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Phosphoinositide 3-kinases as drug targets in cancer

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The past two years have seen phosphoinositide 3-kinases (PI3Ks) move from being seen as potential targets for chemotherapeutics, to one of them — PI3Kα — being generally accepted as validated. A huge amount of work indicated that there was an important role for PI3Ks in tumour progression and, particularly, in the control of proliferation, survival and regulation of the potential oncogene PKB. These links were further strengthened by studies showing that the tumour suppressor, PTEN, is an antagonist of PI3K signalling and that somatic mutations of p110α (PIK3CA) are present in a variety of cancers. We now know that three of the most frequent mutations in cancer constitutively activate PI3Kα and, when expressed in cells, they drive the oncogenic transformation and chronic activation of downstream signalling by molecules such as PKB, S6K and 4E bp1 that is commonly seen in cancer cells. A large body of research into the cellular roles of PI3Ks has also further validated them as potential foci for cancer chemotherapy, with several additional PI3K effectors controlling cell proliferation and apoptosis having been described. Furthermore, molecules important to the processes of metastasis, development of multi-drug resistance, the 'Warburg effect', angiogenesis and cell growth (i.e. distinct to proliferation) have been found to depend upon, or to be driven by, PI3K activity.

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Introduction

Current views of cancer as the outcome of a multi-stage evolving genetic disease have been well described in recent reviews [1]. It is manifest as an increasing mass of cells, driven by proliferation, but potentially caused by the cells failing to die, and cell growth. The genetic aberrations that contribute to cancer progression produce their effects in a variety of ways. They can lead to upregulation of the activity of a gene, a potential oncogene, that favours survival, growth and/or proliferation. This can occur through changes in the sequence or size of a gene product as a result of somatic point mutations, translocations and/or increases in copy number (amplification). Indeed, activating somatic mutations (and somatic mutations without known effect), translocations and amplifications of PI3K (see Glossary) genes have been described in cancers. Several of the signalling targets of PI3Ks can also act as oncoproteins (e.g. PKB). Alternatively, genes that act as antagonists of the process of increasing cell mass — tumour suppressor genes — can, by mechanisms similar to those listed above, be lost. There are several tumour suppressor genes in the PI3K signalling web (e.g. PTEN, TSC1, TSC2, LKB 1 [which can carry germ-line familial mutations], Foxo1a, Foxo3a and possibly PHLPP; see Figures 1, 2 and 3), most of which could render cancers insensitive to PI3K inhibitors (see below). The final class of mutations that can contribute to progression includes those in genes that influence genetic stability and hence the rate of mutation of tumour suppressors and oncogenes; they are not directly relevant here and will not be discussed.

The PI3K signalling web

The family of PI3Ks in mammalian cells can be divided into three classes (see Table 1). Type I PI3Ks are the best understood and are key players in a substantial intracellular signalling network, engaged by many growth and survival factors, which regulates cell proliferation, growth, survival and apoptosis [2]. The pattern of this regulation is hugely in favour of tumourigenesis; cell proliferation, growth and survival are enhanced and apoptosis is suppressed.

Type I PI3Ks integrate a wide variety of signals, precisely which depending on the PI3K subtype, and transduce these signals via their 3-kinase activity into a rate of production of the intracellular PtdIns(3,4,5)P_3 signal. PtdIns(3,4,5)P_3 signals are largely localized to the inner leaflet of the plasma membrane. They are highly dynamic and can increase 50-fold above basal levels within 10 s and be maintained or transient. There are three major phosphatases that degrade PtdIns(3,4,5)P_3 in mammalian cells: SHIP2, PTEN (broadly expressed) and SHIP (haemopoietic cells).

In many respects, the defining moment in the on-going case for PI3K representing an important target for anti-cancer treatments was the realization that the tumour suppressor PTEN was a PtdIns(3,4,5)P_3 3-phosphatase. Until recently, the SHIP genes, which encode PtdIns(3,4,5)P_3 5-phosphatases, were not considered to be tumour

