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Choline Phospholipid Metabolism: A Target in Cancer Cells?

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Abstract The experience of treating cancer over the past several decades overwhelmingly demonstrates that the disease continues to evade the vast array of drugs and treatment modalities available in the twenty-first century. This is not surprising in view of the complexity of this disease, and the multiplicities of pathways available to the cancer cell to enable its survival. Although the progression of cancer arrives at a common end point of cachexia, organ failure, and death, common pathways are rare in cancer. Identifying and targeting common pathways that would act across these levels of multiplicity is essential for the successful treatment of this disease. Over the past decade, one common characteristic consistently revealed by magnetic resonance spectroscopic studies is the elevation of phosphocholine and total choline-containing compounds in cancer cells and solid tumors. This elevation has been observed in almost every single cancer type studied with NMR spectroscopy and can be used as an endogenous biomarker of cancer. In this article, we have summarized some of the observations on the choline phospholipid metabolism of cancer cells and tumors, and make a case for targeting the aberrant choline phospholipid metabolism of cancer cells. J. Cell. Biochem. 90: 525–533, 2003.

Key words: cancer; magnetic resonance; choline phospholipid metabolism

The elevation of phosphocholine (PC) and total choline is one of the most widely established characteristics of cancer cells. Increased choline levels have been detected in breast, prostate, and different types of brain tumors. Some of these data are summarized in Table Ia,b. Numerous in vivo and in vitro ^{1}H and ^{31}P MR spectroscopic studies have detected high levels of PC or phosphoethanolamine (PE), or both, in several cancers (see Table Ia,b), whereas low levels of these metabolites were found in corresponding normal tissues (reviewed in Negendank, 1992; de Certaines et al., 1993). These studies demonstrate the significance of phospholipid precursors and catabolites as biochemical indicators of tumor progression and response to therapy.

Particularly high levels of PC and PE were detected in human breast cancers as compared to normal breast tissue [Negendank, 1992; Leach et al., 1998]. Human breast cancer cells in culture exhibited consistently elevated PC and PE levels [Ting et al., 1996]. Immortalized, oncogene-transformed, or tumor-derived human mammary epithelial cells (HMECs), representing different stages of breast carcinogenesis, exhibited a step-wise increase of PC and total choline levels [Aboagye and Bhujwalla, 1999].

PC is both a precursor and a breakdown product of phosphatidylcholine (PtdCho), the most abundant phospholipid in biological membranes. PtdCho, together with other phospholipids, such as phosphatidylethanolamine and neutral lipids, forms the characteristic bilayer structure of the cell membrane and regulates membrane integrity [Cullis and Hope, 1991]. Biosynthesis and hydrolysis of PtdCho (see Fig. 1) are essential processes for mitogenic signal transduction events in cells [Cai et al., 1993]. Products of choline phospholipid metabolism, such as PC, diacylglycerol, and metabolites synthesized from arachidonic acid may

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TABLE Ia. An Overview of Choline Phospholipid Metabolite Levels Obtained From Cells. Data are From ^{1}H, ^{13}C, and ^{31}P NMR Spectroscopic Studies of Normal and Cancer Cells*

