Pergamon

European Journal of Cancer Vol. 30A, No. 8, pp. 1138–1144, 1994
Elsevier Science Ltd
Printed in Great Britain
0959–8049/94 $7.00+0.00

0959-8049(94)00209-6

Feature Article

Inhibiting Intracellular Signalling as a Strategy for Cancer Chemoprevention

G. Powis and D.S. Alberts

The intracellular signalling pathways that mediate the effects of growth factors and oncogenes on cell growth and transformation offer potential targets for the development of chemopreventive agents that prevent the progression of premalignant cells to invasive cancer. Agents acting on signalling targets would be expected to be cytostatic rather than cytotoxic agents. A number of existing chemopreventive agents exhibit, among their properties, inhibition of intracellular signalling enzymes. It is possible that this activity accounts, at least in part, for their chemopreventive properties.

Key words: intracellular signalling, oncogenes, growth factors, chemoprevention
Eur J Cancer, Vol. 30A, No. 8, pp. 1138–1144, 1994

INTRODUCTION

A CHEMOPREVENTIVE AGENT is a specific natural or synthetic chemical entity used to suppress or reverse the progression of premalignancy to invasive cancer. The concept of chemoprevention is founded in the belief that carcinogenesis is a multistep process that can be halted or even reversed during its preinvasive stages by the use of such agents. There is considerable evidence to support the multistep nature of human carcinogenesis. Epidemiological studies strongly suggest the existence in the diet of inhibitors of human carcinogenesis, and animal studies have shown that certain chemicals can halt or delay the occurrence of chemically- or spontaneously-induced cancers. There is increasing evidence that human carcinogenesis can also be prevented by the use of chemopreventive agents.

Over 500 agents from 20 chemical classes have so far been identified, through epidemiological studies of human populations or studies employing animals, as having some form of chemopreventive activity [1]. Because their use is amenable to hypothesis testing, most interest has focussed on agents that act by mechanistically defined pathways. Chemopreventive agents have been classified into three major mechanistic groups: agents that block metabolic conversion of precarcinogens to carcinogens, agents that prevent carcinogens from reaching or reacting with sensitive tissue sites and agents whose inhibitory action follows exposure to carcinogenic agents [2]. A class of agents in the last group that holds considerable promise as chemopreventive agents for human cancer are the inhibitors of growth factor and oncogene intracellular signalling. This class of compounds form the subject of this review.

Development of chemopreventive drugs

The testing of chemopreventive drugs in human populations is extraordinarily complex, with many ethical issues relating to activity and side-effects that are not seen with cancer chemotherapeutic drugs. The testing is expensive and involves a large number of subjects. The absence of reliable intermediate biomarker endpoints means that the testing often has to be conducted over several years. There is also a requirement for minimal toxicity even with long-term drug use. It is notoriously difficult to predict long-term toxicity of chemicals to humans from animal toxicity studies [3]. Given the competition between agents for selection for human chemoprevention trials, agents that will most likely be chosen are those with a rational mechanism, compelling evidence in appropriate animal models for chemopreventive activity, agents that can be given orally or in depot form, and most importantly, evidence of probable low human toxicity. These are very different requirements than those for potential chemotherapeutic drugs. Since the use of chemicals as drugs in humans is always associated with some side-effects, the benefits of the compound must outweigh its side-effects. This benefit ratio is easier to achieve in populations at high risk of developing cancer due to acquired or genetic risk factors, or subjects at high risk due to previously cured cancers. It is, thus, likely that new chemopreventive agents will be tested in specific populations of relatively high cancer risk. For example, chemoprevention studies for lung and head and neck cancer often limit study subjects to those with a chronic history of cigarette smoking, asbestos exposure and/or prior resection of an early stage cancer. The use of high-risk populations reduces the number of subjects required to observe a significant reduction in tumour incidence. In addition, patients who perceive them-

Correspondence to G. Powis.
The authors are at the Arizona Cancer Center, University of Arizona Health Sciences Center, 1515 North Campbell Avenue, Tucson, Arizona 85724, U.S.A.
Received 8 Apr. 1994; accepted 3 May 1994.

selves to be at an increased risk of cancer may be more compliant and less likely to become early study drop-outs. Finally, since most chemopreventive agents have documented toxicities, the potential risk/benefit ratio is more favourable for individuals with a higher cancer risk.

Study design

Like traditional clinical chemotherapy studies, chemoprevention trials are conducted in phases. However, the objectives of chemoprevention studies differ from their counterparts in cancer therapy.

Phase I study. Phase I studies should only be performed if there are convincing *in vitro* and/or epidemiological data to suggest that an agent has significant chemopreventive activity and there are insufficient clinical data to select an appropriate dose for long-term use. The primary objective of a phase I chemoprevention study is to identify the highest dose of an agent which may be administered with minimal or no toxicity (if *in vitro* data do not show evidence of a dose/efficacy relationship, a dose with no associated toxicity should be selected). As a secondary objective, the study may gather data on the dose/toxicity relationship. Approximately 20 to 25 patients should be treated at each dose level and drug administration should last for several months. Pharmacokinetics studies are often performed in tandem with phase I studies. Additionally, pilot studies of intermediate tumour markers may be performed.

Selection of a dose for further clinical testing is somewhat problematic. Subjects who believe themselves to be at high risk of developing cancer may be more tolerant of side-effects than the general population. In addition, dose selection for chronic administration will be largely based on data from short-term studies. It is important to choose a dose level with no or extremely minimal side-effects since subjects who are asymptomatic from their premalignancy will be reluctant to experience toxicities from treatment of a disease which they may never develop. Since the potential benefit must outweigh the associated risk, severe to moderate toxicities are unacceptable in chemoprevention studies.

Phase II study. Phase II chemopreventive studies may be performed if toxicities associated with an agent proved minimal during initial clinical studies, and epidemiological and laboratory studies continue to provide significant evidence of chemopreventive activity. Phase II chemoprevention studies are randomised, double-blind and generally placebo-controlled studies. The placebo study arm is used to better quantify toxicities which often occur in the general, untreated population (e.g. headaches, gastrointestinal disturbances, dermatological disorders), as well as determine the variability of surrogate endpoint biomarkers over a prolonged time period. Total accrual is generally limited to 100–1000 subjects. Multiple dose levels and drug combinations of chemopreventive agents may be tested. In addition to careful toxicity evaluation, phase II chemoprevention trials may study strategies for improving patient recruitment, retention and compliance, and further evaluate chemopreventive agent pharmacokinetics and the relevance of specific surrogate endpoint biomarkers.

