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) dominate the landscape of the 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 various 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 (PI) phosphate (PIP) kinases (PIPKs). We highlight how PI(4,5)P₂ and PIPKs serve as a proximal node in the phosphoinositide signaling axis and how interaction with cytoskeletal proteins regulates the migratory and invasive nexus of metastasizing tumor cells.

**Phosphoinositides and Phosphoinositide Signaling Axis**

Life is a delicate balance of various 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 that operate in time and space [1]. Among them, phosphoinositide (see Glossary) signaling, initiated by the generation of phosphorylated PI 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 [3,4]. Similarly, PLC hydrolysis of PI(4,5)P₂ and the subsequent generation of diacylglycerol (DAG) and activation of various 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 PIPKs, were mostly considered regulators of basic cellular functioning [7–9]. The inability of PI(4,5)P₂ to directly recruit and activate oncogenic PDK1 and Akt, despite its 10–100-fold greater 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 PIPKs (PIPKI/PIPKII) (Box 1) 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 PIPKI/PIPKII functions are also integral parts of cancer progression.

The 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 PIPK and PI3K, both of which are over-expressed in cancer. PI(4,5)P₂ and PIPK/PIPKI not only regulate cell polarity, motility, and invasion but also control PI3K/Akt activation in cancer. Co-targeting of the PI(4,5)P₂ signaling nexus may enhance the efficacy of canonical anticancer drugs targeting PI3K/Akt.

**Trends**

Although the oncogenic PI3K/Akt/mTORC1 cascade is well established in cancer, PI(4,5)P₂ and PIPK/PIPKI functions are also integral parts of cancer progression.

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control of PI3K/Akt signaling, metabolic stress, cytoskeletal reorganization, and the migratory and invasive nexus are discussed in the subsequent sections of this review, although these represent only a fraction of the cellular functions attributed to P(4,5)P2 signaling. We summarize the phosphoinositide signaling axis in cancer as an integrative role of P(4,5)P2 and P(3,4,5)P3 lipid messengers and the enzymes generating these lipid messengers (Figure 1). We emphasize that P(4,5)P2 and PIPKs are an underappreciated conundrum of the phosphoinositide signaling axis in cancer that deserve attention.

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### Box 1. PIPKs Synthesizing P(4,5)P2

PIPks are responsible for synthesizing P(4,5)P2 in various subcellular compartments in mammalian cells, which is consistent with the diverse cellular functions of the P(4,5)P2 lipid messenger [9,10]. These lipid kinases are classified into type I (PIPKI), type II (PIPKII), and type III (PIPKIII), but PIPKIII is involved in P(3,4,5)P3 synthesis (Figure 1). PIPKII utilizes PtdIns(4)P as a substrate for the generation of P(4,5)P2 and is largely responsible for synthesizing the majority of P(4,5)P2 in mammalian cells; PIPKII utilizes PtdInsP as a substrate to produce P(4,5)P2, and appears to be more important in controlling cellular levels of PtdInsP rather than the production of P(4,5)P2. In mammalian cells, both PIPKI and PIPKII exist in three isoforms: α (PIPKIα, PIPKIIα, and PIPKIIIα), β (PIPKIβ, PIPKIIβ, and PIPKIIIβ), and γ (PIPKIγ, PIPKIIγ, and PIPKIIIγ). These kinases display highly homologous catalytic domains with divergent N and C termini, which is key for their interaction with specific binding partners, differential subcellular targeting, and functional divergence [10]. PIPKIIγ is the most complex isoform among the PIPKs, comprising several splice variants (e.g., PIPKIIγ1, PIPKIIγ2, PIPKIIγ4, PIPKIIγ5) targeted to different subcellular compartments and performing distinct cellular functions [9,44].

