

REVIEW

Regulation of epithelial cell adhesion and repulsion : role of endocytic recycling

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Abstract : A proper balance between cell adhesion and repulsion is essential for cellular morphogenesis during epithelial-mesenchymal transition and mesenchymal-epithelial transition. A number of ligand-receptor pairs including hepatocyte growth factor/scatter factor-Met and semaphorin-plexin are known to control this balance through the complex intracellular signaling pathways. Cell adhesion to other cells and extracellular matrix (ECM) is mediated by cell adhesion molecules (CAMs) and ECM receptors, respectively, which are associated with cytoskeleton through a variety of plaque proteins strengthening and/or weakening adhesion activities. Cell repulsion requires the downregulation of cell adhesion and the extensive changes in cytoskeletal dynamics. The endocytic recycling of CAMs and ECM receptors has recently emerged as an important mechanism to control the balance between cell adhesion and repulsion. Molecule interacting with CasL (MICAL) family proteins are originally identified as a plaque protein associated with ECM receptors integrins and implicated in semaphorin-plexin dependent repulsive axon guidance. We have recently shown that MICAL family protein JRAB/MICAL-L2 functions as an effector protein for Rab family small G protein Rab13 and regulates the endocytic recycling of tight junctional CAM occludin and controls the adhesion and repulsion of epithelial cells. *J. Med. Invest.* 55 : 9-16, February, 2008

Keywords : *Rab13, JRAB/MICAL-L2, cell adhesion, cell repulsion*

INTRODUCTION

A proper balance between adhesion to and repulsion from other cells or extracellular matrix (ECM) determines cellular morphogenesis and plays a central role in a wide variety of physiological and pathological events (1). A number of signals such as hepatocyte growth factor (HGF)/scatter factor (SF) and Semaphorin are able to control the balance between cell adhesion and repulsion through the complex intracellular signaling pathways (2, 3). While

cell adhesion to other cells and ECM is mediated by cell adhesion molecules (CAMs) and ECM receptors, cell repulsion is achieved by the downregulation of cell adhesion and the extensive changes of cytoskeletal dynamics. In order to balance adhesion with repulsion, cells need to regulate the adhesive activities of CAMs and ECM receptors and the cytoskeletal dynamics. Now accumulating evidences are demonstrating the importance of the endocytic recycling of CAMs and ECM receptors to control their adhesive activities (4).

Epithelial cell adhesion or repulsion

Epithelial cell-cell interactions are theoretically controlled by one of the two signals : adhesive and repulsive signals. The ability of epithelial cells to respond to these signals is fundamental to epithelial-

Received for publication December 18, 2007 ; accepted January 10, 2008.

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mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) (Figure 1). EMT is characterized by epithelial cell scattering, which involves the disassembly of cell-cell junctions followed by cell-cell dissociation and acquisition of migratory phenotype, and physiologically observed during embryonic development and organ regeneration. Deregulation of EMT is frequently detected in carcinoma and often associated with progression towards malignancy (5, 6).

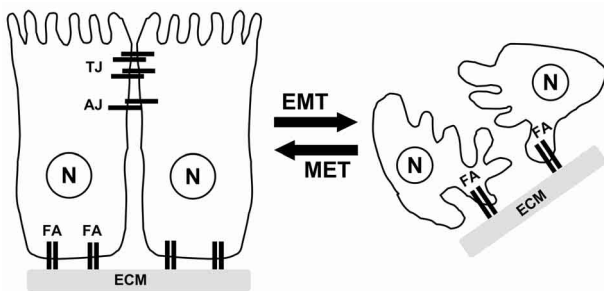


Figure 1. Cell adhesion and repulsion. A dynamic balance between cell adhesion and repulsion in epithelial cells is shown. TJ : tight junction, AJ : adherens junction, FA : focal adhesion, ECM : extracellular matrix, N : nucleus, EMT : epithelial-mesenchymal transition, MET : mesenchymal-epithelial transition.