Phase III study. Phase III chemoprevention trials are large, randomised, placebo controlled studies, generally lasting several years. The primary endpoint of these studies is a reduction in cancer incidence and/or regression of premalignant lesions. Multiple agents and dose levels may be studied using factorial designs. Secondary study endpoints include long-term toxicity evaluations and often, the modulation of surrogate endpoint biomarkers. The sample size of individual studies varies on the basis of statistical concerns (including targeted decrease in precancer recurrence of cancer incidence and estimated subject drop-out rates) and the demographics of the study population being evaluated.

Cancer chemoprevention and signalling pathways

The only known function of oncogenes is to code for protein components of intracellular signalling pathways that mediate the effects of growth factors on cell proliferation. The presence of mutated or overexpressed oncogenes in the cancer cell leads to the constitutive activation of signalling pathways that generate, within the cancer cell, an unrestrained signal to grow. If agents can be developed that block the signalling pathways activated by an oncogene, it might be possible to suppress cancer cell proliferation and invasion. The degeneracy of growth factor intracellular signalling, where alternate pathways can take over the function of an inhibited pathway, suggests that agents that selectively inhibit oncogene function but leave normal cell function unaffected can be developed [4]. This review will describe a number of chemopreventive agents already in clinical trial that may act by inhibiting intracellular signalling.

Signalling pathways

A detailed description of the signalling pathways involved in growth factor and oncogene signalling is beyond the scope of this review, and the reader is referred to more comprehensive treatments of the topic [4, 5]. A major pathway involves the guanine nucleotide binding (G) protein-mediated activation of phosphatidylinositol specific phospholipase C (PtdInsPLC). A mitogenic peptide acts on a membrane-spanning receptor coupled to a specific G protein to activate membrane-bound PtdInsPLC-β [6, 8]. PtdInsPLCs hydrolyse a minor membrane phospholipid, phosphatidylinositol(4,5)bisphosphate, to give the water soluble inositol(1,4,5)trisphosphate and a lipophilic diacylglycerol (DAG) [9]. Inositol(1,4,5)trisphosphate releases Ca^{2+} from non-mitochondrial stores, producing a transient increase in the cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$), while DAG is an activator of a Ca^{2+} and phospholipid-dependent protein serine/threonine kinase, protein kinase C (PKC) [10]. PKC phosphorylates and activates the BZip superfamily of signal-regulated transcription factors [11]. DAG can also be formed by the hydrolysis of phospholipids in addition to PtdIns, most notably phosphatidylcholine [12]. The enzyme primarily responsible for phosphatidylcholine hydrolysis is phospholipase D which gives as products phosphatidic acid and choline [13]. Phosphatidic acid is dephosphorylated by phosphatidate phosphohydrolase to give DAG [14].

In another pathway, the binding of a growth factor, such as platelet-derived growth factor (PDGF) or epidermal growth factor (EGF), to its plasma membrane receptor causes the receptor monomers to dimerise and then to phosphorylate each other on tyrosine residues [15]. This permits a conformational change in the receptor that enhances its protein tyrosine kinase activity toward other substrates [16]. It also provides phosphotyrosine binding sites on the receptor for the recruitment of specific cytoplasmic enzymes that contain a *src*-homology-2 (SH2) domain that binds with high affinity to certain phosphotyrosine-containing sequences [17]. One such enzyme is PtdInsPLC-γ, which becomes phosphorylated on tyrosine and

activated by the ligand-activated receptor [18]. Another enzyme that undergoes SH2 domain binding and is tyrosine phosphorylated is phosphatidylinositol-3-kinase [19]. The mechanism by which phosphatidylinositol-3-kinase activation stimulates cell proliferation is not known.

The adaptor protein, Grb2 (growth factor receptor binding protein 2), undergoes SH2 domain binding to the tyrosine-phosphorylated EGF receptor [20]. Grb2 also binds to the ubiquitously-expressed cytoplasmic protein Sos (son of sevenless) [21]. Sos facilities the exchange of GDP by GTP, thus, activating the small G protein Ras. The movement of Sos from the cytoplasm to the plasma membrane, where Ras is located, results in Ras activation [22]. MAP kinases are a group of protein serine/threonine kinases that are activated by phosphorylation on both a tyrosine and a threonine residues by MAP kinase-kinase or MEK [23, 24]. MEK is itself activated by phosphorylation on a threonine residue by c-Raf protein kinase [25]. c-Raf binds directly to activated Ras in association with MEK [26] and the activity of both c-Raf and MAP kinase is stimulated by Ras [27].

Oncogenes and intracellular signalling

Oncogene protein products relevant to human cancer activate signalling pathways by a number of mechanisms. The first is by acting as a growth factor, thus, setting up an autocrine loop, for example c-*fms* (granulocyte–macrophage colony stimulating factor, GM-CSF). The second is by acting as a receptor protein tyrosine kinase, for example c-*erb*B1 (EGF receptor), c-*erb*B2 (Her2/*neu*), *ret* and *trk* (NGF receptor), or a non-receptor protein tyrosine kinase, for example *src*, *abl* and *met*. The third involves transmission of signals by GTPases such as *ras*. Finally, oncogenes that act as transcription regulators include *myc*, c-*myb*, c-*ets*-1, *hox*-11 and *lyl*.

CHEMOPREVENTIVE DRUGS THAT INHIBIT SIGNALLING PATHWAYS

Inhibitors of protein kinase C

PKCs present a particularly interesting target for chemopreventive drugs because they are a primary receptor for the tumour-promoting phorbol esters, such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) [10, 28]. cDNAs coding for nine different PKC isoenzymes have been isolated (PKC-α, βI, βII, γ, δ, ϵ, ζ, η,θ). PKCs have a catalytic C-terminal domain and an N-terminal regulatory domain. The regulatory domain contains binding sites for Ca^{2+} (PKC-α, βI, βII and δ isoenzymes only), phospholipid and DAG, as well as the autoinhibitory pseudo-substrate amino acid sequence that closely resembles the PKC recognition sequence of its protein substrates [29]. Most activated PKCs are translocated from the cytoplasm to the cell membrane, possibly binding to a membrane receptor [30]. Translocation of PKC to the cell membrane results in its eventual proteolytic degradation and downregulation of the enzyme. However, not all the forms of PKC are membrane associated or activated by Ca^{2+}. Selective localisation of the isoforms PKCβ and PKCη to the nucleus has been reported [31, 32]. PKC levels are altered in many tumour cells [33], and cells that overproduce PKC are susceptible to transformation by v-H-*ras* [34]. Overexpression of PKCβI, PKCγ and PKCϵ in NIH 3T3 cells causes a transformed phenotype [35–37].