Figure I: Phosphatidylinositol (PI) Phosphate (PIP) Kinases (PIPks) Synthesizing P(4,5)P2 in Mammalian Cells. P(4,5)P2 in mammalian cells is primarily synthesized through phosphorylation of the fifth hydroxyl group on the inositol ring of PtdIns(4)P (predominant substrate) by type I PIPK (PIPKI), for which there are three genes: PIPKIα, PIPKIβ, and PIPKIγ. Type II PIPK (PIPKII) synthesizes P(4,5)P2 by phosphorylating the fourth hydroxyl group of PtdInsP (minor substrate), which is increased by metabolic stress or oncogene expression. PIPKII is also further classified as PIPKIα, PIPKIβ, and PIPKIγ. PIPKIγ 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.
The 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 the 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 to PI(3,4,5)P3 by PI3K in growth factor stimulation, G protein-coupled receptor (GPCR) activation, and oncogenic transformation were seminal discoveries that unveiled a new signaling pathway in parallel with PLC-mediated PI(4,5)P2 conversion to DAG and inositol trisphosphate (IP3) [16]. This was followed by the cloning and characterization of different isoforms of PI3K [16,17] and

**Implication of Phosphoinositide Signaling in Cancer**

The discovery 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 to PI(3,4,5)P3 by PI3K in growth factor stimulation, G protein-coupled receptor (GPCR) activation, and oncogenic transformation were seminal discoveries that unveiled a new signaling pathway in parallel with PLC-mediated PI(4,5)P2 conversion to 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 the 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, which turns off PI3K/Akt signaling by dephosphorylating PI(3,4,5)P₃ back to PI(4,5)P₂, was found to be commonly lost or mutated in various 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 cancer [20,21]. As activated Akt directly inhibits the repertoires of proapoptotic proteins (e.g., BAD, BAX, BIM, 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 nutrients (e.g., glucose, ATP, amino acids) in the environment, cell metabolism, and autophagy are emerging areas of PI3K/Akt/mTORC1 signaling and functions [22]. All of these justify PI3K/Akt/mTORC1 signaling as perhaps one of the most common therapeutic targets for cancer treatment [4].

Deregulated PI3K/Akt Activation in Cancer
Various 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) overexpression of PI3K; and (iv) overexpression 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 the 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 (the gene encoding p110α, the catalytic subunit of class I PI3K) to be one of the eight most frequently mutated genes in cancer [26–28]. These mutations occur at the interface between the catalytic (p110α) and adaptor (p85) subunits of PI3K and generally abrogate the inhibitory effect of the adaptor subunit on the catalytic subunit [24,26,27]. More than 75% of these activating mutations reside in either the helical or the catalytic domain of P110α and are called Hot-Spot’ mutation sites [26]. These activating mutations are reported only in the P110α catalytic subunit. Additionally, overexpression or mutational activation of various tyrosine kinase receptors is responsible for aberrant activation of phosphoinositide signaling in cancer [3,23]. These overexpressed RTKs (e.g., PDGF, EGFR, c-MET, IGFR) generally undergo homo- or heterodimerization, even in the absence of ligand binding. This creates the docking sites, which are phosphorylated tyrosine residues in the context of the YXXM motif in their cytoplasmic domains that recruit the PI3K enzyme to the plasma membrane (the SH2 domain of the adaptor subunit p85 specifically binds to these YXXM motifs, mediating the recruitment of the catalytic subunit) [24]. For example, ERBB2/HER2 and ERBB3/HER3 contain multiple docking sites for PI3K and their activation