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The Hidden Conundrum of Phosphoinositide Signaling in Cancer

Narendra Thapa⁵, Xiaojun Tan¹, Suyong Choi⁵, Paul F. Lambert^{2,3,5}, Alan C. Rapraeger^{1,4,5}, and Richard A. Anderson^{1,5,*}

¹Program in Molecular and Cellular Pharmacology, 1300 University Avenue, Madison, WI 53706, USA

²Department of Oncology, 1300 University Avenue, Madison, WI 53706, USA

³McArdle Laboratory for Cancer Research, 1300 University Avenue, Madison, WI 53706, USA

⁴Department of Human Oncology, 1300 University Avenue, Madison, WI 53706, USA

⁵University of Wisconsin-Madison School of Medicine and Public Health, 1300 University Avenue, Madison, WI 53706, USA

Abstract

Phosphoinositide 3-kinase (PI3K) generation of PI(3,4,5)P₃ from PI(4,5)P₂ and the subsequent activation of Akt and its downstream signaling cascades (e.g. mTORC1) dominates the landscape of phosphoinositide signaling axis in cancer research. However, PI(4,5)P₂ is breaking its boundary as merely a substrate for PI3K and phospholipase C (PLC), and is now an established lipid messenger pivotal for different cellular events in cancer. Here, we review the phosphoinositide signaling axis in cancer, giving due weight to PI(4,5)P₂ and its generating enzymes, the phosphatidylinositol phosphate (PIP) kinases (PIPKs). We highlighted how PI(4,5)P₂ and PIP kinases serve as a proximal node in phosphoinositide signaling axis and how its interaction with cytoskeletal proteins regulates migratory and invasive nexus of metastasizing tumor cells.

Keywords

PI(4,5)P₂; PI(3,4,5)P₃; Akt; PIPKI γ ; PI3K

Phosphoinositides and Phosphoinositide Signaling Axis

Life is the delicate balance of different cellular events orchestrated in a highly regulated and coordinated manner, such as cell cycle progression, survival, apoptosis, cell motility and gene expression [1, 2]. These cellular events are the intricate outcome of signaling pathways

*Correspondence: Richard A. Anderson, 3750 Medical Science Center, 1300 University Ave., University of Wisconsin-Madison, School of Medicine and Public Health, Madison, WI 53706. Phone: 608-262-3753, Fax: 608-262-1257 raanders@wisc.edu.

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that operate in time and space [1]. Among them, phosphoinositide signaling, initiated by the generation of phosphorylated phosphatidylinositol lipid moieties- phosphoinositides- unequivocally occupies a central position in health and disease [3-5]. The present and past decades have seen a tremendous surge in the study of phosphoinositide signaling, the deregulation of which is now one of the established culprits in cancer. The phosphorylation of PI(4,5)P₂ into PI(3,4,5)P₃ by PI3K, leading to the recruitment of protein kinase B (PKB)/Akt and 3-phosphoinositide-dependent protein kinase 1 (PDK1) to the plasma membrane and the initiation of downstream signaling cascades (PI3K/Akt/mTORC1), dominates the landscape of phosphoinositide signaling axis in cancer [3, 4]. Similarly, PLC hydrolysis of PI(4,5)P₂ and subsequent generation of diacylglycerol (DAG) and activation of different isoforms of protein kinase C (PKC), has established its own domain in the landscape of cancer biology [6]. As PI3K/Akt/mTORC1 and DAG/PKC cascades have dominated cancer research, PI(4,5)P₂ and its generating enzymes, the phosphatidylinositol phosphate (PIP) kinases, were mostly considered as regulators of basic cellular functioning [7-9]. The inability of PI(4,5)P₂ to directly recruit and activate oncogenic PDK1 and Akt, despite of its 10-100 fold of abundance than PI(3,4,5)P₃, and the lack of oncogenic mutations activating PIPKs largely dampened PI(4,5)P₂ signaling from the limelight of cancer research. However, PI(4,5)P₂ synthesis is highly regulated at different subcellular compartments by distinct isoforms of PIP kinases (PIPKI/PIPKII) (**Box I**) and their diverse interacting partners from cytoskeletal proteins to adhesion receptors and signaling molecules, implicating PI(4,5)P₂ lipid messenger functions in many cellular events that are integral parts of cancer progression [2, 10-12]. PI(4,5)P₂ and PIPK/PIPKII control of PI3K/Akt signaling, metabolic stress, cytoskeletal reorganization, migratory and invasive nexus are discussed in the subsequent parts of this review, though these represent only a fraction of cellular functions attributed to PI(4,5)P₂ signaling. We summarized phosphoinositide signaling axis in cancer as an integrative role of PI(4,5)P₂ and PI(3,4,5)P₃ lipid messengers and the enzymes generating these lipid messengers (**Figure 1**). We emphasized that PI(4,5)P₂ and PIPKs are an underappreciated conundrum of phosphoinositide signaling axis in cancer that deserve due attention.

