Altered choline phospholipid metabolism in pancreatic cancer cells and tumor xenografts

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Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the USA. Early-stage pancreatic cancer is usually clinically silent, and most patients presenting symptoms attributable to pancreatic cancer already have advanced disease. Pancreatic cancer only becomes apparent after the tumor invades surrounding tissues or metastasizes to distant organs. Typical symptoms include abdominal or mid-back pain, obstructive jaundice, and weight loss that can arise from anorexia, maldigestion from pancreatic ductal obstruction, or from cachexia. Better understanding of the disease, effective early detection methods, and new therapeutic targets are urgently needed to improve its outcome. Magnetic resonance spectroscopy (MRS) is being evaluated in the diagnosis of several solid malignancies such as brain, prostate and breast cancer, and for monitoring therapy in brain cancer (1). A hallmark of most solid tumors is the detection of elevated level of phosphocholine (PC) and total choline (tCho) (1). tCho, which is usually seen as single peak *in vivo*, is constituted of three choline-containing metabolites which can be resolved through proton high-resolution spectroscopy into three resonance peaks, namely PC, glycerophosphocholine (GPC) and free choline (Cho). The increase in PC levels in solid tumors has been attributed to high expression of the choline kinase (*Chk)-α* gene (2). Here, our aims were to characterize the metabolism of multiple pancreatic ductal adenocarcinoma cell lines and tumors with *in vitro* high resolution MRS and *in vivo* ^{1}H magnetic resonance spectroscopic imaging (MRSI). We have also characterized the expression of choline kinase (Chk) in the cells and tumor extracts.

Methods

Eight pancreatic adenocarcinoma cell lines and one immortalized pancreatic cell line were used in the present study. Panc1 and BxPC3 were obtained from ATCC (American Tissue Culture Collection). Pa04C, Pa02C, Pa20C, Pa28C, Pa03C, and Pa09C, obtained from the Johns Hopkins pancreatic xenobank, were derived from pancreatic cancer patients and established in nude mice (3). Pa02C and Pa03C were derived from liver metastases, Pa04C from lung metastasis, whereas Panc-1, BxPC-3, Pa09C, Pa28C, Pa03C and Pa20C were primary adenocarcinomas. As a control, we used human pancreatic nestin expressing (HPNE) cells from ATCC. For the *in vivo* experiments, cells were inoculated subcutaneously in SCID male mice. Once tumors reached 500 mm^3, the mice were scanned on a 4.7T spectrometer for ^{1}H MRSI, and were then sacrificed and the tumors excised for immunoblot analyses, and high-resolution ^{1}H MRS. Cells and tumor extracts were carried out using a dual-phase extraction method with methanol/chloroform/water (1/1/1) (4, 5). Fully relaxed ^{1}H MR spectra of the extracts were acquired on a Bruker Avance 500 spectrometer operating at 11.7 T (Bruker BioSpin Corp., Billerica, MA) using a 5-mm HX inverse probe. Integrals of the metabolites of interest were determined from cell extracts and tumors and normalized to the number of cells and to the tumor weight respectively. To determine concentrations, peak integration from ^{1}H spectra for all metabolites studied was compared to the internal standard.

Results and Discussion

We used ^{1}H MRSI and MRS to characterize choline phospholipid metabolism in a panel of pancreatic adenocarcinomas cell lines and tumors.

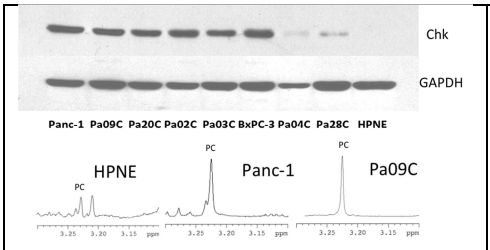

Figure 1a: Representative immunoblots from a panel of pancreatic cell lines showing Chk-α expression (top panel). Representative ^{1}H MR spectra from HPNE, Panc1 and Pa09c pancreatic cancer cell lines (bottom panel).

