Small GTPases 3:4, 213-218; October/November/December 2012; © 2012 Landes Bioscience

Regulation of collective cell migration by RhoGAP myosin IXA

Tatiana Omelchenko
Cell Biology Program; Memorial Sloan-Kettering Cancer Center; New York, NY USA

Collective cell migration is a key process during epithelial morphogenesis, tissue regeneration and tumor dissemination. During collective epithelial migration, anterior-posterior polarity, apical-basal polarity and cell-cell junctions must be dynamically coordinated, but the underlying molecular mechanisms controlling this complex behavior are unclear. Rho GTPases regulate the actin cytoskeleton, in particular protrusive and contractile activities at cell-cell contacts. Recently, a number of regulators—nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs)—have been identified and suggested to provide spatio-temporal control of Rho GTPases at cell-cell contacts. One of these is myosin IXA, a member of class IX, single-headed actin motors having a conserved RhoGAP domain. Using its actin-binding and motor activities, myosin IX interacts with actin filaments and moves toward filament plus ends. At the plasma membrane, myosin IX's RhoGAP activity negatively regulates Rho to facilitate localized reorganization of the actin cytoskeleton. Here, I discuss how myosin IXA regulates Rho and the actin cytoskeleton during the assembly of nascent cell-cell contacts and how this might contribute to collective epithelial migration.

Keywords: collective cell migration, myosin IXA, Rho GTPases, RhoGAP, actin cytoskeleton, cell-cell contact

Submitted: 04/09/12

Accepted: 04/24/12

http://dx.doi.org/10.4161/sgtp.20495

Correspondence to: Tatiana Omelchenko; Email: omelchet@mskcc.org

Commentary to: Omelchenko T, Hall A. Myosin-IXA regulates collective epithelial cell migration by targeting RhoGAP activity to cell-cell junctions. Curr Biol 2012; 22:278–88; PMID:22305756; http://dx.doi.org/10.1016/j.cub.2012.01.014.

Introduction

Collective cell migration is characterized by the maintenance of a physical interaction between cells coupled with coordinated anterior-posterior polarization of individual cells within a migrating monolayer or group (**Fig. 1**). It has a fundamental role in embryonic development, regeneration and cancer metastasis.[1] Collective cell migration has been studied in vitro in migrating epithelial or endothelial monolayers in response to a scratch wound,[2-4] on patterned substrates,[5] in tissue explants of cancer cells, mesoderm or mammary ducts[6-8] and in 3D.[9-11] Examples of in vivo models of collective cell migration are numerous and include developing embryos of fruit fly, zebrafish, mouse and metastatic cancers in mice.[1] The molecular mechanisms underlying such coordinated migration are, however, not well understood.[1]

Morphological features of collective cell migration include basal anterior-posterior cell polarity manifested as unidirectional, actin-rich protrusions at the front of multiple cell rows (**Fig. 1A and B**). This results in the migrating group having a fish scale-like arrangement (**Fig. 1C**). The basal protrusions of following cells penetrate under leading cells and have a distinct cadherin distribution (**Fig. 1B** and underlapping) and actin cytoskeletal organization[12] reflecting complex cell-cell interactions in this region. Highly coordinated regulation of cell-cell junctions (localization and clustering of junctional proteins) and the actin cytoskeleton associated with junctions (affecting stabilization, adhesion strength and protrusive activity) are key features of collective cell migration. Recent experiments have revealed that cell-cell adhesion strength can regulate the directionality of coordinated cell movement, as demonstrated by collective E-cadherin-mediated mesendoderm migration during zebrafish gastrulation.[13] E-cadherin is essential for collective epithelial migration,[14] but excess cell-cell adhesion blocks collective migration, for example in Drosophila