Implication of Phosphoinositide Signaling in Cancer

Discovery of phosphoinositide turnover by the pioneering work of the Hokins in 1953 laid the foundation for the study of phosphoinositide signaling in mammalian cells [13]. In early and late 1990s, a series of key discoveries directly linked phosphoinositide signaling to cancer. The introduction of phosphoinositides phosphorylated at the third hydroxyl group of its inositol ring by the Cantley group [14] and others [15], and the establishment of rapid phosphorylation of PI(4,5)P₂ into PI(3,4,5)P₃ by PI3K upon growth factor stimulation, GPCR activation or oncogenic transformation were seminal discoveries that unveiled a new signaling pathway in parallel with PLC-mediated PI(4,5)P₂ conversion into DAG and inositol trisphosphate (IP3) [16]. This was followed by the cloning and characterization of different isoforms of PI3K [16, 17] and the discovery of serine/threonine kinase Akt as a downstream effector of PI(3,4,5)P₃, and mTORC1 downstream of Akt [18]. Later, the tumor suppressor PTEN that turns off the PI3K/Akt signaling by dephosphorylating PI(3,4,5)P₃ back into PI(4,5)P₂ was found commonly lost or mutated in different cancer types [19].

Furthermore, the discovery of a series of somatic mutations in the components of the PI3K/Akt signaling pathway (e.g. catalytic subunit of PI3K, PDK1 and Akt) further consolidated the significance of the PI3K/Akt/mTORC1 signaling axis in cancers [20, 21]. As activated Akt directly inhibits the repertoires of pro-apoptotic proteins (e.g. BAD, BAX, BIM and Caspase-9) and mTORC1 controls cell growth, activation of PI3K/Akt/mTORC1 is an indispensable signaling node in many cancers [3, 4]. Similarly, responding to the availability of oxygen and nutrient (e.g. glucose, ATP and amino acids) in the environment, cell metabolism, and autophagy are the emerging areas of PI3K/Akt/mTORC1 signaling and functions [22]. All of these justify well that the PI3K/Akt/mTORC1 signaling is perhaps one of the most common therapeutic targets for cancer treatment [4].

Deregulated PI3K/Akt Activation in Cancer

Different repertoires of genetic and epigenetic changes that tumor cells acquire contribute to the evasion of controlled regulation of PI3K/Akt/mTORC1 signaling [3, 4, 23, 24]. The most common mechanisms include (I) loss of the PTEN tumor suppressor, (II) gain of somatic mutations in the components of the PI3K-Akt signaling axis, (III) over-expression of PI3K, and (IV) over-expression or overactivation of receptor tyrosine kinases (RTKs) leading to constitutive recruitment and activation of PI3K. PTEN loss is commonly observed in various cancer types, resulting in aberrant activation of PI3K/Akt [25]. Besides PTEN loss, somatic mutations causing truncation of PTEN protein and loss of its function are reported in tumor-prone germline diseases [25]. The Cancer Genome Atlas (TCGA) genome scale analysis shows PI3KCA (gene encoding p110 α , the catalytic subunit of class I PI3K) as one of the eight most frequently mutated genes in cancers [26-28]. These mutations occur at the interface between the catalytic (p110 α) and adaptor subunit (p85) of PI3K, and generally abrogate the inhibitory effect of the adaptor subunit on the catalytic subunit of PI3K [24, 26, 27]. More than 75% of these activating mutations reside either in the helical or catalytic domain of P110 α and are called “Hot Spot” mutation sites [26]. These activating mutations are reported only in the P110 α catalytic subunit. Additionally, overexpression or mutational activation of different tyrosine kinase receptors is responsible for aberrant activation of phosphoinositide signaling in cancer [3, 23]. These overexpressed receptor tyrosine kinases (e.g. PDGF, EGFR, c-MET, and IGFR) generally undergo homo- or hetero-dimerization, even in the absence of ligand binding. This creates the docking sites which are phosphorylated tyrosine residues in the context of YXXM motif in their cytoplasmic domains that recruit PI3K enzyme to plasma membrane (SH2 domain of adaptor subunit, p85 specifically binds to these YXXM motifs mediating the recruitment of the catalytic subunit) [24]. For example, EGFR/ERBB1/HER1, ERBB2/HER2, ERBB3/HER3 contain multiple docking sites for PI3K and their activation triggers dramatic increase in PI3K/Akt signaling [29]. Additionally, adaptor proteins such as insulin receptor substrate (IRS), Shc, and growth factor receptor-bound protein 2 (Grb2) and the E3 ubiquitin ligase Cbl also provide docking sites for the PI3K adaptor subunit, p85 [3, 4, 23].

Various components of the PI3K/Akt/mTORC1 signaling axis (e.g. PI3K enzyme, PDK1, Akt and mTORC1) are actively targeted for therapeutic treatment of cancers [4, 30, 31]. More than 100 drugs (e.g. Buparlisib, Duvelisib, TGR1202, Copanlisib, BEZ235, RP6530, PWT33597, CUDC-907, PI-103, TG100-115I and NK1117) targeting PI3K/Akt/mTORC1

signaling cascade are undergoing or progressing towards different stages of clinical trials (phase I, II and III) [30, 31]. Everolimus, temsirolimus and idelalisib represent the handful of anticancer drugs that have been approved by FDA so far for therapeutic treatment of cancers. Everolimus and Temsirolimus target mTORC and are used for the treatment of renal cell carcinoma, astrocytoma, and HER2-negative breast cancer, whereas Idelalisib targets P110 δ and is used for leukemia and lymphoma [32, 33]. The readers are referred to <https://clinicaltrials.gov/> to learn more about clinical trials and the outcome of specific drugs in cancer treatments. It is becoming clear that targeting the PI3K/Akt/mTORC signaling axis at multiple points (e.g. PI3K and mTOR in dual therapy) or in combination with inhibitors of TKRs (e.g. HER family tyrosine kinase inhibitors in combination therapies) and other parallel nodal pathways (e.g. MAPK) is more effective to inhibit tumor growth and prevent emergence of regulatory feedback loops and development of drug resistance [34-36].

