Differences in phospholipid and lipid metabolism between cancer cells in culture and in solid tumors

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Introduction: Phosphatidylcholine (PtdCho) is the most abundant phospholipid in eukaryotic cell membranes that regulates membrane integrity and contributes to proliferative growth and programmed cell death [1]. 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 and acidic regions. In our ongoing studies we are characterizing phospholipid and lipid metabolisms of the water soluble (**Figure 1**) and lipid soluble (**Figure 2**) extracts from cells and solid tumors using ^{1}H MR spectroscopy. We have found that phosphocholine (PC):glycerophosphocholine (GPC) ratios in highly malignant breast and prostate cancer cells in culture were significantly higher than in the corresponding solid tumors. Consistently higher CH=CH/CH_2 in fatty acids 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. MDA-MB-231 showed the greatest difference in lipid metabolism between cells in culture and solid tumors. Here, to understand those differences we have compared protein levels of fatty acid synthase (FASN), phospholipase D1 (PLD1), and cytosolic PLA_2 (cPLA_2), and choline kinase-α (Chk-α), which are related to phosphatidylcholine (PtdCho) catabolic and biosynthetic pathways, in cells and solid tumors.

Methods: MDA-MB-231, a triple 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. For solid tumor studies, cells were inoculated in the mammary fat pad (breast cancer cells) or the flank (prostate cancer cells) of severe combined immunodeficient (SCID) mice. For MCF-7 tumors, a 17β-estradiol pellet (Innovative Research of America) was inoculated prior to cancer cell inoculation. Solid tumors were harvested and immediately freeze-clamped when tumor weights were < 0.45 g. Immunoblot analysis was performed using a custom-made Chk-alpha antibody (Proteintech Group, Inc.), cPLA2 and FASN (Santa cruz biotechnology, Inc.), and PLD-1 (Abcam). Anti-GAPDH antibody (Molecular Probes, Eugene) was used for equal loading assessment.

Results and Discussion: We used the 2 breast cell lines, MDA-MB-231 and MCF-7, and 2 prostate cell lines, PC-3 and DU-145 to compare differences in phospholipid and lipid metabolisms between cells in culture and solid tumors. **Figure 3A** shows the immunoblot assay results with Chk-alpha, cPLA2, PLD1, and FASN antibodies. 2 prostate cancer samples, or 2 breast cancer samples were loaded on the same gel. All of cell lines showed higher levels of Chk-alpha protein in cells than in corresponding tumors. This may explain the previous results that PC/GPC values in all cell lines in culture were higher than in corresponding tumors. DU-145, MDA-MB-231 and MCF-7 showed higher levels of cPLA2 in tumors than in corresponding cells, and showed higher levels of PLD1 in cells than in corresponding tumors. PC-3 showed comparable level of cPLA2 in cells and tumors and showed higher level of PLD1 in tumors than in corresponding cells. The levels of FASN were higher in PC-3 cells and MCF-7 cells than in tumors. Since levels of FASN in DU-145 and MDA-MB-231were lower than in other cancer cell lines, and we could not detect visible bands, those samples were loaded again separately (**Figure 3B**). MDA-MB-231 had higher level of FASN protein in cells than in tumors, and DU145 had slightly higher or comparable level of FASN in cells than in tumors. 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. Although both cells in culture and solid tumors are useful tool for investigation, it is important to consider differences between them to unravel microenvironmental influences *in vivo* to understand phospholipid and lipid metabolism in cancer.

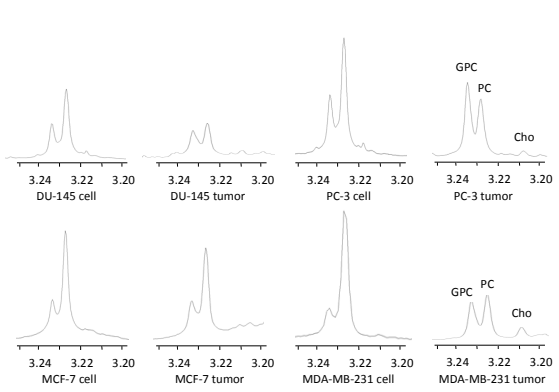

Figure 1. Representative ^{1}H MR spectra obtained from water-soluble extracts. Spectra were acquired on a Bruker Avance 500 spectrometer and are expanded to display signals from 3.20 ppm to 3.25 ppm.

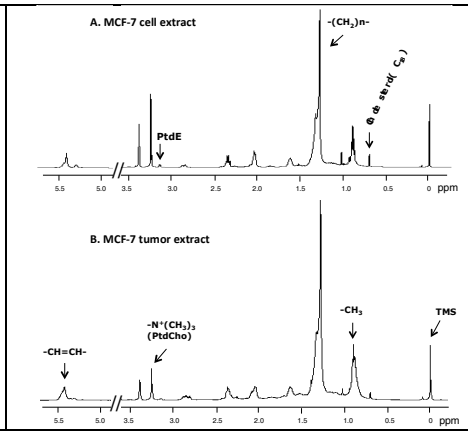

Figure 2. Representative ^{1}H MR spectra obtained from lipid-soluble extracts. Spectra were acquired on a Bruker Avance 500 MR spectrometer.

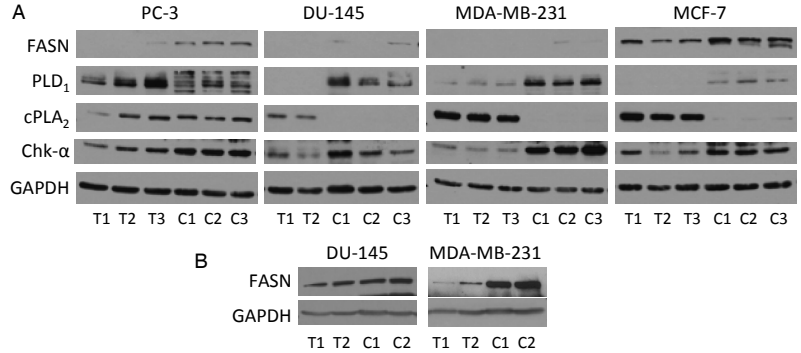

Figure 3. (A) Protein levels of FASN, PLD1, cPLA_2, and Chk-α in cells (notated C) and solid tumors (notated T) determined by immunoblot assay. **(B)** Protein levels of FASN in DU-145 and MDA-MB-231 cells (notated C) and solid tumors (notated T). 50μg of protein on a 10% reducing SDS-PAGE gel.

References and Acknowledgements: [1] Ridgway, N.D., Crit Rev Biochem Mol Biol, 2013. **48**(1): p. 20-38.

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