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Isoform-specific activities of the regulatory subunits of phosphatidylinositol 3-kinases – potentially novel therapeutic targets

Yoshihiro Ito, Jonathan R. Hart, and Peter K. Vogt

The Scripps Research Institute, Department of Molecular Medicine, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

Abstract

Introduction —The main regulatory subunits of Class IA phosphatidylinositol 3-kinase (PI3K), p85 α and p85 β , initiate diverse cellular activities independent of binding to the catalytic subunit p110. Several of these signaling processes directly or indirectly contribute to a regulation of PI3K and could become targets for therapeutic efforts.

Areas covered —This review will highlight two general areas of p85 activity: (1) direct interaction with regulatory proteins and with determinants of the cytoskeleton, and (2) a genetic analysis by deletion and domain switches identifying new functions for p85 domains.

Expert opinion —Isoform-specific activities of regulatory subunits have long been at the periphery of the PI3K field. Our understanding of these unique functions of the regulatory subunits is fragmentary and raises many important questions. At this time, there is insufficient information to translate this knowledge into the clinic, but some tempting targets have emerged that could move the field forward with the help of novel technologies in drug design and identification.

Keywords

Catalytic subunit; regulatory subunit; direct protein-protein interaction; protein truncation; domain switch; protein-protein interactions as therapeutic targets; fragment-based inhibitor screening

1. Introduction

Phosphatidylinositol 3-kinases (PI3Ks) are lipid kinases that regulate a diverse spectrum of cellular activities, including metabolism and aging, proliferation and oncogenicity, motility and invasiveness, vesicle traffic and immune response (1–5). They are grouped into three classes, differing in cellular location and specialized functions (6–9). Class I PI3Ks are mediators of signals from tyrosine kinases, GPCRs and small GTPases and function as regulators of cellular growth, proliferation and metabolism (1–3). They are heterodimeric enzymes composed of a catalytic and a regulatory subunit. Each of these occurs in several

Corresponding author: pkvogt@scripps.edu.

Declaration of interest

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isoforms, encoded by the genes *PIK3CA*, *PIK3CB*, *PIK3CG*, and *PIK3CD* (catalytic) and *PIK3R1*, *PIK3R2*, *PIK3R3*, *PIK3R4*, *PIK3R5* and *PIK3R6* (regulatory). This communication will be confined to isoforms of Class IA, concentrating on the full-length regulatory subunits p85 α and p85 β encoded by *PIK3R1* and *PIK3R2* (10). We will largely ignore the shorter p55 γ as well as the splice variants p50 α and p55 α for which isoform-specific functions remain largely unexplored. PI3Ks of Class IA are most directly linked to cancer. The domain structures of the Class IA isoforms are shown in Figure 1 (1–3, 11, 12). In the basal state of enzymatic activity, regulatory and catalytic subunits are tightly associated. The association stabilizes the enzyme and inhibits its activity. The nSH2 and cSH2 domains of the regulatory subunit make inhibitory contacts with the helical and kinase domain of the catalytic subunit. The iSH2 domain of the regulatory subunit also makes contact with the ABD, helical and C2 domains of the catalytic subunit. In the course of activation, typically by receptor tyrosine kinases (RTKs) or adapter proteins, newly generated phospho-tyrosine residues engage the SH2 domains of p85, resulting in release from inhibition of p110 and phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2) to generate phosphatidylinositol 3,4,5-trisphosphate (PIP3), an important second messenger molecule.

Isoform-specific activities of PI3K have been extensively investigated (2, 3, 13–25). Most of these studies concentrate on functions of the catalytic subunit. In this review, we will focus on isoform-specific activities of the Class IA regulatory subunits *per se*, that are activities conducted by p85 itself.

