Investigation of Metabolic, Pathologic, and Genetic Changes in Prostate Cancer Versus Healthy Prostate Tissues

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Introduction

The combined metabolic, pathologic, and genetic analysis of prostate cancer tissues could lead to improved characterization, therapeutic selection, and monitoring of prostate cancer in individual patients. The purpose of the current study was to quantitatively assess 1) the impact of HR-MAS spectroscopy on histopathologic quality, mRNA integrity and expression levels of post-surgical and biopsy tissues, and 2) the difference in metabolism and gene expression between normal and cancerous prostate tissues.

Methods

21 post-surgical tissues (n=14 healthy, n=7 cancer) and 22 transrectal ultrasound (TRUS) guided biopsies (n=14 healthy, n=8 cancer) were harvested from 26 patients. For each tissue type, half of the healthy and all of the cancer samples underwent HR-MAS analysis prior to pathologic and genetic analysis, while the remaining healthy samples (controls) went on for pathologic and genetic analysis without HR-MAS. At HR-MAS analysis, samples were weighed to 0.01 mg (mean surgical wt: 13.84 ± 4.25 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 sample preparation to refreezing was 28 ± 11 min (range: 12-50 min). ^{1}H HR-MAS data were processed offline using ACD/Labs 1D NMR processor.

Histopathologic and mRNA integrity, 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 19,693 independent genes. The histopathologic integrity was evaluated by two pathologists using a 5 point scale, with 1=poor, 5=excellent. The RNA integrity assay was performed using an Agilent Bioanalyzer 2100, which was also used to assign an RNA integrity number (RIN) from 1 to 10, with 1 = poor, 10 = excellent. The arrays were scanned and analyzed using Cluster and TreeView (Eisen), and GenePix Pro 3.0 (Axon). Pathologic integrity and RIN scores were compared between post-surgical and biopsy tissues, with and without HR-MAS analysis, using a Student's t-test assuming a significance level of $P < 0.05$. Significance Analysis of Microarrays (SAM) was used to identify significantly under/over-expressed genes.

Results

Figure 1 shows representative 1D ^{1}H HR-MAS spectra of healthy predominantly glandular (top) and prostate cancer (bottom) post-surgical tissues. As illustrated in this example, significantly higher levels of choline and glycerophosphocholine plus phosphocholine (GPC+PC), and significantly lower levels of citrate and polyamines (spermine) were observed in prostate cancer versus healthy glandular tissues ($p < 0.05$). Table 1 lists several genes of interest from a total of 232 significantly over-expressed and 76 significantly under-expressed genes in prostate cancer vs. healthy glandular tissues (10% false discovery rate). Included in Table 1 are several genes related to phospholipid, polyamine, and citrate metabolism. To ensure that HR-MAS analysis did not impact pathologic and mRNA integrity and subsequent gene expression, validation studies were performed using 28 healthy samples (14 biopsies, 14 post-surgical), half of which underwent HR-MAS and half of which did not. The mean pathologic integrity scores for post-surgical tissues were 3.4 ± 0.5 (w/ HR-MAS) and 2.9 ± 1.1 (w/o HR-MAS), while the mean pathologic integrity scores for biopsy tissues were 2.7 ± 0.8 (w/ HR-MAS) and 2.3 ± 1.0 (w/o HR-MAS). The mean RIN scores for post-surgical tissues were 6.4 ± 1.0 (w/ HR-MAS) and 6.6 ± 0.7 (w/o HR-MAS), while the mean RIN scores for biopsy tissues were 5.0 ± 1.1 (w/ HR-MAS) and 6.3 ± 1.1 (w/o HR-MAS). There were no significant differences in pathologic integrity or RIN scores between surgical tissues (w/ HR-MAS vs. w/o HR-MAS) or biopsy tissues (w/ HR-MAS vs. w/o HR-MAS).

Figure 1

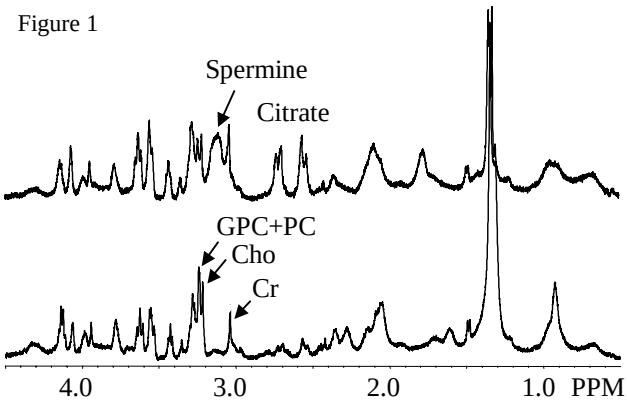

Table 1

Accession#	Gene Name	Overexpressed Genes	Fold Inc.
AA453310	Homosapiens alpha-methylacyl-CoA racemase mRNA		16.47
H65030	Phospholipase A2, group VII		9.16
AA867984	Phospholipase A2, group 1B		9.56
AA195463	ESTs, Highly similar to zinc transporter 1		7.04
AA011215	Spermidine/spermine N-1 acetyltransferase		2.13
AA669545	Spermidine synthase		1.29

Accession#	Gene Name	Underexpressed Genes	Fold Dec.
W72666	Solute carrier family 5 (inositol transporters), member 3		5.88
H17612	Arginase type II		2.37
AA676836	Sphingomyelinase-like phosphodiesterase		1.99
AA678335	Phosphodiesterase 1		1.95
AA136125	Spermine synthase		1.24

Discussion and Conclusions

In this study, significantly higher levels of choline and GPC+PC and lower levels of citrate and spermine were observed in prostate cancer versus healthy glandular tissues. From these same tissues, 232 significantly up-regulated and 76 significantly down-regulated genes were identified, including several genes related to phospholipid, citrate, and polyamine metabolism. Additionally, there were no significant differences in pathologic or mRNA integrity, and subsequent gene expression for post-surgical and biopsy samples that underwent HR-MAS analysis compared to matched control samples. This demonstrates quantitatively for the first time that under careful conditions, HR-MAS spectroscopy can be used in a non-destructive manner to acquire metabolic information from prostate tissues prior to pathologic and genetic analysis of the same tissues. In future studies, these combined metabolic, pathologic, and genetic findings will be correlated with clinical predictors of outcome such as cancer aggressiveness (tumor volume and grade), and local and distant metastasis.