The non-steroidal anti-oestrogen, tamoxifen, is currently used in the adjuvant treatment of breast cancer and for the chemoprevention of breast cancer [38]. Tamoxifen has effects in addition to inhibition of oestrogen receptors, and inhibits DNA synthesis and the proliferation of cells that do not express oestrogen receptors [39–41]. Tamoxifen and its metabolites are weak inhibitors of PKC (IC_{50} 40–100 μm), possibly through a specific binding site on the enzyme [42, 43]. Tamoxifen inhibits TPA stimulation of hydrogen peroxide formation by human neutrophils [44], TPA and DAG-induced histamine release from mast cells [45], and TPA inhibition of bombesin-induced phosphoinositide breakdown in MCF-7 breast cancer cells [46].

Retinoids, such as all-*trans*-retinoic acid, have cell differentiating and chemopreventive properties [47]. Their action has been linked to binding to high affinity nuclear receptors that regulate gene expression. However, retinoids also have effects on intracellular signalling. During cell differentiation induced by retinoids, PKC activity is increased [48–51]. With purified PKC, the retinoids act as partial agonists, activating the enzyme in the absence of DAG [51–53], and blocking the DAG- and TPA-dependant activation of PKC with IC_{50}s around 100 nM [51, 54, 55]. Based on this evidence, it will be interesting to see if another PKC partial agonist, bryostatin 1, that is currently undergoing clinical trials as a chemotherapeutic drug [56], exhibits chemopreventive activity.

α-Tocopherol is a chemopreventive agent for oral leukoplakia in humans [57]. The biological effects of α-tocopherol are usually ascribed to its radical scavenging properties. However, α-tocopherol has been shown to inhibit PKC activity in smooth muscle cells that parallels its antiproliferative effects [58, 59]. β-Tocopherol, which is almost effective as a radical scavenging agent as α-tocopherol, is only a weak inhibitor of PKC and of cell proliferation, which suggests that radical scavenging may not be responsible for the antiproliferative effects of α-tocopherol. α-Tocopherol succinate, although not α-tocopherol acetate or α-tocopherol, inhibits the DNA binding activity of the transcription factor NF-κB [60].

Ether lipid analogues, such as 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET-18-OCH_3, edelfosine), have undergone clinical trials as cancer chemotherapeutic drugs [61, 62]. They are inhibitors of PKC (IC_{50} 10μM) [63] and inhibit PKC in intact cells which has been suggested to be related to their antitumour activity [64–66]. However, the inhibition of PKC in intact cells does not always correlate with the cytotoxicity of ether lipid analogues [67, 68]. As discussed later, ether lipid analogues inhibit other aspects of intracellular signalling including Ca^{2+} release [69], PtdInsPLC [70] and phosphatidylinositol-3-kinase [71]. Clinical studies in cancer patients suggest that ET-18-OCH_3 is a cytostatic rather than a cytotoxic agent, and can be administered orally to patients for extended periods of time with relatively low toxicity [72, 73]. This, together with the observations that ET-18-OCH_3 and other ether lipids have chemopreventive activity against some chemically-induced tumours in animals [74, 75], suggests that the ether lipids should be evaluated as potential chemopreventive agents.

c-AMP-dependant protein kinase A (PKA)

Another protein serine/threonine kinase important for growth factor intracellular signalling that offers a potential target for chemopreventive drugs is c-AMP-dependent protein kinase A (PKA) [76]. PKA is composed of two distinct catalytic and regulatory subunits. Three different catalytic subunits and four regulatory subunits have been identified. Type I and type II PKAs show a common catalytic subunit, but have regulatory subunits of their own distinct class. Increased type I PKA leads to increased cell growth while decreased type I PKA or increased type II PKA leads to cell growth inhibition and differentation.

During retinoid-induced cell differentiation, there is a rapid increase in PKA activity [51, 77] with a preferential increase in type II PKA, compared to type I PKA [51, 78, 79].

Phosphotyrosine signalling

Protein tyrosine phosphorylation is a major event regulating cell growth and transformation [80]. The flavonoid, quercetin, inhibits a number of protein tyrosine kinases [81] and is a chemopreventive agent that prevents the development of chemically-induced colonic and mammary tumours in animals [82]. Depletion of cellular polyamines by the chemopreventive agent, α-difluoromethylornithine, is associated with a decrease in cellular phosphotyrosine levels, apparently due to activation of protein tyrosine phosphatases [83].

Ca^{2+} signalling

Cell membrane Ca^{2+} influx channels are either voltage-dependent or receptor-operated [84]. Inhibitors of voltage-dependent Ca^{2+} channels exhibit growth inhibitory activity against some human tumour cell lines. For example, the growth of HT-39 human breast cancer cells in athymic mice is inhibited by the Ca^{2+} channel antagonists diltiazem, amlodipine and verapamil [85]. CAI, a carboxyamido-triazole, whose lead compound was originally developed as a coccidiostat, has been shown to possess *in vitro* and *in vivo* antitumour and chemopreventive activity [86–88]. CAI inhibits intracellular signalling by blocking voltage-dependent and receptor-operated Ca^{2+} influx channels, arachidonic acid formation and the generation of inositol phosphates [87, 89, 90]. CAI also inhibits nucleotide biosynthesis at the level of phosphoribosyl phosphate synthetase [91]. CAI is currently in phase I clinical trial [92].

Tamoxifen can directly inhibit Ca^{2+} influx channels [93–95], which has been suggested to be responsible for its cell growth inhibitory properties [96]. However, in some cells, tamoxifen prolongs the opening of Ca^{2+} channels that have already been opened by agonists, such as carbachol or K^+ -induced membrane depolarisation, resulting in prolonged increases and oscillations in agonist-induced $[Ca^{2+}]_i$ responses [97]. Tamoxifen also binds to the Ca^{2+}-sensitive regulatory protein, calmodulin, in a Ca^{2+}-dependant manner causing its inhibition [98, 99]. This is probably how tamoxifen increases cyclic-AMP in cells by inhibiting cyclic-AMP hydrolysis by Ca^{2+}-dependant cyclic nucleotide phosphodiesterase [98, 100, 101]. Tamoxifen also attenuates agonist-induced increases in $[Ca^{2+}]_i$ by inhibiting Ca^{2+}-calmodulin-dependent membrane ATPase Ca^{2+} pumps, as well as the calmodulin-independent uptake of Ca^{2+} by the endoplasmic reticulum [94]. How these sometimes conflicting effects of tamoxifen might affect cell proliferation is not known. It should be noted that most of these effects of tamoxifen, as well as inhibition of PKC discussed previously, require tamoxifen concentrations of 10 μM or more, whereas tamoxifen concentrations in the serum of patients being treated chronically with the drug rarely exceed 1 μM [102, 103].