PIPKI/PIPKII Control of PI3K/Akt Activation in Cancer

Given the diversity in the mechanism of PI3K/Akt activation (e.g. repertoire of oncogenic mutations in PI3K to loss of PTEN to overexpression/overactivation of tyrosine kinase receptors), identifying the common node for all the diverse mechanisms could pave the way for developing more effective therapeutic approach for blocking PI3K/Akt signaling axis in cancer. Could the generation of PI(4,5)P₂ substrate at specific sub-cellular compartments provide the common regulatory node upstream of PI3K/Akt activation? Do PI3K collaborate with PIPKI/PIPKII for *de novo* synthesis of PI(4,5)P₂ and PI(3,4,5)P₃, or do they utilize the pre-existing pools of PI(4,5)P₂ in plasma membrane/endo-membranes for PI(3,4,5)P₃ generation and sustenance of PI3K/Akt signaling in cancer? Although, PI(4,5)P₂ is the predominant phosphoinositide in the plasma membrane, the availability of free PI(4,5)P₂ may be rate limiting as PLC hydrolyzes the bulk of PI(4,5)P₂ in the plasma membrane at the vicinity of activated growth factor receptors or adhesion receptors. This could be circumvented by spatial recruitment of PIPKI/PIPKII along with PI3K to the plasma membrane for Akt activation.

Among all PIPKI isoforms, PIPKI α and PIPKI γ appear to regulate PI3K/Akt signaling, though PIPKI β negatively regulates PI3K/Akt signaling [37]. Increased association of PI3K with PIPKI γ upon growth factor stimulation of cells suggests coupling of PI(4,5)P₂ and PI(3,4,5)P₃ synthesis for Akt activation [37]. As upstream activators of PI3K/Akt include integrins and RTKs, PIPKI γ interaction with talin and the proto-oncogene Src facilitates the recruitment of PIPKI γ to the vicinity of integrin-mediated adhesion complexes and activated RTKs, respectively [37]. In both conditions, PIPKI γ potentially provides *de novo* PI(4,5)P₂ to promote and sustain the PI3K/Akt signaling downstream of activated integrins and RTKs [37]. Overexpression of PIPKI γ along with Src sustains the PI3K/Akt signaling and oncogenic growth, where Src serves as a bridging molecule for incorporating PIPKI γ and PI3K into the same complex [37] (**Figure 2**). Alternatively, the assembly of phosphoinositide kinases, PI4K, PIP5KI and PI3K, all into the same complex by a scaffold protein could provide a self-contained mechanism to activate and sustain PI3K/Akt signaling in cancer cells. Such a mechanism is well established in the regulation of MAPK signaling pathway, a companion of PI3K/Akt signaling [38]. IQGAP1 which scaffolds the molecules in MAPK pathway in certain cell types also associate with PI4P, PIPKI and PI3K, and could

potentially serve as scaffolding molecule to streamline and self-sustain PI3K/Akt signaling axis in cancer [39]. A similar mechanism could exist in coupling PI(4,5)P₂ generation with PLC-mediated hydrolysis of PI(4,5)P₂ and PKC activation in cancer. Unlike PIPKI, the contribution of PIPKII enzyme in PI3K/Akt/mTORC1 appears less dominant and counteracting [40] as the majority of PI(4,5)P₂ is synthesized by PIPKI. However, studies in *Drosophila* still lend support to the role of PIPKII in cell growth and Akt/mTORC1 signaling ([41]. Additionally, phosphatidic acid which activates different isoforms of PIPKI inhibits endogenous inhibitor of mTORC1, suggesting that PIPKI provide another level of mechanism in regulating the PI3K/Akt/mTORC1 signaling in cancer [42, 43].

PI(4,5)P₂ and PIPKI/PIPKII in Cancer

As PI(3,4,5)P₃ generation from PI(4,5)P₂ and PLC-mediated PI(4,5)P₂ hydrolysis/PKC activation take the center stage of phosphoinositide signaling axis in cancer, PI(4,5)P₂ and PIPKs largely gained recognition as key regulator of basic cellular functions, such as in the regulation of ion channels and transporters, neuronal transmission, endocytosis, exocytosis, phagocytosis, vesicle trafficking, reorganization of cytoskeletal proteins, cell polarity, gene expression and nuclear events [2, 9, 10, 44]. This reconciles with the fact that PI(4,5)P₂ is the most abundant and an integral lipid moiety of plasma membrane and endo-membranes interrogating with diverse protein-interactomes ranging from ion channels to cytoskeletal proteins and DNA polymerases, and also a substrate for the generation of other second messengers/metabolites [2]. As a result, many disorders, including channelopathies, mental retardation, bipolar diseases, schizophrenia, Alzheimer disease, diabetes, ciliopathies, and Lowe syndrome are associated with deregulation of PI(4,5)P₂ signaling or PI(4,5)P₂ metabolism or loss of PI(4,5)P₂ regulation of protein functions [45, 46]. However, many cellular functions directly attributed to cancer and cancer progression, such as cytoskeletal reorganization and cell motility/invasiveness are under the direct control of PI(4,5)P₂ lipid messenger and PIPKIs as discussed below.

