HR-MAS Spectroscopy of Post-Radiation Prostate Biopsy Tissues

M. G. Swanson[1], K. R. Keshari[1], Z. L. Tabatabai[2], J. P. Simko[2], M-B. Tessem[1], D. J. Joun[1], K. Shinohara[3], and J. Kurhanewicz[1]

[1]Radiology, UCSF, San Francisco, CA, United States, [2]Pathology, UCSF, San Francisco, CA, United States, [3]Urology, UCSF, San Francisco, CA, United States

Introduction

Radiation is commonly applied as a definitive therapy for clinically localized prostate cancer. However, the efficacy of radiation is difficult to determine early on due to the long time to PSA nadir (typically ≥2 years) and radiation induced effects on prostate tissue histology (1). The in vivo identification of recurrent cancer by 3D-MRSI is also challenging because radiation causes a reduction of the healthy ductal metabolites citrate and polyamines, although the presence of elevated choline in 3 or more voxels demonstrates good sensitivity (89%) and specificity (82%) for the diagnosis of local recurrence (2). The goal of this research is to characterize metabolic markers which may predict prostate cancer recurrence at an earlier time point than current clinical markers. One group of potential markers are the choline (Cho) and ethanolamine (Eth) containing metabolites phosphocholine (PC), glycerophosphocholine (GPC), phosphoethanolamine (PE), and glycerophosphoethanolamine (GPE) which are involved in phospholipid membrane synthesis (PC and PE) and degradation (GPC and GPE). In this study 1D and 2D HR-MAS spectroscopy was used for the first time to study changes in citrate, polyamines, and the individual choline and ethanolamine metabolites in post-radiation versus untreated prostate biopsy tissues.

Methods

28 transrectal ultrasound guided biopsies were acquired from 14 post-radiation patients (N=5 external beam; N=6 brachytherapy; N=3 both; median follow up: 60±26 months) and compared to 40 biopsies obtained from 20 untreated patients. HR-MAS data were acquired at 11.7 T, 1°C, and 2,250 Hz spin rate using a Varian INOVA spectrometer equipped with a 4 mm gHX nanoprobe. 3.0 µl of D_2O containing 0.75% TSP (D_2O+TSP) was pipetted into the bottom of a 20 µl zirconium rotor and weighed, after which the tissue samples were weighed (median 5.43±0.97 mg) and added to the rotor. Quantitative 1D spectra were acquired with TR = 4s, 2s presat, AT = 2s, and NT = 64. T_2 filtered (CPMG) spectra were acquired with TE = 144ms and identical parameters except NT = 256 or 512. 2D total correlation spectroscopy (TOCSY) data were acquired using a rotor synchronized adiabatic (WURST-8) mixing scheme with TR = 1.24s, 1s presat, AT = 0.2s, τm = 40ms, NT = 24, SW = 20,000×6,000 Hz, NP = 4096×64 complex points, time = 1.08 hrs. Data were processed and quantified as previously described (3,4). Following HR-MAS, samples underwent routine pathologic processing and staining (H&E) and were evaluated by two pathologists. Data were compared between groups using a two-tailed t-test.

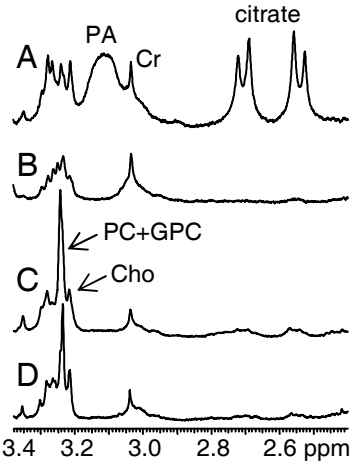

Figure 1. 1D HR-MAS spectra.

Results

Figure 1 shows 1D HR-MAS spectra of A) untreated healthy, B) treated healthy, C) untreated cancer and D) post-radiation cancer tissues. As illustrated in A) and B) significantly higher concentrations (mmol) of citrate (78.0±32.5 vs 12.7±25.1, $p<0.001$) and polyamines (21.2±15.3 vs 5.3±10.0, $p<0.01$) were observed in healthy untreated vs treated tissues. As illustrated in C) and D), prostate cancer tissues demonstrated significantly lower concentrations of citrate (untreated 30.1±22.7, treated 38.8±25.1) and polyamines (untreated 7.8±10.3, treated 8.9±11.6) than healthy untreated tissues ($p<0.01$), however, both cancer groups had significantly higher concentrations of total choline (untreated 18.6±13.2, treated 15.5±8.6) than healthy untreated tissues (9.6±3.0, $p<0.05$). Figure 2 shows the TOCSY CH_2-CH_2 region of A) untreated healthy, B) treated healthy, C) untreated cancer, and D) treated cancer tissues. Untreated healthy tissues demonstrated lower levels of PC and PE and higher levels of Eth than untreated cancer tissues. However, treated healthy tissues demonstrated a dramatic reduction in GPC, GPE, and Eth, and an increase in PE and PC compared to untreated tissues. GPC and GPE levels were also lower in post-radiation cancer vs untreated cancer tissues, but PC was elevated in both groups compared to untreated healthy tissues.

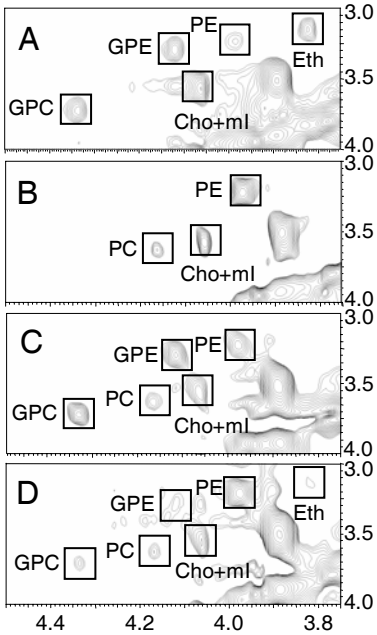

Figure 2. 2D TOCSY spectra.

Discussion and Conclusions

Consistent with the loss of ductal morphology, post-radiation tissues (healthy and cancer) demonstrated significantly lower concentrations of citrate and polyamines than healthy untreated tissues, while cancer tissues (untreated and treated) demonstrated significantly higher concentrations of choline metabolites. The composite choline peak observed in vivo is comprised of Cho, PC, and GPC and contains contributions from Eth, PE, GPE, taurine and myo-inositol (3). This study indicates that at a median of 5 years after radiation, the major contributors to the choline region are Cho, PC and PE in healthy tissues, with higher concentrations of PC, GPC, and GPE in recurrent cancer tissues. Additional metabolic, pathologic, and genetic studies using biopsy tissues acquired at earlier (≤3 years) time points after radiation are needed to better understand these metabolic changes.

References

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