Quantification of Metabolites in HR-MAS Spectra of Human Prostate Biopsy Tissues Using ERETIC and the QUEST Algorithm

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Introduction

High resolution magic angle spinning (HR-MAS) spectroscopy of transrectal ultrasound (TRUS) guided prostate biopsy tissues provides a better metabolic snapshot of in vivo metabolism than post-surgical tissue and is useful for identifying markers of response to hormone deprivation, radiation, and emerging therapies. However, accurate quantification of HR-MAS spectra of prostate biopsy tissue is challenging due to overlapping metabolite peaks, contaminants, and broad background components caused by macromolecules and lipids. While previous studies often relied on relative ratios of metabolites, absolute concentrations are ideal because they facilitate direct comparisons and report how individual metabolites change independently of each other with disease state. The Electronic Reference To access In vivo Concentrations (ERETIC) (1) method is a robust synthetic quantification standard that has recently been used to measure absolute metabolite concentrations in tissue. ERETIC is preferable to internal chemical standards (e.g., TSP), which can leak during rotor assembly or bind to macromolecules. QUEST is a nonlinear minimization algorithm, which utilizes extensive prior knowledge to automatically quantify signals in the time domain based on semi-parametric quantum estimation (2). QUEST requires minimal user input and therefore provides fast and automated spectral quantification without the potential for human bias. In this study, absolute concentrations of 19 metabolites were determined in HR-MAS spectra of prostate biopsy tissues using a combination of ERETIC and QUEST and correlated with the histopathologic findings of the same tissues.

Methods

106 TRUS guided biopsies were acquired from 69 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 D_2O + 0.75% TSP. ^{1}H HR-MAS data were acquired at 11.7T (500 MHz for ^{1}H), 1°C, and 2,250 Hz spin rate using a Varian INOVA spectrometer, equipped with a 4 mm gHX nanoprobe. Quantitative 1D spectra were acquired with 2s presaturation, 2s acquisition (TR = 4s), 40,000 points, 20,000 Hz spectral width, and 128 transients, time = 8 min 40s. The ERETIC signal was generated at a frequency of –0.5 ppm. Data were quantified using a custom version of QUEST 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 QUEST fitting routine. Peaks from known macromolecules and topical anesthetics (contaminants) were also included as part of the basis set. Initial parameters (dampings and frequency shifts) for some metabolites were readjusted to resemble metabolite profiles in tissue. The custom version of QUEST estimated the macromolecule signals using an HLSVD algorithm and iterated between fitting the metabolites and modeling the macromolecules 12 times. Finally, concentrations were calculated relative to the peak area of the ERETIC signal. Following HR-MAS analysis, samples were frozen in OCT, sectioned, and stained (H&E). Slides were reviewed by two pathologists, who determined the percentage of healthy glandular (HG), stomal (HS), and prostate cancer (CA) tissue. A non-parametric pairwise comparison using the Wilcoxon test was applied to all metabolites.

Results

Figure 1a shows a ^{1}H HR-MAS spectrum and QUEST fit results of a healthy glandular prostate biopsy tissue sample. Also shown in the inset is the highly overlapping choline to creatine region from 3.0 to 3.4 ppm. The mean error between original spectra and estimated spectra over the region of interest (0.5ppm to 4.5 ppm) was 0.322%±0.703%. Table 1 lists concentrations of metabolites which were significantly different between healthy glandular (N=38), stromal (N=50), and prostate cancer (N=18) tissues. Phosphocholine (PC), glycerophosphocholine (GPC), glutamate, lactate, and alanine were significantly higher in cancer vs. healthy glandular and healthy stromal tissue, while polyamines were significantly lower in cancer and stromal vs. healthy glandular tissues. phosphoethanolamine (PE) was significantly higher in cancer vs. healthy glandular. Choline and glutamine were significantly higher in stromal tissue vs. cancer. Citrate was significantly higher in healthy glandular vs. stromal but not vs. cancer tissue. Creatine, taurine, sInositol, and glycine were not significantly different between tissue types.

