Importance of characterizing water content in quantifying metabolites in pancreatic cancer and normal pancreas

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Introduction. Most pancreatic cancers are histologically classified as pancreatic ductal adenocarcinoma (PDAC), and have a 5-year survival rate of only 6%. PDAC is an aggressive and lethal disease that develops relatively symptom-free and is therefore advanced at the time of diagnosis. Its poor prognosis is due to a combination of late-stage diagnosis and limited response to chemotherapy and radiotherapy, arising in part from the strong desmoplastic stroma that limits delivery of diagnostic imaging probes and therapeutic agents. The absence of early symptoms and effective treatments has created a critical need for identifying new noninvasive biomarkers and therapeutic targets. Magnetic resonance spectroscopic imaging (MRSI) and magnetic resonance spectroscopy (MRS) are being evaluated in the diagnosis of several solid malignancies including brain, prostate and breast cancer. A hallmark of most solid tumors is the detection of elevated level of phosphocholine (PC) and total choline (tCho). tCho, which is usually seen as single peak *in vivo*, consists of three choline-containing metabolites which can be resolved through high-resolution ^{1}H MRS into three resonance peaks, namely PC, glycerophosphocholine (GPC) and free choline (Cho). We previously observed elevated levels of tCho in several pancreatic cancer cell lines and tumor xenografts (1). However, initial single voxel studies performed in humans suggest that the tCho signal normalized to water may be relatively high in normal pancreas compared to PDAC (2). Our initial high-resolution proton spectra of tumors and pancreatic tissue extracts normalized to the water signal, assuming similar water content, also did not reflect the significantly increased tCho observed *in vivo.* To determine if differences in water content caused this anomaly, we determined the water content in a human pancreatic xenograft and in mouse pancreas, as well as mouse lungs, liver, heart, kidney and muscle for future reference. Our data demonstrate the significantly lower water content in these organs and the importance of considering the water content in the quantification of metabolites when comparing different tissue types.

Methods. We used the human pancreatic adenocarcinoma cell line Panc1 obtained from ATCC (American Tissue Culture Collection) for these studies. For the *in vivo* experiments, tumor pieces were orthotopically implanted onto the pancreas of severe combined immunodeficient (SCID) male mice. Once tumors reached ~500 mm^3, the mice were scanned on a 9.4T spectrometer for ^{1}H MRSI, and were then sacrificed for *ex vivo* ^{1}H MRSI of the tumor tissues and pancreas. To further determine differences in tCho, tumor and pancreas tissue were embedded in agarose in a 50 mL Falcon tube. ^{1}H MRSI was acquired with a 2D CSI sequence (TE 135 ms, TR 1059 ms). A different set of tumors were freeze-clamped and used for high-resolution ^{1}H MRS. Tumor extracts were obtained using a dual-phase extraction method with methanol/chloroform/water (1/1/1) (3,4). Fully relaxed ^{1}H MR spectra of the extracts were acquired on a Bruker Avance 500 spectrometer operating at 11.7 T using a 5-mm HX inverse probe. To determine the tCho concentration, peak integrations from ^{1}H spectra for Cho, PC and GPC were compared to the internal standard. Integrals of the metabolites of interest were determined from tumors and normal pancreas and normalized first to the tissue wet weight. The water content of different organs was estimated as a ratio of the wet weight to the dry weight (measured after 72h of lyophilization), and the tCho concentration in pancreatic tumors and pancreas was corrected using the water content factor.

Results and Discussion. ^{1}H MRSI showed a heterogeneous tCho signal in orthotopically implanted pancreatic tumors as shown in the representative image in **Figure 1**. To further validate differences in the tCho signal between the tumor and the pancreas we performed *ex vivo* ^{1}H MRSI of subcutaneous and orthotopic Panc 1 tumors and of the mouse pancreas (representative images in **Figure 2**).

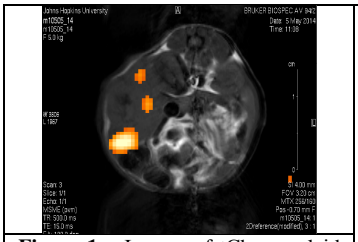

Figure 1: Images of tCho overlaid on anatomic image from mouse bearing an orthotopic Panc1 tumor.

