Structural Effects of Oncogenic PI3Kα Mutations

Sandra B. Gabelli, Chuan-Hsiang Huang, Diana Mandelker, Oleg Schmidt-Kittler, Bert Vogelstein, and L. Mario Amzel

Contents

Abstract Physiological activation of PI3Kα is brought about by the release of the inhibition by p85 when the nSH2 binds the phosphorylated tyrosine of activated receptors or their substrates. Oncogenic mutations of PI3Kα result in a constitutively activated enzyme that triggers downstream pathways that increase tumor aggressiveness and survival. Structural information suggests that some mutations also activate the enzyme by releasing p85 inhibition. Other mutations work by different mechanisms. For example, the most common mutation, His1047Arg, causes a conformational change that increases membrane association resulting in greater accessibility to the substrate, an integral membrane component. These effects are examples of the subtle structural changes that result in increased activity.

S.B. Gabelli (✉) and L.M. Amzel (✉)
Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
e-mail: gabelli@jhmi.edu, mamzel@jhmi.edu

D. Mandelker, O. Schmidt-Kittler, and B. Vogelstein
Ludwig Center for Cancer Genetics and Therapeutics and The Howard Hughes Medical Institute at the Johns Hopkins Kimmel Cancer Center, Baltimore, MD 21231, USA

C.-H. Huang
Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA
Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

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The structures of these and other mutants are providing the basis for the design of isozyme-specific, mutation-specific inhibitors for individualized cancer therapies.

1 Introduction

In 2004, Samuels et al. reported the results of the analysis of the sequences of all known lipid kinase genes in 35 colorectal cancers. *PIK3CA*, which encodes the p110α catalytic subunit of PI3Kα, was the only gene that showed somatic (i.e., tumor-specific) mutations (Samuels et al. 2004). Extension of this analysis to *PIK3CA* in 199 additional colorectal cancers revealed mutations in 32% of the tumors (74 out of 199 tumors). In contrast, in 76 premalignant colorectal tumors only two mutations were found; both these tumors were very advanced tubulovillous adenomas. These observations were interpreted as indicating that *PIK3CA* mutations generally arise late in tumorigenesis, just before or coincident with invasion. In the same study, mutations *PIK3CA* mutations were also identified in several other tumor types, including those of the breast, brain, stomach, and lung. Subsequent studies have identified similar mutations in these and many other tumor types (Parsons et al. 2008; T.C.G.A.R. 2008). Over 1,500 such mutations are now recorded in a cancer mutation database (Wellcome Trust Sanger Institute 2009).

The *PIK3CA* gene codes for p110α, the major catalytic subunit of PI3Kα. This subunit 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 (Cantley 2002; Vanhaesebroeck and Waterfield 1999; Huang et al. 2007; Amzel et al. 2008). The complete enzyme also contains one of several regulatory subunits of similar size and function. The regulatory subunits, such as p85α, also contain five domains: an SH3 domain, a GAP domain, an N-terminal SH2 (nSH2) domain, an inter-SH2 domain (iSH2), and a C-terminal SH2 domain (cSH2) (Escobedo et al. 1991; Otsu et al. 1991). The iSH2 domain is responsible, in part, for binding the catalytic domain, while the nSH2 and cSH2 domains mediate the interactions between PI3Kα and the tyrosine kinase receptors that activate it.

Of the five p110α domains, mutations were initially found in the ABD, C2, helical domain, and the kinase domain. No mutations were found in the Ras binding domain. A large percentage of mutations (∼75%) occurred in two clusters – one in the helical domain and the other in the kinase domain (Samuels et al. 2004). Mutations have since been identified in 140 of the 1068 residues of *PIK3CA*. While some of these mutations are rare, others are commonly observed. The residues with the highest number of observed mutations, so-called "hotspots" are His1047 in the kinase domain (470 occurrences) and Glu545, Glu542 and Gln546 (362, 193, and 51 reported occurrences, respectively).

Samuels et al. also showed that the H1047R mutation resulted in a small but significant increase in enzymatic activity with respect to the wild type (Samuels et al. 2004). Subsequently, it was determined that two additional high frequency mutations, Glu542Lys and Glu545Lys, also increased enzymatic activity (Carson et al. 2008).

