Mechanisms of Indomethacin-Induced Alterations in Choline Phospholipid Metabolism of Non-malignant versus Malignant Human Mammary Epithelial Cells

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Abstract

Distinct differences characterize the choline metabolite profile of malignant human mammary epithelial cells (HMECs) compared to normal HMECs. Treatment with the non-steroidal anti-inflammatory agent indomethacin changes this profile toward a pattern more typical of non-malignant HMECs. Metabolites produced from [1,2-^{13}C]-choline using ^{1}H and ^{13}C NMR spectroscopy, and gene expression levels using microarray technology were analyzed following indomethacin treatment of breast cancer cells. Indomethacin induced diverse changes at the gene expression level. Enzymes involved in choline metabolism were not affected at the transcriptional level. Changes in choline phospholipid metabolites following indomethacin treatment were most likely due to increased membrane turnover.

Introduction

Breast carcinogenesis is accompanied by increasing phosphocholine (PC) and decreasing glycerophosphocholine (GPC) levels [1]. Indomethacin, a non-steroidal anti-inflammatory agent and non-specific cyclooxygenase (COX) inhibitor, has been shown to change this profile toward a pattern more typical of non-malignant HMECs [2]. Indomethacin also reduced the invasive and metastatic behaviour of human breast cancer cells, and decreased tumor angiogenesis and growth [3]. To understand mechanisms underlying the increase of GPC relative to PC following treatment with indomethacin in HMECs, we performed ^{1}H and ^{13}C NMR spectroscopy of cells labeled with [1,2-^{13}C]-choline and as well microarray-based gene expression analysis of breast cancer cells treated with indomethacin. The non-malignant human mammary epithelial cell line MCF-12A was compared with the human breast cancer cell lines MCF-7 and MDA-MB-231. Long-term and short-term incubation with [1,2-^{13}C]-choline was performed to distinguish between anabolic and catabolic pathways of choline metabolism.

Methods

For long-term experiments, cells were exposed to fresh cell culture medium containing 100 µM [1,2-^{13}C]-choline for 24 h to build up a labeled phosphatidylcholine (PtdCho) pool. For short-term experiments, fresh medium without labeled choline was added. Then, cells were incubated with 300 µM indomethacin in medium containing 100 µM [1,2-^{13}C]-choline for 3 h. Lipid and water-soluble cell extract fractions were obtained using a dual-phase extraction method [4]. The samples were dissolved in deuterated solvents. Composite pulse ^{1}H-decoupled ^{13}C NMR spectra and fully relaxed ^{1}H NMR spectra of the water-soluble metabolites and the lipids were measured on a 500 MHz MSL Bruker spectrometer. ^{13}C NMR spectra were corrected for saturation and NOE effects. Total RNA was isolated from MDA-MB-231 cells following 2 h indomethacin treatment. Microarray analysis was perfomed with the Human Genome U133 GeneChip Set containing 39,000 transcripts.

Results

^{13}C and ^{1}H NMR spectra of the water-soluble fractions demonstrated that both breast cancer cell lines, MCF-7 and MDA-MB-231, accumulated high PC levels, depleting most of the free intracellular choline (Cho) (Figure 1) whereas normal HMECs contained similar amounts of PC and free choline (Figure 1). The ^{13}C-enrichment in PC was significantly higher in long-term compared to short-term [1,2-^{13}C]-choline exposure in the breast cancer cells, but not in non-malignant cells. During long-term [1,2-^{13}C]-choline exposure, all HMEC lines built up fractional ^{13}C-enrichment in the PtdCho pool, as calculated from ^{13}C NMR spectra of the lipid fractions. Short-term exposure resulted in no detectable ^{13}C-enrichment in the PtdCho pool. Treatment with 300 µM indomethacin for 3 h caused a significantly decreased [PC]/[GPC] ratio in all three HMEC lines as observed in ^{1}H NMR spectra of long-term as well as short-term experiments. The ^{13}C-enrichment in the PC pool remained relatively constant during indomethacin treatment compared to control in the long-term experiments whereas it decreased in the short-term experiments. Indomethacin treatment also resulted in increased free choline levels in the breast cancer cell lines which was not detected in non-malignant HMECs. Results of the microarray analysis of gene expression after 2 h treatment with indomethacin revealed that 86 cDNA/genes, such as transcription factors, differentiation factors, cytokines, and transporters were least twofold over- or underexpressed after 2 h of indomethacin treatment. Twofold or higher changes were not detected in the messenger RNA expression levels of genes/proteins directly involved in choline phospholipid metabolism.

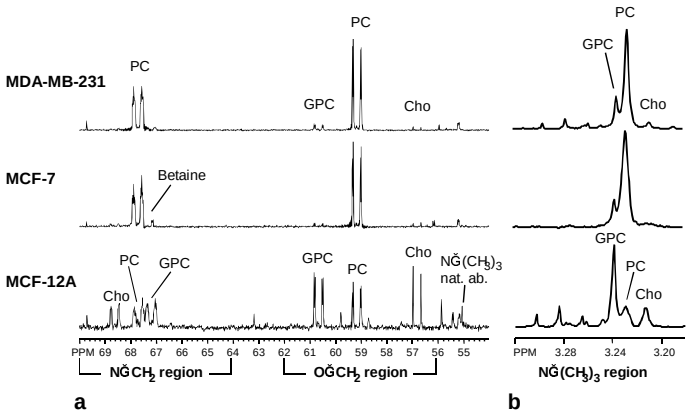

Figure: Representative ^{13}C (a) and ^{1}H (b) NMR spectra of the water-soluble fractions of non-malignant HMECs (MCF-12A: lower panel) compared to human breast cancer cells (MCF-7: central panel, MDA-MB-231: upper panel) after long-term exposure (24+3h) to [1,2-^{13}C]-choline

Discussion

The depletion of free choline and the appearance of PC in MCF-7 and MDA-MB-231 cells support previous observations [5] that breast cancer cells exhibit a higher rate of choline phosphorylation by choline kinase compared to normal HMECs (Figure 1). The reduction of the fractional ^{13}C-enrichment of the PC pool in the short-term experiments in malignant HMECS may be due to a higher phospholipase D or C activity in these cancer cells.

Treatment with indomethacin resulted in a decreased [PC]/[GPC] ratios in both normal and malignant HMECs as reported previously [2]. Free choline that most likely originated from catabolic processes increased after indomethacin treatment in the breast cancer cells but not in the non-malignant HMECs. The decrease in total PC and the absence of free choline in the short-term ^{13}C NMR spectra suggest that indomethacin also upregulated the anabolic pathway.

The microarray-based gene expression analysis revealed that mRNA for none of the enzymes that are directly involved in choline phospholipid metabolism were over- or underexpressed by twofold or higher at the gene expression level. Changes in choline phospholipid metabolites most likely occurred from changes in enzyme activity rather than enzyme expression. However, twofold or higher over- or underexpression was detected in 86 cDNAs/genes after 2 h of indomethacin treatment of MDA-MD-231 human breast cancer cells suggesting that indomethacin causes diverse changes at the transcriptional level.

References

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