Mini review
The orchestrated signaling by PI3Kα and PTEN at the membrane interface

Danai Maria Kotzampasai,a,b,1, Kyriaki Premeti*c,1, Alexandra Papafotikad,e,1, Vasiliki Syropoulouc, Savvas Christoforidisd,e,2, Zoe Courniaa, George Leondaritisc,f,2

A B S T R A C T
The oncogene PI3Kα and the tumor suppressor PTEN represent two antagonistic enzymatic activities that regulate the interconversion of the phosphoinositide lipids PI(4,5)P2 and PI(3,4,5)P3 in membranes. As such, they are defining components of phosphoinositide-based cellular signaling and membrane trafficking pathways that regulate cell survival, growth, and proliferation, and are often deregulated in cancer. In this review, we highlight aspects of PI3Kα and PTEN interplay at the intersection of signaling and membrane trafficking. We also discuss the mechanisms of PI3Kα- and PTEN- membrane interaction and catalytic activation, which are fundamental for our understanding of the structural and allosteric implications on signaling at the membrane interface and may aid current efforts in pharmacological targeting of these proteins.

© 2022 Published by Elsevier B.V. on behalf of Research Network of Computational and Structural Biotechnology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

A R T I C L E  I N F O
Article history:
Received 21 June 2022
Received in revised form 3 October 2022
Accepted 3 October 2022
Available online 7 October 2022

Keywords:
PI3Kα
PTEN
Allostery
Membrane trafficking
Phosphoinositides
Membrane-binding

1. Introduction ........................................... 5608
2. Overview of PM activation of the PI3Kα/Akt/PTEN pathway ........................................... 5608
3. Phosphoinositide turnover interplay between signaling and membrane trafficking .......................... 5608
4. PI3Kα and PTEN structure and regulation of membrane interactions ......................................... 5610
4.1. PI3Kα ........................................ 5610
4.1.1. PI3Kα active state .......................... 5611
4.1.2. PI3Kα membrane interactions .............. 5612
4.2. PTEN ........................................ 5612
4.2.1. PTEN membrane interactions ............... 5613
4.2.2. The open/closed conformation model of PTEN .................................................. 5613
5. Allosteric mechanisms for membrane recruitment and catalysis ........................................... 5614
5.1. Allostery in PI3Kα ................................ 5614
5.2. Allostery in PTEN ................................ 5614
6. Mechanisms for co-regulation of PI3K and PTEN .................................................. 5615
6.1. Role of p85α ..................................... 5615

1 Equal contribution authors.
2 Equal corresponding authors.

https://doi.org/10.1016/j.csbj.2022.10.007
2001-0370© 2022 Published by Elsevier B.V. on behalf of Research Network of Computational and Structural Biotechnology.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

Eukaryotic cells are defined by the presence of a plasma membrane (PM) and a complex endomembrane system. These membraneous compartments exhibit several distinct features that rely on the compartmentalization of specific proteins and membrane lipids and the net charge of the cytosolic face of the membrane bilayer [1]. Amongst these features, relatively non-abundant phospholipids such as phosphoinositides (PIPs) play an essential role in regulating almost all aspects of cell physiology [2,3]. PIPs exhibit a differential subcellular membrane localization, thereby providing a unique PIP code to distinct membrane compartments [4]. These PIP-based switches function as essential drivers of cellular signaling and trafficking at the PM and endomembranes. Phosphatidylinositol (4,5)-biphosphate (PI(4,5)P₂) and Phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P₃) are synthesized and degraded by a family of phosphoinositide-3-kinase (PI3Ks) [5]. Class I, II and III PI3Ks are further classified into four subclasses according to their structure, catalytic activity and substrate preference [6]. Class I PI3Ks, PI3Kα, PI3Kβ and PI3Kγ are ubiquitously expressed and play a significant role in regulating almost all aspects of cell physiology, such as transcription and translation, protein synthesis, and cell cycle progression, among others (Fig. 1) [6,10,11]. PI3Kα, the gene that encodes the catalytic subunit of PI3Kα is the most frequently mutated kinase in human malignancies [12], and the second most frequently-mutated oncogene in all human cancers. Activating mutations that are present in many high mortality cancers are located throughout the PI3Kα primary sequence [13].

The negative regulator of the pathway is PTEN, thus it may come as no surprise that PTEN is also one of the most frequently mutated tumor suppressors in human cancers. PTEN is recruited to PM and functions as a PI(3,4,5)P₃-phosphatase that dephosphorylates the phosphate at position 3 of the inositol ring and converts PI(3,4,5)P₃ to PI(4,5)P₂, thus directly antagonizing PI3Kα [14,15] (Table 1 for kinetic parameters). As a result of low PTEN expression or activity, PI(3,4,5)P₃ downstream signaling pathways are hyperactivated and tumor size increases, while PTEN overexpression brings resistance to tumor growth [14]. Germ-line mutations in the PTEN gene are associated with several cancer predisposition syndromes, collectively referred to as PTEN Hamartoma-Tumor Syndromes (PHTS) [16]. In addition to its tumor suppressor function, PTEN has an important role in the central nervous system and controls neurogenesis, axonal growth, and synaptogenesis [17].

3. Phosphoinositide turnover interplay between signaling and membrane trafficking

Receptor-mediated signaling initiates upon ligand/receptor interaction at the PM. Extensive studies throughout the last two decades have shown that, soon after receptor activation at the cell surface, ligand/receptor complexes are internalized in PM invaginations, leading to formation of cytoplasmic vesicles that deliver their cargo to the early endosome (reviewed in [25]). At the endosomal compartment, sorting of the ligand/receptor complexes determines whether the receptors will be delivered to the lysosome for degradation, or they will return to the PM for another round of activation. Transport from the endosome to the PM can take place directly (quick recycling), via vesicular carriers budding from the endosomal membrane, or after delivery of the cargo to the recycling endosome, which serves as an intermediate station before further transport of the receptor to the PM (slow recycling) [26]. Interestingly, transport of the ligand/receptor complexes through these intricate endosomal compartments, determines not only the fate of the ligand/receptor complexes, but also the exact transient endomembrane stations where the receptor can activate specific downstream signaling molecules [25]. Thus, the specific endocytic routes undertaken by individual ligand/receptor complexes determine the intensity, duration and output of receptor signaling [27].

This bidirectional interplay between signaling and membrane trafficking, relies on a number of molecular players that are shared between these two processes [28]. Typical molecules of this functional interplay are PIPs. Their regulation by signaling molecules, e.g. receptors [29], and trafficking mediators, e.g. Rab GTPases [30], coordinates receptor-mediated signaling with vesicular transport (Fig. 1). More specifically, clathrin-mediated endocytosis (CME) appears to require the presence of a local pool of PI(4,5)P₂, which is necessary for the initial formation of clathrin coated pits.
while the conversion of PI(4,5)P2 to PI(3,4)P2 controls maturation of CCPs [31]. On the other hand, two other endocytic pathways, macropinocytosis (MP) [32] and Fast Endophilin-Mediated Endocytosis (FEME) [33], require the activity of class I PI3Ks that generates PI(3,4,5)P3. Along the endocytic route, PI(3,4,5)P3 is dephosphorylated by 5-phosphatases SHIP1/2 or OCRL to generate PI(3,4)P2, followed by dephosphorylation at the 4’ position by INPP4A/B, to form PI(3)P, the main PIP at the early endosome [34]. HVPS34/p150 complex is recruited to the endosome through interaction with the small GTPase Rab5 [30,35]. Interestingly, Rab5 also mediates the catalytic activation of HVPS34 at the endosomal membrane [7,35], by interacting with the interface between hVPS34 and p150 [7]. As a consequence, both endosomal recruitment and catalytic activation results in generation of membrane domains that are enriched in PI(3)P and Rab5GTP [36], which function as membrane docking sites for a complex network of endosomal proteins containing a specific PI(3)P binding domain, called FYVE [30,36]. Recruitment of the FYVE domain containing proteins