Ras as a target

Mutated Ras proteins play an important role in the development of many human cancers [104]. The carboxy terminus of nascent Ras includes the CAAX motif where the C is a cysteine residue that becomes bound with a farnesyl group derived from the mevalonic acid pathway of cholesterol biosynthesis. This is followed by proteolytic removal of the tipeptide AAX and carboxylation of the farnesylated cysteine residue. At this point, Ras can associate with the plasma membrane and be further modified on other carboxy terminus cysteine residues by palmitic acid [105].

The monoterpene, d-limonene, a major constituent of oil of orange that is widely used as a flavour and fragrance, has been shown to selectively inhibit the isoprenylation of small GTP-binding proteins including Ras, at a point in the mevalonic acid pathway distal to HMG-CoA reductase and to inhibit farnesyl-protein transferase [106, 107]. d-Limonene has shown chemopreventive and chemotherapeutic activity against chemically-induced mammary carcinoma in rat [106, 108], and is currently in phase I clinical trials in patients with advanced breast and gastrointestinal cancer [109].

Phospholipases

PtdInsPLC represents another interesting target for chemopreventive drugs. A number of growth factors, including PDGF and EGF, increase PtdInsPLC activity in cells [110, 111]. Cells transfected with a mutant $G\alpha_q$ subunit, which is normally coupled to PtdInsPLC-β, have elevated PtdInsPLC activity and a transformed phenotype [112]. Transfection of cells with the muscarinic receptors m1, m3 and m5, which potently stimulate PtdInsPLC-β through a G protein-coupled mechanism, causes agonist-dependent cell transformation [113]. Cancer cells expressing the transforming *neu*/HER2 oncogene show constitutive tyrosine phosphorylation of PtdInsPLC-γ, while a non-transforming, kinase-defective mutant of *neu*/HER2 does not cause the tyrosine phosphorylation or association with PtdInsPLC-γ [114]. Studies of specific mutation-restoration of PtdInsPLC-γ binding to a tyrosine-mutated PDGF receptor have shown that binding of PtdInsPLC-γ is sufficient to confer a mitogenic response to PDGF [115]. PtdInsPLC-γ immunoreactive protein is high in human breast cancer compared to benign breast tissue, and is localised to EGF-receptor or Her2/*neu*-expressing cells [116]. PtdInsPLC activity is also elevated in human non-small cell lung cancer, renal cell cancer and glial tumours compared to normal tissue, and is correlated with increased EGF receptor levels in the tumour [117, 118]. The association between increased PtdInsPLC and EGF-receptor or Her2/*neu* expression suggests that PtdInsPLC might be a good surrogate chemopreventive drug target to inhibit the increased activity of these signalling pathways.

The ether lipid antitumour drugs are potent inhibitors of PtdInsPLC. PtdInsPLC-γ is inhibited by ET-18-OCH_3 with an IC_{50} of 0.4 μM [70]. The formation of inositol phosphates in PDGF-stimulated fibroblasts is inhibited by 10 μM ET-18-OCH_3 which is similar to the cytotoxic concentration of ET-18-OCH_3 for this cell [119]. The ether lipid analogue hexadecylphosphocholine inhibits inositol phosphate signalling in fibroblasts [120] and is an inhibitor of PtdInsPLC-δ1 [121]. The *myo*-inositol ether lipid analogue *rac*(3-hexadecyloxy-2-ethoxypropyl)phosphono-*myo*-inositol, has been reported to inhibit human melanoma PtdInsPLC with an IC_{50} of 61 μM and to inhibit human melanoma cell growth with an IC_{50} of 28 μM [122].

Tamoxifen has been reported to bind to phosphatidylinositol and, thus, prevent the feedback product inhibition of phosphatidylinositol kinases [123]. It is possible that, by binding to PtdIns, tamoxifen will inhibit PtdIns PLC in the same way that neomycin, which also binds to PtdIns, inhibits PtdIns PLC [124]. Tamoxifen also inhibits phospholipase A_2 [125]. Retinoic acid, at concentrations that cause HL-60 cell differentiation, inhibits of PtdInsPLC activity by uncoupling its activation by G protein [126].

A newly discovered class of chemopreventive agents are the non-steroidal anti-inflammatory agents (NSAIDs) such as aspirin for the chemoprevention of colon cancer [127]. One of the effects of aspirin and other NSAIDs is the inhibition of phospholipase activity, measured by inhibition of DAG formation, in cells in which they are incubated [128]. This is not a direct effect on phospholipase activity, but appears to be mediated through new protein synthesis. The phospholipases are involved in many aspects of intracellular signalling, and it is an intriguing possibility that they may be a target site for the chemopreventive effects of NSAIDs.

CONCLUSIONS

It is apparent that a number of currently used chemopreventive agents have effects on intracellular signalling pathways. This may reflect the overall importance of the signalling pathways to cell function, and it is not always clear whether the effects on signalling are a primary or secondary effect of the agents. Notwithstanding, in several cases the alterations in signalling are early events that occur at concentrations of the chemopreventive agent that are cytostatic or cause cell differentiation. This argues for more than a casual relationship between the two effects. There are also strong mechanistic reasons to expect signalling inhibitors to show chemopreventive activity. The hypothesis that inhibiting key intracellular signalling pathways involved in oncogene action might provide a safe and effective approach to the chemoprevention of cancer remains to be tested prospectively. However, the evidence presented previously provides a mechanistic basis for classifying some already identified chemopreventive agents as signalling inhibitors, and could aid in the rational development and testing of new types of chemopreventive agents.

