Acta Biochimica Polonica

Vol. 42 No. 4/1995

395–404

QUARTERLY

Text of lecture presented at the 5th International Symposium on ***Molecular Aspects of Chemotherapy***

Advances with phospholipid signalling as a target for anticancer drug development*

Garth Powis[a]**, Margareta Berggren[a], Alfred Gallegos[a], Timothy Frew[a], Simon Hill[a], Alan Kozikowski[b], Rosanne Bonjouklian[c], Leon Zalkow[d], Robert Abraham[e], Curtis Ashendel[f], Richard Schultz[c] and Ronald Merriman[c]

[a]*Arizona Cancer Center, Arizona Health Sciences Center, University of Arizona, 1515 North Campbell Avenue, Tucson, AZ 85724 U.S.A.*
[b]*Georgetown Institute of Cognitive and Computational Sciences, Georgetown University Medical Center, Washington DC 20007 U.S.A.*
[c]*Lilly Research Laboratories, Eli Lilly Corporation, Indianapolis, IN 46285 U.S.A.*
[d]*School of Chemistry, Georgia Institute of Technology, Atlanta, GA 30332 U.S.A.*
[e]*Department of Immunology, Mayo Clinic, Rochester, MN 55905 U.S.A.*
[f]*Department of Medicinal Chemistry, Purdue University, West Lafayette, IN 47907, U.S.A.*

Key words: phosphatidylinositol-3-kinase, phospholipase 3, signalling, cancer drugs, glycosylphosphatidylinositol anchors

The phosphatidylinositol-3-kinases (PtdIns-3-kinase) are a family of enzymes involved in the control of cell replication. One member of the family, the mammalian p110/p85 PtdIns-3-kinase, is a potential target for anticancer drug development because of its role as a component of growth factor and oncogene activated signalling pathways. There are a number of inhibitors of this PtdIns-3-kinase, the most potent being wortmannin (IC_{50} 4 nM). Wortmannin inhibits cancer cell growth and has shown activity against mouse and human tumor xenografts in mice. Other inhibitors of the PtdIns-3-kinase are halogenated quinones which also inhibit cancer cell growth and have some *in vivo* antitumor activity. Some D-3-deoxy-3-substituted *myo*-inositol analogues and their corresponding PtdIns analogues have been synthesized. They may act as *myo*-inositol antimetabolites in the PtdIns-3-kinase pathway and they can inhibit cancer cell growth.

Cancer can be considered a disease of deranged intracellular signalling. A major function of oncogenes is to code for components of intracellular signalling pathways that mediate the effects of growth factors on cell proliferation [1, 2]. When an oncogene is overexpressed or constitutively activated through mutation, the cell is provided with a continuous signal to grow. The combination of a number of oncogene-activated signalling pathways, and the loss of tumor suppressor genes that normally provide a restraint on cell proliferation, leads to the transformation of a normal cell into a cancer cell. The intracellular signalling pathways that

*Supported by NIH Grants CA61015, CA52995 and CA17094.
**Corresponding author address: Dr. Garth Powis, Arizona Cancer Center, 1515 North Campbell Avenue, Tucson, AZ 85724, U.S.A.
Abbreviations: AT, Ataxia telangiectasia; GPI, glycosylphosphatidylinositol; mTOR, mammalian target of rapamycin; PDGF, platelet-derived growth factor; PLC, phospholipase C; PtdIns-3-kinase, phosphatidylinositol-3-kinase.

regulate cell growth and differentiation provide novel targets for the development of new types of cancer chemotherapeutic and chemopreventive drugs.

Using this approach it is not necessary to target the protein product of the oncogene itself, rather a component of the pathway that is activated by the oncogene can be employed as a surrogate target. Targets that occur early in a signalling pathway are more likely to be specific for the pathway than a target that lies downstream and that is, maybe, common to other pathways. Therapeutic selectivity for the cancer cell is possible because of the degeneracy of growth factor signalling. The results of a large number of animal gene knockout studies suggest that degeneracy is a general feature of growth factor signalling and that the animals can survive the complete inhibition of a signalling pathway because other signalling pathways take over some, or all of the normal functions of the inhibited pathway [3, 4]. Thus, inhibiting an oncogene-activated signalling pathway with a drug should leave intact other pathways that are necessary for normal cell function. As examples of antisignalling cancer drugs in preclinical development we will here focus on inhibitors of phosphatidylinositol-3-kinase (PtdIns-3-kinase), a phospholipid signalling enzyme that is a surrogate target for a number of human oncogenes.