The increase in activity produced by the mutations was similar in magnitude to the increase that is observed when PI3Kα is activated by its physiological effectors, including phosphorylated tyrosine kinase receptors and their substrates (Yu et al. 1998a). These data were interpreted as indicating that the observed mutations of p110α result in a constitutively activated enzyme, which in turn regulates cellular pathways that contribute to several aspects of tumor growth. These considerations made PI3Kα an ideal target for the development of chemotherapeutic agents.

PI3Kα is only one of several PI3K enzymes that are central to numerous aspects of metabolism and cell growth. Because of the potential toxicity associated with such pleiotropic enzymes, chemotherapeutic agents that target PI3Kα must be at least isoform-specific, preferentially inhibiting the PI3Kα isoform over the others. The ideal agent would of course be one that inhibited mutant PI3Kα but not normal (wild type or wt) forms of the enzyme. Structural information is crucial for this type of development.

The structure of a major portion of the complete PI3Kα, determined by X-ray diffraction, provided the first look at the overall subunit organization of the enzyme as well as the positions of mutations within the three-dimensional structure (Huang et al. 2007). This structure contains many of the portions of the enzyme most important for the regulation of enzymatic activity and for understanding the effects of common mutations: the complete p110α and two domains of the regulatory subunit p85, viz., nSH2 and iSH2. Of the four regions of p85, iSH2 is absolutely required for binding to p110 and nSH2 is the domain most directly involved in PI3K regulation (Yu et al. 1998b). The iSH2 domain is a long coiled-coil that protects the large subunit from degradation by cellular proteases (Fu et al. 2004). The nSH2 domain has an inhibitory effect on the kinase activity, but this inhibition is relieved when the domain binds to a phosphorylated tyrosine from an activated upstream protein (Yu et al. 1998a). In this regard, physiological activation of PI3Kα is, in reality, removal of the inhibition by nSH2.

In addition, the same portion of the oncogenic H1047R mutant of PI3Ka was determined as the free enzyme and in complex with the covalent inhibitor wortmannin (Mandelker et al. 2009).

2 Description of the Structure

The p110α/niSH2 complex (Huang et al. 2007) is a large sail-boat shaped molecule with a narrow cross section (~80 Å, Fig. 1) (Huang et al. 2007). The base is approximately 100 Å and its height 100 Å. The p110 portion forms the "sail" while the iSH2 of p85 constitutes the "hull" (Fig. 1a). Four of the p110 domains are arranged roughly along the top edge of p85, with the RBD at the top of the molecule. The base of the p110 subunit has the ABD at one end and the kinase domain at the other but permits a direct interaction between these two domains. The helical and C2 domain are closer to the center of the structure, at one side of the kinase domain along the narrow thickness of the subunit.

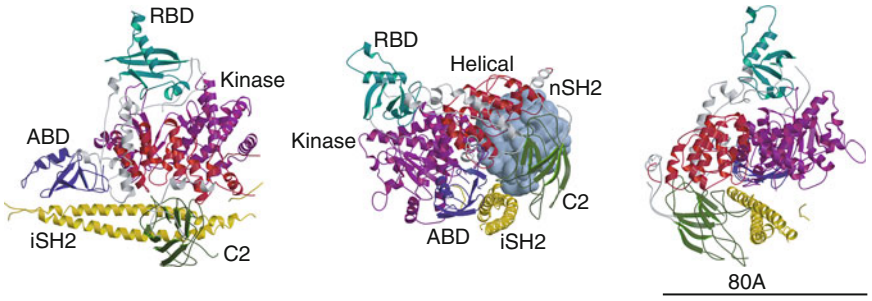

Fig. 1 Structure of the p110α/niSH2 heterodimer. (**a**) Ribbon diagram of the p110α/niSH2 heterodimer. For p110α, ABD is colored *navy blue*, RBD is *turquoise*, C2 is *green*, helical is *red* and kinase is *purple*. p85α iSH2 is *yellow*; all linkers are colored *gray*. (**b**) View of the p110α/niSH2 heterodimer highlighting the iSH2-ABD and iSH2-C2 contacts. The nSH2 domain is modeled in a *light blue* surface. In this orientation of the p110α/niSH2 heterodimer, the kinase, C2 and iSH2 domains are in contact with the membrane. (**c**) View of p110α/niSH2 at 90° from (**a**) that highlights its shape and dimensions

The ABD (residues 1–108) and the RBD (residues 191–291) domains interact with the kinase domain over a large surface area. They both fold with α/β topologies and are connected to each other by an 81-residue linker (109–190) that contains two helices.