Cells	Metabolite	Normal	Cancer
Brain cell extracts, ^{1}H NMR, mean ± SD, [nmol/mg protein]		Schwann cells (rat)	Meningioma cells
[Bhakoo et al., 1996]	PC	9.1 ± 1.7	≥13.0 ± 3.2
	GPC	18.9 ± 2.7	≥4.2 ± 2.2
	tCho	33.1 ± 4.5	
			Glioblastoma cells
	PC		≥6.4
	GPC		≥2.3
			Neuroblastoma cells
	PC		≥10.3
	GPC		≥1.7
HMEC extracts, ^{1}H NMR, mean ± SE, [fmol/μm^3 or mM]	PC	0.027 ± 0.010	≥0.390 ± 0.021
[Aboagye and Bhujwalla, 1999]	GPC	0.0307 ± 0.0067	≥0.037 ± 0.016
	tCho	0.077 ± 0.022	≥0.491 ± 0.017
HMEC extracts, ^{31}P NMR, mean ± SD, [mol%]	PC	0.4 ± 0.2	≥5.8 ± 0.6
[Singer et al., 1995]	PE	6.7 ± 1.9	≥7.5 ± 1.0
	GPC	2.1 ± 0.4	≥3.1 ± 1.4
	GPE	2.2 ± 0.9	≥2.5 ± 1.9
Human prostate epithelial cell extracts, ^{1}H NMR, mean ± SE, [mM]	PC	0.0714 ± 0.0031	≥0.56 ± 0.12
[Ackerstaff et al., 2001]	GPC	0.0244 ± 0.0012	≥0.1437 ± 0.025
	tCho	0.1173 ± 0.0033	≥0.85 ± 0.15
Intact HMECs, ^{31}P and ^{13}C NMR, mean ± SE, [fmol/cell]	PC		>17
[Bogin et al., 1998]	GPC		2.5 ± 0.3
Intact HMECs, ^{31}P NMR, mean ± SE, [Met/NTP]	PC	<0.1	>1.3
[Ting et al., 1996]	PE	<0.2	>0.8
	GPC	<0.3	>0.4
	GPE	<0.2	>0.4
Intact HMECs, ^{31}P NMR, mean ± SD, [mol%]	PC		10.3 ± 3.4
[Singer et al., 1995]	PE		22.2 ± 2.7
	GPC		7.2 ± 2.4
	GPE		6.8 ± 1.0

*Abbreviations used: BPH, benign prostatic hyperplasia; Cho, free choline; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; NAA, N-acetyl aspartate; NTP, nucleoside triphosphate; PC, phosphocholine; PCr, phosphocreatine; PDE, phosphodiester; PE, phosphoethanolamine; Pi, inorganic phosphate; PME, phosphomonoester; tCho, PC + GPC + Cho; tCr, creatine + phosphocreatine.

function as second messengers. These second messengers are essential for the mitogenic activity of growth factors, particularly in the activation of the ras-raf-1-MAPK cascade and protein kinase C pathway [Cai et al., 1993]. The regulation of choline phospholipid metabolism can occur through growth factor stimulation, cytokines [Bogin et al., 1998], oncogenes [Aboagye and Bhujwalla, 1999; Ronen et al., 2001], and chemical carcinogens [Kiss et al., 1993]. Hypoxic and acidic environments, typically found in solid tumors, can also affect water-soluble phospholipid intermediates [Galons et al., 1995]. Choline phospholipid metabolism is also closely related to inflammatory pathways, since arachidonic acid is directly released from membrane PtdCho primarily by phospholipase A2 (PLA2), and secondarily by phospholipase C and phospholipase D (PLD) [Kaiser et al., 1990]. Subsequently, arachidonic acid is converted to a series of cell specific prostaglandins and eicosanoids [Lupulescu, 1996]. These eicosanoids play a role in cell motility, invasion, vascular characteristics, and metastatic dissemination [Noguchi et al., 1995]. The altered choline phospholipid metabolism observed in cancer cells can therefore impact upon several of the phenotypic characteristics of solid tumors.

In this article, we have summarized some of the observations made by us, and by other investigators, demonstrating the importance of choline phospholipid metabolites in cancer progression, invasion, and metastasis.

PROTON AND ^{31}P MRS OF CANCER

High resolution ^{1}H MR spectroscopy (MRS) of tissue and cell extracts can resolve individual choline phospholipid metabolites and can be used to quantify PC, glycerophosphocholine (GPC), and free choline (Cho) (Fig. 2). With ^{1}H MRS of intact cells or solid tumors, an unresolved signal of overlapping PC, GPC, and free choline resonances, referred to as total choline-containing metabolites (tCho) is observed. The increased total choline signal detected in vivo is primarily due to the increased PC signal (Fig. 2). In vivo ^{31}P MRS, although less sensitive, can

