The tumor microenvironment alters choline phospholipid metabolism detected by comparing cancer cells with tumors

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Introduction: An increase of cellular phosphocholine (PC) and total choline-containing compounds (tCho) has been consistently observed in cancer cells and tissue [1-3], and is closely related to malignant transformation, invasion, and metastasis [1, 4]. The study of cancer cells in culture plays an important role in understanding mechanisms leading to altered choline phospholipid metabolism in cancer, as it provides a carefully controlled environment. In the clinic, tCho levels are utilized to distinguish malignant *versus* benign lesions using ^{1}H MRSI [3]. A solid tumor, however, is a complex system with a unique environment frequently containing hypoxic regions. Cancer cell – endothelial cell interactions, which are present in solid tumors, can also alter choline phospholipid metabolism [5]. Human tumor xenograft models in mice are useful to mimic the growth of human cancers. Here we have compared choline phospholipid metabolites of cells in culture and solid tumors to demonstrate the importance of growth conditions *in vivo*, such as hypoxia and cancer cell-stromal/endothelial cell interaction, in influencing choline phospholipid metabolism.

Methods: MDA-MB-231, an estrogen receptor (ER)/progesterone receptor (PR)-negative metastatic human breast cancer cell line and MCF-7, an ER/PR-positive poorly metastatic human breast cancer cell line, as well as PC-3 and DU-145, which are both androgen independent malignant human prostate cell lines were used in this study. Cells were grown in RPMI-1640 medium (MDA-MB-231, PC-3, & DU-145) or Eagle's MEM (MCF-7) supplemented with 10% FBS and antibiotics. Cells were cultured to 80% confluence, and medium was changed 3 h prior to cell collection to avoid any lack of nutrition. Approximately $3x10^7$ to $6x10^7$ cells were harvested for cell extraction. For solid tumor studies, cells were inoculated in the flank of severe combined immunodeficient (SCID) mice. Solid tumors were harvested and immediately freeze-clamped when tumor weights were < 0.4 g. Both lipid and water-soluble cell and tumor extract fractions were obtained using a dual-phase extraction method as described previously [6]. Fully relaxed ^{1}H MR spectroscopy of the water-soluble extracts from both cells in culture and solid tumors was performed on a Bruker Avance 500 NMR spectrometer, using 3-(trimethylsilyl)propionic-2,2,3,3,-d_4 acid (TSP) as an internal concentration standard.

Results: Figure 1 shows choline phospholipid metabolite levels of cells in culture and solid tumors derived from prostate and breast cancer cell lines. PC-3, DU-145, and MDA-MB-231 cells in culture exhibited higher levels of PC as compared to the PC levels in each corresponding solid tumor. MCF-7 cells in culture showed comparable level of PC to the corresponding tumors. Glycerophosphocholine (GPC) levels of cells in culture were either lower (PC-3, MDA-MB-231, & MCF-7) or higher (DU-145) as compared to the corresponding tumors. Total choline (tCho) levels of PC-3 and MCF-7 cells in culture and solid tumors were similar while DU-145, and MDA-MB-231 cells in culture contained a higher level of tCho as compared to solid tumors. The PC/GPC ratio from PC-3 and MDA-MB-231 cell lines showed significantly higher levels in cell culture than in solid tumors as demonstrated in Figures 1 and 2. MCF-7 cells in culture also exhibited a relatively higher PC/GPC ratio than in solid tumors. DU-145 had similar PC/GPC ratios in both cell cultures and tumors. ^{31}P MR spectra confirmed a similar trend of PC/GPC ratios in cells and tumors (data not shown).

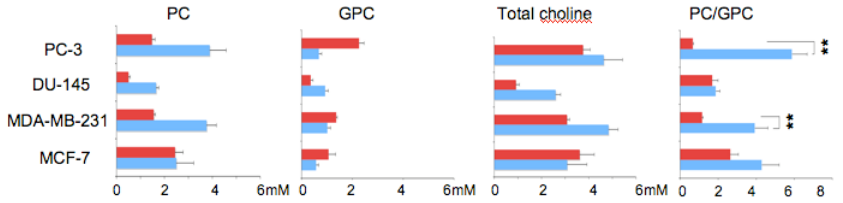

Figure 1: Quantitation of choline phospholipid metabolite levels from
Values are mean ± standard error from more than 3 cell extracts or 4 t

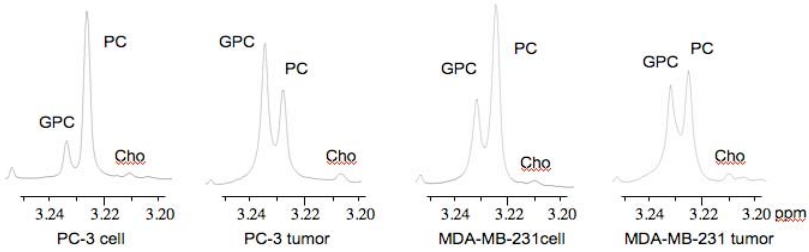

Figure 2: Representative ^{1}H MR spectra (water soluble extract fractions) of the choline phospholipid metabolite region of cells in culture and solid tumors.

Discussion: Our data demonstrate that there are significant differences in choline phospholipid metabolism between cells in culture and the corresponding solid tumors inoculated from the same cell lines in both breast and prostate cancer cell lines. Highly malignant MDA-MB-231 and PC-3 solid tumors exhibited significantly lower PC/GPC ratios as compared to cells in culture, whereas the less malignant MCF-7 and DU-145 lines displayed a smaller PC/GPC difference between cells and tumors. These differences can arise from environmental factors found in solid tumors such as depletion of nutrients and oxygen, changes in pH [7], as well as cancer cell and stromal/endothelial cell interactions [5]. Our observations are consistent with studies, which demonstrate that confluent and post-confluent HeLa and MCF-7 cells in culture showed a decrease of PC and an increase of GPC/PC ratios as compared to proliferating cells [8]. Additional studies are necessary to unravel the tumor microenvironmental factors responsible for the differences observed between cells grown in culture as compared to the same cells grown as solid tumors.

References and Acknowledgements: [1] Aboagye E et al, *Cancer Res*, 59, 80 (1999); [2] Ackerstaff E et al, *Cancer Res* 61, 3599 (2001); [3] Kurhanewicz J et al, *Neoplasia* 2, 166 (2000); [4] Bhujwalla ZM et al, Magn Reson Med 41, 897 (1999) [5] Mori N et al, *Molecular Imaging* 2, 124, (2003); [6] Tyagi RK et al, *MRM* 35, 194 (1996); [7] Galons JP et al, MRM 33, 422, 1995; [8] Rosi A et al, *NMR Biomed* 17, 76 (2004). This work was supported by NIH R01 CA73850 and P50 CA103175. We thank Dr. V.P. Chacko for expert technical support, and Mr. G. Cromwell for inoculating the tumors.