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Role of p21-Activated Kinase 4 in Cell Migration

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Abstract

Cell migration is a cellular process that plays a critical role in various physiological and pathological phenomena, including in cancer metastasis. Understanding at a fundamental level how cancer cells migrate and invade will help to delineate potential targets for the directed development of anti-metastatic therapeutic agents. For example, αv integrins are up-regulated or activated in many migratory cells, and are essential to the processes of wound healing, angiogenesis, and metastasis. Similarly, integrin αvβ5, a vitronectin (VN) receptor, is expressed in most patient carcinoma specimens and is functionally involved in growth factor-induced carcinoma cell migration *in vitro* and metastasis *in vivo*. However, the mechanisms of integrin αvβ5-mediated cell migration are not fully understood. In this project, we aimed to identify proteins that interact with the cytoplasmic tail of integrin β5, and to study their role in cell motility. Firstly, we employed a yeast two-hybrid screening of a mouse embryo cDNA library and thereby identified six proteins specifically interacting with the human integrin β5 cytoplasmic domain. One of the integrin β5-interacting proteins was p21-activated kinase 4 (PAK4), which, in addition to its direct interaction with the integrin β5 cytoplasmic tail, also appeared to functionally regulate αvβ5-mediated migration of the human MCF-7 breast carcinoma cells. Importantly, engagement of integrin αvβ5 by cell attachment to VN led to a redistribution of PAK4 from the cytosol to dynamic lamellipodial structures where PAK4 co-localized with integrin αvβ5. Functionally, PAK4 induced integrin αvβ5-mediated, but not integrin β1-mediated MCF-7 cell migration, without affecting the cell surface levels of integrin αvβ5.

In addition, we found that PAK4 was activated by cell attachment to VN mediated by the PAK4 binding partner integrin αvβ5, and that active PAK4 induced accelerated integrin αvβ5 turn-over within adhesion complexes. Accelerated integrin turn-over was the likely cause of additionally observed PAK4-mediated effects, including inhibited integrin αvβ5 clustering, reduced integrin to F-actin connectivity and perturbed maturation of cell adhesion complexes. These specific outcomes were ultimately associated with reduced cell adhesion capacity and increased cell motility. We thus demonstrate a novel mechanism deployed by cells to tune cell adhesion levels through the auto-inhibitory regulation of integrin-mediated adhesion.

Furthermore, we identified a unique PAK4-binding membrane-proximal β5-SERS-motif in the cytoplasmic tail of β5, and demonstrated a key role for this motif in controlling cell adhesion and migration. We mapped the integrin β5-binding within PAK4 and observed that PAK4 binding to integrin β5 was not sufficient to promote cell migration; instead the PAK4 kinase activity was required for PAK4 promotion of cell motility. In fact, PAK4 specifically phosphorylated the integrin β5 subunit at Ser 759 and Ser 762 within the β5-SERS-motif. Importantly, point mutation of these two serine residues abolished PAK4-mediated promotion of cell migration, indicating a functional role for these phosphorylations in cell migration.

In conclusion, our results demonstrate that PAK4 interacts with and selectively phosphorylates integrin αvβ5 and thereby promotes αvβ5-mediated cell migration, a functional outcome paralleled by a concurrent decrease in total cellular adhesion to VN. Given our finding that PAK4 is activated by αvβ5 ligation to VN, these results delineate an auto-inhibitory negative feedback loop that is initiated by cell adhesion to VN. Binding of integrin αvβ5 to VN drives translocation and activation of PAK4, leading to phosphorylation of αvβ5 and ultimately the limiting of total adhesion between cells and VN and increased cell migration. Thus, our findings provide a new mechanistic characterization of PAK4's role in the functional regulation of integrin αvβ5. This knowledge may ultimately be important for understanding vascular permeability, angiogenesis and cancer dissemination.

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