TABLE Ib. An Overview of Choline Phospholipid Metabolite Levels Obtained From Tissue *In Vivo* and *Ex Vivo*. Data are From ^{1}H and ^{31}P NMR Spectroscopic Studies of Tumors and Corresponding Normal Tissue*

Tissue/Organ	Metabolite	Normal	Cancer
Human brain tissue extracts, ^{1}H NMR		White matter	Astrocytoma III/IV
[μmol/g ww] [Podo, 1999]	tCho	1.79 ± 0.24	1.6 ± 0.5 to 3.0 ± 0.5
		Cortex	Anaplastic astrocytoma
	PE	1.06 ± 0.10	2.05 ± 0.41
	tCho	0.64 ± 0.10	1.326 ± 0.079
Human breast tissue extracts, ^{1}H	PC	0.029 ± 0.025	0.79 ± 0.55
NMR mean ± SD [μmol/g ww]	PE	0.16 ± 0.011	2.1 ± 1.1
[Gribbestad et al., 1999]	GPC	0.042 ± 0.036	0.28 ± 0.20
	tCho	0.087 ± 0.062	1.19 ± 0.64
Human breast tissue extracts, ^{31}P	PC	0.02 to 0.04	0.02 to 2.21
NMR [μmol/g ww] [Podo, 1999]	PE	0.08 to 0.12	0.15 to 2.91
Human liver tissue rxtracts, ^{31}P NMR	PC	0.17 ± 0.11	1.36 ± 0.50
[μmol/g ww] [Bell and Bhakoo, 1998]	PE	0.16 ± 0.10	2.47 ± 0.84
	GPC	2.46 ± 0.37	0.59 ± 0.15
	GPE	2.25 ± 0.46	0.57 ± 0.17
In vivo—clinical studies			
Brain, ^{1}H MRS	Volume of regions with metabolic abnormalities, based on indices obtained from tCho, tCr,		
[Li et al., 2002]	NAA, and lactate/lipid resonances, increased with tumor grade		
Brain, ^{31}P MRS	PME	Normal <astrocytoma / high grade glial cancer	
[Negendank, 1992]	NTP		
	PDE	Normal > high / low grade glial cancer	
	NTP		
Brain, ^{1}H MRSI	tCho	Normal < cancer (no overlap)	
[Kurhanewicz et al., 2000]			
	NAA		
Breast, ^{31}P MRS	PME	Normal < cancer	
[Negendank, 1992]			
Liver, ^{31}P MRS	PME	Normal < cancer	
[Negendank, 1992]	NTP		
	PME	Normal < cancer	
	Pi		
Prostate, ^{1}H MRSI	+ Cho + tCr	Normal ≤ cancer (minimal overlap)	
[Kurhanewicz et al., 2000]	citrate		
Prostate, ^{1}H MRS	tCho/tCr	BPH ≤ cancer	
[Swindle et al., 2003]	lipid/lysine	BPH ≤ cancer	
Prostate, ^{31}P MRS	PME	Normal < cancer	
[Negendank, 1992]	NTP		
	PME	Normal < cancer	
	PCr		

*Abbreviations used: BPH, benign prostatic hyperplasia; Cho, free choline; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; NAA, N-acetyl aspartate; NTP, nucleoside triphosphate; PC, phosphocholine; PCr, phosphocreatine; PDE, phosphodiester; PE, phosphoethanolamine; Pi, inorganic phosphate; PME, phosphomonoester; tCho, PC + GPC + Cho; tCr, creatine + phosphocreatine.

resolve different phosphomonoester (PME) and phosphodiester (PDE) signals. The PME region of ^{31}P MR spectra predominantly consists of PC and PE, while in the PDE region, GPC and glycerophosphoethanolamine (GPE) are the major signals (Fig. 2).