Unlike PI3K, mutational activation of neither PIPKI nor PIPKII has been reported in cancers, although mutational loss of the PIPKI γ kinase activity is found in congenital contractual syndrome type 3 (LCCS3) [47]. Similarly, different from PI3K, ectopic expression of PIPKI or PIPKII alone usually does not induce oncogenic transformation, although overexpression of PIPKI γ variants in cooperation with other oncogenes has been reported to promote oncogenic growth [48]. However, up-regulated expression of PIPKI and PIPKII kinases and their direct implication in cancer progression is emerging as different cellular events regulated by PI(4,5)P₂ and PIPKI/PIPKII lipid kinases are integral parts of cancer progression. In tissue microarrays of breast cancer tissues, increased expression of PIPKI γ correlates with EGFR expression in triple-negative breast cancer tissues [49]. Survival of breast cancer patients inversely correlates with PIPKI γ expression. Corroborating this, xenograft studies in mice show an essential role of PIPKI γ in tumor growth and metastasis [50]. Activation of EGFR phosphorylates tyrosine residue in C-terminus of PIPKI γ (Y639) and this appears essential for the role of PIPKI γ in tumor growth and metastasis [50]. The PIPKI γ /EGFR nexus is further fine-tuned by PIPKI γ i5, a splicing variant of PIPKI γ , which controls the down regulation of EGFR via modulating the SNX5/Hrs lysosomal degradation pathway [51, 52]. However, the functional role of

PIPKI γ i5 regulation of EGFR expression and its impact on cancer progression and metastasis remain to be defined. The catalogue of somatic mutations in cancer (COSMIC) database shows increased copy number of PIPKIA along with PI4KB, PIPK3C2B and AKT3, molecules of the phosphoinositide signaling cascade in breast cancer [53]. More comprehensive studies in the future will define how PIPKIs are involved in regulating oncogenic phosphoinositide signaling nexus in cancer, as overexpression of PIPKI/PIPKII lipid kinases alone is not sufficient in activating and sustaining the PI3K/Akt signaling nexus [48]. Like PIPKIs, deep transcriptome sequencing shows increased expression of PIPKII in cancer cells and cancer tissues [54]. PIP4KII α and PIP4KII β are over-expressed in HER2-positive breast cancer tissues [55], and ACGH array shows the amplification of PIP4KII β gene as part of HER2 amplicon in cancer [56]. These lipid kinases appear essential for tumor growth in the background of p53 loss or mutations. Recent study indicates a novel function of PIP4KII β as a GTP-sensor to regulate cell metabolism in cancer [57]. The use of a shRNA library targeting all known modulators of phosphoinositide metabolism has identified PIP4KII α as gene required for leukemia, indicating PIP4KII α as a potential therapeutic target for hematological malignancies [58]. However, knockdown of PIPKII β is associated with strongly induced basal and insulin-stimulated PI(3,4,5)P₃ level and Akt activation [55]. This points out that, unlike PIPKI, the role of PIPKII in cancer is independent of the PI3K/Akt/mTORC1 signaling axis. Tumor cells encounter oxidative stress as a result of oncogene expression or loss of tumor suppressors that upregulates PI5P levels, and PIPKII lipid kinases are required for its conversion into PI(4,5)P₂ by a non-canonical route [40, 59]. PIP4KII β has also been reported to regulate nuclear PI5P and gene expression [60]. This highlights PIPKII as stress-regulated lipid kinases required for cancer cells to overcome oxidative stress and maintaining the homeostasis of reactive oxygen species [61] (**Figure 3**).

PI(4,5)P₂ and PIPKI/PIPKII Control of Cell Polarity and Cell Motility

Maintenance of cell polarity is one of the most fundamental properties of epithelial cells, and loss of cell polarity is a hallmark of epithelial cancer [62, 63]. Given the ability of PI(4,5)P₂ to provide docking sites for a myriad of lipid-protein interactions on the plasma membrane and the ability of PIPKI/PIPKII lipid kinases to interrogate with a diverse array of protein interactomes, PI(4,5)P₂ and PIPKI/PIPKII serve as integral parts of the epithelial cell polarity program. For example, PI(4,5)P₂ is the landmark phosphoinositide entity of apical surface and an alteration in distribution of PI(4,5)P₂ from apical to basolateral surface in the three-dimensional culture of epithelial cells which disrupts the lumen formation/epithelial morphogenesis and affects the polarized secretion of basement membrane proteins [64, 65]. Many other studies also support a critical role of PI(4,5)P₂ phosphoinositide molecule in maintaining apical cell-polarity [66]. In contrast, PI(3,4,5)P₃ and PI3K function as critical determinant of the basolateral domain of epithelial cells [67]. Epithelial cells lose E-cadherin/cell polarity and gain pro-migratory/pro-invasive phenotypes as results of oncogenic transformation, expression of E-cadherin transcriptional repressors or activation of EMT agonists as seen in many cancer cells [62]. The same PI(4,5)P₂ and PI(3,4,5)P₃ molecules also participate in regulating the myriads of cellular events essential for cell motility and invasive program. This indicates that PI(4,5)P₂ and PI(3,4,5)P₃ function at