Table 1

| Enzyme | Kinetic parameter | Substrate | References |
|--------|------------------|-----------|------------|
| PI3K   | Km (μM)          | ATP       | 24.8 ± 4.2 | [18]       |
|        |                  | PI(4,5)P2 | 2.0 ± 0.5  | [19]       |
|        | iKm (μM)         | PI(4,5)P2 | 62–69      | [18-20,21] |
|        |                  |           | 1.8 ± 0.03 | [19]       |
| PTEN   | Km (μM)          | soluble PI(3,4,5)P3 | 23–70 | [22,23] |
|        | iKm (μM)         | PI(3,4,5)P3 in lipid vesicles | 0.04–0.6 | [22,24] |

* Km, the concentration of substrate at half of maximum enzymatic activity; iKm, apparent Km constant. Note that the values may differ depending on the type of assay used, the concentrations of co-substrates or on the form of substrate presentation (i.e., soluble or membrane bound PIPs).
to endosomes plays an important role in endocytic transport, thereby controlling receptor trafficking and signaling [36].

The Receptor Tyrosine Kinase (RTK)-dependent recruitment of PI3Ks, generation of PI(3,4,5)P3 from PI(4,5)P2 and subsequent activation of Akt have been traditionally assigned to take place at the PM. Yet, it is now clear that activation of Akt takes also place at endosomes [28–37,41]. Interestingly, although PI3K/PI(3,4,5)P3/Akt signaling can take place both at the PM and intracellularly, different cellular functions appear to depend on activation of this signaling pathway in restricted cellular locations, rather than globally. For example, chemotactic migration of macrophages relies on PM activation of PI3K/Akt [42], while angiogenesis depends on activation of this signaling axis at the endosomes [43,44].

One of the proposed mechanisms for PI3Kα-mediated generation of PI(3,4,5)P3 at the endosomes involves membrane recruitment of the lipid kinase by interaction with the microtubule-binding protein MAP4 [37]. The question that arises from this proposed regulatory mechanism concerns PI(4,5)P2 availability, which appears to be absent from early endosomes. Thus, according to this mechanism, the de novo synthesis of endosomal PI(4,5)P2 would be required. Batrouni and Baskin hypothesized that phosphorylation of phosphatidylinositol (PI) by PI4KIIIα and PI5P3K is a possible source of PI(4,5)P2 [37,45,46]. An additional mechanism that contributes to the production of PI(3,4,5)P3 at the endosomes relies on Rab5-dependent recruitment of the class I PI3K p85α/p110β [34], which is also enriched at clathrin coated vesicles [30] (Fig. 1). Subsequently, PI(3,4,5)P3 is hydrolyzed by 5- and 4-phosphatases, thus contributing to an enrichment of PI(3)P at the endosomal membrane [34]. The presence of class I [34] and class II [47] PI3Ks in endocytic compartments could result in a transient production of PI(3,4,5)P3 or PI(3,4)P2, which further supports a local activation of the Akt pathway in the endomembranes [48]. Interestingly, PTEN, besides PI(3,4,5)P3, dephosphorylates also PI(3,4)P2 and its association with endosomes via a PI(3)P docking site results in termination of endosomal Akt activation [51]. These regulatory mechanisms may ensure that Akt activation is limited to endocytic vesicles that reside in the cell periphery, while mature endosomes that are distant from the PM are restrictive in Akt-signaling. Besides the above mentioned catalytically-mediated role of PTEN at the endosome, endosomal PTEN plays an enzymatically-independent role in Glut1 recycling. More specifically, binding of PTEN to SNX27, an adaptor of various cargo molecules, prevents interaction of SNX27 to VPS26 retromer complex, which disables recycling of GLUT1 to the PM, thus leading to impaired cellular glucose uptake [52]. This PTEN-mediated regulation of glucose metabolism could be part of the PI3K-independent mechanisms that control this metabolic pathway.

Our understanding on the role of endocytosis in receptor signaling and PIP turnover has been greatly facilitated by the development of excellent inhibitors of the various endocytic routes [55–57]. However, as small molecule inhibitors could bind to multiple targets, complementary experiments, e.g. knockdowns or knockouts, are usually undertaken to back up experimental data generated with these inhibitors [58–64].

4. PI3Kα and PTEN structure and regulation of membrane interactions

4.1. PI3Kα

PI3Kα is a heterodimer composed of a catalytic (p110α) and a regulatory subunit (p85α), encoded by PIK3CA and PIK3R1, respectively (Fig. 2a). The catalytic subunit consists of an N-terminal adaptor binding domain (ABD) that mediates binding to the SH2 domain of p85α, a Ras binding domain (RBD) that interacts with switch I and II regions of RAS to stabilize PI3Kα on the membrane [65,66], a C2 domain (C2) that participates in membrane binding, a helical domain which interacts with nSH2, and a kinase domain which hosts functionally important regions, including the P(4,5)P2 and the ATP binding pockets (Fig. 2a and 2b). PIK3CA, the gene encoding the catalytic subunit of PI3Kα, is one of the most frequently mutated genes in solid tumors. The vast majority of the oncogenic mutations are located in either one of two hotspots; (i) E545K and E542K located at the interface between nSH2 and helical domain and (ii) H1047R located at C-terminal tail of the kinase domain. The regulatory subunit contains two Src homology 2 domains, nSH2 and cSH2, that bind to the pYXXM motifs of the phosphorylated RTK, and a coiled-coiled domain (iSH2) between them, which mediates the high-affinity interaction to the catalytic subunit. These domains are preceded by a Src homology 3 domain (SH3), a Bar cluster region homology domain (BH), and two proline-rich regions, PR1 and PR2 surrounding the BH domain (Fig. 2a) [6,67]. Cheung et al. reported that SH3 and BH domains along with the PR regions may facilitate the homodimerization of p85α [68]. Besides p85α, PIK3R1 also encodes two functional splice variants, p55α and p50α, which lack the domains required for homodimerization (they lack the proline-rich regions, BH and SH3 domains) (Fig. 2c).

Post-translational modifications of p85α regulate the catalytic activity of PI3Kα (Fig. 2d). Direct phosphorylation of S361 in nSH2, and S652 in cSH2, by Protein Kinase C (PKC), prevents PI3Kα from binding to the activated receptor [69,70]. It should be noted, however, that PKCα can also indirectly stimulate PI3K/Akt signaling by phosphorylating MARCKS to displace it from PI(4,5)P2, thereby generating free PI(4,5)P2, the lipid substrate of PI3K, required for PI(3,4,5)P3 production [71,72]. Another post-translational modification that prevents the binding of cSH2 to the phosphorylated RTKs is the phosphorylation of S690 in cSH2, by IkB kinase (IKK). On the contrary, phosphorylation of Y688 in cSH2 may activate PI3Kα by facilitating an intramolecular interaction with nSH2. Moreover, phosphorylation of S83 in the SH3 domain by protein kinase A (PKA) promotes p85 binding of 14–3–3z protein, which leads to increased membrane binding and subsequently to increased p110α activation [69,70,73]. The catalytic subunit, p110α can phosphorylate the regulatory subunit, p85α, at S608, although this autophosphorylation is not a significant regulator of the lipid kinase activity of PI3Kα [74]. Moreover, p85α is phosphorylated on Y508 by the Platelet-derived growth factor receptor (PDGFR) [75]. Cruz-Herrera et al. suggest that SUMO1 and SUMO2 modulate the p85 function by reducing the levels of tyrosine-phosphorylated-p85. Different lysine residues located at the iSH2 domain are putative SUMOylation sites [76].

