Neoplasia • Vol. 8, No. 9, September 2006, pp. 758–771 758

www.neoplasia.com

Mechanisms of Indomethacin-Induced Alterations in the Choline Phospholipid Metabolism of Breast Cancer Cells[1]

Kristine Glunde, Chunfa Jie[†] and Zaver M. Bhujwalla**

*MR Oncology Section, Division of MR Research, The Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; [†]McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Abstract

Human mammary epithelial cells (HMECs) exhibit an increase in phosphocholine (PC) and total choline-containing compounds, as well as a switch from high glycerophosphocholine (GPC)/low PC to low GPC/high PC, with progression to malignant phenotype. The treatment of human breast cancer cells with a non-steroidal anti-inflammatory agent, indomethacin, reverted the high PC/low GPC pattern to a low PC/high GPC pattern indicative of a less malignant phenotype, supported by decreased invasion. Here, we have characterized mechanisms underlying indomethacin-induced alterations in choline membrane metabolism in malignant breast cancer cells and nonmalignant HMECs labeled with [1,2-^{13}C]choline using ^{1}H and ^{13}C magnetic resonance spectroscopy. Microarray gene expression analysis was performed to understand the molecular mechanisms underlying these changes. In breast cancer cells, indomethacin treatment activated phospholipases that, combined with an increased choline phospholipid biosynthesis, led to increased GPC and decreased PC levels. However, in nonmalignant HMECs, activation of the anabolic pathway alone was detected following indomethacin treatment. Following indomethacin treatment in breast cancer cells, several candidate genes, such as *interleukin 8*, *NGFB*, *CSF2*, *RHOB*, *EDN1*, and *JUNB*, were differentially expressed, which may have contributed to changes in choline metabolism through secondary effects or signaling cascades leading to changes in enzyme activity.

Neoplasia (2006) **8,** 758–771

Keywords: Breast cancer, choline compounds, anti-inflammatory agent, phospholipids, magnetic resonance spectroscopy.

Introduction

Proton and ^{31}P magnetic resonance spectroscopy (MRS) studies have detected high levels of phosphocholine (PC), phosphoethanolamine (PE), or both in most cancers, including breast cancer, whereas low levels of these metabolites have been found in corresponding normal tissues [1]. Consistently elevated PC and PE levels were observed in human breast cancer cells in culture [2,3], with PC and total choline-containing compounds (tCho) progressively increasing with malignancy [3]. An increased malignancy of breast cancer cells also resulted in higher levels of PC relative to glycerophosphocholine (GPC), as reflected by an increased PC/GPC ratio [3]. These increased PC levels in breast cancer cells can be attributed to an increased expression and/or activity of choline kinase [4,5], phospholipase D (PLD), or phospholipase C (PLC) [5,6], and/or to increased choline transport [7]. Transfection of malignant breast cancer cells by the metastasis-suppressor gene *nm23* significantly decreased the PC/GPC ratio [8], whereas an increase in PC levels was detected in NIH 3T3 cells transfected with the mutant *ras* oncogene [9], providing further evidence of a close link between choline phospholipid metabolites and malignancy. Treatment with antimicrotubule drugs significantly increased cellular GPC levels in several breast cancer cell lines [10], as did treatment with the non-steroidal anti-inflammatory agent, indomethacin [11,12]. Indomethacin increased GPC levels and decreased PC levels in breast cancer cells and in nonmalignant human mammary epithelial cells (HMECs). These data suggest that diverse genes and drugs profoundly alter choline phospholipid metabolism and result in common endpoints of change in PC and GPC.

The increase of GPC and the decrease of PC in indomethacin treatment suggest that choline compounds may be linked to inflammatory pathways [11,12]. Brain ^{1}H MRS studies of multiple sclerosis (MS) have demonstrated that an elevated choline signal was observed in inflammatory disease states [13]. Proton MRS of neuroblastoma cells treated with cyclooxygenase (COX) inhibitors demonstrated depletion of choline compounds [14]. Indomethacin is a nonsteroidal anti-inflammatory drug (NSAID) and a nonspecific COX (EC 1.14.99.1) inhibitor. Indomethacin inhibits COX-1 and COX-2 time-dependently by

Abbreviations: Cho, free choline; COX, cyclooxygenase; GPC, glycerophosphocholine; HMEC, human mammary epithelial cell; NSAID, nonsteroidal anti-inflammatory drug; MR, magnetic resonance; MRS, magnetic resonance spectroscopy; PC, phosphocholine; PE, phosphoethanolamine; PLA_2, phospholipase A_2; PLC, phospholipase C; PLD, phospholipase D; PtdCho, phosphatidylcholine; tCho, total choline-containing compounds

Address all correspondence to: Zaver M. Bhujwalla, PhD, Department of Radiology, Johns Hopkins University School of Medicine, 208C Traylor Building, 720 Rutland Avenue, Baltimore, MD 21205. E-mail: zaver@mri.jhu.edu

[1]This work was supported by the National Institutes of Health (R01 CA82337).

Received 24 February 2006; Revised 16 June 2006; Accepted 19 June 2006.

DOI 10.1593/neo.06187

noncovalently binding to the COX active site [15]. Treatment with indomethacin reduces the invasive and metastatic behaviors of human breast cancer cells [16]. Indomethacin was also shown to reduce angiogenesis [17] and tumor growth [18].

