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Rho GTPases
Masters of cell migration

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Since their discovery in the late eighties, the role of Rho GTPases in the regulation of cell migration has been extensively studied and has mainly focused on the hallmark family members Rho, Rac, and Cdc42. Recent technological advances in cell biology, such as Rho-family GTPase activity biosensors, studies in 3D, and unbiased RNAi-based screens, have revealed an increasingly complex role for Rho GTPases during cell migration, with many inter-connected functions and a strong dependency on the physical and chemical properties of the surrounding environment. This review aims to give an overview of recent studies on the role of Rho-family GTPase members in the modulation of cell migration in different environments, and discuss future directions.

Introduction

Cell migration is a complex, dynamic process that involves continuous remodeling of the cellular architecture, which is needed in order for the cell to move and adapt to changes in the surrounding environment. It requires rapidly activated and spatiotemporally regulated signaling networks that enable cellular responses to external cues. Rho-family GTPases are key components of these signaling networks, most of them acting as molecular switches that cycle between a GTP-bound (active) and GDP-bound (inactive) form.[1] The activity of Rho-family GTPases is tightly regulated by guanine nucleotide exchange factors (GEFs) that activate Rho-family GTPases by promoting the release of GDP, allowing the binding of GTP. GTPase-activating proteins (GAPs) inactivate Rho-family GTPases by stimulating the hydrolysis of GTP.[2] The inactive Rho GTPases are sequestered in the cytosol by the RHO-specific guanine nucleotide dissociation inhibitors (GDIs) which, upon binding the GTPases C-terminal prenyl group, prevent their membrane association.[3] The molecular switch characteristic of the Rho GTPases enables them to regulate signals in a transient and localized fashion, and such dynamic regulation is crucial for effective cell migration. However, ten of the 20 members of the Rho-family GTPases are constitutively bound to GTP, and hence constitutively activated, and therefore regulated by alternative mechanisms. Recently, new insights into the regulation of constitutively active Rho family members have been generated through the finding that Rnd3/RhoE is phosphorylated by the kinases ROCK and PKC, with subsequent binding to 14-3-3 proteins leading to its translocation from the plasma membrane to the cytosol.[4]

Over 80 GEFs and more than 70 GAPs have been reported,[5] suggesting that Rho-family GTPase regulation is complex and that activity and localization can be modulated by a multitude of signaling pathways depending on the spatiotemporal context. Regulation may integrate both the physical properties of the environment (rigidity, confinement, homogeneity, and shear stress) as well as its chemical characteristics (ligands, gradients, and redox status).

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Interplay between Rho-Family GTPases during Cell Migration

Rac, Rho, and Cdc42 in 2-D environments

The concept of Rho GTPase involvement in the rearrangement of cellular architecture during cell migration in 2-D environments is built on landmark findings by Hall, Ridley, and Nobes, who showed that the Rho GTPase Rac promotes lamellopodia formation in response to PDGF stimulation,[6] whereas RhoA stimulates the formation of contractile actomyosin fibers (i.e., stress fibers) downstream of LPA signaling.[7] Cdc42 was later shown to promote filopodia and to activate Rac.[8] Rac promotes lamellopodia formation through binding to the SCAR/Wave Regulatory Complex (WRC) components Sra1 and WAVE1 which generates a conformational change that unmasks the VCA motif (verprolin homology, cofilin homology, and acidic region) in WAVE1 leading to the activation of the Arp2/3 complex and actin assembly,[9,10] thereby promoting cell migration. RhoG (a Rho family GTPase closely related to Rac) has been shown to function upstream of Rac in some systems by recruiting its effector ELMO, bound to members of the DOCK family of Rac GEFs, to regulate Rac-driven actin remodeling and migration.[11]

In addition to Rac, activation of the Arp2/3 complex can be triggered by Cdc42 through binding to, and activation of, N-WASP.[12] Interestingly, recent work from the Machesky group has shown that inhibition of SCAR/Wave-dependent Arp2/3 activation, through knockdown of the SCAR/Wave Regulatory

