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Emerging role of ILK and ELMO2 in the integration of adhesion and migration pathways

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Integrins and their associated proteins are essential components of the cellular machinery that modulates adhesion and migration. In particular, integrin-linked kinase (ILK), which binds to the cytoplasmic tail of $\beta 1$ integrins, is required for migration in a variety of cell types. We previously identified engulfment and motility 2 (ELMO2) as an ILK-binding protein in epidermal keratinocytes. Recently, we investigated the biological role of the ILK/ELMO2 complexes, and found that they exist in the cytoplasm. ILK/ELMO2 species are recruited by active RhoG to the plasma membrane, where they induce Rac1 activation and formation of lamellipodia at the leading edge of migrating cells. A large number of growth factors and cytokines induce keratinocyte migration. However, we found that formation of RhoG/ELMO2/ILK complexes occurs selectively upon stimulation by epidermal growth factor, but not by transforming growth factor- $\beta 1$ or keratinocyte growth factor. Herein we discuss the relevance of these complexes to our understanding of the molecular mechanisms involved in cell migration, as well as their potential functions in morphogenesis and tissue regeneration following injury.

Introduction

Cell migration is a key element in nearly every biological process (for a review, see ref. 1). Unicellular organisms use it to find optimal growth and/or survival environments. In multicellular organisms, cell

migration is necessary from the earliest embryonic stages and throughout organogenesis, as cell progenitors must move to appropriate regions to give rise to all tissues in the body. During postnatal life, cell migration becomes very important in homeostatic processes, such as tissue repair and inflammation. Migration mechanisms can also be co-opted and abnormally regulated during the development of multiple and widely diverse pathological conditions, including chronic autoinflammatory disorders, such as asthma and psoriasis, atherosclerosis and vascular disease, as well as carcinoma invasion and metastasis.

Forward cell movement occurs as a series of distinct and coordinated stages.² First, the cell establishes front-rear polarity based on directional cues from its surrounding environment. Polarization involves remodeling of the cytoskeleton and formation of focal adhesions to generate a leading edge. The cell protrusions at the leading edge form as a result of local actin polymerization. Associated with cell extensions, focal adhesions assemble to mediate membrane attachment to the extracellular substratum, providing traction. Finally, adhesions are disassembled at the rear of the cell, which retracts allowing translocation of the cell body. Myriad proteins modulate directional migration, and central among them are chemotactic growth factors, integrins and their associated signaling factors, as well as the Rho family of GTPases.³⁻⁵ How all these factors work coordinately to regulate cell motion and adhesion remains a fundamental question in cell biology.

Keywords: cell migration, ILK, keratinocyte, epidermal growth factor, integrin-linked kinase, ELMO2, Rho GTPases, integrins

Abbreviations: EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ELMO2, engulfment and cell motility 2; Eph, ephrin; ILK, integrin-linked kinase

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ILK/EGF Crosstalk in Acquisition of Cell Polarity and Migration

Persistent cell migration requires recruitment and activation of Rho GTPases to regions adjacent to the plasma membrane, to induce formation of stable lamellipodia and to maintain the orientation of the leading edge.² Recently, a signaling module linking epidermal growth factor receptor (EGFR) stimulation with translocation and activation of RhoG to lamellipodia was identified in migrating keratinocytes.^{6,7} In response to a migratory stimulus, active RhoG in turn recruits cytoplasmic species containing engulfment and cell motility-2 (ELMO2) and integrin-linked kinase (ILK; Fig. 1). In this novel complex, ELMO2 serves as a bridge that links active RhoG with ILK, and the latter is essential for activation of Rac1 upon epidermal growth factor (EGF) stimulation. Further, the RhoG/ELMO2/ILK complex is involved in Rac1-dependent lamellipodia formation in response to EGF, and consequent development of front-rear polarity and cell migration. It is significant that ILK itself does not show polarized cellular distribution, as it can be found both at the front and at the rear of migrating cells. However, the plasma membrane regions that show colocalization of ILK and ELMO2 concentrate at the lamellipodia on the leading edge (Figs. 1 and 2), emphasizing the concept that polarized distribution of heteromeric complexes, and not necessarily of single proteins, is important for forward cell movement.

Coordinated responses to growth factors and the extracellular matrix (ECM), mediated by receptors and integrins, respectively, are essential for cell proliferation and migration.⁸ Not surprisingly, EGF-induced keratinocyte polarization and directional migration also require expression of $\beta 1$ integrins.⁷ It will be important to determine the contribution of $\beta 1$ integrins to optimal EGFR clustering for signaling and activation of Rho GTPases, relative to their role in the formation of focal contacts to generate traction forces necessary for lamellipodia formation and migration.

