Tumor microenvironment alters choline phospholipid metabolite levels

N. Mori[1], K. Glunde[1], Z. M. Bhujwalla[1]
[1]Radiology Department, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Introduction

The increase of cellular phosphocholine (PC) and total choline-containing compounds (tCho) is one of the most widely established characteristics of cancer [1-3]. This elevation is closely related to malignant transformation, invasion, and metastasis. 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 between malignant *versus* benign lesions using ^{1}H MRSI. A solid tumor, however, is a complex system with a unique environment frequently containing hypoxic regions. Cancer cell – endothelial cell interactions can also alter choline phospholipid metabolism [4]. Human tumor xenograft models in mice are useful to mimic the growth of human cancers. A comparison of choline phospholipid metabolites of cells in culture and solid tumors can reveal the importance of growth conditions *in vivo*, such as hypoxia and cancer cell-stromal/endothelial cell interaction, in influencing choline phospholipid metabolism.

Methods

Malignant human prostate cell lines PC-3 and DU-145 were purchased from American Type Culture Collection and grown in RPMI-1640 medium supplemented with 10% FBS and penicillin (100 units/ml) and streptomycin (100 ug/ml). Both cell lines were maintained in a humidified atmosphere with 5% CO_2 in air, at 37 ºC. PC-3 and DU-145 were cultured to 80 % confluence. Approximately 3 to 5x10^7 cells were harvested for cell extraction. PC-3 and DU-145 cells were inoculated in the flank of severe combined immunodeficient (SCID) mice. Solid tumors were extracted and freeze clamped immediately when tumor weights were 2.7 ± 0.05 g (PC-3, n=4) and 3.4 ± 0.04 g (DU-145, n=4). Both lipid and water-soluble cell and tumor extract fractions were obtained using a dual-phase extraction method as described previously [5]. 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 PC-3 and DU-145 cells. Both PC-3 and DU-145 cells in culture exhibited higher levels of PC as compared to solid tumors. Glycerophosphocholine (GPC) levels of cells in culture were either lower (PC-3) or comparable (DU-145) to the corresponding tumors. Total choline (tCho) levels of PC-3 cells in culture and solid tumors were similar while DU-145 cells contained a higher level of tCho as compared to solid tumors. The PC/GPC ratio from both PC-3 and DU-145 cell lines showed much higher levels in cell culture than in solid tumors as demonstrated in Figures 1 and 2.

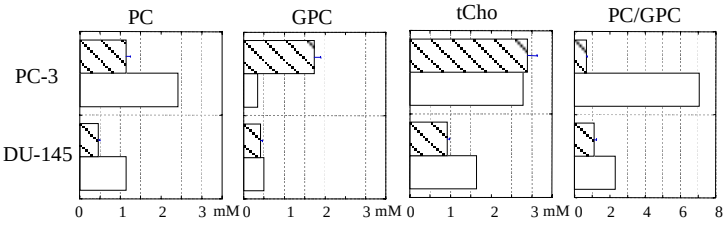

Figure 1: Quantitation of choline phospholipid metabolite levels from the ^{1}H MR spectra of PC-3 and DU-145 cells in culture (□) and solid tumors (▨). Values are mean ± standard error.

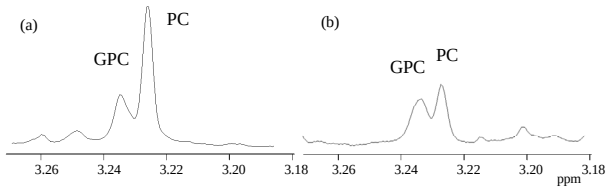

Figure 2: Representative ^{1}H MR spectra displaying the choline phospholipid metabolite region of DU-145 cells in (a) culture and (b) solid tumors.

Discussion

Our data demonstrate the significant differences of choline phospholipid metabolism between cells in culture and solid tumors. Solid tumors had lower levels of PC/GPC as compared to cells in culture. These differences can arise from environmental differences such as depletion of nutrients and oxygen, changes in pH [6], as well as cancer cell and stromal/endothelial cell interactions [4]. 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 [7].

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] Mori et al, *Molecular Imaging* **2**, 124, (2003); [5] Tyagi RK et al, *MRM* **35**, 194 (1996); [6] Galons JP et al, MRM 33, 422, 1995; [7] Rosi A et al, *NMR Biomed* **17**, 76 (2004). This work was supported by NIH 2RO1 CA 73850 and P50 CA103175. We thank Dr. V.P.Chacko for expert technical support and Mr. Gary Cromwell for inoculating the tumors.