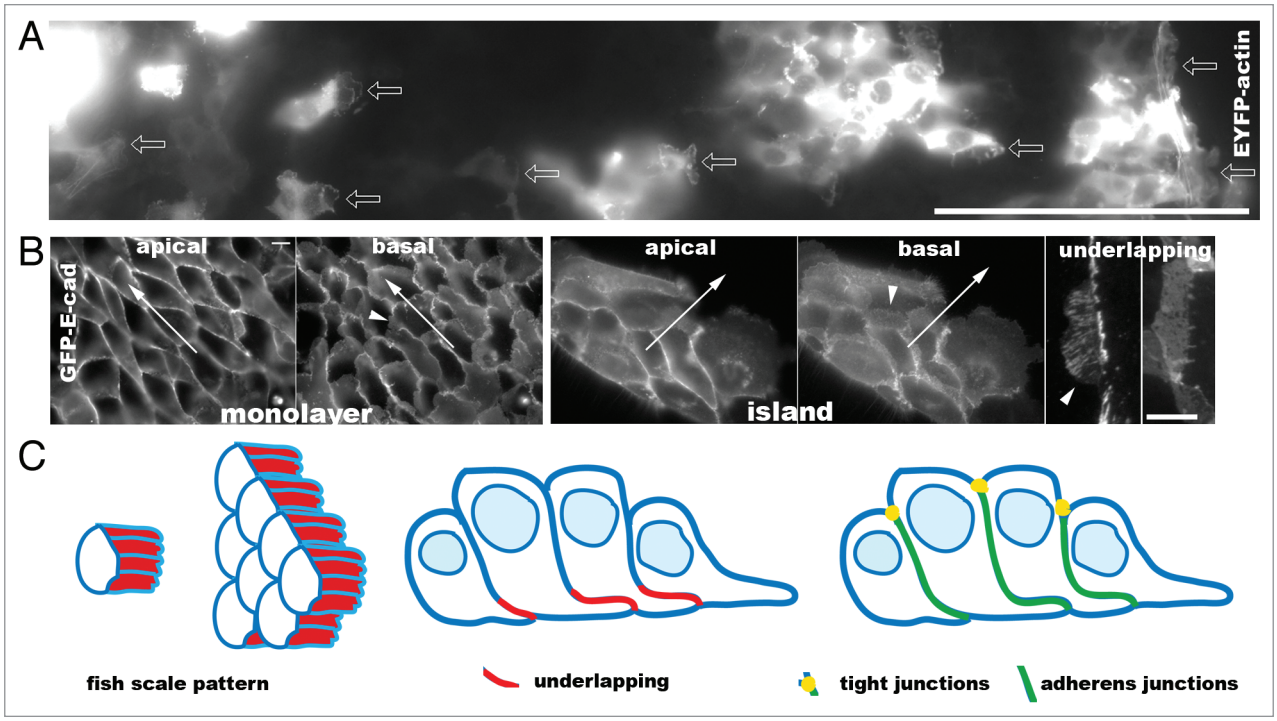

Figure 1. Morphological features of collective epithelial cell migration. (**A**) Anterior-posterior polarity in 16HBE cells developed in response to a wound scratch. Wound edge is at the right. Actin-rich protrusions (arrows) visualized by EYFP-actin expressed in a mosaic pattern in a 16HBE epithelial monolayer have unidirectional orientation many rows behind the scratch. Bar is 100 μm. (**B**) Protrusions found at the basal plane (arrowheads) visualized by GFP-E-cadherin expression in a monolayer or islands of 16HBE cells face the direction of migration (arrows). Note, the protrusions in the back row cells underlap the front rows cells (arrowheads). Bar is 10 μm. (**C**) Schematic of migrating epithelial cell island profile. Cell-cell interaction zones at the basal plane labeled with red lines (underlapping protrusions) and the lateral sides marked with tight and adherens junctions. Protrusions face direction of migration (to the right).

border cells or in mouse mammary epithelial cells.[14,15]

The Rho family of small GTPases are major regulators of the actin cytoskeleton, with protrusive lamellipodial activity promoted by Rac, filopodia formation by Cdc42 and contractile actin-myosin activity by Rho.[16] They also regulate cell-cell junction dynamics (adherens and tight junctions), both directly (transport and clustering) and indirectly (through the associated actin cytoskeleton).[17,18] Rho GTPases are molecular switches and are themselves controlled by interconvertion between active GTP-bound, and inactive GDP-bound states. When active, GTPases bind specific effector proteins to stimulate downstream signaling. Rho GTPases are activated by guanine nucleotide exchange factors (GEFs) [19] and inactivated by GTPase activating proteins (GAPs).[20] Some 150 genes encode mammalian GEFs and GAPs, and most are not well characterized. It is likely that these regulators play a central role in defining the spatio-temporal activity of Rho GTPases during migration.

In a recent study, we described a role for myosin IXA, a Rho-specific GAP, in the collective migration of human bronchial epitheliocytes, 16HBE cells.[21] These cells, which show remarkably coordinated collective migration in culture (**Fig. 1**), were used in an siRNA-based screen to identify GEFs and GAPs involved in collective cell migration. We found that in the absence of myosin IXA, 16HBE cells failed to form stable adherens junctions during migration resulting in cell scattering and subsequent random migration. More careful analysis revealed that remodeling of the actin cytoskeleton at cell-cell contacts in response to cadherin-mediated adhesion was defective in myosin IXA-depleted cells. Here, I will discuss our current ideas about how the regulation of Rho by myosin IXA likely contributes to effective collective migration of these epithelial cells.