Glossary
AMP-PK: AMP-activated protein kinase
ATM: Ataxia telangiectasia mutated complex
BAD: Bcl-1-associated death promoter
DNA-PK: DNA-dependent protein kinase
FOXO: Forkhead box class O transcription factor
HIF: Hypoxia-inducible factor
PDK-1: Phosphoinositide-dependent kinase 1
PHLPP: PH domain leucine-rich repeat protein phosphatase
PI3K: Phosphoinositide 3-kinase
PKB: Protein kinase B
PtdIns(3,4,5)P_3: Phosphatidylinositol 3,4,5 trisphosphate
PTEN: Phosphatase and tensin homolog deleted on chromosome 10
SHIP: SH2 domain-containing inositol 5-phosphate
Tor: Target of rapamycin
TSC: Tuberous sclerosis complex
VEGF: Vascular endothelial growth factor
YAP: Yes-associated protein

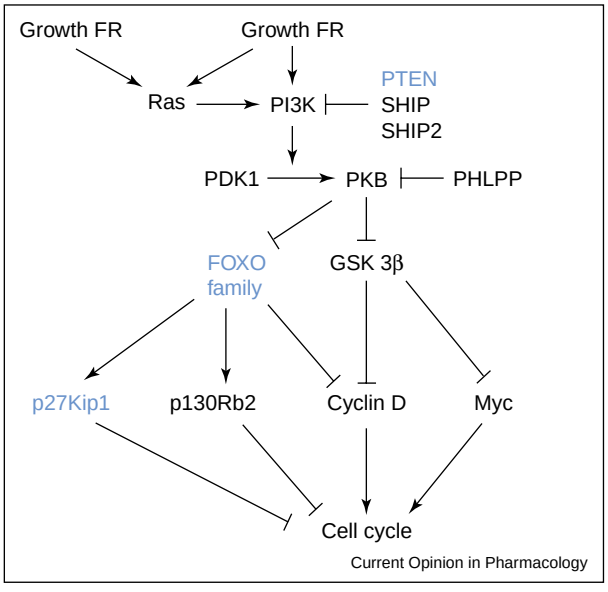
suppressors; however, a recent study identified a high proportion of acute, myeloid and lymphoblastic leukaemias with mutations in *SHIP* (Inpp5d) [3••].

PtdIns(3,4,5)P_3 signals at the membrane are interpreted by a large group of proteins that can specifically bind to PtdIns(3,4,5)P_3, often via pleckstrin homology (PH) domains, and become activated in the process.

PKB is a PH-domain-containing protein that translocates to sites of PtdIns(3,4,5)P_3 in cells [4]. It is a major transducer of PI3K signals and can be mutated to yield an oncogene that is often amplified in tumours. As a result of localising at the site of PI3K activity, it becomes phosphorylated (and hence activated) at two sites, T308 (numbers in PKBα) and S473, through the action of PDK-1 [4] and possibly DNA-PK [5•] and/or mTOR/Rictor (this could still be indirect) [6•], respectively. Recent work indicates that phospho-S473 is dephosphorylated by a PP2C-family phosphatase, and candidate tumour suppressor, PHLPP [7••]. Although phosphorylation of T308 is key for the activation of PKB, there is evidence indicating that phosphorylation of S473 can augment phosphorylation of T308 by PDK-1; in combination with the above evidence pointing to the complex control of S473, and earlier work showing that S473 was needed for full activation of PKB, this suggests that the site should be seen as an important 'gain-control handle' on PKB activity. Antibodies recognizing phospho-S473 are frequently used to immunostain tumour samples to assess their PI3K activity [8]. Although this can be an indicator of elevated PI3K activity, it can also be the result of changes in a variety of other molecules that might be insensitive to PI3K inhibitors.

Through the application of the small-molecule PI3K inhibitors LY294002 and wortmannin, and use of reagents such as constitutively active or dominant-negative PI3Ks or PtdIns(3,4,5)P_3-phosphatases, it is now clear that type I PI3Ks are involved in many different cell functions relevant to tumourigenesis.

Figure 1



Cell proliferation. PI3K signals act amongst many others to regulate cell proliferation. A large variety of growth factor receptors drive activation of type I PI3Ks either directly or via associated tyrosine kinases, G-protein βγ subunits or activation of Ras. Potentially tumourigenic PI3K signals can be antagonised by the PtdIns(3,4,5)P_3 phosphatases PTEN and SHIP. The PtdIns(3,4,5)P_3 effector PKB regulates a variety of molecules controlling the cell cycle. PKB-mediated phosphorylation of the FOXO family of transcription factors enables 14:3:3 proteins to bind and sequester them into the cytosolic compartment. In the nucleus, the FOXO proteins act to repress cyclin D1 expression and promote expression of cell cycle inhibitors such as p27Kip1. These signals are complemented by the ability of PKB to phosphorylate and inhibit GSK-3β, a repressor of cyclin D1 expression. The products of tumour suppressor genes are marked in blue.