THE PtdIns-3-KINASE FAMILY

The PtdIns-3-kinases are a family of proteins found in yeast and mammalian cells (Fig. 1). The signature amino-acid sequence of the family is (V)LG(I)GDRH-15-D(F)G. The original member of the family identified as a PtdIns-3-kinase was mammalian p110/p85 PtdIns-3-kinase [5]. The p110 catalytic subunit of human PtdIns-3-kinase consists of a 1070 amino-acid peptide [6] which exhibits considerable sequence similarity (> 57%) to a PtdIns-3-kinase in yeast, Vps34, that is involved in the sorting of vacuolar proteins [7]. There is also sequence similarity to mTOR, a mammalian protein related to yeast proteins required for cell cycle progression [8], as well as to the cell cycle check point *MEC1* gene product of budding yeast [9]. Recently a partial cDNA clone derived from the human gene for Ataxia telangiectasia (AT) has been found to encode a putative protein that is a new member of the PtdIns-3-kinase family [10]. AT is an autosomal recessive disorder, affecting 1 in 40 000 to 1 in 100 000 of the population. The homozygous condition is characterized by growth retardation, neuromotor deterioration, deficiencies of the humoral and cellular immune response, increased incidence of certain forms of cancer, acute sensitivity to ionizing radiation, and premature aging and death during the second or early third decade of life [11]. Approximately 0.5% to 1.4% of the population have one defective AT gene and manifest an increased risk of developing cancer as well as having mild radiation sensitivity [12]. Epidemiological studies suggest that female carriers with one copy of the defective AT gene have a four to five-fold increased risk of breast cancer compared to noncarriers, and it could account for up to 13% of all breast cancer [12, 13]. This would make AT mutation the most

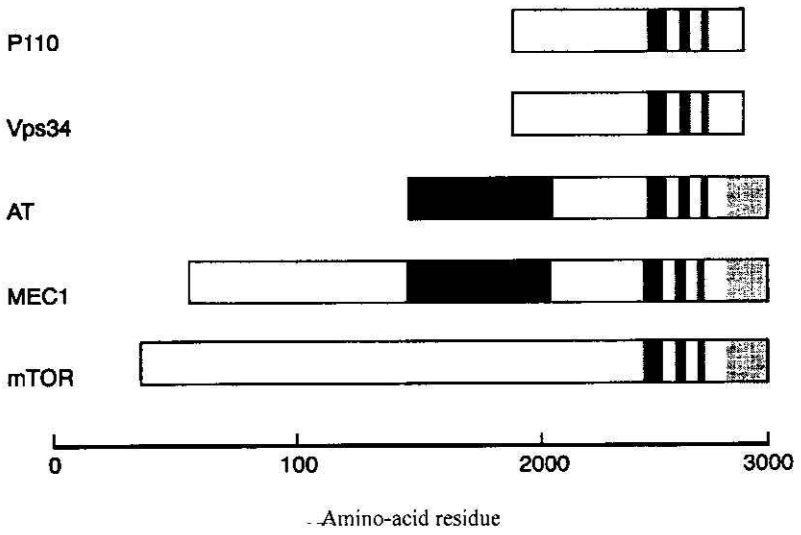

Fig. 1. The PtdIns-3-kinase family of proteins.
p110 is human PtdIns-3-kinase, Vps34 is a yeast protein involved in vacuolar trafficking, AT is the human Ataxia telangiectasia protein, MEC1 is a yeast cell cycle checkpoint protein and mTOR is a mammalian cell cycle protein acting at the G1 checkpoint. The PtdIns-3-kinase sequence similarities are shown by dark shading. The large filled areas are areas of weak similarity to *rad3*, a DNA repair protein in yeast (see text).

common single cause of hereditary breast cancer. More controversially, it has been suggested that radiation received during mammography may be a contributing factor to breast cancer in women heterozygous for the AT mutation [13]. In addition to its similarity to p110 PtdIns-3-kinase the AT protein also has weak similarity to a portion of the *rad*3 gene product of the fission yeast *Schizosaccharomyces pombe*. Rad3 monitors the completion of DNA damage repair and is essential for the correct coupling of mitosis to DNA synthesis [14]. Interestingly, mutation of the *rad*3 protein in *S. pombe* [14] and of MEC1 in budding yeast [9] results in radiation sensitivity. Thus, it appears that the AT gene product is a member of a family of proteins with potentially PtdIns-3-kinase activity and DNA repair activity. Whether the AT gene product and the other family members all have PtdIns-3-kinase activity and just how this activity is related to DNA repair is presently unknown. The apparent involvement of PtdIns-3-kinases in DNA repair provides a new mechanism for the action of this family of enzymes.

Recently other PtdIns-3-kinase activities have been described in mammalian cells. These include a guanine nucleotide binding (G) protein-dependent PtdIns-3-kinase activity that is inactive in the absence of added $G_{\beta\gamma}$ subunits [15].

p110/p85 PtdIns-3-KINASE

Mammalian p110/p85 PtdIns-3-kinase (hereafter referred to just as PtdIns-3-kinase) is found in cellular complexes with ligand activated growth factor receptor and oncogene protein tyrosine kinases [16]. The enzyme consists of two subunits, an 85 kDa regulatory subunit and a 110 kDa catalytic subunit [17]. The 85 kDa regulatory subunit acts as an adaptor protein that allows the 110 kDa catalytic subunit of PtdIns-3-kinase to interact with growth factor receptors and tyrosine phosphorylated proteins [18]. The p85 subunit contains two SH2 domains, one SH3 domain, and a domain homologous to the carboxy terminus of the breakpoint cluster region (*bcr*) gene product [19]. The p110 subunit is widely expressed and is known to exist in distinct isoforms [20]. In addition to its PtdIns-3-kinase activity, the p110 subunit also possesses a protein serine kinase activity, which specifically phosphorylates $serine^{608}$ of the p85 subunit, resulting in down-regulation of PtdIns-3-kinase activity *in vitro* [21]. Downstream targets regulated by p110/p85 PtdIns-3-kinase are the protooncogene serine/threonine kinase Akt [22], $pp70^{S6K}$ kinase [23], and protein kinase Cζ (PKCζ) [24].