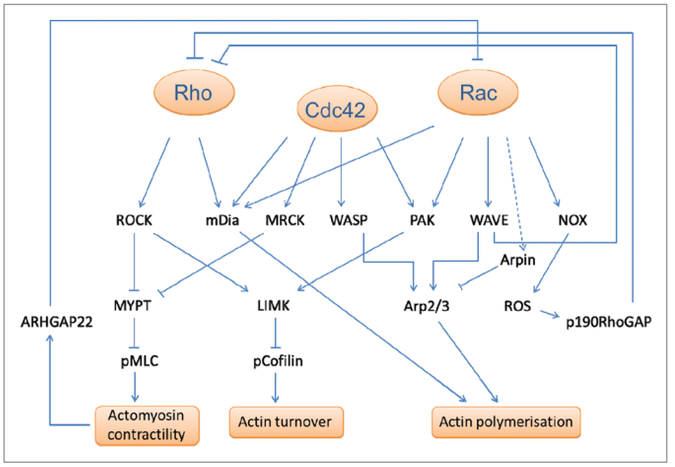

Figure 1. Crosstalk between classical Rho-family GTPases regulates actin remodeling during cell migration. Rho promotes actomyosin contractility through ROCK-dependent phosphorylation, and subsequent inhibition, of MLC phosphatase MYPT. ROCK also phosphorylates LIMK, leading to inhibition of cofilin activity. Another effector of Rho is the formin mDia, which promotes actin polymerization during cell migration. Rho antagonizes Rac-mediated signaling through ROCK/contractility-dependent activation of the RacGAP ARHGAP22. Rac promotes actin polymerization and lamellopodia formation through activation of WAVE and PAK and controls directionality through recruitment of Arpin, which inhibits WAVE-dependent activation of Arp2/3. Rac also antagonizes Rho-mediated signaling through a WAVE-dependent mechanism, and through activation of Nox-depednent ROS production, which promotes activation of p190RhoGAP, leading to inhibition of Rho. Cdc42 connects with both Rac and Rho, promoting actomyosin contractility through activation of MRCK, which phosphorylates MTPT and induces filopodia formation via WASP-mediated activation of Arp2/3.

Complex components Sra1 and Nap1, inhibits movement in 2-D but promotes 3-D invasion, indicating that different mechanisms drive cell movement in 2D vs. 3D. This study reported that, upon depletion of the WRC in a 3-D environment, enhanced FAK activation leads to the recruitment and activation of N-WASP at the invasive front, promoting Arp2/3-driven invasion. However, in 2-D WRC depletion slowed down migration and did not promote accumulation of N-WASP or Arp2/3 at the tip of cells.[13]

Concomitantly to WAVE/Arp2/3 activation, a new study has shown that Rac-dependent signaling recruits and activates the newly characterized protein Arpin (for Arp inhibitor) that binds to Arp2/3 but is unable to activate it, since Arpin lacks the VCA motifs, and therefore acts as a competitive inhibitor of the Arp2/3 complex. The study shows that Rac activation recruits Arpin to the tip of lamellipodia, where it inhibits Arp2/3, leading to a reduction in migration speed and a subsequent change in direction. The authors concluded that Rac-dependent recruitment of Arpin is needed for steering of migrating cells.[14]