1. Malone WF, Kellnoff GJ, Pierson H, Greenwald P. Chemoprevention of bladder cancer. *Cancer* 1987, **60**, 650–657.
2. Costa A. Breast cancer chemoprevention. *Eur J Cancer* 1993, **29A**, 589–592.
3. Grieshaber CK. Prediction of human toxicity of new antineoplastic drugs from studies in animals. In Powis G, Hacker MP, eds. *The Toxicity of Anticancer Drugs*. New York, Pergamon Press, 1991, 10–27.
4. Powis G. Signalling pathways as targets for anticancer drug development. *Pharmacol Ther* 1994, in press.
5. Powis G. Signalling targets for anticancer drug development. *Trends Pharmacol Sci* 1991, **12**, 188–194.
6. Smrcka AV, Hepler JR, Brown KO, Sternweis PC. Regulation of polyphosphoinositide-specific phospholipase C activity by purified G_q. *Science* 1991, **281**, 804–827.
7. Taylor SJ, Chae HZ, Rhee SG, Exton JH. Activation of the β1 isozyme of phospholipase C by α subunits of the G_q class of G proteins. *Nature* 1991, **350**, 516–518.
8. Wu D, Lee CH, Rhee SG, Simon MI. Activation of phospholipase C by the α subunits of the Gq and G_{11} proteins in transfected Cos-7 cells. *J Biol Chem* 1992, **267**, 1811–1817.
9. Berridge MJ, Irvine RF. Inositol phosphates and cell signalling. *Nature* 1989, **341**, 197–205.
10. Kikkawa U, Nishizuka Y. The role of protein kinase C in transmembrane signalling. *Ann Rev Cell Biol* 1986, **2**, 149–178.
11. Karin M, Smeal T. Control of transcription factors by signal transduction pathways: the beginning of the end. *Trends Biochem Sci* 1992, **17**, 418–422.
12. Cook SJ, Wakelam MJO. Phospholipases C and D in mitogenic signal transduction. *Science* 1992, **119**, 14–38.
13. Cook SJ, Wakelam MJO. EGF increases sn-1,2-diglyceride levels and activates phospholipase D-catalysed phosphatidylcholine breakdown in Swiss 3T3 cells in the absence of inositol lipid hydrolysis. *Biochem J* 1992, **281**, 597–600.
14. Moolenaar WH, Jalink K, VanCorven EJ. Lysophosphatidic acid: a bioactive phospholipid with growth factor-like properties. *Rev Physiol Biochem Pharmacol* 1992, **119**, 48–63.
15. Bishayee S, Majumdar S, Khire J, Das M. Ligand-induced dimerization of the platelet-derived growth factor receptor. *J Biol Chem* 1989, **264**, 11699–11705.
16. Nishimura J, Huang JS, Deuel TF. Platelet-derived growth factor stimulates tyrosine specific protein kinase activity in Swiss mouse 3T3 cell membranes. *Proc Natl Acad Sci USA* 1982, **79**, 4303–4307.
17. Margolis B. Protein with SH2 domains: transducers in the tyrosine kinase signalling pathway. *Cell Growth Differ* 1992, **3**, 73–80.
18. Wahl MI, Oleshaw NE, Nishibe B, Rhee SG, Pledger WJ, Carpenter G. Platelet-derived growth factor induces rapid and sustained tyrosine phosphorylation of phospholipase C-γ in quiescence BALB/c 3T3 cells. *Molec Cell Biol* 1989, **9**, 2934–2943.
19. Downes CP, Carter AN. Phosphoinositide 3-kinase: a new effector in signal transduction? *Cell Signal* 1991, **3**, 501–507.
20. Egan SE, Giddings BW, Brooks MW, Buday L, Sizeland AM, Weinberg RA. Association of Sos Ras exchange protein with Grb2 is implicated in tyrosine kinase signal transduction and transformation. *Nature* 1993, **363**, 45–51.
21. Bowtell D, Fu P, Simon M, Senior P. Identification of murine homologues of the *Drosophila* Son of sevenless gene: potential activation of *ras*. *Proc Natl Acad Sci USA* 1992, **89**, 6511–651
22. Buday L, Downward J. Epidermal growth factor regulates p21*ras* through the formation of a complex of receptor, Grb2 adapter protein, and Sos nucleotide exchange factor. *Cell* 1993, **73**, 611–620.
23. Crews CM, Alessandrini A, Erikson RL. The primary structure of MEK, a protein kinase that phosphorylates the *ERK* gene product. *Science* 1992, **258**, 478–480.
24. Thomas G. MAP kinase by any other name smells just as sweet. *Cell* 1992, **68**, 3–6.
25. Kyriakis JM, App H, Zhang X-F, *et al.* Raf-1 activates MAP kinase-kinase. *Nature* 1992, **358**, 417–419.
26. Moodie SA, Willumsen BM, Weber MJ, Wolfman A. Complexes of ras-GTP with raf-1 and mitogen-activated protein kinase kinase. *Science* 1993, **260**, 1658–1661.
27. Wood KW, Sarnecki C, Roberts TM, Blenis J. *ras* mediates nerve growth factor receptor modulation of three signal transducing protein kinases: Map kinase, Raf-1 and RSK. *Cell* 1992, **68**, 1041–1048.
28. Hug H, Sarre TF. Protein kinase C isoenzymes: divergence in signal transduction? *Biochem J* 1993, **291**, 329–343.
29. Alvarez E, Northwood IC, Gonzalez FA, *et al.* Pro-Leu-Ser/Thr-Pro is a consensus primary sequence for substrate protein phosphorylation. Characterization of the phosphorylation of c-*myc* and c-*jun* proteins by an epidermal growth factor receptor threonine 669 protein kinase. *J Biol Chem* 1991, **266**, 15277–15285.
30. Mochly-Rosen D, Khaner H, Lopez J. Identification of intracellular receptor proteins for activated protein kinase C. *Proc Natl Acad Sci USA* 1991, **88**, 3997–4000.
31. Hocevar BA, Fields AP. Selective translocation of β protein kinase C to the nucleus of human promyelocytic (HL60) leukemia cells. *J Biol Chem* 1991, **266**, 28–33.
32. Greif H, Ben-Chaim J, Shimon T, Bechor E, Eldar H, Livneh E. The protein kinase C-related PKC-L(*eta*) gene product is localized in the cell nucleus. *Molec Cell Biol* 1992, **12**, 1304–1311.
33. Barr LF, Mabry M, Nelkin BD, Tyrler G, May WS, Baylin SB. c-*myc* gene induced alterations in protein kinase C expression: a possible mechanism facilitating *myc-ras* gene complementation. *Cancer Res* 1991, **51**, 5514–5519.
34. Borner C, Guadagno SN, Hsieh LL, Hsiao WL, Weinstein IB. Transformation by a *ras* oncogene causes increased expression of protein kinase C-alpha and decreased expression of protein kinase C-epsilon. *Cell Growth Differ* 1990, **1**, 653–660.
35. Housey GM, Johnson MD, Hsiao WLW, *et al.* Overproduction of protein kinase C causes disordered growth control in rat fibroblasts. *Cell* 1988, **52**, 343–354.
36. Persons DA, Wilkison WO, Bell RM, Finn OJ. Altered growth regulation and enhanced tumorigenicity of NIH 3T3 fibroblasts transfected with protein kinase C-1 cDNA. *Cell* 1988, **52**, 447–458.
37. Mischak H, Goodnight J, Kolch W, *et al.* Overexpression of protein kinase C-δ and -ε in NIH 3T3 cells induces opposite effects on growth, morphology, anchorage dependence, and tumorigenicity. *J Biol Chem* 1993, **268**, 6090–6096.
38. Leis HP, Jr. The role of tamoxifin in the prevention and treatment

