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PIP2 signaling, an integrator of cell polarity and vesicle trafficking in directionally migrating cells

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Cell migration is a fundamental cellular process required for embryonic development to wound healing and also plays a key role in tumor metastasis and atherosclerosis. Migration is regulated at multiple strata, from cytoskeletal reorganization to vesicle trafficking. In migrating cells, signaling pathways are integrated with vesicle trafficking machineries in a highly coordinated fashion to accomplish the recruitment and trafficking of the trans-membrane proteins toward the leading edge. Different signaling molecules regulate cell migration in different physiopathological contexts, among them, phosphatidylinositol-4,5-biphosphate (PIP2) is an integral component of the plasma membrane and pleiotropic lipid signaling molecule modulating diverse biological processes, including actin cytoskeletal dynamics and vesicle trafficking required for cell migration. In this commentary, we provide a brief overview of our current understandings on the phosphoinositide signaling and its implication in regulation of cell polarity and vesicle trafficking in migrating cells. In addition, we highlight the coordinated role of PIPKIγi2, a focal adhesion-targeted enzyme that synthesizes PIP2, and the exocyst complex, a PIP2-effector, in the trafficking of E-cadherin in epithelial cells and integrins in migrating cancer cells.

Keywords: phosphoinositides, integrins, exocyst complex, cell migration

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PIP2 Signaling in Cell Polarity and Vesicle Trafficking

Cell migration occurs as a result of multiple coordinated events, including external signaling cues, cytoskeletal reorganization, cell polarity and polarized trafficking of signaling molecules.[1,2] Defects in any of these steps impair cell migration. In directionally migrating cells, establishment and maintenance of cell polarity and polarized trafficking are indispensable and different signaling molecules play role in these processes.[1-3] Among them, PIP2 and its phosphorylated product, phosphatidylinositol 3,4,5-triphosphate (PIP3), are the crucial players in both maintaining cell polarity and the polarized trafficking of signaling molecules.[4-8] The spatio-temporal generation/accumulation of these phosphoinositides at the leading edge of migrating cells imparts morphological and functional asymmetry to migrating cells.[8,9] These phosphoinositides are directly involved in the recruitment/activation of effectors or signaling molecules, such as WAVE2,[10] RhoA, Rac1, Cdc42,[11,12] N-WASP, PKA and IQGAP1,[13] to the leading edges of migrating cells is the commonly understood mechanism of phosphoinositide regulation of cell polarity and cell migration. An asymmetric distribution of phosphoinositides is required for migrating cells, which is achieved by polarized recruitment and activation of PIP2- and PIP3-generating enzymes at the leading edges.[7,14,15] However, the functional role of PIP2-generating enzymes in maintaining PIP2/PIP3 pool at the leading edge is poorly defined except in leukocytes where some PIPKI isoforms are known to maintain phosphoinositide asymmetry during cell migration.[7,14] Additionally, the expression of different PIPKIs isoforms in cells and the cell type involved may also give rise to differences in the PIPKI enzymes involved in this process.

Localized PIP2 generation is crucial for the proper sorting of vesicles to intracellular