PI3Kα can be activated by phosphorylated RTKs, their adaptor proteins (e.g. IRS-1) and a broad spectrum of RAS family members (e.g. HRAS). RTKs activate PI3Kα through binding of the PI3Kα SH2 domains to the pYyr residues (pYXXM motifs) located in the cytosolic domain of the activated RTK. This phosphopeptide sequence binds to the nSH2 domain, disrupting the charge-charge interaction between the nSH2 domain and the helical domain of p110α [67]. pYyr also binds to cSH2, however, cSH2 does not have an inhibitory role in PI3Kα [78]. Two conserved FLVR motifs (res. 355–358 in nSH2 and 646–649 in cSH2 domain) are critical for binding to the phosphorylated RTK [70]. The receptor and the RAS protein synergistically activate PI3Kα. In fact, it was shown by Buckles et al. that the activation of PI3Kα by RAS is mediated primarily via the membrane recruitment mechanism, rather than allosterically. Thus, the association of receptor pYyr residues with the inhibitory SH2 domains of p85 make p110 accessible for PI(4,5)P2 and binding of p110 to HRAS contributes to the activation promoting the membrane recruitment [79]. Upon activation, p110α is recruited to the membrane to syn-
thesize PI(3,4,5)P$_3$ by transferring the $\gamma$-phosphate group of ATP to PI(4,5)P$_2$. However, the ATP pocket (active site) and the PI(4,5)P$_2$ binding site of PI3K$_\alpha$ are 6–7 Å apart in crystal structures of the inactive state (e.g. PDB ID: 4OVV [80]). For the phosphorylation to take place, PI(4,5)P$_2$ needs to approach ATP. This is mediated through a sequence of allosteric motions that are triggered by nSH2 disengagement. Hence, the surface of the kinase domain becomes accessible for interaction with the membrane [81].

4.1.1. PI3K$_\alpha$ active state

The natural transition of PI3K$_\alpha$ from an inactive cytosolic state to an activated state on membranes entails four distinct events. Apart from the breaking of the nSH2–helical interface described above (event 1), three other events have been described by Burke et al. [82]. These events include disrupting the iSH2–C2 interface (event 2), movement of the ABD domain relative to the kinase domain (event 3), and interaction of the kinase domain with the membrane (event 4). Based on HDX-MS data and protein-lipid FRET assays, oncogenic mutations upregulate the enzyme by enhancing one or more of these dynamic events [82]. Moreover, a recent cryo-electron microscopy (cryo-EM) study showed that the phosphopeptide-bound structure of PI3K$_\alpha$ consists of a stable core, where electron density for ABD and the regulatory subunit is absent (Fig. 3a) [83]. Indeed, in a previous study Zhao et al. had shown that the deletion mutant of PI3K$_\alpha$ lacking the ABD domain ($\Delta$ABD) and the entire regulatory subunit, efficiently activates PI3K signaling [84].
4.1.2. PI3Kα membrane interactions

As mentioned, mutations are distributed throughout the primary sequence with two main hot spots in key regulatory regions, one in the helical domain (E545K) and one in the C-terminus of the kinase domain (H1047R). The PI3Kα C-terminus plays both an auto-inhibitory role for the kinase, but it is also a key element for binding to the cell membrane, where PI3Kα receives its substrate, the lipid PI(4,5)P2, and converts it to PI(3,4,5)P3, which starts a signaling cascade for cell proliferation. It has been shown in the literature that PI3Kα C-terminal mutants such as the H1047R prevalent PI3Kα mutation cause the kinase to be overactivated by altering the PI3Kα interaction with the cell membrane [82,85–87]. According to HDX-MS data of the overactivated H1047R oncogenic mutant, the membrane-binding regions are 716–744 (membrane binding loop1), 848–859 (active site), 859–872 (membrane binding loop 2), 930–956 (activation loop) and 1039–1068 (C-terminus) (Fig. 3b) [82,88,89]. To generate a model of the PI3Kα active state we used the recent cryo-EM structure with PDB ID: 7MYN [83] as a reference [83] (a) Proposed active state model of PI3Kα in which electron density for the ABD and the p85α is absent (b) The AABD model on a model cell membrane based on the PDB ID 7MYN structure [83]. Membrane interacting residues are colored green. (c) Residues 405–420 of the nSH2 domain (colored red) are in close proximity to the membrane. The AABD model based on the 7MYN structure is colored blue and the superimposed AABD + nSH2 structure of the ES45K mutant with a detached nSH2 domain, as observed in our simulations, is colored green. The AABD model was placed on a model membrane at a distance of 5 Å and was rotated to match the referred membrane interactions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

HDX-MS experiments show that the 405–420 region of nSH2 exhibits decreased hydrogen–deuterium exchange upon membrane binding with HRAS-coupled vesicles, suggesting that nSH2 binds not only to the RTKs but also to the membrane [89,91]. Full activation of PI3Kα requires recruitment to membrane-bound HRAS, which greatly leads to the formation of a stable, membrane-bound PI3Kα complex [79].

4.2. PTEN

PTEN is a cytosolic 403aa protein consisting of five functional domains. The N-terminus contains the PI(4,5)P2-binding domain (PBD), which folds into an α-helix conformation [92–94], the catalytic phosphatase domain, which includes the active site composed by the P loop with the catalytic CX5R sequence, the WPD loop, the TI loop, and a positively charged Arginine loop [94–97], the C2 domain, which contains CBR1, CBR2 and CBR3 loops that are responsible for PTEN binding to the membrane [94–96,98] and the C-terminal tail (CTT), which is crucial for PTEN regulation [99,100]. At the end of the CTT a PDZ-binding region is located, which facilitates the interaction with other proteins [17,101]. HDX-MS experiments show that the 405–420 region of nSH2 exhibits decreased hydrogen–deuterium exchange upon membrane binding with HRAS-coupled vesicles, suggesting that nSH2 binds not only to the RTKs but also to the membrane [89,91]. Full activation of PI3Kα requires recruitment to membrane-bound HRAS, which greatly leads to the formation of a stable, membrane-bound PI3Kα complex [79].

PTEN is under hierarchical control by transcriptional, post-transcriptional and post-translational mechanisms, and protein–protein interactions [14]. Post-translational modifications are heavily studied and include phosphorylation [102], oxidation...
4.2.1. PTEN membrane interactions

According to HDX-MS data of the active dephosphorylated PTEN in the presence and absence of cholesterol and P(4,5)P₂-containing lipid vesicles the membrane-interacting regions were residues, 4–21, 35–42 (Arginine loop), 82–99 (WPD loop), 155–177 (TI loop), 201–215 (CBR1 loop), 259–273 (CBR3 loop), 319–342 (Cox2) (Fig. 4b) [96]. The WPD and TI loops, together with the P loop, create a negatively charged, wide and deep pocket, allowing access to the bulky P(3,4,5)P₃ in the membrane [94]. From functional mutagenesis studies the dominant PTEN membrane binding sites include: (a) the C-terminus of the phosphatase domain that interact with P(4,5)P₂ with residue K13 being required for binding [93,115]; (b) the positively charged Arginine loop in the phosphate domain that boosts the interaction with acidic phospholipids including PI (3,4,5)P₃ [96,97,114]; (c) the CBR3 and Cxx2 loops in the C2 domain that bind to both zwitterionic and anionic membrane phospholipids [92,116]. Interestingly, molecular dynamics simulation of the phosphatase-C2 domains of PTEN suggests that binding to the membrane proceeds through electrostatic interactions and results in changes in the relative orientation of the phosphatase and C2 domain [117].

