Breast Cancer Research and Treatment **36**: 299–306, 1995.

Report

Tamoxifen elicits rapid transmembrane lipid signal responses in human breast cancer cells

Myles C. Cabot, Zu-Chuan Zhang and Armando E. Giuliano
John Wayne Cancer Institute at Saint John's Hospital and Health Center, 2200 Santa Monica Boulevard, Santa Monica, CA 90404, USA

Key words: tamoxifen, phospholipase, phosphatidic acid, second messengers, signal transduction

Abstract

The antiestrogen tamoxifen competes with estrogen for receptor occupancy, although reports indicate that not all effects of tamoxifen are mediated via this specific interaction. In the present study we sought to determine whether tamoxifen can initiate transmembrane lipid signals. Lipid signaling is a prominent mode by which hormones, growth factors, and phorbol diesters transduce messages. Using the human mammary carcinoma cell line MDA-MB-231, phospholipid metabolism was analyzed in cells prelabeled with ^{3}H-fatty acid. After short-term (10 min) exposure to tamoxifen (10 μM), cellular phosphatidic acid (PA) increased by approximately 50%. Dose-response kinetics for PA formation were obtained over a tamoxifen range of 2.5–20 μM. Treatment of MDA-MB-231 cells with phorbol diester (12-O-tetradecanoylphorbol-13-acetate, TPA) also elicited PA generation (60% above control). Interestingly, addition of tamoxifen, a purported protein kinase C inhibitor, to TPA-treated cells, caused further increase in PA (approximately 100% above control). PA, a second messenger lipid produced upon effector-receptor coupling, shares a prominent role in signal transduction events that govern cellular proliferation. It is therefore suggested that some actions of tamoxifen are mediated by promoting production of second messenger lipids that elicit transmembrane signal transduction cascades. This view is in line with ideas on non-estrogen receptor associated actions of tamoxifen by way of alternate binding sites.

Introduction

Tamoxifen, a synthetic nonsteroidal triphenylethylene derivative, known for its estrogen, antiestrogen, and/or antitumoral action, is widely used for treatment of Stage I and II breast cancer. A major clinical trial to determine if tamoxifen can aid in the prevention of breast cancer in healthy women is under way [1]. Although the antitumoral character of tamoxifen is largely due to antagonism of estrogen via competitive binding to the estrogen receptor, several reports indicate that not all tamoxifen-related biological effects are mediated by estrogen receptor interactions (reviewed in 2). Other potentially significant estrogen receptor-independent interactions of tamoxifen have been revealed. Tamoxifen has been shown to induce TGF-β_1 in human breast cancer [3], and inhibit PKC activity *in vitro* and in intact cells [4, 5]. Stimulated proliferation of MCF-7 cells has been demonstrated with tamoxifen and *trans*-hydroxytamoxifen [6, 7]. In patients presenting with progressive disease, evidence

Address for offprints: M.C. Cabot, Department of Breast Cancer Research, John Wayne Cancer Institute, 2200 Santa Monica Blvd., Santa Monica, CA 90404, USA

of tamoxifen-stimulated tumor growth of breast cancer has been observed [8, 9], and studies using an MCF-7 tumor variant have revealed that this type of tamoxifen-dependent growth can be inhibited by pure antiestrogen treatment [10]. Further support that not all tamoxifen effects stem from estrogen receptor interaction are gleaned from work showing that various biological effects of tamoxifen cannot be reversed by estrogen [11, 12]. Alternate binding sites for tamoxifen have been described previously [13–16]; however, the relevance of the estrogen receptor-independent activity of tamoxifen to cellular proliferation is not clear.

Many cell processes are initiated and directed by the interaction of hormones and growth factors with specific cell surface receptors [17, 18]. The resulting signal transduction cascade is the molecular transfer system by which gene transcription, protein production, and metabolic pathways are regulated. Any modified event along the signal cascade has potential to ultimately impact on cell behavior and proliferation. Lipids play a principal role in signal transduction across membranes. This is clearly evidenced by work showing that binding of cellular effectors to specific receptors elicits membrane phospholipid hydrolysis. The phospholipid hydrolysis products serve as second messengers for governing myriad cellular events [18, 19]. The second messenger PA exhibits growth factor-like properties [20], and may link signaling to *Ras* that is critical in control of cell proliferation [21, 22]. DG (diacylglycarol), another important second messenger, is a cellular activator of PKC [18, 19]. Lipid second messengers arise by several enzymatic pathways which enlist the action of various cellular phospholipases and DG-kinase. The activation and integration of these enzymatic pathways is crucial in governing downstream events regulated by PA and DG second messengers.

