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Class I PI3K in oncogenic cellular transformation

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Abstract

Class I phosphoinositide 3-kinase (PI3K) is a dimeric enzyme, consisting of a catalytic and a regulatory subunit. The catalytic subunit occurs in four isoforms designated as p110α, p110β, p110γ and p110δ. These combine with several regulatory subunits; for p110α, β and δ the standard regulatory subunit is p85, for p110γ it is p101. PI3Ks play important roles in human cancer. *PIK3CA*, the gene encoding p110α, is mutated frequently in common cancers, including carcinoma of the breast, prostate, colon and endometrium. Eighty percent of these mutations are represented by one of three amino acid substitutions in the helical or kinase domains of the enzyme. The mutant p110α shows a gain of function in enzymatic and signaling activity and is oncogenic in cell culture and in animal model systems. Structural and genetic data suggest that the mutations affect regulatory inter- and intramolecular interactions and support the conclusion that there are at least two molecular mechanisms for the gain-of-function in p110α. One of these mechanisms operates largely independently of binding to p85, the other abolishes the requirement for an interaction with Ras. The non-alpha isoforms of p110 do not show cancer-specific mutations. However, they are often differentially expressed in cancer and, in contrast to p110α, wild-type non-alpha isoforms of p110 are oncogenic when overexpressed in cell culture. The isoforms of p110 have become promising drug targets. Isoform-selective inhibitors have been identified. Inhibitors that target exclusively the cancer-specific mutants of p110α constitute an important goal and challenge for current drug development.

Keywords

PI3K; PTEN; Akt; Ras; p85

Introduction

This contribution will present a brief review of Class I phosphatidylinositol 3-kinases (PI3Ks) and their oncogenic activities, focusing on cancer-specific mutations and on differential expression of the four catalytic subunits of this enzyme family.

Class I PI3Ks phosphorylate phosphatidylinositol 4,5 bisphosphate (PIP_2) at the 3 position of the inositol ring. The product, phosphatidylinositol 3,4,5-trisphosphate (PIP_3), functions as a second cellular messenger that controls cell growth, survival, proliferation, motility and morphology (Bader *et al.*, 2005; Cantley, 2002; Deane and Fruman, 2004; Engelman *et al.*, 2006; Hawkins *et al.*, 2006; Katso *et al.*, 2001; Okkenhaug and Vanhaesebroeck, 2003; Vanhaesebroeck *et al.*, 2001; Vanhaesebroeck and Waterfield, 1999; Vivanco and Sawyers, 2002). The phosphatase PTEN (phosphatase and TENsin homolog deleted on chromosome 10) hydrolyzes PIP_3 to PIP_2, thus acting as the catalytic antagonist of PI3K (Maehama and Dixon, 1998; Myers *et al.*, 1998; Stambolic *et al.*, 1998). Mutational activation and overexpression of class I PI3K and genetic or epigenetic inactivation of PTEN result in enhanced PI3K signaling which is associated with oncogenic cellular transformation and cancer (Ali *et al.*, 1999; Bachman *et al.*, 2004; Bader *et al.*, 2005; Broderick *et al.*, 2004;

Campbell *et al.*, 2004; Cantley, 2002; Cully *et al.*, 2006; Eng, 2003; Fruman, 2004; Hartmann *et al.*, 2005; Kang *et al.*, 2005b; Lee *et al.*, 2005; Leslie and Downes, 2004; Levine *et al.*, 2005; Li *et al.*, 2005; Maehama *et al.*, 2001; Saal *et al.*, 2005; Salmena *et al.*, 2008; Samuels *et al.*, 2004; Simpson and Parsons, 2001; Vogt *et al.*, 2007; Wang *et al.*, 2005; Wishart and Dixon, 2002). Because of the enzymatic antagonism of PI3K and PTEN, it is tempting to equate loss of PTEN with gain in PI3K function. However, there is mounting evidence that a loss of PTEN results in cellular changes that are quite different from those induced by a gain of function in PI3K (Blanco-Aparicio *et al.*, 2007). The enzymatic antagonism is not the only determining factor that characterizes the balance of PTEN and PI3K in the cell. The cellular distribution of the two proteins is different, and these differences can be enhanced by external and internal stimuli. Interaction with other proteins could also gravely affect the balance between PTEN and PI3K. Tumors that have lost PTEN often show drug sensitivities that are different from those that have a direct gain of PI3K function (Salmena *et al.*, 2008).

Only Class I PI3Ks are involved in cancer; there are no data linking Class II PI3Ks or Class III PI3K (Vsp34p) to oncogenesis. This fact probably reflects the different product and substrate specificities of the three classes of PI3K. Only Class I PI3Ks can use PIP_2 to generate PIP_3, Class II PI3Ks produce the 3,4-bisphosphate and the 3-monophosphate of inositol lipids, and Class III can only make the 3-monophosphate. PIP_3 is a critical component in the control of cell growth and replication, and the ability to produce this important second messenger molecule confers oncogenic potential to the lipid kinase. Class I PI3Ks have both lipid and protein kinase activities (Dhand *et al.*, 1994; Foukas *et al.*, 2004; Foukas and Shepherd, 2004). Genetic experiments have shown that lipid kinase is essential for oncogenic activity; p110 engineered to have only protein kinase activity is non-oncogenic (Kang *et al.*, 2006). Whether protein kinase plays a role in conjunction with lipid kinase is not known.