2. The interaction between PTEN and p85 α

The most important function of p85 α independent of p110 is arguably its interaction with PTEN. PTEN is a phosphatase with a preference for lipid substrates. By dephosphorylating PIP3 to generate PIP2, PTEN functions as the enzymatic and regulatory antagonist of PI3K and as a tumor suppressor (26–28). PTEN is frequently lost or mutated in cancer resulting in constitutive activation of the PI3K pathway. The stability of the PTEN protein is controlled by the E3 ligase WWP2; ubiquitination by WWP2 initiates the proteolytic degradation of PTEN (29). p85 α binds to the unphosphorylated, enzymatically active conformation of PTEN. This binding competes with access of WWP2 and thus stabilizes PTEN (30–33). A secondary effect of p85 α binding to PTEN is recruitment to the plasma membrane which could contribute to higher enzymatic activity by improving access to the substrate. However, binding of p85 α to PTEN leads to an increase in enzymatic activity independent of cellular localization (32, 34). Enhanced stability and activity of PTEN translate into attenuation of PI3K signaling. Only p85 α but not p85 β regulates PTEN in this fashion.

A genetic analysis has revealed details of the p85 α -PTEN interaction (34). Homodimerization of p85 α is a prerequisite for PTEN binding. This homodimerization involves the binding of the SH3 domain of one partner to the PR1 domain of the other in trans, and the dimer is further stabilized by interactions between the two BH domains (also referred to as RhoGAP domains). Mutations that disrupt the contacts between these domains interfere with dimerization and with binding to PTEN. Details of the p85 α dimer structure have also been revealed in a study using truncations and applying analytic

ultracentrifugation, fluorescence fluctuation spectroscopy and small-angle X-ray scattering (12). Important results from this work are the identification of a new binding contact of the p85 α dimer in the cSH2 domain and the conclusion that the structure of the p85 α dimer is highly flexible, allowing the existence of multiple conformational states. These can be expected to show distinct affinities for the interaction with different protein partners. The critical contacts that bind the p85 α dimer to the phosphatase domain of PTEN are located in the PR2 and BH domains. Mutations in BH or PR2 interfere with binding to PTEN, although they do not affect the ability to dimerize (34).

All engineered or patient-derived mutants of p85 α that reduce PTEN binding activate PI3K (33–35). They have enhanced oncogenic potential, and some of these mutants show overt cell-transforming activity in culture. The abilities of p85 α mutants to dimerize, to interact with PTEN, to compete with WWP2 binding and to stabilize PTEN are highly correlated. The experimental data on the PTEN-p85 α interaction have been integrated in a hypothetical structural model (34).

3. Interaction of p85 α and p85 β with XBP1 and BRD7

The second important activity of both p85 isoforms without involvement of p110 is the binding to critical members of the unfolded protein response, XBP1 and BRD7 (36–38). BRD7 is a bromodomain-containing protein, part of a multi-protein complex involved in chromatin remodeling and in the regulation of transcription and differentiation (39–41). XBP1 is a b-ZIP transcription factor that controls the response to ER stress (42). BRD7 binds to the iSH2 domain of p85 α with its evolutionarily conserved C-terminal region, and XBP1 depends on the BH domain of p85 for the binding interaction. There is little sequence similarity between the p85 α and p85 β BH domains, but they have similar structures (PDB 2XS6 and 6D81). Identification of critical residues in both p85 isoforms that mediate this BH-XBP1 domain interaction would strengthen this finding. In this context, p85 α and p85 β have been reported to form heterodimers that become dissociated by insulin signaling and then bind as monomers to XBP1 and BRD7. It should be noted that a direct interaction of p85 α and p85 β has not been demonstrated with the purified proteins, and the presence of other proteins in this heterodimer has not been ruled out. The complex of p85, BRD7 and XBP1 is imported to the nucleus using the BRD7 NLS. This nuclear sequestration depletes p85 α from the cytosol and probably deprives newly synthesized p110 of its essential stabilizer. Lower levels of p85 α can translate into reduced PI3K signaling.

4. Interaction of p85 α and SHP2

A third direct protein-protein interaction involving p85 α independent of p110 is the binding to the tyrosine phosphatase SHP2. This affinity is conserved from drosophila to humans (43). It is a direct binding with SHP2 recruiting p85 into a ternary complex that also includes the adapter protein GAB2. p85 α binds to SHP2 with its SH2 domains but not with the iSH2 domain as suggested by co-immunoprecipitation using the respective tagged p85 α domains. The ternary complex of SHP2-p85 α -GAB2 is inactive in PI3K signaling. It is disrupted by insulin signaling setting p85 α free to interact with p110, stabilizing p110 and initiating PI3K activity. The effects of the p85 α -SHP2 interaction on the activities of PI3K and of SHP2

have not been investigated in detail. A sequestration of p85 α by SHP2 could have similar consequence as that by BRD7. SHP2 is a critical component of the signal transduction pathway from RTKs to RAS, and mutant KRAS-driven tumors depend on SHP2 (44, 45). The consequences of the binding of p85 α to SHP2 for RAS signaling remain to be determined.