Epithelial cell adhesion

Cell-cell adhesion—In epithelial cells, cell-cell adhesion is mediated through apical junctional complex (AJC). The organization of the AJC is defined by tight junction (TJ), which seals the intercellular space and delineates the boundaries between the apical and basolateral membranes, and adherens junction (AJ), which principally initiates and maintains cell-cell contacts (Figure 1) (7). At TJ, the transmembrane proteins claudins function as essential CAMs (8, 9). Claudins comprise claudin family consisting of at least 24 members in mammalian cells (10). Two other transmembrane proteins, occludin and junction adhesion molecules (JAMs), have been identified as CAMs at TJ (11, 12). Occludin is the first identified CAM, whose physiological function remains to be established, whereas JAMs are involved in accumulation of cell polarity proteins including Par-3/Par-6/atypical protein kinase C (aPKC) (13). At AJ, the transmembrane protein E-cadherin is a key CAM. E-cadherin is a member of cadherin superfamily that comprises over 100 members, each of which is expressed in non-epithelial cells as well as in epithelial cells (14, 15). Nectins are other IgG superfamily CAMs at AJ and involved in the organization of AJ either in co-

operation with or independently of E-cadherin (16, 17). These transmembrane CAMs are clustered by AJC plaque proteins, which in turn bind to actin cytoskeleton. AJC plaque proteins form an organizing platform for a variety of scaffolding, signaling, and membrane traffic proteins, including ZO proteins (ZO-1, ZO-2, and ZO-3), catenins, Rab3B, Rab8, and Rab13 (18). As exemplified in EMT/MET, AJC is very plastic cellular structure ; it is subjected to remodeling and highly motile even within apparently stable, confluent cultured monolayers (19, 20).

Cell-ECM adhesion—Cell-ECM adhesion is formed by the interaction of integrins with ECM proteins, such as collagen, fibronectin, laminin, and vitronectin (Figure 1). In the cytoplasm, integrins are linked to the actin cytoskeleton and recruit a large number of plaque proteins that are assembled into macromolecular structures termed focal adhesion (FA) (21). They include focal adhesion kinase (FAK), Src, and Crk-associated substrate (Cas) family proteins that comprise p130Cas, Crk-associated substrate in lymphocyte/human enhancer of filamentation 1/neural precursor cell-expressed, developmentally down-regulated gene 9 (CasL/HEF1/NEDD9), and embryonal fyn substrate/Src interacting (Efs/Sin). Cas family members have conserved domain structure and many, but not all, functional interactions, and serve as key scaffolding proteins bridging integrins and the actin cytoskeleton (22). CasL/HEF1/NEDD9 is identified independently as a highly phosphorylated 105-kDa protein after β_1 integrin stimulation in lymphocytes, a human protein that regulates filamentous budding in yeast, and a developmentally down-regulated gene in mouse brain (23-26). CasL/HEF1/NEDD9 is shown to regulate the scattering of Jurkat T cells and epithelial MCF7 cells (27, 28), and is recently identified as a metastasis gene for melanoma and implicated in the invasion of glioblastoma (29, 30).

Epithelial cell repulsion

Cell repulsion requires the downregulation of cell adhesion to other cells and to ECM and the extensive changes in cytoskeletal dynamics (1). Typical cell repulsion can be observed in the epithelial cell scattering. A variety of extracellular signals such as HGF/SF and Semaphorin are shown to induce cell repulsion through the complex intracellular signaling pathways (2, 3).

Extracellular signals

Among various extracellular signals, HGF/SF

plays a major role in epithelial cell repulsion. HGF and SF are discovered independently as a potent growth stimulator for primary hepatocytes kept in culture ("HGF") (31) and as a factor capable of inducing scatter of epithelial cells ("scatter factor") (32), respectively, and proved to be identical. HGF/SF receptor Met has tyrosine kinase activities and is identified as a proto-oncogene product (33). Activation of Met by HGF/SF evokes pleiotrophic biological effects in addition to epithelial cell scattering, and constitutive activation of HGF/SF-Met signaling results in the development and malignant progression of carcinoma, particularly in invasiveness and metastatic potential (2). In addition to HGF/SF, Semaphorins can induce scattering of epithelial cells. Semaphorins are a family of secreted or membrane-associated glycoproteins identified initially through their role in axon guidance (3). They have more than 20 members and are divided into 1-7 classes according to their structural features. Plexins are a family of Semaphorin receptors having an intrinsic GTPase activating protein (GAP) activity for R-Ras and are grouped into A-D classes on the basis of overall homology. Membrane-associated Semaphorins bind directly to Plexins, whereas secreted Semaphorins also require Neuropilins (Nrp1 and Nrp2) as obligate co-receptors. While HGF/SF belongs to plasminogen family, Met, Semaphorins, and Plexins are members of semaphorin superfamily based on the presence of a conserved Sema domain, an atypical motif made by over 500 amino acids, in the extracellular moiety (34, 35). There are functional interdependences between Met and Plexins. Members of Plexin B interacts constitutively with Met, and stimulation of Plexin B1 with its natural ligand Semaphorin 4D induces Plexin clustering and consequent HGF/SF-independent Met activation (36, 37).