The canonical PI3K signaling pathway

In normal cells, the activity of class I PI3Ks is tightly controlled. Upstream signals recruit the cytosolic PI3Ks to the plasma membrane. This relocation is mediated by interactions with receptor tyrosine kinases (RTK) (Skolnik *et al.*, 1991) or G-protein-coupled receptors (GPCR) (Stephens *et al.*, 1994). Interaction with Ras also contributes to the activation of PI3K (Chan *et al.*, 2002; Rodriguez-Viciana *et al.*, 1994; Rodriguez-Viciana *et al.*, 1996) (Fig. 1). The product of class I PI3K, PIP_3, recruits proteins that contain a pleckstrin homology (PH) domain to cellular membranes (Corvera and Czech, 1998). Among these are the serine-threonine kinase Akt (cellular homolog of murine thymoma virus Akt8 oncoprotein), as well as its activating kinase PDK1 (3-phosphoinositide-dependent kinase 1). PDK1 phosphorylates and thereby activates Akt at threonine 308 (Alessi *et al.*, 1997). Signals originating from Akt control the initiation of protein synthesis through a cascade of interactions that proceeds through the tuberous sclerosis complex (TSC), Rheb (Ras homolog enriched in brain) and TOR (target of rapamycin) to two critical downstream targets, S6K (p70 S6 kinase) and 4EBP (eukaryotic initiation factor 4E-binding protein) (Bader and Vogt, 2004; Garami *et al.*, 2003; Inoki *et al.*, 2003; Inoki *et al.*, 2002; Tee *et al.*, 2003; Zhang *et al.*, 2003). Akt signals also regulate transcription, inducing phosphorylation-dependent degradation of FOXO1 (forkhead box O transcription factor) (Biggs *et al.*, 1999; Brunet *et al.*, 1999; Kops *et al.*, 1999; Takaishi *et al.*, 1999; Tang *et al.*, 1999) and inactivation of GSK3β (glycogen synthase kinase-3β) (Cross *et al.*, 1995). Important targets of FOXO1 are the growth-attenuating p27(Kip1) (Medema *et al.*, 2000) and p21(Cip1) (Seoane *et al.*, 2004), and pro-apoptotic BIM (Bcl-2 interacting mediator of cell death) proteins (Arden, 2004; Gilley *et al.*, 2003; Stahl *et al.*, 2002). GSK3β regulates the potentially oncogenic transcription factors Jun (cellular homolog of the Jun oncoprotein of avian retrovirus ASV17) and Myc (cellular homolog of the avian myelocytoma retroviral oncogene) (de Groot *et al.*, 1993; Gregory *et al.*, 2003; Nikolakaki *et al.*, 1993; Sears *et al.*, 2000; Wei *et al.*, 2005). In a positive feedback loop, TOR, in complex with the Rictor

(Rapamycin-insensitive companion of TOR) protein phosphorylates and thereby additionally activates Akt at serine 473 (Sarbassov *et al.*, 2005). S6K can introduce an inhibitory phosphorylation on IRS1 (insulin receptor substrate 1), mediating a negative feedback loop (Harrington *et al.*, 2004). Ras is also linked to the PI3K pathway. Activated Ras enhances the activities of PI3K; in turn, the product of PI3K, PIP_3, stimulates Ras activation (Chan *et al.*, 2002; Rodriguez-Viciana *et al.*, 1994; Rodriguez-Viciana *et al.*, 1996). The overall effect of the combined PI3K signals is to enhance the stimulation of cellular replication and survival and to reduce growth inhibition and apoptosis.

Class I PI3Ks are heterodimeric proteins that consist of a catalytic subunit and a regulatory subunit, also referred to as an adaptor (Fig. 2). There are four isoforms of the catalytic subunit: p110α, p110β, p110δ and p110γ. They share the same domain composition: an amino-terminal adaptor-binding domain (ABD) that provides the principal interaction surface with the regulatory subunit, a Ras-binding domain (RBD) that mediates the interaction between p110 and Ras-GTP and contributes to the stimulation of PI3K and to the Ras-driven signaling pathway, a C2 (protein-kinase-C homology-2) domain with affinity for lipid membranes, a helical domain acting as a scaffold for other domains of p110, and a carboxyl-terminal kinase domain (Walker *et al.*, 1999). Class I PI3Ks are further subdivided according to their regulatory subunits (Vanhaesebroeck *et al.*, 1997a). Class IA, encompassing p110α, p110β and p110δ, associates with the regulatory subunits p85α, p85β, p55α, p55γ and p50α. Class IB, consisting only of p110γ, binds the regulatory subunits p101, p84 and p87PIKAP. The representative regulatory subunit, p85α, contains several modular protein-protein interaction domains: a Src-homology 3 (SH3) domain, a breakpoint clustered homology (BH) domain, two Src-homology 2 (SH2) domains, and an inter-SH2 (iSH2) domain. The iSH2 domain is the primary p110-binding domain (Dhand *et al.*, 1994;Klippel *et al.*, 1994). Regulatory subunits link p110 to upstream signals, interacting with RTKs and GPCRs. In the absence of upstream signals, the regulatory subunits stabilize p110 and suppress its catalytic activities (Luo *et al.*, 2005;Yu *et al.*, 1998).