5. p85, the cytoskeleton and small GTPases

The BH domain of p85 interacts with small GTPases, CDC42, RAB5, RAB4, and Rac, functioning as GAP (GTPase-activating protein) (11, 46–48). This interaction does not compete with the binding to PTEN (34, 35). In response to PDGF, p85 α -bound Rac induces membrane ruffling (49). A second, coordinated cytoskeletal change, the disassembly of stress fibers and the reduction of focal adhesion complexes, requires p85 α but is not inhibited by inhibitors of PI3K signaling and is therefore also mediated by p85 α *per se*. These changes are correlated with enhanced cell migration (49). The GAP activity of p85 α for RAB5 affects endocytosis and is important for the control of PDGFR signaling, as RAB5 facilitates PDGFR degradation. Cellular expression of a p85 α mutant that has lost RabGAP activity induces oncogenic transformation (50). This process appears to depend on the MAPK pathway; the activity of PI3K in the transformed cells is not significantly elevated. However, the levels of phosphorylated AKT are increased, and the mechanism of this AKT activation has not been determined. The available data support the conclusion that the p85 α -RAB5 interaction is tumor-suppressive; precise molecular mechanisms remain to be explored. There is also still a lack of knowledge about *specialized* functions that can be linked to the interactions of p85 α with individual small GTPases. p85 β also affects the actin network, inducing the formation of “invadopodia”, which could facilitate tumor invasion (51). These observations show that there are p85-based signaling pathways that can control substantial portions of the signal-initiated and signal-independent cytoskeletal changes without assistance by p110. Extensive gene knock-out experiments have been conducted that result in the inactivation of p85 α , p85 β , p55 and p50 singly and in combination in vivo and in vitro (11, 52). The phenotypes of p85 knock-out mice and mouse fibroblasts are in accord with the studies on p85 and the cytoskeleton. However, these gene inactivation studies also raise questions about the exclusive role of the p85 α N-terminal domains in the control of the cytoskeleton as some cytoskeletal defects observed with p85 α knock-outs can be rescued by the *PIK3R1* splice variant p50 α (52).

An overview of important protein-protein interactions involving p85 α is given in Figure 2.

6. Other protein-protein interactions involving p85 β

Whereas many details of the significant protein-protein interactions involving p85 α are known, our knowledge of p85 β is much more fragmentary. One exception is the interaction with the XBP1-BRD7 complex (38). A second one is the binding to the F-box protein FBXL2 and the phosphatase PTLP1 which control cellular p85 β levels by mediating proteolytic degradation. This mechanism is specific for p85 β and is not observed with p85 α (53). Possibly related to this p85 β regulating pathway which results in inhibition of PI3K signaling is an interaction between PTEN and p85 β (54). PTEN has been reported to

dephosphorylate p85 β . Phosphorylation of p85 β at Y655 interferes with FBXL2 binding and stabilizes p85 β , and dephosphorylation would enhance degradation of p85 β . Additional p85 β activities were characterized in B- and T-cells where knockout of p85 β has an unexpected positive effect on signaling and cell proliferation (55–57).

An interaction that is peripheral to the topic of this review but is interesting and instructive takes place in influenza A virus infection between the NS1 protein and p85 β , resulting in an activation of the PI3K pathway (58). The binding site for NS1 in p85 β is the iSH2 domain, and this interaction appears to weaken the inhibitory contacts between p110 and the nSH2 domain of p85 β . It has been proposed that the complex also includes p110 (59, 60).

