Choline Metabolism in Breast Cancer; The Influence of the Microenvironmental conditions

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Introduction: Choline is a dietary component essential for normal function of all cells. It directly affects cell signaling and lipid metabolism while its metabolites assure the structural integrity and signaling functions of cell membranes. The most abundant soluble metabolites of choline in mammalian cells are phosphocholine (PCho) and glycerophosphocholine (GPCho) which are a precursor and a degradation product, respectively, of choline phospholipids. MRS studies of choline metabolism in human breast cancer cells, breast biopsy specimens and breast lesions of patients have suggested that malignant transformation of the breast is associated with the accumulation of water soluble choline metabolites. Indeed, the presence and changes in the composite choline signal in breast lesions helps to improve diagnosis and therapy management of this disease. We have previously explored the molecular origins and biochemical pathways responsible for the augmentation of choline transport and metabolism in human breast cancer cells (1). Inhere we present MRS studies aimed at characterizing choline metabolism in human breast cancer tumors implanted in the mammary gland of mice and the effect of the microenvironmental conditions on this metabolism.

Methods: Human breast cancer cells (MDA-MB-231, MCF7, ZR-75-1, and T47D) were cultured under standard conditions (1). Orthotopic tumors of these cells were implanted in the mammary gland of SCID mice and were grown for several weeks before extracting the tumors. Extracts of breast cancer cells and tumors were prepared using the dual phase method (2). ^{1}H and ^{31}P high-resolution spectra of the extracted tumors were recorded on a DMX-500 spectrometer (Bruker, Germany). ^{31}P NMR spectra were recorded at 202.4 MHz, using 45° excitation pulse and a repetition time of 2 sec. The concentrations of PCho, GPCho, and NTP were determined relative to an internal reference, phenylphosphonic acid (PPA). In order to correct for T_1 saturation effects, ^{31}P T_1 relaxation rates of PCho, GPCho, and PPA were measured independently by the inversion-recovery method. High-resolution ^{1}H NMR spectra of the same extracts were recorded at 500 MHz, using 90^0 pulses, with repetition time of 10 sec (fully relaxed conditions). The concentrations of choline, PCho, and GPCho were determine in reference to an external standard, 3-(trimethylsilyl)-propionic acid (TSPA). The influence of the microenvironmental conditions (i.e. acidosis, hypoxia, nutrient deprivation, and confluency) on choline metabolism was studied in extracted cells and in living cells cultured on agarose beads and perfused in the spectrometer at 36^0C.

Results and discussion: Quantification of PCho and GPCho in the ^{31}P spectra of all tumors yielded an average of 1.8 ± 0.16 and 3.3 ± 0.55 μmol/g wt, respectively (n=25). ^{1}H spectra enabled us to detect choline, however, in most tumors this signal was below detection level or at very low intensity. Unexpectedly, GPCho signal was higher then that of PCho in three out of the four types of tumors (except T47D tumors), and varied between the different types of tumors (Fig 1A). This finding was in contrast to the results obtained in the extracted cell lines, which exhibited high level of PCho and very low level of GPCho. This distinct difference between cells and tumors of the same origin could stem from the different microenvironment conditions of cancer cells cultured in a dish as a monolayer and cancer cells growing as a tumor *in vivo,* affected by heterogeneous perfusion. Hence, we further studied the influence of the microenvironmental conditions on choline metabolism in perfused MDA-MB-231 and MCF7 cells, as well as in extracts of these cells. When acidic conditions were applied to the cells (pH ~6), PCho level decreased whereas GPCho level increased as compared to standard conditions. These changes were more prominent in MDA-MB-231, which also showed a decrease in NTP level upon acidification, and a substantial decrease in the internal pH. When these cells were subjected to hypoxic conditions PCho level increased while GPCho level decreased as compared to standard conditions. Moreover, perfusion of MCF7 cells with glucose free medium, maintaining the hypoxic conditions, further elevated PCho level and decreased GPCho level below detection, whereas NTP decreased by less than 30%. Similar results were obtained in extracted cell lines subjected to acidosis and hypoxia (Figure 1B). Moreover, in MDA-MB-231 and MCF7 cells cultured under confluence conditions PCho decreased and GPCho increased as was found in the tumors. In conclusion, the heterogeneous microenvironmental conditions in tumors affect choline metabolism via altered activity of transporters and enzymes involved in the anabolism and catabolism of phospholipids leading to variations in the levels of PCho and GPCho and to dramatic modulations in their concentration ratio. The changes in the level of each metabolite appear to be independent of each other. In addition, this study reveals the importance of defining growth conditions in metabolic studies of cultured cells *in vitro* and tumors *in vivo*.

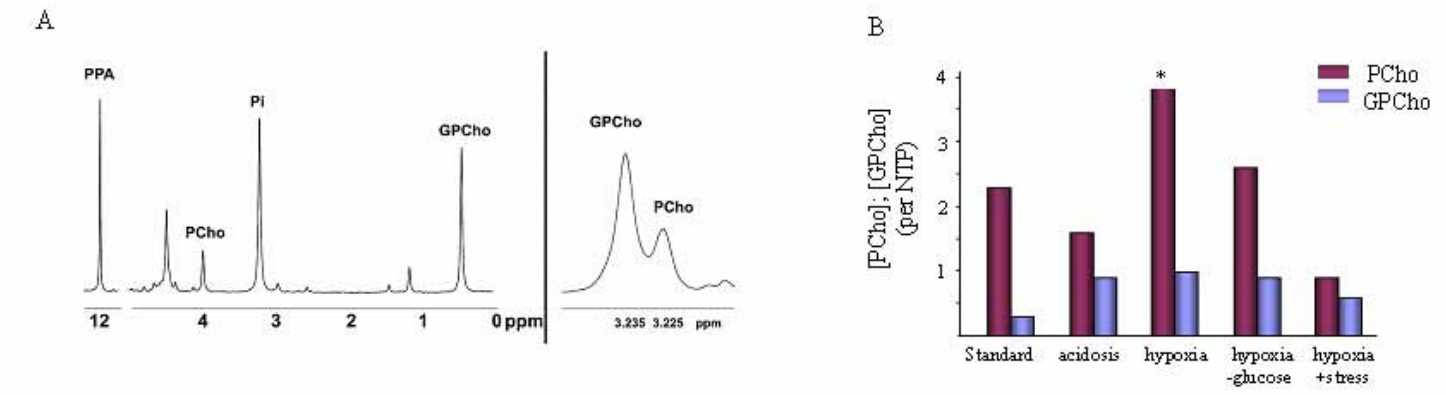

Figure 1: PCho and GPCho level in MDA-MB-231. A - ^{31}P (left) and ^{1}H (right) spectra of orthotopic human breast tumor implanted in SCID mice, focus on choline-derived metabolites. PPA – phenylphosphonic acid; PCho – phosphocholine; Pi – free phosphate; GPCho – glycerophosphocholine. B – PCho and GPCho in cultured cells subjected to acidosis (12 h), hypoxia (12 h), hypoxia in glucose free medium (4 h), and hypoxia in stressed medium (12 h) as compared to these cells cultured under standard conditions. (* - The level of PCho/NTP under hypoxia was ~ 7).

References:

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