4.2.2. The open/closed conformation model of PTEN

A breakthrough in our understanding of the membrane-associated PTEN active state has been the discovery that PTEN can assume two conformations: an "open" (active) and a "closed" (inactive) state (Fig. 4b). When S380, T382, T383, and S385 of the CTT are phosphorylated, PTEN assumes the compact "closed" state associated with increased membrane interaction and phosphatase activity [96,118]. The closed state is associated with increased stability and it is strengthened by intramolecular interactions between the phosphorylated CTT that folds over the active-site and the C2 domain [22,118,119]. Chen et al using X-ray scattering, found that phosphorylated C-tail interacted with the Cxx2 and CBR3 loops of the C2 domain but also with the Arginine loop of the phosphatase domain [120]. These results are in agreement with Masson et al. 2016 mass spectrometry (HDX-MS) experiments [96] and also compatible with crystallography studies on semisynthetic PTEN-like molecules [121]. Residues R41, E73, N262, and N329 in the phosphatase and C2 domains play important roles in the intramolecular interaction with the CTT and specific mutations in these residues result in increased membrane association and increased phosphatase activity of PTEN [122].

Thus, phosphorylated PTEN assumes a "closed" autoinhibited form that is mostly cytoplasmic, whereas upon dephosphorylation of the CTT, the autoinhibition is released, the active site is exposed, thus permitting access to P(3,4,5)P₃ in the membrane [123]. Apparently, the released unphosphorylated CTT does not interact with membranes nor interfere with PTEN membrane binding per se [114,116]. Mutation of S/T residues in the 380–385 stretch of CTT to alanine results in an "open" PTEN conformation, localized at the membrane with increased catalytic activity [105]. The details of how this phosphorylation-induced conformational changes happen are missing and the fact that PTEN CTT is consid-
erased an IDR, makes it difficult to create an accurate structural model of the proposed closed state [112,121,124,125]. While several kinases, most prominently CK2, have been postulated to phosphorylate PTEN and thus induce the conformational shift to the autoinhibited state [126], the involvement of protein phosphatases has been understudied. In this context, it has been suggested that PTEN may release this auto-inhibitory state by its protein phosphatase activity. Although this notion is compelling, and it is supported by some experimental data [111,127] very little is known about the mechanism of this regulatory step.

It has been proposed that formation of PTEN dimers is an additional step towards its full activation [129,130]. Homodimerized PTEN is in the open conformation and exhibits maximal lipid phosphatase activity [129]. PTEN dimers seem to assume a more compact conformation than monomers, and the CTT is highly associated with the stability of the configuration. This compact conformation permits increased cooperativity between the catalytic and C2 domains that may affect the efficiency of the phosphatase activity [130]. The biological significance of PTEN dimer formation has been demonstrated in vitro and in vivo; it has been proposed that mutated PTEN alleles have a dominant negative function on the wild type protein via dimer formation [129]. Dimerization appears to be inhibited by the E3 ligase WWPP1 via K27-linked polyubiquitination of PTEN [131]. Accordingly, pharmacological inactivation of WWPP1 reactivates PTEN and suppresses PI3K/Akt-dependent tumorigenesis [131].

5. Allostery in PTEN

5.2. Allostery in PTEN

An important aspect in order to appreciate allosteric regulation of PTEN relies on its mode of interfacial catalysis kinetics. PTEN likely operates via a mixed scooting/hopping model [24,114]. Pl(4,5)P2, the product of PTEN, appears to be a major determinant of catalytic activation by imposing a positive feedback loop upon interaction with membranes. Early studies showed that catalytic activity is stimulated by 5-8fold when assayed with lipid vesicles containing variable amounts of Pl(4,5)P2 [24,115]. Interestingly, this Pl(4,5)P2-dependent increase of catalytic activity is observed also with monodisperse PIPs, which are typically water-soluble, suggesting that interaction with membrane per se is not the decisive factor [136]. At higher Pl(3,4,5)P3 concentrations the kinetic curves are sigmoidal and do not follow Michaelis-Menten models.
This indicates that enzymatic activity increases as the reaction progresses, apparently due to the increase of its product, P(4,5)P2 [136]. Furthermore, P(4,5)P2 may additionally regulate the membrane lateral diffusion of PTEN. Thus, individual PTEN molecules may exhibit temporal changes in their lateral diffusion mobility as the local P(4,5)P2 density changes on the membrane whereas the spatial distribution of P(4,5)P2 also changes depending on the local density of PTEN, since P(4,5)P2 density increases due to the enzymatic activity [137]. These findings imply multiple feedback loop mechanisms operating at the level of membrane between PTEN and P(4,5)P2.

Detailed analysis using supported lipid bilayers coupled to TIRF imaging has proposed that PTEN kinetics can be described by a combination of both recruitment and allosteric activation effects by P(4,5)P2 [138]. Interestingly, this P(4,5)P2 positive feedback loop is necessary and sufficient for the reaction size-dependency of PTEN catalysis that is readily observed in restricted supported lipid bilayers [139].

Recent studies have updated and informed the open-closed conformation notion and the allosteric P(4,5)P2 positive feedback loop notion [116,121]. The temporal sequence of allosteric modes embedded in the activation of PTEN and the interaction with membrane are: (a) phosphorylation of the CTT (S380, T382, T383, S385 stretch) results in intramolecular closure with CTT contacting the CBR3 loop and Csx2 segment in C2 domain and likely extending into the phosphatase domain; at the same time this closure disrupts the N-terminal α-helix of PBD [121], (b) dephosphorylation of PTEN results in the open conformation which uncovers the active site in the phosphatase domain and regains the α-helical motif in PBD. (c) binding to the membrane proceeds via the positively charged arginine loop and CBR3 loop in the phosphatase and C2 domains respectively [116], (d) the initial PBD α-helical motif is rapidly disrupted and converted into an unstructured region which anchors the protein in the lipid bilayer via strong salt bridge interactions between anionic lipids and a polybasic patch (R11, K13, R14, and R15), resulting in the coordination of two P(4,5)P2 lipids [116]. It is likely that the coordination of P(3,4,5)P2 to the P loop may allosterically promote PBD unfolding to stabilize PTEN binding to the membrane [116].