PtdIns-KINASE AS A DRUG TARGET

Evidence that PtdIns-3-kinase plays a role in mitogenesis and cell transformation comes from situations where mutated tyrosine kinases fail to associate with and activate PtdIns-3-kinase. Polyoma middle T mutants that associate with and activate $pp60^{c\text{-}src}$ tyrosine kinase but that fail to activate PtdIns-3-kinase are nontransforming [25]. The levels of cellular PtdIns-3-phosphates are elevated by transforming mutants of polyoma middle T but not by transformation-defective mutants [26]. Transformation defective $pp60^{v\text{-}src}$ with mutations in the SH3 domain, show decreased association with PtdIns-3-kinase [27]. Cells transfected with mutant PDGF (platelet-derived growth factor) receptors that retain protein tyrosine kinase activity but that do not associate with or activate PtdIns-3-kinase fail to show a mitogenic response to PDGF, unlike cells transfected with the wild-type PDGF receptor [28]. Specific mutation restoration of PtdIns-3-kinase binding to a tyrosine mutated PDGF receptor is sufficient to restore a mitogenic response to PDGF [29]. A transforming Her2/*Neu* oncoprotein is found constitutively coupled to PtdIns-3-kinase while nontransforming kinase-defective or carboxyl-terminal deleted versions show no constitutive association with PtdIns-3-kinase [30].

The mechanism by which the products of PtdIns-3-kinase, the PtdIns-3-phosphates, affect cell growth and transformation is not known. It has been variously suggested that this may occur through PtdIns-(3,4,5)-trisphosphate binding causing modulation of actin assembly, thus, affecting cytoskeleton function [31], alteration in the activity of PKCζ [24], effects on protein trafficking [6] and as intermediates in the synthesis of glycosylphosphatidylinositol (GPI) membrane protein anchors [32] (see following).

INHIBITORS OF PtdIns-3-KINASE

Quercetin and its analogues are relatively weak inhibitors of PtdIns-3-kinase [33]. Quercetin is also an inhibitor of other signalling enzymes, including protein tyrosine kinases and topoisomerase II [34, 35]. One quercetin analogue, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one, has been found to produce a complete and specific inhibition of PtdIns-3-kinase activity (IC_{50} 1.4 μM) without inhibiting protein tyrosine and lipid kinases or PtdIns-4-kinase [36]. Lovostatin inhibits the insulin and PDGF-stimulated formation of PtdIns-3-phosphates in NIH 3T3 cells (IC_{50} 10 μM), although whether this is a direct effect on PtdIns-3-kinase is not known [37]. The ether lipid analogues, such as ET-18-OCH_3 are weak inhibitors of PtdIns-3-kinase (IC_{50} 36 μM) and inhibit PDGF-stimulated PtdIns-3-phosphate formation in intact *v-sis* transformed NIH 3T3 cells [38]. Benzoyolstaurosporine (CGP-41,251), an inhibitor of protein kinase C [39], is also an inhibitor of PtdIns-3-kinase (IC_{50} 9 μM) [38]. We have found that the anticancer drug suramin is an inhibitor of PtdIns-3-kinase (IC_{50} 8 μM). We have also recently identified some more potent inhibitors of PtdIns-3-kinase, namely: asperuloside (IC_{50} 2 μM); utronin A (IC_{50} 1 μM); and hypericin, (IC_{50} 0.2 μM) [40].

WORTMANNIN

We and others have identified the fungal metabolite wortmannin (Fig. 2, I) as a potent and specific inhibitor of p110 PtdIns-3-kinase with an IC_{50} of about 4 nM [41, 42]. Interestingly, the yeast Vps34 protein PtdIns-3-kinase is not inhibited by wortmannin [42]. Inhibition of PtdIns-3-kinase by wortmannin is irreversible and PtdIns-4-kinase activity is unaffected [41]. We have found that in MCF-7 breast cancer cells less than 30% of the total PtdIns-3-kinase activity is inhibited by 10 nM wortmannin suggesting that there are other wortmannin insensitive PtdIns-3-kinases (unpublished observations). We have examined the cytotoxic activity of wortmannin against several human tumor cell lines [43]. The most sensitive lines were GC3 colon carcinoma, IGROV1 ovarian carcinoma and CCRF-CEM leukemia with IC_{50}s for wortmannin ranging from 0.3 to 0.9 μg/ml. Two human breast cancer cell lines with Her2/*neu* amplification were insensitive to wortmannin (IC_{50} > 20 μg/ml). The optimal schedule of wortmannin showing *in vivo* antitumor activity against the C3H mammary carcinoma was p.o. at 2.5 mg/kg on days 1, 3, 5, 7, which gave 80% inhibition of tumor growth [43]. The human BxPC3 pancreatic carcinoma xenograft showed 66% inhibition of