Collective Cell Migration and the Role of Rho-Dependent Actin-Myosin Contractility

A major contributor to collective cell migration is thought to be a mechanical force. Actin-myosin contractile forces regulate cell shape and the stability of cell-matrix and cell-cell junctional adhesions.[22-25] The forces generated by actin-myosin contractile filament bundles associated with cell-cell junctions can also be transmitted throughout migrating cell groups to regulate collective behavior, as seen in monolayers[26] and tissues.[27]

Two spatially and functionally distinct actin populations have been reported at cell-cell contacts in epithelial cells: junctional or radial actin, and tangential contractile thin bundles.[28,29] The collision of two protruding lamellipodia stimulates local clustering of E-cadherins (primordial junctions) accompanied by the formation of junctional actin. The junctional actin associates with and stabilizes E-cadherins

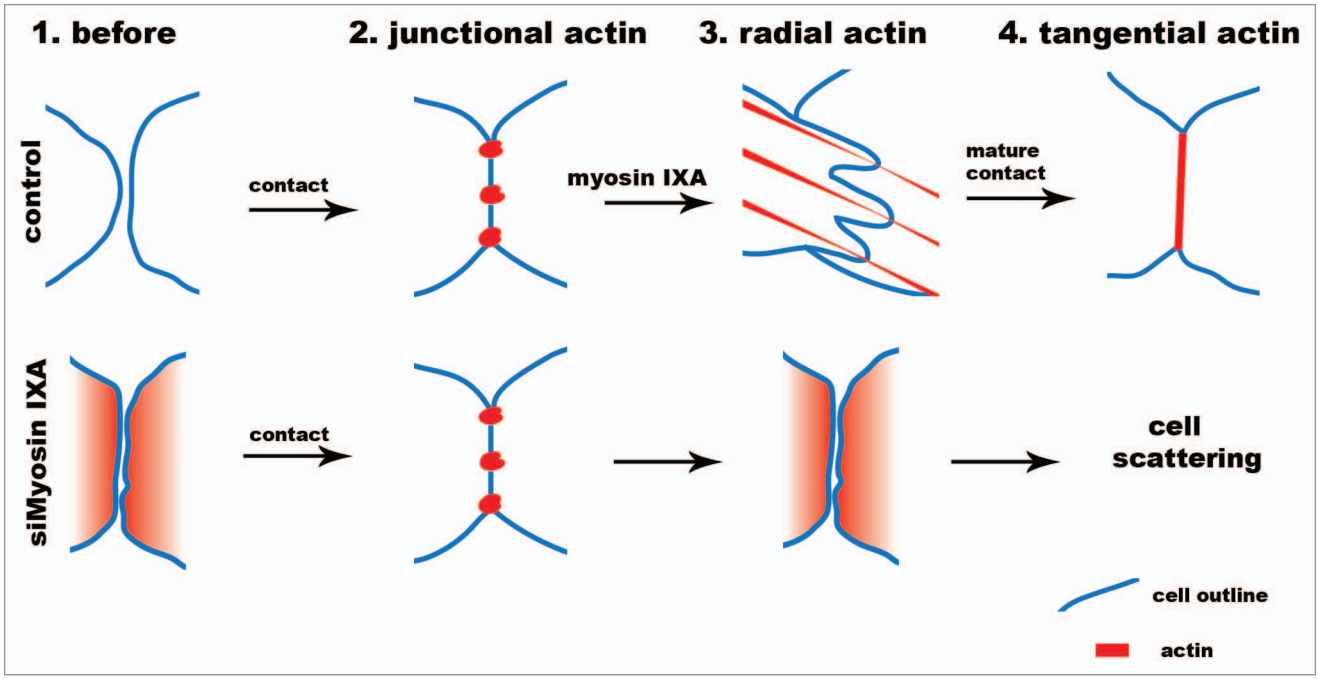

Figure 2. Cell-cell contact formation in 16HBE cells is controlled by myosin IXA. Protrusive lamellipodia collide and overlap leading to accumulation of junctional actin (red dots). Myosin IXA is recruited at this time and the GAP domain reduces Rho activity at contact zones allowing the formation of radial actin bundles (red diagonal lines). Maturation of the cell-cell contact leads to tangential actin bundles. In myosin IXA depleted cells, high Rho activity prevents lamellipodial overlapping, formulation of radial actin and stabilization of cell-cell contacts. Colliding lamellipodia retract leading to cell scattering.

and primordial junctions.[28] During junctional maturation, actin filaments become tangential, circumferential bundles (**Fig. 2**). These are contractile and co-align with linear tangential E-cadherin-mediated adhesions and function to increase the height of the lateral surface of cell-cell contact.[28] In 16HBE epitheliocytes, actin puncta and radial actin finger bundles are associated with primordial junctions, and this later reorganizes into circumferential bundles and mature junctions (**Fig. 2**).[21,30]