In this study we sought to determine whether tamoxifen can initiate transmembrane lipid signals. Using an estrogen receptor negative human mammary carcinoma cell line, we report that tamoxifen treatment elicits rapid (within minutes) increases in cellular PA, a property shared with tumor promoting phorbol diesters. This transmembrane response may be key in dissecting the estrogen receptor-independent activities of tamoxifen. At present it is not known whether the PA response to tamoxifen is proliferative or antiproliferative in MDA-MB-231 cells. Because of its widespread use, the non-genomic aspects of antiestrogen action must be thoroughly understood.

Materials and methods

Cells

MDA-MB-231 cells (adeno-carcinoma, breast, plural effusion, human) were obtained from the American Type Culture Collection (Rockville, MD) and grown in Dulbecco's modified Eagle's medium (DMEM)/F12, 50/50 mix (Mediatech, Inc., Herndon, VA), containing 10% fetal bovine serum (Hyclone, Logan, UT), L-glutamine (584 mg/L), penicillin (100 units/ml), and streptomycin (100 µg/ml), GIBCO BRL, Grand Island, NY. Cells were passaged using 0.05% trypsin, 0.53 mM EDTA (Mediatech). Stock cultures were maintained in Costar 75 cm^2 tissue culture flasks (Cambridge, MA) and subcultured into Corning 6-well plates (Corning, NY) for experiments.

Chemicals

Tamoxifen (free base) TPA, and fatty acid-free bovine serum albumin were purchased from Sigma (St. Louis, MO). Phenol red-free DMEM was from GIBCO BRL. [^{3}H]myristic acid (16–30 Ci/mmol) was purchased from Du Pont, New England Nuclear, and EcoLume scintillation cocktail was from ICN, Costa Mesa, CA. Silica gel G TLC plates were purchased from Analtech, Inc., and HPTLC silica gel 60 plates were from EM Industries. Reagent grade solvents were from Fisher Scientific. Phospholipid standards for TLC were purchased from Avanti (Alabaster, AL), and neutral lipids were from NU Chek Prep (Elysian, MN). HEPES (1 M, Ultrapure, pH 7.3) was from USB (Cleveland, OH).

Cell culture, radiolabeling, and treatment

MDA-MB-231 cells, seeded into 6-well plates, were labeled at 1-day preconfluence with [^{3}H]myristic acid (1.0 μCi/ml DMEM/F-12, 5% fetal bovine serum) for 24 h. After removal of labeling medium, monolayers were gently rinsed and equilibrated for 5 h at 37° C with phenol red-free, serum-free, BSA-containing (0.3 mg/ml) medium buffered with HEPES (10 mM, pH 7.3). TPA (1.45 mM) and tamoxifen (20 mM) were stored as stock solutions in acetone at –20 °C. Compounds were diluted using fresh equilibration medium. Acetone vehicle was present at 0.05% or less in the experiments. Following cell treatment, the media were aspirated and total cellular lipids were extracted by the method of Bligh and Dyer [23] modified as described [24].

Lipid analysis

[^{3}H]PA, [^{3}H]DG, and [^{3}H]PEt (phosphatidylethanol) were resolved from total cellular lipids by TLC employing solvent systems previously described [24]. Two different solvent systems were used to resolve and identify PA. PA was separated from other cell lipids on HPTLC silica gel 60 layers developed in the organic phase of ethyl acetate/isooctane/acetic acid/water (110:50:20:100, v/v) and as previously described [24] using silica gel G layers developed in chloroform/pyridine/70% formic acid (50:25:7, v/v).