conjunction of epithelial cell polarity as well as pro-migratory/pro-invasive nexus of cancer cells. How are these two seemingly opposite cellular functions regulated by phosphoinositide signaling? In this regard, PIPKI γ and PI(4,5)P₂ deserve special attention as it provides the molecular platform for the assembly of not only E-cadherin-mediated adherens junctions at cell-cell contact sites in epithelial cells but also integrin-mediated adhesion complex at the interface of cell-extracellular matrix interaction sites [68, 69].

In epithelial cells, cell polarity is maintained by E-cadherin-mediated adherens junctions between the adjacent cells [70]. The precise regulation of targeting, recycling and endocytosis of E-cadherin molecules control the integrity of adherent junctions and epithelial cell polarity [70]. PIPKI γ i2, a specific variant of PIPKI γ , integrates the clathrin adaptor protein AP1B and the evolutionarily conserved vesicle trafficking protein complex, exocyst, to regulate basolateral trafficking of E-cadherin for cell polarity and epithelial morphogenesis [68, 71-73] (**Figure 4**). In this process, PIPKI γ functions as a molecular scaffold by bridging E-cadherin molecules with AP1B and the exocyst complex [68]. This facilitates targeting and trafficking of E-cadherin cargo to adherens junctions. Tyrosine-based sorting motifs in the context of YXXQ in the C-terminus of PIPKI γ (YSPL and YSAQ in PIPKI γ i2) recruit the adaptor protein AP1B for basolateral sorting of recycling endosomes. However, these motifs also recruit AP2 clathrin adaptor protein to mediate endocytosis of E-cadherin from the plasma membrane [68]. The recruitment of AP2 clathrin adaptor protein to YXXQ motif in PIPKI γ is favored when the tyrosine residue in the motif is unphosphorylated [68, 70]. However, the precise mechanism that governs the phosphorylation of these motifs and selective recruitment of AP1B or AP2 remains poorly understood. Along with clathrin adaptor proteins, exocyst complex is another direct interacting partner of PIPKI γ that mediates the basolateral targeting of E-cadherin molecules in polarized epithelial cells [73]. The ability of two exocyst subunits, Sec3 and Exo70 to interact with both PI(4,5)P₂ and PIPKI γ on the plasma membrane/endo-membrane facilitates the basolateral targeting of E-cadherin molecules in recycling or synthetic cargo [8]. The expression of Exo70-mutant deficient in PI(4,5)P₂ binding severely impairs the E-cadherin targeting to developing adherens junctions [73]. Further, a specific variant of PIPKI γ , PIPKI γ i5, in coordination with SNX5, controls the lysosomal sorting and degradation of E-cadherin molecules [74], although the implication of PIPKI γ i5/SNX5 complex in E-cadherin degradation and their role in cancer remains poorly understood.

As epithelial cells lose E-cadherin-mediated adherens junctions and cell polarity, PIPKI γ and PI(4,5)P₂ engage in the development of dynamic focal adhesion complexes, and control the migratory and invasive nexus of different cancer cells [39, 49, 71, 72, 75] (**Figure 4**). One of the key functions of PI(4,5)P₂ lipid messenger and PIPKI γ is to promote the recruitment of cytoskeletal proteins at developing adhesion complex. Talin, vinculin and FAK, all harbor the patches of basic residues that bind to PI(4,5)P₂. Additionally, the spatial generation of PI(4,5)P₂ at adhesion complex promotes the recruitment and activation of these molecules by relieving intra-molecular constraints imposed to them, thus establishing structurally and functionally competent adhesion complex [76]. PIPKI γ i2, the focal adhesion targeting and talin interacting variant of PIPKI γ putatively provides PI(4,5)P₂ at developing nascent adhesion complexes in adhering and migrating cells. However, PIPKI γ i2

also competes with $\beta 1$ integrin for talin binding, indicating that the assembly of PIPKI γ 2, talin and integrin at the adhesion complex is a tertiary complex facilitated by PI(4,5)P₂ lipid messenger. Specifically, the recruitment of talin to cytoplasmic domain of $\beta 1$ integrin at adhesion complexes is a key for initiation of “inside-out” and “outside-in” signaling, an integral part of focal adhesion signaling in adhering and migrating cells [77]. Additionally, PIPKI γ association with the exocyst complex and talin, and PI(4,5)P₂ generation facilitates the polarized delivery of integrins required for developing nascent adhesion complexes at leading edge of migrating cells [71, 72]. PIPKI γ also co-works with its interacting partner IQGAP1 to promote actin polymerization and cell migration [39]. These illustrate PIPKI γ and PI(4,5)P₂ function at the conjunction of the epithelial cell polarity program and migratory/invasive nexus of cancer cells.