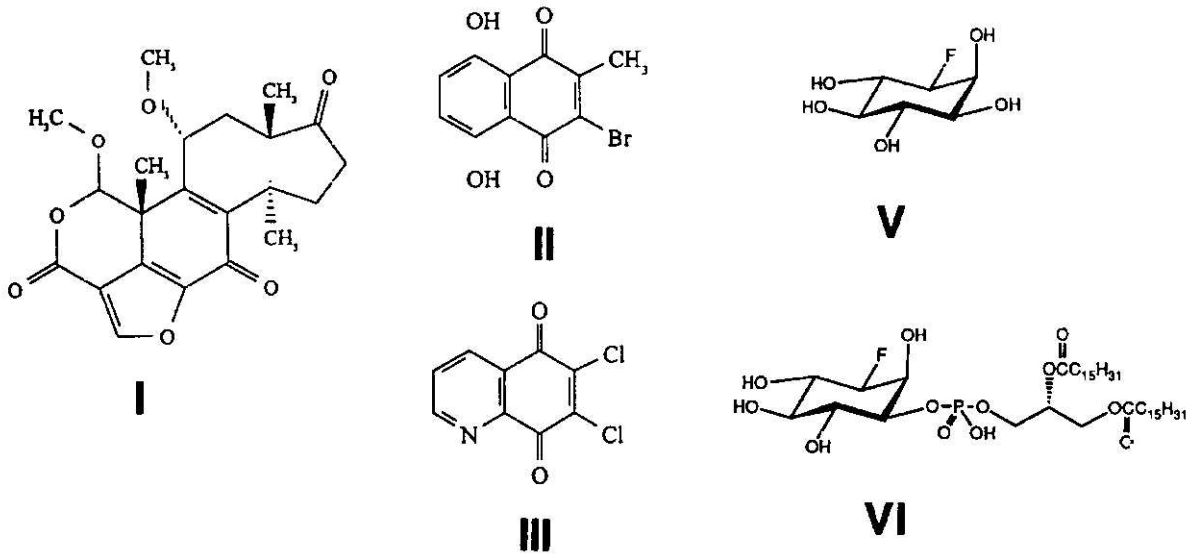

Fig. 2. Structures of some inhibitors of PtdIns-3-kinase.
I, Wortmannin; II, halogenated naphthoquinone; III, halogenated quinoline quinone; IV, D-3-deoxy-3-fluoro-*myo*-inositol; and VI, D-3-deoxy-3-fluoro-PtdIns.

tumor growth. Wortmannin did not have significant activity against other human carcinoma xenografts including HC1, GC3, VRC5 and CX1 colon carcinomas, MX1 mammary carcinoma and LX1 lung carcinoma.

INHIBITION OF GPI-ANCHOR FORMATION BY WORTMANNIN

GPI anchors provide a method of attaching membrane proteins to the lipid bilayer. Instead of a polypeptide chain containing hydrophobic domains, as is found in transmembrane-spanning anchored proteins, GPI anchored proteins utilize a glycosylated phosphatidylinositol lipid structure covalently bound to the C-terminus of the protein [44]. GPI-anchored proteins are found in many eukaryotic cell types and a variety of proteins are anchored in this way. Examples range from protozoal coat surface proteins to mammalian neural cell adhesion molecules. Despite the wide variety of proteins and species utilizing this anchoring method the structure of the GPI core is highly conserved [45]. During synthesis, GPI precursors are acylated on the phosphatidylinositol ring rendering them resistant to cleavage by phospholipase C (PLC) [46]. Following completion of synthesis and presentation on the cell surface, many GPI anchored proteins remove this palmitoyl tail, thus, allowing for PLC cleavage and protein release. Other GPI anchored proteins retain the palmitoyl tail and, thus, their resistance to cleavage.

The fact that PtdIns-3-phosphates are not substrates for PLC suggested to us that PtdIns-3-kinase might be involved in the synthesis of GPI-precursors for acylation. This was reinforced by some initial observations with D-3-deoxy-3-substituted-*myo*-inositol analogues (see later), which caused SAOS-2 osteosarcoma cells to detach from the culture surface, possibly due to the loss of GPI-anchored adhesion factors, without affecting cellular viability. Further studies showed that wortmannin produced dose-dependent and time-dependent decreases in GPI-anchored surface alkaline phosphatase on SAOS-2 cells and also MT-1, a human mammary tumor cell line (Fig. 3).