7. Oncogenic activity of mutant and wild type p85 isoforms

The two isoforms, p85 α and p85 β , differ in their oncogenic potential and potency. Expression of wild type p85 α does not induce oncogenic cellular changes. This ability is restricted to mutated and truncated p85 α , and such mutations have been identified in numerous human cancers (33, 61–63). Many of these mutations are clustered in the nSH2 and iSH2 domains and weaken or eliminate p110-p85 α contacts, reducing the inhibitory activity of p85 α on p110 (33, 34). Studies with isoform-specific small molecule inhibitors of PI3K suggest that these oncogenic p85 α mutants depend exclusively on p110 α but not on p110 β for their effectiveness (61).

There is also an interesting group of p85 α mutations mapping to the N-terminal portion of p85 α . Since the N-terminal region of p85 α can interact with several protein partners (64), genetic lesions in these domains could have pleiotropic effects. However, some of these patient-derived mutations diminish the binding and interaction with PTEN or with RAB5 (33), and these reductions in p85 α -mediated inhibitory pathways could activate PI3K.

In contrast to p85 α , expression of wild type p85 β can induce oncogenic changes in cell culture, enhancing cell growth and stimulating PI3K signaling (65). These changes were observed in cultures of chicken embryo fibroblasts and could be dismissed as a unique response of avian cells to p85 β . But wild type p85 α fails to elicit such a response in avian cells, and the oncogenic activity of p85 β has also been observed in Ba/F3 murine cells (33, 34). These oncogenic activities of p85 β depend on interaction with p110 and on the activity of the TOR kinase pathway. But in distinction to the oncogenic mutants of p85 α , wild type p85 β can use both p110 α and p110 β to produce its oncogenic effect. The oncogenic activity of p85 β observed in cell culture is in accord with activity in vivo that shows enhancement of tumor progression by p85 β (66). The oncogenicity of wild type p85 β could reflect the failure of this isoform to stabilize and activate PTEN. In contrast, overexpressed p85 α could be tied up in the association with PTEN or BRD7-XBP1 which would diminish PI3K activity. However, much of the enhanced activity of p85 β can be transferred to p85 α by substituting the p85 α cSH2 domain with that of p85 β (see Section 9). Therefore, the inability to bind PTEN cannot be the only reason for the high activity of p85 β .

8. N-terminal deletions of p85 α and p85 β

Truncations of the N-terminal SH3 and of the combined SH3-BH domains lead to notable changes in p85 protein activity. For p85 α , deletion of the SH3 domain, not including PR1, has a strong activating effect that is seen in cellular transformation and in PI3K signaling. For p85 β , the functional consequences of the SH3 truncation are not significant (67).

The activating effect of the SH3 deletion on p85 α could be connected to the interaction with PTEN. According to the current model, homodimerization of p85 α is a prerequisite for PTEN binding (34). When p85 α is present in the cell in excess over p110, it undergoes spontaneous homodimerization (12, 33, 68). An essential contact in the dimer assembly is located in the SH3 domain. A deletion of this domain would abrogate dimerization, PTEN binding and PTEN stabilization and activation. This could lead to increased PI3K activity. In contrast, p85 β does not interact with PTEN. This is a hypothetical cause for its inherently enhanced oncogenic and signaling activity. However, an alternative explanation that is supported by experimental evidence assigns the determinant for elevated activity to the cSH2 domain of p85 β (see Section 9). A deletion of the SH3 domain in p85 β does not affect its elevated activity.

Deletion of the combined SH3 and BH domains leads in p85 α to loss of the elevated activity seen with the SH3 truncation, and in p85 β eliminates the enhanced oncogenic and signaling activities characteristic of the wild type protein (67). These truncations in essence create proteins that closely resemble the splice variants p50 α and p55 α and the p55 γ isoform respectively of the regulatory subunits encoded by *PIK3R1* and *PIK3R3*. These constructs include the PR2 domain and the nSH2-iSH2-cSH2 domains. The combined SH3-BH domain deletions in p85 α and p85 β therefore generate proteins whose activities are reduced to a basal set of functions of p85: binding to p110 with the ensuing stabilization, inhibition and RTK-triggered activation (32). The molecular mechanisms by which the SH3-RhoGAP deletions affect p85 α and p85 β probably involve the BH domains and would be different in the two isoforms, because these domains show only minimal homology between p85 α and p85 β .