Diverse mechanisms control actin-myosin contractility, but a major player, particularly during cell migration, is Rho.[31] Activation of Rho by a variety of GEFs results in activation of the effector Rho kinase (ROCK). ROCK in turn activates myosin II directly, by phosphorylation of myosin light chain II, and probably more importantly indirectly, by inactivating myosin light chain phosphatase. Bundled filaments of active myosin II motors pull on the F-actin network to generate contractile force.[31]

Rho is an important player in the collective migration of 16HBE cells. Overexpression of constitutively active or dominant negative forms of RhoA has been reported to alter the actin cytoskeleton and inhibit monolayer migration.[32] A more detailed analysis revealed that the depletion of RhoA by RNAi inhibits the formation of mature cell-cell contacts and the formation of tangential actin bundles, but does not prevent the assembly of junctional actin and radial bundles associated with primordial junctions.[30] On the contrary, it seems likely that the initial stages of cell-cell contact establishment, including the formation of radial actin fingers, are inhibited by contractile forces associated with Rho and myosin II activity. Direct evidence to support this has emerged from studies on the spatio-temporal activity of RhoA using FRET-based biosensors in various epitheliocytes. They reveal that active RhoA accumulates at the lateral expanding edges of cell-cell contacts, but not at the overlapping central area.[33,34] This raises the possibility that RhoA is locally inhibited and an obvious mechanism for this would be through the recruitment of a RhoGAP.

A number of Rho family regulators including RhoGAP domain containing proteins have been characterized in the context of cell-cell contact regulation.[17] In fibroblasts, N-cadherin-mediated cell-cell junctions are regulated by p190RhoGAP-mediated inhibition of RhoA. It was shown that Rac1 activation promoted recruitment of p190RhoGAP to adherens junctions where p190RhoGAP transiently interacted with p120catenin leading to local inhibition of RhoA.[35] However, the exact role of p190RhoGAP in epithelial cells remains unclear.[17] In epithelial cells, ARHGAP10 was found to localize at cell-cell contacts and interact with α-catenin. In siRNA depletion experiments ARHGAP10 was necessary for recruitment of α-catenin. Overexpression of C-terminal RhoGAP-containing fragment led to disassembly of the actin stress fibers.[36] ARHGAP12 was recently identified and cloned as a new junctional protein localized to E-cadherin-mediated junctions of intestinal epithelia.[37] It seems likely that the negative regulation of Rho GTPases underlies the dynamic behavior of cell-cell contacts, and it will be interesting to investigate the functional roles of these different GAPs at junctions.