triggers a 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, mTORC1) are actively targeted for treatment of cancer [4,30,31]. More than 100 drugs (e.g., buparlisib, duvelisib, TGR1202, copanlisib, BEZ235, RP6530, PWT33597, CUDC-907, PI-103, TG100-115I, NK1117) targeting the PI3K/Akt/mTORC1 signaling cascade are undergoing or progressing towards various stages of clinical trials (Phase I, II, and III) [30,31]. Everolimus, temsirolimus, and idelalisib represent the handful of anticancer drugs that have so far been approved by the FDA for treatment of cancer. Everolimus and temsirolimus target mTORC and are used for the treatment of renal cell carcinoma, astrocytoma, and HER2-negative breast cancer, whereas idelalisib targets PI110α and is used for leukemia and lymphoma [32,33].
Readers are referred to ClinicalTrials.gov (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 in inhibiting tumor growth and preventing the 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 mechanisms of PI3K/Akt activation (e.g., from the repertoire of oncogenic mutations in PI3K to loss of PTEN to overexpression/overactivation of tyrosine kinase receptors), identifying the common node for all of these diverse mechanisms could pave the way for developing more effective therapeutic approaches to blocking the PI3K/Akt signaling axis in cancer. Could the generation of P(4,5)P2 substrate at specific subcellular compartments provide a common regulatory node upstream of PI3K/Akt activation? Does PI3K collaborate with PIPKI/PIPKII for de novo synthesis of P(4,5)P2 and P(3,4,5)P3 or are the preexisting pools of P(4,5)P2 in the plasma membrane/endomembranes utilized for P(3,4,5)P3 generation and sustenance of PI3K/Akt signaling in cancer? Although P(4,5)P2 is the predominant phosphoinositide in the plasma membrane, the availability of free P(4,5)P2 may be rate limiting as PLC hydrolyzes the bulk of the P(4,5)P2 in the plasma membrane in 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 PIPKI isoforms, PIPKια and PIPKιγ appear to regulate PI3K/Akt signaling, although PIPKιβ negatively regulates PI3K/Akt signaling [37]. Increased association of PI3K with PIPKιγ in growth factor stimulation of cells suggests coupling of P(4,5)P2 and P(3,4,5)P3 synthesis for Akt activation [37]. As upstream activators of PI3K/Akt include integrins and RTKs, PIPKιγ interaction with talin and the proto-oncogene Src facilitates the recruitment of PIPKιγ to the vicinity of integrin-mediated adhesion complexes and activated RTKs, respectively [37]. In both conditions, PIPKιγ potentially provides de novo P(4,5)P2 to promote and sustain the PI3K/Akt signaling downstream of activated integrins and RTKs [37]. Overexpression of PIPKιγ along with Src sustains the PI3K/Akt signaling and oncogenic growth, where Src serves as a bridging molecule for incorporating PIPKιγ and PI3K into the same complex [37] (Figure 2). Alternatively, the assembly of all of the phosphoinositide kinases PI4K, PIP5Kι, and PI3K 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 the MAPK signaling pathway, a companion of PI3K/Akt signaling [38]. IQGAP1, which scaffolds the molecules in the MAPK pathway in certain cell types, also associates with PI4P, PIPKI, and PI3K and could potentially serve as a scaffolding molecule to streamline and self-sustain the PI3K/Akt signaling axis in cancer [39]. A similar mechanism could exist in coupling P(4,5)P2 generation with PLC-mediated hydrolysis of P(4,5)P2 and PKC activation in cancer. Unlike PIPKI, the contribution of the PIPKII enzyme to PI3K/Akt/mTORC1 signaling appears less dominant and counteracting [40] as the majority of P(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 various isoforms of PIPKI, inhibits endogenous inhibitor of mTORC1, suggesting that PIPKI provides another level of mechanism regulating PI3K/Akt/mTORC1 signaling in cancer [42,43].