A short helix and a long coil (residues 292–329) connects the RBD to the C2 domain (residues 330–480), which folds as a β-sandwich composed of 2 four-stranded antiparallel β-sheets. C2 interacts not only with the helical and kinase p110 domains but also with the iSH2 domain of p85: H-bonds between Asp560 and Asn564 of iSH2 to Asn345 of C2 form the major interaction sites. The topology of the C2 domain of the p110 domains in class I PI3K enzymes appears to be highly conserved despite the fact that this domain has lower sequence homology than the other four domains (27% identity between α and γ). The differences between the C2 domains of p110α and p110γ are concentrated in the loops connecting the β-strands and include insertions/deletions: the loop spanning residues 406–424, for example, is ten residues shorter in p110α than in p110γ (Fig. 2). Differences in conformation between the loops of the two isoforms may be a consequence of the interaction of the C2 domain of p110α with the iSH2 domain of the regulatory subunit p85 (p85 is not the regulatory subunit of p110γ). Importantly, the conformation that the loops adopt in p110γ makes it impossible for the iSH2 coiled-coil to fit in a position equivalent to that of the p110α/niSH2 complex.

The helical domain is connected through a linker (residues 481–524) to the C2 domain and through another linker (residues 525–696) to the kinase domain (Fig. 1). The kinase domain (residues 696–1068) folds as an α/β structure composed of two subdomains separated by a cleft that harbors the catalytic site of the enzyme, in an arrangement reminiscent of other kinases. This similarity allows assignment of the catalytic and the activation loops of p110α to residues 912–920 and 933–957. The structure of this domain is conserved among Class I PI3Ks (rmsd between

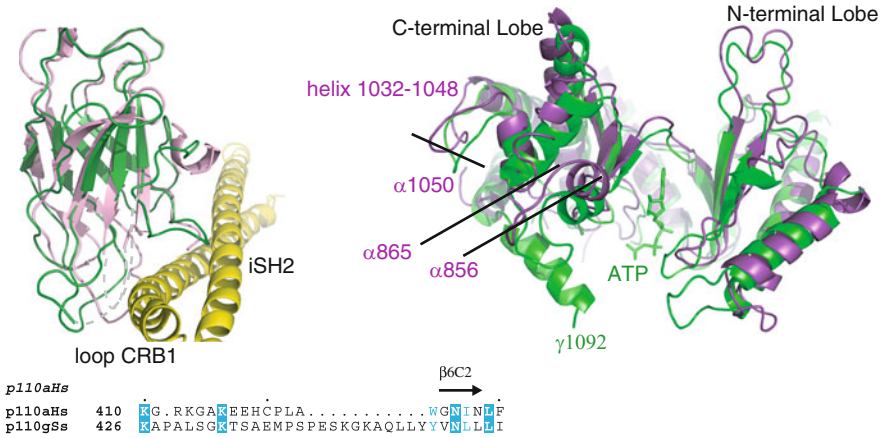

Fig. 2 Structural alignment and overlap of human p110α/niSH2 heterodimer (PDB id 2RD0) and wild boar p110γ (PDB id 1E8X) in relationship to the iSH2 domain of p110α. (**a**) Ribbon diagram of the p110α/niSH2 C2 (*green*) and p110γ C2 (*lavender*) domains showing the differences in loops and CRB2. (**b**) Structural overlap of the kinase domains of structure 2RD0 (*purple*) and 1E8X (*green*). Helices that show the largest differences as well as the ATP are shown. Observed C-terminal residues in both structures are also shown (1050 and 1092)

p110α and p110γ for 288 Cα atoms is 1.8 Å), especially for residues surrounding the binding pocket. The largest differences occur in the helices spanning residues 856–865, a region that lines the ATP binding site, as well as in residues 1032–1048 (rmsd 3.2 Å), a region that contains two positions that are mutated with high frequency in cancers (Fig. 2b).

