Simultaneous metabolic, histopathologic, and genetic analysis of prostate biopsy tissues.

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Synopsis
A protocol was developed for ¹H high resolution magic angle spinning (HR-MAS) spectroscopic analysis of prostate biopsy samples and optimized to preserve tissue integrity for subsequent histopathological and genetic analyses of the same tissues. Prostate cancer tissues (n=3) demonstrated significantly higher (GPC+PC)/Cr (p=0.0001), and significantly lower citrate/Cr (p=0.03) and polyamine/Cr (p=0.0002) ratios relative to benign tissues (n=16). Additionally, lactate was not appreciably observed in any biopsy tissues, indicating minimal tissue degradation during harvesting and data acquisition. Sufficient levels of RNA were also obtained from biopsy tissues following HR-MAS analysis to perform cDNA gene expression arrays on the same tissues.

Introduction
Previous ex vivo NMR studies involving post-surgical tissues have identified specific metabolic differences between prostate cancer and benign glandular and stromal tissues. The use of biopsy tissues for HR-MAS analysis allows for faster tissue harvesting and also allows tissues to be studied from patients who select therapies other than surgery (e.g., hormone deprivation and radiation therapy). In this study, a ¹H HR-MAS spectroscopy protocol was developed for the rapid analysis of quick frozen prostate biopsy samples and optimized to preserve tissue and RNA integrity for subsequent histopathological and gene array analyses.

Methods
Nineteen biopsy specimens (~15 mm × 1 mm in size) were obtained from 10 patients (median age 66 ± 9 years) using an 18 gauge needle under transrectal ultrasound (TRUS) guidance. Typically, 2 samples were acquired from contra-lateral sides of the gland, transferred to cryovials, and frozen under dry ice pellets (≤20s). At HR-MAS analysis, samples were rinsed with ice cold D₂O to remove excess blood and briefly examined using a dissection microscope (50×) to identify and separate any obvious periprostatic fat (≤45s). Samples were then transferred to a 4 mm (O.D.) cell containing 5.0 ul of D₂O plus 1% TSP and inserted into a gHX nanoprobe maintained between −5 and 0 °C (≤30s). Data were acquired using an 11.5 T Varian Inova NMR spectrometer at a spinning rate of 2250Hz. An automated routine was used to shim on the FID (~5 min), after which the water linewidth was determined and a 1D “presat” spectrum (NT = 64, TR = 6s, SW=10kHz, NP =14.2k) was acquired (~6min). CPMG spectra were also acquired when large lipid resonances were present. Following HR-MAS, tissues were imbedded in OCT and processed, during which every 8th slice was stained with hematoxylin and eosin (H&E), and the intermediate slices were used for genetic analysis. ¹H HR-MAS data were quantitated by Lorentzian-Gaussian peak fitting using MacNuts software, and metabolite to creatine (Cr) ratios were compared between benign and malignant tissues using a Student’s t-test, assuming a significance level of p≤0.05.

Results and Discussion
Figure 1 shows an axial T2-weighted MR image (A) and corresponding MRSI spectral array (B) from a region of prostate cancer (circled). A ¹H HR-MAS spectrum (C) was obtained from a 6.5 mg TRUS guided biopsy sample containing Gleason(4+3) prostate cancer obtained from the region of the MRI/MRSI abnormality. Although appreciable lipids can be seen in this spectrum, elevated levels of GPC+PC and choline, and decreased levels of citrate can be clearly observed. Additionally, polyamines, which resonate between choline and creatine, are not observed in this spectrum.

Sixteen out of the 19 samples analyzed were spectroscopically interpretable. Of these samples, 13 were benign, two contained Gleason(3+3), and one contained Gleason(4+3) prostate cancer. (GPC+PC)/Cr ratios were significantly higher (median 3.06±0.18 vs. 1.34±0.29, p=0.0001), while citrate/Cr (median 1.59±0.84 vs. 3.76±1.00, p=0.03) ratios were significantly lower in prostate cancer vs. benign tissues. Free choline/Cr ratios were also higher (but not significantly) in prostate cancer samples than in benign samples (median 1.44±0.54 vs. 0.93±0.39). Polyamines were not observed in any prostate cancer samples and one benign sample, and the polyamine/Cr ratio was significantly higher in benign tissue vs. prostate cancer (median 4.78±3.94 vs. 0.0±0.0, p=0.0002). Lactate was not appreciably observed in any biopsy sample as evidenced by the lack of methyne and methyl proton signals at 4.12 and 1.33 ppm, respectively. In the presence of strong lipids, the absence of lactate was verified using long echo time CPMG experiments (τm =500 ms). This indicates minimal sample degradation during tissue harvesting and data acquisition. Additionally, UDP sugars were observed at 5.34 ppm in 10 samples, and at 5.8 ppm in 3 samples. Viable levels of RNA were also detected in biopsy samples following HR-MAS spectroscopy, and studies are currently underway to correlate the metabolic patterns observed by HR-MAS with the gene expression profiles of the same tissues.

Conclusions
A protocol was developed for the rapid and non-destructive ¹H HR-MAS analysis of prostate biopsy samples, which allows for the simultaneous metabolic, histopathologic, and genetic analysis of the same tissues.