Prospects for phosphoinositide 3-kinase inhibition as a cancer treatment

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Abstract
The phosphoinositide 3-kinases (PI3-kinases) are a family of lipid kinases that have a key role in the regulation of many cellular processes including proliferation, survival, carbohydrate metabolism, and motility. There is now strong evidence that some members of the PI3-kinase family have an important role in cancer. Emerging evidence for functional specialisation of PI3-kinase isoforms suggests that isoform selective inhibitors, in contrast to the existing non-selective inhibitors wortmannin and LY294002, may prove to be useful anticancer drugs.

Introduction
The phosphoinositide 3-kinases (PI3-kinases) are increasingly considered to have a key role in intracellular signal transduction in health and disease. In particular the PI3-kinases generate and convey signals that have an important role in cancer. PI3-kinases are ubiquitously expressed, are activated by a high proportion of cell surface receptors, especially those linked to tyrosine kinases, and influence a bewildering variety of cellular functions and events. Although some PI3-kinase activity is likely to be essential for cellular health, the PI3-kinases are a rather diverse group of enzymes for which there is increasing evidence of functional specialisation. This opens up the possibility of developing isoform-selective inhibitors that could be used to treat cancer with limited toxicity.

The PI3-kinase family
The primary enzymatic activity of the PI3-kinases is the phosphorylation of inositol lipids (phosphoinositides) on the 3-position of the inositol headgroup (Fig. 1). Different members of the PI3-kinase family generate different lipid products. To date four 3-phosphorylated inositol lipids have been identified in vivo. These lipids are bound by proteins that contain the appropriate lipid recognition module and which either act as effectors or transmit the PI3-kinase signal onwards.

The most familiar form of PI3-kinase is a heterodimeric complex, consisting of a 110 kDa catalytic subunit now known as p110α and an 85 kDa regulatory/adapter subunit, p85α. There are, however, a total of eight mammalian PI3-kinases, which have been divided into three main classes (Fig. 2) on the basis of sequence homology, in vitro substrate preference and method of activation and regulation. It is likely that all mammalian cells express representatives of each class. A single member of each of the three classes is found in Drosophila (MacDougall et al. 1995).

Aspects of PI3-kinase function and signalling that are relevant to consideration of the potential consequences and therapeutic applications of PI3-kinase inhibition are described below. Much of this material has been reviewed in detail by others (Fruman et al. 1998, Wymann & Pirola 1998, Vanhaesebroeck & Waterfield 1999, Vanhaesebroeck et al. 2001).

Class I kinases
Four class I enzymes are found in humans; they are divided into two subclasses (Ia and Ib) on the basis of their mechanism of activation. The principal lipid generated by the class I kinases in vivo is phosphatidylinositol 3,4,5-triphosphate (PtdIns(3,4,5)P3). In addition, the class I kinases can act as serine/threonine protein kinases although the significance of this is currently unclear.

