Quantification of choline and ethanolamine containing phospholipids in healthy and malignant prostate tissues

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INTRODUCTION:

Choline and ethanolamine metabolites are major cytosolic precursors and degradation products of phospholipid membrane assembly and catabolism(1). Currently, there is interest in utilizing the concentrations of individual choline and ethanolamine metabolites to assess cancer aggressiveness and monitor therapy because of the information they contain about cellular proliferation, apoptosis, and expression of key enzymes. In traditional one-dimensional spectroscopy it is difficult to quantify the choline and ethanolamine containing compounds due to overlapping resonances. However, ethanolamine (Eth), phosphoethanolamine (PE), glycerophosphoethanolamine (GPE), phosphocholine (PC), and glycerophosphocholine (GPC) can be resolved from overlapping resonances using *ex vivo* 2D high-resolution magic angle spinning (HR-MAS)total correlation spectroscopy (TOCSY). The purpose of this study was to use a quantitative HR-MAS TOCSY approach to quantify changes in choline and ethanolamine metabolites between healthy and malignant prostate tissues acquired at surgery.

MATERIALS AND METHODS:

Forty prostate tissues were removed from 22 biopsy proven prostate cancer patients at surgery and snap frozen on dry ice. Spectral data were acquired at 11.7T (500 MHz), 1°C, and a 2,250 Hz spin rate using a Varian INOVA spectrometer equipped with a 4 mm gHX nanoprobe and a custom designed 35 ul rotor. Quantitative adiabatic TOCSY spectra were acquired for tissue samples (mean = 17.36 ± 3.71 mg) with D_2O+0.75% TSP as a standard (Sigma-Aldrich, St. Louis, MO). Adiabatic (WURST-8) TOCSY spectra were acquired with a 2s HOD presaturation/relaxation delay, 0.2s acquisition time, nt = 24, ni = 64, and tm = 40 ms (2). Quantification of 2D cross peaks for Eth, PE, GPE, PC, and GPC required calibration with standard solutions of metabolites to optimize the mixing time for maximum CH_2-CH_2 cross peak detection and correct the cross peak volumes relative to 1D spectral areas. Choline was not quantified due to overlap with myo-inositol. Following HR-MAS, tissue samples were frozen in OCT, sectioned, H&E stained and examined by two pathologists. Pathologists determined that 29 samples were healthy and 11 samples contained cancer (≥ 20% of the sample, Gleason grade N=1(4+4), N= 2 (3+4) and N=8 (3+3)). Concentrations (mM) were statistically compared using a Student's T-test, with $p<0.05$ considered significant.

RESULTS:

Figure 1 shows 2D TOCSY spectra of (a) healthy and (b) malignant prostate tissues demonstrating representative changes in the CH_2-CH_2 cross peaks of the choline and ethanolamine containing metabolites. Overall, the concentrations of ethanolamine containing compounds were significantly higher than choline containing compounds in both healthy and malignant tissues (Figure 2). All phosphorylated choline and ethanolamine metabolites increased in cancer relative to healthy tissue. The significant increase in [PC] (≈ 6 fold) in cancer was more dramatic than the increase in [GPC] (≈2 fold) yielding a dramatically increased [PC]/[GPC] ratio in cancer (3.47±4.48) relative to healthy (0.034±0.10). Moreover, PC cross peaks were absent in a majority (24/29) of the healthy tissue samples and present in the majority (8/11) of the cancer samples. [PE] was also significantly increased (≈1.3 fold) but less than PC resulting in minimum overlap of the PC/PE ratio in healthy versus malignant tissues (Figure 3).

Fig. 1

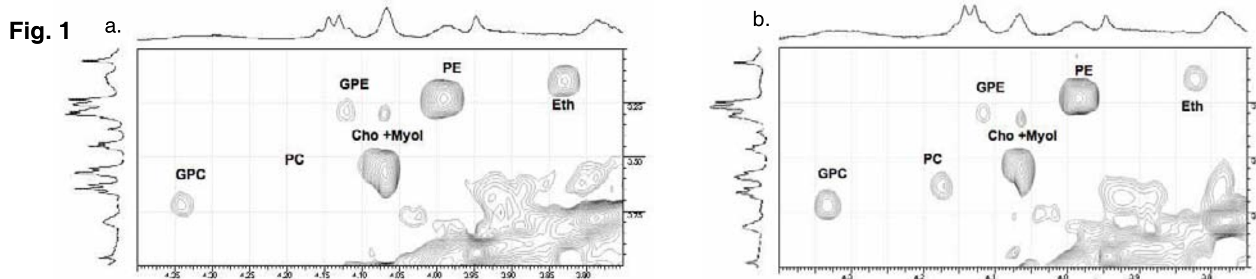


Fig. 2

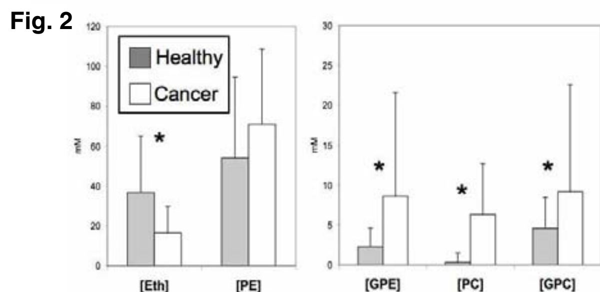
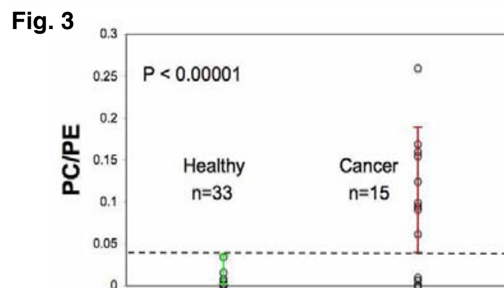


Fig. 3



DISCUSSION AND CONCLUSIONS:

In this study, concentrations of choline and ethanolamine phospholipid intermediates were determined in intact healthy and malignant prostate tissues using an adiabatic HR-MAS TOCSY sequence. Similar to prior ^{31}P studies of prostate cancer, there were higher levels of ethanolamine than choline containing metabolites in healthy tissue and prostate cancer (3). Consistent with elevated total choline being a biomarker of prostate cancer in in vivo MRSI studies of prostate cancer, we observed an increase in all phosphorylated phospholipid intermediates (4). Also consistent with the literature, the most dramatic change in prostate cancer was an elevation of [PC] resulting in large increases in the [PC]/[GPC] (5) and [PC]/[PE] (1) ratios. The large variability of [PC]/[GPC] ratio resulted in substantial overlap of individual [PC]/[GPC] ratios between healthy and cancer tissues, while there was minimal overlap of the [PC]/[PE] ratio. Ongoing studies involve determining whether there are grade dependent changes in concentrations of phospholipid intermediates in a larger patient cohort.

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ACKNOWLEDGEMENTS: National Health Institute, R01-CA102751, K01-CA96618, and UC Discovery grants LSIT01-10107 and ITL-BIO04-10148.