6. Mechanisms for co-regulation of PI3K and PTEN

Although PI3Kα and PTEN have been studied extensively, due to their importance in diseases including cancer, there are still significant gaps in our understanding of how these two proteins co-regulate and orchestrate interconversion of P(3,4,5)P3 and P(4,5)P2. Both are cytosolic proteins, which implies that their recruitment on the membrane plays a fundamental role in their mechanism of action. Although membrane recruitment and catalytic activation of PI3Kα is agonist-induced and receptor-operated, this is not the case for PTEN. Several receptors of the tyrosine kinase and G protein-coupled receptor families have been proposed to bind and recruit PTEN to the PM although it is not always clear if these interactions are direct or even agonist-induced [17,108]. Regardless, these interactions may function to prime PTEN towards PI3K-generated P(3,4,5)P3 domains [17]. It has to be emphasized that although recruitment and activation of PTEN to sites of PI3K/P(3,4,5)P3 signaling may be important for fine tuning of P(3,4,5)P3, it may be counterproductive in other settings, particularly when a polarized steep P(3,4,5)P3 distribution on the PM is necessary for cell functions. For example, PTEN may be still recruited but inhibited at the PM in order to attain high levels of P(3,4,5)P3 for induction of filopodia and branches along neuronal axons [140,141], or even actively excluded from PM regions with intense synthesis of P(3,4,5)P3 to ensure continuous and robust cell polarization and chemotaxis [142,143]. Below we briefly discuss different modes of interaction and co-regulation of PI3K and PTEN.

6.1. Role of p85α

It has been reported that p85 plays a dual function in regulation of P(3,4,5)P3 upon growth factor receptor activation. In addition to its role as a regulatory subunit of p110-PI3K, p85α binds directly to unphosphorylated PTEN and enhances its stability and phosphatase activity [144,145]. Consequently, p85α would be an ideal candidate for the temporal co-regulation of PI3K and PTEN activities towards P(4,5)P2 and P(3,4,5)P3 upon acute signaling. Binding proceeds via interactions of the BH domain of p85 with the phosphatase and C2 domains of PTEN [146]. Interestingly, the BH domain of p85α binds with low affinity to monophosphorylated PIPs in vitro; the binding to P(3)P specifically [146], might also help to localize p85α to endosomal vesicles containing PTEN [51]. Cancer-related mutations in the p85α-BH domain, however, do not consistently correlate with changes in PTEN activity [147]. Cheung et al. have suggested that p85α dimers bind and stabilize PTEN by preventing ubiquitination and degradation by the E3 ligase WWP2 [68]. The p85α homodimer includes intermolecular interactions between SH3:PR1 in trans and BH: BH interactions between the monomers. The homodimerized p85α binds PTEN at least partly through the PR2 domain and through several residues of the BH domain (I127, I133, E137) [68].

This model of dual regulation of p110α and PTEN entails the presence of free p85α in the cytoplasm that is able to either form homodimers, or stabilize and activate p110α and PTEN. The presence of excess free p85α has been verified and indeed it modulates PI3K signaling with the effect being dominant at low levels of receptor activation [148]. Also, the balance between the amount of p85α and the amount of the splice variants, p50α and p55α, which are not able to stabilize p110α as strongly as p85α and at the same time cannot form homodimers, can affect PI3Kα signaling. Although the direct regulation of PTEN by free p85 in the monomeric or dimeric form provides an intriguing explanation for the known effects of partial loss of p85α on PI3K signaling, these results have not been verified by other groups [149]. Thorpe et al. have suggested that PTEN downregulation may represent a secondary (and not a direct) effect of p85α loss [149]. It is likely, however, that direct p85α-PTEN interactions might play an important role under certain conditions for example in specific cell types or specific growth factor receptors.

6.2. Localized P(4,5)P2 and P(3,4,5)P3 pools

Intriguingly, recent advances in lipid kinase and phosphatase assays on restricted supported bilayer systems have revealed a reaction size-dependency of catalytic activity of several enzymes, including PTEN [139,150]. In essence, reaction size-dependency of PTEN suggests that PTEN will dephosphorylate faster large domains of P(3,4,5)P3 compared to small domains on the PM. Studies with P(4,5)P2 phosphatase and P(4)P kinase pairs have shown that competition may result in formation of a compositional pattern of P(4)P and P(4,5)P2 that exhibits bistability when the membrane reaction environment is geometrically confined [150]. This model has been also proposed for PI3K and PTEN competition, thus imposing size restriction and bistability to P(3,4,5)P3 and P(4,5)P2 [139,150].

These fundamental properties of antagonizing lipid kinases and phosphatases may relate also to the distribution of PIP-rich domains in the PM. Recent super resolution microscopy of P(4,5)P2 and P(3,4,5)P3 in live cells or membrane sheets have advanced our understanding of the segregation, fractional partitioning and
distinct properties of PIP domains in the membrane. For example, PI(4,5)P₂ and P(3,4,5)P₃ appear to mark distinct nanoscale domains within the PM of PC12 cells under basal conditions [151]. PI(4,5)P₂ has been observed in nanoscale domains within the PM in several cellular settings (reviewed in [152]). Considering the biophysical properties of distinct PI(4,5)P₂ domains, these may correspond to liquid-ordered, cholesterol-rich "raft-like" or liquid-disordered "non-raft" regions of the membrane [152]. Interestingly, raft and non-raft PI(4,5)P₂ domains may experience differential dynamics upon receptor activation of Phospholipase C signaling [153]. The spatial segregation, recruitment, and signaling of PI3K and PTEN in lipid raft vs non-lipid raft membrane has also been proposed [154], with PTEN primarily localized in non-raft domains. This segregation, however, may relate to the activation of PI3Kβ isoform in lipid rafts rather than the PI3Kα isoform [155].

Although intuitively-one assumes that PI3K and PTEN are co-regulating the same PI(3,4,5)P₃ domains in the PM, we still lack the temporal and spatial insight on whether this is indeed the case. Paradoxically, in chemoattractant-induced migration of Dictyostelium slime molds and immune cells, PTEN and PI(3,4,5)P₃ exhibit mutually exclusive localization at the PM [142,143]. This segregation corresponds to PI(3,4,5)P₃-enriched/PTEN-excluded and PTEN-enriched/PI(3,4,5)P₃-rich but PI(3,4,5)P₃-excluded states. This apparent segregation suggests that, at least upon strong activation of PI3Kα, PTEN may be actively excluded from PI(3,4,5)P₃-rich PM regions [143].

7. Pharmacological targeting of PI3Kα and PTEN

PI3Kα has emerged as a promising therapeutic target due to its importance in cancer. Extensive efforts have been made in the last decades to develop drugs able to regulate PI3Kα signaling. Inhibitors targeting PI3Kα can be classified into pan-PI3K, dual PI3K/mTOR and isoform-specific inhibitors. Pan-PI3K inhibitors are ATP-competitive inhibitors that target all four isoforms of class I PI3K and thus, due to their non-selectivity, are associated with several adverse reactions. An example of a pan-PI3K inhibitor is Copanlisib, which is FDA-approved for treatment of adult patients with relapsed follicular lymphoma [156]. Dual PI3K/mTOR inhibitors target and block both all PI3K isoforms and mTOR kinase. They exhibit poor tolerance when given systemically and therefore none of them has been approved by the FDA [157,158]. Isoform-specific PI3K inhibitors selectively inhibit specific PI3K isoforms and four FDA-approved isoform-specific inhibitors are currently on the market; Alpelisib, which is selective against PI3Kα [159], Idelalisib [160] and Umbralisib selective against PI3Kα [161] and Duvelisib, which is a dual PI3Kγ/PI3Kδ inhibitor [162]. Although the development of PI3K inhibitors has progressed rapidly with many drugs entering clinical trials in the last few years, intrinsic and acquired resistance limits their therapeutic efficacy [163]. Resistance to PI3K inhibitors often occurs through numerous feedback loops involved in the PI3K/Akt signaling network, rebalancing the inhibitory effects of the drugs [158]. Other factors that can lead to resistance in PI3K inhibitors include inactivation or loss of PTEN activity [164], mutations and amplification of PI3K [165], and non-coding RNAs (ncRNAs) [166].