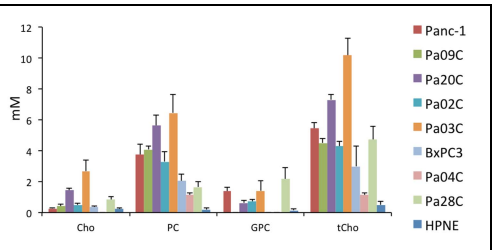

Figure 1b: Quantitative data for various choline containing compounds in a panel of pancreatic cell lines. (Cho: free choline, tCho: total choline, GPC: glycerophosphocholine). (mean ± sd) (n = 3).

We identified differences in tCho content in both the pancreatic cell lines and the tumor xenografts. In the panel of cell lines, the differences in tCho levels were associated with differences in Chk expression. PC and tCho levels were significantly elevated in the pancreatic cancer cell lines compared to the immortalized pancreatic cell line ($p < 0.05$), and the Chk enzyme was overexpressed in the cancer cell lines (**Figures 1a-b**). *In vivo* studies revealed that tCho could be detected in tumors derived from the 4 cell lines tested, with higher levels of tCho in Panc1 tumors (**Figures 2a-b**). The *in vivo* results were confirmed by *ex vivo* MRS analysis of the tumor extracts (**Figure 3**). The high level of tCho observed in Panc1 tumors was mainly due to an increase of PC, and correlated with high Chk-α expression observed in the immunoblots obtained from tumor extracts (data not shown). These data support including the use of ^{1}H MRS to noninvasively detect pancreatic cancer. Moreover, the aberrant choline metabolism may provide novel targets in the treatment of pancreatic cancer.

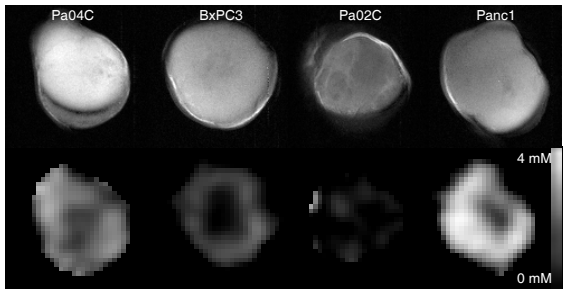

Figure 2a: Representative anatomical images of Pa04C, BxPC3, Pa02C and Panc1 tumors (top row), and corresponding representative tCho maps (bottom row).

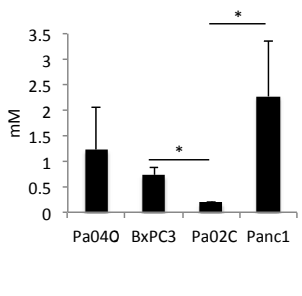

Figure 2b: Total choline concentration measured *in vivo* with ^{1}H MRSI (mean ± sd) (n = 3, *$p < 0.05$)

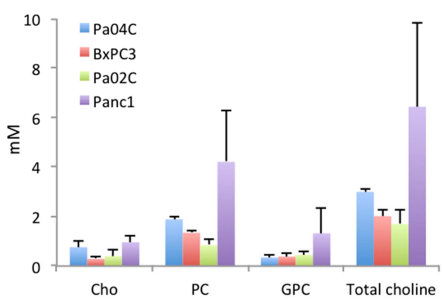

Figure 3: Metabolite quantification in tumor extracts with high-resolution ^{1}H MRS (mean ± sem) (n = 3)

Acknowledgement This work was supported by NIH P50CA103175. *Equal contribution.

References (1) Glunde *et al.,* Nature reviews (2006). (2) Ramirez de Molina *et al*., Biochem Biophys Res Commun (2002). (3) Jones *et al*., Science (2008). (4) Shah *et al*., NMR Biomed (2012). (5) Glunde *et al.,* Cancer Res (2005).