Endocytic recycling of CAMs and ECM receptors

Endocytosis is a complex, multistep process, which involves invagination/budding of the plasma membrane, and formation of membrane vesicles followed by their delivery and fusion with specific intracellular compartments (38). Endocytosis occurs by one of four major mechanisms. The first is the formation of large actin-coated vacuolae that are responsible for uptake of liquids from the cell exterior and is referred to as macropinocytosis. The second involves polymerization of a specific coat protein, clathrin, on the intracellular face of the plasma membrane resulting in formation of clathrin-coated

pits and is referred to as clathrin-dependent endocytosis. The third involves invagination of cholesterol-enriched microdomains within the plasma membrane that may contain a coat protein, caveolin, and is referred to as caveolin-dependent endocytosis. The fourth does not form actin-coated vacuolae, clathrin-coated pits, and caveolin-containing invagination and is referred to as clathrin- and caveolin-independent endocytosis. After separation from the plasma membrane, endocytic vesicles first fuse with a juxtamembrane cytosolic compartment, early endosomes. Then, internalized proteins may directly return to the plasma membrane (short-loop recycling), enter recycling endosomes for the subsequent recycling (long-loop recycling), or be delivered to late endosomes where they become targeted for degradation in lysosomes (Figure 2).

These pathways are strictly regulated by soluble *N*-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) proteins that drive intermembrane fusion (39). SNARE proteins have 36 members in humans and a characteristic SNARE motif, an evolutionarily conserved stretch of 60-70 amino acids arranged in heptad repeats. Each endosomal vesicle would have carried a specific 'v-SNARE' which interacts with a cognate 't-SNARE' on the target membrane. In polarized epithelial cells, two major t-SNARE proteins, syntaxin 3 and 4 appear to be spatially segregated into different plasma membrane domains with syntaxin 3 confined to the apical surface, and syntaxin 4 confined to the lateral plasma membrane (40). While the apical targeting requires the tetanus neurotoxin (TeNT)-resistant v-SNARE TI-VAMP (VAMP7), the basolateral targeting is recently shown to involve the TeNT-sensitive v-SNARE cellubrevin (VAMP3) (41, 42).

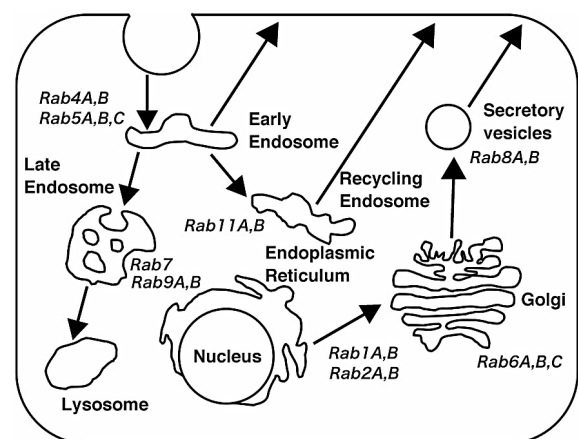


Figure 2. Rab family small G proteins. Subcellular localization of human Rab proteins is shown.

The critical components of regulatory machinery for the endocytic recycling of CAM and ECM receptors include ARF and Rab family small G proteins (43). ARF proteins have six members in mammalian cells and regulate membrane traffic and organella structures. Each member of ARF proteins localizes to distinct subsets of intracellular membranes and uses the guanine nucleotide-dependent switch mechanism to carry out its specific function. While ARF6 activation results in the internalization of E-cadherin from AJC into early endosomes, blocking ARF6 activation inhibits its internalization and prevents cell-cell dissociation (44). ARF6 and its GAP ACAP1 also regulate the endocytic recycling of β 1 integrin (45, 46).