The class Ia group consists of the classical p110α and two additional, closely related enzymes, p110β and p110δ. The p110α and p110β isoforms both have a nearly ubiquitous tissue distribution in adults, whereas that of p110δ is rather more restricted, with prominent expression in particular in leucocytes. All class Ia enzymes are constitutively associated with a ‘p85’ regulatory/adapter subunit to form a heterodimeric complex. At least eight adapter subunit isoforms, encoded by three genes (which generate a number of splice variants) have been identified.
Figure 1  Inositol lipids. The basic structure of the phosphoinositides consists of diacyl glycerol linked by phosphodiester bond to an inositol head group. The acyl chains of diacyl glycerol (typically stearyl-arachidonyl) are inserted into the inner leaflet of the membrane bilayer. The inositol head group, which is exposed to the cytosol, has a ‘chair’ configuration. A variety of enzymes are able to phosphorylate and de-phosphorylate the inositol head group. The PI3-kinases phosphorylate inositol lipids on the D3 position of the inositol head group. There are four known lipid products of the PI3-kinases. The mono-phosphorylated lipid, phosphatidylinositol 3-phosphate (PtdIns(3)P) is readily detectable in all mammalian cells. The total cellular content of the lipid, which is apparently invariant, is approximately 10% of that of the bisphosphorylated PtdIns(4,5)P2, the dominant inositol lipid in most cells. PtdIns(3,5)P2 is present at low levels in many quiescent cells, but has been shown to increase in response to osmotic stress in both yeast and mammalian cells. In contrast, PtdIns(3,4)P2 and PtdIns(3,4,5)P3 are virtually undetectable in quiescent cells, but after ligand stimulation, concentrations increase rapidly, reaching a peak of 5–10% of that of PtdIns(3)P after 1–10 min. Thereafter, they decay away to low levels over 10–60 min. These are characteristics that might be expected of a second messenger. The 3-phosphorylated inositol lipids are metabolised by conversion to other inositol lipids through a process of dephosphorylation. This is accomplished by a network of phosphoinositide phosphatases. These include 5′-phosphatases, the action of which on the tri-phosphorylated PtdIns(3,4,5)P3 is probably the major source of cellular PtdIns(3,4)P2 (which increases with a slight lag after ligand stimulation) and the tumour suppresser gene PTEN (phosphatase and tensin homologue detected on chromosome 10), which is a 3′-phosphatase. The 3-phosphorylated inositol lipids are not substrates for phospholipase C; in contrast to PtdIns(4,5)P2, which can be cleaved to form diacylglycerol and the freely soluble inositol tri-phosphate (Ins(1,4,5)P3 or IP3). PIP-kinase, phosphoinositide 4-kinase; PIP-kinase, phosphatidyl inositol phosphate kinase.

The sole member of class Ib is p110γ, expression of which is confined largely to leucocytes, and which associates with the p101 adapter subunit.

Activation of class I PI3-kinases

The majority of tyrosine kinase coupled trans-membrane receptors will activate class Ia PI3-kinases (Wymann & Pirola 1998) through binding of the SH2 domains found in all p85 isoforms, although there is clearly a variation in the strength and duration of the resulting signal. Thus some receptors such as the insulin and platelet derived growth factor (PDGF) receptors activate PI3-kinases strongly. In contrast, epidermal growth factor (EGF) receptor (EGFR) has a weak and inconsistent effect; it seems likely that PI3-kinase activation is achieved through an indirect association with this receptor (Rodrigues et al. 2000). There does not appear to be preferential association of any of the p110s with particular p85 adapter subunit isoforms, but there is some variation in the tissue distribution of adapter subunits, which is likely to have functional significance.

In contrast to the class Ia enzymes, the p101–p110γ complex is activated by βγ subunits of heterotrimeric G proteins, which are released on activation of 7-transmembrane receptors.
Figure 2 The PI3-kinase superfamily. Dendrogram showing the degree of sequence homology between the catalytic domains of the PI3-kinases and related kinases. The catalytic subunits of the PI3-kinases have additional regions of homology which are lacking in the other related kinases (and which have not been used in the construction of this figure). The phosphoinositide (PI-) kinases are divided into classes on the basis of catalytic domain homology, in addition to other structural features and binding partners/adapters, their mode of activation and their *in vitro* substrate specificity (not shown in this diagram).

In addition to activation mediated through the adapter subunits, class I kinases can be activated *in vitro* by GTP bound (activated) ras, which interacts directly with the catalytic subunit. The mechanism of this activation has been explained by X-ray crystallography (Pacold et al. 2000). The contribution made by ras-mediated activation to total PI3-kinase activity in ligand-stimulated cells is uncertain at present.

**Class II kinases**

The three members of class II kinases form the least understood group of PI3-kinases. Enzymes of this class are...
involved in the control of the protein synthesis machinery this to cell cycle control (Cliby et al. 1998, Rotman & Shiloh 1998). The cellular functions of the class II kinases are unknown.

Class III kinases

The prototype of the solitary class III PI3-kinase is the Saccharomyces cerevisiae enzyme, vps34p, which is believed to be a constitutively active enzyme. The S. cerevisiae vps34p associates with a serine/threonine protein kinase, vps15p, and has an essential role in protein trafficking through the vacuole. The pathway and function are conserved in mammalian cells, in which vps34p is involved in the traffic of proteins through the lysosome, which is the mammalian equivalent of the vacuole. The mammalian vps34 is probably responsible for generating the majority of the mono-phosphorylated lipid PtdIns(3)P, its only lipid product.

