Monitoring the Microenvironmental Effects on Choline Metabolism in Human Breast Cancer Cells

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Introduction: The most abundant soluble metabolites of choline in mammalian cells are phosphocholine (PCho) and glycerophosphocholine (GPCho), which serve as a precursor and a degradation product of phospholipids, respectively. MRS studies of choline metabolism in human breast cancer cells, in biopsy specimens and in patients have indicated that malignant transformation of the breast is associated with the accumulation of these water soluble choline metabolites. We have previously explored the molecular basis and biochemical pathways responsible for the augmentation of PCho in various human breast cancer cell lines (1). Further studies of choline metabolites in orthotopic breast cancer xenografts have indicated that the microenvironmental conditions affect choline metabolism leading to changes in PCho and GPCho levels. We therefore focus herein on real-time ^{31}P MRS measurements of the level of these metabolites in perfused breast cancer cells subjected to microenvironmental changes such as acidosis and hypoxia.

Methods: MCF7 and MDA-MB-231 breast cancer cells were cultured on agarose polyacrolein microcarrier bead, precoated with collagen as previously described (2). The cells were continuously perfused with a perfusion system described earlier (2). ^{31}P high-resolution spectra of the perfused cells were recorded on a DMX-500 Bruker Avance (Bruker, Karlsruhe, Germany), operating at 11.7 Tesla, using 60° excitation pulse, a repetition time of 2 sec, time domain of 16K, acquisition time of 0.8 sec and continuous composite pulse proton decoupling. The ^{31}P NMR spectra were analyzed for intensity, line width and area using the DMFIT program (3). The extracellular and intracellular pHs were determined using pH calibration curves of Pi and PCho chemical shift, respectively. Cells and tumor extracts were obtained as previously described (1, 4). ^{31}P spectra were recorded on AV-500 Bruker Avance using the same sequence parameters as for the perfused cells.

Results: Analysis of choline metabolites levels in extracts of tumors and cells of the same origin cultivated under standard and well controlled conditions have indicated major differences between the levels *in vivo* and *in vitro* (Figure 1A and B). To overcome variations due to the use of extracts we have monitored the same cells keeping them viable and metabolically active throughout the experiments. This enabled us to quantify the level of PCho, GPCho and NTP as well as monitor the extra and intracellular pH of the cells during standard conditions, acidosis and hypoxia. Acidic conditions induced two fold decrease in PCho level as compared to standard conditions (Figure 2A). Upon returning to standard perfusion conditions the level of the PCho also returned to the initial control values. Further application of hypoxic conditions induced a small (~30%) but significant increase in PCho level (Figure 2A), whereas NTP level remained stable throughout the entire experiment. The level of GPCho was low of the order of the noise level in most of the spectra and thus, no significant change could be detected. The change in the external basic pH under hypoxia (due to the lack of CO_2) did not significantly change the internal pH (Figure 2B) and did not affect NTP level. When cells were perfused under hypoxic conditions with glucose free medium PCho continued to increase and NTP decreased by ~ 30%. Our MRS studies of extracted breast cancer cells subjected to 12 h of acidosis or hypoxia indicated similar changes for PCho and also provided enough S/N to quantify changes in GPCho. Under acidosis, PCho level decreased and GPCho level increased while under hypoxia the levels of both PCho and GPCho increased.

Conclusion: The microenvironmental conditions in tumors can markedly modulate the levels of choline metabolites, in addition to their regulation by gene expression of choline transporters and of the anabolic and catabolic enzymes involved in choline derived phospholipids synthesis in the course of breast malignant transformation. Interestingly, acidosis and hypoxia differentially affect choline metabolism suggesting different mechanisms of action for these stressful conditions.