Rab family small G proteins

Rab family small G proteins comprise the largest family of the Ras superfamily small G proteins (47-49). To date, 63 Rab proteins have been identified in human and phylogenetic analyses allow their arrangement into eight Rab functional groups (50). Rab proteins behave as membrane-associated molecular switches, cycling between GTP-bound active and GDP-bound inactive states. This switch is controlled by guanine nucleotide exchange factor (GEF), which trigger the binding of GTP, and GAP, which accelerate hydrolysis of the bound GTP to GDP. Rab proteins also undergo a membrane association and dissociation cycle, which is coupled to the GTP/GDP cycle. Most Rab proteins are post-translationally modified with geranylgeranyl moieties that enables their membrane association. GDP dissociation inhibitor (GDI) binds to geranylgeranylated Rab proteins in their GDP-bound form, masking their geranylgeranyl anchor and thereby maintaining the Rab proteins in the cytosol. Membrane attachment of Rab proteins therefore requires the function of a GDI displacement factor (GDF). Once dissociated from GDI, Rab proteins are converted to their GTP-bound form by their specific GEFs. The active, membrane-bound Rab proteins are then able to fulfill their various functions in membrane traffic by binding to their specific effectors. After inactivation by their specific GAP, the GDP-bound Rab proteins can be extracted from the membrane by GDI and recycled back to the cytosol.

A vital aspect of Rab function is the specific localization of each Rab protein to a particular subcellular compartment, and its involvement in a specific transport step. In other words, each subcellular compartment has a unique combination of Rab

proteins, which have been frequently used as markers of the particular organella (Figure 2). For example, Rab5 is associated with early endosomes, whereas Rab4 and Rab11 are markers for recycling endosomes and Rab7 and Rab9 are primarily associated with the late endosomal compartment. However, the molecular mechanisms controlling Rab localization are not fully understood. A unique, hypervariable C-terminal domain of Rab proteins is first thought to be the signal sequences for the correct targeting of Rab proteins to their specific membranes. However, accumulating evidences suggest that multiple factors including GDI, GDF, GEF, and effector proteins contribute to the specificity of Rab localization and the close coordination of membrane targeting with Rab activation (47-49).

Several members of Rab proteins have been implicated in the regulation of endocytic recycling of CAMs and ECM receptors. Rab5 regulates the endocytosis of E-cadherin in response to HGF/SF-Met activation in MDCK cells (51, 52). We have revealed that Rab13 regulates the endocytic recycling of occludin and the formation of functional TJ in epithelial cells (53, 54). For the endocytic recycling of integrins, Rab4 and Rab11 are implicated in their short-loop and long-loop recycling, respectively, and are known to influence the cell adhesion and migration (45, 55). Recently, Rab5, Rab21, and Rab25 are also implicated in the endocytic recycling of integrins (Figure 4) (56, 57).

MICAL family proteins

Molecule interacting with CasL (MICAL) is originally identified as a novel binding protein of a FA plaque protein CasL/HEF1/NEDD9 that regulates the scattering of epithelial cells and the progression and metastasis of cancer cells (58). MICAL is conserved from flies to mammals, with two MICAL family genes (D-MICAL and D-MICAL-L) identified in *Drosophila* and five (MICAL-1, MICAL-2, MICAL-3, MICAL-L1, and JRAB/MICAL-L2) found in mammals (59). While there seems to exist several splicing variants for each MICAL family member, their functions remain to be determined except for MICAL-2 isoforms (PVa and PVb) that are recently shown to be involved in the progression of prostate cancer (60). MICAL family proteins are large, multidomain, cytosolic proteins expressed in specific neuronal and non-neuronal cells both during development and in adulthood. Sequence analysis reveals that MICAL family proteins contain calponin homology (CH) and LIM domains, protein-protein

interaction domains implicated in signal transduction and cytoskeletal organization, plus coiled-coil (CC) domain. D-MICAL, MICAL-1, MICAL-2, and MICAL-3 also possess a flavin-adenine dinucleotide (FAD)-binding monooxygenase domain (Figure 3).

In a search for a mediator of Semaphorin 1-Plexin A signaling in *Drosophila*, D-MICAL is identified as a Plexin A-interacting protein. D-MICAL, MICAL-1, and MICAL-2 bind to the cytoplasmic region of Plexin A via its C-terminus containing the CC domain (59). D-MICAL-Plexin A interaction and monooxygenase activity of D-MICAL are required for Semaphorin 1-induced motor axon repulsion in the developing *Drosophila*. MICAL-1, MICAL-2, and MICAL-3 also function downstream of Semaphorin 3 receptor Plexin A and the selective monooxygenase inhibitor (-)-epigallocatechin gallate (EGCG) abrogates Semaphorin 3A-mediated repulsion of rat sensory axons *in vitro* (61). Consistent with the need of the extensive changes in cytoskeletal dynamics in cell repulsion, association of MICAL proteins with vimentin and microtubules has been reported (58, 62). We have also shown that JRAB/MICAL-L2 is displaced from TJ upon actin depolymerization and is distributed along radiating actin cables and stress fibers in Ca²⁺-depleted epithelial and fibroblastic cells, respectively (53).