Table 1. Incorporation of myristic acid into lipids of MDA-MB-231 breast cancer cells. MDA-MB-231 cells were radiolabeled (24 hr) with [^{3}H] myristic acid (1.14 μCi/ml medium) at 1-day preconfluence. Following cell equilibration in isotope-free, serum-free medium, lipids were extracted and analyzed by TLC using a solvent system containing chloroform/methanol/acetic acid/water (50:30:7:3, v/v)

Lipid	Incorporation (% total lipid ^{3}H)
Lysophosphatidylcholine	1.3
Sphingomyelin	9.3
Phosphatidylcholine	62.1
Phosphatidylinositol + phosphatidylserine	6.9
Phosphatidylethanolamine	13.2
PA + neutral lipid	7.0

The individual lipids, identified by co-migration with commercial standards, were visualized in iodine vapor. After iodine subliming, radioactivity was determined in the silica gel scrapings by liquid scintillation spectrometry [24]. The indicated areas of the gel were first scraped into 0.5 ml water, 4 ml Ecolume was added, and the plastic minivials were vortex mixed before counting.

Data

All data points represent the mean ± S.D. from at least three separate cultures. All experiments were repeated three times or more, and the experiments yielded similar results.

Results

Sustained formation of second messenger lipids generally results from cellular hydrolysis of PC (phosphatidylcholine), not from the polyphosphoinositides [25]. Preliminary experiments were conducted to determine if myristic acid, which is preferentially incorporated into PC in many cell types [24, 25], would be of utility to radiolabel complex glycerophospholipids of MDA-MB-231 cells. After a 24 h incubation, 70–86% of the [^{3}H] myristic acid added to the culture medium was taken up by the cells. Of this, approximately 95% was incorporated into cellular phospholipids (Table 1). PC tritium accounted for 62% of total lipid radioactivity, whereas phosphatidylethanolamine was labeled to a lesser extent (13%). The combined inositol- and serine-containing phospholipids accounted for approximately 7.0% of total cell tritium, and sphingomyelin contained 9.0% of the counts.

In addition to functioning as an estrogen receptor antagonist, we sought to determine whether tamoxifen interacts with transmembrane lipid signal events. Because of its high affinity for cellular proteins, lipophilic nature, and the presence of a dimethylethanolamine function, our idea was that tamoxifen would modify cellular phospholipase activity. In preliminary experiments using MDA-MB-231 cells radiolabeled with [^{3}H] myristic acid,

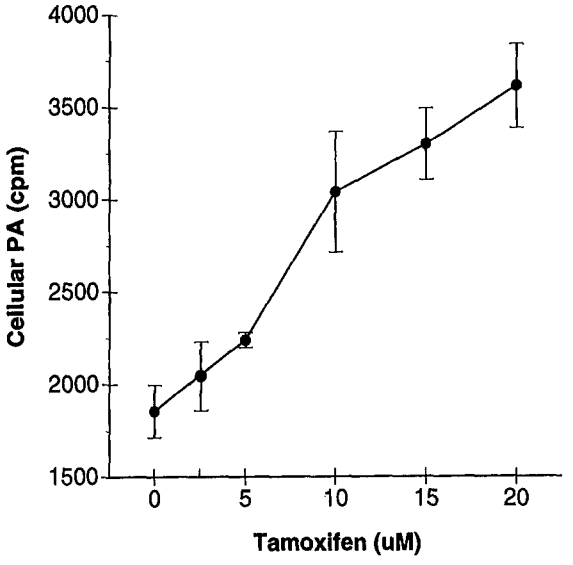

Fig. 1. Influence of tamoxifen concentration on formation of [^{3}H]PA in MDA-MB-231 cells. Prelabeled and equilibrated cells were exposed to the indicated concentrations of tamoxifen for 30 min. All wells contained acetone vehicle (0.05%). Counts (y-axis) represent one-fifth of the total PA in the cultures.