Clinically, the total choline signal has been employed for proton magnetic resonance spectroscopic imaging (MRSI). MRSI is typically performed in conjunction with high-resolution anatomic MR imaging and can significantly improve the diagnosis and the assessment of cancer location and aggressiveness. Pre- and post-therapy studies have demonstrated the potential of combined MRI and MRSI to provide a direct measure of the presence and spatial extent of cancer, as well as the time course and mechanism of therapeutic response [Leach et al., 1998; Kurhanewicz et al., 2000]. The use of elevated choline levels to detect cancer with MRS or MRSI has been demonstrated for prostate [Kurhanewicz et al., 2000], brain [Li et al., 2002], breast [Gribbestad et al., 1999], and other cancers [Negendank, 1992]. Ex vivo MRS analysis of biopsied tissue samples can also be clinically useful to detect cancer [Swindle et al., 2003].

METASTASIS AND CHOLINE PHOSPHOLIPID METABOLITES IN A HUMAN BREAST CANCER MODEL

Transfection of invasive and metastatic human breast cancer cells with a metastasis

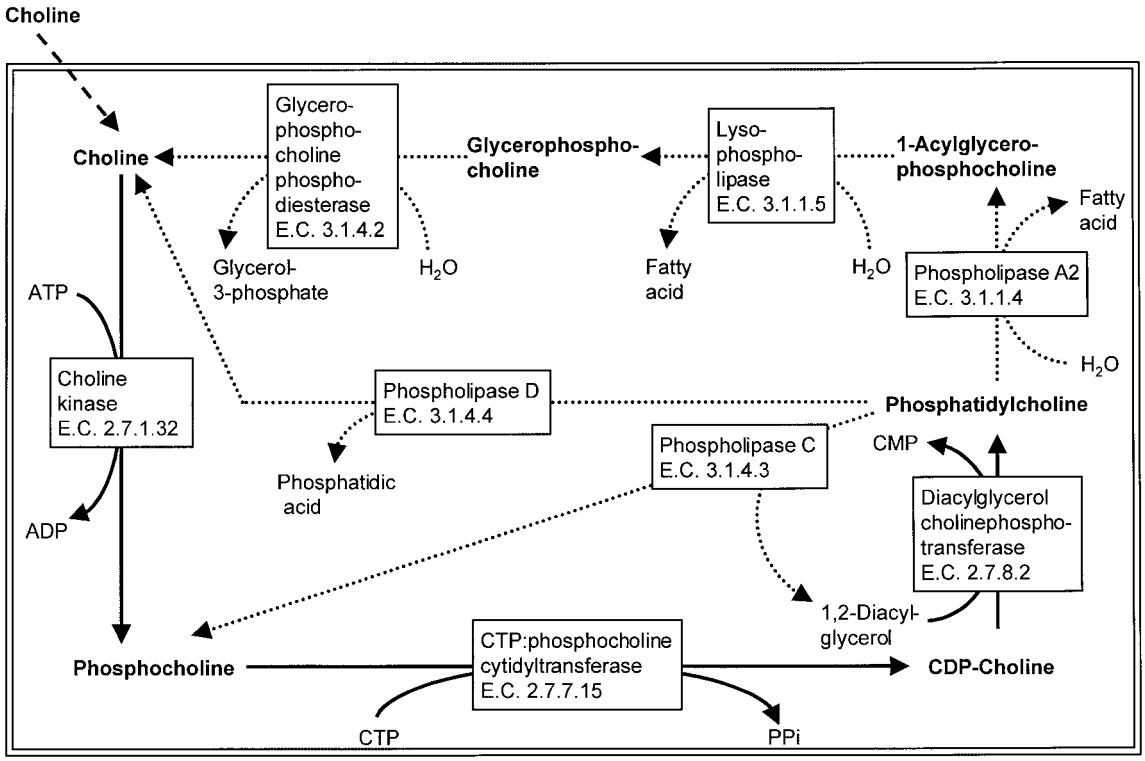

Fig. 1. Biosynthetic (solid lines) and catabolic (dashed lines) pathways of phosphatidylcholine (PtdCho) metabolism. Metabolites are given in bold letters and enzymes of PtdCho metabolism with their EC numbers are given in boxes.