sites and/or for the fusion of these vesicles with the plasma membrane.[5,6] Several studies have demonstrated the PIP2-induced assembly of actin-containing complexes propelling endosomal vesicles toward the plasma membrane.[16-18] PIPKIγ knockout studies also support the role of PIP2 on various aspects of endosomal vesicle trafficking.[6,19] Cell polarity and polarized vesicle trafficking are inter-dependent cellular processes.[3,4] The role of endosomal vesicle trafficking in regulating cell polarity and cell migration is emerging with several studies depicting the coordinated role of these two processes in cell migration,[2,4,20] such as FAK/RACK1[21] and ESCRT complexes,[22,23] integrate the endosomal trafficking with cell polarity and migration. Recently, we demonstrated the coordinated role of the PIP2-generating enzyme PIPKIγi2, with talin and the exocyst complex. All are recruited to the leading edge of migrating cells, controlling cell polarity and cell migration in tumor cells.[4] Interestingly, PIPKIγi2 and the exocyst complex are also essential components in maintaining epithelial cell polarity by regulating E-cadherin trafficking.[24,25] Thus, PIPKIγi2 and PIP2-signaling are potential integrators of vesicle trafficking and cell polarity in both polarized epithelial cells and directionally migrating cells (illustrated in **Fig. 1**). In migrating cells, these complexes controlled the polarized trafficking of integrin molecules required for nascent focal adhesion formation at the leading edge of the migrating cells.[4] In this complex, talin and exocyst are PIP2-interacting and PIP2-regulated molecules playing key roles in cell polarity and vesicle trafficking. The individual knockdown of PIPKIγi2, talin or exocyst complex displayed the same phenotypic defect on cell polarity and polarized recruitment of integrin molecules to the leading edges. However, the precise mechanism how PIPKIγi2, exocyst complex and talin accomplish the polarized trafficking and exocytosis of integrin molecules at the leading edge membrane is not clear and needs further investigation. In addition, how PIP2- and PIP3-generating enzymes coordinate with each other to regulate the localized pool of PIP2/PIP3 needed for controlling cell polarity and cell migration still remains sketchy. PIPKIγi2 is specifically recruited to focal adhesions[26] and is solely responsible for PIP2-generation at focal adhesion sites.[27] PIPKIγi2 has clear advantage over other PIPKIs isoforms in its recruitment to nascent focal adhesion sites at the leading edge of migrating cells due to its intimate association with talin, a cytoskeletal protein recruited to the focal adhesion sites.[26] Both talin and PIPKIγi2 are inter-dependently recruited to the nascent focal adhesion sites.[4,28] However, the functional role of PIP2 synthesis in vesicle trafficking in and out of focal adhesion sites during cell adhesion and migration is not precisely understood. Unlike cell migration and polarized integrin recruitment, the knockdown of PIPKIγi2 or exocyst complex displayed minor defects in cell adhesion, indicating phosphoinositide-dependent vesicle trafficking at focal adhesions could be differentially regulated during cell adhesion and cell migration, although cell adhesion requires vesicle trafficking and regulated exocytosis for the assembly of functional adhesion complex. Investigation of PIPKIγi2 in the regulation of RhoGTPases and vice versa is also an important aspect of cell polarity and vesicle trafficking in PIPKIγi2-regulated cell migration as RhoA, Rac1 and Cdc42 are upstream target molecule of type I PIPKI.[29]

PIP2 Signaling and Exocyst Complex in Polarized Recruitment of β1-Integrins at Leading Edge of the Migrating Cells

How is polarized integrin recruitment/trafficking achieved at focal adhesion sites during cell adhesion and migration? Leading edge extension and its attachment to the surrounding ECM protein is a crucial initial event in cell migration.[2,3] The activated β1-integrin localized at leading edge lammellipodium guides migration and regulates the migration force.[30] The polarized recruitment/trafficking of integrin molecules, formation of new adhesion sites at the leading edge and dissolution of adhesion sites at the trailing edge is the widely believed dogma in cell migration.[2,3,20,31] However, signaling molecules intimately involved in polarized trafficking of integrin molecules required for nascent focal adhesion formation at leading edge of migrating cells is also poorly defined. Similarly, complexity lies in defining polarized integrin trafficking in migrating cells, as integrin molecules throughout the cell surface of even a single cell undergo endo-exocytic trafficking at varying rates and by different mechanisms[31] and defining this phenomenon is technically challenging. PIPKIγi2 recruitment to nascent focal adhesion sites at the leading edge of migrating cells and its regulation on polarized recruitment of β1-integrins via its association with exocyst complex could be one of the mechanisms migrating cells utilize during cell migration. PIPKIγi2 is required for talin and exocyst complex recruitment, both PIP2-regulated and PIP2-interacting proteins, to the leading edge.[4] The precise mechanisms for how PIPKIγi2 and exocyst complex coordinate with each other to accomplish the polarized integrin trafficking/exocytosis at nascent focal adhesion sites needs further investigation. Does it participate in a core machinery of vesicle exocytosis by generating the localized pool of PIP2 at focal adhesion building sites? How do PIPKIγi2-mediated PIP2 generation and exocyst complex recruitment at membrane couple with the complex mechanism of exocytosis? Does PIPKIγi2 provide the discreet pool of PIP2-required for recruitment of cargo-laden vesicle-associated exocyst complex at leading edge? The exocyst complex consists of eight different subunits (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84) and its two subunits Sec3 and Exo70 directly interact with PIP2 via conserved basic residues in their C-terminus.[32] PIPKIγi2 and the exocyst complex extensively co-localize at focal adhesions, and the association of exocyst complex with PIPKIγi2 at focal adhesion sites could be a mechanism to facilitate the localized PIP2-generation and polarized exocytosis.