The ATP binding site was identified by aligning the structure of the kinase domain of p110α with that of the same domain in the structure of the complex of p110γ with ATP. The high degree of similarity between the two structures in this region allowed an unambiguous localization of the ATP.

3 Association with the Lipid Membrane

Although PI3Ks are not integral membrane proteins, their substrate, PIP_2 is mainly found as a plasma membrane component. To gain access to their substrates, PI3Ks must be recruited to the plasma membrane. The C2 domain within the p110 sequence is thought to provide a locus for the association of PI3Ks with membranes. In PI3Kα, iSH2 binds between the C2 domain and the rest of the p110 in such a way that if C2 and the kinase domain interact with the membrane, iSH2 must also interact (Fig. 3). In this arrangement, iSH2 would provide a large contact surface lined with positively charged residues lysines 447, 448, 480, 530, 532, 551, and 561, and arginines 461, 465, 472, 480, 523, 534, 543, and 544 (Fig. 3c). Residues 723–729 and 863–867 of the kinase domain, which includes positively

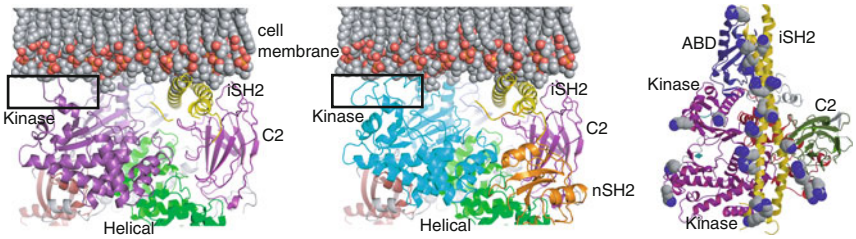

Fig. 3 Model of the association of the p110α/niSH2 heterodimer with the lipid membrane. (**a**) Model of the lipid membrane with a ribbon diagram of the p110α/niSH2 structure. A *black box* highlights the loops that move in the mutant structure. (**b**) Model of the lipid membrane with a ribbon diagram of the p110α H1047R/niSH2. A *black box* highlights the loops that change conformation (residues 864–874 and 1050–1062). (**c**) Face of the p110α/niSH2 heterodimer that interacts with the membrane; the iSH2 is shown as *yellow ribbons*. Positively charged residues, such as lysines and arginines, are shown in *black* as ball and stick representations

charged residues Lys723, Lys729, Lys863, and Lys867 complete this surface. Arg349, Lys410, Arg412, Lys413, and Lys416 of the C2 domain of p110 may also interact with the membrane.

4 Cancer-Specific Mutations

The cancer-associated mutations that have been identified in the ABD, C2, helical, and kinase domains of p110α were believed to act through unrelated mechanisms (Zhao and Vogt 2008a,b) but these hypotheses were difficult to interpret in the absence of structural information. The structure of the p110α/niSH2 suggests specific mechanisms through which these mutations increase kinase activity (Huang et al. 2007, 2008; Vogt et al. 2007).

As the ABD domain was known to interact with p85, ABD mutations Arg38Cys, Arg38His, and Arg88Gln were initially thought to disrupt the interaction between ABD and iSH2. However, the structure of the complex between ABD and iSH2 showed that these mutations are not located at the interface between the two domains (Miled et al. 2007). In the structure of the p110α/niSH2 heterodimer, Arg38 and Arg88 are located at a contact surface between the ABD and the kinase domains, at hydrogen bonding distance (<3.2 Å) of Gln738, Asp743, and Asp746 of the N-terminal lobe of the kinase domain (Fig. 4a, b). Thus, mutations of Arg38 and Arg88 are likely to disrupt these interactions, resulting in a conformational change of the kinase domain that alters enzymatic activity.