suppressor gene (*nm23*) led to increased GPC and decreased PC levels compared to empty vector-transfected control cells, consistent with a reduction of the metastatic phenotype of the nm23-transfected cells [Bhujwalla et al., 1999]. We performed a ^{31}P MR spectroscopic study of tumors formed in the mammary fat pad of SCID mice by MDA-MB-435 human breast carcinoma cells transfected with cDNA encoding wild-type nm23-H1 protein. Tumors formed by MDA-MB-435 cells transfected with empty vector alone were used as controls. In vivo ^{31}P MR spectra of transgene tumors formed by nm23-H1–transfected MDA–MB–435 cells exhibited significantly higher amounts of PDE relative to PME, when compared with control tumors [Bhujwalla et al., 1999]. Nm23 expressing MDA-MB-435 cells exhibited significantly lower PC levels and higher GPC levels compared to vector-transfected or wild-type MDA-MB-435 cells, as studied by high resolution ^{1}H MRS of perchloric acid cell extracts [Bhujwalla et al., 1999]. The effect of nm23 transfection on the attenuation of metastasis was confirmed by histological analysis of lung sections obtained from tumor bearing animals [Bhujwalla et al., 1999]. These changes in phospholipid metabolism detected following nm23 transfection suggest that choline phospholipid metabolites are involved in invasion and metastasis.

MALIGNANT PROGRESSION AND CHOLINE PHOSPHOLIPID METABOLITES

We assessed PC, GPC, and choline levels in a number of epithelial cell lines derived from reduction mammoplasty (normal) tissues and neoplastic lesions, and investigated the effects of immortalization and oncogene transformation on levels of phospholipid precursors. This model was employed to evaluate the stepwise progression in mammary epithelium from normal to malignant phenotype [Aboagye and Bhujwalla, 1999]. Our data suggests that phenotypic changes in phospholipids probably commence early in carcinogenesis and may, as with most other neoplastic phenotypes, be regulated by an interplay of cellular

