Metabolic profiling of pancreatic cancer and matched uninvolved biopsies reveals a dramatic decrease in lipid levels associated with disease.

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Introduction. Pancreatic cancer is the fourth leading cause of cancer-related death in the United States. Because over 80% of pancreatic cancer cases are diagnosed after the disease has reached an advanced stage, the overall one-year survival rate is only 20% and drops to a dismal 4% at five years for all stages of the disease. However, the five-year survival rate can rise to about 25% if the tumor is diagnosed and surgically removed before it has spread to lymph nodes. Novel, non-invasive methods for early diagnosis of pancreatic cancer are therefore needed. Here we characterize the metabolic profile of human pancreatic cancer and matched uninvolved biopsies using high resolution magic angle spinning (HR-MAS) magnetic resonance spectroscopy (MRS) to evaluate the feasibility of exploiting MR spectroscopic imaging to help diagnose and stage pancreatic cancer at an early stage.

Methods. Seven patient-matched cancerous and uninvolved needle core biopsies (14 samples in total) were analyzed in this study. Tissue samples were obtained during or immediately following resection. Six of the paired samples were obtained from Whipple procedures (tumor in head of pancreas) and one paired sample from a distal pancreatectomy (tumor in tail of pancreas). Cancerous biopsies were taken from the palpated tumor while uninvolved biopsies were taken from regions of the pancreas distal to the tumor. Samples were placed in cryogenic vials snap frozen, and stored at -140 ºC until the HR-MAS MRS analysis. Immediately prior to the analysis each sample was cut to fit into a leak-proof, wide-mouth zirconia HR-MAS rotor. Samples weighed between 3.3 and 31.2 mg. 3 µL of deuterium oxide containing 0.75%wt 3-(trimethylsilyl) propionic-2-2-3-3-d$_4$ acid were also added inside the rotor to serve as a chemical shift reference. One-dimensional spectra were acquired on all samples at 500 MHz. Samples were spun at 2,250 Hz. Single pulse experiments were acquired using a 90° pulse with 20 kHz spectral width, 2 s acquisition time and 2 s water presaturation delay, 128 transients and 4 steady state pulses. In addition, to minimize the broad signal contributions from lipids and macromolecules, a Carr-Purcell-Meiboom-Gill (CPMG) sequence was used with 144 ms echo time and 512 transients. The Electronic Reference To access In-vivo Concentrations (ERETIC) method was used to provide a reference for metabolite quantification. Data acquisition was performed at 1 °C to minimize tissue degeneration and the total time for sample preparation and data acquisition was kept to a minimum (between 60 and 90 minutes). Following standard processing steps spectra were normalized to the ERETIC signal and to sample mass, and data analyzed using Matlab. Principal component analysis (PCA) was performed on CPMG spectral regions containing signals from small metabolites (excluding lipid signals). Prior to PCA the selected spectral datapoints were normalized to total spectral area and each patient-matched dataset was mean centered separately.

Results. The single pulse spectra acquired on both cancerous and uninvolved samples were characterized by intense lipid signals (representative spectra in **Fig. 1A**). However, the comparison of spectral profiles indicated a very significant overall decrease in the intensity of these lipid signals in the cancerous specimens to approximately 35% (p<0.03) of the uninvolved tissue. Importantly, when directly comparing patient-matched samples, the drop in lipid levels was even more significant. The 1.3 ppm lipid peak in cancerous samples fell to 28%±17% of the uninvolved samples (p<0.007) and the other lipid peaks dropped on average to 34%±14% (p<0.007) of the matched uninvolved samples. Additional changes in levels of small metabolites between cancerous and uninvolved tissue samples were evaluated from the CPMG spectra (**Fig. 1B**) and using PCA (**Fig. 1C**). Taurine was found to be significantly increased in cancerous samples, up to ~325% (p<0.02) of uninvolved samples. Lactate was detected only in 2 out of the 7 uninvolved samples analyzed and in all of the cancerous samples. Choline containing metabolites were not significantly altered.