During cell migration, the GTPase Rho is involved in both actin polymerization and force generation, through binding and activation of the formin mDia and the kinase ROCK, respectively.[15] In the classic model of cell migration in 2D, it has been assumed that Rac and Cdc42 are active at the leading edge, in order to promote protrusion formation, whereas Rho would be active only in the cell body and at the rear, so as to provide the actomyosin-mediated force needed for rear retraction and forward movement (**Fig. 1**). The use of FRET-based Rho GTPase activity biosensors showed this model to be incomplete by demonstrating that Rho is also active at the leading edge,[16] and activated before Rac and Cdc42.[17] These findings highlight the complexity and inter-connectivity of Rho GTPase-mediated signaling during cell migration. It is not clear whether Rho activation at the front and rear of the cell is mediated by two different GEFs, one targeting Rho to the leading edge to initiate actin polymerization at the onset of the protrusion-retraction cycle,[17] and the second localizing it to the rear of the cell to increase tension-induced detachment of the rear.[18] Interestingly, Vega et al. have shown that the closely related homologs RhoA and C have different roles during cell migration by acting through different downstream targets. The study shows that RhoA, through activation of the kinase ROCK, inhibits formation of multiple protrusions and promotes tail retraction, whereas RhoC inhibits lamellopodia broadening through activation of the formin FMNL3.[19] However, other studies suggest that Rho A, B and C act redundantly to generate actomyosin contractility.[20] Early work on RhoB reported that it localizes to endocytic vesicles[21] where, upon activation by Vav2,[22] it signals through PRK1 to regulate the kinetics of intracellular EGF trafficking to the lysosome.[23] Interestingly, work from Rodriguez et al. revealed that RhoB activates NFκB, independently from PRK1, in a ROCK-I dependent manner.[24] More recent work from Ridley's group has shown that the GTPase RhoB plays a key role in the uPA/uPAR-mediated migration and invasion of prostate cancer cells. The study shows that RhoB mediates the uPAR-induced upregulation of surface integrin levels as well as the uPAR-dependent adhesion to vitronectin. Depletion of RhoB induces a decrease in the uPAR-induced phosphorylation of paxillin, Akt, and cofilin, and reduces the association of uPAR with integrins, leading to a decrease in migration and invasion of prostate cancer cells.[25] Since uPAR is known to drive migration through a DOCK180-Rac pathway,[26] these studies show how signaling via RhoB can link to Rac activity.

Rac, Rho, and Cdc42 in 3-D environments

Studies in 3D environments show that Rho GTPases can coordinate different modes of movement, where cells move through collagen-rich connective tissue with variable physical and chemical properties. In such environments single cancer cells can either adopt a round, highly contractile, Rho-driven mode of movement, or an elongated, lower contractility Rac-dependent mode of migration.[27,28] These two modes are inter-convertible since, in a permissive environment, inhibition of components of the signaling pathway that promotes a given mode of movement switches cells to the other mode of migration.[29] Importantly both types of movement rely on actomyosin contractility to generate the force needed for migration, but differ in the levels of contractility required. Actomyosin contractility is driven by canonical Rho/ROCK signaling, where Rho activates ROCK, which phosphorylates (and thus inactivates) the myosin light chain phosphatase (MYPT) leading to the activation of the myosin-II.[30] Phosphorylation and inactivation of MYPT can also be triggered by MRCK (myotonic dystrophy kinase-related