As mentioned, only one PI3Kα-targeting drug is currently in the clinic, alpelisib, which has been approved by the FDA for breast cancer due to its selectivity and pharmacokinetics [159]. Another orthosteric small molecule PI3Kα inhibitor, Inavolisib (GDC-0077), leads to degradation of the E545K and H1047R mutant p110α in cells [167] and is in clinical trials [168]. Both these ATP-competitive inhibitors exhibit severe concentration-dependent adverse effects due to the fact that the ATP binding pocket is very similar across the different PI3K isoforms [169]. Understanding the allosteric pathways that exist in PI3Kα and its mechanism of action (see Section 5.1) will enable the design of mutant-specific inhibitors that will selectively regulate the membrane-binding and activation. Targeting the protein-membrane interface emerges as a new promising therapeutic strategy, as the activation of PI3Kα is directly linked to the membrane recruitment. The membrane-binding regions are expected to communicate with the active site and thus it can be modulated allosterically.

In contrast to PI3Kα, less attention has been given to development of effective drugs that modify the activity of PTEN, likely because it is considered undruggable [116,125]. However, positive or negative modulators of PTEN may find use in cancer and neurodevelopmental diseases or neurodegenerative and nerve injury conditions, respectively [170]. Unfortunately, currently used 1st generation PTEN inhibitors suffer from specificity, selectivity and reversibility issues [171] and unexpected adverse effects (Premeti, Syropoulou, Leondaritis, unpublished data). Most 1st generation PTEN inhibitors are complexes of peroxo-V (V), o xo-V (VI) and vanadyl- (VO-) with organic substituent, and they are proposed to cause oxidative inhibition of PTEN, at least in vitro [128,171,172]. However, it is unclear whether all these compounds act by oxidative inhibition in cells (Premeti, Syropoulou, Leondaritis, unpublished data). Nevertheless, some of these compounds have exhibited promising results in preclinical animal studies assessing tissue survival and regeneration after trauma [173].

As previously discussed, the interaction with the membrane has a crucial role for enzymatic activity of PTEN [116,174]. Considering the allosteric mechanisms that affect PTEN membrane interaction and catalytic activation (see Section 5.2), could we design reversible allosteric modifiers/modulators to shift the equilibrium between active and inactive states or influence the formation of dimers [116,125]? Recently, PTEN reactivation by small molecules that restore its dimerization via inhibition of polyubiquitination has proved the validity of these approaches to treat cancer [131]. In alternative approaches, peptides targeting several regions and domains of PTEN have proven their validity in modulating (inhibiting) PTEN membrane interactions and activity in neurodegenerative and nerve injury conditions [175,176]. On a more general note, direct PTEN modulators will certainly provide us with unprecedented insight on the acute roles of PTEN upon activation of PI3K signaling; so far, we have almost exclusively relied on genetic means of manipulating PTEN activity or localization to membrane.

Although controlling PI3K signaling through PTEN activity is crucial for the regulation of vital cellular mechanisms, the function of PTEN as a tumor suppressor gene complicates the use of existing drugs as well as the design of new potential modulators. Considering PTEN’s association with a multitude of other diseases and pathological phenotypes beyond cancer, it is increasingly recognized that we need to better study and understand the complications and benefits of PTEN’s pharmacological targeting.

8. Summary and outlook

As described herein, our knowledge of the mechanistic and structural details of PI3Kα and PTEN membrane interactions and catalytic activation have progressed at a rapid pace over the last decade. However, the molecular mechanisms of how PI3Kα and PTEN communicate and co-regulate remain unclear. Thus, we lack fundamental insight into how they antagonize, or even synergize, to create spatially and temporally dynamic pools of signaling PI (3,4,5)P₃ in the rich PI(4,5)P₂ environment of the PM inner leaflet. Do PI3Kα and PTEN display bistability upon competition on mem-
branes as has been suggested for other pairs of PIP kinases and phosphatases [150]? What is the role of PI(4,5)P2 or PI(3,4,5)P3 binding proteins [3] that may limit free binding/substrate sites available for PI3K and PTEN? Can their effect be quantified and modeled? Are PI3K and PTEN segregated upon intense receptor signaling? This segregation may relate to the need for establishing steep opposing gradients of PI(3,4,5)P3 and PI(4,5)P2 along the PM that may define polarity upon cell migration [143].

Significant questions about their allosteric regulation remain unanswered. Concerning PI3Kα, how does the catalytic cycle close and PI3Kα returns to its inactive state? What is its active conformation? If ΔABD p110α is indeed the active state of PI3Kα, how does ABD domain interact again with p85α (iSH2)? Does p85α have a dual role, stabilizing the inactive state of PI3Kα and at the same time homodimerizing and stabilizing PTEN on the membrane? p85α/p110 and p85α/PTEN coincide on RTKs? Are there any phospholipid recognition regions in the p85α subunit? Concerning PTEN, how are PTEN dimers integrated in the conformational model of PTEN membrane interaction and catalysis?

Given the distinct PIP composition between PM and endosomes, the mechanisms controlling the balance between PI3K and PTEN activities in these prominent subcellular compartments also remain unexplored. So far, simulations and experiments have been biased towards a typical PM lipid composition. The different membrane interface in endosomal compartments may alter the rules of engagement. For PTEN specifically, PI(3)P seems to be an additional C2-directed docking site to endosomal membranes. Furthermore, it is intriguing to address whether PI3K/PTEN balance is affected by the exact coordinates of individual endosomal compartments, i.e. proximal to the PM versus distant ones, or by their maturation state, their shape, size and cargo content, and how these parameters affect the final output towards Akt signaling?

Finally, complexity of PI3Kα and PTEN catalytic kinetics and interaction with membranes coupled with the inherent redundancy in PI(4,5)P2 and PI(3,4,5)P3 metabolism necessitates the use of innovative computational tools in parallel with current and future experimental approaches. Such computational tools ranging from predictive kinetic modelling [50,138], molecular dynamics simulations [86,87,90,125,177] to membrane interaction modelling and new cryo-EM structures will be of crucial importance in better understanding the interplay between these important signaling enzymes.

CRediT authorship contribution statement

Danai Maria Kotzampasi: Writing – original draft, Writing – review & editing, Visualization. Kyriaki Premeti: Writing – original draft, Writing – review & editing, Visualization. Alexandra Papafothia: Writing – original draft, Writing – review & editing, Visualization. Vasiliki Syropoulou: Writing – original draft, Writing – review & editing. Sawsaw Christoforidis: Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. Zoe Cournia: Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing. George Leonariditis: Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the “1st Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant” (Project Number: 1780), awarded to Z. Cournia. This project has also received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 956314.

Work in the group of G. Leondaritis is supported by project BIOMED-20 (MISS047236) implemented under the Action “Reinforcement of the Research and Innovation Infrastructure” funded by the Operational Programme “Competitiveness, Entrepreneurship and Innovation” (NSRF 2014-2020) and European Regional Development Fund and grant 02667 funded by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the “2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers”.

Work in the group of S. Christoforidis is supported by grants EDBM34, co-financed by Greece and the European Union (European Social Fund-ESF) in the context of the project MISS004410, and project “Biomaging-GR: The Greek Research Infrastructure for the Imaging and Monitoring of Fundamental Biological Processes” (MIS 5002755).

Figures were created with BioRender.com and with PyMol.