Cancer-specific mutations in *PIK3CA*

The gene coding for p110α, *PIK3CA*, is mutated in various human cancers (Samuels and Ericson, 2006; Samuels *et al.*, 2004). The mutations are non-synonymous, arising from single-nucleotide substitutions. They occur in around 30% of several common cancers, including carcinoma of the breast, the colon, endometrium and prostate (Catalogue of Somatic Mutations in Cancer, http://www.sanger.ac.uk/genetics/CGP/cosmic). These cancers carry a single p110α mutation, and 80 % of the mutated proteins contain one of three “hot spot” mutations. Two of these hot spot mutations map to the helical domain of p110α, and the third resides in the kinase domain. The hot spot mutations induce a gain of function in p110α. The lipid kinase activity of the mutant protein is significantly upregulated (Ikenoue *et al.*, 2005; Kang *et al.*, 2005a; Samuels *et al.*, 2004). Mutant-expressing cells show constitutive downstream signaling detectable by el“evated phosphorylation of Akt, S6K, 4EBP and GSK3β. p110α carrying one of the hot spot mutations shows oncogenic activity. It can transform primary fibroblasts in culture, induce anchorage-independent growth and cause tumors in animal model systems (Bader *et al.*, 2006; Ikenoue *et al.*, 2005; Isakoff *et al.*, 2005; Kang *et al.*, 2005a; Zhao *et al.*, 2005). This oncogenic potential probably contributes to the neoplastic phenotype of the human cancer cells carrying mutant p110α; the mutations can therefore be regarded as driver” mutations. The clustering of p110α mutations in hot spots suggests that the mutations provide a selective growth advantage to the cell. In addition to the three hot spot mutations which account for four fifths of the p110α mutations, numerous different rare cancer-specific mutations have been identified (for selected examples see Fig. 3). They are widely distributed over the coding sequence and occur in all domains of p110α except the RBD. Most of these rare mutations also show a gain of function (Gymnopoulos *et al.*, 2007; Ikenoue *et al.*, 2005).

However, quantitative measurements of oncogenic activity show that these rare mutants are far less potent than the hot spot mutants. This lower level of oncogenic activity may translate into a weaker selective advantage for the mutant-carrying cell and may explain the rarity of these marginally oncogenic mutations. A recurring theme in the gain-of-function mutations of p110α is substitution of an acidic or neutral residue with a basic residue and location of the substituted residue on the surface of the protein. Indeed, some of the engineered point mutations of p110α that meet these criteria show a gain of function (Gymnopoulos *et al.*, 2007)

Structural data

The X-ray crystal structure of p110α in complex with a portion of p85α has been solved (Huang *et al.*, 2008; Huang *et al.*, 2007). The data show that the ABD of p110α not only binds to the iSH2 domain of p85 (the region responsible for high affinity binding between p85 and p110), but also interacts with the kinase domain and a linker region between ABD and RBD. R38 and R88 of p110α form hydrogen-bonds with Q738, D743 and D746 of the N-terminal lobe of the kinase domain. The rare cancer-specific mutations, R38H, R38C and R88Q possibly disrupt these interactions, resulting in a conformational change of the kinase domain. An ABD deletion mutant of wild-type p110α shows enhanced lipid kinase activity compared to its full-length counterpart (Zhao *et al.*, 2005; Zhao and Vogt, 2008). This increase in activity may be due to the loss of an inhibitory interaction between ABD and the kinase domain of p110α, or it may reflect a relief of the inhibition that is caused by the binding of the N-SH2 (N-terminal SH2) domain of p85 to the helical domain of p110 (see below). Deletion of part of the p85-binding domain in wild-type p110α also reveals a low level of oncogenic transforming activity that is readily demonstrable in cell culture (Zhao and Vogt, 2008).

The C2 domain has been postulated to facilitate recruitment of p110 to the plasma membrane (Nalefski and Falke, 1996; Newton and Johnson, 1998; Rizo and Sudhof, 1998). This function is evident in the crystal structure of p110γ (Walker *et al.*, 1999). Positively charged amino acids are critically involved in membrane binding (Heo *et al.*, 2006). Cancer-specific mutations (N345K and C420R) in the C2 domain of p110α increase the positive surface charge of the domain and were thought to mediate improved binding to the cell membrane, making lipid kinase activity independent of signals transmitted through the regulatory subunit (Gymnopoulos *et al.*, 2007). However in the co-crystal structure of p110α and p85, N345 of p110α forms a hydrogen bond with N564 and D560 in the iSH2 domain of p85. Therefore, N345K is likely to disrupt this interaction of the C2 and iSH2 domains and thus alter the regulatory effect of p85 on p110α (Huang *et al.*, 2007). The electron density of the C420 residue in the C2 domain of p110α is not seen in the co-crystal structure. The C420R mutation may function to increase the affinity of p110α for lipid membranes as previously proposed (Gymnopoulos *et al.*, 2007). Another C2 domain mutation, E453Q, also disrupts the interaction of the C2 domain with iSH2, similar to the N345K mutation (Huang *et al.*, 2007).