PI(4,5)P₂ Control of Cytoskeletal Reorganization

Although PI(4,5)P₂ could not shine on par with PI(3,4,5)P₃ lipid messenger in growth signaling in cancer, PI(4,5)P₂ signaling has been established itself as a key regulator of cytoskeletal machinery and cytoskeletal reorganization in both physiological and pathological conditions [78, 79]. Many excellent reviews ([78] serve as great resources for understanding PI(4,5)P₂ regulation of cytoskeleton associated and regulatory proteins. Importantly, cancer cells display upregulated expression of many cytoskeleton associated and regulatory proteins and they are essential for their migratory and invasive property as one third of proteins that are induced in metastatic cancers are related to adhesion and cytoskeletal reorganizations [80]. Spatial and temporal reorganization of actin cytoskeleton controls different cellular events involved in metastatic cascades, such as conversion of indolent epithelial cells into mesenchymal state, detachment from the primary tumor, migration/invasion and extravasation for secondary/tertiary growth [81]. The first evidence of intimate association between cytoskeletal protein and phosphoinositide was demonstrated by Anderson and Marchesi [82]. The subsequent studies established PI(4,5)P₂ as the most dominant lipid moiety in regulating cytoskeletal organization via modulating the activities of diverse arrays of cytoskeleton associated and regulatory proteins [78]. For example, PI(4,5)P₂ inhibits the actin binding and depolymerizing activity of ADF/cofilin. Similarly, PI(4,5)P₂ inhibits capping proteins like gelsolin, which prevent the addition and loss of actin monomers from the end of actin polymer. Besides these examples, PI(4,5)P₂ also directly regulates a plethora of proteins involved in regulating cytoskeletal reorganization such as spectrin, dynamin, myosin X, ezrin, radixin, spectrin, gelsolin, profilin, actin, N-WASP, myosin, MARCKS, annexins and α -actinin [11]. The fine-tuned activity of these actin binding and regulating proteins controls the nucleation/formation of actin filaments and their polymerization. Furthermore, the coordinated interplay between these processes controls the formation as well as the geometry of actin filaments in cellular machineries like cell adhesion complex, lamellipodia, filopodia and invadopodia which are essential for migrating and invading tumor cells [81].

PI(4,5)P₂ regulation of actin nucleating activity of Arp2/3 protein complex via Wiskott-Aldrich syndrome protein (WASP) and Rho family small GTPases is the most extensively studied in the context of tumor cells migration/invasion and metastasis [81, 83-85]. This depends upon PI(4,5)P₂ along with active Cdc42 and Rac1 binding to the N-terminus of N-

WASP and PI(4,5)P₂ binding to basic motifs [84, 85]. This leads to exposition of intramolecularly masked VCA domain in N-WASP which promotes the binding of VCA domain to Arp2/3 and G-actin and initiates the nucleation of actin polymer from the sides of existing actin filaments. Furthermore, Rho GTPases interacts with PIPKI and activates the synthesis of PI(4,5)P₂ lipid messenger required for the activation of Arp2/3 complex, establishing the self-contained molecular complex for Arp2/3 mediated actin nucleation and polymerization (**Figure 4**). This process plays a pivotal role in the formation of lamellipodia and invadopodia, key cellular machineries employed by migrating and invading tumor cells. Consistently, the Arp2/3 complex, along with Rho GTPases, is elevated in the majority of cancers [86], although correlation between up-regulated expression of cytoskeleton associated and regulatory proteins with PI(4,5)P₂ and PIPKI remains poorly defined. As tubulin-targeting has been a successful approach in cancer therapy [87] intervening PI(4,5)P₂ and PIPKI functions in actin cytoskeleton could be similarly exploited in developing drugs targeting the cytoskeletal machinery for tumor therapy.

Concluding Remarks

Phosphoinositide signaling represents one of the most fundamental signaling nexus involved in many cellular functions in health and disease. Given the broad horizons and extraordinary surges in phosphoinositide study over the past decades, it has become a tough job to emphasize particular aspects of phosphoinositide signaling and its cellular functions that are important in cancer. It is plausible that PI(4,5)P₂ and PIPKIs have been overshadowed by the well-known PI3K/(3,4,5)P₃/Akt/mTORC1 axis in cancer. However, it is also important to notice and pinpoint the aspects of PI(4,5)P₂ and PIPKI/PIPKII that are directly and indirectly implicated in cancer progression. In this review, we emphasized and attempted to incorporate the functional role of PI(4,5)P₂ and generating enzymes in the context of cancer and oncogenic phosphoinositide signaling axis. As discussed here, PI(4,5)P₂ and PIPKI/PIPKII are companions of the PI3K/PI(3,4,5)P₃/Akt/mTORC1 signaling axis in cancer. However, many other aspects of phosphoinositide signaling that are equally as important in cancer, such as cell cycle regulation, cell survival, apoptosis, anoikis evasion, cancer stem cells, autophagy and nuclear signaling, remain uncovered in this review. Importantly, the future attempt to target the phosphoinositide signaling nexus in cancer should also consider PI(4,5)P₂ lipid messenger and relevant lipid kinases (see Outstanding Question Box). Targeting the spatiotemporal generation of PI(4,5)P₂ lipid messenger could be exploited to target PI3K/Akt/mTORC1 axis in cancer as this would impair the most proximal node of oncogenic phosphoinositide signaling axis in cancer, and it might overcome the possible variations in the efficacy of anti-cancer drugs targeting PI3K and Akt due to the bewildering arrays of mutations in these target molecules. Optimism remains high in phosphoinositide research as drugs targeting specific catalytic subunit of PI3K may also come into clinic within couple of years. However, the precise understanding of mechanism regulating spatial activation of PI3K/Akt/mTORC1 signaling in cancer would provide an important avenue for the development of more effective therapeutic approaches for cancer treatment.