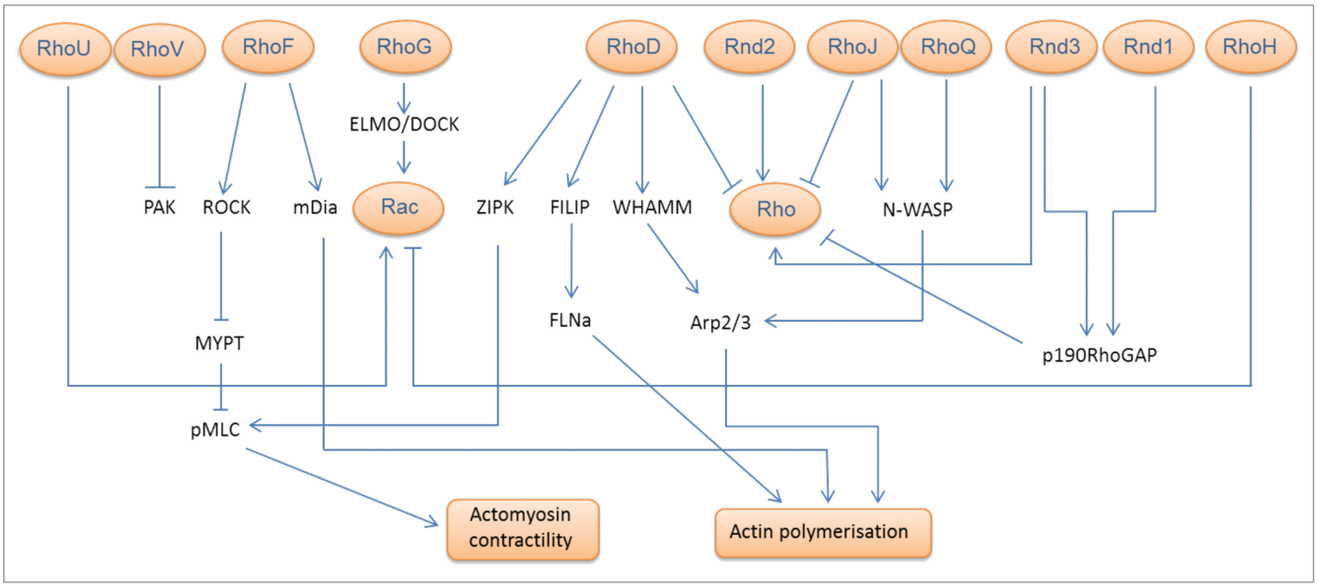

Figure 2. Interplay between atypical and classical Rho-family GTPases during cell migration. RhoD, Rnd1, Rnd3, and RhoJ antagonize the Rho-mediated actin remodelling during cell migration. In endothelial cells Rnd2 and Rnd3 promote RhoB-induced stress fiber formation. RhoD regulates the reorganization of actin through the activation of Arp2/3 and ZIPK. RhoG activates Rac by recruiting the ELMO/DOCK complex. RhoU promotes cell migration through activation of Rac, whereas RhoH antaintergonizes Rac-mediated actin reorganization and cell migration. RhoV also antagonizes cell migration by promoting PAK degradation. RhoF activates mDia and is involved in the targeting of active ROCK to the cell cortex.

Cdc42-binding kinase) downstream of Cdc42. Wilkinson et al. showed that Rho and Cdc42 cooperate in order to generate the actomyosin contractility needed for elongated movement. The study shows that either of the Rho/ROCK- or Cdc42/MRCK-dependent pathways could phosphorylate and subsequently inhibit MYPT. Interestingly, most of the actomyosin contractility needed for rounded movement is generated downstream of the canonical Rho/ROCK pathway.[31] Alternatively, activation of Cdc42 downstream of DOCK10 can promote actomyosin contractility through activation of the kinase Pak2, which directly phosphorylates the Myosin Light Chain (MLC) at Ser19, leading to activation of myosin-II.[32] In a co-culture system of collective migration of squamous cell carcinoma (SCC) and stromal fibroblasts, Gaggioli et al. showed that cancer-associated fibroblasts remodel the extracellular matrix, through actomyosin-driven traction force that generates tracks which are followed by the carcinoma cells. This study highlighted a new aspect of the cooperation between Rho and Cdc42 for the control of actomyosin contractility, as it showed that the carcinoma cells used Cdc42 and MRCK-driven actomyosin contractility to follow the tracks that have been generated by the Rho/ROCK dependent-actomyosin contractility in fibroblasts.[33]