Biochemical and structural modeling studies provide evidence for an interaction between the helical domain of p110α and the N-SH2 domain of p85 (Miled *et al.*, 2007; Shekar *et al.*, 2005; Wu *et al.*, 2007). In the co-crystal structure of p110α/p85, the N-SH2 domain is not highly ordered. However, a structural model can be generated if biochemically identified interactions are taken into account. In this model, the N-SH2 domain of p85 binds to the interface between the kinase and the helical domains of p110α (Huang *et al.*, 2007). These interactions may be responsible for the p85-induced inhibition of p110α. The helical domain mutations (E542K and E545K) could interfere with this p85-p110α interaction and thus could relieve the inhibition. The phosphorylated insulin receptor substrate activates the lipid kinase activity of wild-type p110α, presumably by engaging the N-SH2 domain of p85 and thus lifting the inhibitory hold of N-SH2 on the helical domain. This activation is not seen with the helical domain mutants suggesting that the inhibitory interaction with the N-SH2 domain of p85 has

been weakened or interrupted by the mutations (Carson *et al.*, 2007). However, helical domain mutations that carry an ABD truncation show significantly higher oncogenic activity than the truncated wild-type p110α (Zhao and Vogt, 2008). Since both have lost p85 binding, the difference in oncogenic potency suggests an effect of the helical domain mutations that goes beyond interference with p85 binding. The relevant intra- and intermolecular interactions are schematically summarized in Fig. 2.

The genetics of cancer-specific mutations in *PIK3CA*

Genetic experiments provide insight into the molecular mechanisms of mutant-induced gain of function in p110α (Kang *et al.*, 2005a; Liu and Roberts, 2006; Zhao *et al.*, 2005; Zhao and Vogt, 2008). The location of the hot spot mutations in two different domains of the protein, E542K and E545K in the helical and H1047R in the kinase domain, suggests that they operate by different mechanisms. This proposal is supported by the observation that combining helical and kinase domain hot spot mutations in the same molecule has a strongly synergistic effect on downstream signaling and on oncogenic potency. The double mutant, E545K/H1047R, has also been found in human cancer (Lee *et al.*, 2005). The case for mechanistic differences between helical and kinase domain mutations is further strengthened by the interactions with p85 and Ras (Zhao and Vogt, 2008). A truncation of the p85-binding domain that eliminates the interaction with p85 does not silence oncogenic and signaling activities of the helical domain mutants, but completely abolishes oncogenicity in the kinase domain mutant. Curiously, the thus incapacitated kinase-domain mutant still signals through Akt and TOR, albeit at lower levels. Disabling the Ras-p110α interaction by the K227E mutation in the RBD has the opposite effect on the hot spot mutants of p110α. Interaction of GTP-bound Ras with wild-type p110α is known to augment the activity of p110α (Chan *et al.*, 2002), possibly by inducing a conformational change in the substrate-binding site (Pacold *et al.*, 2000). In turn, PI3K is an important Ras effector, mediating the proliferative and survival functions of Ras (Rodriguez-Viciana *et al.*, 1994; Rodriguez-Viciana *et al.*, 1996). Introducing the Ras-binding mutation into the hot spot mutants causes a complete loss of oncogenic potency in the helical domain mutations together with a cessation of signaling, whereas the kinase domain mutant is unaffected by the absence of Ras-binding (Figs. 3 and 4). The kinase domain mutant is even able to rescue helical domain mutants that were incapacitated by the absence of Ras-binding, restoring oncogenic and signaling activities to the synergistic levels seen with helical-kinase domain double mutants. The kinase domain mutation maps close to the activation loop and may affect the conformation of the loop, altering the interaction with the substrate (Huang *et al.*, 2008; Huang *et al.*, 2007). Previous structural studies on the p110γ-Ras complex have demonstrated a change in the conformation of the substrate-binding site as a result of the interaction with Ras (Pacold *et al.*, 2000). The kinase domain mutation H1047R may induce a similar conformational change in the absence of Ras and thus gain Ras-independence. The data on p85 and Ras interaction strongly support the existence of two distinct molecular mechanisms for the mutation-induced gain of function in p110α. The data are compatible with the suggestion that the helical domain mutations lift the inhibitory interaction between N-SH2 of p85 and the helical domain of hot spot mutants of p110α and that the kinase domain mutation mimics the conformational change that is triggered by the interaction with Ras (Zhao and Vogt, 2008). These straight-forward interpretations ascribe the effect of the mutations in the p85 and Ras interacting domains to the specific elimination of p85 and Ras-binding respectively. However, the possibility that these mutant effects are caused by some conformational change that is independent of the targeted protein-protein interactions has not been ruled out. For instance, the co-crystal structure of p110α and p85 also reveals an unexpected interaction between p85-binding domain and kinase domain, which would be affected by the truncation of the p85-binding region (Huang *et al.*, 2007). The ultimate test of these ideas will be the co-crystal structure of mutant p110α bound to the full-length p85 regulatory subunit.