Discussion and Conclusions. The metabolic significance of our observations remains to be determined. Nonetheless, our results indicate that the high intensity lipid signals detected in uninvolved pancreatic tissue are significantly reduced in tumors and therefore represent a candidate biomarker for the detection and possibly staging of pancreatic cancer. Spectroscopic imaging in the abdomen requires breath hold or respiratory gating in order to overcome motion artifacts. Nonetheless, this easily detectable, high-signal biomarker could help in the early diagnosis of pancreatic cancer.

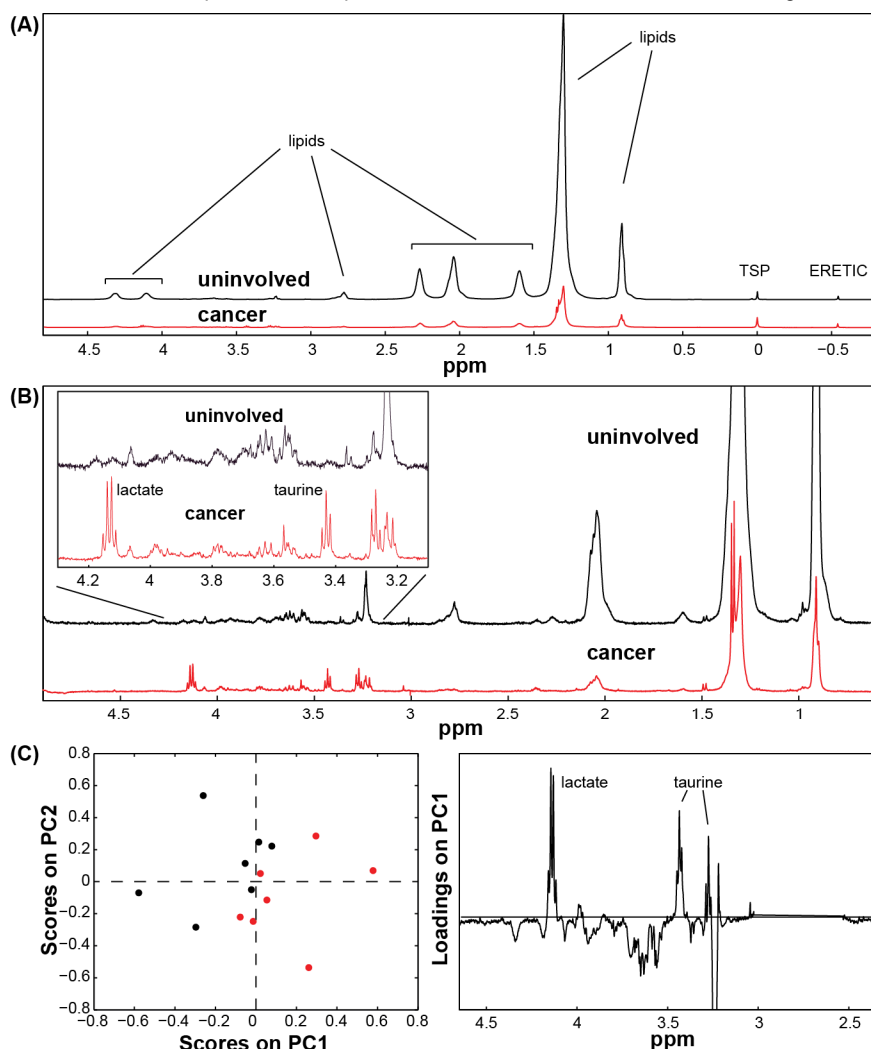**Figure 1.** Representative single pulse **(A)** and CPMG **(B)** HR-MAS MR spectra. **(C)** Scores and loadings plots obtained from the PCA performed on CPMG spectra.