Related lipid and protein kinases

A number of other enzymes have been identified in which the catalytic domains closely resemble those of the PI3-kinases. These include the PI4-kinases (currently divided into two classes), which generate PtdIns(4)P in vivo. The phosphoinositide phosphate (PIP)-kinases (i.e. enzymes that will phosphorylate mono-phosphorylated inositol lipids on either the 4 or 5 position) curiously have very little sequence similarity to the other phosphoinositide kinases. The PI4- and PIP-kinases are clearly important in maintaining the phosphoinositide cycle, but probably have a comparatively minor direct role in signal transduction.

A further group of enzymes that belong to the PI3-kinase ‘superfamily’ have a related kinase domain with a distinctive carboxyl terminal region but no other regions of homology; with sizes of 200–400kDa, they are larger than the PI-kinases. Members of this group appear to act as protein kinases only. The best characterised of these is the DNA-dependent protein kinase (DNA-PK) that, with two other members of this group, ataxia telangiectasia mutated (ATM) and ataxia telangiectasia related (ATR), is involved in the activation of DNA repair machinery and the linking of this to cell cycle control (Ciliby et al. 1998, Rotman & Shiloh 1999, Smith & Jackson 1999).

A fourth member of the group, mTOR, is the target of the immunosuppressant (and emerging anti-cancer) drug, rapamycin (Dennis et al. 1999). Functionally, mTOR is involved in the control of the protein synthesis machinery through activation of a number of targets, including p70S6 kinase.

PI3-kinase signalling

Extensive functional characterisation of the class I, and particularly class Ia, kinases has been performed, with mapping of some downstream signalling pathways. The contribution, if any, to these processes by ligand activation of the class II kinases is unknown. In contrast, the class III PI3-kinases seem to be constitutively active and have a role that is confined to intracellular protein trafficking. Paradoxically little is known about the role of PI3-kinases in human disease, in part because of the difficulty in detecting and quantifying the concentrations of inositol lipids in cells and tissues, and also because of a paucity of good antibodies against the class I kinases.

The physiological consequences of class I enzyme activation fall into three broad categories, namely cell growth/survival, intracellular trafficking, and cellular motility. In addition, class Ia PI3-kinases are strongly activated by insulin and many of the metabolic actions of insulin, including the translocation of the GLUT4 glucose transporter to the cell membrane, have been linked to PI3-kinase signalling (Shepherd et al. 1998).

Targets of class I PI3-kinases

A detailed discussion of the molecules that have been identified as downstream targets of the class I PI3-kinases is beyond the scope of this article; several reviews have recently been published (Fruman et al. 1998, Wymann & Pirola 1998, Vanhaesebroeck & Alessi 2000, Vanhaesebroeck et al. 2001). It is now clear that the majority of known functional effects of PI3-kinase activation can be explained at least in part on the basis of those protein molecules that are known or believed to be PI3-kinase signalling targets (Fig. 3).

One pathway that does merit a brief discussion is the now very well characterised pathway involving protein kinase B (PKB) which, as the mammalian homologue of the oncogene v-akt, is also known as akt. There is close homology between the kinase domains of PKB and those of protein kinases A and C (PKA and PKC). These three kinases, together with PKG, p70S6 kinase and a number of other related kinases are sometimes termed the AGC kinases. PKB/akt is now known to be activated by the phosphoinositide-dependent kinase (PDK1) after PI3-kinase activation. Downstream of PKB/akt lie a number of enzymes implicated as effectors of the actions of insulin on glucose metabolism and protein synthesis and of PI3-kinase signalling on cell survival.