Atypical and other Rho-family GTPases

Work on less well characterized members of the Rho-family has revealed new levels of complexity and inter-connectivity in Rho-family GTPase signaling during cell migration. This subfamily includes RhoF, RhoD, RhoQ, RhoJ, and the non-cycling family members Rnd 1–3, RhoH, RhoV, RhoU, and RhoBTB (**Fig. 2**). However, recent work from the Ahmadian group has shown that the Rho GTPases RhoD and RhoF are to be considered atypical as they exhibit a high intrinsic exchange activity and hence bound GTP under equilibrium and quiescent conditions.[34] RhoF plays an important role in the organization of cell shape and cell migration. An overexpression approach showed that RhoF stimulates the formation of Cdc42-independent filopodia, through activation of the formin mDia-2.[35] Recent work extended this finding and showed that RhoF interacts with mDia-1 to promote filopodia formation independently from the canonical Cdc42/WASP/Arp2/3 pathway.[36] RhoF can also trigger the formation of actin stress fibers in epithelial cells in a ROCK-dependent fashion. The study shows that, in the absence of its effector mDia1, RhoF regulates the distribution of active ROCK at the cell cortex without affecting the overall activity of the kinase or the phosphorylation status of its effectors MLC and MYPT.[37] RhoD localizes to the plasma membrane and the early endocytic compartment.[38] It has been shown to promote the alignment of early endosomes along the actin fibers[39] and to induce disassembly of focal adhesions and loss of the actin stress fibers by antagonizing RhoA, leading to impairment of cell migration.[40] Recent work reported that expression of active RhoD induces the formation of filopodia and promotes the assembly of actin filament bundles and that knock down of RhoD decreases cell migration.[41] This work showed that RhoD impacts on cell migration and adhesion by coordinating the Arp2/3-dependent and Filamin A (FLNa)-driven regulation of actin dynamics. RhoD regulates Arp2/3-mediated actin organization, through binding to the actin nucleation factor WASP homolog associated with actin Golgi membranes and microtubules (WHAMM), and regulates FLNa-dependent mechanisms through interaction with the FLNa-interacting protein FILIP.[41] Recent work reported that RhoD also interacts with Zipper-Interacting Protein Kinase (ZIPK), in a GTP-dependent manner, to modulate the

reorganization of actin and focal adhesions.[42] RhoQ (TC10) and RhoJ (RhoT/TCL) belong to the Cdc42 subfamily of Rho GTPases and, like Cdc42, RhoQ and RhoJ bind to N-WASP and induce Arp2/3-mediated actin polymerization. Overexpression of RhoQ and RhoJ induce the formation of long filopodial protrusions in fibroblasts and promote neurite outgrowth in PC12 cells.[43] In endothelial cells, RhoJ is activated by vascular endothelial growth factor, and is required for endothelial cell migration and tube formation through modulation of actomyosin contractility and focal adhesion numbers.[44] Similarly, in human corneal epithelial cells, RhoJ regulates polarization and migration speed in a wound healing assay[45]

The three Rnd proteins Rnd1, Rnd2, and Rnd3/RhoE lack the ability to hydrolyze GTP and so are constitutively bound to GTP. They have been implicated in the regulation of cell migration. Rnd1 and 3 have been shown to induce loss of stress fibers and cell rounding in several cell types,[46] potentially by antagonizing Rho/ROCK-driven actomyosin contractility. It has been shown that both Rnd1 and Rnd3 interact with p190RhoGAP, which increases the GAP activity of p190RhoGAP toward active RhoA, leading to reduced cellular levels of active RhoA and a decrease in actomyosin contractility.[47] Work from the Sahai group showed that Rnd3, through binding to a DDR-Par3-Par6 complex, is targeted to cell-cell contact regions during collective migration, antagonizing the Rho-driven actomyosin contractility that induces disruption of cell-cell cohesion.[48] However, Rnd3 function appears to be cell type- and context-dependent, since recent work in endothelial cells revealed that Rnd3 stimulates stress fiber formation by inducing an increase in the level of RhoB expression, leading to activation of RhoB/ROCK-driven actomyosin contractility.[49] Rnd2-driven stimulation of cell contraction, through activation of a Rho/ROCK-dependent signaling pathway, has been reported by the work of Tanaka et al., who showed that Rnd2 interacts with its effector pragmin to augment the levels of RhoA activity.[50] Moreover, the cytoplasmic localization of Rnd2 makes it unable to affect the activity of RhoA through binding to p190RhoGAP, as recent work reported that targeting of Rnd1 and Rnd3 to lipid rafts is required for the activation of p190RhoGAP. This work showed that Rnd2 lacks the N-terminal KERRA (Lys-Glu-Arg-Arg-Ala) sequence of amino acids needed for the targeting to lipid rafts.[51]