In normal tissues, arachidonic acid, a key mediator of inflammation, is released from membrane phosphatidylcholine (PtdCho) by phospholipase A_2 (PLA_2) (Figure 1) in response to tissue injury. Two isoforms of COX, COX-1 and COX-2, catalyze the conversion of arachidonate to prostaglandin endoperoxide H_2 (PGH_2) in a two-step reaction: by acting as a COX and then by exhibiting peroxidase activity. PGH_2 is used as an immediate substrate for a series of cell-specific prostaglandin and thromboxane synthases, which eventually synthesize different eicosanoids [19,20]. The constitutive form of COX, COX-1, is significantly overexpressed in malignant *versus* nonmalignant HMECs [11]. The inducible form of COX, COX-2, which is regulated by cytokines, growth factors, tumor promoters, and hypoxia, was shown to have high expression levels in a wide variety of human and animal tumors [21]. Increasing evidence suggests that COX-2 overexpression is caused by disturbances of cellular signaling cascades, such as the Ras–Raf–MAPkinase cascade, due to oncogenic gene mutations [21].

Recently, it was shown that the effect of indomethacin on choline metabolite profile in HMECs may be partly mediated through the upregulation of the metastasis-suppressor gene *nm23* [11]. Previous studies have demonstrated the utility of [1,2-^{13}C]choline, in combination with ^{13}C MRS, to the study of choline metabolism [5,22]. In this study, the ^{1}H and ^{13}C MRS of HMECs labeled with [1,2-^{13}C]choline was performed to further understand the mechanisms underlying the increase of GPC relative to PC, following treatment with indomethacin in breast cancer cells and HMECs. The spontaneously immortalized nonmalignant HMEC line MCF-12A was compared with the estrogen receptor–negative, highly invasive, and metastatic human breast cancer line MDA-MB-231. Long-term and short-term incubations with [1,2-^{13}C]choline were performed to distinguish between the anabolic and catabolic pathways of choline metabolism, as previously described [5]. A microarray-based gene expression analysis with the Human Genome U133 Set (Affymetrix, Inc., Santa Clara, CA) was performed to probe more than 39,000 transcripts derived from approximately 33,000 well-substantiated human genes [5]. This microarray analysis using the Affymetrix set was used to determine changes in gene expression profiles between control and indomethacin-treated MCF-12A HMECs and MDA-MB-231 breast cancer cells.

Methods

Cell Lines

The spontaneously immortalized nonmalignant HMEC line MCF-12A, established from MCF-12M mortal cells [23],

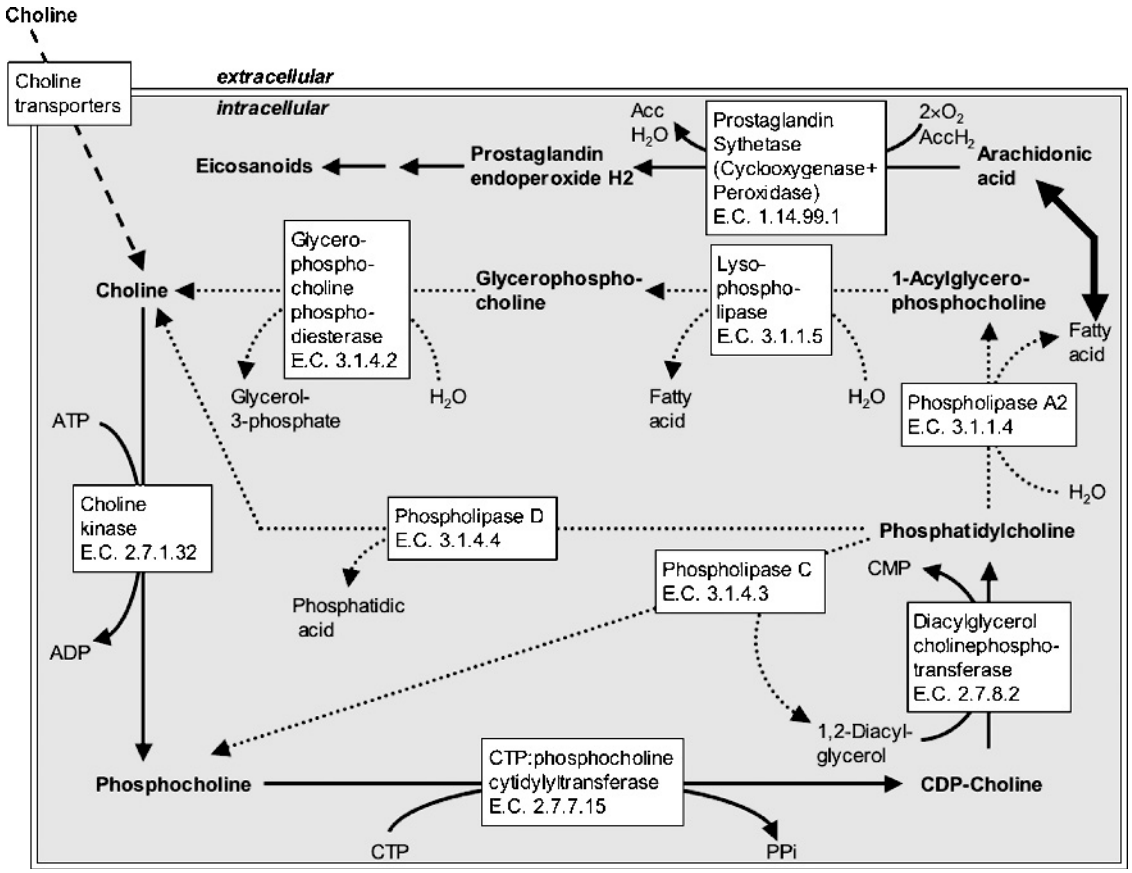

Figure 1. *Biosynthetic (solid lines) and catabolic (dashed lines) enzymatic reactions in PtdCho and arachidonic acid metabolism. CDP, cytosine diphosphate; CMP, cytosine monophosphate; CTP, cytosine triphosphate; PPi, pyrophosphate.*