Published in final edited form as:

Adv Enzyme Regul. 2011 ; 51(1): 273–279. doi:10.1016/j.advenzreg.2010.09.013.

Capitalizing on Tumor genotyping: Towards the design of mutation specific inhibitors of Phosphoinositide-3-Kinase

Sandra B. Gabelli^{*}, Krisna Duong-Ly^{*}, Evan T. Brower⁼, and L. Mario Amzel^{*}

^{*} Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

⁼ Ludwig Center for Cancer Genetics and Therapeutics, and The Howard Hughes Medical Institute at The Johns Hopkins Kimmel Cancer Center, Baltimore, Maryland, USA.

Abstract

Phosphatidylinositol-3-kinases (PI3K) PI3K α , is a lipid kinase frequently mutated in several cancer types. Under normal physiological conditions it initiates a number of signaling pathways by recruiting other kinases, such as Akt, to the plasma membrane. The mutations observed in tumors increase PI3K kinase activity, leading to increased cell survival, cell motility, cell metabolism, and cell cycle progression. The structure of the complex between the catalytic subunit of PI3K α , p110 α , and a portion of its regulatory subunit, p85 α reveals that many of the oncogenic mutations occur at the interfaces between p110 α domains and between p110 α and p85 α domains. At these positions, mutations may disrupt interactions that result in changes in the kinase domain that may increase enzymatic activity. Other mutations may dislodge the nSH2 domain from its inhibitory position in the complex. The structure also suggests that interaction with the membrane is mediated by a region of the heterodimer that includes one of the p85 α domains (iSH2). The structure of the most common oncogenic mutation, H1047R, shows differences in p110 α with the wild-type that indicate that this mutation may increase its interaction with the cellular membrane resulting in activation by increasing accessibility to the substrate. These findings may provide novel structural *loci* for the design of new isoform-specific, mutant-specific anti-cancer drugs.

Keywords

PIK3CA; PI3K; p110 α ; p85 α ; p110 α /p85 α ; lipid kinase; PIP3; somatic mutation; hot spot; drug design

Introduction

Phosphoinositide phosphates are membrane components that participate in the early steps of signaling cascades. Phosphoinositide-3,4,5-triphosphate (PIP3) is one of the most important members of this family (Fruman, Meyers et al. 1998). When produced in the cell membranes, it recruits proteins that contain a pleckstrin homology (PH) domain, such as AKT and PDK-1 that in turn initiate signaling pathways that control important cellular functions including cell proliferation, cell motility, metabolism, and survival.

© 2010 Elsevier Ltd. All rights reserved.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Phosphoinositide-3-kinases (PI3K) catalyze the phosphorylation of phosphoinositide-4,5-bisphosphate (PIP₂) to produce PIP₃ (Fruman, Meyers et al. 1998). As such, these enzymes are key players in the initiation of cellular responses to extracellular signals. Class IA PI3K enzymes are activated by binding phosphorylated tyrosine residues present in activated tyrosine kinase receptors or their phosphorylated substrates. Understanding the molecular mechanism of the 2- to 4-fold activation of PI3K α , a class I A PI3K, by phosphotyrosine motifs of activated receptor tyrosine kinases is a key step in the characterization of this important signaling activity (Carson, Van Aller et al. 2008).

Human PI3K α is composed of two subunits: the catalytic subunit p110 α , and a regulatory subunit, p85 α (Katso, Okkenhaug et al. 2001). After cell stimulation, PI3K α is recruited to the membrane and activated by binding, through an SH2 domain (nSH2) of p85, to phosphotyrosine motifs of activated membrane receptors or their phosphorylated substrates. In vitro, phosphorylated peptides carrying the cognate sequences (p-Y-pep) also activate the enzyme. The PIP₃ lipids produced by activated PI3Ks act as membrane docking sites for pleckstrin homology domain (PH)-containing proteins such as the AKT serine/threonine kinases (also known as protein kinase B, PKB) and the 3-phosphoinositide-dependent protein kinase-1 (PDK1) (Vanhaesebroeck and Alessi 2000). Through this activity, class I PI3Ks link cell-surface receptors including epidermal growth factor receptor, insulin-like growth factor receptor, and platelet growth factor receptor to signaling networks that control cell growth, proliferation, survival, apoptosis, differentiation, motility, migration, and adhesion (Katso, Okkenhaug et al. 2001; Cantley 2002; Vivanco and Sawyers 2002; Bader, Kang et al. 2005).

