Quantification of Labile Metabolites in Prostate Biopsy Tissues Using HR-MAS Spectroscopy

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Introduction

Previous high resolution magic angle spinning (HR-MAS) spectroscopy studies involving post-surgical prostate tissues have identified distinct metabolic changes in cancer versus healthy glandular and stromal tissues[1]. However, post-surgical tissues are acquired in an unknown state of metabolic degradation because the gland is removed from its blood supply up to 3 hours prior to tissue harvesting. Consequently, the reliability of certain key labile metabolites (e.g., choline compounds, glucose, alanine, and lactate) in post-surgical samples has been questioned. Since biopsy tissues can be harvested and frozen in seconds, they should yield the best possible ex vivo "snapshot" of in vivo cancer metabolism. In this study, HR-MAS spectroscopy was used to quantify and compare the concentrations of the major metabolites, including several labile metabolites, observed in healthy and malignant prostate biopsy tissues.

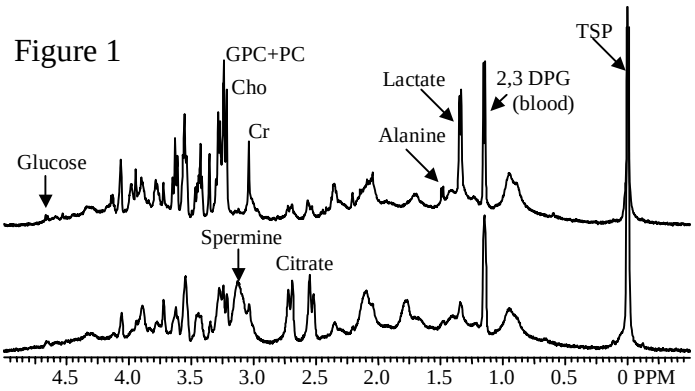
Methods

28 transrectal ultrasound (TRUS) guided biopsies were harvested from 14 patients, frozen on dry ice, and stored at -80°C until ready for use. At HR-MAS analysis, ~1mm was cut and removed from both sides of the core in order to remove periprostatic fat. Samples were then weighed to 0.01 mg (mean wt: 5.75 ± 1.49 mg) and transferred to a 4 mm (O.D.) cell containing a previously weighed 3.0 μl aliquot of D_2O plus 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 equipped with a 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 submitted for pathologic analysis. The mean total time from beginning sample preparation to refreezing was 29 ± 13 min (range: 12-55 min). ^{1}H HR-MAS data were processed offline using ACD/Labs 1D NMR Processor. Data were zerofilled to 65K points, apodized with a matched exponential filter, Fourier transformed, phase and baseline corrected. Metabolite peak areas were determined by Lorentzian-Gaussian peak fitting, after which metabolite concentrations (mmol/kg) were calculated using the masses of tissue and D_2O+TSP. At pathologic analysis, the percentage of healthy glandular, healthy stromal, and prostate cancer tissue were recorded. Metabolite concentrations were compared between pathologic groups using a Student's t-test.

Results

Figure 1 shows representative 1D ^{1}H HR-MAS spectra and the major metabolites observed in healthy glandular (bottom) and prostate cancer (top) biopsy tissues. On pathology, 18 samples were healthy (10 predominantly glandular, 8 predominantly stromal) and 10 samples contained >20% prostate cancer. Metabolically, significantly higher concentrations of free choline (cho) [8.9 ± 3.0 vs. 3.4 ± 1.9, p=0.005], glycerophosphocholine plus phosphocholine (GPC+PC)[11.9 ± 3.9 vs. 4.5 ± 2.4, p = 0.005], creatine [14.8 ± 5.6 vs 7.8 ± 3.0, p = 0.031], glucose [9.8 ± 5.1 vs. 4.2 ± 1.4, p = 0.043], alanine [12.8 ± 3.5 vs. 5.6 ± 1.7, p = 0.003], and lactate [32.0 ± 11.4 vs. 18.5 ± 8.2, p = 0.050] were observed in prostate cancer compared to healthy glandular tissues. Significantly higher concentrations of spermine [33.2 ± 10.1 vs. 11.3 ± 10.3, p = 0.006] were observed in healthy glandular tissue compared to prostate cancer. However, interestingly, citrate concentrations were not significantly different between healthy glandular and prostate cancer tissues [p > 0.05] presumably due to tissue heterogeneity.

Figure 1



Discussion and Conclusions

There are advantages and disadvantages to using both surgical and biopsy tissues for HR-MAS studies of prostate cancer metabolism. Post-surgical tissues are typically obtained in several hundred milligram quantities allowing for multiple analyses and the potential to study homogeneous tumor foci. However, in surgical tissue, glucose quickly converts to pyruvate, then lactate and alanine, and the choline containing compounds GPC and PC degrade to free choline over time. Conversely, biopsy tissues better reflect in vivo metabolism and can be used to study non-surgical patients after therapy, but are much smaller, fragile, and more heterogeneous. In this study, significantly higher concentrations of the labile metabolites glucose, alanine, lactate, free choline, and GPC+PC were observed in prostate cancer versus healthy glandular tissues; whereas significantly higher levels of spermine were observed in healthy glandular compared to prostate cancer tissues. These studies suggest that prostate biopsy tissues can be used to more reliably study the metabolic effects of aberrant glycolysis and altered phospholipid metabolism in prostate cancer than post-surgical prostate tissues.

References

[1] Swanson MG, et al. Magn Reson Med 50, 944 (2003).