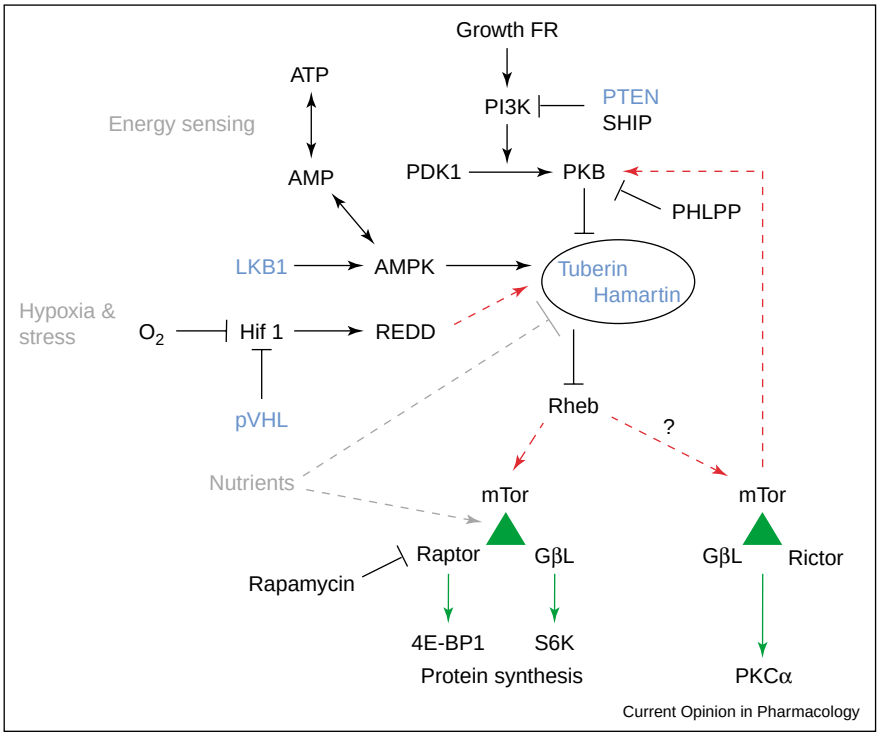
Cell responses dependent upon PI3K activity and relevant to tumour progression or treatment

It is possible to divide the cellular responses dependent on, or driven by, type I PI3K activity into various functional classes relevant to tumourigenesis or its treatment, even if the underpinning signalling pathways overlap or are not fully understood. However, none of the responses below are solely regulated by PI3K activity.

Proliferation

Cell proliferation, size and growth have to be tightly coordinated and hence the mechanisms and molecules regulating them would be expected to overlap. In many contexts, the proliferation of mammalian cells depends upon PI3K activity. The strongest influences are probably exerted through activation of PKB, inhibition of glycogen synthetase kinase-3β and hence control of cyclinD1 levels (Figure 1) [9]. Although some growth factors do not directly activate type I PI3Ks, stimulation of Ras, an extremely potent mitogenic signal, leads directly to