9. Observations on C-terminal deletions and on domain exchanges of p85 further document functional differences between p85 α and p85 β .

The effects of C-terminal deletions on PI3K signaling and on oncogenic transformation in cell culture have been described recently (67). In p85 α , deletion of the cSH2 domain induces significant activation of signaling and of oncogenicity. This activating effect is virtually abolished if the truncation includes the iSH2 domain or the iSH2 and nSH2 domains. In p85 β , deletion of the cSH2 domain greatly diminishes oncogenic transformation and partially reduces signaling. Truncation of the iSH2/cSH2 or of the nSH2/iSH2/cSH2 segments abolishes the elevated signaling that is characteristic of p85 β and also greatly reduces oncogenic activity.

Exchange of the cSH2 domains between p85 α and p85 β strengthens the results obtained with the cSH2 deletion. p85 α with the cSH2 domain of p85 β is strongly activated, and p85 β

with the cSH2 domain of p85 α loses the inherently elevated activity seen with the wild type protein. The exchange of the iSH2 domains has a more moderate effect that goes in the same direction as the exchange of cSH2 domains: activating in the p85 α /iSH2 β combination and inactivating in the p85 β /iSH2 α combination. The iSH2 domains of both isoforms expressed as protein fragments have an activating effect on oncogenicity and on signaling. These different activities of truncations and domain substitutions are not correlated with protein stability (67).

The observations on cSH2-deleted p85 β suggest that the cSH2 domain is required for the elevated activity of p85 β . This activating function of the p85 β cSH2 is in apparent contradiction to a set of solid data that show the p85 β cSH2-mediating inhibition of PI3K enzyme activity (69, 70). However, these results could be reconciled by hypothesizing that the cSH2 domain of p85 β can also interact with a yet to be identified regulatory protein that affects p85 β activity.

The analysis of p85 α and p85 β activities by truncation and domain exchange is summarized in Figure 3.

10. Conclusion

A comprehensive review publication on p85 activities lists more than twenty proteins that can bind to an isoform of p85 (64). Many of these studies use co-immunoprecipitation as the main tool, and direct binding would have to be confirmed with purified proteins. The interactions of p85 α with PTEN, BRD7, SHP2 and small GTPases involve such direct binding, and genetic analyses have identified some critical contact residues in the partner proteins.

The genetic analysis of p85 α and p85 β reveals additional features that distinguish the two isoforms. These features, like the binding to PTEN, BRD7, SHP2 and CDC42, are initiated by p85 *per se*, without a primary involvement of p110, or, in the case of PTEN, even require the exclusion of p110 from the interacting complex. However, the signaling chains initiated by these activities can eventually involve p110 and affect its function (64).

11. Expert opinion

The importance of PI3K in cancer has led to intense efforts in industry and in academic institutions aimed at identifying therapeutically effective inhibitors of this pivotal signaling activity. Numerous small molecule inhibitors have been disclosed and characterized, and about twenty are currently being evaluated in clinical trials. Yet, no breakthrough drug has been discovered (71, 72). The main reason for this slow progress is embedded in the nature of the PI3K signaling pathway and its essentialness in cell viability (73). However, the strategy for therapeutic control of PI3K has been very narrow. With the exception of rapamycin and its derivatives, it is based on ATP-competitive compounds. Most of these produce significant adverse reactions, some of which may be off-target effects. It is therefore time to consider alternative strategies that may be worthy of additional exploration.

The diverse activities of the PI3K regulatory isoforms p85 α and p85 β could offer opportunities for such alternative strategies, because some of the p110-independent interactions of p85 α affect PI3K activity. We can examine whether our present knowledge of these activities justifies an expectation of such an alternative strategy.

All p110-independent activities of p85 α involve protein-protein interactions and together with the interaction with p110 they will affect each other as they occur in the same cell. Collectively, these interactions form a network (Fig. 2). The key properties of this network are virtually unknown. These include the relative affinities of the individual protein-protein interactions and the distribution of p85 subunits among the various complexes. How is the expression of p85 regulated? Is there a limited abundance of p85 subunits? An additional factor is cellular distribution which commonly changes as the result of interacting with partner proteins. Understanding the complexity of this network requires measuring the interactions in situ. Available techniques for this task rely on read-outs of downstream effects (30–33, 37, 38, 43, 46, 47). Because of these limitations, the p85 network as a whole remains a mainly speculative entity.

