies, particularly high grade, and combining all of the metabolic changes using multivariate approaches will be necessary to determine the true prognostic value of metabolic changes to predict aggressive prostate cancer.

Reference: 1) Kurhanewicz J. et al. (2002) *Magn Reson Med* 16:451-463; 2) Tessem M. et al. (2008) *Magn Reson Med* 60:510-516; 3) Swanson M. et al. (2006) *Magn Reson Med* 55:1257-1264; 4) Ratiney H. et al. (2005) *NMR Biomed* 18(1):1-13; 5) Albers M. et al. (2008) *Magn Reson Med* "in press"; 6) Zhang V. et al. (2008) 16th Scientific Meeting and Exhibition of ISMRM, Toronto, Ontario 3-9 May 2008 p3803; 7) Albers M. et al. (2008) *Cancer Res.* 68(20):8607-15; 8) Gallapther et al. (2008) *Magn Reson Med* 60:253-267

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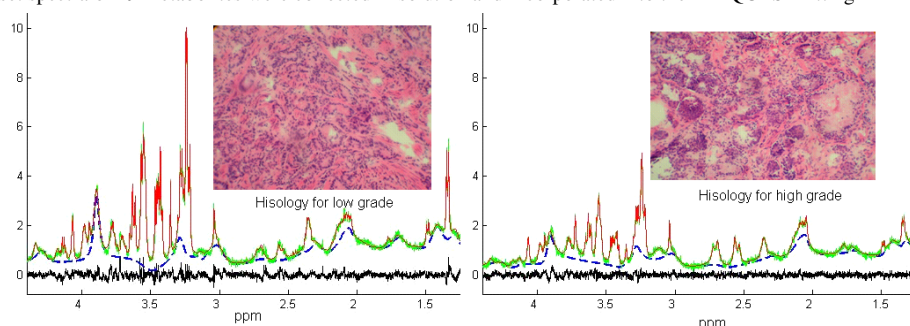


Figure 1: Low grade cancer biopsy sample (left) and high grade cancer sample (right) showing the original spectrum (green), the HR-QUEST fitted spectrum (red), the estimated baseline (blue) and the residual (black)

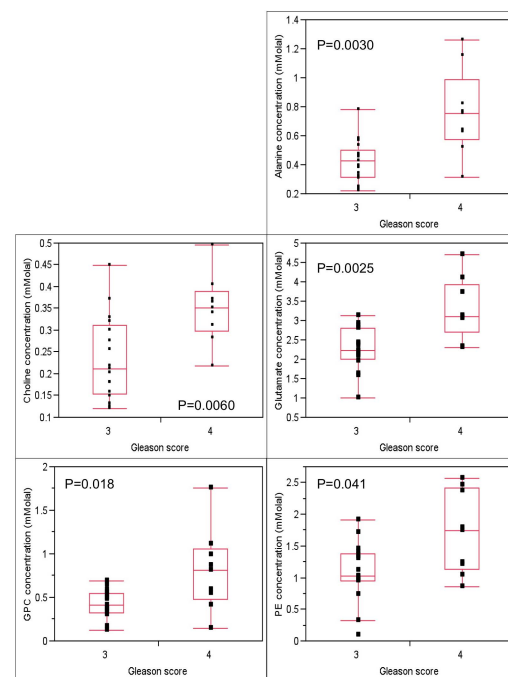


Figure 2: Calculated metabolite concentration (mmol/kg) for low grade cancer (Gleason score 3+3) and high grade cancer (Gleason score $\geq 3+4$)