PDK1 potentially has an important role in the activation, priming, or both, of other AGC kinases. The activation of most of these enzymes requires multiple phosphorylation events

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Figure 3  Major class I PI3-kinase signalling pathways and functions. Many additional downstream targets of class I PI3-kinases have been identified; those shown here have particularly well-defined roles and probably represent the major functional pathways for transmission of PI3-kinase signals. Enzymes marked with a star have been identified as oncoproteins; underlining indicates known tumour suppressor function. MEK, mitogen-activated protein kinase kinase; ERK, extracellular regulated kinase; PDK1, phosphoinositide-dependent kinase 1; PKB, PKC, protein kinases B and C; Casp9, caspase 9; BAD, bcl2 antagonist of cell death; FKHLR1, forkhead transcription factor; IKK, IκB kinase; GSK3, glycogen synthase kinase 3; PLCγ, phospholipase C-γ; Btk/Tec, Bruton's (and related) tyrosine kinase. For further detail, see text.

including PI3-kinase-dependent events. In the case of p70S6 kinase, PKB/akt itself also contributes to activation, possibly acting in concert with the PI3-kinase related protein kinase, mTOR. It is commonly speculated that p70S6 kinase, the major function of which is the initiation of protein synthesis, mediates many of the PI3-kinase-induced effects on cellular proliferation.

Isoform dependence of class Ia PI3-kinase signalling

Historically there has been a tendency to treat all class Ia kinases as equal, largely because of the paucity of good immunoblotting antibodies for the kinase subunits. However, evidence largely obtained by microinjection of inhibitory antibodies into cells suggests that there may be some functional specialisation between the isoforms. Thus in fibroblasts, p110a mediates PDGF, insulin and EGF-induced mitogenic responses, whereas p110b also mediates insulin- but not PDGF-induced mitogenesis (Roche et al. 1994, 1998). In endothelial cells it has been reported that PDGF-induced actin rearrangement requires p110a, whereas insulin achieves the same effect through p110b signalling (Hooshmand-Rad et al. 2000). In macrophages, colony stimulating factor 1 (CSF1) regulates the actin cytoskeleton and cell migration through the activation of p110b and p110d, but p110a transmits the CSF1 mitogenic response (Vanhaesebroeck et al. 1999).

There are also some hints of specificity in the interactions of class Ia kinases with cellular proteins. Thus p110b, but not p110a, has been reported to be recruited to the endosomal compartment, where the cellular GLUT4 glucose transporter pool resides, on insulin stimulation (Wang et al. 1998). This is possibly mediated through a direct association with the transport protein, rab5 (Christoforidis 1999). Similarly, there may be some preferences for particular receptors to interact with a subset of 85 adapters, as in the case of the insulin receptor (Inukai et al. 1997, 2001).

These data suggest that the class Ia kinases are to some extent functionally distinct. This is likely to be mediated through a combination of expression patterns and perhaps a
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preferential association of certain kinases (or combinations of kinases and adapter subunits) with particular receptors. An example is the possible preference of the insulin receptor for p110β. It is almost certain that both the extent and exact nature of functional specialisation will vary from tissue to tissue.

Consequences of disrupting PI3-kinase signalling in vivo

A number of attempts have been made to generate PI3-kinase knockout mice. The consequences of disruption of the class Ib kinase p110γ are reasonably clear-cut. Mice that lack p110γ develop and live normally in standard animal housing facilities. However, they have a number of deficiencies in immune and inflammatory responses (reviewed by Condiffe & Hawkins 2000) that demonstrate the importance of this PI3-kinase isoform for immune system function. It has also been reported that one strain of p110γ are susceptible to development of colonic cancers (Sasaki et al. 2000) but this has apparently not been observed in other strains (Vanhaesebroeck et al. 2001), raising the possibility that this is an artefact of the manner in which the knockout was created.

The interpretation of the class Ia kinase knockouts is much more difficult. Deletion of p110a results in embryonic death midway through the gestational period (Bi et al. 1999). Interpretation of the result, however, is complicated by the observation that there was over-expression of the p85 regulatory subunits in these animals, which is likely to have had a dominant-negative effect on all class Ia PI3-kinase signalling in these animals (Vanhaesebroeck et al. 2001). Disruption of the α p85 adapter subunit gene (which is likely to have some effects on all three class Ia PI3-kinases) results in abnormalities in immune system function and glucose metabolism (Suzuki et al. 1999, Terauchi et al. 1999, Fruman et al. 2000) but, again, detailed interpretation is difficult (Vanhaesebroeck et al. 2001).