References

[1] Harayama T, Riezman H. Understanding the diversity of membrane lipid composition. Nat Rev Mol Cell Biol 2018;19:281–96. https://doi.org/10.1038/nrm.2017.138.
[2] Balla T. Phosphoinositides: tiny lipids with giant impact on cell regulation. Physiol Rev 2013;93:1019–137. https://doi.org/10.1152/physrev.00282.2012.
[3] Hammond GRV, Burke JE. Novel roles of phosphoinositides in signaling, lipid transport, and disease. Curr Opin Cell Biol 2020;63:57–67. https://doi.org/10.1016/j.eceh.2019.12.007.
[4] Di Paolo G, De Camilli P. Phosphoinositides in cell regulation and membrane dynamics. Nature 2006;443:651–7. https://doi.org/10.1038/nature05185.
[5] Jiang N, Dai Q, Su X, Fu J, Feng X, Peng J. Role of PI3K/PTK pathway in cancer: the framework of malignant behavior. Mol Biol Rep 2020;47:4587–629. https://doi.org/10.1007/s11033-020-05472-0.
[6] Vadas O, Burke JE, Zhang X, Berndt A, Williams RL. Structural basis for activation and inhibition of class I phosphoinositide 3-kinases. Sci Signal 2011;4:re2. https://doi.org/10.1126/scisignal.2002165.
[7] Trench S, Ohashi Y, Morado DR, Bertram J, Perissi O, Brandt LTL et al. Structural basis for VPS34 kinase activation by Rab1 and Rab6 on membranes. Nat Commun 2021;12:1564. https://doi.org/10.1038/s41467-021-21695-2.
[8] Bayasacas JR, PK1: the major transducer of PI 3-kinase actions. Curr Top Microbiol Immunol 2010;346:9–28. https://doi.org/10.1007/82_2010_43.
[9] Jiang Q, Zhang X, Dai A, Han S, Wu X, Wang L et al. Stk6Mediated phosphorylation of PKD1 impairs AKT kinase activity and oncogenic functions. Nat Commun 2022;13:1548. https://doi.org/10.1038/s41467-022-28510-8.
[10] Manning B, Toker A. AKT/PKB signaling: navigating the network. Cell 2017;169:381–405. https://doi.org/10.1016/j.cell.2017.04.001.
[11] Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. Cell 2017;168:960–76. https://doi.org/10.1016/j.cell.2017.02.004.
[12] Liu P, Cheng H, Roberts TM, Zhao J. Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov 2009;8:627–44. https://doi.org/10.1038/nrd2925.
[13] Paddock MN, Field SJ, Cantley LC. Treating cancer with phosphatidylinositol-3-kinase inhibitors: increasing efficacy and overcoming resistance. J Lipid Res 2019;60:747–52. https://doi.org/10.1194/jlr.R902310.
[14] Lee Y-R, Chen M, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor: new modes and prospects. Nat Rev Mol Cell Biol 2018;19:547–62. https://doi.org/10.1038/s41580-018-0015-0.
[15] Garcia-Cao I, Song MS, Hobbs RM, Laurent G, Giorgi C, de Boer VJC et al. Systemic elevation of PTEN induces a tumor–suppressive metabolic state. Cell 2012;149:45–62. https://doi.org/10.1016/j.cell.2012.02.030.
[16] Yehia L, Keel E, Eng C. The clinical spectrum of PTEN mutations. Annu Rev Med 2020;71:103–16. https://doi.org/10.1146/annurev-med-052118-132543.
[17] Kreis P, Leonariditis G, Lieberam I, Eckholt Bj. Subcellular targeting and dynamic regulation of PTEN: implications for neuronal cells and neurological disorders. Front Mol Neurosci 2014;7:23. https://doi.org/10.3389/fnmol.2014.00023.
Lee JY, Chiu Y-H, Asara J, Cantley LC. Inhibition of PI3K binding to activators reveals conformational changes during inhibition and activation. Proc Natl Acad Sci U S A 2005;102:18443–8. https://doi.org/10.1073/pnas.0501664102

Cordier F, Chaffoteau A, Terrien E, Préhaud C, Theillet F-X, Delepierre M, et al. Identification of intrinsically disordered regions in PTEN and delineation of its function via a network approach. PLoS ONE 2012;7:e32591. https://doi.org/10.1371/journal.pone.0032591

Al-Khouri AM, Ma Y, Togo SH, Williams S, Mustelin T. Cooperative phosphorylation of PTEN by casein kinases and glycogen synthase kinase 3beta. J Biol Chem 2005;280:35195–202. https://doi.org/10.1074/jbc.M503453200

Tibarewal P, Zilidis G, Spinelli L, Schurch N, Maccario H, Gray A, et al. Ordered phosphorylation events in two independent cascades of the PTEN C-terminal tail revealed by NMR. J Am Chem Soc 2012;134:20533–43. https://doi.org/10.1021/ja307101g

Malaney P, Uversky VN, Davé V. The mechanism of activation of class IA phosphoinositide 3-kinases (PI3Ks) by PKC and MARCKS: single-molecule analysis of a reconstituted signaling pathway. Biophys J 2016;104:1161–25. https://doi.org/10.1016/j.bpysj.2018.02.004

Lee JY, Chiu Y-H, Asara J, Cantley LC. Evidence for regulation of the PI3K tumor suppressor by a membrane-localized multi-PI3K domain containing scaffold protein MAGI-2. Proc Natl Acad Sci U S A 2000;97:4233–8. https://doi.org/10.1073/pnas.97.9.4233

Vazquez F, Grossman SR, Takahashi Y, Rokas MV, Nakamura N, Sellers WR. Phosphorylation of the PTEN tumor suppressor by PKCα driven by reorientation of the ABD, p85 and C-terminus. Cell 2007;128:129–39. https://doi.org/10.1016/j.cell.2007.02.026

Mandelker D, Gabelli SB, Schmidt-Kittler O, Zhu J, Cheong I, Huang C-H, et al. Ordered phosphorylation events in two independent cascades of the PTEN C-terminal tail revealed by NMR. J Am Chem Soc 2012;134:20533–43. https://doi.org/10.1021/ja307101g

Das S, Dixon JE, Cho W. Membrane-binding and activation mechanism of PTEN. Proc Natl Acad Sci U S A 2003;100:7491–6. https://doi.org/10.1073/pnas.1337353100
[118] Rahdar M, Inoue T, Meyer T, Zhang J, Vazquez F, Devreotes PN. A phosophorylation-dependent intramolecular interaction regulates the membrane association and activity of the tumor suppressor PTEN. Proc Natl Acad Sci U S A 2002;99:8880–5. https://doi.org/10.1073/pnas.1221120100.

[119] Odriozola I, Singh G, Hoang T, Chan AM. Regulation of PTEN activity by its carboxyl-terminal autoinhibitory domain. J Biol Chem 2007;282:23206–15. https://doi.org/10.1074/jbc.M704179200.

[120] Chen Z, Dempsey DR, Thomas SN, Hayden D, Bolduc DM, Cole PA. Molecular features of phosphatase and tensin homolog (PTEN) regulation by C-terminal phosphorylation. J Biol Chem 2016;291:14160–9. https://doi.org/10.1074/jbc.M116.730205.

[121] Dempsey DR, Viennet T, Iwase R, Park E, Henriquez S, Chen Z, et al. Engineering PTEN, an enhanced PTEN with increased tumor suppressor activities. Proc Natl Acad Sci U S A 2014;111. https://doi.org/10.1073/pnas.1316043111.

