1D High Resolution Magic Angle Spinning Total Correlation Spectroscopy of Prostate Cancer Tissues

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Introduction

Spectral overlap is a major problem in 1D high resolution magic angle spinning (HR-MAS) spectroscopy studies of prostate cancer tissues. Specifically, the phosphocholine (PC) and glycerophosphocholine (GPC) singlets cannot be resolved from each other at 11.7T, ethanolamine (Eth), phosphoethanolamine (PE), and glycerophosphoethanolamine (GPE) resonate underneath the highly congested choline region (3.05 to 3.35 ppm), and lactate (Lac) and alanine (Ala) are difficult to quantify in the presence of lipids. Although these metabolites can be resolved and quantified using 2D total correlation spectroscopy (TOCSY) (1-3), the time required for 2D frequency encoding can severely degrade the pathologic and RNA integrity of the tissue. In this study, a selective excitation 1D TOCSY sequence was developed to detect 1) the $CH_2 \rightarrow CH_2$ responses of Eth, PE, and GPE, 2) the side chain $CH_2 \rightarrow CH_2$ responses of PC and GPC, and 3) the $CH_3 \rightarrow CH$ responses of Lac and Ala in healthy glandular and prostate cancer tissues.

Materials and Methods

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. Studies were carried out on phantom solutions containing approximate physiological concentrations of prostate metabolites and on post-surgical healthy glandular (n=15) and prostate cancer (n=11) tissues. A quantitative 1D "presat" spectrum was acquired on each sample prior to starting the TOCSY experiments. A rotor-synchronized double pulse field gradient spin echo (DPFGSE) sequence (4) was used to selectively excite three regions [Figure 1 top: B) 4.12–4.40, C) 3.05–3.35, and D) 1.25–1.55 ppm] and suppress unexcited regions, after which a rotor-synchronized WURST-8 adiabatic mixing train was used to transfer magnetization to the $^3J_{HH}$ coupled resonances of B) PC (4.18→3.62 ppm) and GPC (4.34→3.71 ppm), C) Eth (3.15→3.83 ppm), PE (3.22→3.99 ppm), and GPE (3.30→4.12 ppm), and D) Lac (1.33→4.14 ppm) and Ala (1.49→3.79 ppm). Mixing times (τ_m) were arrayed from 4.44 to 133.2 ms to study the $^3J_{HH}$ modulation behavior and determine the τ_m that yielded the maximum signal intensity for each spin system [B) τ_m = 57.72 ms, C) τ_m = 48.84 ms, D) τ_m = 66.66 ms]. Following optimization, 1D TOCSY data were acquired with 1s presat, AT = 0.5s, SW = 20,000 Hz, NP = 10,000 (2Hz/pt), NT = 512 (C and D) or 1024 (B), total experiment time ≈ 13.5 (C and D) or 27 min (B). Following HR-MAS, tissues underwent routine H&E staining and were evaluated by a pathologist. Data were processed offline using ACD 8.0 1D NMR processor (ACD/Labs, Toronto). Data were apodized with a shifted Gaussian function, zero-filled to 32K data points, Fourier transformed, phased and frequency corrected, and quantified using Lorentzian-Gaussian peak fitting. PE/Eth, GPE/Eth, PC/GPC, and Lac/Ala ratios were calculated and compared between groups using a Student's t-test.

Results

Figure 1A (top) shows a representative 1D HR-MAS spectrum of Gleason 3+3 prostate cancer tissue; dashed lines indicate the selectively excited regions for B) PC and GPC, C) Eth, PE, and GPE, and D) Lac and Ala. Figure 1 (bottom) shows the corresponding responses detected after isotropic mixing. PE levels were typically much higher than Eth and GPE levels, while GPC levels were typically much higher than PC levels, regardless of tissue type. PE/Eth (7.09±7.94 vs. 1.47±1.57, p=0.046), GPE/Eth (1.86±1.19 vs. 0.74±0.52, p=0.027), and Lac/Ala (10.4±4.4 vs. 5.6±2.0, p=0.037) ratios were all significantly higher in prostate cancer vs. healthy glandular tissues. PC/GPC ratios were also higher in prostate cancer vs. healthy glandular tissues (0.36±0.17 vs. 0.23±0.035), but the difference did not reach statistical significance (p=0.099).

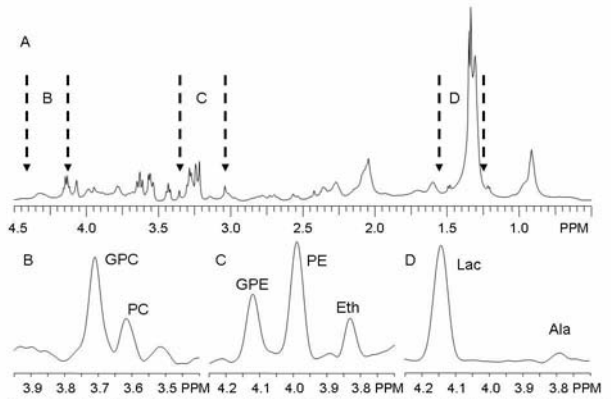

Figure 1. Top: A) 1D HR-MAS spectrum of Gleason 3+3 prostate cancer tissue; dashed lines show the selectively excited regions of B) PC and GPC, C) Eth, PE, and GPE, and D) Lac and Ala. Bottom: B,C,and D) corresponding signals detected using 1D TOCSY.

Discussion and Conclusions

There is much interest in exploiting the specific choline and ethanolamine containing metabolites as markers of cellular proliferation (PC and PE) and apoptosis (GPC and GPE), and Lac and Ala as markers of increased glycolysis, in MRS studies of cancer. In this study, a selective excitation 1D TOCSY sequence was developed to detect each of these metabolites while carefully avoiding the excitation and transfer of unwanted signals (e.g., myo-inositol). Ethanolamine, PE, and GPE, and Lac and Ala could be routinely detected and quantified in 13.5 min; however, due to their lower concentrations, 27 min acquisition times were needed to detect PC and GPC routinely. This study demonstrates that PC/GPC, PE/Eth, GPE/Eth, and Lac/Ala ratios are elevated in prostate cancer and that the ethanolamine containing metabolites, particularly PE, are major contributors to the composite choline peak observed in vivo. The selective excitation 1D TOCSY sequence developed in this study allows the detection of PC, GPC, PE, and GPE without the need to perform ^{31}P spectroscopy, and could be translated to in vivo MRS studies of prostate cancer patients at high field (≥3T).

References

1) Morvan D et al, Magn Reson Med 2003;49:241-248, 2) Swanson MG et al, Proc. ISMRM 2004;12:383, 3) Zektzer AS et al, Magn Reson Med 2005;53:41-48, 4) Kover KE et al, J Magn Reson 1998; 130:162-168.