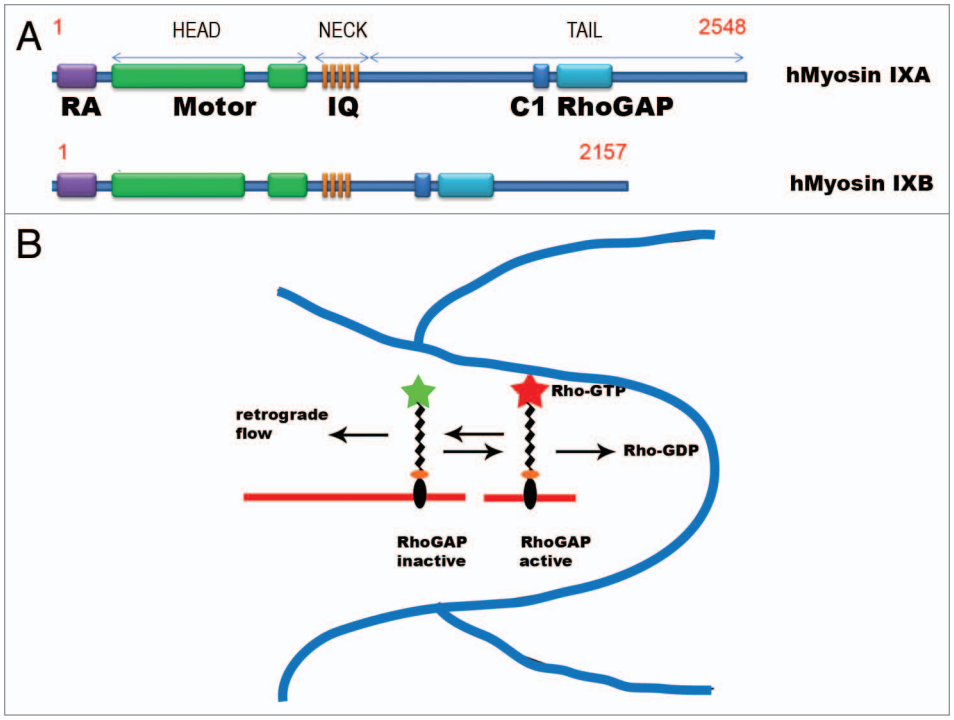

Figure 3. Possible mechanism of myosin IXA function at cell-cell contacts. (**A**) Schematic diagram of the domain organization of the human class IX myosins. The domains are RA (Ras-associated domain, purple), MOTOR (motor domain divided by loop 2 insertion, green), IQ (five IQ motifs, a light chain binding region, yellow), C1 domain (dark blue) and RhoGAP domain (blue). Sequence length is in aminoacids (red numbers). (**B**) Model for myosin IXA RhoGAP function at cell-cell contact site. Targeted to the cell-cell collision sites, myosin IXA maintains low Rho-GTP levels at the plasma membrane. Low Rho favors the formation of thin actin fingers associated with primordial junctions. Myosin IXA RhoGAP activity is later attenuated, perhaps by binding to actin filaments and removal by retrograde flow.

In our work, using an RNAi-based screen to identify Rho family GTPase regulators required for the collective migration of human bronchial epithelial cells, we identified RhoGAP myosin IXA. High-resolution time-lapse imaging and structure-function analysis of RhoGAP myosin IXA allowed us to investigate how RhoGAP activity regulates the junctional actin cytoskeleton and cell-cell adhesions in collectively migrating cells. In 16HBE epitheliocytes, FRET ratio imaging experiments revealed high levels of RhoA activity at primordial cell-cell contacts after myosin IXA depletion, resulting in destabilization of overlapping lamellipodia and inhibition of radial actin bundle formation. Experimentally reducing Rho-dependent contractility, using a ROCK inhibitor or expressing the RhoGAP domain-containing fragment of myosin IXA, increased cell-cell contact stability in myosin IXA-depleted cells.[21] These data support the idea that local inhibition of Rho-dependent actin-myosin contractility is important for actin reorganization supporting cell-cell contact stability during collective epithelial migration.

Regulation of Rho at Cell-Cell Contacts by Myosin IXA

Myosin IXA (Myo9a) and myosin IXB (Myo9b) are the only class IX myosins in mammals.[38] These two proteins share similar domains: N-terminal RA (Ras-associated) domain, the motor domain composed of two parts separated by loop 2 insertion (the head), multiple IQ motifs (a light chain binding region, the neck), an atypical C1 domain and a RhoGAP domain (the tail) (**Fig. 3A**). The motor domain has a unique structure allowing myosins IX to attach to actin filaments and move processively toward the plus end of F-actin.[39,40] The loop 2 insertion has been shown to be required for actin filament attachment.[41] The RhoGAP domain is highly conserved and shows specificity to RhoA/B/C in vitro and in vivo.[42,43] The IQ motifs play a regulatory role by binding calmodulin, but the function of the RA and C1 domains is not clear.

In contrast to myosin IXB, whose biochemical and motor properties have been characterized in some detail, little is known about myosin IXA.[38,44] A myosin IXA knockout mouse revealed defects in cell-cell junctions and cell morphology of brain ependymal epithelial cells, indicating the physiological importance of myosin IXA function at junctions.[38,45] We found that EGFP-myosin IXA localizes to actin bundles through its motor domain, and in a punctate fashion similar to myosin II.[21] These EGFP-myosin IXA puncta were able to translocate along actin bundles, as observed by TIRF microscopy in live epitheliocytes (data not published), and to move together by retrograde flow. Mutations abolishing motor activity or actin-binding (loop 2 insertion deletion) of myosin IXA led to the loss of puncta and a diffuse localization of the protein into the cytoplasm. Thus, motor and actin-binding activities are important for junctional localization of myosin IXA.

More detailed imaging analysis will be required to understand how myosin IXA is delivered to primordial cell-cell junctions. It will also be interesting to investigate the nature of EGFP-myosin IXA puncta, for example whether they have actin cross-linking activity.

Inactivation of RhoGAP activity, by a point mutation in the conserved arginine finger at the catalytic site, revealed an important role for GAP activity at cell-cell contacts in 16HBE cells.[21,46] Low-level expression of the RhoGAP domain alone partially rescued the cell scattering phenotype induced by myosin IXA depletion. Together with the FRET imaging data, this indicates that the GAP domain of myosin IXA acts as a localized, negative regulator of Rho to control contractile forces during the early stages of cell-cell contact formation.[21]

How might myosin IXA regulate Rho activity at cell-cell junctions? It seems likely that during lamellipodial collisions, myosin IXA is targeted to the membrane at nascent cell-cell contacts, through its actin-binding and motor properties. Low Rho/ROCK activity at junctions is needed to maintain lamellipodia overlap and radial actin finger formation during these early stages of junction formations. Once thin actin bundles are formed, myosin IXA RhoGAP activity could either be inactivated (perhaps through phosphorylation) or removed by retrograde flow (**Fig. 3B**). The removal of this Rho GAP activity would allow a buildup of Rho activity, to promote subsequent maturation of junctional contacts.