RhoH is an atypical Rho GTPase widely expressed in hematopoietic cells, where it has little effect by itself on actin reorganization and cell[52]migration. The general consensus is that RhoH antagonizes the classical Rho GTPase-mediated signaling, since it has been shown to inhibit the activation of NFκB and p38 induced by overexpression of constitutively active Cdc42, Rac1, and RhoA-,[53] and to antagonize Rac activation, Rac-mediated actin reorganization and cell migration.[54] Genetic deletion of RhoH in hematopoietic cells is associated with an increased Rac activity and Rac-mediated migration, chemotaxis, and cortical F-actin assembly.[55] Although it has been suggested that RhoH regulates membrane targeting of Rac,[55] little is known about the mechanism by which RhoH represses the activation of Rac and signaling mediated by other Rho GTPases. Wnt-1-regulated Cdc42 homolog-1 (Wrch-1), also known as RhoU, and Cdc42-homologous protein Chp and Wrch2 (RhoV), are other examples of atypical Rho family GTPases. They both have an N-terminal proline-rich domain, allowing them to interact with proteins harboring SH3 domains such as Nck and Grb2.[56,57] Unlike the atypical Rho GTPases Rnd, RhoH, and RhoBTB, which have amino acid substitutions that prevent GTP hydrolysis and are therefore constitutively active, RhoU has a normal GTP hydrolysis activity, but exhibits a high intrinsic exchange activity and therefore has high levels of bound GTP[58]. RhoU has been shown to localize to podosomes in osteoclasts and c-Src-expressing cells, and to focal adhesions in HeLa cells and fibroblasts.[59,60] Overexpression of RhoU disrupts focal adhesions, reduces stress fibers and induces multiple filopodial protrusions,[56] whereas its depletion by RNAi increases focal adhesion formation and inhibits cell migration.[60] Interestingly, In addition to the well described Wnt-1 pathway, RhoU has been shown to be regulated by the gp130/STAT3 pathway, and its expression could be induced in several cell lines by stimulation with the cytokines OSM or IL6. In the context of melanoma and cancer-associated fibroblasts, it has been shown that cytokine-dependent activation of the gp130/STAT3 pathway stimulates actomyosin contractility and promotes round "amoeboid-like" movement,[61] suggesting that RhoU might play a role in the regulation of different modes of cell migration in 3-D. Recent work in *Xenopus* further strengthened the role of RhoU in the regulation cell migration, as it showed that RhoU is expressed in, and required for the migration of, cranial neural crest (CNC) cells, and that RhoU knockdown impaired CNC migration both in vitro and in vivo. Interestingly, this study shows that overexpression of RhoU also impairs CNC cell migration, confirming that the level of RhoU is critical to this process. Mechanistically, RhoU regulates CNC cell migration by activation of the Rac/PAK pathway, since expression of dominant negative PAK could rescue the impairment of migration induced by overexpression of RhoU, and overexpression of Rac could rescue the decrease in migration upon RhoU inhibition.[62] Few studies support a role for Chp2/RhoV in the regulation of cell migration, perhaps explained by its weak expression across tissues.[63] However, when overexpressed in Jurkat T-cells, RhoV reduced SDF1-stimulated migration by promoting ubiquitin-dependent degradation of Pak1.[64] RhoBTBs (1 and 2) are the most distantly related Rho-family GTPases, as they are much larger than the classical GTPases and contain additional domains.[65] RhoBTBs are believed to act as tumor suppressors through regulating ubiquitinylation,[66] and have no reported direct effect on cell migration.