A series of recent studies revealed the potential role of MICAL family proteins in the regulation of a proper balance between cell adhesion and repulsion. MICAL-1 can interact with CasL/HEF1/NEDD9 that is implicated in the regulation of the adhesive

activities of integrins and responsible for the progression and metastasis of melanoma and glioblastoma (29, 30, 58). MICAL-2 isoforms (PVa and PVb) are implicated in the progression of prostate cancer (60). We have identified JRAB/MICAL-L2 as a Rab13 effector protein and found that Rab13 and JRAB/MICAL-L2 mediate the scattering of epithelial cells in response to 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (Figure 4) (53, 63).

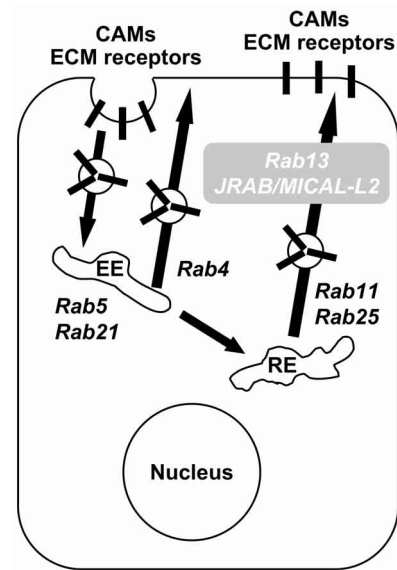


Figure 4. Rab13 and JRAB/MICAL-L2. Role of Rab13 and JRAB/MICAL-L2 in regulating the endocytic recycling of CAMs is shown. CAMs : cell adhesion molecules, ECM : extracellular matrix, EE : early endosome, RE : recycling endosome.

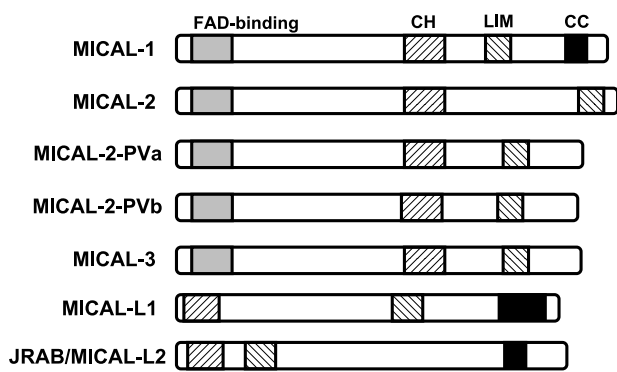


Figure 3. MICAL family proteins. Domain architecture of human MICAL proteins is shown. For simplicity, the representative isoforms of each human MICAL family member are presented [MICAL-1 (NM_022765), MICAL-2 (NM_014632), MICAL-2-PVa (AB 110785), MICAL-2-PVb (AB 110786), MICAL-3 (NM_015241), MICAL-L1 (NM_033386), and JRAB/MICAL-L2 (NM_182924)]. FAD-binding : flavin-adenine dinucleotide-binding domain, CH : calponin homology domain, LIM : LIM domain, CC : coiled-coil domain.

CONCLUSIONS

Recent studies have begun to reveal the crucial role of endocytic recycling of CAMs and ECM receptors in regulating cell adhesion and repulsion. We have revealed that JRAB/MICAL-L2 functions as a Rab13 effector protein and mediates the endocytic recycling of occludin, the formation of functional TJ, and the scattering of epithelial cells. While Rab family small G proteins are key regulators of the endocytic recycling pathways, MICAL family proteins interact with receptors for cell repulsion signals, FA plaque proteins, and cytoskeletons. Given the importance of regulating the endocytic recycling of CAMs and ECM receptors and the cytoskeletal dynamics for the proper balance between cell adhesion and repulsion, JRAB/MICAL-L2 may serve as a central scaffold to switch from adhesion to repulsion and/or *vice versa*.

ACKNOWLEDGEMENTS

This study was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, Culture, and Technology of Japan.

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