PI3K α is mutated in a large number of cancers (Samuels, Wang et al. 2004; Cancer and Network 2008; Parsons, Jones et al. 2008). Mutated enzymes show increased enzymatic activity similar to that resulting from physiological activation by phosphorylated tyrosine kinase receptors (Gymnopoulos, Elsliger et al. 2007; Zhao and Vogt 2008; Zhao and Vogt 2008).

Given the biological importance of the processes controlled by PI3Ks, it is imperative to determine the various mechanisms of PI3K activation and to understand how these modes of activation impact the kinetics, structure, and regulation of the enzyme.

The 3-D structures of the complex of p110 α with an important portion of its regulatory partner p85, the fragment nSH2, containing the N-terminal SH2 domain (nSH2) and the inter-SH2 domain (iSH2), determined in the last few years represent an important first step toward characterizing the activation of the enzyme at the molecular level (Huang, Mandelker et al. 2007; Amzel, Huang et al. 2008; Mandelker, Gabelli et al. 2009), (Fig 1). Inclusion of the nSH2 and iSH2 domains of p85 α in these structures was key for providing insight into PI3K activation. The iSH2 domain is necessary for stabilizing p110 α , and the nSH2 was shown to have an inhibitory effect on the activity of PI3K (Yu, Wjasow et al. 1998; Yu, Zhang et al. 1998). These structures suggest that binding the phosphotyrosine of the activated receptors interferes with the interaction of the nSH2 domain of p85 α the p110 α subunit and relieves the inhibitory effect of nSH2 (Mandelker, Gabelli et al. 2009). They showed, in addition, that most mutations occur at interfaces between p110 α domains or between p110 α and p85 α domains, in positions that have the potential to modify the conformation of residues in the catalytic site and/or release inhibition by the nSH2 of the regulatory domain in a manner similar to physiological activation by phosphorylated receptors. One mutation in p110 α (H1047R), however, increases PI3K activity by changing the interaction of the enzyme with the membrane (Gabelli, Mandelker et al. ; Mandelker, Gabelli et al. 2009).

Structure of the p100 α /niSH2 complex of PI3K α

The p110 α subunit of PI3K α is composed of five domains: an adaptor binding domain (ABD), a ras binding domain (RBD), a C2 domain, a helical domain, and a kinase domain. The kinase domain contains the catalytic site of the enzyme. The p85 α subunit also contains five domains: an SH3 domain, a GAP domain, an nSH2 domain, an iSH2 domain, and a cSH2 domain. The nSH2 and the cSH2 domains are both typical SH2 domains, while the iSH2 folds as a coiled-coil.

The p110/niSH2 structure shows that the complex is a compact assembly of p110 α and p85 α domains, (Fig. 1). The overall shape of the complex is triangular with the RBD at the tip and the iSH2 forming the elongated side at the bottom. The iSH2 interacts with several of the p110 α domains, in particular with the ABD and the C2 domains. The helical domain interacts with the ABD and RBD domains and even more extensively with the kinase domain. These interactions are without doubt important for the control of the activity of the enzyme.

Activation by phosphotyrosine peptide (pY-pep) binding

Although the structure of the complex of PI3K α with a cognate pY-pep was not determined, the structure of the nSH2 domain of the enzyme with a pY-pep from the platelet derived growth factor (PDGFR) has been known since 1996 (Nolte, Eck et al. 1996). Structural alignment of this structure (pdb id 2IU1) with that of the nSH2 in the structure of the p110 α /niSH2 complex (pdb id 3HHM) was used to characterize the effect of pY-pep binding on the activation of PI3K α (Figure 2)