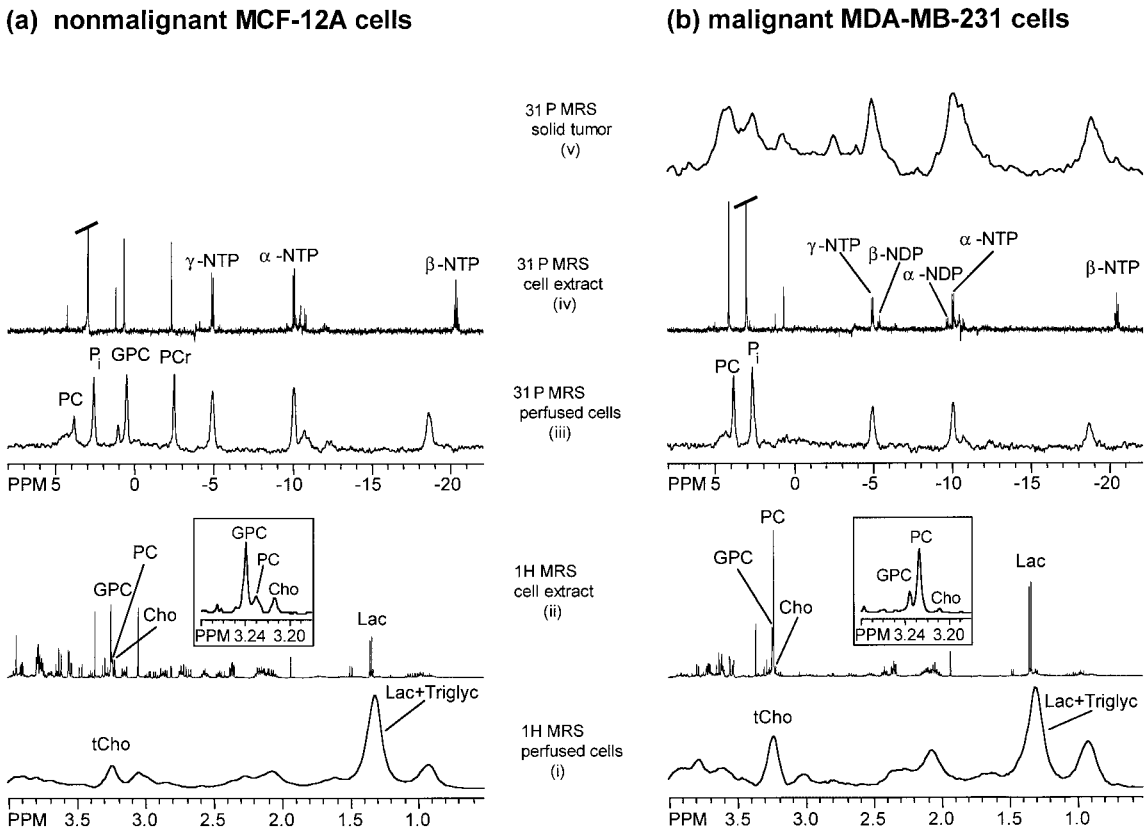

Fig. 2. ^{1}H MR spectra and ^{31}P MR spectra of (**a**) nonmalignant human mammary epithelial cells MCF-12A and (**b**) malignant, invasive/metastatic MDA-MB-231 human breast cancer cells. Diffusion-weighted, water-suppressed ^{1}H MR spectra shown in **panel (i)** and ^{31}P MR spectra shown in **panel (iii)** were acquired from intact cells perfused in our MR-compatible cell perfusion system. ^{1}H MR spectra shown in **panel (ii)** and ^{31}P MR spectra shown in **panel (iv)** are high resolution spectra of cell extracts. An in vivo ^{31}P NMR spectrum from a MDA-MB-231 tumor in a SCID mouse is shown in **panel (v)**. These spectra (insets display zoomed regions) demonstrate that nonmalignant human mammary epithelial cells shown in (**a**) contain high glycerophosphocholine (GPC) levels, low phosphocholine (PC) levels, and low levels of total choline-containing metabolites (tCho), whereas human breast cancer cells shown in (**b**) exhibit low GPC levels, high PC levels, and high levels of total choline-containing metabolites. Assignments made in the MR spectra are: Cho, free choline; GPC, glycerophosphocholine; Lac, lactate; NDP, nucleoside diphosphate; NTP, nucleoside triphosphate; PC, phosphocholine; PCr, phosphocreatine; Pi, inorganic phosphate; tCho, total choline-containing metabolites; Lac + Triglyc, lactate + triglycerides.

immortalization and oncogene transformation. A GPC to PC switch appeared to be an early phenotypic change during carcinogenesis as observed in benzo(α)pyrene immortalized cells where instead of GPC, PC became the major choline phospholipid metabolite. Our findings suggest that normal human mammary epithelium has low steady state levels of total choline-containing metabolites and that GPC was the major metabolite in the normal HMECs. However, despite this 'switch,' total choline-containing metabolite levels remained low in these immortalized cells. Transformation of 184B5 immortal cells by overexpression of the erbB2 oncogene resulted in a dramatic increase in both PC/GPC ratio and total choline levels compared to the benzo(α)pyrene immortalized cells, although total choline-containing metabolites and PC levels were still less than those of tumor-derived cells. ErbB2 is an important (proto)oncogene which is amplified in 20–30% of breast cancer cases and is associated with poor prognosis; amplification of this oncogene is thought to occur late in tumor progression [Pierce et al., 1991]. Transformation of 184B5 by erbB2 results in the ability of these cells to form colonies in semi-solid medium, and to form small, low frequency tumors with high latency in vivo [Pierce et al., 1991]. All of the breast tumor cell lines showed the GPC to PC switch.

In addition to this switch, all breast tumor cells showed significantly higher total choline-containing metabolite levels ($p < 0.05$). The increased total choline-containing metabolite level was mainly due to an increase in PC levels and, to a lesser and variable extent, an increase in GPC levels.

