High-resolution Magic Angle Spinning (HRMAS) 1H MRS Detects Biomarkers in Pancreatic Cancer

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Introduction

Approximately 30,000 people die each year in the United States from pancreatic cancer, the fourth leading cause of cancer deaths (1). Pancreatic cancer is fatal, with a 3% prognosis for 5-year survival, and a median survival of less than 6 months, following initial diagnosis (2). Early diagnosis could greatly improve prognosis. To this end, a method to directly evaluate the biological behavior of individual cancers and predict aggressiveness is needed to advance pancreatic cancer management. Our underlying hypothesis is that Magnetic Resonance Spectroscopy (MRS) can identify biomarkers of predictive value. To improve current approaches for pancreatic cancer diagnosis, we developed a novel approach using state-of-the-art, high-resolution Magic Angle Spinning (HRMAS) ^{1}H MRS to analyze intact biopsies from pancreatic cancer patients. This is the first report using HRMAS ^{1}H MRS on intact pancreatic biopsies.

Materials and Methods

Our recruitment is from approximately 150 and 180 pancreatic procedures per year. Resected specimens were brought to the Pathology Department frozen section room for sample collection. HRMAS ^{1}H MRS were performed on a Bruker Bio-Spin Avance NMR spectrometer (proton frequency at 600.13 MHz, 89 mm Vertical Bore) using a 4mm triple resonance (^{1}H, ^{13}C, ^{2}H) HRMAS probe (Bruker). The temperature was controlled at 4 °C by a BTO-2000 unit in combination with a MAS pneumatic unit (Bruker). The tissue was placed into 4mm zirconium oxide (Zirconia, Bruker) rotors with spherical inserts. 10 µl D_2O containing 50 mM TSP (trimethylsilyl propionic-2,2,3,3-d4 acid, Mn=172, δ=0ppm) was added to the rotor with the sample to serve as the deuterium lock reference and external chemical shift reference respectively. The MAS speed was stabilized at 4.0 ± 0.001 kHz by a MAS speed controller. The one dimensional ^{1}H MRS spectra were acquired on all samples using a rotor synchronized Carr-Purcell-Meiboom-Gill (CPMG) spin echo pulse sequence, [90°-(τ-180°-τ)n-acquisition], which works as a T_2 filter to remove the spectral broadening. The inter-pulse delay (τ) was synchronized to the MAS speed to 250µs. The value for n was 20 (2nτ = 10ms). The relaxation delay was set to 5s. The number of transients was 256 with 32,768 (32k) data points. A line-broadening apodization function of 1.0Hz was applied to all HRMAS ^{1}H FIDs prior to Fourier transformation. The spectra are curve-fitted using Lorentzian and Gaussian functions before integration of resonance intensities of metabolites peaks. Two-dimensional ^{1}H-^{1}H TOCSY using an MLEV-17 mixing sequence for homonuclear Hartman-Hahn transfer was acquired with water suppression. The spectra bandwidth was 14ppm. 256 increments were applied along the first axis and 4K data points along the second axis. The number of scans was 8 and the repetition time was 2s, corresponding to a total acquisition time of 80 minutes. The mixing time was set to 75ms. The data were weighted with a square sine bell function before Fourier transformation.

Results

Representative HRMAS ^{1}H NMR spectra of normal human pancreas and pancreatic cancer tissue are shown in figure 1. The spectra were normalized to the intensity of alanine peaks at 1.48ppm. Increased lipid peaks were observed in tumor versus normal. Phosphorylcholine (PCho), glycerophosphocholine (GPC), phosphoethanolamine (PE) and free Cho (Cho) were only distinguished from one another in TOCSY NMR (fig. 2).