Growth factor stimulation in mesenchymal and epithelial cells also induces formation of dorsal ruffles.⁹ These actin-based transient structures can form at the leading edge of a cell, and facilitate the generation of lamellipodia in preparation for forward movement. Significantly, treatment of kidney fibroblast cell lines with EGF induces formation of dorsal ruffles through mechanisms that involve stimulation of $\alpha 5\beta 1$ integrin and require expression of ILK.¹⁰ ILK does not localize to dorsal ruffles. Rather, it is involved in recruitment to and activation of the Src tyrosine kinase at focal adhesions, which occurs upon joint stimulation of EGFR and $\alpha 5\beta 1$ integrins. This signaling pathway regulated by ILK contrasts with its modulation of EGF-induced lamellipodia formation in keratinocytes, as the ILK/ELMO2 species involved in the latter appear to be excluded from paxillin-containing focal adhesions (Fig. 2).

Role of EGF Signaling through Eph Receptors, RhoG and ELMO2 in Cell Migration

Ephrins are membrane-bound proteins that elicit biological responses in a paracrine manner, by activating cognate receptors on adjacent cells.¹¹ There are two subfamilies of Eph receptor tyrosine kinases, which can be distinguished by their relative ability to preferentially bind ephrin-A or -B. Ephrin/Eph receptor pathways play important roles in development, cell survival and migration. In particular, the Eph receptor EphA2 can function as a downstream effector of EGF receptor stimulation independently of ephrin ligands. In this context, EGF induces binding of EphA2 to Ephexin 4, which then recruits and activates RhoG at the cell membrane.¹² Active RhoG then binds to ELMO2 and Dock 4, which locally activate Rac1 to promote formation of lamellipodia and subsequent migration and invasion in mammary epithelial carcinoma cells. Similarly, EphA2 can also bind to Ephexin 4 to activate RhoG in HeLa cells.¹³ It will be important to determine whether Ephexin 4 is also an upstream activator of the RhoG/ELMO2/ILK complex formed in response to EGF in epidermal keratinocytes.

Rho GTPases and ILK in the Modulation of Adhesion Turnover

Continuous cycles of formation and disassembly of cell-ECM contacts allow forward cell movement. Central to the regulation of adhesion assembly are Rho GTPases, which themselves are modulated through signals generated at adhesion sites. The networks involved in these processes couple responses to integrin stimulation with those due to activation of receptor tyrosine kinases. Several focal adhesion proteins function upstream of Rho GTPases to regulate adhesion turnover. For example, ILK, as well as focal adhesion kinase working in conjunction with Src, are necessary to limit RhoA and promote Rac1 activities. This results in reduced Rho-mediated stabilization of adhesions, while increasing Rac1-dependent adhesion turnover and cell motility.¹⁴⁻¹⁶ To what extent these two pathways are redundant or complementary remains to be established. However, the fact that, at least in some circumstances, ILK modulates Src activation at focal adhesion sites, in addition to the other mechanisms whereby it activates Rac1, places ILK as a key hub for Rho GTPase regulation.

Adhesion turnover, regulation of Rho GTPases and persistent cell migration are also influenced by endocytic-exocytic transport of integrins, and RhoG appears to play important roles in both Rac1 activation and integrin trafficking (for a review, see ref. 17). Specifically, RhoG has been associated with activation of Rac1 via association with ELMO/Dock or ELMO2/ILK species, regulating cell polarity and motility.^{6,7,18} RhoG was also recently found to form complexes with and promote endocytosis of $\beta 1$ integrins upon cell stimulation by ECM substrates.¹⁹ Significantly, RhoG-null fibroblasts and keratinocytes exhibit impaired migration. Given that ILK also binds to $\beta 1$ integrins,²⁰ the investigation of potential additional links between RhoG, ELMO2 and ILK in integrin endocytosis will help resolve the key question of how interdependence of integrin stimulation, GTPase activation and receptor trafficking regulates cell adhesion and migration.

