Altered choline phospholipid metabolism in a panel of pancreatic cancer cell lines

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths in both men and women in US (1). Pancreatic cancer has a very poor prognosis, in part due to its diagnosis at late stages of the disease and to limited response to chemotherapy and radiotherapy. Currently, there is no specific marker available for their early diagnosis and there is an urgent need for biomarkers with enough sensitivity and specificity to help diagnose pancreatic cancer early. Development of novel therapeutic strategies is also critically important. Magnetic resonance spectroscopy (MRS) is being evaluated in the diagnosis of other solid malignancies such as brain, prostate and breast cancer, and for monitoring therapy in brain cancer (2). A hallmark of most solid tumors is the detection of elevated level of phosphocholine (PC) and total choline (tCho) (2). This increase in PC has been attributed to high expression of choline kinase (Chk)–α gene (3). Here we have used ^{1}H MRS to characterize choline phospholipid metabolism in a panel of pancreatic adenocarcinomas cell lines.

Material and Methods: Eight pancreatic adenocarcinoma cell lines and one immortalized pancreatic cell line were used to obtain cell extracts for high resolution ^{1}H MRS. Information about the origin of the cell lines in provided in Table 1. Human Pancreatic Nestin Expressing (HPNE) cells stably expressing human telomerase reverse transcriptase (hTERT) - hTERT-HPNE cells, Panc-1 and BxPC-3 were obtained from ATCC (American Tissue Culture Collection). A6L, Panc-198, Panc-215, JD13D, 3.014 were obtained Johns Hopkins pancreatic xenobank derived from pancreatic cancer patients and established in nude mice. All the pancreatic cancer cell lines were provided by Dr. Maitra. The hTERT-HPNE cell line is derived from human pancreatic duct by transduction with a retroviral expression vector containing the hTERT gene. For immunoblots, whole-cell extracts were prepared by lysing cells with RIPA buffer supplemented with protease inhibitor cocktail. Total cellular protein was resolved on SDS-PAGE and specific detection of *Chk-α* on immunoblots by a custom-designed *Chk-α* antibody. GAPDH was used as a loading control.
MR spectroscopy of cell extracts: Approximately $1.5x10^7$ cells were harvested and water-soluble cell extracts were obtained using a dual-phase extraction method based on methanol/chloroform/water (1/1/1; v/v/v) as previously described (4). The samples were dissolved in deuterated solvent containing 3-(trimethylsilyl) propionic-2,2,3,3-d_4 acid (TSP) which served as a concentration standard and chemical shift reference. Integrals of various water-soluble metabolites were determined to estimate their absolute concentration relative to TSP.

Results and Discussion

Here we have characterized the choline phospholid metabolites profile in a panel of malignant pancreatic cell lines together with and a nonmalignant pancreatic cell line. Figure 1 shows immunoblots from these cell lines and shows different level of *Chk-α* overexpression relative to hTERT-HPNE cells. Figure 2 shows quantitative data for choline containing metabolites obtained from water soluble cell extracts. Elevated PC and total choline were present in all adenocarcinomas relative to the immortalized pancreatic cells ($p<0.05$). Differences were also observed within the adenocarcinoma cell lines. While Panc-1, Panc-215 and Panc-198 showed the highest amounts of PC, Panc-198 and A10.7 pancreatic cancer cells showed significantly high free choline compared to all other cell lines ($p<0.05$).

Here we have identified altered choline phospholipid metabolism in a panel of pancreatic cancer cell lines. These data support the development of ^{1}H MRS to detect pancreatic cancer and monitor response to treatment. The aberrant choline metabolism observed here in pancreatic cancer cells may also provide novel targets in the treatment of pancreatic cancer.

Table 1: Origin of cell lines

Cell line	Origin	Source/tissue
htert-HPNE	Normal human pancreatic duct, Male (ATCC)	Obtained by transducing with a retroviral expression vector containing the hTERT gene
Panc-1	Male, ATCC	Ductal adenocarcinoma
Panc-215	Female	Ductal adenocarcinoma
Panc-198	Male	Ductal adenocarcinoma
A6l	Male	Ductal adenocarcinoma/ Lung metastasis
BxPC-3	Female, ATCC	Ductal adenocarcinoma
A10.7	Male	Ductal adenocarcinoma/ Lung metastasis
JD13D	Male	Ductal adenocarcinoma/ Lung metastasis
3.014	Male	Ductal adenocarcinoma

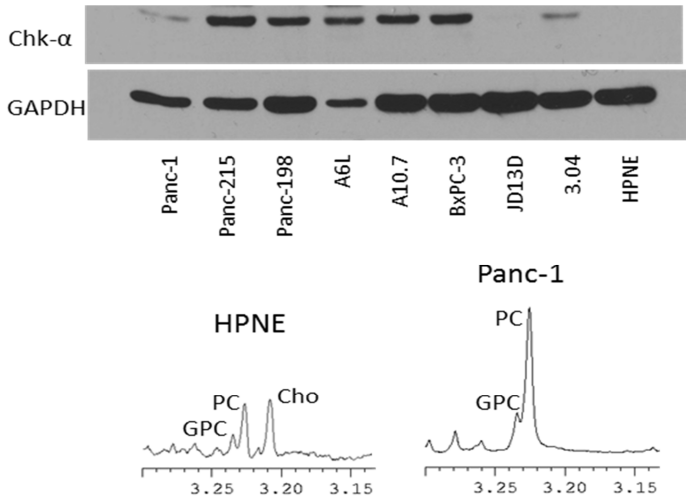

Figure 1: Representative immunoblots from a panel of pancreatic cell lines showing Chk-α expression (top panel). (b) Representative proton spectra of HPNE and Panc-215 pancreatic cancer cell lines (bottom panel) from an expanded region containing choline phospholipid metabolites.

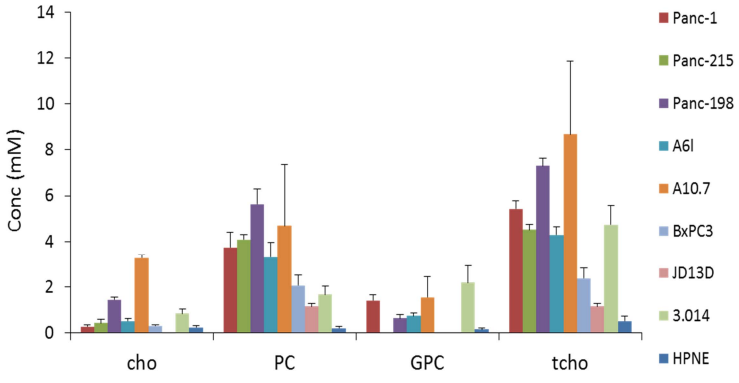

Figure 2: Quantitative data for various choline containing compounds in a panel of pancreatic cell lines. (Cho: free choline, GPC: glycerophosphocholine)Values are Mean ± SD (n=3).

References: (1) American Cancer Society 2012 Facts and figures. (2) Glunde K, Bhujwalla ZM, Ronen M, Nat Rev Cancer 2011;11:835-48. (3) Ramírez de Molina A *et al*. Biochem Biophys Res Commun 2002; 296:580-3. (4) Glunde *et al.* Can Res 2008;68:172-80. (5) Poptani H *et al.* J Cancer Res Clin Oncol. 1999;125:343-9.
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