Characteristics of existing PI3-kinase inhibitors

A number of compounds that inhibit PI3-kinases have been identified. Foremost amongst these are wortmannin (Powis et al. 1994) which was originally isolated from soil bacteria and is toxic to fungi, the closely related but much less studied demethoxyviridin (Woscholski et al. 1994), and LY294002, a morpholino derivative of the broad-spectrum kinase inhibitor quercetin (Vlahos et al. 1994) (Fig. 4, Table 1).

Wortmannin and demethoxyviridin (which are unstable in aqueous media) are both irreversible inhibitors of all susceptible kinases so far examined. There is evidence that wortmannin alkylates a lysine residue at the putative ATP binding site of p110α (Wymann et al. 1996). LY294002, in contrast, is a pure competitive inhibitor of ATP. The X-ray structure of wortmannin, LY294002 and several broad-spectrum kinase inhibitors, including quercetin in complex with p110γ, confirms the mechanism of inhibition and offers a basis for designing more specific compounds (Walker et al. 2000).

It is important to emphasise that wortmannin and, particularly, LY294002 display little selectivity within the PI3-kinase family (Table 1). Most isoforms are inhibited with concentrations comparable to those required to inhibit p110/ p85α, although PI3KC2α is significantly less sensitive. Those PI3-kinase related protein kinases for which information is available are also inhibited by LY294002, which displays little selectivity for most of these enzymes against p110α/p85α (Brunn et al. 1996, Withers et al. 1997, Izzard et al. 1999, Banin et al. 1998). Wortmannin inhibits this group of enzymes also, but rather less potently. Both compounds lose specificity at high concentrations. In a recent systematic screening of both compounds for activity against a broad range of kinases, LY294002 was reported to inhibit casein kinase 2 with a sensitivity comparable to that of p110α/p85α (Davies et al. 2000). This observation is important, because casein kinase 2 is involved in many cell regulatory processes.

A number of chemical modifications of both wortmannin and LY294002 have been made, a few of which are significantly more potent than the parent compound. There are no data available on their isoform selectivity. In addition, other unrelated compounds have been shown to inhibit PI3-kinase in vitro, but in general their potency and selectivity are low.

Both wortmannin and LY294002 have been extensively applied to cultured cells and, indeed, have been among the major tools used to elucidate the biological functions of PI3-kinase activation at a cellular level in short-term assays. Both compounds inhibit growth in concentrations that would be expected to inhibit class Ia PI3-kinases in a cellular environment and will induce widespread apoptosis in greater concentrations (Yao & Cooper 1995). It is possible, however, that some of these effects are mediated through inhibition of related kinases. Similarly, there are some data on the use of wortmannin and analogues in the treatment of xenografted tumours in immunodeficient mice (Norman et al. 1996). The therapeutic index of the compounds is reported to be low but, again, it is unclear whether this is the consequence of broad-spectrum inhibition of the class I PI3-kinases or results from inhibition of other classes of PI3-kinase and the related kinases.

The lack of selectivity of these compounds, together with the instability of wortmannin and the insolubility of LY294002, means that neither has very promising pharmaceutical potential.

A number of other compounds are known to inhibit PI3-kinases, but these have not been investigated in detail, are comparatively weak, or are the subject of unpublished patent applications. Compounds that have been described
**Figure 4** Existing PI3-kinase inhibitors. Wortmannin and demethoxyviridin are products of soil bacteria that irreversibly inhibit PI3-kinases at the ATP binding site. Quercetin is a broad-spectrum inhibitor of many protein kinases, including PI3-kinase, and LY294002 is a derivative that is selective for PI3-kinases; both are competitive inhibitors of ATP binding. All inhibit the majority of known PI3-kinases and other members of the PI3-kinase ‘superfamily’.