Turning to individual p85 interactions, we also deal with a level of understanding that is still incipient and evolving.

Several aspects of the interaction between p85 α and BRD7–XBP1 remain unexplored. What is the function of p85 α in the BRD7–XBP1 complex? Is p85 α merely a structural component or does it have specific nuclear functions? The sequestration of p85 α in the nucleus depletes cytoplasmic supplies of p85 α that are needed for the stabilization of p110 and causes a downregulation of PI3K activity. But reduced cytoplasmic p85 α levels could also affect the interaction with PTEN which would increase PI3K activity.

The prime question in the interaction between p85 α and SHP2 pertains to the effect on the RAS signaling pathway. This question has taken on special importance because of the dependence of RAS-mutated tumors on SHP2 (44, 45). An allosteric small molecule inhibitor for SHP2 is effective in mutant RAS-driven preclinical models. The binding of p85 α to SHP2 also raises the same issues about sequestration as the binding to BRD7: what is the relative effect on p110 stabilization versus PTEN stabilization?

The p85 function that has been investigated in greatest detail and that is arguably the most consequential is binding, stabilizing and activating PTEN. It represents an axis of regulation that should be considered an integral part of the PI3K pathway. Key findings on the PTEN interaction have emerged from an analysis of cancer-associated mutations in p85 α (33–35) and have been used to build a cogent structural model of the PTEN-p85 α interaction (34). Additionally, extensive new analyses also reveal that the occurrence of cancer-specific mutations in p85 α is strongly correlated with the occurrence of loss-of-function mutations in PTEN, suggesting that these two genetic changes induce complementary or additive oncogenic traits. This co-occurrence is highly significant (p value of 10^{-23}) (74).

Our knowledge of the p85 α -PTEN axis also sheds light on the observation that p85 α can function as a tumor suppressor (75–77). Reduced expression of p85 α has been observed in several types of cancer. In such cells, there is an increase of active p110-p85 α dimers and

augmented PI3K signaling. The reduction in p85 α levels would also affect the interaction with PTEN and lower the p85 α -induced stabilization and activation of PTEN leading to enhanced PI3K activity. This suggestion raises again the question of balance between the p110-p85 α and the PTEN-p85 α interactions and underlines the importance of determining the relative affinities of these two complexes. It also brings up the issue of seemingly contradictory results that probably reflect cellular context. To cite an example, in breast cancer, p85 α can function as tumor suppressor, and low levels of p85 α are connected to elevated PI3K activity (75). In contrast, the depletion of cytoplasmic p85 α by BRD7 reduces PI3K activity in several cultured cell lines (37).

This review has excluded the p85 α splice variants p55 and p50, because little is known about their p110-independent activities. But they are members of the p85 α network, and their abundance can affect the activities of p85 α (Fig. 2). They can bind to p110 and induce stabilization and activation, but they cannot interact with the proteins that bind to the N-terminal region of p85 α , including PTEN, XBP1 and small GTPases. The short forms of p85 could have significant importance in oncogenesis. A telling and instructive reminder of this fact has emerged from a recent analysis of human metastatic cancers: tumors with wild type p85 α often show amplification of that portion of the gene that encodes the C-terminal half of the molecule, equivalent to the p50 or p55 splice variants of p85 (74). These amplifications probably contribute to the oncogenic cellular phenotype. They could function similar to mutations that reduce PTEN binding and induce elevated PI3K activity. Conversely, the amplifications suggest an alternative explanation for some of the cancer-associated mutations in the N-terminal half of p85 α . These could induce a shift of the active transcriptional start site of p85 α to that of p55 or p50. The oncogenicity of these mutants could then be due to this shift of transcriptional start site rather than a reduced interaction with PTEN.