of benign and malignant breast lesions: a chemopreventive. *Int Surg* 1993, **78**, 176–182.
39. Grenman SE, Worsham MJ, Van Dyke DL, *et al.* Establishment and characterization of UM-EC-2, a tamoxifen-sensitive, estrogen receptor-negative human endometrial carcinoma cell line. *Gynecol Oncol* 1990, **37**, 188–199.
40. Pollack IF, Randall MS, Kristofik MP, Kelly RH, Selker RG, Vertosick JR. Effect of tamoxifen on DNA synthesis and proliferation of human malignant glioma lines *in vitro*. *Cancer Res* 1990, **50**, 7134–7138.
41. MacNeil S, Dawson RA, Crocker G, *et al.* Antiproliferative effects on keratinocytes of a range of clinically used drugs with calmodulin antagonist activity. *Br J Dermatol* 1993, **128**, 143–150.
42. O'Brian CA, Housey GM, Weinstein IB. Specific and direct binding of protein kinase C to an immobilized tamoxifen analogue. *Cancer Res* 1988, **48**, 3626–3629.
43. Edashige K, Sato EF, Akimaru K, Yoshioka T, Utsumi K. Nonsteroidal antiestrogen suppresses protein kinase C—its inhibitory effect on interaction of substrate protein with membrane. *Cell Struct Funct* 1991, **16**, 273–281.
44. Lim JS, Frenkel K, Troll W. Tamoxifen supresses tumor promoter-induced hydrogen peroxide formation by human neutrophils. *Cancer Res* 1992, **52**, 4969–4872.
45. Chakravarty N. The roles of calmodulin and protein kinase C in histamine secretion from mast cells. *Agents Act* 1992, **36**, 183–191.
46. Patel KV, Schrey MP. Evidence for a role for protein kinase C in the modulation of bombesin-activated cellular signalling in human breast cancer cells. *Mol Cell Endocrin* 1992, **85**, 215–225.
47. Tallman MS, Wiernik PH. Retinoids in cancer treatment. *J Clin Pharmacol* 1992, **32**, 868–888.
48. Kraft AS, Anderson WB. Characterization of cystolic calcium-activated phospholipid-dependent protein kinase activity in embryonal carcinoma cells. *J Biol Chem* 1983, **158**, 9178–9183.
49. Niles RM, Loewy BP. Induction of protein Kinase C in mouse melanoma cells by retinoic acid. *Cancer Res* 1989, **49**, 4483–4487.
50. Makowske M, Ballester R, Cayres Y, Rosen OM. Immunochemical evidence that three protein kinase C isozymes increase in abundance during HL-60 differentiation induced by dimethyl sulfoxide and retinoic acid. *J Biol Chem* 1988, **263**, 3402–3410.
51. Anderson WB, Thomas TP, Plet A, Brion DE. Effect of retinoic acid on cyclic AMP-dependent and calcium, phospholipid-dependent protein kinase activities: Counteraction to phorbol ester tumor promoter. In Saurat R, ed. *Retinoids: New Trends in Research and Therapy*. Geneva, Retinoid Symposium, 1984, 40–47.
52. Ohkubo S, Yamada E, Endo T, Itok H, Hidaka H. Vitamin A-induced activation of Ca^{2+}-activated, phospholipid-dependent protein kinase. *Biochem Biophys Res Commun* 1984, **118**, 460–466.
53. Cope FO, Boutwell RK. A role for retinoids and a tumor-promoting phorbol ester in the control of protein phosphorylation. In Saurat R, ed. *Retinoids: New Trends in Research and Therapy*. Geneva, Retinoid Symposium, 1984, 106–124.
54. Sun S. The inhibitory effect of a third generation retinoid, R8605, on protein kinase C activity *in vitro*. *Chung Kuo I hsueh Ko Hsueh Yuan Hsueh Pao* 1991, **13**, 267–270.
55. Ghalayini A, Eichberg J. Purification of phosphatidylinositol synthetase from rat brain by CDP-diacylglycerol affinity chromatography and properties of the purified enzyme. *J Neurochem* 1985, **44**, 175–182.
56. Prendiville J, Crowther D, Thatcher N, *et al.* A phase I study of intravenous bryostatin 1 in patients with advanced cancer. *Br J Cancer* 1993, **68**, 418–424.
57. Benner SE, Winn RJ, Lippman SM, *et al.* Regression of oral leukoplakia with alpha tocopherol: a community clinical oncology program chemoprevention study. *J Natl Cancer Inst* 1993, **85**, 44–47.
58. Chatelain E, Boscoboinik DO, Bartoli GM, *et al.* Inhibition of smooth muscle cell proliferation and protein kinase C activity by tocopherols and tocotrienols. *Biochim Biophys Acta* 1993, **1176**, 83–89.
59. Ozer NK, Palozza P, Boscoboinik D, Azzi A. d-alpha-tocopherol inhibits low density lipoprotein induced proliferation and protein kinase C activity in vascular smooth muscle cells. *FEBS Lett* 1993, **322**, 307–310.
60. Suzuki YJ, Packer L. Inhibition of NF-κB DNA binding activity by α-tocopheryl succinate. *Biochem Molec Biol Int* 1993, **31**, 693–700.