Figure 2



Cell growth and size control. PI3K signals act amongst a range of others defining the appropriateness of the environment for growth. mTor acts as an integrator of these signals and activates a variety of steps involved in protein synthesis, but particularly favouring production of key molecules such as Myc and cyclin D and the ribosomal proteins [48••]. The tumour suppressor complex, comprising Tuberin and Hamartin (the gene products of TSC2 and TSC1, respectively), acts as a suppressor of the growth-promoting signals produced by mTor. The tuberin component has GAP activity against, and therefore functionally antagonises, the small GTPase, Rheb. GTP-bound Rheb activates, probably indirectly, mTor. PKB can phosphorylate and inhibit the TSC2 gene product Tuberin and hence de-represses mTor activity. Recent work has shown that Tor is part of two distinct complexes that are conserved between yeast and mammals. One complex contains Raptor and mLST8 [14] (GβL [49]); the other, mAVO3 [50•] (Rictor) and mLST8. The latter complex is rapamycin-insensitive and regulates both PKCα and possibly phosphorylation of S473 in PKB [6•]. A variety of signals, indicating whether the metabolic circumstances of the cell are appropriate for growth, impinge on mTor. ATP levels are read by AMPK, which is phosphorylated and activated by the tumour suppressor LKB-1 only when AMPK is bound to AMP. AMPK can phosphorylate and activate Tuberin. Nutrients, particularly amino acids, have an important role in regulating mTor/Raptor association and activity; the mechanism is unclear but probably both direct and via the TSC complex. Hypoxia, and several other cellular stresses, can regulate mTor. Oxygen inhibits the accumulation of the HIF family of transcription factors by promoting degradation of their α-subunits, in a process involving the product of the VHL tumour suppressor. Important targets of HIF are the REDD family [16•] (along with VEGF, see above) and these proteins drive activation of the TSC complex, probably indirectly. The products of tumour suppressor genes are marked in blue. The identities of the most important regulators of the rapamycin-insensitive mTor complex are unclear. The high sensitivity of the phosphorylation of S473 in PKB to wortmannin indicates that a signal through the PI3K/PKB/Tuberin/Rheb-pathway is likely to be important because mTor is relatively insensitive to Wortmannin. This suggests a potential positive feed back loop could exist that would tend to switch mTor signals to either high or, if conditions for growth are poor, low levels.

activation of type I PI3Ks (with the possible exception of PI3Kβ [10•]) and, in some cases, it is clear that PI3Ks, and not the MEK/ERK pathway, are the most important mediators of the transforming activity of oncogenic Ras [11].

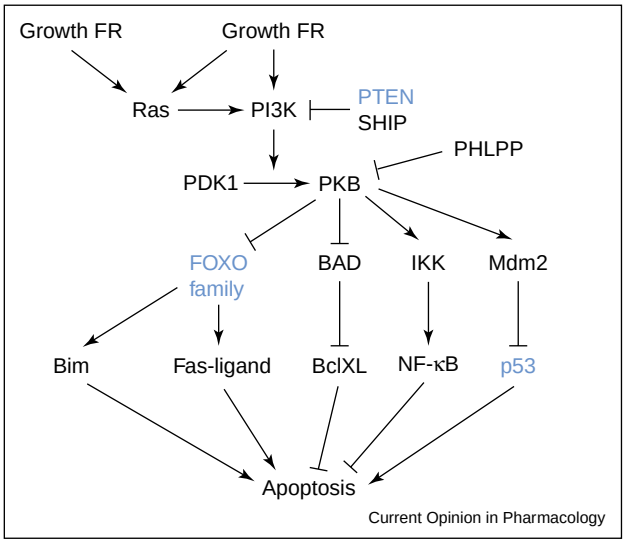
Growth and cell size

Growth of mammalian cells is controlled most powerfully by the activity of the protein kinase mTor [12] and its targets through their ability to regulate protein synthesis and cell size. mTor, in complexes containing Raptor, but not Rictor/mAVO3, is sensitive to the drug rapamycin [13–15], which is currently being trialled as a cancer chemotherapy. A huge range of inputs from hypoxia, [16•], stress [17•], nutrient- [15] or cellular energy-sensing [18•] pathways and growth factor receptors all converge on mTor. PI3K/PDK-1/PKB signals operate amongst these environmental signals to favour increases in cell growth and size. Significantly, four different tumour suppressor genes act to suppress mTor activity (TSC1, TSC2 [19], LKB-1 [20•] and VHL [21], see Figure 2).

Survival/inhibition of apoptosis

PI3K activity is an essential part of the signalling network that blocks programmed cell death (apoptosis) and enables cells to survive when they are in their correct