Concluding Remarks

Collective cell migration plays a major role during morphogenetic movements in development and disease. Collective cell migration requires dynamic rearrangements of cell-cell junctions and the associated actin cytoskeleton and this likely involves the localized and coordinated action of Rho family GEFs and GAPs. In agreement with this, a number of Rho family GAP and GEF domain containing proteins have been characterized in the context of cell-cell contact regulation. The Rho GAP activity of myosin IXA negatively regulates Rho-dependent actin-myosin contractility during early stages of cell-cell contact formation, but during junctional maturation Rho activity is restored, presumably though recruitment of specific GEFs. The spatio-temporal recruitment of Rho family GEFs and GAPs is likely to be a major driving force for collective epithelial cell migration.

Acknowledgments

I thank Alan Hall for critical reading and editing of the manuscript. I thank Fanny Jaulin and Joanne Durgan for useful comments. The work was supported by a National Institutes of Health (NIH) grant GM081435 to A. Hall and a National Cancer Institute core center grant (P30-CA 08748).

References

1. Friedl P, Gilmour D. Collective cell migration in morphogenesis, regeneration and cancer. Nat Rev Mol Cell Biol 2009; 10:445-57; PMID:19546857; http://dx.doi.org/10.1038/nrm2720.
2. Farooqui R, Fenteany G. Multiple rows of cells behind an epithelial wound edge extend cryptic lamellipodia to collectively drive cell-sheet movement. J Cell Sci 2005; 118:51-63; PMID:15585576; http://dx.doi.org/10.1242/jcs.01577.
3. Omelchenko T, Vasiliev JM, Gelfand IM, Feder HH, Bonder EM. Rho-dependent formation of epithelial "leader" cells during wound healing. Proc Natl Acad Sci USA 2003; 100:10788-93; PMID:12960404; http://dx.doi.org/10.1073/pnas.1834401100.
4. Vitorino P, Hammer M, Kim J, Meyer T. A steering model of endothelial sheet migration recapitulates monolayer integrity and directed collective migration. Mol Cell Biol 2011; 31:342-50; PMID:20974808; http://dx.doi.org/10.1128/MCB.00800-10.
5. Poujade M, Grasland-Mongrain E, Hertzog A, Jouanneau J, Chavrier P, Ladoux B, et al. Collective migration of an epithelial monolayer in response to a model wound. Proc Natl Acad Sci USA 2007; 104:15988-93; PMID:17905871; http://dx.doi.org/10.1073/pnas.0705062104.
6. Hegerfeldt Y, Tusch M, Bröcker EB, Friedl P. Collective cell movement in primary melanoma explants: plasticity of cell-cell interaction, beta1-integrin function and migration strategies. Cancer Res 2002; 62:2125-30; PMID:11929834.
7. Carmona-Fontaine C, Theveneau E, Tzekou A, Tada M, Woods M, Page KM, et al. Complement fragment C3a controls mutual cell attraction during collective cell migration. Dev Cell 2011; 21:1026-37; PMID:22118769; http://dx.doi.org/10.1016/j.devcel.2011.10.012.
8. Ewald AJ, Brenot A, Duong M, Chan BS, Werb Z. Collective epithelial migration and cell rearrangements drive mammary branching morphogenesis. Dev Cell 2008; 14:570-81; PMID:18410732; http://dx.doi.org/10.1016/j.devcel.2008.03.003.
9. Wolf K, Wu YI, Liu Y, Geiger J, Tam E, Overall C, et al. Multi-step pericellular proteolysis controls the transition from individual to collective cancer cell invasion. Nat Cell Biol 2007; 9:893-904; PMID:17618273; http://dx.doi.org/10.1038/ncb1616.