Before the determination of the structure of the p110α/niSH2 complex, the C2 domain was considered to be the main locus of interaction of PI3K with the membrane. Not surprisingly, mutations in the C2 domain were thought to change the affinity of p110α for the lipid membrane (Vogt et al. 2007). In the structure of the complex, however, Asn345, which is mutated to Lys in some cancers, is within

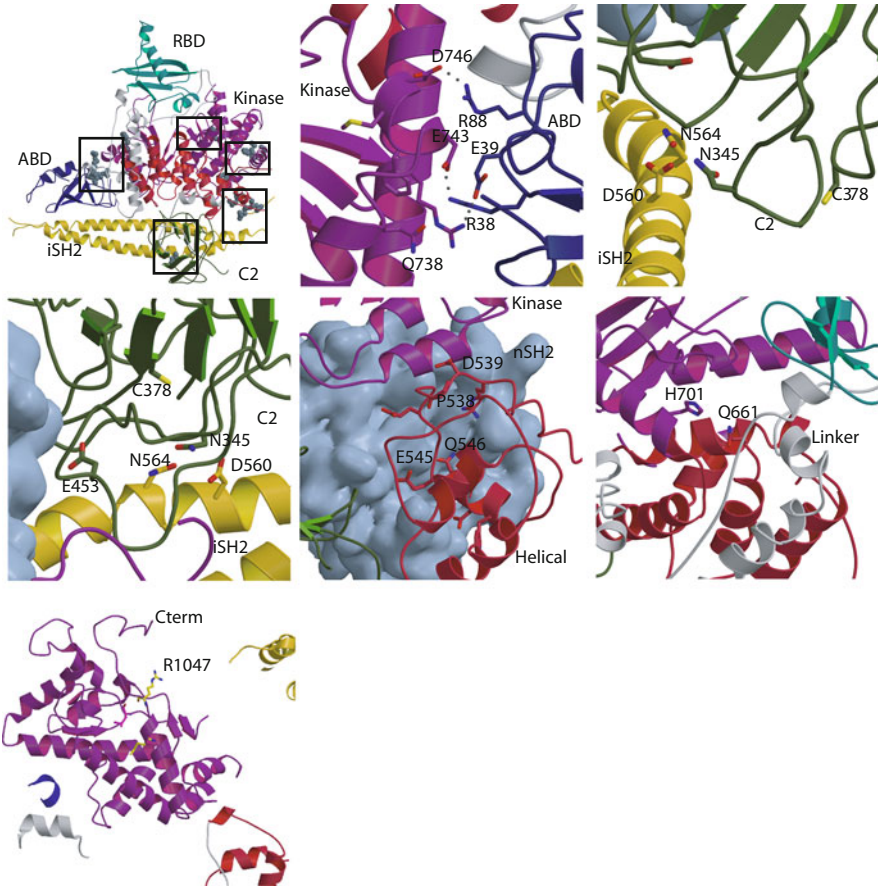

Fig. 4 Somatic mutations of p110α identified in human cancers localize to domain interfaces. (**a**) Location of representative mutations within p110α and niSH2. Amino acids mutated in cancers are shown as CPK models and framed with a *black box*. (**b**) ABD Arg38 and Arg88 mutations at the interface of the ABD and kinase domains. (**c**) C2 Asn345 mutation at the interface with iSH2. The C378R mutation is also shown. (**d**) C2 E453N mutation at the interface of C2 with iSH2 on one side and modeled nSH2 on the other side. (**e**) Mutations in the helical domain (Glu542, Glu545, and Gln 546) are located at the interface with nSH2 (*light blue surface*). (**f**) Helical Gln661 mutation is located across from kinase domain residue His701, which also is independently mutated. (**g**) Kinase Met1043 and His1047 located near the C-terminal end of the protein, shown in relationship to the helical domain (*red*), the iSH2 domain (*yellow*) as observed in the p110α H1047R/niSH2 structure

hydrogen bonding distance (2.8 Å and 3.0 Å) of Asn564 and Asp560 of iSH2, suggesting that mutation of Asn345 would disrupt the interaction of the C2 domain with iSH2 (Fig. 4a, c). This mutation may alter the regulatory effect of p85 on p110α rather than disrupt the interaction between p110α with the membrane. Another mutation identified in cancers, Glu453Gln, is also located at the interface between C2 and iSH2 (Fig. 4d). A recently identified mutation (Cys378Arg) most

likely increases the positive charge of the surface proposed to interact with the membrane, as it is contiguous with the surface of the iSH2 domain (Fig. 4c, d).