Despite the intriguing information on p110-independent activities of p85, it is clear that at this time the knowledge base is insufficient to allow realistic plans of drug development but invites speculation. In the sphere of the p85 α network, the utmost concern would be that we are dealing with protein-protein interactions. But protein-protein interactions are no longer “forbidden territory” for intervention with small molecules. The clinical success of Venetoclax, an inhibitor of BCL2 (78–80), has validated a strategy for regulating protein-protein interactions following fragment-based screening for the identification of useful small molecules. In the p85 α network, the target that presently appears most promising is the p85 α homodimer in the conformation that binds to PTEN. Although the standard goal of controlling protein-protein interactions is to identify disruptors, a ligand could, in principle, also act as a stabilizer. In case of a homodimer, making a ligand of the monomer bivalent is a chemically simple task. Additionally, a monovalent ligand could be effective by locking the protein into a conformation that favors and stabilizes dimerization. The flexibility of the p85 α homodimer structure could be an important factor in this process (12).

For a well-grounded attempt to control protein-protein interactions, detailed knowledge of the interacting surfaces is mandatory. In case of the p85 α homodimer, we know domains and residues critical for dimerization and have a model of the contact surfaces including PTEN (34). A possible answer to the need to identify molecular pockets and grooves could be

provided by a cryo-EM study. The PTEN-p85 α complex is within the resolving power of current cryo-EM technology, and high-resolution cryo-EM information could become an enabling factor for successful drug development.

The p85 interaction network opens a new chapter for PI3K research. Identifying small molecule drugs that can control critical aspects of these protein-protein interactions requires better mechanistic understanding and significantly more structural information. Basic research and translational applications in this area remain urgent and challenging tasks with high risks and potentially high rewards.

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Article highlights

- This review draws attention to isoform-specific activities of the regulatory PI3K subunits, p85 α and p85 β . These proteins can enter into important protein-protein interactions that are initiated without binding to p110 and could function as ultimate regulators of PI3K activity.
- The interactions of p85 α with PTEN, BRD7 and SHP2 belong into this category that has the potential of forming a PI3K regulatory network.
- The remarkable ability of p85 β to induce oncogenic transformation in cell culture is also reflected by its tumor-enhancing effects on the cytoskeleton.
- Isoform-specific features are further revealed by dissecting p85 α and p85 β with N- and C-terminal truncations. Domain exchanges support the results of the deletion analysis.
- The most promising targets for therapeutic intervention among the p85 activities are protein-protein interactions. Protein-protein interactions have for a long time been a formidable challenge to drug development, but recent successes and new methods have made the control of protein-protein interactions a realistic possibility.

Class I A: p110 α , p110 β , p110 δ

Figure 1.

Domain structure of the Class IA PI3K catalytic (top) and regulatory (bottom) subunits. p110: p85-binding domain (also referred to as ABD), RAS-binding domain, C2 domain, helical domain, kinase domain. p85: SH3 domain, PR1 (proline-rich) domain 1, BH (BcR homology) domain (also referred to as RhoGAP domain), PR2 (proline-rich) domain 2, nSH2, N-terminal SH2 domain, iSH2, inter-SH2 domain, cSH2, C-terminal SH2 domain.

Figure 2.

Snapshot of protein-protein interactions involving p85α (the “p85α network”).

The binding to PTEN and to BRD7-XBP1 has a negative effect on PI3K activity (red color), the first by stabilizing PTEN, the second via p85α depletion (32, 34, 36–38). The possible consequences of such a depletion on the p85α-PTEN interaction are not known. It is noteworthy that both p85α and p85β can bind to BRD7-XBP1. The interaction between p85α and SHP2 could impinge on the RTK-RAS pathway as well as on PI3K; molecular mechanisms remain to be determined (43). The binding and GAP activity of p85α on small GTPases RAC, CDC42, and RAB5 affects the cytoskeleton and endocytosis (46, 47, 49, 52, 64). Failure of p85α to bind to RAB5 induces oncogenic cellular transformation (50). The p85α splice variants p55 and p50 are discussed only briefly in this review. For the sake of simplicity, the figure ignores the distinction between p55α and p55γ.

Cell-transforming activity¹ **Figure 3.**

A condensed summary of the deletion and domain exchange analysis of p85α (blue) and p85β (green) (65).

¹These represent estimates summarizing the results of multiple observations. Signaling activities as measured by the phosphorylation of AKT at S473 or of ribosomal protein S6 are in accord with the cell-transforming activities.