10. Hidalgo-Carcedo C, Hooper S, Chaudhry SI, Williamson P, Harrington K, Leitinger B, et al. Collective cell migration requires suppression of actomyosin at cell-cell contacts mediated by DDR1 and the cell polarity regulators Par3 and Par6. Nat Cell Biol 2011; 13:49-58; PMID:21170030; http://dx.doi.org/10.1038/ncb2133.
11. Ewald AJ, Huebner RJ, Palsdottir H, Lee JK, Perez MJ, Jorgens DM, et al. Mammary collective cell migration involves transient loss of epithelial features and individual cell migration within the epithelium. J Cell Sci 2012; PMID:22344263; http://dx.doi.org/10.1242/jcs.096875.
12. Kametani Y, Takeichi M. Basal-to-apical cadherin flow at cell junctions. Nat Cell Biol 2007; 9:92-8; PMID:17159998; http://dx.doi.org/10.1038/ncb1520.
13. Arboleda-Estudillo Y, Krieg M, Stühmer J, Licata NA, Muller DJ, Heisenberg CP. Movement directionality in collective migration of germ layer progenitors. Curr Biol 2010; 20:161-9; PMID:20079641; http://dx.doi.org/10.1016/j.cub.2009.11.036.
14. Li L, Hartley R, Reiss B, Sun Y, Pu J, Wu D, et al. E-cadherin plays an essential role in collective directional migration of large epithelial sheets. Cell Mol Life Sci 2012; PMID:22410739; http://dx.doi.org/10.1007/s00018-012-0951-3.
15. Melani M, Simpson KJ, Brugge JS, Montell D. Regulation of cell adhesion and collective cell migration by hindsight and its human homolog RREB1. Curr Biol 2008; 18:532-7; PMID:18394891; http://dx.doi.org/10.1016/j.cub.2008.03.024.
16. Hall A. Small GTP-binding proteins and the regulation of the actin cytoskeleton. Annu Rev Cell Biol 1994; 10:31-54; PMID:7888179; http://dx.doi.org/10.1146/annurev.cb.10.110194.000335.
17. Citi S, Spadaro D, Schneider Y, Stutz J, Pulimeno P. Regulation of small GTPases at epithelial cell-cell junctions. Mol Membr Biol 2011; 28:427-44; PMID:21781017; http://dx.doi.org/10.3109/09687688.2011.603101.
18. Baum B, Georgiou M. Dynamics of adherens junctions in epithelial establishment, maintenance and remodeling. J Cell Biol 2011; 192:907-17; PMID:21422226; http://dx.doi.org/10.1083/jcb.201009141.
19. Rossman KL, Der CJ, Sondek J. GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. Nat Rev Mol Cell Biol 2005; 6:167-80; PMID:15688002; http://dx.doi.org/10.1038/nrm1587.
20. Bos JL, Rehmann H, Wittinghofer A. GEFs and GAPs: critical elements in the control of small G proteins. Cell 2007; 129:865-77; PMID:17540168; http://dx.doi.org/10.1016/j.cell.2007.05.018.
21. Omelchenko T, Hall A. Myosin-IXA regulates collective epithelial cell migration by targeting RhoGAP activity to cell-cell junctions. Curr Biol 2012; 22:278-88; PMID:22305756; http://dx.doi.org/10.1016/j.cub.2012.01.014.
22. Sahai E, Marshall CJ. ROCK and Dia have opposing effects on adherens junctions downstream of Rho. Nat Cell Biol 2002; 4:408-15; PMID:11992112; http://dx.doi.org/10.1038/ncb796.
23. Ayollo DV, Zhitnyak IY, Vasiliev JM, Gloushankova NA. Rearrangements of the actin cytoskeleton and E-cadherin-based adherens junctions caused by neoplasic transformation change cell-cell interactions. PLoS One 2009; 4:8027; PMID:19956566; http://dx.doi.org/10.1371/journal.pone.0008027.
24. Liu Z, Tan JL, Cohen DM, Yang MT, Sniadecki NJ, Ruiz SA, et al. Mechanical tugging force regulates the size of cell-cell junctions. Proc Natl Acad Sci USA 2010; 107:9944-9; PMID:20463286; http://dx.doi.org/10.1073/pnas.0914547107.