We also examined a panel of normal human prostate cells (HPCs) and tumor cells derived from prostate metastases, by ^{1}H MRS, to determine if malignant transformation of HPCs results in similar alteration of choline compounds [Ackerstaff et al., 2001]. HPCs derived from metastases exhibit significantly higher PC and total choline levels compared to normal prostate epithelial and stromal cells. However, HPCs did not exhibit the 'switch' detected in HMECs from high GPC, low PC to low GPC, high PC with malignant progression. These data suggest that PC is primarily related to malignancy.

Several other studies have also shown elevation of PC with malignant transformation. Several members of the *ras* oncogene family are frequently mutated in human cancers [Downward, 2003], thereby constitutively activating Ras. In ^{31}P MR spectra of mutant *H-ras* transfected NIH 3T3 fibroblasts, PC levels were significantly elevated (fourfold) as compared to NIH 3T3 fibroblasts [Ronen et al., 2001]. High PC levels were observed in ^{31}P MR spectra of three breast cancer cell lines (21PT, 21NT, and 21MT-2) established from the same patient when compared to a normal breast epithelial cell strain (76N) [Singer et al., 1995]. These changes were reflected as a significant decrease in the GPC/PC ratios in the primary (21PT, 21NT) and metastatic tumor (21MT-2) cell lines in comparison with the normal cell strain. 21MT-2, the metastatic cell line, also showed a significant decrease in GPC/PC ratio compared to the primary breast cell lines, 21PT and 21NT. Similarly, a high PC/GPC ratio was observed for a series of human tumor lines of neuronal origin when compared to primary cultures of the central and peripheral nervous system or to normal tissue obtained from brain biopsy extracts [Bhakoo et al., 1996]. Conversely, growth arrest has been shown to reduce the PC/GPC ratio. Treatment of MCF-7 cells with tumor necrosis factor-alpha, which induces cell cycle arrest and apoptosis, resulted in a decrease of PC [Bogin et al., 1998].

THE EFFECT OF AN ANTI-INFLAMMATORY AGENT ON CHOLINE PHOSPHOLIPID METABOLITES

Previous studies have shown that the non-steroidal anti-inflammatory agent indomethacin can inhibit the invasive and metastatic behavior of human breast cancer cells [Rozic et al., 2001]. We therefore treated malignant as well as nonmalignant HMECs with indomethacin, and determined its effect on choline phospholipid metabolism. Real-time monitoring of choline compounds of intact breast cancer cells was performed during treatment with 200 μM indomethacin using our MR-compatible cell perfusion system [Glunde et al., 2002]. Additionally, ^{1}H MR spectra were obtained from extracts of indomethacin-treated breast cancer cells in tissue culture [Natarajan et al., 2002]. In these extract studies, cells were treated with 50 μM indomethacin for 18 h or 300 μM indomethacin for a period of 3 h [Natarajan et al., 2002]. Control experiments of untreated cells were performed over the same time period for the isolated perfused cell studies as well as the extract studies.

Treatment with indomethacin resulted in a significant reduction of PC and an increase of GPC; control spectra obtained over a similar time period showed no changes. In intact MDA-MB-231 cells, the drop in PC and increase in GPC was evident within 80 min following addition of indomethacin to the cell perfusion medium [Glunde et al., 2002]. Consistent with the study on intact cells, ^{1}H MR spectra obtained from cell extracts of MDA-MB-231 cells exhibited a significant increase of GPC and decrease of PC following treatment with indomethacin [Natarajan et al., 2002]. Additionally, treatment with indomethacin resulted in increased expression of nm23 in nonmalignant MCF-12A cells and MCF-7 breast cancer cells [Natarajan et al., 2002]. A significant correlation was observed between cyclooxygenase (COX)-1, but not COX-2, levels and total choline in HMECs [Natarajan et al., 2002]. These data suggest that choline phospholipid metabolites may be related, in part, to the inflammatory state of human breast cancer cells. We are currently using ^{13}C NMR spectroscopy of labeled choline in combination with cDNA microarray analyses to further understand alterations in choline phospholipid metabolites following treatment with indomethacin.