**P(4,5)P2 and PIPKI/PIPKII in Cancer**

As P(3,4,5)P3 generation from P(4,5)P2 and PLC-mediated P(4,5)P2 hydrolysis/PKC activation takes the center stage of the phosphoinositide signaling axis in cancer, P(4,5)P2 and PIPKIs have largely gained recognition as key regulators of basic cellular functions such as 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 integral lipid moiety of the plasma membrane and endomembranes, interrogating 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 the PI(4,5)P₂ lipid messenger and PIPKIs, as discussed below.

Unlike PI3K, mutational activation of neither PIPKII nor PIPKII has been reported in cancer, although mutational loss of the PIPKIγ kinase activity is found in congenital contractural syndrome type 3 (LCCS3) [47]. Similarly, different from PI3K, ectopic expression of PIPKII or PIPKII alone usually does not induce oncogenic transformation, although overexpression of PIPKII variants in cooperation with other oncogenes has been reported to promote oncogenic growth [48]. However, upregulated expression of PIPKI and PIPKII kinases and their direct implication in cancer progression is emerging, as various 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 PIPKII correlates with EGFR expression in triple-negative breast cancer tissues [49]. Survival of breast cancer patients inversely correlates with PIPKII expression. Corroborating this, xenograft studies in mice show an essential role of PIPKII in tumor growth and metastasis [50]. Activation of EGFR phosphorylates a tyrosine residue in the C terminus of PIPKIIγ (Y639) and this appears to be essential for the role of PIPKII in tumor growth and metastasis [50]. The PIPKIIγ/EGFR nexus is further fine-tuned by PIPKIIδ, a splicing variant of PIPKII, which controls the downregulation of EGFR via modulating the SNX5/Hrs lysosomal...
degradation pathway \[51,52\]. However, the functional role of PIPK\(g\) 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 numbers of PIPKIA along with P14KB, 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 the oncogenic phosphoinositide signaling nexus in cancer, as over-expression of PIPKI/PIPKII lipid kinases alone is not sufficient to activate and sustain the PI3K/Akt signaling nexus \[48\]. Like PIPKIs, deep transcriptome sequencing shows increased expression of PIPKII in cancer cells and cancer tissues \[54\]. PIP4KII\(a\) and PIP4KII\(b\) are overexpressed in HER2-positive breast cancer tissues \[55\] and ACGH array shows amplification of the PIP4KII\(b\) gene to be part of the HER2 amplicon in cancer \[56\]. These lipid kinases appear to be essential for tumor growth in the background of p53 loss or mutation. A recent study indicates a novel function of PIP4KII\(b\) as a GTP sensor in the regulation of cell metabolism in cancer \[57\]. The use of a shRNA library targeting all known modulators of phosphoinositide metabolism has identified PIP4KII\(a\) as a gene required for leukemia, indicating PIP4KII\(a\) as a potential therapeutic target for hematological malignancies \[58\]. However, knockdown of PIPKII\(b\) is associated with strongly induced basal and insulin-stimulated PI(3,4,5)P\(_3\) levels and Akt activation \[55\]. This indicates that, unlike PIPKI, the role of PIPKI 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 upregulate PI5P levels, and PIPKII lipid kinases are required for its conversion to PI(4,5)P\(_2\) by a noncanonical route \[40,59\]. PIP4KII\(b\) has also been reported to regulate nuclear PI5P and gene expression \[60\]. This highlights PIPKII as a stress-regulated lipid kinase required for cancer cells to overcome oxidative stress and maintain homeostasis of reactive oxygen species \[61\] (Figure 3).

**PI(4,5)P\(_2\) 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)P\(_2\) to provide docking sites for myriad lipid–protein interactions on the plasma membrane and the ability of PIPKI/PIPKII lipid kinases to interrogate a diverse array of protein interactomes, PI(4,5)P\(_2\) and PIPKII/PIPKII serve as integral parts of the epithelial cell polarity program. For example, PI(4,5)P\(_2\) is the landmark phosphoinositide entity of the apical surface and an alteration in distribution of PI (4,5)P\(_2\) from the apical to the basolateral surface in 3D culture of epithelial cells disrupts lumen formation/epithelial morphogenesis and affects the polarized secretion of basement membrane proteins \[64,65\]. Many other studies also support a critical role of the PI(4,5)P\(_2\) phosphoinositide molecule in maintaining apical cell polarity \[66\]. By contrast, PI(3,4,5)P\(_3\) and PI3K function as critical determinants of the basolateral domain of epithelial cells \[67\]. Epithelial cells lose E-cadherin/cell polarity and gain promigratory/prominvasive phenotypes as a result 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)P\(_2\) and PI(3,4,5)P\(_3\) molecules also participate in regulating the myriad cellular events essential for cell motility and the invasive program. This indicates that PI(4,5)P\(_2\) and PI(3,4,5)P\(_3\) function at the conjunction of epithelial cell polarity as well as the promigratory/prominvasive nexus of cancer cells. How are these two seemingly opposite cellular functions regulated by phosphoinositide signaling? In this regard, PIPK\(g\) and PI(4,5)P\(_2\) deserve special attention as they provide the molecular platform for the assembly of not only E-cadherin-mediated adherens junctions at cell–cell contact sites in epithelial cells but also the integrin-mediated adhesion complex at the interface of cell–extra-cellular matrix interaction sites \[68,69\].

