Tumor microenvironmental alterations of lipid metabolism detected by comparing cancer cells with tumors

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Introduction: Cancer cells in culture and human tumor xenografts in mice are important models to study cancer biology. 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 some metabolisms such as choline phospholipid metabolism [1]. An increase of cellular phosphocholine (PC) and total choline-containing compounds (Cho) has been consistently observed in cancer cells and tissue [2-4], and is closely related to malignant transformation, invasion, and metastasis [2,5]. Previously, we have shown that PC/glycerophosphocholine (GPC) ratios in highly malignant cancer cells in culture were significantly higher than in the corresponding solid tumors, indicating the importance of the tumor microenvironment in choline phospholipid metabolism. Lipid soluble cell or tumor extracts include lipids such as fatty acids, cholesterol, and phospholipids. These lipids can function as energy storage molecules, structural components of cell membranes, and signaling molecules involved in cell growth, inflammation and immunity [6,7]. Here we have compared lipid components in cells in culture and solid tumors using 1H MR spectroscopy to understand the importance of growth conditions in vivo, such as hypoxia and cancer cell-stromal/endothelial cell interaction, in influencing lipid components.

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. All cell lines were grown in RPMI-1640 medium supplemented with 10% FBS and antibiotics. Cells were cultured to about 80% confluence, and medium was changed 3 h prior to cell collection to avoid any lack of nutrition. Approximately 3x10^6 to 6x10^6 cells were harvested for cell extraction. For solid tumor studies, cells were inoculated in mammary fat pad (breast cancer cells) or the flank (prostate cancer cells) of severe combined immunodeficient (SCID) mice. Solid tumors were harvested and immediately freeze-clamped when tumor weights were < 0.44 g. Both lipid and water-soluble cell and tumor extract fractions were obtained using a dual-phase extraction method as described previously [8]. The lipids were dissolved in 0.4 ml of deuterated chloroform and 0.2 ml of deuterated methanol for 1H MRS analysis. Fully relaxed 1H MR spectroscopy of the lipid-soluble extracts from both cells in culture and solid tumors was performed on a Bruker Avance 500 MR spectrometer, using tetramethylsilane (TMS) as an internal concentration standard. Chemical shifts were assigned using the internal standard as reference [9].

Results and Discussion: Representative examples of lipid spectra from MCF-7 cells and tumor are shown in Figure 1. The ratio of lipid peaks from lipid-soluble cell or tumor extracts derived from prostate and breast cancer cell lines are shown in Figure 2. CH2/ CH3 is related to the length of fatty acids, CH3/CH3 shows fatty acid amount versus cholesterol. PC-3, MDA-MB-231, and DU-145 cells had higher level of PtCho/PtE than solid tumors, but PC-3 cells had lower level of PtCho/PtE than solid tumors. Both cells and solid tumors contained similar lengths of fatty acid (CH2/CH3) in MCF-7, DU-145, and PC-3. As shown in Figure 3, in solid DU-145, MCF-7 and MDA-MB-231 tumors, there was less PLA2 protein than in cells, whereas PC-3 tumors had a lower level of PLA2 than cells. PLA2 is an enzyme that catalyzes phospholipids and releases lysophospholipids and arachidonic acid [10]. There was no consistent relationship between PtCho/PtE and PLA2 protein level. The differences between cell and tumors can arise from environmental factors found in solid tumors such as depletion of nutrients and oxygen, changes in pH, as well as cancer cell and stromal/endothelial cell interactions. 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.