Figure 3



Cell survival/inhibition of apoptosis. PI3K signals are critical for cell survival and PKB is a critical link. Sequestration of FOXO family members leads to reduced expression of pro-apoptotic proteins like Bim and Fas-ligand. PKB-mediated phosphorylation of Bad leads to its sequestration by 14:3:3 proteins and as a result de-repression of the pro-survival molecule BclXL. Similar negative regulation of YAP (not shown) leads to repression of the p53 related transcription factor p73 and reduced expression of the pro-apoptotic protein Bax. Phosphorylation of IKK and Mdm2 lead to functionally complementary, reciprocal changes in the classic NF-κB pro-survival pathway and the pro-apoptotic p53 pathway. PKB activation also leads to a stimulation of the rate of glycolysis and production of lactate, without any change in oxygen consumption, and this effect probably underlies the observation that cell transformation leads to an acceleration of glycolytic rate (the 'Warburg' effect). The products of tumour suppressor genes are marked in blue. Counter-intuitively, this phenomenon appears to result from increased glucose uptake and primary utilization over and above the immediate demands for growth; the outcome is increased production of potential biosynthetic intermediates [51•].

cellular context. These pathways are typically wired so that a continuous survival signal from a growth factor or adhesion receptor is required to block apoptosis; if the signal is lost, perhaps because the cell moves out of reach of a source of growth factor or becomes detached from its substrate, apoptosis is de-repressed. PKB is an most important target of PI3Ks in these contexts because it phosphorylates and regulates a wide variety of proteins implicated in cell survival/death decisions (see Figure 3). A striking theme in the regulation of these PKB targets, particularly BAD, FOXO family members [4] and YAP [22], is that upon phosphorylation they are sequestered into complexes with 14:3:3 proteins, preventing them from interacting with their targets. Recent evidence suggests that PKB is also the key signal eliciting the long-known 'Warburg effect', where the rate of glycolysis and glucose uptake is accelerated in transformed cells [23••]. This phenomenon is now known to be paralleled by increased uptake of other nutrients; it is seen as an integral part of the anti-apoptotic survival signals delivered by growth factors that can be usurped during tumour progression (see legend to Figure 3).

Metastasis

Although driven by cell motility, metastasis also depends upon cells avoiding apoptotic responses normally elicited by loss of survival signals supplied by their home environment (see above). PI3K signalling also promotes cell motility. This occurs through a wide range of mechanisms, including activation of the small GTPases of the Rac family. Rac can drive the polymerisation of peripheral actin and hence the protusion of lamellipodia. Several Rac activators (GEFs) can mediate these effects of PI3K activity on Rac; one of these, Tiam1, was originally identified in a screen for genes inducing an invasive phenotype [24]. Hence through its roles in both survival

Table 1

Mammalian PI3Ks.

	Catalytic subunits		Regulatory subunits	
	Name	Gene	Name	Gene
Type 1A PI3Ks				
PI3Kα	p110α	*PIK3CA*	p85α	*PIK3R1*[a]
PI3Kβ	p110β	*PIK3CB*	p85β	*PIK3R2*
PI3Kδ	p110δ	*PIK3CD*	p55γ	*PIK3R3*
Type 1B PI3Ks				
PI3Kγ	p110γ	*PIK3CG*	p101	*PIK3R5*
			p84	(not yet assigned)
Type II PI3Ks				
Type II PI3Kα		*PIK3C2A*	None known	
Type II PI3Kβ		*PIK3C2B*	None known	
Type II PI3Kγ		*PIK3C2G*	None known	
Type III PI3K				
Type III PI3K	hVps 34	*PIK3C3*	p150	*PIK3R4*

[a] There are five splice variants created from PIK3R1.

and motility, upregulation of PI3K signalling is a powerful force driving metastasis.

Angiogenesis

Vascularisation of tumours is a key marker of progression. VEGF-A is a critical regulator of endothelial cell survival, motility and proliferation. Its production can be stimulated by; reductions in oxygen levels, tumour necrosis factor-α, interleukin-1β and insulin via their ability to promote stabilization and accumulation of the α-subunit of the HIF-1 transcription factor and hence production of VEGF-A message (Figure 2). PI3Ks have been shown to have important roles at three levels in this cascade: insulin-, interleukin-1β- and hypoxia-induced accumulation of HIF-1α [25] (although some aspects of this have been contested [26]), the accumulation of VEGF-A [25] and the effects of VEGF-A on cells [27].

Drug resistance

Expression of the multi-drug resistance transporter MRP-1 has been shown to be dependent upon PI3K activity in carcinoma of the prostrate [28].

Specific roles for individual type I PI3Ks

All of the above could be seen as potentially beneficial, anti-tumour outcomes of inhibiting PI3K activity. However, the lack of selectivity of the reagents used in much of the above work means that the roles/importance of individual PI3Ks remains unclear.