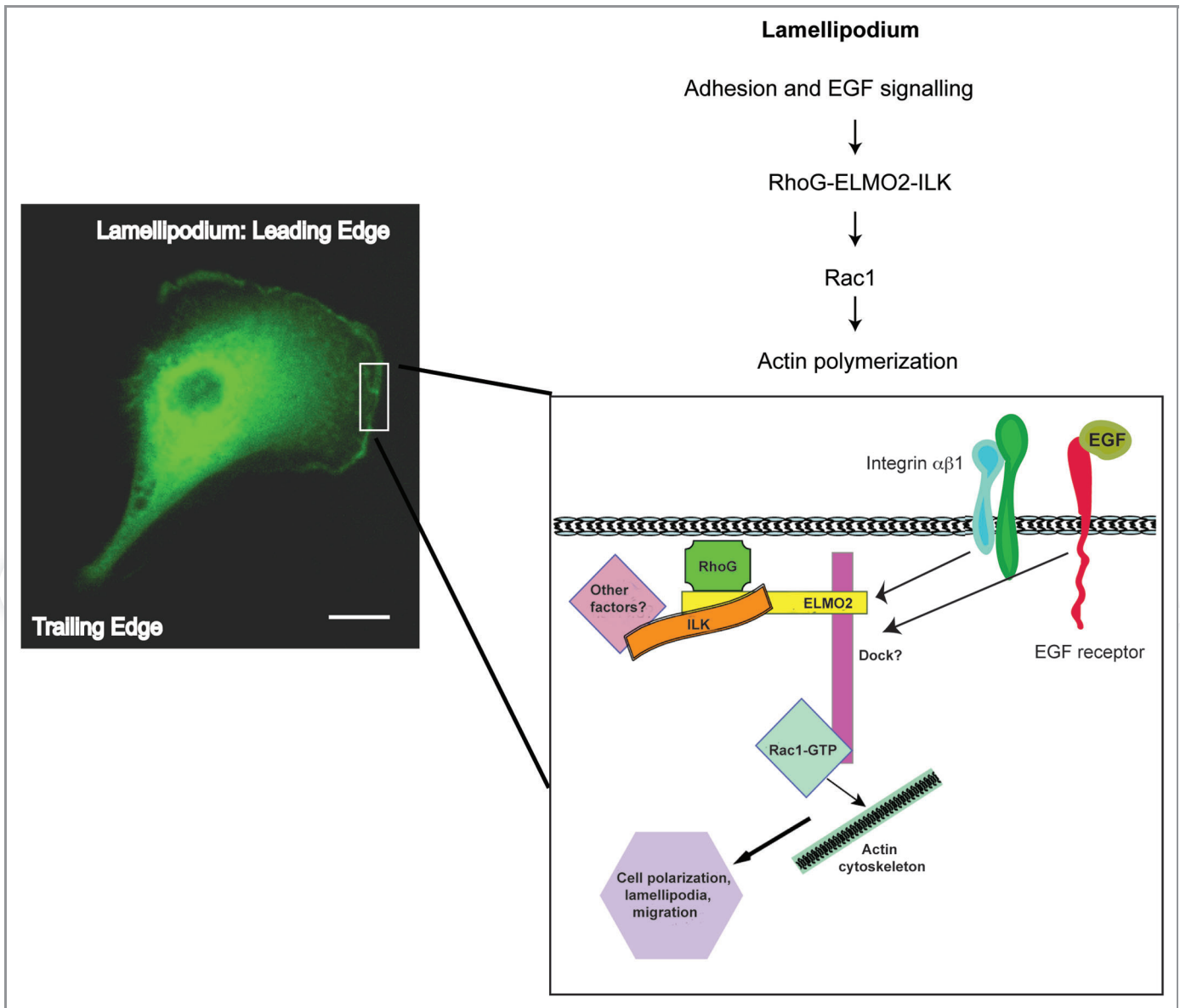


Figure 1. Recruitment of RhoG, ELMO2 and ILK to leading edges of migrating cells in response to EGF. Cultured epidermal keratinocytes were transiently transfected with a vector encoding GFP-tagged ELMO2, and treated with EGF. The migrating cell shown was visualized by time-lapse videomicroscopy, and exhibits localization of ELMO2 to the lamellipodium that defines the leading edge. In addition to ELMO2, RhoG and ILK interact and localize to the cell front in response to EGF, as depicted in the cartoon. This complex is necessary for normal Rac1 activation and forward movement. Whether Rac1 is directly activated by Dock proteins, or by other guanine exchange factors in association with the RhoG-ELMO2-ILK complex remains to be determined. Bar, 20 μm .

ILK Regulation of Keratinocyte Motility In Vivo

The mechanisms of RhoG- and ILK-dependent cell motion described thus far are directly relevant to directional movement in two dimensions, as the latter is generally characterized by lamellipodial protrusion coupled with myosin-driven retraction at the rear of the cell, and assembly/disassembly of cell adhesions.

However, when cells move through 3D matrices in vivo, they face a substantially distinct environment, and therefore behave differently. For example, instead of using lamellipodia, they form larger, rounder pseudopods and blebs to squeeze through extracellular matrix fibers (for a review, see ref. 21). Significantly, ILK can also modulate 3D cell adhesion and movements within various tissues, as illustrated below for the epidermis and epidermal appendages.