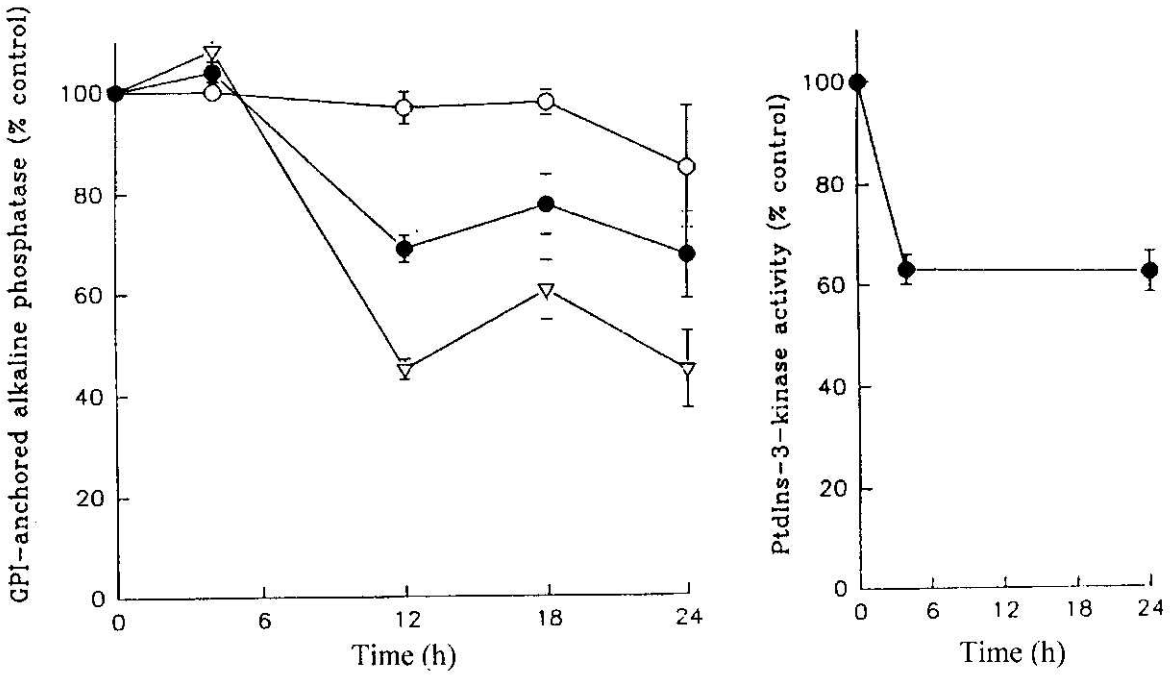

Fig. 3. Time and dose dependent loss of GPI-anchored alkaline phosphatase on SAOS-2 cells exposed to wortmannin and corresponding inhibition of cellular PtdIns-3-kinase activity.

Left panel: cells were exposed to 0.1 μg/ml (○), 1 μg/ml (●) or 10 μg/ml (▽) wortmannin and incubated for varying time periods. Following incubation, GPI-anchors were cleaved by treatment with bacterial phospholipase C and released levels of alkaline phosphatase determined using a diagnostic spectrophotometric-based assay kit (Sigma, St. Louis, MO). Right panel: cell lysates from cells treated with 1 μg wortmannin/ml were incubated with sonicated PtdIns substrates and γ-[^{32}P]ATP as described [47]. Cellular PtdIns-3-kinase activity was then measured by thin-layer chromatography and quantification of radiolabelled PtdIns-3-phosphate quantified by phosphorimager analysis.

These decreases, in turn, correlated with the inhibition of cellular PtdIns-3-kinase activity by wortmannin exposure suggesting a role for PtdIns-3-kinase in GPI-anchor synthesis, although the exact nature of this role is still to be elucidated.

HALOGENATED NAPHTHOQUINONES

We have found that halogenated quinones related to methyljuglone (Fig. 2, II and III) inhibit PtdIns-3-kinase at micromolar concentrations [48]. PtdIns-3-kinase inhibition was time dependent and could be prevented by added thiols. The compounds were only weak inhibitors of PtdIns-4-kinase. Some of the compounds also inhibited PKC, but c-Src protein tyrosine kinase was not inhibited. In intact cells, PtdIns-3-kinase was only partly inhibited by concentrations of the halogenated quinones that inhibited cell growth. Some of the halogenated quinones were found to have *in vivo* antitumor activity. The most sensitive tumor was C3H mammary carcinoma, which showed up to 63% growth inhibition, and B16 melanoma. In the same *in vivo* systems methyljuglone was without antitumor activity. Thus, it is possible that inhibition of PtdIns-3-kinase contributes to the cytotoxic and antitumor effects of the halogenated quinones.

D-3-DEOXY-*myo*-INOSITOL ANALOGUES

We have investigated D-3-deoxy-3-substituted *myo*-inositol analogues (Fig. 2, V) as potential antimetabolites of *myo*-inositol in the PtdIns-3-kinase signalling pathway. The compounds show cell growth inhibitory activity that is selective for *v-sis* transformed NIH 3T3 cells compared to wild-type NIH 3T3 cells. The most potent analogues we found were D-3-deoxy-3-chloro-*myo*-inositol, IC_{50} for *v-sis* NIH 3T3 cells 0.39 mM, and D-3-deoxy-3-azido-*myo*-inositol, IC_{50} 0.04 mM [49]. The growth inhibitory activity of the analogues was, as expected for *myo*-inositol antimetabolites, antagonized by *myo*-inositol, but this antagonism occurred even at physiological concentrations (40 μM) of *myo*-inositol. Thus, the analogues themselves are unlikely to be useful *in vivo* antitumor agents. In order to circumvent the antagonism of the *myo*-inositol analogues by *myo*-inositol we are investigating synthetic PtdIns containing D-3-deoxy-3-substituted *myo*-inositol, that should bypass the uptake and synthesis into PtdIns, where we believe the competition by *myo*-inositol occurs [50]. The first such PtdIns's we have studied, D-3-deoxy-PtdIns and D-3-deoxy-3-fluoro-PtdIns (Fig. 2, VI), exhibited growth inhibitory activity against both wild-type NIH 3T3 and *v-sis* transformed NIH 3T3 cells with IC_{50}s of 100 μM, and against HT-29 colon carcinosarcoma cells with IC_{50s} of around 35 μM [47, 51]. *myo*-Inositol in the medium has no effect on growth inhibition by the compounds. D-3-Deoxy-PtdIns did not inhibit PtdIns-3-kinase but D-3-deoxy-3-fluoro-PtdIns was an inhibitor. The mechanism of inhibition of cell growth by the compounds remains to be established.