Over the past few years, a steadily accumulating mass of data has begun to dissect the individual roles of PI3Ks. Murine knockouts of PI3Ks have shown that α and β subtypes are essential in early development and that δ, although not essential, has a critical role in the regulation of proliferation and development of the immune compartment [29]. Results following micro-injection of PI3K-specific anti-catalytic antibodies into macrophages have suggested specific roles for PI3Kα in cell proliferation and for PI3Ks β and δ in chemotaxis (although the phenotype of the murine PI3Kδ knockout did not confirm this conclusion); similar earlier experiments with another cell line also suggested a role for α in proliferation. These results are supported by accumulating evidence indicating that PIK3CA is amplified in several carcinomas and primary cell lines.

The breakthrough in this area came, however, with the report that a remarkably high proportion (32%) of colorectal cancers contained somatic mutations in *PIK3CA* [30••] Importantly, this work sequenced all of the coding exons of *PIK3CA* and identified several hot-spots in the amino terminal p85-binding region and the C2 domain, and in the helical and kinase domains. One further study also sequenced all of the coding exons of *PIK3CA* in tumours, confirming the distribution of mutations found previously [31•,32] (Figure 4). Further work has focused on the helical and kinase domains, encoded by exons 9 and 20, and collectively found somatic mutations in *PIK3CA* in a wide range of tumours: breast (the proportions of tumours with mutations in individual studies were 8%, 25%, 27% and 40%), glioblastomas (5 and 27%), medulloblastoma (5%), lung (4%), ovarian (6.6%), liver (35%), acute leukaemia (1%) and gastric (6.5 and 25%) [32–34].

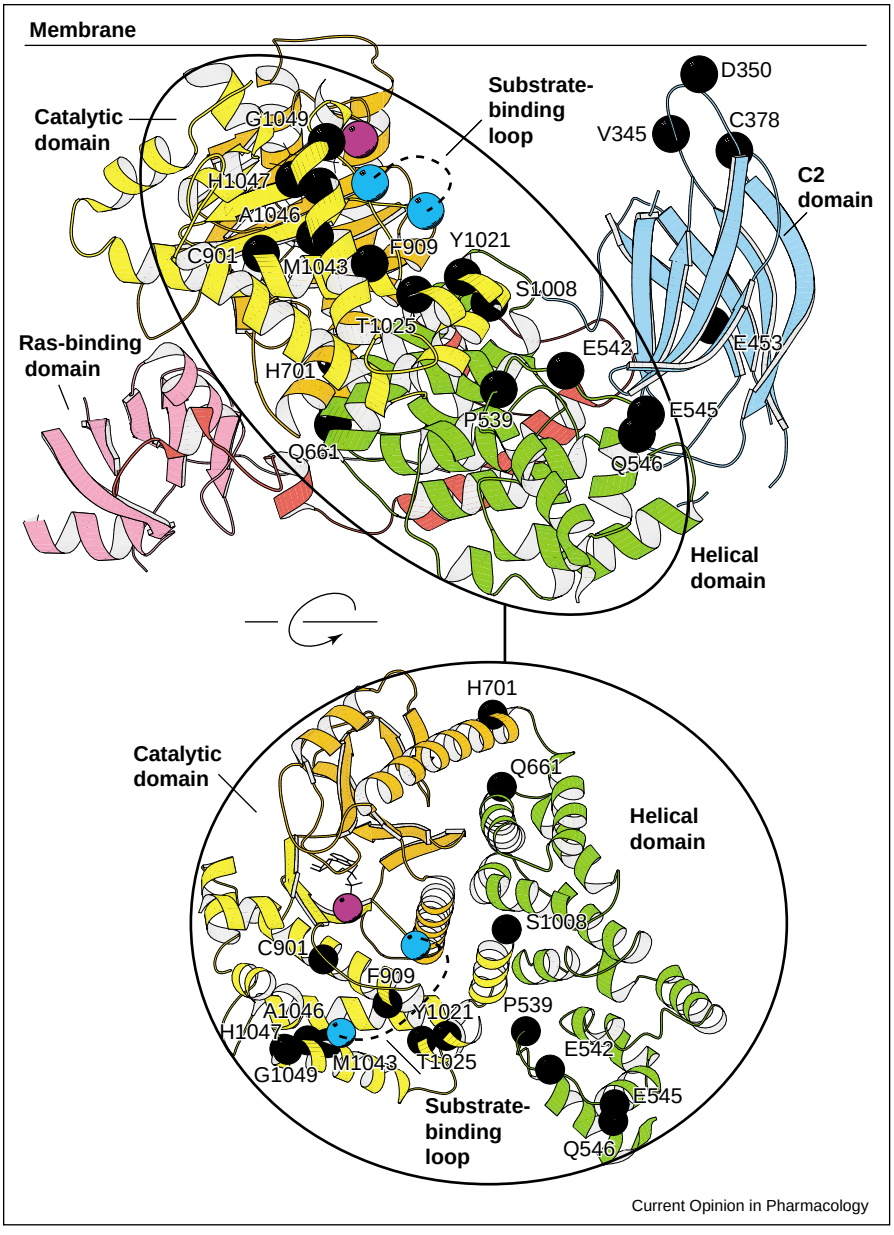
Importantly, only a small proportion (3%) of low-grade colorectal tumours compared with advanced cases (32%) carried *PIK3CA* mutations, suggesting that the acquisition of *PIK3CA* mutations may be an important event in progression into the more dangerous invasive phenotype. Similarly, a recent study of amplification of the *PIK3CA* locus during progression of lung cancer showed that amplification appeared in high-grade, but not in low-grade, pre-invasive lesions, suggesting that gain of PI3K function represented a commitment to tumour progression [8]. By contrast, in gastric and liver cancers, *PIK3CA* mutations were found in both early and advanced specimens, which could indicate an important role in the early evolution of these tumour types [32]. Secondly, in keeping with studies of mutations in other classes of cancer-progressing genes, there is clear evidence of an inverse relationship between the occurrence of *PIK3CA* mutations and amplification; a tumour that has already taken advantage of a constitutive rise in PI3Kα signalling does not take much advantage from a gain-of-function in the same pathway delivered by a separate mechanism.

