

¹H HR-MAS Spectroscopic Analysis of Post-Surgical Prostate Tissue Targeted Using 3D MRI/MRSI.

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Purpose

The purpose of this study was to use ¹H HR-MAS NMR spectroscopy to evaluate metabolic differences between healthy and malignant post-surgical prostate tissue targeted using 3D MRI/MRSI.

Introduction

In MRSI studies of the *in situ* prostate, the *in vivo* choline resonance is elevated in regions of prostate cancer (PCA), while citrate is reduced [1]. The choline resonance is comprised of several compounds involved in phospholipid metabolism which cannot be resolved *in vivo*. There is also evidence that these compounds may correlate with cellular proliferation and differentiation. ¹H HR-MAS spectroscopy has recently demonstrated the ability to provide detailed metabolic information on intact tissue samples [2]. To identify the specific compounds responsible for the *in vivo* elevation of choline and to identify new chemical markers, ¹H HR-MAS studies were performed on post-surgical prostate tissue targeted using pre-surgical *in vivo* 3D MRI/MRSI data.

Methods

Twenty seven patients with biopsy proven PCA and no prior therapy underwent an MRI and 3D MRSI examination within 6 months of radical prostatectomy. MR studies were performed on a 1.5T GE Signa scanner using the body coil for excitation and combined endorectal and pelvic phased array coils for signal reception. The MR imaging protocol used has been previously described [1]. MRSI data were acquired using a water and lipid suppressed (BASING-PRESS) sequence [3]. Data sets were acquired as 16x8x8 phase encoded spectral arrays with a spectral resolution of 0.24-0.34 cc.

For MRSI voxels which contained at least 75% peripheral zone (PZ) tissue, (choline+creatine)/citrate (CC/C) ratios were calculated and defined as either healthy (H) (CC/C < 0.5), possible cancer (P) (0.5 < CC/C < 0.8), or cancer (C) (CC/C > 0.8). Low signal intensity on T2W images was used to define cancer on MRI images.

Tissue samples (n=53) were collected from the PZ within 30 minutes of prostate removal. MRI/MRSI reports were used to target tissue collection to within a sextant of the prostate. Typically, two samples were obtained from contra-lateral regions and stored in liquid nitrogen until ¹H HR-MAS analysis.

¹H HR-MAS spectra were acquired on a 9.4 T Bruker Avance NMR spectrometer. Samples were spun at 2.5 kHz using a 4 mm ¹H/¹³C HR-MAS probe. 1D parameters: 20 °C; 16K points; 8kHz spectral width; zgpr pulse sequence with HOD presaturation. For selected samples, 2D J-Resolved and TOCSY experiments ($\tau_m = 65$ ms) were also performed. After ¹H HR-MAS spectroscopy, samples were weighed (mean: 7.68±1.79 mg), transferred to cassettes, and submitted in formalin for histology.

During pathologic analysis, samples were imbedded in paraffin wax and 3 level sections were reviewed for each. Standard hemotoxylin and eosin staining was used. Sample compositions were reported in terms of percent PCA, benign tissue, glandular components, stroma, prostatitis, fibrosis, and atrophy.

¹H HR-MAS data were analyzed off-line using MacNUTS (Acorn NMR). Data were apodized with a 0.3 Hz exponential function prior to FT. Lactate was used as an internal chemical shift reference ($\delta = 1.33$ ppm). Resonances were assigned from previously reported chemical shift values, comparison to standards, and analysis of 2D crosspeaks. Peak areas were determined by Lorentzian and Gaussian curve-fitting following baseline correction of the region of interest.

Results and Discussion

¹H HR-MAS data (Figure 1) were categorized into three groups based on pathology. Forty one samples were benign (n=29>50% glandular, n=12>50% stromal tissue), and 12 samples contained PCA (mean: 52±34%). Healthy glandular tissue showed high levels of polyamines (PA) and citrate (Cit) with low choline (Cho) and phosphocholine (PC); stromal tissue exhibited decreased (or no) PA and Cit; and PCA showed elevated Cho and PC with decreased PA and Cit. Creatine (Cr) levels did not significantly change between tissue types.

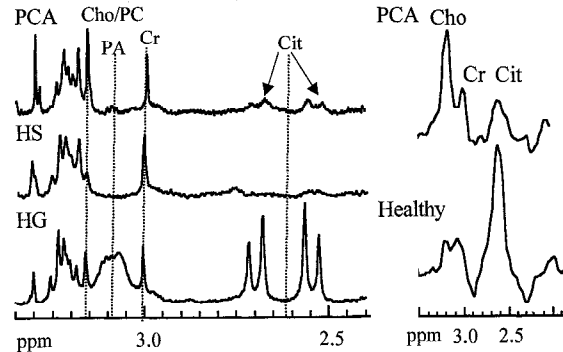


Figure 1. ¹H HR-MAS spectra (left) of healthy glandular (HG, 100%), healthy stromal (HS, 100%), and prostate cancer (PCA, 100%) tissue; and *in vivo* MRSI spectra (right) of healthy and PCA voxels.

¹H HR-MAS findings correlated with metabolic changes observed *in vivo*, and showed that the *in vivo* elevation of the composite choline peak is primarily due to an elevation of Cho and PC (Figure 2). Interestingly, PA were significantly reduced or absent in PCA. There was metabolic overlap between stromal tissue and PCA (Figure 2) due to a reduction of citrate in both tissues. Tissue atrophy was also associated with decreased metabolite S/N ratios.

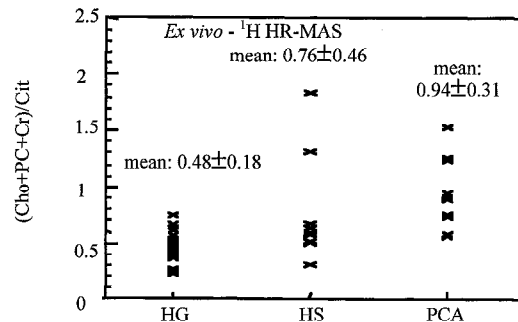


Figure 2. *Ex vivo* (Cho+PC+Cr)/Cit ratios for healthy glandular (HG), stromal (HS), and prostate cancer (PCA) tissue.

Conclusion

The use of 3D MRI/MRSI to guide tissue collection combined with ¹H HR-MAS analysis allows us to better understand the mechanisms of metabolic changes observed *in vivo*, identify new markers for *in vivo* studies, and correlate metabolic changes with promising biomarkers (PSA, morphometric, proliferative, and nuclear markers).

References

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