CONCLUSIONS

To adequately test the hypothesis that growth factor and oncogene signalling pathways such as the PtdIns-3-kinase pathway offer target sites for anticancer drug development the drugs will have to be tested in patients. This process can take several years. There are, however, compounds in clinical development as cancer preventive and therapeutic agents that may act by inhibiting growth factor signalling pathways. In most cases the activity of the compounds on intracellular signalling pathways was discovered after their identification as anticancer agents. In contrast, PtdIns-3-kinase inhibitors have been specifically developed as inhibitors of growth factor signalling, although their selectivity for tumor cells compared to normal tissue remains to be investigated fully in animal tumor models. Clearly there are several challenges to developing antisignalling anticancer drugs. The drugs need to be tested in appropriate animal tumor models before they can be given to human subjects. This means that the animal tumor should be shown to express the oncogene or signalling pathway that is to be inhibited. Ultimately, it may be more appropriate for the antisignalling drugs to classify human tumors by their complement of oncogenes and tumor suppressor genes, rather than by their histopathological type. It is also possible that a single antisignalling drug

by itself may not have the power to completely inhibit tumor growth and a combination of drugs may be needed. It may also take a combination of drugs to prevent the emergence of resistance.

The excellent secretarial assistance of Madelon Cook is gratefully acknowledged.

REFERENCES

1. Powis, G. (1991) Signalling targets for anticancer drug development. *Trends Pharmacol. Sci.* **12**, 188–194.
2. Powis, G. & Kozikowski, A. (1994) Inhibitors of *myo*-inositol signalling; in *New Targets for Cancer Chemotherapy* (Kerr, D.J. & Workman, P., eds.) pp. 81–95, CRC Press, Baton Rouge.
3. Hooper, C. (1992) Genetic knockouts: Surprises, lessons, and dreams. *J. Natl. Inst. Health Res.* **4**, 34–35.
4. Brookfield, J. (1992) Can genes be truly redundant? *Curr. Biol.* **2**, 553–554.
5. Whitman, M., Downes, C.P., Keeler, M., Keller, T. & Cantley, L. (1988) Type 1 phosphatidylinositol kinase makes a novel inositol phospholipid, phosphatidylinositol-3-phosphate. *Nature (London)* **332**, 644–646.
6. Hiles, I.D., Otsu, M., Volinia, S., Fry, M.J., Gout, I., Dhand, R., Panayatou, G., Ruiz-Larrea, F., Thompson, A., Tatty, N.F., Hsuan, J.J., Courtneidge, S.A., Parker, P.J. & Waterfield, M.D. (1992) Phosphatidylinositol 3-kinase: Structure and expression of the 110 kd catalytic subunit. *Cell* **70**, 419–429.
7. Schu, P.V., Takegawa, K., Fry, M.J., Stack, J.H., Waterfield, M.D. & Emr, S.D. (1993) Phosphatidylinositol 3-kinase encoded by yeast *VPS*34 gene essential for protein sorting. *Science* **260**, 88–91.
8. Sabers, C.J., Martin, M.M., Brunn, G.J., Williams, J.M., Dumont, F.J., Wiederrecht, G. & Abraham, R.T. (1995) Isolation of a protein target of the FKB12-rapamycin complex in mammalian cells. *J. Biol. Chem.* **270**, 815–822.
9. Kato, R. & Ogawa, H. (1994) An essential gene, *ESR1*, is required for mitotic cell growth, DNA repair and meiotic recombination in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **22**, 3104–3112.
10. Savitsky, K., Bar-Shira, A., Gilad, S., Rotman, G., Ziv, Y., Vanagaite, L., Tagle, D.A., Smith, S., Uziel, T., Sfez, S., Ashenazi, M., Pecker, I., Frydman, M., Harnik, R., Patanjali, S.R., Simmons, A., Clines, G.A., Sartiel, A., Gatti, R.A., Chessa, L., Sanal, O., Lavin, M.F., Jaspers, N.G.J., Taylor, A.M.R., Arlett, C.F., Miki, T., Weissman, S.M., Lovett, M., Collins, F.S., Shiloh, Y. (1995) A single ataxia telangiectasia gene with a product similar to PI-3-kinase. *Science* **268**, 1749–1753.
11. Bundey, S. (1994) Clinical and genetic features of ataxia-telangiectasia. *Int. J. Radiat. Biol.* **66** (Suppl.), 23–29.
12. Easton, D.F. (1994) Cancer risks in A-T heterozygotes. *Int. J. Radiat. Biol.* **66** (Suppl.), 177–182.
13. Swift, M., Morrell, D., Massey, R.B. & Chase, C.L. (1991) Incidence of cancer in 161 families affected by ataxia-telangiectasia. *New Engl. J. Med.* **325**, 1831–1836.
14. Seaton, B.L., Yucel, J., Sunnerhagen, P. & Subramani, S. (1992) Isolation and characterization of the *Schizosaccharomyces pombe rad3* gene, involved in the DNA damage and DNA synthesis checkpoints. *Gene* **119**, 83–89.
15. Stephens, L., Smrcka, A., Cooke, F.T., Jackson, T.R., Sternweis, P.C. & Hawkins, P.T. (1994) A novel phosphoinositide 3 kinase activity in myeloid-derived cells is activated by G protein $\beta\gamma$ subunits. *Cell* **77**, 83–93.
16. Cantley, L.C., Auger, K.R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R., Soltoff, S. (1991) Oncogenes and signal transduction. *Cell* **64**, 281–302.
17. Shibaski, F., Homma, Y., Takenawa, T. (1991) Monomer and heterodimer forms of phosphatidylinositol-3-kinase. *J. Biol. Chem.* **266**, 8108–8114.
18. Margolis, B. (1992) Protein with SH2 domains: Transducers in the tyrosine kinase signaling pathway. *Cell Growth Differ.* **3**, 73–80.
19. Kapeller, R. & Cantley, L.C. (1994) Phosphatidylinositol 3-kinase. *BioEssays* **16**, 565–574.
20. Carpenter, C.L., Duckworth, B.C., Auger, K.R., Cohen, B., Schaffhausen, B.S. & Cantley, L.C. (1990) Purification and characterization of phosphoinositide 3-kinase from rat liver. *J. Biol. Chem.* **265**, 19704–19711.
21. Dhand, R., Hiles, I., Panayotou, G., Roche, S., Fry, M.J., Gout, I., Totty, N.F., Truong, O., Vicendo, P., Yonezawa, K., Kasuga, M., Courtneidge, S.A. & Waterfield, M.D. (1994) PI-3-kinase is a dual specificity enzyme: autoregulation by an intrinsic protein-serine kinase activity. *EMBO J.* **12**, 522–533.
22. Franke, T.F., Yang, S., Chan, T.O., Datta, K., Kazlauskas, A., Morrison, D.K., Kaplan, D.R. & Tsichlis, P.N. (1995) The protein kinase encoded by the *Akt* proto-oncogene is a target of the