**Table 1** Actions of existing PI3-kinase inhibitors. Comparative inhibition of kinases is mostly based on IC\textsubscript{50} data performed in different laboratories under a variety of conditions, usually without direct comparison with p110\textalpha and is therefore very approximate.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Wortmannin</th>
<th>LY294002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site of action</td>
<td>ATP binding site</td>
<td>ATP binding site</td>
</tr>
<tr>
<td>Mode of action (class 1 PI3-kinase)</td>
<td>Irreversible (alkylates a lysine in the ATP binding site)</td>
<td>Pure competitive inhibitor (K\textsubscript{i} = 1 \mu M)</td>
</tr>
<tr>
<td>\textit{In vitro} IC\textsubscript{50} of p110\textalpha</td>
<td>2–5 nM</td>
<td>0.5–1.5 \mu M</td>
</tr>
<tr>
<td>Sensitivity of other PI3-kinases</td>
<td>As p110\textalpha except: P13KC2\textalpha (IC\textsubscript{50} = 400 nM) P13KC2\gamma (unknown)</td>
<td>As p110\textalpha except: P13KC2\textalpha (IC\textsubscript{50} = 20 \mu M) P13KC2\gamma (unknown)</td>
</tr>
<tr>
<td>Sensitivity of PI3-kinase protein kinase activity</td>
<td>As for lipid kinases (where documented)</td>
<td>As for lipid kinases (where documented)</td>
</tr>
<tr>
<td>Inhibition of related kinases – sensitivity in comparison with p110\textalpha</td>
<td>PI4K\textalpha, PI4K\beta \approx 2% ATM, DNA-PK \approx 2% mTOR \approx 5% ATR \approx 0.1%</td>
<td>PI4K\beta \approx 1% mTOR \approx 100% DNA-PK = 20% (K = 6 \mu M)</td>
</tr>
<tr>
<td>Inhibition of unrelated kinases – sensitivity in comparison with p110\textalpha</td>
<td>Smooth muscle myosin light chain kinase \approx 2%</td>
<td>Casein kinase 2 \approx 100%</td>
</tr>
<tr>
<td>Concentration required for inhibition of cellular PI3-kinases</td>
<td>20–50 nM (reasonably selective for broad PI3-kinase inhibition)</td>
<td>10–20 \mu M (poorly selective)</td>
</tr>
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include ether lipids and steroid derivatives that are likely to act at the lipid binding site (Hu et al. 2000, Xie et al. 2000).

**A role for PI3-kinase in the pathogenesis of cancer?**

The known functional effects of the PI3-kinases suggest possible therapeutic targets. Foremost amongst these is a potential role in the treatment of malignant diseases. It is also highly likely that PI3-kinase inhibition will prove valuable in treatment of inflammatory disease, but detailed consideration of the evidence for this lies outside the scope of this article.

**PI3-kinases and cell proliferation/survival**

The class Ia PI3-kinases have a very well documented role in the transmission of proliferation and survival signals in a wide variety of cells. Ligand-induced mitogenic responses have been shown to be sensitive to blockade of PI3-kinase activation, by means of techniques such as selective mutation of tyrosine residues in receptors to block the association of PI3-kinase with activated receptors such as PDGF receptor (PDGFR), and the microinjection of selective neutralising antibodies (Valius & Kazlauskas 1993, Roche et al. 1994, 1998). Constitutively active PI3-kinases have been shown to induce entry into S-phase (Klippel et al. 1998). There is evidence that class Ia PI3-kinase activity is biphasic after receptor stimulation and that only the late (3–7 h) phase is required for mitogenesis (Jones et al. 1999). In transgenic Drosophila, there is a variation in both cell number and size with class I PI3-kinase ‘dose’ in target organs (Leevers et al. 1999). In a similar analysis, variation in p110α dose in the murine heart resulted in variation in cardiac myocyte size; reliable measurement of cell number was not possible in this study (Shiota et al. 2000).

Although the use of non-selective PI3-kinase inhibitors suggests an involvement of the enzyme family in the mitogenic response to activation of tyrosine-kinase coupled receptors in a range of cell types, it is unclear how universal this is. Other data obtained by a variety of techniques clearly show that the simultaneous activation of a number of different signalling pathways is required for a full mitogenic response and that the exact pattern of pathway activation required depends on both the receptor and cell type studied.