Furthermore, the functional role of PIPKIγi2 in cell migration is largely defined in 2D migration where focal adhesions are the predominant structural components and their functional role is more pronounced. However, in more physiologically relevant conditions such as 3D systems, where focal adhesions are less predominant, the significance of PIPKIγi2-regulated

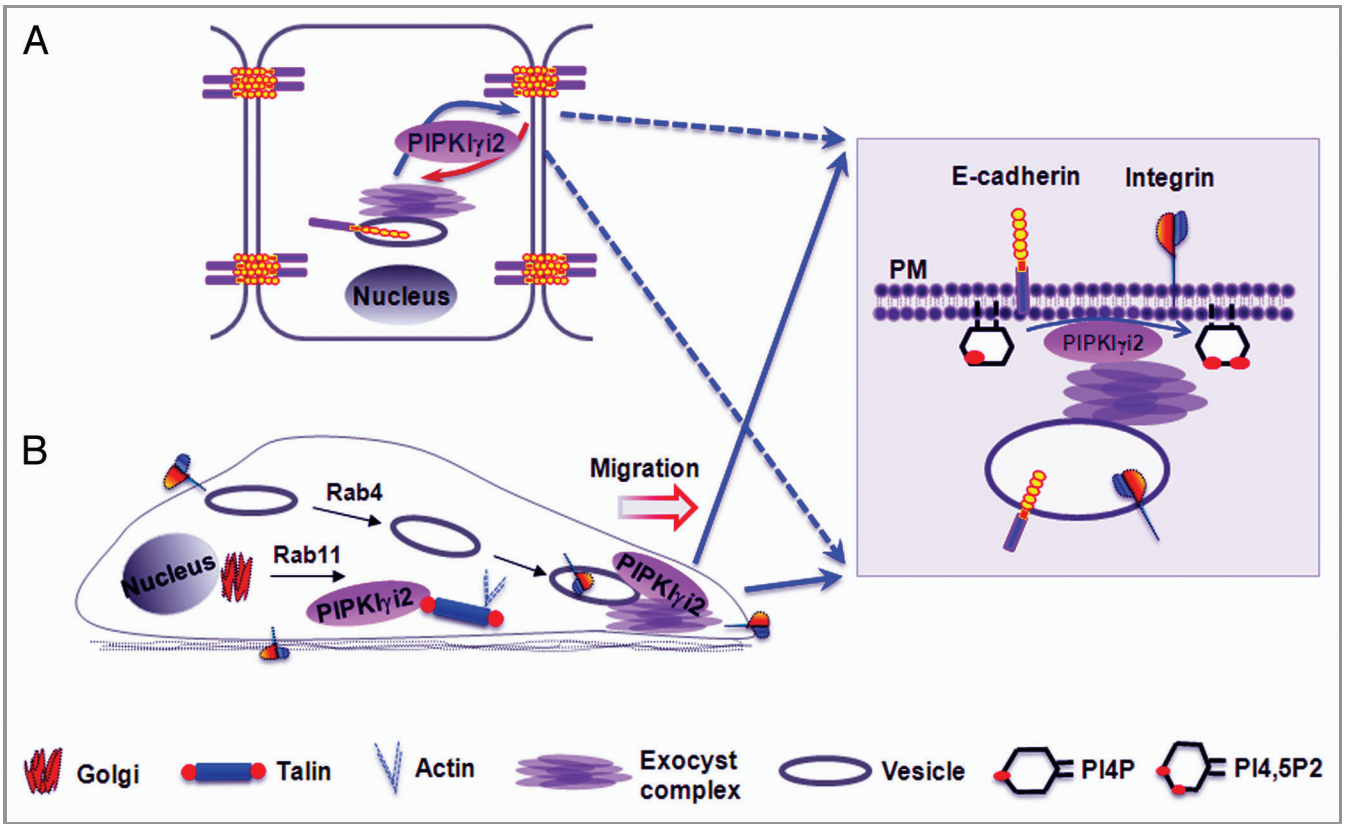

Figure 1. Proposed model depicting the role of PIPKIγi2 and exocyst complex in polarized E-cadherin and integrin trafficking. (A) In normal epithelial cells, PIPKIγi2 association with exocyst complex mediate the polarized trafficking of E-cadherin molecules to maintain the adherent junctions at cell-cell contact sites. The loss of either PIPKIγi2 or exocyst complex results in defect in E-cadherin transport to adherent junction and loss of cell polarity. (B) In migrating tumor cells that have already lost E-cadherin, the PIPKIγi2 and exocyst complex mediate polarized recruitment/trafficking of integrin molecules toward the direction of cell migration. Cell migration induces the integration of PIPKIγi2, talin and β1-integrin into the complex either in plasma membrane or in intracellular recycling compartments. The PIP2 generated in the complex facilitates the assembly of the exocyst complex. Thus, the coordinated activity of PIPKIγi2 and the exocyst complex in concert with talin promotes the polarized recruitment and trafficking of integrin molecules to leading edge plasma membrane. Loss of PIPKIγi2 or the exocyst complex or talin compromises the polarized recruitment/trafficking of integrin impairing cell polarization and directional cell migration.

cell migration and polarized signaling has yet to be investigated.

PIP2-Generating Enzymes in Cancer

Type I PIPKI enzymes are largely responsible for PIP2 generation in mammalian cells.[4] Different isoforms including PIPKIα, PIPKIβ and PIPKIγ regulate cell migration in different cell types.[4,7,14,28,34,35] Most migration and/or invasion studies we and other laboratories conducted defining the role of PIPKIγ and PIPKIγi2 are cancer cells such as MDA-MB-231, HeLa, MDA-MB-435, SKBR3 and HCT116.