In epithelial cells, cell polarity is maintained by E-cadherin-mediated adherens junctions between adjacent cells \[70\]. The precise regulation of targeting, recycling, and endocytosis of E-cadherin molecules controls the integrity of adherens junctions and epithelial cell polarity \[70\]. PIPK\(g\)2, a
specific variant of PIPKιγ, integrates the clathrin adaptor protein AP1B and the evolutionarily conserved vesicle trafficking protein complex, the exocyst, to regulate basolateral trafficking of E-cadherin for cell polarity and epithelial morphogenesis [68,71–73] (Figure 4). In this process, PIPKιγ 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 PIPKιγ (YSPL and YSAQ in PIPKιγ2) recruit the adaptor protein AP1B for basolateral sorting of recycling endosomes. However, these motifs also recruit the AP2 clathrin adaptor protein to mediate endocytosis of E-cadherin from the plasma membrane [68]. The recruitment of the AP2 clathrin adaptor protein to the YXXQ motif in PIPKιγ 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 the selective recruitment of AP1B or AP2 remains poorly understood. Along with clathrin adaptor proteins, the exocyst complex is another direct interacting partner of PIPKιγ 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 PIPKιγ
on the plasma membrane/endomembrane facilitates the basolateral targeting of E-cadherin molecules in recycling or synthetic cargo [8]. The expression of an Exo70 mutant deficient in PI (4,5)P2 binding severely impairs E-cadherin targeting to developing adherens junctions [73]. Further, a specific variant of PIPK\(_\gamma\)1, PIPK\(_\gamma\)5, in coordination with SNX5, controls the lysosomal sorting and degradation of E-cadherin molecules [74], although the involvement of the PIPK\(_\gamma\)5/SNX5 complex in E-cadherin degradation and its role in cancer remain poorly understood.

As epithelial cells lose E-cadherin-mediated adherens junctions and cell polarity, PIPK\(_\gamma\) and PI (4,5)P2 engage in the development of dynamic focal adhesion complexes and control the migratory and invasive nexus of various cancer cells [39,49,71,72,75] (Figure 4). One of the key functions of the PI(4,5)P2 lipid messenger and PIPK\(_\gamma\) is to promote the recruitment of cytoskeletal proteins at developing adhesion complexes. Talin, vinculin, and FAK all harbor patches of basic residues that bind to PI(4,5)P2. Additionally, the spatial generation of PI(4,5)P2 at adhesion complexes promotes the recruitment and activation of these molecules by relieving the intramolecular constraints imposed on them, thus establishing structurally and functionally competent adhesion complexes [76]. PIPK\(_\gamma\)2, the focal adhesion-targeting and talin-interacting variant of PIPK\(_\gamma\), putatively provides PI(4,5)P2 at developing nascent adhesion complexes in adhering and migrating cells. However, PIPK\(_\gamma\)2 also competes with \(\beta1\) integrin for talin binding, indicating that the assembly of PIPK\(_\gamma\)2, talin, and integrin at the adhesion complex is a tertiary complex facilitated by the PI(4,5)P2 lipid messenger. Specifically, the recruitment of talin to the cytoplasmic domain of \(\beta1\) integrin at adhesion complexes is key for the initiation of ‘inside-out’ and ‘outside-in’ signaling, an integral part of focal adhesion signaling in adhering and migrating cells [77]. Additionally, PIPK\(_\gamma\)’s association with the exocyst complex and talin and PI(4,5)P2
generation facilitate the polarized delivery of integrins required for developing nascent adhesion complexes at the leading edge of migrating cells [71,72]. PIPK\(\gamma\) also works with its interacting partner IQGAP1 to promote actin polymerization and cell migration [39]. These illustrate PIPK\(\gamma\) and PIP(4,5)P\(_2\) function at the conjunction of the epithelial cell polarity program and the migratory/invasive nexus of cancer cells.

**PI(4,5)P\(_2\) Control of Cytoskeletal Reorganization**

Although PI(4,5)P\(_2\) could not shine on par with the PI(3,4,5)P\(_3\) lipid messenger in growth signaling in cancer, PI(4,5)P\(_2\) signaling has been established as a key regulator of the cytoskeletal machinery and cytoskeletal reorganization under both physiological and pathological conditions [78,79]. Many excellent reviews [78] serve as great resources for understanding PI(4,5)P\(_2\) regulation of cytoskeleton-associated and regulatory proteins. Importantly, cancer cells display upregulated expression of many cytoskeleton-associated and regulatory proteins and these are essential for their migratory and invasive properties, as one-third of proteins that are induced in metastatic cancers are related to adhesion or cytoskeletal reorganization [80]. Spatial and temporal reorganization of the actin cytoskeleton controls various cellular events involved in metastatic cascades, such as conversion of indolent epithelial cells to the mesenchymal state, detachment from the primary tumor, and migration/invasion and extravasation for secondary/tertiary growth [81].