61. Daniel LW. Ether lipids in experimental cancer chemotherapy. In Hickman JA, Tritton TR, eds. *Cancer Chemotherapy*. Oxford, Blackwell Sci., 1993, 146–177.
62. Andreesen R. Ether lipids in the therapy of cancer. *Prog Biochem Pharmac* 1988, **22**, 118–131.
63. Zheng A, Oishi K, Shoji M, *et al.* Inhibition of protein kinase C, (sodium plus potassium)-activated adenosine triphosphatase, and sodium pump by synthetic phospholipid analogues. *Cancer Res* 1990, **50**, 3025–3031.
64. Helfman DM, Barnes KC, Kinkade JM, Vogler WR, Shoji MM, Kuo JF. Phospholipid-sensitive Ca^{2+} dependent protein phosphorylation system in various types of leukemic cells from human patients and in human leukemic cell lines HL60 and K562, and its inhibition by alkyl-lysophospholipid. *Cancer Res* 1983, **43**, 2955–2961.
65. Marasco CJ, Jr., Piantadosi C, Ishaq KS, *et al.* Synthesis and biological activity of novel quaternary ammonium derivatives of alkylglycerols as potent inhibitors of protein kinase C. *J Med Chem* 1990, **33**, 985–991.
66. Shoji M, Raynor RL, Berdel W, Vogler WR, Kuo JF. Effects of thioether phospholipid BM 41.440 on protein kinase C and phorbol ester-induced differentiation of human leukemia HL60 and KG-1 cells. *Cancer Res* 1988, **48**, 6669–6673.
67. Salari H, Dryden P, Davenport R, Howard S, Jones K, Bittman R. Inhibition of protein kinase C by ether-linked lipids is not correlated with their antineoplastic activity on WEHI-3B and R6X-B15 cells. *Biochim Biophys Acta* 1992, **1134**, 81–88.
68. Morris-Natschke S, Meyer KL, Marasco CJ Jr, *et al.* Synthesis of quaternary amine ether lipids and evaluation of neoplastic cell growth inhibitory properties. *J Med Chem* 1990, **33**, 1812–1818.
69. Oishi K, Zheng B, Kuo JF. Inhibition of Na, K-ATPase and sodium pump by protein kinase C regulators sphingosine, lysophosphatidylcholine, and oleic acid. *J Biol Chem* 1990, **265**, 70–75.
70. Powis G, Seewald MJ, Gratas C, Melder D, Riebow J, Modest EJ. Selective inhibition of phosphatidylinositol phospholipase C by cytotoxic ether lipid analogues. *Cancer Res* 1992, **52**, 2835–2840.
71. Berggren M, Gallegos A, Dressler LA, Modest EJ, Powis G. Inhibition of the signalling enzyme phosphatidylinositol-3-kinase by antitumor ether lipid analogues. *Cancer Res* 1993, **53**, 4297–4302.
72. Berdel WE, Fink U, Rastetter J. Clinical phase I pilot study of the alkyl lysophospholipid derivative ET-18-CH_3. *Lipids* 1987, **22**, 967–969.
73. Khanavkar B, Ulbrich F, Gatzemeier U, *et al.* Treatment of non-small cell lung cancer with the alkyllysophospholipid edelfosine—intermediate data analysis. *Contrib Oncol* 1989, **37**, 224–235.
74. Berdel WE, Andreesen R, Munder PG. Synthetic alkylphospholipid analogs; a new class of antitumor antigens. In Kuo J, ed. *Phospholipids and Cellular Regulation*. Boca Raton, CRC Press, 1985, 42–73.
75. Berger MR, Schmähl D. Modulation of chemical carcinogenesis in rats by alkyllysophospholipids. *Lipds* 1987, **77**, 935–942.
76. Cho-Chung YS. Suppression of malignancy targeting cyclic AMP signal transducing proteins. *Biochem Soc Trans* 1992, **20**, 425–430.
77. Ludwig K, Loewy B, Niles R. Retinoic acid increases cyclic AMP-dependent protein kinase activity in murine melanoma cells. *J Biol Chem* 1980, **255**, 5999–6002.
78. Abou-Issa H, Duruibe V. *In vitro* and *in vivo* inhibition of breast cancer growth by retinoids via cyclic AMP-dependent protein kinase. *FASEB J* 2, A859.
79. Sporn M, Roberts A. Role of retinoids in differentiation and carcinogenesis. *Cancer Res* 1983, **43**, 3034–3040.
80. Glenney JR Jr. Tyrosine-phosphorylated proteins: mediators of signal transduction from the tyrosine kinases. *Biochim Biophys Acta* 1992, **1134**, 113–127.
81. Burke TR Jr. Protein-tyrosine kinase inhibitors. *Drugs Future* 1992, **17**, 119–131.
82. Ip C, Ganther HE. Combination of blocking agents and suppressing agents in cancer prevention. *Carcinogenesis* 1991, **12**, 365–367.
83. Oetken C, Pessa-Morikawa T, Autero M, Andersson LC, Mustelin T. Reduced tyrosine phosphorylation in polyamine-starved cells. *Exp Cell Res* 1992, **202**, 370–375.
84. Putney JW, Jr. Receptor-regulated calcium entry. *Pharmacol Ther* 1990, **48**, 427–434.
85. Taylor JM, Simpson RU. Inhibition of cancer cell growth by calcium channel antagonists in the athymic mouse. *Cancer Res* 1992, **52**, 2413–2418.