In addition to their effect on mitogenesis, the PI3-kinases have an important role in transmitting survival signals from cell surface receptors such as the insulin-like growth factor-I receptor and integrins. There is abundant evidence that these effects require the activation of PKB/akt (Kauffmann Zeh et al. 1997, Khwaja et al. 1997). A number of PKB/akt targets including the bcl2 antagonist of cell death (BAD), caspase 9, forkhead transcription factors and IκB kinases have been implicated as effectors (Vanhaesebroeck & Alessi 2000).

**PI3-kinases in cancer**

Taken in its entirety the case is convincing that class Ia PI3-kinases play an important part in some human cancers, although at present it is difficult to estimate how widespread and how frequent this is and which cancer types are particularly affected.

Direct evidence that activating mutations of the enzyme exist in human cancer is lacking. However, a fully functional retroviral oncogene, v-p3k, an orthologue of p110α, has been isolated from the avian sarcoma virus ASV16 that induces haemangiosarcomas in chickens, and from ASV8905 (Aoki et al. 2000). The v-p3k protein is N-terminally fused to gag. This results in membrane localisation that is probably the mechanism of its constitutive activation. The activity of v-p3k is independent of association with either a p85 adapter or ras. A number of membrane-targeted ‘synthetic’ p110α constructs are also known to be constitutively active, and in appropriate conditions these have been shown to transform transfected fibroblasts (Klippel et al. 1998).

A second activated PI3-kinase has been isolated from a radiation-induced murine lymphoma (Jimenez et al. 1998). These tumour cells in contrast to ASV16–induced tumours contain an apparently normal p110α associated with a truncated p85α, p65, which lacks the carboxyl-terminal SH2 domain. The p65/p110α complex has been shown to be constitutively active in lymphoma cells, although the mechanism is not clear at present. Transgenic mice that express p65 in T lymphocytes display reduced apoptosis and are prone to develop a lymphoproliferative condition and autoimmune disease (Borlado et al. 2000). T cell lymphoma develops at an early age when the p65-transgene is expressed in animals with a p53 null background. It seems likely that PKB/akt activation is essential for the effects of both v-p3k (Aoki et al. 2001) and p65/p110α.

In humans, amplification of PIK3CA, the gene encoding p110α on chromosome 3q26, has been reported in some ovarian cancer cell lines and primary ovarian cancers (Shayesteh et al. 1999) and also in cervical cancers (Ma et al. 2000). At present, the functional significance of this is not fully elucidated. Evidence for protein over-expression and enzymatic activation of class Ia PI3-kinases in a proportion of colorectal tumours has been presented, although at present the mechanism of this is unknown (Phillips et al. 1998).

In addition to a direct oncogenic role as is implied above, it is apparent that PI3-kinase lies in a network of oncoproteins (Fig. 3). Downstream of PI3-kinase, akt2 (PKBβ) has been shown to be amplified and over-expressed in some ovarian cancers (Bellacosa et al. 1995). Similarly akt3 (PKBγ) has been reported to be over-expressed in some steroid hormone-insensitive breast cancers (Nakatani et al. 1999). On currently available evidence, however, activation of PKB/akt seems more likely to reflect PI3-kinase activity,
as is suggested by an analysis of akt2 activation in ovarian cancer specimens (Yuan et al. 2000), rather than being the consequence of primary over-expression.

PI3-kinases are known to be activated by many cell surface receptors with an established oncogenic role, such as the PDGFR and EGFR families. PI3-kinase signalling is essential for the transforming activity of some cytoplasmic oncoproteins, including v-src, the polyoma middle T antigen and bcr-abl in Ph+ chronic myeloid leukaemia (Vanhaesebroeck et al. 1996). In the case of bcr-abl, mutants that are unable to activate PI3-kinase have been shown to lose leukaemogenic potential and dominant-negative PKB/akt is able to reverse bcr-abl induced leukaemia development in mice with severe combined immunodeficiency (Skorski et al. 1997).