25. Taguchi K, Ishiuchi T, Takeichi M. Mechanosensitive EPLIN-dependent remodeling of adherens junctions regulates epithelial reshaping. J Cell Biol 2011; 194:643-56; PMID:21844208; http://dx.doi.org/10.1083/jcb.201104124.
26. Trepat X, Fredberg JJ. Plithotaxis and emergent dynamics in collective cellular migration. Trends Cell Biol 2011; 21:638-46; PMID:21784638; http://dx.doi.org/10.1016/j.tcb.2011.06.006.
27. Gorfinkiel N, Blanchard GB. Dynamics of actomyosin contractile activity during epithelial morphogenesis. Curr Opin Cell Biol 2011; 23:531-9; PMID:21764278; http://dx.doi.org/10.1016/j.ceb.2011.06.002.
28. Zhang J, Betson M, Erasmus J, Zeikos K, Bailly M, Cramer LP, et al. Actin at cell-cell junctions is composed of two dynamic and functional populations. J Cell Sci 2005; 118:5549-62; PMID:16291727; http://dx.doi.org/10.1242/jcs.02639.
29. Ivanov AI. Actin motors that drive formation and disassembly of epithelial apical junctions. Front Biosci 2008; 13:6662-81; PMID:18508686; http://dx.doi.org/10.2741/3180.
30. Wallace SW, Magalhaes A, Hall A. The Rho target PRK2 regulates apical junction formation in human bronchial epithelial cells. Mol Cell Biol 2011; 31:81-91; PMID:20974804; http://dx.doi.org/10.1128/MCB.01001-10.
31. Katoh K, Kano Y, Noda Y. Rho-associated kinase-dependent contraction of stress fibres and the organization of focal adhesions. J R Soc Interface 2011; 8:305-11; PMID:20826475; http://dx.doi.org/10.1098/rsif.2010.0419.
32. Desai LP, Aryal AM, Ceacareanu B, Hassid A, Waters CM. RhoA and Rac1 are both required for efficient wound closure of airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 2004; 287:1134-44; PMID:15298851; http://dx.doi.org/10.1152/ajplung.00022.2004.
33. Yamada S, Nelson WJ. Localized zones of Rho and Rac activities drive initiation and expansion of epithelial cell-cell adhesion. J Cell Biol 2007; 178:517-27; PMID:17646397; http://dx.doi.org/10.1083/jcb.200701058.
34. Yamazaki Y, Umeda K, Wada M, Nada S, Okada M, Tsukita S, et al. ZO-1- and ZO-2-dependent integration of myosin-2 to epithelial zonula adherens. Mol Biol Cell 2008; 19:3801-11; PMID:18596233; http://dx.doi.org/10.1091/mbc.E08-04-0352.
35. Wildenberg GA, Dohn MR, Carnahan RH, Davis MA, Lobdell NA, Settleman J, et al. p120-catenin and p190RhoGAP regulate cell-cell adhesion by coordinating antagonism between Rac and Rho. Cell 2006; 127:1027-39; PMID:17129786; http://dx.doi.org/10.1016/j.cell.2006.09.046.
36. Sousa S, Cabanes D, Archambaud C, Colland F, Lemichez E, Popoff M, et al. ARHGAP10 is necessary for alpha-catenin recruitment at adherens junctions and for Listeria invasion. Nat Cell Biol 2005; 7:954-60; PMID:16184169; http://dx.doi.org/10.1038/ncb1308.
37. Matsuda M, Kobayashi Y, Masuda S, Adachi M, Watanabe T, Yamashita JK, et al. Identification of adherens junction-associated GTPase activating proteins by the fluorescence localization-based expression cloning. Exp Cell Res 2008; 314:939-49; PMID:18241859; http://dx.doi.org/10.1016/j.yexcr.2007.11.009.
38. Bähler M, Elfrink K, Hanley PJ, Thelen S, Xu Y. Cellular functions of class IX myosins in epithelia and immune cells. Biochem Soc Trans 2011; 39:1166-8; PMID:21936783; http://dx.doi.org/10.1042/BST0391166.
39. Post PL, Tyska MJ, O'Connell CB, Johung K, Hayward A, Mooseker MS. Myosin-IXb is a single-headed and processive motor. J Biol Chem 2002; 277:11679-83; PMID:11801597; http://dx.doi.org/10.1074/jbc.M111173200.
40. Liao W, Elfrink K, Bähler M. Head of myosin IX binds calmodulin and moves processively toward the plus-end of actin filaments. J Biol Chem 2010; 285:24933-42; PMID:20538589; http://dx.doi.org/10.1074/jbc.M110.101105.
41. Struchholz S, Elfrink K, Pieper U, Kalhammer G, Honnert U, Grützner A, et al. Functional role of the extended loop 2 in the myosin 9b head for binding F-actin. J Biol Chem 2009; 284:3663-71; PMID:19059909; http://dx.doi.org/10.1074/jbc.M808338200.
42. Müller RT, Honnert U, Reinhard J, Bähler M. The rat myosin myr 5 is a GTPase-activating protein for Rho in vivo: essential role of arginine 1695. Mol Biol Cell 1997; 8:2039-53; PMID:9348541.
43. Post PL, Bokoch GM, Mooseker MS. Human myosin-IXb is a mechanochemically active motor and a GAP for rho. J Cell Sci 1998; 111:941-50; PMID:9490638.
44. Coluccio LM, BÄhler M. Class Ix Myosins. Myosins 2008; 391-401.
45. Abouhamed M, Grobe K, San IV, Thelen S, Honnert U, Balda MS, et al. Myosin IXa regulates epithelial differentiation and its deficiency results in hydrocephalus. Mol Biol Cell 2009; 20:5074-85; PMID:19828736; http://dx.doi.org/10.1091/mbc.E09-04-0291.
46. van den Boom F, Düssmann H, Uhlenbrock K, Abouhamed M, Bähler M. The Myosin IXb motor activity targets the myosin IXb RhoGAP domain as cargo to sites of actin polymerization. Mol Biol Cell 2007; 18:1507-18; PMID:17314409; http://dx.doi.org/10.1091/mbc.E06-08-0771.