PDGF-activated phosphatidylinositol 3-kinase. *Cell* **81**, 727–736.

23. Chung, J., Grammer, T.C., Lemon, K.P., Kazlauskas, A. & Blenis, J. (1994) PDGF- and insulin -dependent $pp70^{S6k}$ activation mediated by phosphatidylinositol-3-OH kinase. *Nature (London)* **370**, 71–75.
24. Nakanishi, H., Brewer, K.A. & Exton, J.H. (1993) Activation of the zeta isozyme of protein kinase C by phosphatidylinositol 3,4,5-trisphosphate. *J. Biol. Chem.* **268**, 13–16.
25. Kaplan, D.R., Whitman, M., Schaffhausen, B., Pallas, D.C., White, M., Cantley, L. & Roberts, T.M. (1987) Common elements in growth factor stimulation and oncogenic transformation: 85 kd phosphoprotein and phosphatidylinositol kinase activity. *Cell* **50**, 1021–1029.
26. Serunian, L.A., Auger, K.R., Roberts, T. & Cantley, L.C. (1989) Production of novel polyphosphoinositides *in vivo* is linked to cell transformation by polyomavirus middle T antigen. *J. Virol.* **64**, 4718–4725.
27. Wages, D.S., Keefer, J., Rall, T.B. & Weber, M.J. (1992) Mutations in the SH3 domain of the *src* oncogene which decrease association of phosphatidylinositol 3'-kinase activity with $pp60^{v\text{-}src}$ and alter cellular morphology. *J. Virol.* **66**, 1866–1874.
28. Coughlin, S.R., Escobedo, J.A. & Williams, L.T. (1989) Role of phosphatidylinositol kinase in PDGF receptor signal transduction. *Science* **243**, 1191–1194.
29. Valius, M. & Kazlauskas, A. (1993) Phospholipase C-γ1 and phosphatidylinositol 3 kinase are the downstream mediators of the PDGF receptor's mitogenic signal. *Cell* **73**, 321–334.
30. Peles, E., Lamprecht, R., Ben-Levy, R., Tzahar, E. & Yarden, Y. (1992) Regulated coupling of the Neu receptor to phosphatidylinositol 3'-kinase and its release by oncogenic activation. *J. Biol. Chem.* **267**, 12266–12274.
31. Eberle, M., Traynor-Kaplan, A.E., Sklar, L.A. & Norgauer, J. (1990) Is there a relationship between phosphatidylinositol trisphosphate and F-actin polymerization in human neutrophils. *J. Biol. Chem.* **265**, 16725–16728.
32. Hill, S.R., Berggren, M.M. & Powis, G. (1995) The involvement of phosphatidylinositol-3'-kinase in the synthesis of glycosylphosphatidylinositol anchors. *Proc. Am. Assoc. Cancer Res.* **36**, 55.
33. Matter, W.F., Brown, R.F. & Vlahos, C.J. (1992) The inhibition of phosphatidylinositol 3-kinase by quercetin and analogs. *Biochem. Biophy.s Res. Commun.* **186**, 624–631.
34. Yamashita, Y., Kawada, S. & Nakano, H. (1990) Induction of mammalian topoisomerase II dependent DNA cleavage by nonintercalative flavonoids, genistein and orobol. *Biochem. Pharmacol.* **39**, 737–744.
35. Hagiwara, M., Inoue, S., Tanaka, T., Nunoki, K., Ito, M. & Hidaka, H. (1988) Differential effects of flavonoids as inhibitors of tyrosine protein kinases and serine/threonine protein kinases. *Biochem. Pharmacol.* **37**, 2987–2992.
36. Vlahos, C.J., Matter, W.F., Hui, K.Y. & Brown, R.F. (1994) A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *J. Biol. Chem.* **269**, 5241–5248.
37. McGuire, T., Corey, S. & Sebti, S.M. (1993) Lovostatin inhibits insulin- and PDGF-induced activation of phosphatidylinositol-3'-kinase. *Proc. Am. Assoc. Cancer Res.* **34**, 528.
38. Berggren, M., Gallegos, A., Dressler, L.A., Modest, E.J. & Powis, G. (1993) Inhibition of the signalling enzyme phosphatidylinositol-3-kinase by antitumor ether lipid analogues. *Cancer Res.* **53**, 4297–4302.
39. Meyer, T., Regenass, U., Fabbro, D., Alteri, E., Rösel, J., Müller, M., Caravatti, G. & Matter, A. (1989) A derivative of staurosporine (CGP 41 251) shows selectivity for protein kinase C inhibition and *in vitro* anti-proliferative as well as *in vivo* anti-tumor activity. *Int. J. Cancer* **43**, 851–856.
40. Frew, T., Powis, G., Berggren, M., Abraham, R.T., Ashendel, C.L., Zalkow, L.H., Hudson, C., Qazia, S., Gruszecka-Kowalik, E., Merriman, R. & Bonjouklian, R. (1994) A multiwell assay for inhibitors of phosphatidylinositol-3-kinase and the identifcation of natural product inhibitors. *Anticancer Res.* **14**, 2425–2428.
41. Powis, G., Bonjouklian, R., Berggren, M.M., Gallegos, A., Abraham, R., Ashendel, C., Zalkow, L., Matter, W.F., Dodge, J., Grindey, G. & Vlahos, C.J. (1994) Wortmannin, a potent and selective inhibitor of phosphatidylinositol-3-kinase. *Cancer Res.* **54**, 2419–2423.
42. Woscholski, R., Kodaki, T., McKinnon, M., Waterfield, M.D. & Parker, P.J. (1994) A comparison of demethoxyviridin and wortmannin as inhibitors of phosphatidylinositol 3-kinase. *FEBS Lett.* **342**, 109–114.
43. Schultz, R.M., Merriman, R.L., Andis, S.L., Bonjouklian, R., Grindey, G.B., Rutherford, P.G., Gallegos, A., Massey, K. & Powis, G. (1995) *In vitro* and *in vivo* antitumor activity of the phosphatidylinositol-3-kinase inhibitor, wortmannin. *Anticancer Res.* (in press).