The p85 α nSH2 domain interacts with three p110 α domains, C2, helical and kinase, (Fig. 1). A loop of the helical domain containing residues 541 to 546 fits in a groove of the nSH2 domain providing a strong interaction that holds the nSH2 in its inhibitory position (Mandelker, Gabelli et al. 2009). Aligning the structure of the isolated nSH2 with bound peptide to the same domain in the p110/niSH2 structure shows that the peptide binds to the nSH2 domain in exactly the same position occupied by residues 541-546 of the helical domain, (Fig. 2). Thus, pY-pep binding competes with the major interaction between the nSH2 domain and the p110 α helical domain, dislodging the nSH2 domain and thereby relieving the nSH2 inhibition resulting in activation of the enzyme. The loss of this interaction is not sufficient to dissociate p85 α from p110 α due to the strong interactions of the ABD and C2 with the iSH2 of p85.

Cancer Associated mutations

Mutations in the ABD, C2, helical, and kinase domains of p110 α are observed with high frequency in cancers. All mutations tested show enhanced enzymatic activity (Gymnopoulos, Elsliger et al. 2007; Zhao and Vogt 2008; Zhao and Vogt 2008). The structure of the p110 α /niSH2 and the H1047R mutant show the location of these cancer-associated mutations in the PI3K α structure (Huang, Mandelker et al. 2007; Mandelker, Gabelli et al. 2009), (Figures 3, 4). In tumors, Arg38 and Arg88 are frequently mutated in the ABD domain. The mutations most commonly present at these positions include Arg38Cys, Arg38His, and Arg88Gln (Figure 4). Before the structure of the p110 α /p85 α became available, these mutations were proposed to break the interaction of the ABD with the iSH2 domain of p85. However, the structure of the p110 α /niSH2, as well as the structure of the complex ABD/iSH2, showed that residues 38 and 88 of ABD are not at the ABD/iSH2 interface (Gabelli, Huang et al. ; Huang, Mandelker et al. 2007; Miled, Yan et al. 2007)}. The structure of the p110 α /niSH2 complex showed that these residues occur at an interface between the ABD and the kinase domain in which the two ABD residues are at H-bonding distance from residues in the

kinase domain: Arg38 is hydrogen bonded to Gln 738 and Asp743, and Arg88 is hydrogen bonded to Asp746. It was proposed that mutation of either arginine residue would disrupt the corresponding H-bond. Loss of any one of these H-bonds due to the mutations might change the conformation of the kinase domain in a way that affects the enzymatic activity. An equally likely possibility is that the weakening of the ABD/iSH2 interaction will change the orientation of the iSH2 so that the nSH2 will be dislodged, relieving inhibition.

In tumors, C2 residue Asn345 is frequently mutated to lysine. Before the publication of the p110 α /niSH2 structure, the main function of C2 was thought to be promotion of the interaction of PI3K with the membrane. Based on this assumption it was proposed that the mutation weakened the interaction of the enzyme with the membrane, lessening the access to its substrate PIP2. The structure of the p110 α /niSH2, however, showed that these mutations have a different effect. Asn354 is at H-bonding distance of two residues of the iSH2 domain of p85, Asn564 and Asp560; therefore its mutation affects the interaction of p110 α with iSH2. In agreement with this observation, a recent sequence of a glioblastoma cell line showed that residues Asp560 and Asn564 of p85 α are also highly mutated in tumors (TCGRN 2008). The effect of mutating these residues is equivalent to mutating Asn354 of the C2 domain. Glu453Gln, another common C2 mutation, is also present at the interface between C2 and iSH2 and most likely alters the interaction between the C2 domain of p110 α and the iSH2 domain of p85 α .

Two residues of the helical domain, Glu542 and Glu545, are frequently mutated in cancers. The most common substitution is to lysine, which reverses the charge of the original residues. These residues are within the loop (541-546) that was described above as a major participant in the interaction between the helical domain of p110 α and the nSH2 domain of p85 α (Mandelker, Gabelli et al. 2009), Figure 2. Previous biochemical information as well as the structures of the p110 α /niSH2 showed that Glu542 and Glu545 of p110 α interact with Lys379 and Arg340 of the nSH2 of p85 α . Mutation of either Glu 542 or Glu545 may disrupt these interactions and affect the activity of the enzyme. Mutations of either of these residues resulted in an increase in PI3K activity and rendered the enzyme insensitive to activation by the addition of a phosphorylated peptide (Carson, Van Aller et al. 2008). Furthermore, this data suggested that activation by mutations in this region of p110 α and activation by phosphorylated peptides proceeds through a similar mechanism.

