Correlation of Choline-Containing Metabolites with Gene Expression of Kennedy Cycle Enzymes

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Introduction

The elevation of choline-containing metabolites [phosphocholine (PC), glycerophosphocholine (GPC) and free choline (Cho)] and the over and under-expression of key enzymes in the Kennedy cycle have been associated with the progression and therapeutic response of a variety of human cancers (breast, prostate, and brain) (1). Additionally in prior HR-MAS studies of prostate biopsy tissues we have observed that although total choline is elevated in prostate cancer, the concentration of free choline tends to be higher, and GPC and PC lower than that observed for breast cancers (2). These findings warrant further investigation since many lower pathologic grade (Gleason 3+3) prostate cancers never metastasize. The aim of this study is to better understand the relationship between the levels of Cho, PC, and GPC in normal and cancerous prostate tissues and the expression of key enzymes in the Kennedy cycle.

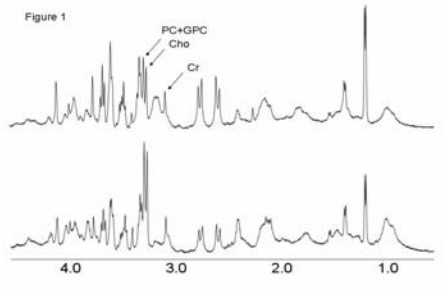
Methods

Sixteen transrectal ultrasound (TRUS) guided biopsies (n=10 healthy, n=6 Gleason 3+3 cancers) were harvested from 16 patients. All tissues underwent HR-MAS spectroscopic analysis prior to pathologic and genetic analysis of the same tissue. At HR-MAS analysis, samples were weighed to 0.01 mg (mean biopsy wt: 5.07 ± 0.95 mg) and then transferred to a 4 mm (O.D.) cell containing 3.0 µl of D_2O+0.75% TSP (Sigma-Aldrich). Data were acquired at 1°C and a 2,250 Hz spin rate using an 11.7 T (500 MHz for ^{1}H) Varian INOVA NMR spectrometer and gHX nanoprobe. An automated routine was used to shim on the FID (<10 min), after which a 1D "presat" spectrum (NT = 64 to 128, TR = 6s, AT = 2s, SW = 20kHz, NP = 40k complex) was acquired (~6.5 to 13 min). Following HR-MAS, tissues were placed in cryo-molds, frozen in OCT (Sakura), and stored at -80°C until sectioning. The mean total time from beginning of sample preparation to refreezing was 28 ± 11 min (range: 12-50 min). It has previously been demonstrated that no significant loss of pathologic or RNA integrity occurs within this time frame (3). ^{1}H HR-MAS data were processed offline using ACD/Labs 1D NMR processor. Each peak in the range from 3.0 ppm to 3.4 ppm was quantified using Lorentzian-Gaussian peak fitting, and resulting peak areas for PC, GPC, and Cho, and creatine (Cr) were determined. GPC+PC/Cr, Cho/Cr, and GPC+PC/Cho peak area ratios were statistically compared using a Student's t-test.

Histopathologic and Gene Expression Analysis: For each sample, sixteen 14 µm frozen sections were placed on individual slides, and every 8th slide was stained with H&E. The remaining slides were used for genetic analysis as described at www.microarrays.org. Briefly, extracted total RNA underwent two rounds of linear amplification, conversion to cDNA, coupling to Cy3 or Cy5 dyes, and hybridization to cDNA spotted microarrays representing 41,000 independent genes. The arrays were scanned and analyzed using Cluster and TreeView (Eisen), and GenePix Pro 3.0 (Axon). Statistical analysis using Significance Analysis of Microarrays (SAM) (false discovery rate of 10%) was used to identify significantly under/over-expressed genes of key enzymes in the Kennedy cycle between cancer and healthy.

Results

Figure 1 shows representative 1D ^{1}H HR-MAS spectra of healthy predominantly glandular (top) and Gleason 3+3 prostate cancer biopsy tissues (bottom). As illustrated in this example, significantly higher levels of Cho and GPC+PC were observed in prostate cancer vs. healthy glandular tissue. Specifically, as shown in Figure 2, the peak area ratios of GPC+PC/Cr (3.8 ± 1.1 vs. 1.5 ± 0.3) and Cho/Cr (3.3 ± 1.7 vs. 1.6 ± 0.7) were significantly higher in cancer than in healthy tissue. However, no difference was found between the GPC+PC/Cho ratio in cancer (1.3 ± 0.03) vs. healthy tissue (1.3 ± 0.7). Table 1 lists only those Kennedy cycle genes that were significantly over and under-expressed in prostate cancer (Gleason 3+3) vs. healthy glandular tissue. Of particular interest, choline kinase, the enzyme that catalyzes conversion of free Cho to PC, was not significantly over-expressed in prostate cancer (cancer mean expression=0.40, normal mean expression=0.53). Additionally, phospholipase D1, which is responsible for the regeneration of Cho from phosphatidylcholine, was found to be the most over-expressed Kennedy enzyme in prostate cancer. The choline transporter CDW92 antigen was also found to be over-expressed. Furthermore, phospholipase C was found to be the most under-expressed Kennedy enzyme.



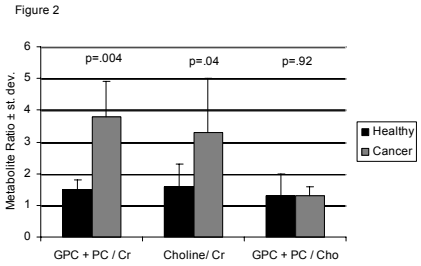


Table 1

Accession#	Gene Name	Overexpressed Genes	Fold Inc.
AA626014	Phospholipase D1, phophatidylcholine-specific		2.14
AA703582	CDW92 antigen		1.64

Accession#	Gene Name	Underexpressed Genes	Fold Dec.
AA411387	Phospholipase C-like 1		4.22
AA460363	Glycerophosphodiester phosphodiesterase domain 3		2.67
AA446893	Phosphate cytidylyltransferase 1, choline, alpha isoform		1.55
T61323	Phospholipase A2, group IIA (platelets, synovial fluid)		1.47
N32019	Choline/ethanolamine phosphotransferase 1		1.43

Discussion and Conclusions

Previous studies involving breast cancer cell lines have shown that the over-expression of choline kinase and phospholipase C, and the underexpression of phospholipase D, leads to elevated PC levels relative to Cho (1). In the current study, the increased Cho uptake (CDW92 antigen), regeneration of Cho through the over-expression of phospholipase D1, and the lack of over-expression of choline kinase all may account for the relatively higher levels of Cho and lower levels of PC+GPC observed in prostate cancer relative to breast cancers. Although this study focused on Gleason 3+3 prostate cancers, which are most commonly observed in the clinic, ongoing studies will investigate the relationship between the expression of these key enzymes and Cho, PC, and GPC levels observed in more aggressive prostate cancers (Gleason ≥7).

References

1. Glunde K et al. Molecular Causes of the Aberrant Choline Phospholipid Metabolism in Breast Cancer. Cancer Res 2004;64:4270-6.
2. Swanson MG et al. Proc. ISMRM 2005; 13:263.
3. Gbegnon AA et al. Proc. ISMRM 2005; 13:185.