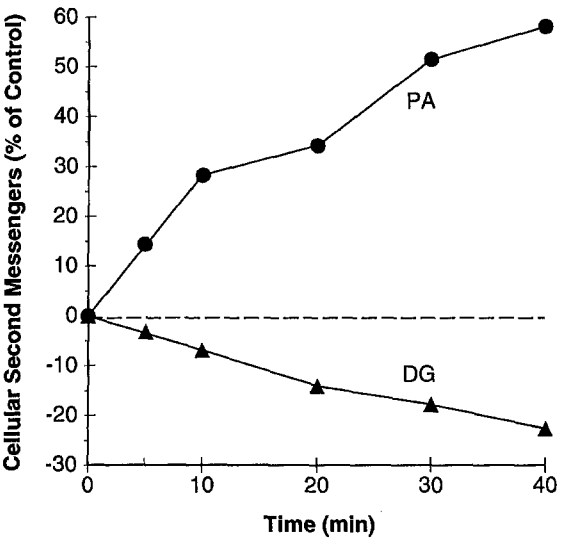

Fig. 2. Effects of incubation time on tamoxifen-induced changes in MDA-MB-231 cell PA and DG. Cells were exposed to tamoxifen (10 μM) for the times indicated.

we found that tamoxifen treatment for 10 min (10 μM) increased cellular PA radioactivity by approximately 50%. The parameters of this response were subsequently characterized. Although tamoxifen caused the cells to become slightly more rounded, cell attachment and viability at 4 and 24 h after tamoxifen (10 μM) addition was similar with control (minus tamoxifen).

A positive correlation between increased drug concentration and labeled PA content was revealed. The data of Fig. 1 demonstrate that intracellular PA increased with increasing concentrations of tamoxifen as tested over a range of 2.5 to 20 μM. With 20 μM tamoxifen, cell PA increased nearly 100% above control (untreated) values. The lipid response to tamoxifen was also time-dependent as illustrated by Fig. 2. Increased exposure time (5–40 min) resulted in an increase in the formation of cellular [^{3}H]PA. The data of Fig. 2 also show that as cellular [^{3}H]PA increases, the amount of [^{3}H]DG decreases in a time-dependent fashion.

TPA, a potent and specific agonist of PKC, has been shown to elicit PA formation in numerous cell types [24]. It was therefore of interest to determine whether TPA treatment would induce PA formation in MDA-MB-231 cells, similar to the tamoxifen-elicited response. Figure 3 demonstrates that TPA stimulated the formation of PA in MDA-MB-231 cells (60% above control), similar to tamoxifen. Interestingly, when cells were exposed to tamoxifen plus TPA, PA generation was stimulated to a greater extent (100% above control) than when

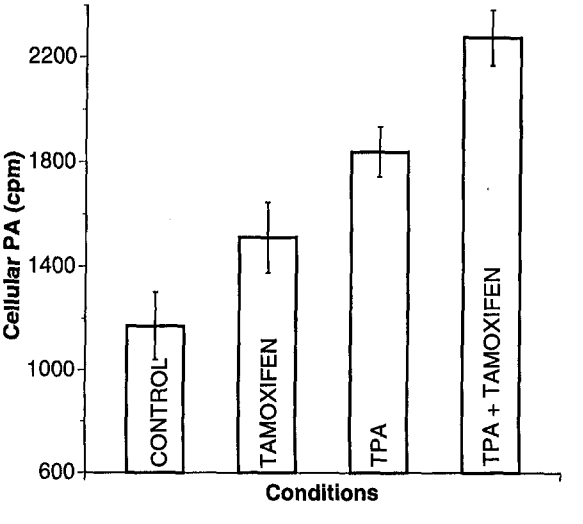

Fig. 3. Effects of tamoxifen and TPA on the production of [^{3}H]PA in MDA-MB-231 cells. Culture cells were exposed to tamoxifen (10 μM), TPA (20 nM), or a combination of the two compounds for 20 min. Control cultures contained acetone vehicle.

either compound was given singly (Fig. 3). A comparison of the data reveals that the influence of both agents (on PA generation) is nearly additive.

We have previously demonstrated that TPA provokes PA formation from phosphatidylcholine in cells by a pathway involving PLD (phospholipase D) [26]. PLD activity can be measured in intact cells by a transphosphatidylation reaction catalyzed by PLD; in the presence of a strong nucleophile, ethanol in this case, PEt is generated at the expense of PA [24]. The experiment in Fig. 4 was conducted using ethanol-supplemented culture medium to determine if tamoxifen or TPA promote formation of PEt in MDA-MB-231 cells. The appearance of PEt would be consistent with activation of a PLD. Figure 4 shows that whereas tamoxifen stimulated the formation of PA (40% above control) it did not stimulate the generation of PEt (in the presence of ethanol). In contrast TPA markedly promoted the formation of PEt (60% above control) and elicited a concomitant 20% increase in cellular PA. The data of Fig. 4 suggest that tamoxifen and TPA stimulate the formation of cellular PA by disparate pathways.