One common mutation in cancers is the substitution of His1047 of the kinase domain of p110 α by arginine. The structure of the H1047R mutant of the p110 α /p85 α complex was determined alone and complex with wortmanin, a promiscuous kinase inhibitor (Mandelker, Gabelli et al. 2009). The structures show that this mutation dramatically changes the conformation of the C-terminal loop (residues 1046 to 1062). In the wild-type structure, amino acids past residue 1047 are not ordered. In the mutant, however, the same residues are folded in such a way that part of this loop is now able to interact with the membrane. Increased interaction with the membrane provides the mutant enzyme greater accessibility to the PIP2 substrate and therefore higher enzymatic activity. If this is the mechanism of activation by this mutation, it can be expected that the activity of the H1047R mutant can be further increased by binding pY-pep. Carson *et al.* have shown that this is indeed the case, further supporting this mechanism (Carson, Van Aller et al. 2008).

Summary

PI3Ks catalyze the phosphorylation of the inositol hydroxyls of phosphoinositide membrane components. The changes in phosphorylation of the inositides recruit proteins to the plasma membrane that initiate important signaling cascades.

PI3K α , one of the class IA PI3Ks, is highly mutated in cancers. All mutations analyzed result in an increase in enzymatic activity. The structures of this enzyme determined by X-ray diffraction, provide a framework for analyzing the possible structural effect of these mutations and their effect on the enzymatic activity. Many of the mutations occur at domain interfaces where they can affect domain interactions and relieve the inhibition of the wild-type enzyme by the nSH2 domain of p85. This mechanism is analogous to the mechanism of physiological activation by activated tyrosine-kinase receptors in which the phosphorylated tyrosine of the receptor (or their substrates) dislodges the nSH2 from its inhibitory position in the complex by competing with its binding to a loop in the helical domain. Other mutations in the kinase domain can directly affect the conformation of the catalytic site. One mutation, His1047Arg, uses a completely different mechanism: it changes the conformation of the C-terminal loop in such a way that it increases the interaction of the enzyme with the membrane, granting increased access to the phosphoinositide substrates.

Taking advantage of the reliance of some cancers on the increased activity of mutated PI3K α , will require the development of isoform-specific, mutant-specific inhibitors. The structural, biochemical and physiological data that are becoming available for PI3Ks are an important first step in this direction.

REFERENCES

- Amzel LM, Huang CH, et al. Structural comparisons of class I phosphoinositide 3-kinases. *Nat Rev Cancer*. 2008; 8(9):665–9. [PubMed: 1863356]
- Bader AG, Kang S, et al. Oncogenic PI3K deregulates transcription and translation. *Nat Rev Cancer*. 2005; 5(12):921–9. [PubMed: 16341083]
- Cancer and GAN Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature*. 2008; 455(7216):1061–8. [PubMed: 18772890]
- Cantley LC. The phosphoinositide 3-kinase pathway. *Science*. 2002; 296(5573):1655–7. [PubMed: 12040186]
- Carson JD, Van Aller G, et al. Effects of oncogenic p110 α subunit mutations on the lipid kinase activity of phosphoinositide 3-kinase. *Biochem J*. 2008; 409(2):519–24. [PubMed: 17877460]
- Fruman DA, Meyers RE, et al. Phosphoinositide kinases. *Annu Rev Biochem*. 1998; 67:481–507. [PubMed: 9759495]
- Gabelli SB, Huang CH, et al. Structural Effects of Oncogenic PI3K α Mutations. *Curr Top Microbiol Immunol*.
- Gabelli SB, Mandelker D, et al. Somatic mutations in PI3K α : structural basis for enzyme activation and drug design. *Biochim Biophys Acta*. 1804(3):533–40. [PubMed: 19962457]
- Gymnopoulos M, Elsliger MA, et al. Rare cancer-specific mutations in PIK3CA show gain of function. *Proc Natl Acad Sci U S A*. 2007; 104(13):5569–74. [PubMed: 17376864]
- Huang CH, Mandelker D, et al. The structure of a human p110 α /p85 α complex elucidates the effects of oncogenic PI3K α mutations. *Science*. 2007; 318(5857):1744–8. [PubMed: 18079394]
- Katso R, Okkenhaug K, et al. Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. *Annu Rev Cell Dev Biol*. 2001; 17:615–75. [PubMed: 11687500]
- Mandelker D, Gabelli S, et al. A frequent kinase domain mutation that changes the interaction between PI3K α and the membrane. *PNAS*. 2009 X(&).
- Mandelker D, Gabelli SB, et al. A frequent kinase domain mutation that changes the interaction between PI3K α and the membrane. *Proc Natl Acad Sci U S A*. 2009; 106(40):16996–7001. [PubMed: 19805105]
- Miled N, Yan Y, et al. Mechanism of two classes of cancer mutations in the phosphoinositide 3-kinase catalytic subunit. *Science*. 2007; 317(5835):239–42. [PubMed: 17626883]