During embryogenesis, ectodermal cells mature to give rise to the stratified epidermis, and to epidermal appendages, such as the hair follicles.^{22,23} Hair follicles begin to form as epithelial cell placodes, which then grow and invaginate into the dermis. Inactivation of ILK in the embryonic epidermis does not impair placode formation. However, it results in substantial alterations in the capacity of keratinocytes in the developing follicle

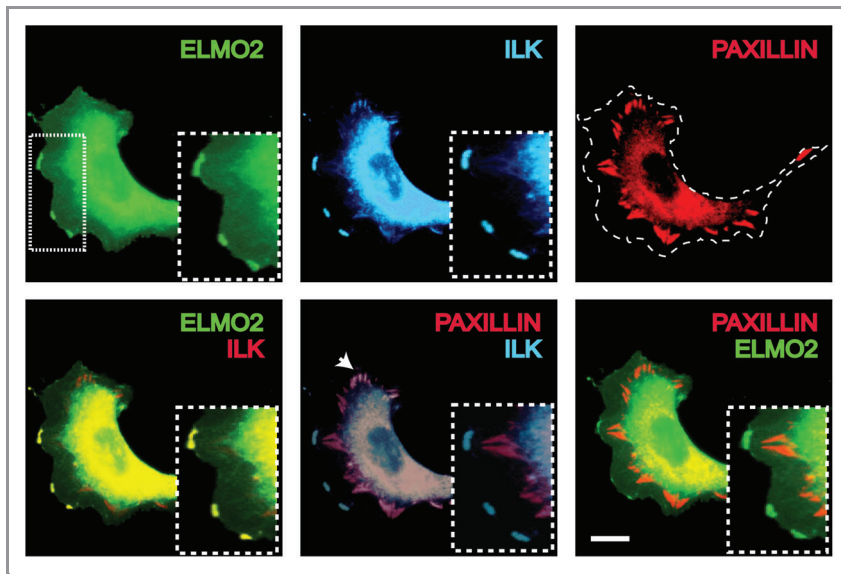


Figure 2. ELMO2 co-localizes with ILK, but not with paxillin, at the edge of lamellipodia in migrating cells. Cultured epidermal keratinocytes were transiently transfected with vectors encoding dsRed-tagged paxillin, V5-tagged ILK and GFP-tagged ELMO2. The cells were seeded onto laminin 332 matrix-coated culture dishes to allow migration, and processed for immunofluorescence microscopy 2 h after plating. The boxed area is shown at higher magnification in the insets, and the dotted line in the photomicrograph showing paxillin-associated fluorescence represents the outline of the cell. ELMO2 fluorescence localizes to the cell edges in the boxed area, whereas paxillin-containing focal adhesions are found away from the cell borders. Notice that areas where ILK and ELMO2 co-localize exclude paxillin, but a different pool of ILK co-localizes with paxillin, as shown by the arrowhead. Bar, 20 μ m.

to invaginate and move into the underlying mesenchyme.^{24,25} As a result, ILK-deficient epidermis exhibits severely impaired hair follicle morphogenesis.

After birth, hair follicles undergo continuous cycles consisting on sequential regression, resting and growth phases. In the growth phase, the hair follicle regenerates and extends into the dermis, in a process similar, but not identical, to that observed in the embryo. This process requires that hair follicle stem cells, localized to a permanent part of this appendage termed the bulge, migrate out and produce progeny that invaginates into the dermis. Notably, and in stark contrast with its role during embryonic hair follicle morphogenesis, ILK is not required for migration of postnatal hair follicle stem

cells out of their niche, or for invagination of their progeny,²⁶ suggesting that distinct and/or redundant mechanisms for keratinocyte movement into the dermis may be in place after birth.

A different scenario is observed during wound healing. Following epidermal injury, keratinocytes in the epidermis and hair follicles adjacent to the wound edges are activated to divide and migrate to cover the denuded area.²⁷ These cells acquire an elongated morphology and form protrusions. When ILK-deficient hair follicle stem cells are activated following cutaneous injury, their descendants are able to leave the bulge and move up the hair follicle toward the epidermis, but very few continue and migrate over the denuded area. The lower contribution of

hair follicle stem cell descendants to the newly formed epithelium is associated with significantly impaired wound repair.²⁶ Notably, EGF receptors are upregulated in keratinocytes at the leading edge of the healing wound border, and their stimulation promotes cell migration during re-epithelialization.²⁸ Further, RhoG-null epidermis exhibits important delays in wound repair, associated with reduced migratory capacity of keratinocytes.¹⁹ Given the coupling of EGFR stimulation with RhoG activation and recruitment of ILK/ELMO2 species to induce migration, it is likely that these same complexes play key roles in keratinocyte forward movements during epidermal regeneration after injury.

Conclusion and Perspectives

A large number of proteins that interact with ILK have been identified, increasing the realization that ILK serves as a scaffold that mediates multiple, diverse cell functions. In particular, through its association with ELMO2, ILK plays key roles in the regulation of Rho GTPases and cross-talk pathways between adhesion and growth factor receptors. In the context of tissue repair, EGF plays important roles for re-epithelialization.²⁹ Thus, regulation of the EGF/RhoG/ELMO2/ILK module may find important applications in therapies aimed at improving impaired wound healing.

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