The two residues of the helical domain that are most frequently mutated in cancers are Glu542 and Glu545 (Fig. 4e). In the majority of cases, these two residues are mutated to Lys, causing a charge reversal. These residues as well as the less frequently mutated Gln546 are located on an exposed region of the helical domain (Fig. 4e). Biochemical studies suggested that they interact with Lys379 and Arg340 of the p85 nSH2 domain and that this interaction inhibited the activity of the catalytic subunit (Miled et al. 2007). Though nSH2 was included in the wild type p110α/niSH2 protein complex, it was not highly ordered in the crystal. However, in the crystal structure of the same construct carrying the His1047Arg mutation, the nSH2 domain was clearly visible (Mandelker et al. 2009). In this structure, the nSH2 is located close to the interface between the kinase and the helical domain, in a manner that allows it to interact with both domains as well with the C2 domain of p110α. Biochemical experiments showed that mutations at residues 542, 545, and 546 abrogate the inhibitory effect of nSH2 (Miled et al. 2007). The structure suggests a mechanism through which the mutations may have this effect: they could modify the interaction of nSH2 with the helical and the kinase domains in a manner similar to that achieved by binding of the phosphotyrosine residue of physiological activators to nSH2. Other examples of somatic mutations at the interface between domains are provided by those at His701 of the kinase domain and Gln661 at the helical domain (Fig. 4f).

His1047 in the kinase domain is another hot spot for somatic mutations in cancer and is associated with an unfavorable clinical prognosis breast cancers (Lai et al. 2008; Lerma et al. 2008; Kalinsky et al. 2009). It is interesting that His1047 is mutated to Arg in the majority of cases, yet arginine is normally present at the homologous position in human p110γ (Fig. 4g). In the structure of the p110α/niSH2 p85 complex of the wild type enzyme, His1047 is located within a helix of the C-terminal lobe of the kinase domain and is close to the C-terminal end of the activation loop, making a hydrogen bond with the main chain carbonyl of Leu956 within the activation loop. In the structure of the His1047Arg mutant with and without wortmannin (Mandelker et al. 2009) (Fig. 5), the orientation of the arginine side chain of residue 1047 is perpendicular to that of the histidine residue in the wild type. In this orientation, Arg1047 occupies a crevice in the kinase domain and points toward the membrane (Fig. 4g). Additional effects of this mutation include ordering of the C-terminal residues of p110α 1050–1062, which were disordered in the wild type (Fig. 3a). The new orientation of this loop places some of its residues directly on the surface that was proposed to interact with the membrane (Fig. 3b). The conformation of another loop (residues 864–874) that also interacts with the membrane assumes different conformations in the wild type and the mutant (Fig. 3a, b). Taken together, these changes suggest that the gain of function that results from the His1047Arg is a result of stronger interaction of the mutant with the membrane that, in turn, provides increased accessibility to the PIP_2 substrate. This mode of increasing enzymatic activity is compatible with the observation that binding to a cognate phosporylated peptide further increases the activity of this

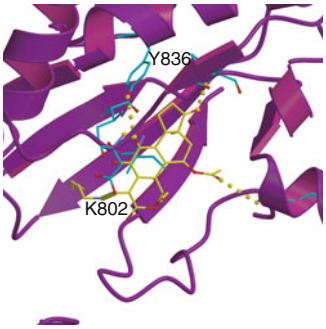

Fig. 5 Wortmannin bound to the p110α H1047R/niSH2. The kinase domain is shown as *purple ribbons* with the wortmaninn carbons in *yellow*. The covalent bond between K802 and wormannin is shown as a *thicker line* to distinguish it from other bonds. Residues at hydrogen bonding distance Q859, Y836, V851 are shown in turquoise

mutant (Carson et al. 2008). Another less frequently observed mutation, Met1043Ile, is located on the same helix and may exert its effect through changes in the activation loop (Fig. 4g).

In addition to mutations in p110α, mutations in p85α have been observed, particularly in brain tumors (Parsons et al. 2008; T.C.G.A.R. 2008). Residues Asn564 and Asp560 of iSH2, which make H-bonds with Asn345, were found to be mutated. These iSH2 mutations probably affect PI3K activity by the same mechanism proposed above for the Asn345 mutations, i.e., by disrupting the interaction between the iSH2 and C2 domains.