- Nolte R, Eck M, et al. Crystal structure of the PI 3-kinase p85 amino-terminal SH2 domain and its phosphopeptide complexes. *Nat Struct Biol.* 1996; 3(4):364–374. [PubMed: 8599763]
- Parsons DW, Jones S, et al. An integrated genomic analysis of human glioblastoma multiforme. *Science.* 2008; 321(5897):1807–12. [PubMed: 18772396]
- Samuels Y, Wang Z, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science.* 2004; 304(5670):554. [PubMed: 15016963]
- TCGRN; CGARN. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature.* 2008; 455(7216):1061–8. [PubMed: 18772890]
- Vanhaesebroeck B, Alessi DR. The PI3K-PDK1 connection: more than just a road to PKB. *Biochem J.* 2000; 346(Pt 3):561–76. [PubMed: 10698680]
- Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. *Nat Rev Cancer.* 2002; 2(7):489–501. [PubMed: 12094235]
- Yu J, Wjasow C, et al. Regulation of the p85/p110alpha phosphatidylinositol 3'-kinase. Distinct roles for the n-terminal and c-terminal SH2 domains. *J Biol Chem.* 1998; 273(46):30199–203. [PubMed: 9804776]
- Yu J, Zhang Y, et al. Regulation of the p85/p110 phosphatidylinositol 3'-kinase: stabilization and inhibition of the p110alpha catalytic subunit by the p85 regulatory subunit. *Mol Cell Biol.* 1998; 18(3):1379–87. [PubMed: 9488453]
- Zhao L, Vogt PK. Class I PI3K in oncogenic cellular transformation. *Oncogene.* 2008; 27(41):5486–96. [PubMed: 18794883]
- Zhao L, Vogt PK. Helical domain and kinase domain mutations in p110alpha of phosphatidylinositol 3-kinase induce gain of function by different mechanisms. *Proc Natl Acad Sci U S A.* 2008; 105(7):2652–7. [PubMed: 18268322]

Figure 1.

Assembly of the heterodimer p110 α /niSH2 p85 α . The surface of the ABD, RBD, C2, and helical domains of p110 α are colored grey with the kinase domain as green ribbons. The nSH2 and iSH2 domains of p85 α are shown as orange ribbons. The arrow shows how the nSH2 domain can swing up to bind to phosphorylated receptors.

Figure 2. Recognition of phosphorylated peptides by the nSH2 domain. A. Surface of the nSH2 domain colored according to the electrostatic surface potential with the PDGF peptide as shown bound in PDB id 2IUI. B. Same surface with residues 539-545 of the helical domain as shown in the structure of 3HHM.

Figure 3.
Oncogenic mutations in the p85 α subunit. A. Some of the mutations along the iSH2 domain are shown in red. B. Mutation G376R of the nSH2 domain is at the interface with C2 domain. C. Scheme of the domains of p85.

Figure 4. Oncogenic mutations in p110 α subunit shown in the structure of the p110 α /nSH2. A. Some of the most common mutations shown as black dots are at the interfaces between domains. B. Scheme of the domains of p110 α with the mutations observed in the structure in black.