[122] Nussinov R, Zhang M, Tsai C-J, Jiang H. Phosphorylation and drover mutations in PI3Kα and PTEN autoinhibition. Mol Cancer Res 2021;19:543–8. https://doi.org/10.1158/1541-7786.MCR-20-0818.

[123] Malaney P, Uversky VN, Dave V. The PTEN Long N-tail is intrinsically disordered: increased viability for PTEN therapy. Mol BioSyst 2013;9:877–88. https://doi.org/10.1039/c3mb30267a.

[124] Smith IN, Dawson JE, Krieger J, Thacker S, Bahir I, Eng C. Structural and dynamic effects of PTEN C-terminal tail phosphorylation. Biophysics 2022;11:6. https://doi.org/10.1002/bip.21535.

[125] Miller SJ, Lou DY, Seldin DC, Lane WS, Neel BG. Direct identification of PTEN-associated complex. Mol Cell 2009;29:5377–88. https://doi.org/10.1016/j.molcel.2009.07.012.

[126] Zhang XC, Piccini A, Myers MP, Van Aelst L, Tonks NK. Functional analysis of phosphatidylinositol 3-kinase. J Biol Chem 2016;291:14160–9. https://doi.org/10.1074/jbc.M115.70267X.

[127] Yoshioka D, Fukushima S, Koteishi H, Okuno D, Ide T, Matsuoka S, et al. Allosteric regulation of PTEN activity by its carboxyl-terminal autoinhibitory domain. Mol Cell Biol 2009;29:5377–88. https://doi.org/10.1099/jgc.0.015926-0.

[128] Chakrabarti M, Gabelli SB, Amzel LM. Allosteric activation of PI3K by bisperoxidovanadium complexes. Angew Chem Int Ed Engl 2020;59:17631–4. https://doi.org/10.1002/anie.202001966.

[129] Marshall JDS, Mellor P, Ruan X, Whitecross DE, Moore SA, Anderson DH. Insight into the PTEN - p85 interaction and lipid binding properties of the p85β BH domain. Oncotarget 2018;9:36975–92. https://doi.org/10.18632/oncotarget.26437.

[130] Corrà F, Agnoletto C, Minotti L, Baldassari F, Volinia S. The network of non-coding RNAs in cancer drug resistance. Front Oncol 2018;8:327. https://doi.org/10.3389/fonc.2018.00327.

[131] Thorne LM, Spangler JM, Ohlson CE, Chen Z, et al. PI3K-phλ10α mediates the oncogenic activity induced by loss of the novel tumor suppressor PI3K-phλ5α. Proc Natl Acad Sci U S A 2017;114:7995–1000. https://doi.org/10.1073/pnas.1625712114.

[132] Chen Z, Dempsey DR, Viennet T, Iwase R, Park E, Devreotes PN, et al. Direct positive regulation of PTEN by the p85 subunit of phosphatidylinositol 3-kinase. Proc Natl Acad Sci U S A 2010;107:5471–6. https://doi.org/10.1073/pnas.0908993107.

[133] Song KW, Edgar KA, Hanan EJ, Hafner M, Oeh J, Merchant M, et al. RTK-activated mTORC1, a novel PI3K/Akt signaling pathway. Oncogenesis 2015;4:e26431. https://doi.org/10.3390/ijms22073464.

[134] Gao X, Lowry PR, Zhou X, Depry C, Wei Z, Wong GW, et al. PI3K/Akt signaling and positive feedback enable enzyme kinetics at the membrane to sense dynamic effects of PTEN C-terminal tail phosphorylation. Biophysics 2022. https://doi.org/10.1002/bip.21535.

[135] Myeong J, Park C-G, Suh B-C, Hille B. Compartmentalization of phosphatidylinositol 4,5-bisphosphate metabolism in plasma membrane liquid-ordered/raft domains. Proc Natl Acad Sci U S A 2011;108:202534118.

[136] Lannutti BJ, Meadows SA, Herman SEM, Kashishian A, Steiner B, Johnson AJ, et al. The network of non-coding RNAs in cancer drug resistance. Front Oncol 2018;8:327. https://doi.org/10.3389/fonc.2018.00327.

[137] Marshall JDS, Mellor P, Ruan X, Whitecross DE, Ross RL, Knowles MA, et al. Patient-derived mutations within the N-terminal domains of p85α impact PTEN or Rab5 binding and regulation. Sci Rep 2018;8:7110. https://doi.org/10.1038/s41598-018-25487-5.

[138] Miller SJ, Lou DY, Seldin DC, Lane WS, Neel BG. Direct identification of PTEN-associated complex. Mol Cell 2009;29:5377–88. https://doi.org/10.1038/onc.2011.532.

[139] Winkler DG, Faia KL, DiNitto JP, Ali JA, White KF, Brophy EE, et al. PI3K inhibitor refractory chronic lymphocytic leukaemia and lymphoma: an open-label, single-arm trial. Blood 2011;117:591–4. https://doi.org/10.1182/blood-2010-03-275305.

[140] Alpelisib MA. First global approval. Drugs 2019;79:1249–53. https://doi.org/10.1007/s40266-019-00216-2.

[141] Borus HA, Flinn IW, Patel MR, Fenske TS, Dong C, Brander DM, et al. Umbralisib, a novel PI3Kδ and casine kinase-1c inhibitor, in relapsed or refractory chronic lymphocytic leukaemia and lymphoma: an open-label, phase 1, dose-escalation, first-in-human study. Lancet Oncol 2018;19:486–96. https://doi.org/10.1016/S1470-2045(18)30083-2.

[142] Hanner AB, Kaklamani V, Arteaga CL. Challenges for the clinical development of PI3K inhibitors: strategies to improve their impact in solid cancers. Cancer Discov 2019;9:482–91. https://doi.org/10.1158/2155-9255.CD-18-1175.

[143] Juric D, Castel P, Griffith M, Griffith OL, Won HH, Ellis H, et al. Convergent loss of PI3Kα and PI3Kβ in colorectal cancer progression. J Clin Investig 2015;125:451–65. https://doi.org/10.1172/JCI75431.

[144] Babini S, Rojas L, autonomously computing. J Cell Sci 2013;126:1364–74. https://doi.org/10.1242/jcs.127546.

[145] vanhaesebroeck B, Perry MWD, Brown JR, André F, Okkenhaug K. PI3K inhibitors are finally coming of age. Nat Rev Drug Discov 2021;20:741–69. https://doi.org/10.1038/s41573-021-00209-1.

[146] Corrà F, Agnoletto C, Minotti L, Baldassari F, Volinia S. The network of non-coding RNAs in cancer drug resistance. Front Oncol 2018;8:327. https://doi.org/10.3389/fonc.2018.00327.

[147] Umbralisib, a novel PI3Kδ and casine kinase-1c inhibitor, in relapsed or refractory chronic lymphocytic leukaemia and lymphoma: an open-label, phase 1, dose-escalation, first-in-human study. Lancet Oncol 2018;19:486–96. https://doi.org/10.1016/S1470-2045(18)30083-2.

[148] Babini S, Rojas L, autonomously computing. J Cell Sci 2013;126:1364–74. https://doi.org/10.1242/jcs.127546.

[149] Babini S, Rojas L, autonomously computing. J Cell Sci 2013;126:1364–74. https://doi.org/10.1242/jcs.127546.

[150] Babini S, Rojas L, autonomously computing. J Cell Sci 2013;126:1364–74. https://doi.org/10.1242/jcs.127546.