86. Hupe DJ, DiSalvo J, Schaeffer MT. A novel antiproliferative and antimetastasis agent which interferes with calcium signal transduction. *Cancer Chemother Pharmac* 1989, **24**, S85.
87. Kohn EC, Liotta LA. L651582: a novel antiproliferative and antimetastasis agent. *J Natl Cancer Inst* 1990, **82**, 54–60.
88. Kohn EC, Sandeen MA, Liotta LA. *In vivo* efficacy of a novel inhibitor of selected signal transduction pathways including calcium, arachidonate, and inositol phosphates. *Cancer Res* 1992, **52**, 3208–3212.
89. Felder CC, Ma. AL, Liotta LA, Kohn EC. The antiproliferative and antimetastatic compound L651582 inhibits muscarinic acetylcholine receptor-stimulated calcium influx and arachidonic acid release. *J Pharmacol Exp Ther* 1991, **257**, 967–971.
90. Hupe DJ, Boltz R, Cohen CJ, *et al.* The inhibition of receptor-mediated and voltage-dependent calcium entry by the antiproliferative L-651, 582. *J Biol Chem* 1991, **266**, 10136–10142.
91. Hupe DJ, Behrens ND, Boltz R. Anti-proliferative activity of L-651, 582 correlates with calcium-mediated regulation of nucleotide metabolism at phosphoribosyl pyrophosphate synthetase. *J Cell Physiol* 1990, **144**, 457–466.
92. Cole K, Kohn E. Calcium-mediated signal transduction: biology, biochemistry and therapy. *Cancer Metast Rev* 1994, **13**, 31–44.
93. Lipton A, Morris ID. Calcium antagonism by the antioestrogen tamoxifen. *Cancer Chemother Pharmac* 1986, **18**, 17–20.
94. Malva JO, Lopes CF, Vale GP, Carvalho AP. Action of antiestrogens on the $(Ca^{2+} + Mg^{2+})$-ATPase and Na^+/Ca^{2+} exchange of brain cortex membranes. *Biochem Pharmacol* 1990, **40**, 1877–1884.
95. Greenberg DA, Carpenter CL, Messing RO. Calcium channel antagonist properties of the antineoplastic antiestrogen tamoxifen in the PC12 neurosecretory cell line. *Cancer Res* 1987, **47**, 70–74.
96. Salomon DS, Zwiebel JA, Bano M, Losonczy I, Fehnel P, Kidwell WR. Presence of transforming growth factors in human breast cancer cells. *Cancer Res* 1984, **44**, 4069–4077.
97. Morley P, Whitfield JF. Effect of tamoxifen on carbachol-triggered intracellular calcium responses in chicken granulosa cells. *Cancer Res* 1994, **54**, 69–74.
98. Lopes CF, Vale GP, Carvalho AP. Ca^{2+}-dependent binding of tamoxifen to calmodulin isolated from bovine brain. *Cancer Res* 1990, **50**, 2753–2758.
99. Bouhoute A, Leclerq G. Antagonistic effect of triphenylethylenic antiestrogens on the association of estrogen receptor to calmodulin. *Biochem Biophys Res Commun* 1992, **184**, 1432.
100. Rowlands MG, Parr IB, McCague R, Jarman M, Goddard PM. Variation of the inhibition of calmodulin dependent cyclic AMP phosphodiesterase amongst analogues of tamoxifen; correlations with cytotoxicity. *Biochem Pharmacol* 1990, **40**, 283–289.
101. Lam H-YP. Tamoxifen is a calmodulin antagonist in the activation of cAMP phosphodiesterase. *Biochem Biophys Res Commun* 1984, **118**, 27–32.
102. Furr BJA, Jordan VC. The pharmacology and clinical uses of tamoxifen. *Pharmacol Ther* 1984, **25**, 127–205.
103. Bratherton DG, Brown CH, Buchanan R, *et al.* A comparison of two doses of tamoxifen (Nolvadex) in postmentrual women with advanced breast cancer: 10 mg bd versus 20 mg bd. *Br J Cancer* 1984, **50**, 199–205.
104. Barbacid M. *Ras* genes. *Ann Rev Biochem* 1987, **56**, 779–827.
105. Gibbs JB. Ras C-terminal processing enzymes—new drug targets? *Cell* 1991, **65**, 1–4.
106. Crowell PL, Kennan WS, Haag JD, Ahmad S, Vedejs E, Gould MN. Chemoprevention of mammary carcinogenesis by hydroxylated derivatives of d-limonene. *Carcinogenesis* 1992, **13**, 1261–1264.
107. Crowell PL, Chang RR, Ren ZB, Elson CE, Gould MN. Selective inhibition of isoprenylation of 21-26-kDa proteins by the anticarcinogen *d*-limonene and its metabolites. *J Biol Chem* 1991, **266**, 17679–17685.
108. Haag JD, Lindstrom MJ, Gould MN. Limonen-induced regression of mammary carcinomas. *Cancer Res* 1992, **52**, 4021–4026.
109. Coombes RC. Limonene, inhibitor of ras-farnesylation, in the treatment of mammary tumours and phase I study in patients with cancer. *Cell Signal Cancer Treat Symposium* 1993, Dec. 5–9, b-6.
110. Takuwa N, Takuwa Y, Bellag WE, Rasmussen H. The effects of bombesin on polyphosphoinositide and calcium metabolism in Swiss 3T3 cells. *J Biol Chem* 1987, **262**, 182–188.
111. N»nberg E, Rozengurt E. Temporal relationship btwen inositol polyphosphate formation and increases in cytosolic Ca^{2+} in quiescent 3T3 cells stimulated by platelet-derived growth factor, bombesin and vasopressin. *EMBO J* 1988, **9**, 2741–2747.
112. Kalinec G, Nazarali AJ, Hermouet S, Xu N, Gutklind JS. mutated α subunit of the G_q protein induces malignant transformation in NIH 3T3 cells. *Molec Cell Biol* 1992, **12**, 4687–4693.
113. Gutkind JS, Novotny EA, Brann MR, Robbins KC. Muscarinic acetylcholine receptor subtypes as agonist dependent oncogenes. *Proc Natl Acad Sci USA* 1991, **88**, 4703–4708.
114. Peles E, Levy RB, Or E, Ullrich A, Yarden Y. Oncogenic forms of the *neu*/HER2 tyrosine kinqase are permanently coupled to phospholipase C-γ. *EMBO J* 1991, **10**, 20778–2086.
115. Valius M, Kazlauska A. Phospholipase C-γ1 and phosphatidylinositol 3 kinase are the downstream mediators of the PDGF receptor's mitogenic signal. *Cell* 1993, **73**, 321–334.
116. Grisham JW. Use of hepatic cell cultures to detect and evaluate the mechanism of action of toxic chemicals. *Int Rev Exp Pathol* 1979, **20**, 123–209.
117. Haas N, McDanel h, Godwin A, Humphrey P, Bigner S, Wong A. Specific expression of phospholipase C-γ in human gial tumors. *Proc Am Assoc Cancer Res* 1991, **32**, 17.
118. Robertson JB, Hurd SD, Koch MO, Arteaga CL. Increased phospholipase C(PLC)-γ, activity in human non-small cell lung (NSCLC) and renal cell (RCC) carcinomas correlates with elevated EGF receptor levels. *Proc Am Assoc Cancer Res* 1992, **33**, 89.
119. Seewald MJ, Olsen RA, Sehgal I, Melder DC, Modest EJ, Powis G. Inhibition of growth factor-dependnt inositol phosphate Ca^{2+} signalling by antitumor ether lipid analogues. *Cancer Res* 1990, **50**, 4458–4463.
120. Uberall F, Oberhuber H, Maly K, Zaknun J, Demuth L, Grunicke HH. Hexadecylphosphocholine inhibits inositol phosphate formation and protein kinase C activity. *Cancer Res* 1991, **51**, 807–812.
121. Pawelczyk T, Lowenstein JM. Inhibition of phospholipase Cδ by hexadecylphosphorylcholine and lysophospholipids with antitumor activity. *Biochem Pharmacol* 1993, **45**, 493–497.
122. Perrella FW, Piantadosi C, Marasco CJ, Modest EJ. Inhibition of phospholipqase C and cell growth by ethr lipid analogues of phosphatidylinositol. *Proc Am Assoc Cancer Res* 1990, **31**, 409.
123. Friedman ZY. Tamoxifen and vanadate synergize in causing accumulation of polyphosphoinositides in GH_4C_1 membranes. *J Pharmacol Exp Ther* 1993, **267**, 617–623.
124. Gabev E, Kasianowicz J, Abbott T, McLaughlin S. Binding of neomycin to phosphatidylinositol 4,5-bisphosphate (PIP_2). *Biochim Biophys Acta* 1989, **979**, 105–112.
125. Fayard J-M, Chanal S, Fanidi A, Pageaux J-F, Lagarde M, Laugier C. *In vivo* inhibition of basal and estrogen-induced phospolipase A_2 activities by the triphenylethylene antiestrogen, tamoxifen, in immature quail oviduct. *Eur J Pharmacol* 1992, **216**, 127–130.
126. Geny B, Cost H, Barreau P, *et al.* The differentiating agent, retinoic acid, causes an early inhibition of inositol lipid-specific phospholipase C activity in HL-60 cells. *Cell Signal* 1991, **3**, 11–23.
127. Thun MJ, Calle EE, Namboodiri MM, *et al.* Risk factors for fatal colon cancer in a large prospective study. *J Natl Cancer Inst* 1992, **84**, 1491–1500.
128. Bomalaski JS, Hirata F, Clark MA. Aspirin inhibits phospholipase C. *Biochem Biophys Res Commun* 1986, **139**, 115–121.

Acknowledgements—Work conducted in the authors' laboratories was supported by NIH Grants CA42286 and CA52995 (GP) and CA27502 and CA41108 (DSA). The excellent secretarial assistance of Madelon Cook is gratefully acknowledged.