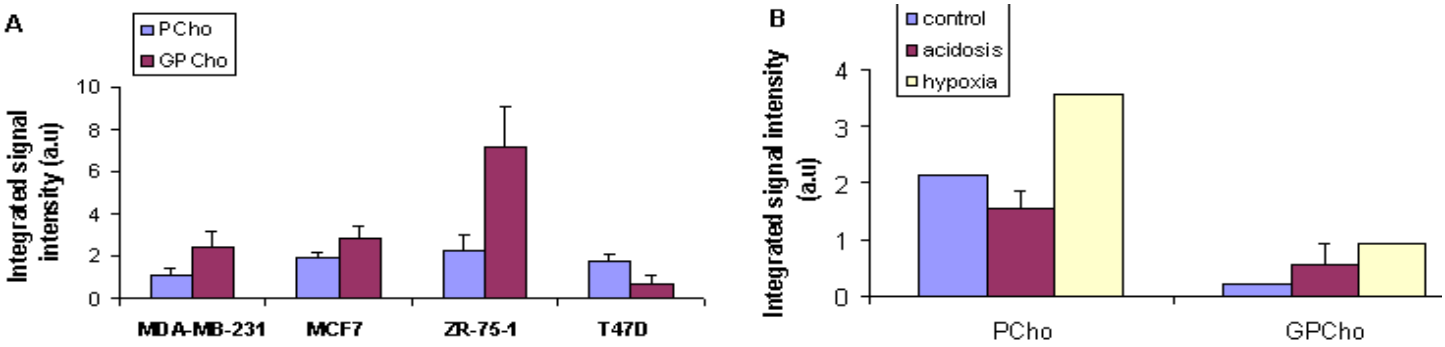

Figure 1: PCho and GPCho levels. A – in extracted orthotopic breast cancer xenografts. B – in extracted MDA-MB-231 breast cancer cell line.

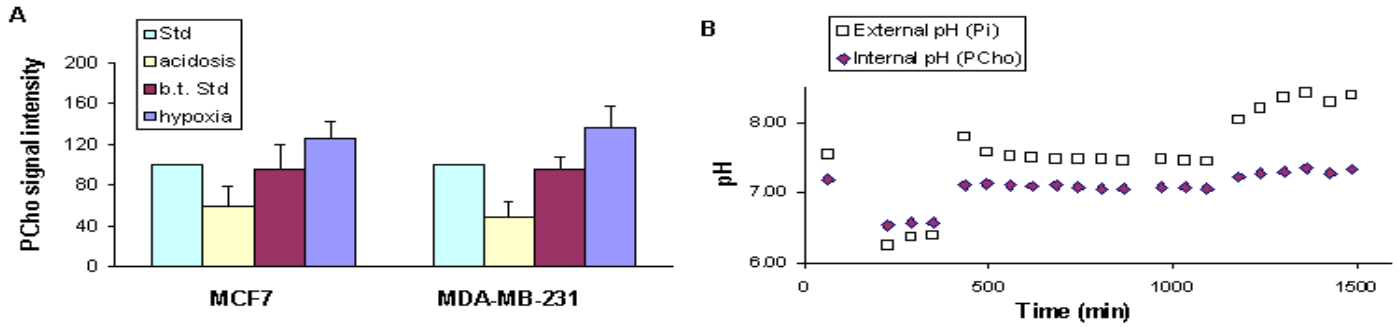

Figure 2: PCho level and pH under standard (Std), acidic and hypoxic conditions in perfused MDA-MB-231 cells cultivated on beads. A – PCho integrated signal intensity as determined by the DMFIT program in MCF7 and MDA-MB-231. B – External and internal pH values as determent by the chemical shift of Pi and PCho, respectively.

References:

1. Eliyahu G et al. International Journal of Cancer 120(8):1721-30 (2007); 2. Degani H et al. NMR in Physiology and Biomedicine, Academic Press 329-351 (1994); Massiot D et al. Magnetic Resonance in Chemistry 40(1): 70-76 (2001); **4.** Katz-Brull R et al. Cancer Research 62 (7): 1966-1970 (2002).