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

N. Mori[1], K. Glunde[1], T. Takagi[1], L. Xiong[1], F. Wides[1], and Z. Bhujwalla[1]

[1]JHU ICMIC Program, The Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MD, United States

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 (tCho) 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 ^{1}H 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 $3x10^7$ to $6x10^7$ 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 0.4ml of deuterated chloroform and 0.2ml of deuterated methanol for ^{1}H MRS analysis. Fully relaxed ^{1}H 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]. Immunoblot analysis was performed using monoclonal cytosolic PLA_2 antibody (Santa Cruz Biotechnology Inc.).

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. CH_2/CH_3 is related to the length of fatty acids, CH_2/Cholesterol shows fatty acid amount *versus* cholesterol, $CH{=}CH/CH_2$ is related to the degree of unsaturation of fatty acids, and PtCho/PtE shows phosphatidylcholine amount *versus* phosphatidylethanolamine. Consistently higher $CH{=}CH/CH_2$ was observed in tumors compared to cells for all four human cancer models, indicating that solid tumors had a higher degree of unsaturation in fatty acids than cells in culture. PC-3, MDA-MB-231, and DU-145 cells had higher level of PtCho/PtE than solid tumors, but MCF-7 cells had lower level of PtCho/PtE than solid tumors. Both cells and solid tumors contained similar lengths of fatty acid (CH_2/CH_3) 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 more PLA_2 protein than in cells in culture, whereas PC-3 tumors had a lower level of PLA_2 than cells. PLA_2 is an enzyme that catalyzes phospholipids and releases lysophospholipids and arachidonic acid [10]. There was no consistent relationship between PtCho/PtE and PLA_2 protein level. The differences between cells 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.

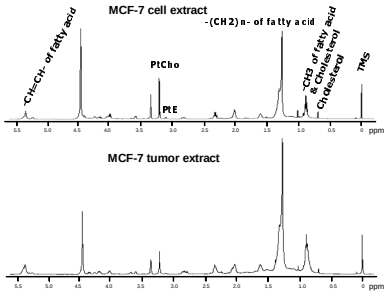

Figure 1: Representative ^{1}H MR spectra of lipid-soluble extract fractions from cells in culture and solid tumors.

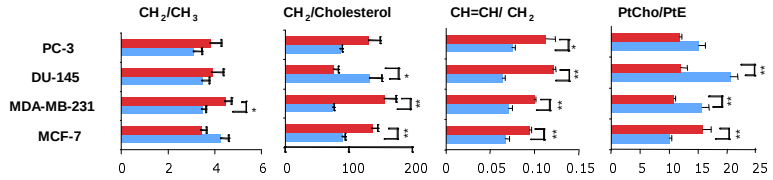

Figure 2: Ratios of lipid peaks from the ^{1}H MR spectra of cells in culture (■) and solid tumors (■). Values are mean ± standard error from more than 3 extracts. *: P < 0.5, ** < 0.01 (cells *versus* tumors)

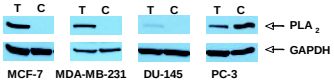

Figure 3: PLA_2 protein expression level. 40 µg of protein was loaded on 10% reducing SDS-PAGE gel. GAPDH: loading control. T: tumor, C: cell

References and Acknowledgements: [1] Mori N et al, *Molecular Imaging* 2, 124 (2003); [2] Aboagye E et al, *Cancer Res*, 59, 80 (1999); [3] Ackerstaff E et al, *Cancer Res* 61, 3599 (2001); [4] Kurhanewicz J et al, *Neoplasia* 2, 166 (2000); [5] Bhujwalla ZM et al, Magn Reson Med 41, 897 (1999) [6] Varga T et al, *Eur. J. Clin. Invest.* 38, 695 (2008); [7] Eyster K.M. *Adv. Physiol. Edu.* 31, 5 (2007); [8] Tyagi RK et al, *MRM* 35, 194 (1996); [9] Le Myoyec L et al, *NMR Biomed,* 13, 92 (2000); [10] Leslie CC, *J. Biol. Chem.* 272, 16709 (1997). This work was supported by NIH R01 CA73850 and P50 CA103175. We thank Dr. V.P. Chacko for expert NMR technical support, and Mr. G. Cromwell for assistance with the animal studies.