An alternative mechanism for activation of PI3-kinase signalling is a failure to degrade the phosphoinositide second messengers generated by the enzymes. The gene PTEN encodes a lipid phosphatase with the ability to remove the 3’ phosphate from PtdIns(3,4)P2 and PtdIns(3,4,5)P3 – an activity which is essential for its role as a tumour suppressor (reviewed by Di Cristofano & Pandolfi 2000). Germline loss of PTEN is responsible for Cowden’s disease and related conditions, which are characterised by the development of hamartomas and a susceptibility to breast cancer. Loss of PTEN function is now considered to be one of the more common somatic mutations in human cancer, occurring particularly in glioblastoma, prostatic, endometrial and endometroid ovarian cancer (Ali et al. 1999). This may well prove to be the single most significant contributor to PI3-kinase activation in human cancer.

**Prospects for PI3-kinase inhibition in cancer treatment**

The evidence discussed in this review suggests that the consequences of inhibiting class I PI3-kinase signalling may be beneficial in the treatment of cancer. The ubiquitous distribution of class I PI3-kinases and their role in regulating many key cellular functions, particularly in mediating insulin action, however, means that broad-spectrum inhibitors would almost certainly have unacceptable toxicity if administered continuously. Limited information obtained using the existing PI3-kinase inhibitors in vivo, and also the class Ia knockout mouse experiments, support this view. Furthermore, as is evident from the properties of the existing compounds, there is a risk that inhibitors will affect the class III PI3-kinase and related kinases such as DNA-PK and ATM. Chronic inhibition of these enzymes that are involved in protein trafficking and in DNA repair and cell cycle checkpoint control is likely to be undesirable. The potential toxicity of PI3-kinase inhibitors can probably best be limited by developing compounds of which the activity is restricted to a narrow range of isoforms.

The PI3-kinase isoform that is most clearly associated with cell proliferation and survival and is most likely to be implicated in human cancer is p110α. The suggestion that the p110β isoform may be preferentially involved in insulin signalling offers the prospect that isoform-selective inhibitors may have acceptable toxicity. Thus p110α might be considered as a potential target cancer treatment and also (on the basis of mouse knockout experiments), immuno-suppression. The early clinical success of the rapamycin analogue, CCI 779 (Hidalgo & Rowinsky 2000), the target of which, mTOR, lies downstream of the class I PI3-kinases but is not otherwise known to have any special role in cancer, lends strong additional support to the argument. The pharmacodynamic effects of p110α and mTOR inhibition will be different; the relative merits of the two approaches remain to be determined.

The potential role of PI3-kinase inhibitors in cancer treatment may be considered both in terms of both the proliferative and anti-apoptotic effects of the PI3-kinases. If the fundamental activity arises from targeting proliferation, then drugs are much more likely to be effective if administered continuously. Although the frequency with which PI3-kinase signalling is activated in cancer is unknown, there are grounds for suspecting that it is common. There is also the possibility that class Ia PI3-kinases may be involved in transmitting angiogenic signalling in endothelial cells, which raises the possibility of a dual mode of action of class Ia inhibitors, with potential application in the treatment of all solid tumours. Continuous treatment would also have the effect of removing survival signals from tumour cells. However, it might also prove possible to exploit the potential pro-apoptotic effects of selective class Ia inhibitors in intermittent (pulsed) treatment. Patients are far more tolerant of drug toxicity arising from intermittent than from continuous administration, as is also the case with cancer treatment using cytotoxic drugs. It is possible that a pro-apoptotic effect of PI3-kinase inhibition could be best exploited by intermittent co-administration with conventional cytotoxic drugs. As cytotoxic drugs achieve their effect through the induction of DNA damage, in this setting, it may even be beneficial to develop drugs that inhibit not only class Ia PI3-kinases, but also DNA-PK or ATM. Thus, even if it does not prove possible to develop highly selective and non-toxic PI3-kinase inhibitors, cancer is still an attractive therapeutic target. The case for therapeutic development of PI3-kinase inhibitors is very powerful.

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**References**

Stein: PI3-kinase inhibition as a cancer treatment


Aoki M, Blazek E & Vogt PK 2001 A role of the kinase mTOR in cellular transformation induced by the oncoproteins P3k and Akt. PNAS 98 136–141.


Stein: PI3-kinase inhibition as a cancer treatment