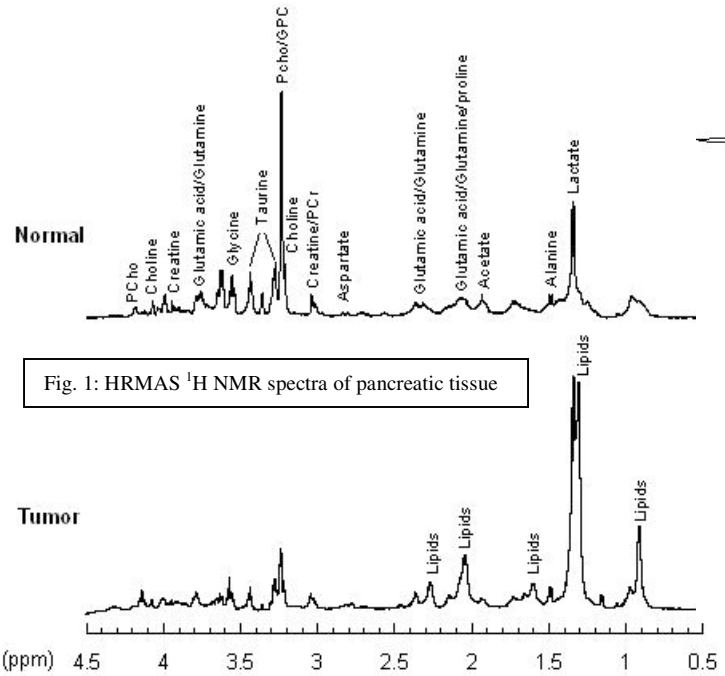
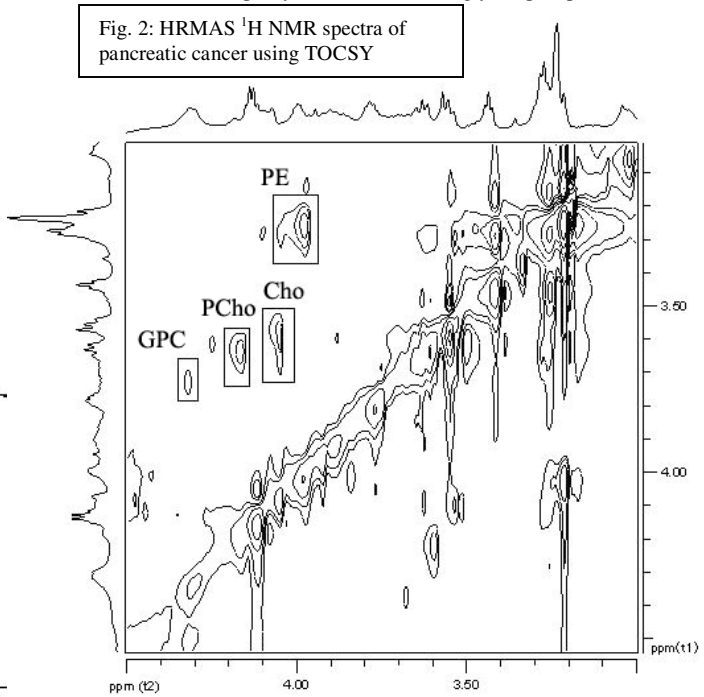


Fig. 1: HRMAS ^{1}H NMR spectra of pancreatic tissue

Fig. 2: HRMAS ^{1}H NMR spectra of pancreatic cancer using TOCSY



Discussion

HRMAS ^{1}H NMR permits the identification of novel pancreatic tumor biomarkers. Other investigators in a previous study using extracts from animal tissues did not identify the prominent increase in lipids possibly due to losses resulting from extraction procedures; however, they did identify increased taurine, lactate and creatine (3). A recent human study using lower resolution in vivo MRS is in agreement with our findings although these investigators of this study could not identify biomarkers other than lipids due to dynamic range effects (4). Ultimately, we expect HRMAS ^{1}H NMR to identify metabolic biomarkers of predictive value, and thus significantly shift and advance the existing paradigm for the management of pancreatic cancer patients, and permit tailored therapies for individual patients.

References

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