Most other mutations in p85α have involved truncations or deletions starting at or near residue 571 (Fig. 6). In particular, a truncation mutant known as p65, that lacks all amino acids C-terminal to residue 571 leads to constitutively activated PI3Kα activity (Jimenez et al. 1998). It is possible, that residues 581–593 constrain the location of the inhibitory nSH2 domain and that the deletion removes this constraint. Based on the crystal structure of p110/niSH2, however, an alternative possibility appears more likely: truncation at residue 571 might destabilize the iSH2 coiled-coil around residues 560 and 564 that make an important contact with Asn345 of the C2 domain (Fig. 6). Thus, the effect of this truncation may also be equivalent to that of the Asn345 mutation discussed above.

Another intriguing mutation identified in p85α is Gly376Arg. Residue 376 is within the nSH2 domain in a tightly packed volume at the interface between nSH2 and the C2 domain of p110α. It forms a close contact with Glu365 of C2 and substitution of the glycine at residue 376 with a bulky positively, charged arginine residue would disrupt this contact.

In sum, most of the p85α mutations described to date, whether they be subtle point mutations or large deletions, appear to disrupt the interaction between nSH2 and iSH2 and the C2 domain of p110α, thereby relieving inhibition of the kinase domain by nSH2. Others, such as His1047Arg may increase interaction with the plasma membrane.

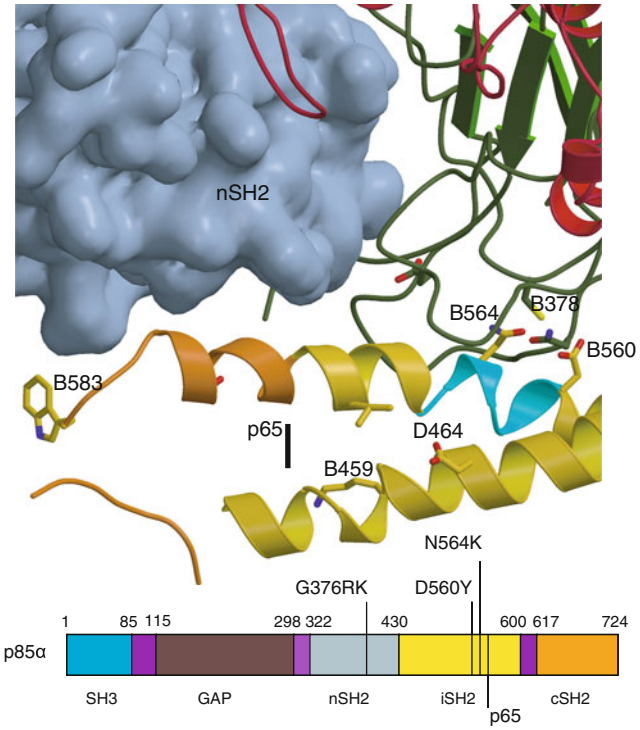

Fig. 6 Somatic mutations of p850α identified in human cancers. (**a**) Modeled structure of nSH2 domain shown as a surface in relation to C2, iSH2 and helical domains. Single mutations observed in the iSH2 are shown as stick and ball representations (Lys459, Asp464, Glu560, Asn564, Trp583); the indel mutation is shown in turquoise (DKRMNS560del) and p65 (deletion from 571) is shown in *orange*. (**b**) Location of mutations with respect to the indicated domains with p85α

5 Summary and Conclusions

PI3Kα is mutated in many cancers and biochemical analyses have shown that they often result in constitutively activated enzymes. Some mutants, such as His1047Arg, can be further activated by tyrosine phosphorylated peptides derived from their physiological effectors. The increased PI3Kα activity of the mutants activate downstream processes that control cell growth, survival, apoptosis, differentiation, motility, migration, and adhesion. Structural information on PI3Kα has provided an initial look at possible mechanisms through which the oncogenic mutations may result in enzyme activation. Most commonly, mutations occur at the interfaces between p110α domains or between p110α and p85 domains, disrupting the negative regulatory influences that results from the interfacial contacts. Other mutations, such as His1047Arg, may affect the activity of PI3Kα by changing the interaction of the protein with the membrane.

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