We have begun experiments to determine the influence of tamoxifen on MDA-MB-231 cell growth. Growth characteristics in the presence of tamoxifen are highly dependent on the type of culture media and supplements employed. We are currently pursuing media conditions to evaluate tamoxifen-induced PA effects on cell growth. In addition, our preliminary experiments reveal that the ability of tamoxifen to elicit rapid changes in the levels of second messengers is not limited to MDA-MB-231 cells. Using CCD986Sk cells (human breast normal fibroblast) we have observed that tamoxifen causes rapid generation of PA and DG (unpublished data), very similar to lipid signaling elicited by hormones, growth factors, and phorbol diesters [24, 25].

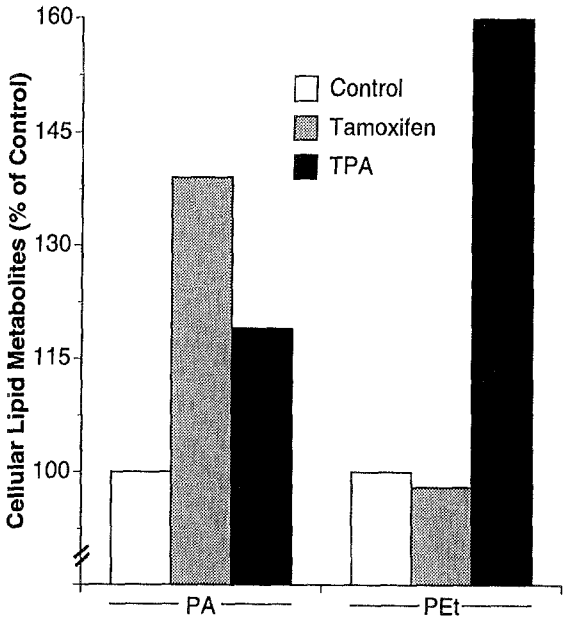

Fig. 4. Influence of tamoxifen and TPA on phosphatidylethanol formation in MDA-MB-231 cells. Cells were exposed to tamoxifen (10 μM) or TPA (80 nM) in medium containing 1.0% ethanol for 40 min.

Discussion

In addition to prevailing therapeutic use for treatment of breast cancer, tamoxifen administration to normal woman is currently being considered to determine the preventive efficacy of this synthetic antiestrogen. Recent information, however, on uterotrophic properties [27] and associated endometrial neoplasia [1], necessitates that we further examine molecular actions of tamoxifen. The present communication reveals several points worthy of notice: i) tamoxifen elicited PA formation in human breast cancer cells; ii) phorbol diesters, activators of PKC, likewise stimulated the formation of PA; iii) estrogen receptor negative cells [28] were used in the experiments; iv) tamoxifen is known to bind to sites distinct from the estrogen receptor.

In close rivalry with DG, PA has been shown to occupy an important role as a cellular second messenger. PA can be generated directly by hormone, growth factor, or TPA stimulation of PLD [24–26, 29, 30] or indirectly by stimulation of phospholipase C followed by DG-kinase [25]. PA has been shown to induce calcium mobilization, proto-oncogene expression, and DNA synthesis in cultured cells [20], and lyso-PA, the *sn*-2 deacylated metabolite of PA, induces cell proliferation in fibroblasts by G protein mediated signal transduction [21]. Additional work proposes that PA links growth factor signaling to *Ras* that is critical in control of cell proliferation

[22], and Rice *et al.* [31] suggest that PA metabolism has clinical potential for treatment of sepsis and septic shock. Therefore, tamoxifen-induced increases in cellular PA may provoke responses of a varied nature.