The non-alpha isoforms of class I PI3K

Although the four isoforms of class I PI3K have identical enzymatic activities, they have different, non-redundant cellular functions. Their patterns of expression are distinct, ubiquitous for the p110α and p110β isoforms and largely leukocyte-specific for p110γ and p110δ (Sawyer *et al.*, 2003; Vanhaesebroeck *et al.*, 1997b). Genetic inactivation of p110α and p110β in mice leads to early embryonic lethality (Bi *et al.*, 2002; Bi *et al.*, 1999); p110γ and p110δ knock-out mice are viable but show defective immune responses (Ali *et al.*, 2004; Clayton *et al.*, 2002; Hirsch *et al.*, 2000; Jou *et al.*, 2002; Laffargue *et al.*, 2002; Li *et al.*, 2000; Okkenhaug *et al.*, 2002; Rodriguez-Borlado *et al.*, 2003; Sasaki *et al.*, 2000). Conditional and tissue-specific mutations of the p110 isoforms and experiments with isoform-specific antibodies have generated a steadily increasing catalog of diverse isoform-specific activities (Ali *et al.*, 2008; Bony *et al.*, 2001; Foukas *et al.*, 2006; Graupera *et al.*, 2008; Hooshmand-Rad *et al.*, 2000; Ji *et al.*, 2007; Leverrier *et al.*, 2003; Suire *et al.*, 2006; Vanhaesebroeck *et al.*, 2005; Vanhaesebroeck *et al.*, 1999; Yip *et al.*, 2004). The general conclusions emerging from this work place p110γ and δ firmly in the realm of the immune system, assign p110α to cell growth and reveal an interesting connection between p110β and blood clotting (Ono *et al.*, 2007; van der Meijden *et al.*, 2008). Class I p110α has attracted much attention because of its involvement in cancer, documented by the frequent occurrence of gain-of-function, cancer-specific mutations. No such cancer-specific mutations have been identified in the non-alpha isoforms. Yet there is evidence that non-alpha isoforms of p110 are involved in the development and progression of malignancies. Consistent overexpression of p110δ is seen in acute myeloblastic leukemia (Sujobert *et al.*, 2005). Inhibitors of p110δ specifically interfere with the growth of the leukemic cells, suggesting that p110δ can function as an oncoprotein (Sadhu *et al.*, 2003). Elevated expression of p110γ is observed in chronic myeloid leukemia (Hickey and Cotter, 2006; Skorski *et al.*, 1997). Further data suggest a role of non-alpha isoforms in cancers of the bladder, brain and colon (Benistant *et al.*, 2000; Knobbe *et al.*, 2005; Mizoguchi *et al.*, 2004). Overexpression of non-alpha isoforms in cancer is significant in view of observations in cell culture. Unlike wild-type p110α which lacks oncogenic activity when expressed in primary fibroblasts, the wild-type non-alpha isoforms are oncogenic. This surprising activity of p110β, γ and δ was first documented in avian cells (Kang *et al.*, 2006), but has now been observed in rodent cells as well (Ueno and Vogt, 2007, unpublished). The absence of cancer-specific mutations in the non-alpha isoforms may therefore reflect an inherent oncogenic potential that can be activated by differential expression in the absence of mutation.

A study of cells transformed by p110 isoforms has revealed striking isoform-specific activities that group p110β and p110γ together, placing them apart from p110α and p110δ (Fig. 5) (Denley *et al.*, 2007). In PI3K-transformed cells, wild-type p110δ signals constitutively as does the H1047R mutant of p110α. Transformation by p110β and p110γ does not result in constitutive downstream signaling. This deficiency can be remedied by the addition of a myristylation signal to p110β and p110γ which also results in an enhancement of oncogenic activity (Fig. 4). Additional criteria also show similarities between p110β and p110γ: oncogenicity and signaling of these isoforms require interactions with Ras. The disabling mutation of the RBD K227E eliminates transforming and signaling activities of p110β and p110γ. In contrast, the activities of p110α H1047R and of wild-type p110δ are not affected by this mutation in the RBD. Again, myristylation of p110β and p110γ can substitute for Ras-binding, restoring oncogenic and signaling potential (Fig. 6A). This observation suggests that an essential function of Ras in PI3K signaling is the recruitment of p110β and p110γ to the cell membrane. This recruitment function of Ras may also explain the Ras-independence of p110δ. p110δ has a unique accumulation of basic residues in its C2 domain which probably mediate a direct interaction with the cell membrane. Mutating these basic residues results in an inactive protein. The dependence of p110β and p110γ on Ras is also seen in their sensitivity

to inhibitors of the MAP kinase pathway. The Raf inhibitor BAY43-9006 and the MEK1/2 inhibitor U0126 interfere with oncogenicity and signaling of p110β and p110γ (Fig. 6B).

Evolving small molecule inhibitors of p110

The distinctive properties of the p110 isoforms lead to the question of small molecule inhibitors and their specificity. The standard PI3K inhibitors for experimental work, Wortmannin and LY294002, are not isoform-specific. However, there are several inhibitors that show significant selectivity for one of the isoforms (Denley *et al.*, 2007) (Fig. 7) However, they are ATP-competitive inhibitors, and because ATP-binding pockets of different kinases are structurally similar, such inhibitors usually show activities against several kinases. Of the ones shown in the figure, PI 103 also inhibits TOR, and TGX221 is effective against class III PI3K. All four p110 isoforms are promising targets for small molecule inhibitors, each isoform is linked to a specific set of clinical indications. The identification and development of such inhibitors with drug-like properties and therapeutic potential has proceeded at a rapid pace in recent years (for example, see (Aftab *et al.*, 2008; Bruce *et al.*, 2007; Folkes *et al.*, 2007; Knight *et al.*, 2006; Knight and Shokat, 2007; Mutton and Pass, 2007; Quattropani *et al.*, 2007; Raynaud *et al.*, 2007)). The success of these efforts and the initiation of clinical trials will require a careful examination of side effects, important especially in long-term use. For p110α, the cancer-specific mutations offer a solution to this potential problem: the identification and development of mutant-specific inhibitors. These could interfere with the oncogenic versions of p110α and leave the important normal functions of wild-type p110α untouched. The genetic and biochemical data on the p110α mutants suggest that mutant-specificity could be attainable. A crystal structure of the mutant proteins would provide decisive guidance in this effort.