Consequences of mutations in PI3Kα

Three of the most common mutant versions (helical domain mutants E542K and E545K and the catalytic domain mutant H1047R) of *PIK3CA* in tumours have been expressed in chicken embryo fibroblasts [35••]. All three caused oncogenic transformation and led to constitutive increases in phosphorylation of PKB, S6K and 4E-bp1, whereas wild-type p110α had no effect. These important results demonstrate the somatic mutations are likely to contribute directly to tumour progression.

In vitro kinase assays with the mutant constructs indicated that they were constitutively active (also demonstrated for H1047 in [30••]). How these estimates of activity will translate into a cell is not yet clear; however, they are sufficient to lead to activation of PKB. Although it is easiest to think of the mutants achieving this result because of their catalytically efficient conformation, perhaps through shifts in the catalytic core in the case of the catalytic domain mutants, or through improved membrane association in the case of the C2 domain mutants, it is also possible that at least some of these mutations increase the thermal stability of p110α. The result of this stabilization for an enzyme such as p110α, which is

Figure 4



Somatic mutation of p110α in tumours. The black spheres mark positions of the equivalent residues in the closely related p110γ for which there is a known structure. The top panel illustrates a view of the subunit oriented on a lipid membrane (suggested by the horizontal line above the subunit). All but one of the C2 domain mutations are in loops that would be in contact with the lipid membrane, and mutation of these residues to a basic residue would be expected to cause preferential localization to lipid membranes. The bottom panel is an expanded view of the catalytic and helical domains viewed from the membrane surface. The bound ATP is shown in stick representation with the γ-phosphate shown as a large purple sphere. To visualize the region interacting with lipid substrates, the ends of the substrate-binding (activation) loop are indicated by cyan spheres (this loop is disordered in the crystal structure determined in the absence of a bound phosphoinositide substrate). The mutations in the catalytic domain are predominately in the C-terminal lobe (yellow) and involve residues in the helices forming a scaffold for the substrate-binding loop. The mutations in the helical domain are not near the active site, but instead lie along the interface between the catalytic domain and the helical domain as well as on a loop at the N-terminal end of the domain. None of the helical domain mutations would be expected to be in contact with the lipid membrane or phosphoinositide substrate.

sensitive to denaturation, would be that a higher proportion of the molecules in the cell would be catalytically competent; in effect, the mutants would be present at a higher concentration.