TPA, which has been shown to induce cell PA and DG formation via a PKC-directed pathway [25, 26], also elicited PA formation of MDA-MB-231 cells. In view of the results in Fig. 3, this is of particular interest because tamoxifen has long been regarded as a PKC inhibitor [4, 5]. When we tested the effect of tamoxifen on TPA-induced PA formation (Fig. 3), we demonstrated that tamoxifen did not inhibit TPA action but instead had an additive influence on PA formation. Although these data suggest that TPA and tamoxifen induce PA formation by dissimilar pathways, the results show that tamoxifen is not effective as a PKC inhibitor in this system. In accord with this idea, opposite effects of tamoxifen on PKC activity, effects proposed to be dependent on the phospholipid environment of PKC, have been shown *in vitro* and in intact cells [2]. Structure-activity relationship studies of the di- and triphenylethylene antiestrogens for PKC interaction suggest that the basic amino side-chain interacts with the regulatory domain and the 1,1-bis (*p*-hydroxyphenyl) ethylene moiety interacts with the catalytic domain of PKC [32]. We have not yet determined whether tamoxifen elicits PA formation through a PKC-associated mechanism in MDA-MB-231 cells.

Results from the experiment shown in Fig. 4 imply that tamoxifen does not elicit PA formation by a PLD pathway, as previously described for phosphatidylcholine degradation [24–26, 29]. The failure of tamoxifen to elicit PEt formation by PLD catalyzed transphosphatidylation supports this notion. In cells PA is readily formed by the action of DG kinase. We have previously demonstrated (in intact fibroblasts) vasopressin-induced phospholipase C, forming DG, with subsequent conversion to PA by DG-kinase [25]. The data of Fig. 2 suggest that PA formation induced by tamoxifen is in part derived by the action of DG-kinase. Although the increase in PA and decrease in DG is not stoichiometric, this may be related to radiospecific activity of labeled cellular lipids and the contribution of monoacylglycerolphosphate acyltransferase to PA formation.

The amount of tamoxifen required to induce rapid formation of PA in MDA-MB-231 cells is in the low μM range. Although this is not in line with the pharmacology of steroid receptor action, serum tamoxifen levels of patients undergoing therapy have been observed in the μM range [4, 5]. Also of relevance to the issues of pharmacological activity are reports demonstrating the existence of multiple binding sites for the antiestrogens [2, 13, 33, 34]. A broader spectrum of specific binding sites would clearly be indicative of multiple modes of action. Among the interesting properties of tamoxifen suggestive of a polyvalent nature, are interactions with PKC [2, 4, 5, 32], agonistic effects on MCF-7 cell proliferation during short-term absence of estrogen [6], uterotrophic actions at the level of insulin-like growth factor expression [27], induction of TGF-β in human breast stromal components [3], and modulation of cisplatin sensitivity in human malignant melanoma [35].

It will be beneficial to learn whether tamoxifen-induced PA formation alters cell growth. At present we are examining appropriate culture media to conduct these experiments. Activation of phosphatidylcholine hydrolysis via PKC-direct avenues [26] is not solely associated with mitogenicity. In the human breast cancer cell line MCF-7, activation of PKC is associated with inhibition of cell proliferation [2]. Little is known about the relationship of late phase signaling to cell proliferation. In our previous studies we have described a correlation between late phase lipid signaling and cellular homeostasis [36]. A recent report [37] showed that long-term exposure of MDA-MB-231 cells to tamoxifen had no effect on cell doubling time. However, it is difficult to compare the biology of a signal induced within minutes, as described herein, to influences elicited over a period of eight weeks [37].

The fact that tamoxifen itself stimulates PA formation in estrogen receptor negative breast cancer cells is intriguing. At present the relationship of this new property to cell proliferation, uterotrophic effects, and endometrial neoplasia is not known; however, it is clear from previous studies that nongenomic aspects of antiestrogen biology may be important determinants of action. The ability of tamoxifen to induce PA formation is not limited to

MDA-MB-231 cells; tamoxifen is most effective in inducing PA and DG generation in normal breast stromal cells (unpublished data). Therefore, tamoxifen appears to share some of the signaling properties common to growth factors, hormones, and phorbol diesters [24–26]. Examination of these actions may provide ideas for design of improved endocrine therapies.