44. Low, M.G. (1989) The glycosylphosphatidylinositol anchor of membrane proteins. *Biochim. Biophys. Acta* **988**, 427–454.

45. Ferguson, M.A. (1992) Colworth Medal Lecture. Glycosyl-phosphatidylinositol membrane anchors: The tale of a tail. *Biochem. Soc. Trans.* **20**, 243–256.

46. Ferguson, M.A.J. (1992) Site of palmitoylation of a phospholipase C-resistant glycosylphosphatidylinositol membrane anchor. *Biochem. J.* **284**, 297–300.

47. Kozikowski, A.P., Kiddle, J.J., Frew, T., Berggren, M. & Powis, G. (1995) Synthesis and biology of 1D-3-deoxyphosphatidylinositol: A putative antimetabolite of phosphatidylinositol-3-phosphate and an inhibitor of cancer cell colony formation. *J. Med. Chem.* **38**, 1053–1056.

48. Frew, T., Powis, G., Berggren, M., Gellegos, A., Abraham, R.T., Ashendel, C.L., Zalkow, L.H., Hudson, C., Gruszecka-Kowalik, E., Burgess, E.M., Benedetti-Doctorovich, V., Kerrigan, J.E., Lambropoulos, J., Merriman, R. & Bonjouklian, R. (1995) Novel quinone antiproliferative inhibitors of phosphatidylinositol-3-kinase. *Anti-Cancer Drug Design* **10**, 347–359.

49. Kozikowski, A.P., Fauq, A.H., Powis, G. & Melder, D.C. (1991) D-3-Modified *myo*-inositol analogues: Synthesis and growth inhibitory properties. *Med. Chem. Res.* **1**, 277–282.

50. Brunn, G., Fauq, A.H., Chow, S., Kozikowski, A.P., Gallegos, A. & Powis, G. (1994) Cellular pharmacology of D-3-deoxy-*myo*-inositol, an inhibitor of phosphatidylinositol signaling having antiproliferative activity. *Cancer Chemother. Pharmacol.* **35**, 71–79.

51. Kozikowski, A.P., Tückmantel, W. & Powis, G. (1992) Synthesis and biological activity of D-3-deoxy-3-fluorophosphatidylinositol — A new direction in the design of non-DNA targeted anticancer agents. *Angew. Chemie* **31**, 1379–1381.