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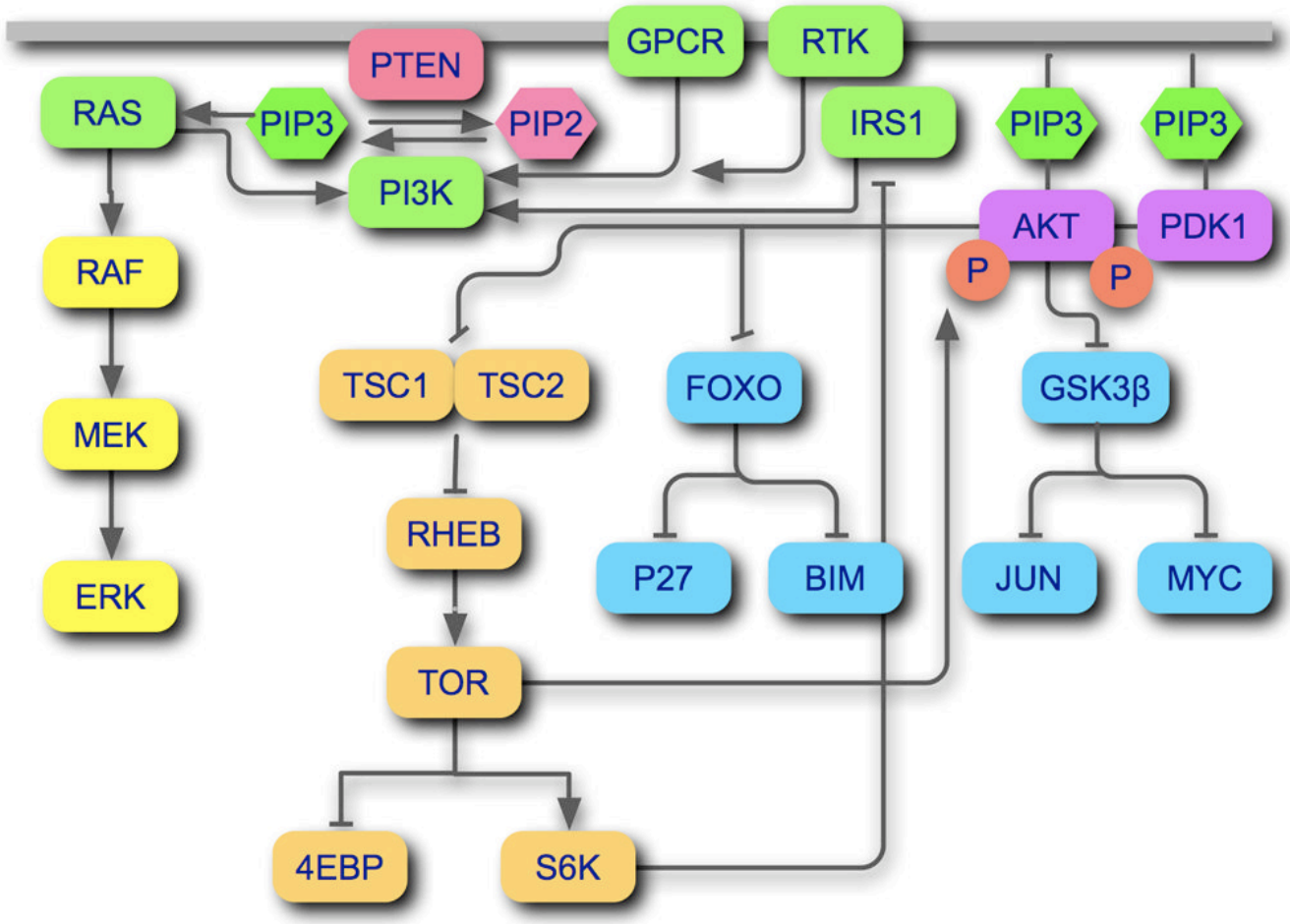

Figure 1.

The canonical PI3K signaling pathway. PI3Ks can be activated by RTKs (with or without adaptors such as IRS1) or GPCRs. Ras is an additional positive regulator of PI3K, probably by facilitating membrane localization. The phosphatase PTEN dephosphorylates the product of PI3K, PIP_3 at the 3-positiion and thus acts as the exact enzymatic antagonist of PI3K. PIP_3 initiates downstream signaling by recruiting the serine-threonine kinases AKT and PDK1. PDK1 phosphorylates and thereby activates Akt. Three major signaling branches originate from Akt. Akt-mediated phosphorylation of GSK3β and of FOXO directly and indirectly controls transcriptional activities and cellular growth and survival (blue icons). The signal proceeding through the TSC complex, RHEB, and TOR affects primarily protein synthesis (beige icons). A positive feed-back loop extends from the TOR-RICTOR complex to Akt, resulting in additional activating phosphorylation of Akt. A negative feed-back loop consists of the S6K-mediated phosphorylation of IRS1.

