# High Phosphocholine in Breast Cancer; Metabolic-Molecular Elucidation

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**Introduction:** Magnetic resonance spectroscopy (MRS) revealed the presence of high level of phosphocholine (PCho) in various human breast cancer cells relative to human mammary epithelial cells (HMEC) (1-3) as well as in breast cancer biopsies relative to benign breast lesions (4). It was further shown using proton MRS that the methyl-choline signal is elevated in breast cancer and can serve as a diagnostic marker for differentiating malignant from benign lesions (5 and references cited therein). Although there have been several investigations of the biochemical origin of these observations (6-8), the molecular basis and importance of PCho in malignant transformation are still not fully understood. We present here comprehensive investigations of the biochemical and molecular processes (Figure 1) that regulate PCho levels in various human breast cancer cells and HMEC.

**Methods:** Breast cancer cell lines: MCF-7, T47D, ZR-75-1 (ER positive), MDA-MB-231, SKBR-3 (ER negative), as well as HMEC were cultured routinely using standard media. PCho levels were determined in extracts of the breast cancer cells and HMEC, cultivated for 24 h before the extraction with medium containing 100μM choline in order to create saturated conditions for choline transport (2).

High resolution $^{31}$P NMR spectra were recorded on a DMX-400 spectrometer (Bruker) at 162 MHz by applying $45^0$ pulses, a repetition time of 5 s, and continuous composite pulse proton decoupling. Quantification was based on measuring the area under the PCho peak / the area under γNTP.

Choline transport rates were measured by adding various concentrations of choline (1–175μM) to the medium and trace amounts of [*methyl*-$^{14}$C]choline chloride. Following measurements of initial transport rates the kinetic parameters were calculated using Stein's method for zero-*trans* transport (2, 9). Choline transporter expression was determined by RT-PCR using specific primers for hCHT1, OCT-1, OCT-2 and CTL1. Choline kinase activity was measured using [*methyl*-$^{14}$C]choline chloride, and a phase separation method (10). Phospholipase D *(PLD) activity* was determined *in vitro* with fluorescent-phosphatidylcholine ($C_6$-NBD-PtdCho) as substrate, measuring the production of $C_6$-NBD-phosphatidylpropanol ($C_6$-NBD-PPr) (11). CTP**:**phosphocholine cytidylyltransferase (CCT) activity was performed as described by Bodennec et al (12).

Results and discussion: $^{31}$P NMR data confirmed the presence of high PCho level in all human breast cancer cells as compared to HMEC. The elevation of PCho relative to HMEC varied from 5 to 16 fold, depending on the cell line, although the external choline concentration was high and constant (Figure 2A). Choline kinase activity increased by 2-5 fold in the breast cancer cells relative to HMEC (Figure 2B). Choline transport measurements revealed the presence of facilitated transport (with distinct Km and Vmax of transport) and diffusional like transport. The cancer cells exhibited increased maximum velocity of choline transport ($V_{max}$) relative to HMEC (Figure 2C). HMEC exhibited a high diffusion constant, which was effective only in non physiological conditions. However, only minor differences in the activity of PLD and CCT were found between the cancer cells and HMEC. Hence, increased choline transport rate and choline kinase activity were found to be the main contributors to the elevated level of PCho in breast cancer cells. Moreover, choline transport was found to be the rate limiting step of PCho synthesis and consequently, Vmax of transport and PCho levels were highly correlated (Figure 2D). In contrast, choline kinase activity did not show a significant correlation with PCho level. In view of the importance of the up regulation of choline transport in breast cancer we focused on investigating the four known choline transporters: hCHT1, High-affinity choline transporter, typical to cholinergic neurons and the additional choline transporters, OCT-1, OCT-2 and CTL1 known to be expressed in various tissues. Semi quantitative RT-PCR studies of all choline transporters showed that the high-affinity choline transporter hCHT1 was expressed in breast cancer cells but not in HMEC. The other choline transporters OCT-1, OCT-2 and CTL1 were variably expressed in both the normal and cancerous breast cells.

**Conclusion:** Malignant transformation of breast epithelial cells results in the up-regulation of choline transport and choline kinase activity leading to the presence of elevated PCho. The transport is the rate limiting step which determines PCho level. Its upregulation in breast cancer appears to be associated with induced expression of the high affinity choline transporter. These results suggest that differences in the integrated intensity of the choline signal in breast cancer lesions reflect variations in the choline transporter expression.

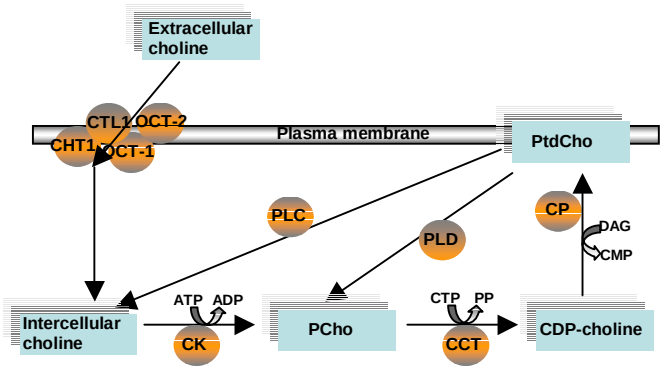


**Figure 1:** Transport and metabolism of choline through the Kennedy pathway: PCho- phosphocholine, PtdCho- phosphatidylcholine, CK- choline kinase, CCT- CTP**:**phosphocholine Cytidylyltransferase, CP- cholinephosphotransferase, PLD- phospholipase D, PLC- Phospholipase.

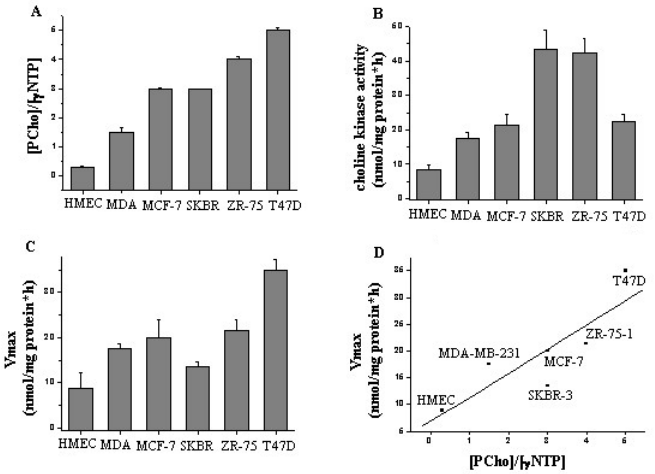


**Figure 2:** PCho level (**A**), choline kinase activity (**B**), maximum velocity (Vmax) of choline transport (**C**), and the correlation between Vmax and PCho levels ($R^2$=0.74) (**D**) in various human breast cancer cell lines and in HMEC.

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