PI4,5P$_2$ REGULATES THE POLARIZED TRAFFICKING OF INTEGRINS THROUGH EXO70 FOR DIRECTIONAL CELL MIGRATION

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Introduction. The polarized delivery of integrin adhesion molecules to the leading edge is required for cell migration and has been linked to cancer cell invasion, cancer aggressiveness, and poor patient outcome (1,2). Thus, regulators of integrin traffic may represent new molecular targets to inhibit tumor progression. However, identifying the regulatory networks that control integrin traffic has posed a big challenge, because, in contrast to the internalization of other cargoes, integrins take diverse routes, depending on their specific ligand, activation state, type of adhesion structure and cell type assayed.

Materials and methods. To analyze integrin trafficking during cell migration, we used conformation-specific antibodies that distinguish active from inactive integrins, as well as photoactivatable fluorescent integrin probes. By combining these probes with confocal microscopy in live and fixed cells and biochemical analyses, we were able to visualize and quantitate the intracellular movement of specific integrin subpopulations.

Results and discussion. By using these approaches, we discovered that integrins are endocytosed during the turnover of focal adhesions and recycled back to the leading edge via the Rab5 endosome and Rab11 recycling vesicles for reinsertion into newly forming adhesions. Notably, this polarized recycling pathway not only delivers integrins, but also critical regulators of actin assembly, including the Arp2/3 complex. To identify the regulatory molecules that mediate this transport, we investigated how components of the recycling machinery are spatially targeted to the leading edge. Significantly, we discovered that Type I phosphatidylinositol 4-phosphate 5-kinase beta PIPK1P is a critical regulator of this process. At the leading edge, PIPK1P generates a PI4,5P$_2$ pool that appears to target recycling vesicles to the plasma membrane through binding the exocyst complex component Exo70. By using a mutant PIPK1P protein that selectively abrogates PIPK1 B localization to the leading edge, but leaves the localization of PIPK1 B to focal adhesions and its function in focal adhesion disassembly intact, we further show that the vectorial transport of alpha5eeta1 integrins and actin regulators is essential for actin-dependent leading edge formation and directional cell migration. Collectively, these studies reveal how cells employ vesicular transport pathways to spatially control and coordinate focal adhesion dynamics and actin assembly and thereby regulate cell polarity, adhesion, and migration.

Conclusions. These studies provide a molecular basis for how PIPK1 B regulates directed cell migration and further identify PIPK1e as a potential therapeutic target to inhibit integrin trafficking.

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References.

1. Caswell PT, *et al.* (2009). *Nature reviews. Molecular cell biology* 10(12):843-853.
2. Mosesson Y, *et al.* (2008). *Nature reviews. Cancer* 8(11):835-850.