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Glossary

Phosphatidylinositol and Phosphoinositides

Phosphatidylinositol (PI) is a lipid signaling molecule in the inner leaflet of plasma membrane. It is comprised of two fatty acid chains linked to a glycerol moiety and a water soluble, inositol head group. The hydroxyl groups (at the –3, –4 and –5 positions) in the inositol ring can be phosphorylated in different combinations resulting in seven distinct and inter-convertible phosphoinositides: PI3P, PI4P, PI5P, PI(3,4)P₂, PI(3,5)P₂, PI(4,5)P₂ and PI(3,4,5)P₃. This is achieved by different phosphatidylinositol or phosphoinositide kinases and phosphatases that are expressed in the cells. These molecules account for less than 1-5% of the total phospholipid content of the mammalian cells. Phosphatidylinositol phosphorylated on 4th position (PI4P) of inositol ring is the most abundant of these phosphoinositides in the cell. It is followed by phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂), which is phosphorylated at 4th and 5th positions.

Phosphatidylinositol Phosphate Kinases (PIPK)

These are lipid kinases that phosphorylate phosphoinositides (e.g. PI3P, PI4P and PI5P) in the cells and are classified into type I, II and III. Type I and type II PIPK generate PI(4,5)P₂ in the cells.

Phosphatidylinositol -4,5-Bisphosphate 3-Kinases (PI3K)

These are lipid kinases that phosphorylate 3-OH group of the inositol ring in phosphatidylinositol to form PI3P, PI(3,4)P₂ and PI(3,4,5)P₃. Depending upon their structure and substrate specificity, these are categorized into class I, II and III. Class I is the most dominant member in cell signaling and cancer biology, and are activated downstream of receptor tyrosine kinases, G-protein coupled receptors and adhesion receptors. Class IA PI3K are heterodimeric molecules composed of regulatory (five variants: p85α, p85β, p85γ, p55α and p50α) and catalytic (three variants: p110α, p110β and p110δ) subunits. Class IB PI3K is composed of p110γ catalytic subunit. PI3K enzymes with catalytic subunits p110α and p110β are the most ubiquitously expressed. Class II PI3K produces PI3P and PI(3,4)P₂ whereas class III PI3K produces only PI3P.

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Box I: Phosphatidylinositol Phosphate Kinases (PIPKs) Synthesizing PI(4,5)P₂

PIP kinases (PIPKs) are responsible for synthesizing PI(4,5)P₂ in different sub-cellular compartments in mammalian cells, which is consistent with diverse cellular functions of PI(4,5)P₂ lipid messenger [9, 10]. These lipid kinases are classified into type I PIPK (PIPKI), type II PIPK (PIPKII) and type III PIPK (PIPKIII), but PIPKIII is involved in PI(3,5)P₂ synthesis (**Figure I**). PIPKI utilizes PI4P as a substrate for the generation of PI(4,5)P₂ and is largely responsible for synthesizing the majority of PI(4,5)P₂ in mammalian cells. PIPKII utilizes PI5P as a substrate to produce PI(4,5)P₂ and it appears more important in controlling cellular levels of PI5P rather than the production of PI(4,5)P₂. In mammalian cells, both PIPKI and PIPKII exist in three isoforms, α , β and γ (PIPKI α , PIPKI β , PIPKI γ , PIPKII α , PIPKII β and PIPKII γ). These kinases display highly homologous catalytic domains, with divergent N- and C-termini, which is key for their interaction with specific binding partners, differential sub-cellular targeting, and functional divergence [10]. PIPKI γ is the most complex isoform among PIPKs, consisting of several splice variants (e.g. PIPKI γ i1, PIPKI γ i2, PIPKI γ i4 and PIPKI γ i5) targeted to different sub-cellular compartments and performing distinct cellular functions [9, 44].

Outstanding Question Box

- How is PI(3,4,5)P₃ generation regulated for the sustenance of PI3K/Akt/mTORC1 signaling in cancers? Does it always need a spatiotemporal production of PI(4,5)P₂ as a substrate for PI3K to activate Akt? Could the spatiotemporal generation of PI(4,5)P₂ by PIPKI serve as a common upstream node for the oncogenic PI3K/Akt/mTORC1 pathway?
- How do PI(4,5)P₂, PI(3,4,5)P₃ and their generating enzymes work together to control different aspects of cellular functions crucial for cancer progression? The precise mechanisms of PI(4,5)P₂ and PI(3,4,5)P₃ regulation of cell cycle, cell survival, apoptosis, anoikis evasion and stemness trait would provide the important platform for uncovering the collective role phosphoinositide lipid messengers in cancers.
- Does phosphatidic acid suppresses the endogenous mTORC1 inhibitor by activating PIPKI? Could PIPKI activate mTORC1 independently of PI(3,4,5)P₃ generation or does it always activate mTORC1 signaling through the PI3K/Akt cascades?
- Does constitutive activation of PI3K/Akt/mTORC1 depend upon the molecular assembly of PI3K and PIPKI? What are the factors that regulate the molecular assembly of PIPKI and PI3K? Are PIPKI and PI3K assembled in a unique way in cancers addicted to PI3K/Akt/mTORC1 signaling? How to specifically target these unique factors for cancer treatments?