PI3Kα as a target for chemotherapy

Clearly, the evidence discussed above represents a reasonable case for considering PI3Kα a potential target for chemotherapy; indeed, several companies have already

accepted the evidence and are exploring this possibility. Although a variety of approaches could be considered, screening for small-molecule inhibitors of the catalytic ATP binding site appears to be the most practical. Interfering with activation of PI3Kα via its SH2 domains would be challenging and limited by the promiscuous use of adaptors by the type IA PI3Ks; similarly, there is no president for a selective Ras-effector binding-site inhibitor.

Currently, a variety of small molecules that are partially selective for different type I PI3Ks have been described as potential inhibitors of inflammation or thrombosis, as well as cancer chemotherapies, but none are specific for PI3Kα [8,36–39]. It appears that inhibitors are tending to separate out into those that target PI3Kβ or PI3Kδ, which is unsurprising as these have the most closely related catalytic domains. Compounds with greater specificity for α and γ are more difficult to isolate; however, there are notable exceptions. Interestingly, the major off-target activity of these compounds seems to be inhibition of DNA-PK [40]. The toxicity of non-specific PI3K inhibitors implies that selectivity is essential, but the precise amount of crossover that could be tolerated is not clear.

Regardless of the method of selective inhibition, however, several issues are still unresolved. PI3Kα is essential for mammalian development; although this does not prove it is essential in post-natal life, it is likely that PI3Kα is performing important functions in non-tumour cells. Currently, we must hope that cancer cells will, in accord with the 'addiction hypothesis' [41], be more dependent upon the signalling pathways that have been upregulated during disease progression for their survival.

Designing inhibitors to selectively block signalling that has been activated through somatic mutation or amplification is consistent with the recent emphasis on profiling individual cancers to enable the correct treatment to be selected. Currently, it would seem that such profiling requires sequencing, copy number analysis and/or expression studies of PIK3CA, PTEN and possibly p85α (PIK3R1).

There are now several examples of somatic and oncogenic mutations in *p85* that usually focus into a small 'hot spot', resulting in mutant proteins that retain their ability to bind p110, but lose their C-terminal SH2 domains, and result in constitutive activation of the heterodimer [42–44]. Although this upregulation of PI3K activity would not be restricted to p110α, it is not clear whether the oncogenic consequences of these mutations are all mediated by PI3Kα.

Similarly, although it is true that many of the consequences of loss of PTEN function can be reversed by inhibition of PI3K activity and that it is PI3Kα which is most commonly mutated in tumours, it is currently unclear whether PI3Kα is the most important oncogenic driver of PtdIns(3,4,5)P_3 signalling in the absence of PTEN.

Such profiling would not focus on downstream markers such as PKB, S6K or 4E-bp1 in isolation. This is because of the huge number of responses in which PI3Ks play an important role but which are also regulated by several other tumour suppressors and oncogenes that can drive similar activation of downstream signalling; however, these additional regulators would be expected to be insensitive to PI3K inhibitors.

What of the other PI3Ks?

None of the other PI3K catalytic subunits nor any of the PI3K-related protein kinases (e.g. mTor, ATM) have been found to carry somatic mutations in tumours [30••,34]. However, it should be appreciated that work to date has focused on their catalytic exons and, although some mutations were found in α, it is now clear that mutations are more common in other none-catalytic exons. Hence it remains possible that potentially significant mutations may occur in other PI3Ks. In keeping with this possibility, there is evidence of amplification of PI3Kδ (PIK3CD) in glioblastomas [45] and of rises in PI3Kβ (PIK3CB) expression and activity in colon and bladder tumours, relative to adjacent non-diseased tissue [46]. Moreover, PI3Kβ has been reported to have roles in cell proliferation and invasive cell growth [47].

Conclusions

Key practical issues for the future remain:

1. What level of PI3K selectivity, if any, will be most effective?
2. Do PTEN wild-type and null cells have similar dependencies on PI3K (α) activity?
3. Precisely what is the frequency with which cancers acquire other activating mutations, which might confer resistance to PI3K inhibitors, in the PI3K pathway?
4. Do cancers tend to acquire PI3K mutations in specific windows in their progression?

However, selective small-molecule inhibitors of PI3Kα have tremendous potential as novel cancer chemotherapies.

Acknowledgements

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