Acknowledgements

We wish to thank Kimberley Robinson for excellent assistance in the preparation of this manuscript. This research was supported by the Ben B. and Joyce E. Eisenberg Foundation.

References

1. Marshall E: Tamoxifen: Hanging in the balance. Science (Washington D.C.) 264: 1524–1527, 1994
2. Issandou M, Faucher C, Bayard F, Darbon JM: Opposite effects of tamoxifen on *in vitro* protein kinase C activity and endogeneous protein phosphorylation in intact MCF-7 cells. Cancer Res 50: 5845–5850, 1990
3. Butta A, MacLennan K, Flanders KC, Sacks PM, Smith I, McKinna A, Dowsett M, Wakefield LM, Sporn MB, Baum M, Colletta AA: Induction of transforming growth factor β_1 in human breast cancer *in vivo* following tamoxifen treatment. Cancer Res 52: 4261–4264, 1992
4. Horgan K, Cooke E, Hallett MB, Mansel RE: Inhibition of protein kinase C mediated signal transduction by tamoxifen. Biochem Pharmacol 24: 4463–4465, 1986
5. O'Brien CA, Liskamp RM, Solomon DH, Weinstein IB: Inhibition of protein kinase C by tamoxifen. Cancer Res 45: 2462–2465, 1985
6. Katzenellenbogen BS, Kendra KL, Norman MJ, Berthois Y: Proliferation, hormonal responsiveness, and estrogen receptor content of MCF-7 human breast cancer cells grown in the short-term and long-term absence of estrogens. Cancer Res 47: 4355–4360, 1987
7. Wakeling AE, Newboult E, Peters SW: Effects of antiestrogens on the proliferation of MCF-7 human breast cancer cells. J Molec Endocrinol 2: 225–234, 1989
8. Tormey DC, Simon RM, Lippman ME, Bull JM, Meyer CE: Evaluation of tamoxifen dose in advanced breast cancer: a progress report. Cancer Treat Rep 60: 1451–1459, 1976
9. Plotkin D, Lechner JJ, Jung WE, Risen PJ: Tamoxifen flare in advanced breast cancer. JAMA 240: 2644–2646, 1978
10. Gottardis MM, Jiang SY, Jeng MH, Jordan VC: Inhibition of tamoxifen-stimulated growth of an MCF-7 tumor variant in athymic mice by novel steroidal antiestrogens. Cancer Res 49: 4090–4093, 1989
11. Reddel RR, Murphy LC, Sutherland RL: Effects of biologically active metabolites of tamoxifen on the proliferation kinetics of MCF-7 human breast cancer cells *in vitro*. Cancer Res 43: 4618–4624, 1983
12. Sutherland RL, Hall RE, Taylor IW: Cell proliferation kinetics of MCF-7 human mammary carcinoma cells in culture and effects of tamoxifen on exponentially growing and plateau phase cells. Cancer Res 43: 3998–4006, 1983
13. Lerner LJ, Jordan VC: Development of antiestrogens and their use in breast cancer: eighth Cain memorial award lecture. Cancer Res 50: 4177–4189, 1990
14. Sutherland RL, Murphy LC, Foo MS, Green MD, Wybourne AM, Krozowski ZS: High affinity antioestrogen binding site distinct from the oestrogen receptor. Nature Lond 288: 273–275, 1980
15. Sudo K, Monsma FJ, Katzenellenbogen BS: Antiestrogen binding sites distinct from the estrogen receptor: subcellular localization ligand specificity and distribution in tissues of the rat. Endocrinology 112: 425–434, 1983
16. Watts CKW, Murphy LC, Sutherland RL: Microsomal binding sites for nonsteroidal anti-estrogens in MCF-7 human mammary carcinoma cells. J Biol Chem 259: 4223–4229, 1984
17. Cabot MC, McKeehan WL (eds): Mechanisms of Signal Transduction by Hormones and Growth Factors. Progress in Clinical and Biological Research 249. New York: Alan R Liss Inc, 1987
18. Asaoka Y, Nakamura SI, Yoshida K, Nishizuka Y: Protein kinase C, calcium and phospholipid degradation. TIBS 17: 414–418, 1992
19. Farooqui AA, Farooqui T, Yates AJ, Horrocks LA: Regulation of protein kinase C activity by various lipids. Neurochem Res 6: 449–511, 1988
20. Moolenaar WH, Kruijer W, Tilly BC, Verlaan I, Bierman AJ, de Laat SW: Growth factor-like action of phosphatidic acid. Nature (Lond) 323: 171–173, 1986
21. van Corven EJ, Groenink A, Jalink K, Eichholtz T, Moolenaar WH: Lysophosphatidate-induced cell proliferation: identification and dissection of signaling pathways mediated by G proteins. Cell 59: 45–54, 1989
22. Fukami K, Takenawa T: Phosphatidic acid that accumulates in platelet-derived growth factor stimulated Balb/c 3T3 cells is a potential mitogenic signal. J Biol Chem 267: 10988–10993, 1992
23. Bligh EG, Dyer WT: A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917, 1959
24. Huang C, Cabot MC: Phorbol diesters stimulate the accumulation of phosphatidate, phosphatidylethanol, and diacylglycerol in three cell types. J Biol Chem 265: 14858–14863, 1990
25. Huang C, Cabot MC: Vasopressin-induced polyphosphoinositide and phosphatidylcholine degradation in fibroblasts. J Biol Chem 265: 17468–17473, 1990
26. Cabot MC, Welsh CJ, Zhang ZC, Cao HT: Evidence for a