The first evidence of an intimate association between cytoskeletal protein and phosphoinositide was demonstrated by Anderson and Marchesi [82]. Subsequent studies established PI(4,5)P\(_2\) as the 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)P\(_2\) inhibits the actin-binding and -depolymerizing activity of ADF/cofilin. Similarly, PI(4,5)P\(_2\) inhibits capping proteins like gelsolin, which prevent the addition and loss of actin monomers from the end of actin polymers. Besides these examples, PI(4,5)P\(_2\) also directly regulates a plethora of proteins involved in regulating cytoskeletal reorganization such as spectrin, dynamin, myosin X, ezrin, radixin, gelsolin, profilin, actin, neural Wiskott–Aldrich syndrome protein (N-WASP), myosin, MARCKS, annexins, and \(\alpha\)-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 complexes, lamellipodia, filopodia, and invadopodia, which are essential for migrating and invading tumor cells [81].

PI(4,5)P\(_2\) regulation of the actin-nucleating activity of the Arp2/3 protein complex via WASP and Rho family small GTPases is the most extensively studied in the context of tumor cell migration/invasion and metastasis [81,83–85]. This depends on PI(4,5)P\(_2\) along with active Cdc42 and Rac1 binding to the N terminus of N-WASP and PI(4,5)P\(_2\) binding to basic motifs [84,85]. This leads to exposure of the intramolecularly masked VCA domain in N-WASP, which promotes the binding of the 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 interact with PIPKI and activate the synthesis of the PI(4,5)P\(_2\) lipid messenger required for activation of the 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 the correlation between upregulated expression of cytoskeleton-associated and regulatory proteins and PI(4,5)P\(_2\) and PIPKI remains poorly defined. As tubulin targeting has been a successful approach in cancer therapy [87], intervening in PI(4,5)P\(_2\) and PIPKI functions in the actin cytoskeleton could be similarly exploited in developing drugs targeting the cytoskeletal machinery for tumor therapy.
Concluding Remarks
Phosphoinositide signaling represents a fundamental signaling nexus involved in many cellular functions in health and disease. Given the broad horizons of and extraordinary surges in phosphoinositide studies over the past decades, it has become difficult 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/PI(3,4,5)P3/Akt/mTORC1 axis in cancer. However, it is also important to note the aspects of PI(4,5)P2 and PIPK/IPIKII 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 the oncogenic phosphoinositide signaling axis. As discussed here, PI(4,5)P2 and PIPK/IPIKII 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 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, future attempts to target the phosphoinositide signaling nexus in cancer should also consider the PI(4,5)P2 lipid messenger and relevant lipid kinases (see Outstanding Question). Targeting the spatiotemporal generation of the PI(4,5)P2 lipid messenger could be exploited to target the PI3K/Akt/mTORC1 axis in cancer as this would impair the most proximal node of the oncogenic phosphoinositide signaling axis in cancer and might overcome possible variations in the efficacy of anticancer 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 the specific catalytic subunit of PI3K may also come into clinic within a few years. However, a precise understanding of the 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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Outstanding Questions
How is PI(3,4,5)P3 generation regulated for the sustenance of PI3K/Akt/mTORC1 signaling in cancers? Does it always need 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 PIPK 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 the various aspects of cellular functions crucial for cancer progression? The precise mechanisms of PI(4,5)P2 and PI(3,4,5)P3 regulation of the cell cycle, cell survival, apoptosis, anoikis evasion, and the stemness trait would provide an important platform for uncovering the collective role of phosphoinositide lipid messengers in cancer.

Does phosphatidic acid suppresses the endogenous mTORC1 inhibitor by activating PIPK? Could PIPKI activate mTORC1 independently of PI(3,4,5)P3 generation or does it always activate mTORC1 signaling through the PI3K/Akt cascade?

Does constitutive activation of PI3K/Akt/mTORC1 depend on the molecular assembly of PI3K and PIPK? What are the factors that regulate the molecular assembly of PIPK and PI3K? Are PIPK and PI3K assembled in a unique way in cancers addicted to PI3K/Akt/mTORC1 signaling? How can we specifically target these unique factors for cancer treatment?
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