We also characterized changes in invasion of the invasive human breast cancer cell line MDA-MB-435 following exposure to indomethacin, using our MR-compatible cell invasion assay and detected a significant reduction of invasion following treatment with indomethacin [Ackerstaff et al., 2003].

PARACRINE FACTORS SECRETED BY CANCER CELLS ALTER CHOLINE PHOSPHOLIPID METABOLITES OF ENDOTHELIAL CELLS

Since endothelial cells form a key component of tumor vasculature, we used MRS to characterize the choline phospholipid phenotype of human umbilical vein endothelial cells (HUVECs). We determined the effect of conditioned media obtained from a malignant cell line on choline phospholipids. Treatment with conditioned medium obtained from MDA-MB-231 cancer cells increased PC and decreased GPC levels of HUVECs [Mori et al., 2003]. These results suggest that cancer cells secrete growth factors and/or other molecules that influence the choline phospholipid metabolism of endothelial cells. These data also suggest that cancer cell–endothelial cell interaction may occur through phospholipid signaling.

UNDERLYING MECHANISMS AND POTENTIAL TARGETS IN CHOLINE PHOSPHOLIPID METABOLISM

Some of the mechanisms underlying the increased PC levels observed in cancer cells include increased expression and activity of choline kinase [Ramirez de Molina et al., 2002a,b], a higher rate of choline transport [Katz-Brull and Degani, 1996], and increased PLD [Noh et al., 2000], and PLA2 [Guthridge et al., 1994] activity.

Choline kinase is the enzyme at the first step of the Kennedy pathway and is responsible for generating PC from choline (Fig. 1). Various growth factors, chemical carcinogens, and oncogenes [Hernandez-Alcoceba et al., 1999] enhance the activity/expression of choline kinase in mammalian cells. Convincing evidence of the role of choline kinase in breast cancer was recently published in a study which demonstrated an increase in choline kinase activity in tumoral tissue when compared to normal tissue [Ramirez de Molina et al., 2002a]. A significant association between choline kinase enzymatic activity with histological tumor grade and with ER-negative tumors was also observed in these studies. Overexpression of choline kinase has also been detected in several human breast cancer cell lines, including the ones used by us, as well as in lung, prostate, and colorectal human cancers [Ramirez de Molina et al., 2002b]. Targeting choline kinase with choline kinase inhibitors resulted in an anti-mitogenic and anti-proliferative effect [Hernandez-Alcoceba et al., 1999].

Membrane-associated phospholipase A2 (M-PLA2), an enzyme that hydrolyses the sn-2 fatty acyl ester bond of phosphoglycerides (Fig. 1), has been investigated as prognostic marker for breast cancer [Yamashita et al., 1994]. M-PLA2 enzyme levels correlated strongly with clinicopathological factors, and were significantly higher in patients with distant metastasis as compared to patients without metastasis. Breast cancer patients with high M-PLA2 concentrations exhibited significantly shorter disease-free and overall survival than those with low M-PLA2 concentration [Yamashita et al., 1994]. Therefore, targeting M-PLA2 may prove useful in cancer.

PLD, an enzyme that hydrolyses phosphoglycerides to phosphatidic acid and choline (Fig. 1), was shown to have an elevated activity in MDA-MB-231 human breast cancer cells [Zhong et al., 2003]. Inhibiting PLD activity in these cells rendered them sensitive to an apoptotic insult of serum withdrawal indicating that elevated PLD activity generates survival signals allowing cells to overcome default apoptosis programs [Zhong et al., 2003]. PLD may therefore be another valid target for breast cancer treatment.

CONCLUSIONS

Overall, these data indicate that diverse molecular alterations such as metastasis-suppressor gene expression, oncogene expression, and malignant transformation arrive at common endpoints in choline phospholipid metabolism, and demonstrate the important role of choline phospholipid metabolites in cancer diagnosis. Targeting enzymes involved in choline metabolism may prove to be highly effective against cancer cells. MR spectroscopic imaging would be ideally suited to determine the effectiveness of such targeting, experimentally and clinically.

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