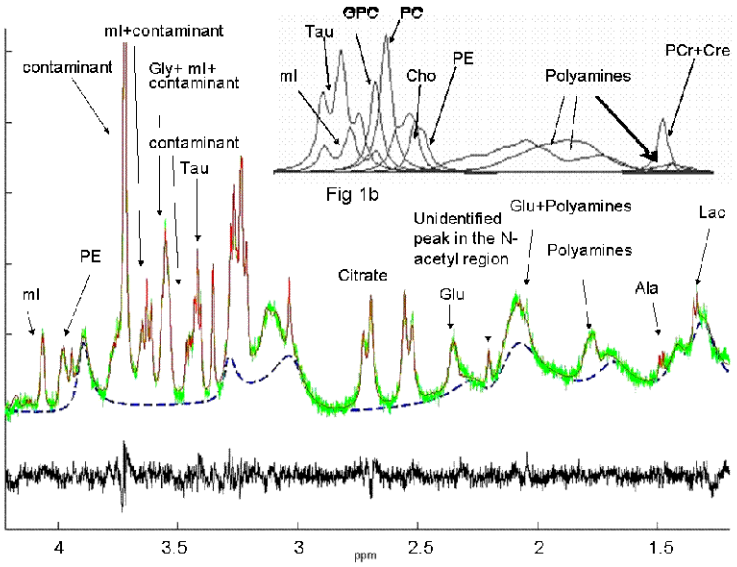

Figure1: a) healthy predominantly glandular prostate biopsy sample showing the original spectrum (green), the QUEST fitted spectrum (red), the estimated baseline (blue) and the residual (black); b) Overlapping choline to creatine region (3.0-3.4ppm).

Metabolites	HG	HS	CA	P value		
				HG vs. CA	HS vs. CA	HG vs. HS
PC	0.364±0.181	0.445±0.225	0.766±0.382	0.0075	0.0381	0.0006
GPC	0.499±0.189	0.332±0.169	0.990±0.619	0.0033	<0.0003	0.0063
Choline	0.308±0.157	0.237±0.131	0.410±0.177	0.1344	0.0042	0.1989
PE	1.87±0.618	1.98±0.868	2.57±0.812	0.0159	0.1332	1.00
Lactate	1.14±0.622	0.999±0.622	1.70±0.589	0.0018	0.0006	1.00
Alanine	0.438±0.154	0.368±0.143	0.657±0.271	0.0024	0.0003	1.00
Citrate	9.41±4.07	5.06±3.73	6.85±2.68	0.3675	0.186	0.0015
Polyamines	1.14±0.896	0.432±0.570	0.543±0.627	0.0405	1.00	0.0036
Glutamate	3.06±0.878	2.81±0.829	3.96±1.14	0.0078	0.0033	1.00
Glutamine	0.506±0.614	0.283±0.277	0.517±0.422	0.5856	0.0489	1.00

Table1: Calculated metabolite concentrations (mmol/kg) for healthy glandular, stromal, and prostate cancer biopsy tissues.

Discussion and Conclusions

The combination of QUEST and ERETIC provided robust quantification of absolute metabolite concentrations in prostate biopsy samples even in the presence of contaminants from macromolecules and topical anesthetics. Inclusion of macromolecule peaks in prior knowledge stabilized the background spectra and increased the consistency of metabolite estimates, whereas failure to include macromolecules in the basis set would result in an over-estimation of metabolite concentrations. Several metabolites were significantly different between healthy glandular, stromal, and prostate cancer biopsy tissues, consistent with previous surgical studies (3). Future studies will incorporate multivariate analysis to examine all metabolite concentrations simultaneously to predict the presence, percentage, and grade of prostate cancer in the biopsy.

References

1) Ziarelli F and Caldarelli S. (2006) Solid State Nucl Magn Reson 29: 214-218; 2) Ratiney H, et al. (2005) NMR Biomed 18(1): 1-13; 3) Swanson MG et al. (2006) Magn Reson Med 55(6): 1257-1264.