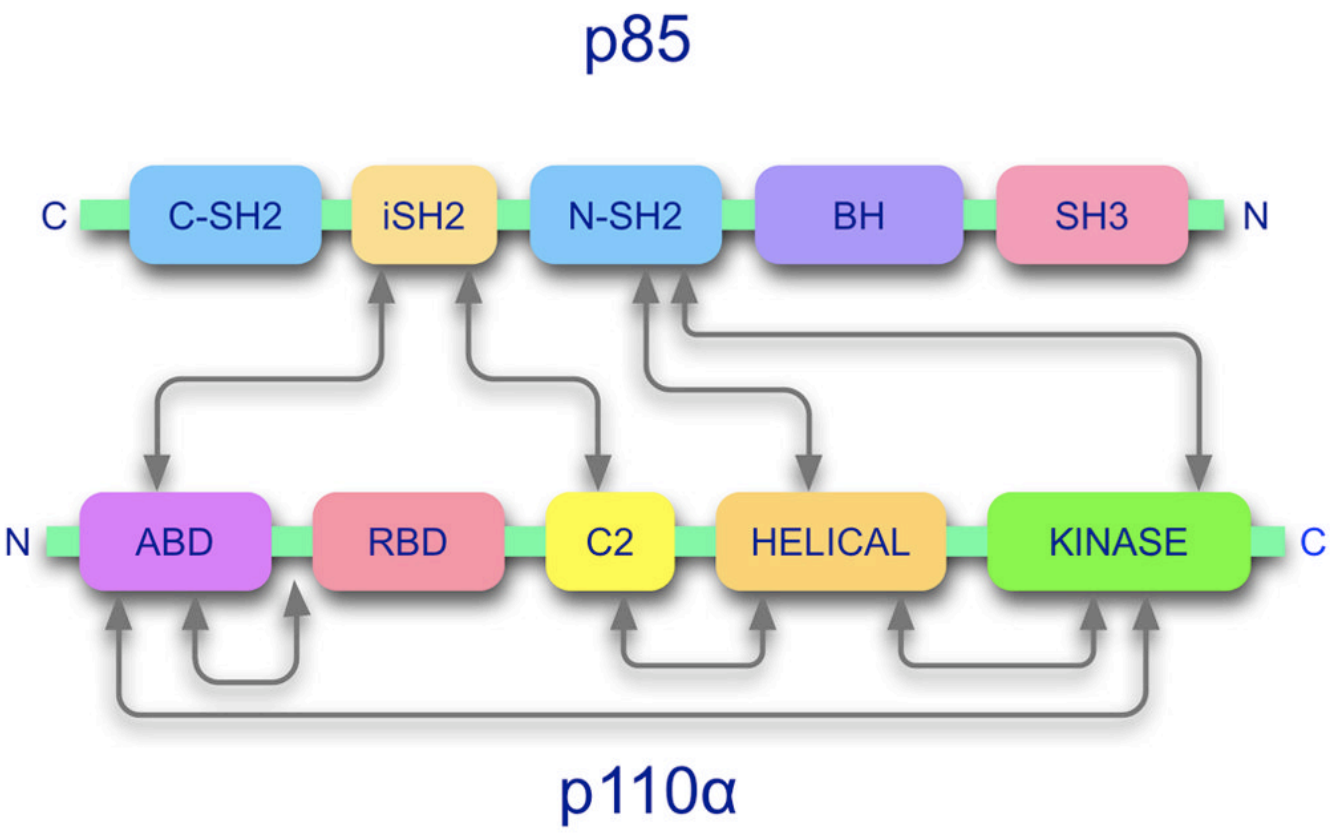

Figure 2.
PI3K is a dimeric enzyme. The figure shows the domain structure and domain interaction map of the standard regulatory subunit, p85 and the catalytic subunit, p110 (Huang *et al.*, 2007; Miled *et al.*, 2007; Pacold *et al.*, 2000; Shekar *et al.*, 2005; Walker *et al.*, 1999).