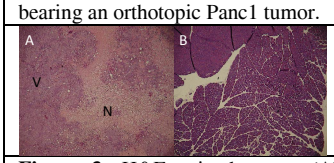

Figure 3: H&E-stained tumor (A) and pancreas (B) sections. (N-necrotic area, V-viable tissue)

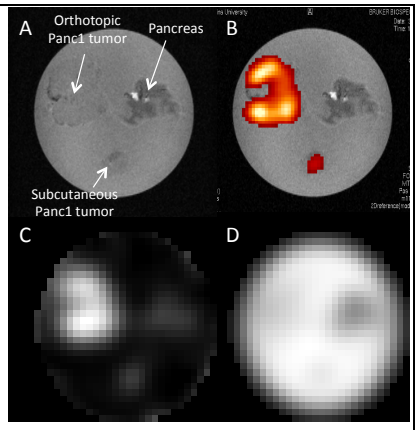

Figure 2: *Ex vivo* T_1-weighted image (A), tCho density map (B), tCho concentration map (C), water density map (D).

Concentrations of tCho were 3.38 ± 0.95 mM in orthotopic tumors, 1.32 ± 0.59 mM in subcutaneous tumors, and 1.27 ± 0.52 mM in normal pancreas (n = 2), when using the water signal for normalization, showing that despite a much higher tCho signal in the subcutaneous tumors (**Figure 2B**), tCho concentration was comparable to the pancreas (**Figure 2C**). The *ex vivo* study revealed a lower water content in the pancreas compared to tumor tissue (**Figure 2D**) that increased the tCho/water ratio in this tissue. Histological analysis revealed the presence of necrosis in the orthotopic tumor (**Figure 3**) that could explain the heterogeneity in the tCho signal, highlighting the importance of acquiring ^{1}H MRSI instead of single voxel MRS. Tumor heterogeneity can also complicate biopsy sample analysis, or extract analysis, if only part of the tumor is used for analysis. To more precisely assess the water content in tumors, pancreas, and other organs, we obtained dry weight values by lyophilizing them and calculated wet weight to dry weight ratio for each tissue. The results confirmed a higher water content in tumors compared to the normal pancreas (**Figure 4**). The tCho concentrations measured with high-resolution ^{1}H MRS from an orthotopic tumor and normal pancreas before and after taking into account differences in water content are shown in **Figure 5**. The data demonstrate a two-fold difference in tCho concentration between tumor tissue and pancreas when the correction for the water content is applied. These data support the use of ^{1}H MRSI that provides a tCho map rather than the placement of single voxels to address heterogeneities in the pancreas and in pancreatic cancers. The results highlight the importance of including the water content in the calculation of metabolite concentration when comparing different tissues characterized by different water concentration.

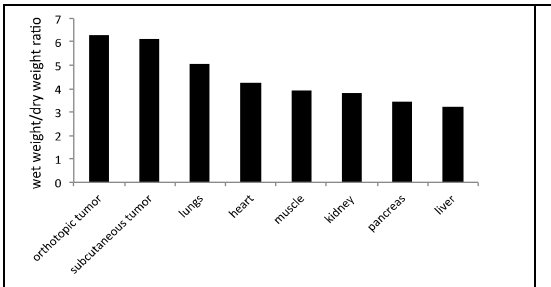

Figure 4: Wet weight over dry weight ratio from different organs showing higher water content in tumors compared to the pancreas and other organs.

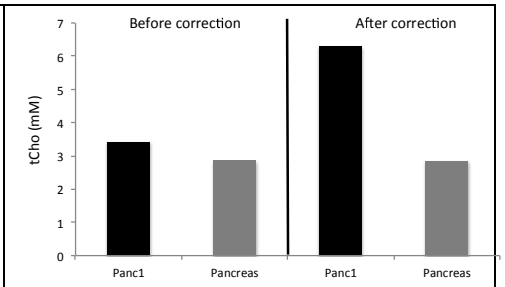

Figure 5: tCho concentrations derived from high-resolution ^{1}H MRS before and after correction with the water content of the tissue.

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References (1) Penet *et al.*, Clinical Cancer Research (2014). (2) Ma *et al.,* Journal of computer assisted tomography (2011). (3) Shah *et al.*, NMR Biomed (2012). (4) Glunde *et al.,* Cancer Res (2005).