Comparison of fatty acid and phosphatidylcholine levels in breast and prostate cancer cells and tumors

N. Mori[1], F. Wides[1], T. Takagi[1], K. Glunde[1], and Z. M. 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: Increased lipid biosynthesis is one of the characteristic features of cancer. Elevated *de novo* fatty acid synthesis is necessary for rapidly proliferating tumor cells to continually provide lipids such as phospholipid for membrane production. Fatty acid synthase (FASN) is one of the important lipogenic enzymes in multiple mechanisms. FASN overexpression has been reported in many human cancers including breast, prostate, colorectum, bladder, ovary [1,2]. 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. 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 [3,4]. Here we have compared total fatty acid and phosphatidylcholine (PtCho) levels 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. Also, since most of fatty acid can be from *de novo* synthesis by FASN in cancer cells, we compared the FASN protein expression levels in nonmalignant and malignant human mammalian epithelial cells.

Methods: MCF-12A, nonmalignant human mammalian epithelial cells (HMEC), 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. MCF-12A cells were grown in DMEM-Ham's F12 medium with supplements as described [5]. All other 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 < 0.42 g. Both lipid and water-soluble cell and tumor extract fractions were obtained using a dual-phase extraction method as described previously [5]. The lipids were dissolved in 0.4 ml of deuterated chloroform and 0.2 ml 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 [6]. Total fatty acid and PtCho levels were quantified as A.U./cell or A.U./g by dividing the integrals of the 1.3 ppm and 3.2 ppm signals in the ^{1}H NMR spectra, respectively, by the integral of the standard signal and the respective cell number or tumor weight (g). Immunoblot analysis was performed using polyclonal FASN antibody (Abcam Inc.).

Results and Discussion: Representative examples of lipid spectra from MDA-MB-231 and PC-3 grown as cells and tumors are shown in Figure 1. The levels of PtCho and total fatty acid from lipid-soluble cell or tumor extracts derived from breast and prostate cancer cell lines are shown in Figure 2. Patterns of PtCho and fatty acid levels were similar in cells, but different in tumors. Both PtCho and fatty acid levels were significantly higher in MCF-7 cells compared to MCF-12A and MDA-MB-231 cells. Interestingly, the levels of PtCho and fatty acid in nonmalignant MCF-12A cells and malignant MDA-MB-231 cells were comparable. However, the level of fatty acid in MDA-MB-231 tumors was significantly higher than in MCF-7 tumors. It has been known that hypoxia and low pH conditions activate FASN activity [2]. FASN or/and other lipogenic enzymes in MDA-MB-231 tumors may be more sensitive to those microenvironmental factors. The fatty acid level of DU-145 cells was significantly higher compared with PC-3 cells. However, both PtCho and fatty acid levels in PC-3 and DU-145 tumors were not significantly different. As shown in Figure 3, FASN protein levels in MCF-12A and MCF-7 cells were not significantly different *in vitro*. Since MCF-12A medium contains epidermal growth factor, which activates FASN, we need to further investigate the involvement of FASN without its activators and with only *de novo* fatty acid. Our results revealed that fatty acid levels of cells and tumors do not show the same pattern. 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 in lipid metabolism observed between cells grown in culture as compared to the same cells grown as solid tumors.

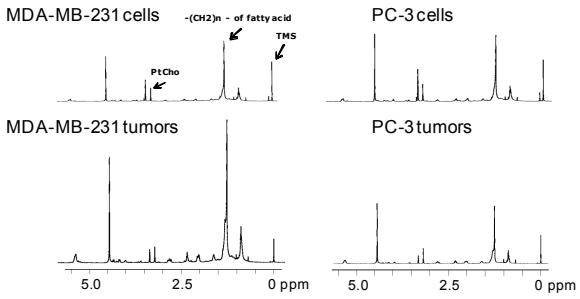

Figure 1: Representative ^{1}H MR spectra of lipid-soluble extract fractions from cells in culture and solid tumors.

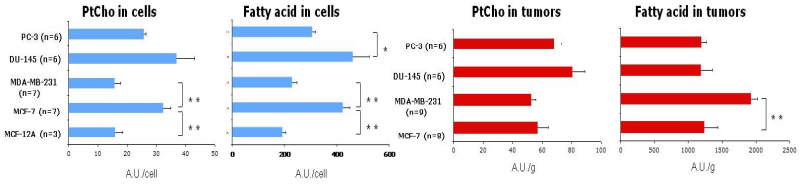

Figure 2: The levels of PtCho and total fatty acid of lipid signals from the ^{1}H MR spectra of cells in culture (■) and solid tumors (■). Values are mean ± standard error from more than 3 extracts. *: P < 0.05. ** < 0.01

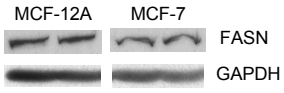

Figure 3: FASN protein expression levels in 2 cell lysates/cell line. 30 µg of protein was loaded on 7.5% reducing SDS-PAGE gel. GAPDH: loading control.

References and Acknowledgements: [1] Menendez JA et al, Nature reviews 7, 763 (2007), [2] Mashma T et al, British J of Cancer 100, 1369 (2009), [3] Varga T et al, *Eur. J. Clin. Invest*. 38, 695 (2008); [4] Eyster K.M. *Adv. Physiol. Edu.* 31, 5 (2007); [5] Glunde K et al, *Cancer Res*, 65, 11034 (2005); [6] Le Myoyec L et al, *NMR Biomed,* 13, 92 (2000); 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.