Trends Box

1. Although the oncogenic PI3K/Akt/mTORC1 cascade is well established in cancer, PI(4,5)P₂ and PIPKI/PIPKII functions are also integral parts of cancer progression.
2. Phosphoinositide signaling axis in cancer is collectively regulated by PI(4,5)P₂ and PI(3,4,5)P₃ lipid messengers and their generating enzymes PIPKs and PI3K, both of which are overexpressed in cancers.
3. PI(4,5)P₂ and PIPKI/PIPKII not only regulate cell polarity, motility, and invasion, but they also control PI3K/Akt activation in cancers.
4. Co-targeting of the PI(4,5)P₂ signaling nexus may enhance the efficacy of canonical anti-cancer drugs targeting PI3K/Akt.

Figure 1. Collective Role of PI(4,5)P₂ and PI(3,4,5)P₃ Lipid Messengers and Phosphoinositide Kinases in Cancer Progression

PI(4,5)P₂, PIPKs, and PI(4,5)P₂ effectors regulate different cellular functions, including cytoskeletal reorganization, cell migration, invasion, and basic cellular functioning. PI(4,5)P₂ is predominantly synthesized by type I PIP kinases (PIPKI α , PIPKI β and PIPKI γ) and it functions as a substrate of PI3K for PI(3,4,5)P₃ generation downstream of activated receptors. PI(3,4,5)P₃ generation and activation of Akt/mTORC1 is critical for cell growth, survival, metabolism and inhibition of the repertoire of proteins involved in cell apoptosis. Different cellular events involved in cancer progression is collectively regulated

by PI(4,5)P₂ and PI(3,4,5)P₃ lipid messengers together with their generating enzymes.
GPCR, G-protein coupled receptor; RTK, receptor tyrosine kinase.

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Figure 2. PIPKI γ Assembly with Activated Receptor Tyrosine Kinases and Adhesion Receptors for PI(4,5)P₂ Synthesis and PI3K/Akt Activation

PIP KI γ recruitment to activated integrins at the adhesion complex and activated receptor tyrosine kinases (RTKs) may provide the mechanism for *de novo* synthesis of PI(4,5)P₂ for PI(3,4,5)P₃ generation and Akt activation. PIP KI γ interaction with talin provides selective advantage for PIP KI γ among other PIP KI isoforms to be recruited at the vicinity of activated integrins upon cell stimulation with extracellular matrix proteins. Similarly, interaction with Src promotes PIP KI γ recruitment to activated RTKs for PI(4,5)P₂ and PI(3,4,5)P₃ generation and Akt activation.

Figure 3. Distinct Roles of PIPKI and PIPKII in Cancer Progression

As PIPKI is predominantly involved in PI(4,5)P₂ generation, its role in cancer is coupled with PI(3,4,5)P₃ generation and Akt activation. PI(4,5)P₂ and its effectors, along with PI(3,4,5)P₃, activated Akt and its downstream effector molecules, control various cellular functions, including cell survival, cell growth, apoptosis inhibition, and cytoskeletal reorganization, all of which are required for cancer progression. PIPKII function in cancer is associated with balancing the metabolic stress. The loss of tumor suppressor or oncogenic insults results in increased PI5P generation which is then converted into PI(4,5)P₂ by PIPKII for stress alleviation. In the absence of PIPKII, increased accumulation of PI5P promotes cell senescence.

Figure 4. PIPKI γ Plays Key Role in Epithelial Polarity, Reorganization of Actin Cytoskeleton and Formation of Adhesion Complexes

PIPKI γ , in association with polarized vesicle trafficking complex (AP1B and exocyst), controls E-cadherin trafficking to adherens junctions in polarized epithelial cells. However, in malignant cells, which have lost cell polarity, PIPKI γ and PI(4,5)P₂ play pivotal roles in controlling cytoskeletal reorganization and adhesion complexes, which are key for migrating and invading tumor cells.

Type I PIP Kinase (PIPKI)

Type II PIP Kinase (PIPKII)

Figure I. PIP kinases synthesizing PI(4,5)P₂ in mammalian cells

PI(4,5)P₂ in mammalian cells is primarily synthesized through phosphorylation of the fifth hydroxyl group on the inositol ring of PI(4)P (predominant substrate) by type I PIP kinases (PIPKI) of which there are three genes, PIPKI α , PIPKI β and PIPKI γ . Type II PIP kinases (PIPKII) synthesize PI(4,5)P₂ by phosphorylating the fourth hydroxyl group of PI(5)P (minor substrate) which is increased by metabolic stress or oncogene expression. PIPKII is also further classified into PIPKII α , PIPKII β and PIPKII γ . PIPKI γ is the most complex isoform of PIPKI and displays various post-transcriptional splicing variants differing in their C-tails which specify their interactions with distinct binding partners.