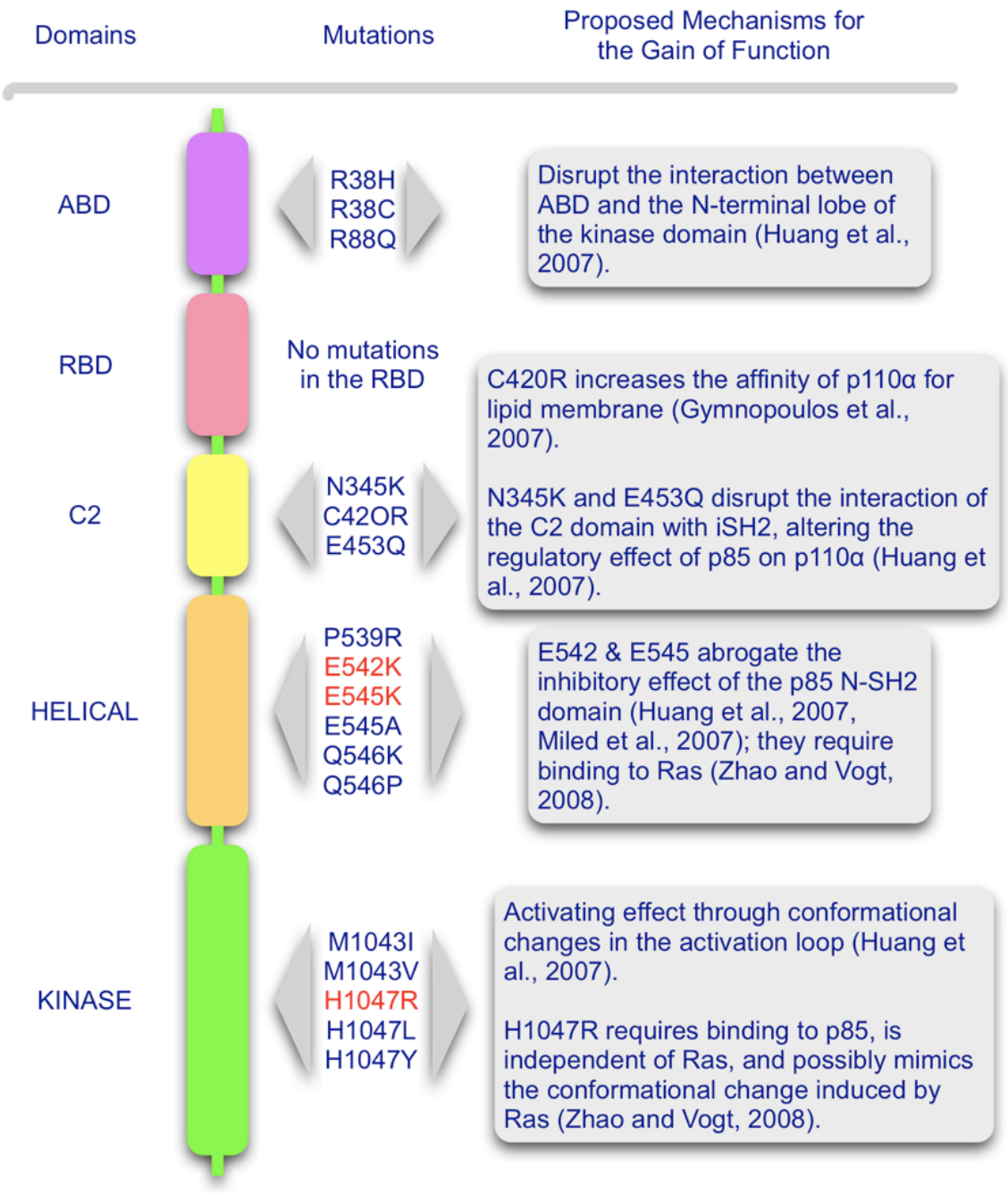

Figure 3.
A map of selected cancer-specific gain-of-function mutations in p110α. Suggested mechanisms for the gain of function are listed at the right. The three hot-spot mutations are in red.

Interaction disabled	Oncogenicity and constitutive signaling	
	Helical domain mutations E542K, E545K	Kinase domain mutation H1047R
p85	+	-
Ras	-	+

Figure 4.

The interactions with p85 and with Ras define two distinct molecular mechanisms for the gain of function seen in the hot spot mutations in p110α. The helical domain mutations are largely but not completely independent of binding to p85 but require the interaction with Ras. The kinase domain mutation completely depends on the interaction with p85 but is not affected by a loss of Ras-binding. However, the kinase domain mutation still shows residual signaling activity in the absence of p85-binding.

Indicators of signaling	Constitutive signaling myr-α α-H1047R wt-δ myr-β myr-γ myr-δ	No constitutive signaling wt-β wt-γ
pAkt-T308	+	-
pAkt-S473	+	-
pGSK3β-S9	+	-
Presence of FOXO	-	+
p4EBP-S65	+	-
pS6K-T389	+	-

Figure 5.
Cells transformed by the four isoforms of Class I p110 show distinct patterns of constitutive downstream signaling that group p110α–H1047R together with p110δ, as both constitutively activate Akt and downstream components of the pathway. In contrast, p110β–and p110γ–transformed cells do not show this constitutive activation of Akt, but this deficiency can be remedied by linking a myristylation signal to the N-terminus of p110.

	Sensitive	Not sensitive
Loss of Ras-binding	wt-β wt-γ	myr-β myr-γ α-H1047R wt-δ

Inhibitor	Sensitive	Not sensitive
MEK 1/2 U0126	wt-β wt-γ	α-H1047R wt-δ
RAF BAY 43-9006		

Figure 6.

(A) Loss of Ras-binding inactivates wild-type p110β and p110γ, but not p110α–H1047R and p110δ. A myristylation signal can substitute for Ras-binding in p110β and p110γ, suggesting that Ras functions as membrane anchor. (B) The dependence on Ras is also reflected by the sensitivity of p110β and p110γ to inhibitors of the MAP kinase pathway.

Inhibitor	Selectivity for	IC50 (μM) for oncogenic transformation in cell culture			
		α-H1047R	wt-β	wt-γ	wt-δ
PI 103	α	0.01	>1	>5	>1
TGX-221	β	>1	0.035	>0.5	0.3
AS 604850	γ	>10	9	1.2	>20
ICB 7114	δ	>10	8	>10	0.6

Figure 7.
Isoform-selective inhibitors of PI3K. The IC50 values were determined by measuring oncogenic activity in cell culture (Denley *et al.*, 2007).