protein kinase C-directed mechanism in the phorbol diester-induced phospholipase D pathway of diacylglycerol generation from phosphatidylcholine. FEBS Lett 245: 85–90, 1989
27. Huynh H, Pollak M: Uterotrophic actions of estradiol and tamoxifen are associated with inhibition of uterine insulin-like growth factor binding protein 3 gene expression. Cancer Res 54: 3115–3119, 1994
28. Cailleau R, Young R, Olive M, Reeves WJ Jr: Breast tumor cell lines from pleural effusions. J Natl Cancer Inst 53: 661–674, 1974
29. Exton JH: Signaling through phosphatidylcholine breakdown. J Biol Chem 265: 1–4, 1990
30. Daniel LW, Huang C, Strum JC, Smitherman PK, Greene D, Wykle RL: Phospholipase D hydrolysis of choline phosphoglycerides is selective for the alkyl-linked subclass of Madin-Darby canine kidney cells. J Biol Chem 268: 21519–21526, 1993
31. Rice GC, Brown PA, Nelson RJ, Bianco JA, Singer JW, Bursten S: Protection from endotoxic shock in mice by pharmacologic inhibition of phosphatidic acid. Proc Natl Acad Sci USA 91: 3857–3861, 1994
32. Bignon E, Pons M, Doré JC, Gilbert J, Ojasoo T, Miquel JF, Raynaud JP, De Paulet AC: Influence of di- and tri-phenylethylene estrogen/antiestrogen structure on the mechanisms of protein kinase C inhibition and activation as revealed by a multivariate analysis. Biochem Pharmacol 42: 1373–1383, 1991
33. Wiseman H: Tamoxifen: New membrane-mediated mechanisms of action and therapeutic advances. TIPS 15: 83–89, 1994
34. Gross C, Yu M, Van Herle AJ, Giuliano AE, Juillard JF: Presence of a specific antiestrogen binding site on human follicular thyroid carcinoma cell line (UCLA RO 82 W-1): Inhibition by an endogenous ligand present in human serum. J Clin Endocrinol Metab 77: 1361–1366, 1993
35. McClay EF, Albright KD, Jones JA, Christen RD, Howell SB: Tamoxifen modulation of cisplatin sensitivity of human malignant melanoma cells. Cancer Res 53: 1571–1576, 1993
36. Cabot MC: Cancer cells display attenuated signaling via the phosphatidylcholine pathway. In: Lipid Mediators in Health and Disease. Freund Publishing House, Ltd., London, in press
37. Sipila PE, Wiebe VJ, Hubbard GB, Koester SK, Emshoff VD, Maenpaa JU, Wurz GT, Seymour RC, DeGregorio MW: Prolonged tamoxifen exposure selects a breast cancer cell clone that is stable *in vitro* and *in vivo*. Eur J Cancer 29A: 2138–2144, 1993