[4,28,34,35] These studies suggest pro-tumorigenic function of the PIPKIγ and PIPKIγi2 in cancer as cell migration and invasion are the pivotal cellular processes required for cancer metastasis. Corroborating with pro-migratory and pro-tumorigenic function of PIPKIγ in cancer cells, the survival of breast cancer patients inversely correlates with increased PIPKIγ expression,[34] although thorough investigation of different PIPKIγ isoform expression and their role in breast and other cancers need to be investigated. Furthermore, the role of PIPKIγi2 in the polarized trafficking of integrin molecules has the potential to impact our understanding of the patho-biological mechanisms of cancer metastasis. Several molecules implicated in tumorigenesis, such as Rab11, ARF6 and mutant-p53, modulate the integrin trafficking, resulting in enhanced migration associated with metastasizing tumor cells.[31,33] Understanding how PIPKIγ and PIPKIγi2 in association with exocyst complex regulate the vesicle trafficking process can shed light into how highly metastatic tumor cells can exploit phosphoinositide signaling in novel ways.

Contradicting with their pro-migratory function, PIPKIγ and PIPKIγi2 are also involved in epithelial morphogenesis by regulating E-cadherin trafficking at adherens junctions.[24,25] Similarly, exocyst complex are also involved in E-cadherin transport in epithelial cells and integrin trafficking in migrating tumor cells.[4,25] The integrity of E-cadherin-mediated cell-cell contact formation is crucial for maintaining cell polarity and has anti-migratory/anti-tumorigenic functions in epithelial tissues. In epithelial cancers, loss of E-cadherin-mediated cell-cell contact facilitates tumor metastasis. Thus,

PIPKIγ/PIPKIγi2 and exocyst complex have opposing roles in normal epithelial vs. tumor cells (see **Fig. 1**). The investigation of how this protein complex functions with other regulatory mechanisms in epithelial-mesenchymal transition (EMT) is going to be an extremely interesting area of research in cancer biology.

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