RhoGTPases Sensors of Physical Environment

In vivo, migratory cells have to adapt to variations in the physical properties of the surrounding environment, be it a change in rigidity, density, or organization of the surrounding matrix. Variations in the physical properties of the environment activate cellular mechano-sensors, which can generate transcriptional responses in the cell through activation of transcriptional regulators like YAP1, in the hippo pathway,[67] as well as

components of the SRF pathway,[68] impacting on cell fate,[69,70] cell shape, and migration.[71] Rho-family GTPases play a key role in integrating intracellular signals downstream of mechano-sensors, promoting re-organization of the actin cytoskeleton that is needed for the change in cell shape and, eventually, the mode of migration in a given environment. In endothelial cells subjected to shear stress, the formation of an integrin β1/Caveolin mechano-signaling complex induces the inactivation of p190RhoGAP and the subsequent induction of RhoA activity, leading to an increase in the formation of actin stress fibers which will increase the resistance of endothelial cells to hemodynamic stress, as in the case of hypertension.[72] Recent work from the Sahai group demonstrated that Rho/ROCK-driven actomyosin contractility, and activation of Src, are required for the activation of YAP in response to increased matrix stiffness. Activation of Yap and its downstream signaling, including the stabilization of MLC levels, is required for the generation and maintenance of the highly contractile cancer-associated fibroblast phenotype.[73] During melanoma migration on a deformable substrate, increasing Rho/ROCK-driven actomyosin contractility switches the cells from an elongated to a round mode of movement through actomyosin contractility-mediated activation of ARHGAP22, which specifically inactivates the Rho-family GTPase Rac.[29] In order to understand how strain on the actin cytoskeleton generates intracellular signals that determine cell behavior, recent work has identified FLNA as a central mechano-transduction element of the cytoskeleton.[74] This work showed in vitro that the application of either external shear or myosin-induced contraction of FLNA-bound actin filaments, in the presence of two FLNA-binding partners, the cytoplasmic tail of β-integrin, and FilGAP (an ARHGAP22 family member), results in increased integrin binding to FLNA and dissociation of FilGAP.[74] In cells, dissociated FilGAP relocates to the plasma membrane where it inactivates Rac.[75] This work provides the molecular basis for the observation made by Shifrin et al., who reported that Rac activity is force-regulated by a FilGAP-FLNA interaction.[76]

In vivo, migratory cells experience varying degrees of physical confinement as they have to go through pores and channels with cross-sectional areas ranging from 3 to >400 μm.[2,77] How the physical dimensions of the ECM, such as pore size, influence cell migration is of great interest. Recent work on cells migrating through micro-channel devices with varying diameters ranging from 3 microns, a constricted physical environment, to 50 microns (an unconfined environment), reported that Rac activation downstream of α4β1 integrin is compulsory for migration in unconfined 3D environments, whereas migration in constricted environments requires myosin-II-driven contractility that is further increased by the inhibition of Rac activity, suggesting a switch from Rac-driven protrusive movement in an unconfined environment to Rho-ROCK-dependent, high actomyosin contractility-driven movement in constricted environments.[78] Interestingly, computational modeling of cell migration in different matrix geometries and confinements predicted that confined environment modifies the contractility-velocity relationship for optimal migration. The model shows that—in contrast to migration on an unconfined surface, where increasing actomyosin contractility slows down movement through cell detachment—migration in confined environments favors high levels of actomyosin contractility. This is because, in such physical environments, the decrease in velocity due to actomyosin contractility-mediated cell detachment is reduced, and high actomyosin contractility promotes hydrostatic pressure-driven bleb formation, which enables high actomyosin contractility to drive fast, bleb-driven migration.[79]

Concluding Remarks and Future Directions

The field of Rho-family GTPases, and their role in cell migration, is evolving rapidly. This is in part due to the interest in different modes of migration, and in part to studies being expanded beyond the canonical family members. It is likely that studies of how Rho-family GTPase signaling interprets the physical environment, in addition to the chemical environment, will be of particular interest. Crucial to our understanding of Rho-family GTPases will be continued expansion of studies to in vivo models, and the capacity to image the activation of Rho-family GTPases and their signaling pathways in such models.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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References

1. Jaffe AB, Hall A. Rho GTPases: biochemistry and biology. Annu Rev Cell Dev Biol 2005; 21:247-69; PMID:16212495; http://dx.doi.org/10.1146/annurev.cellbio.21.020604.150721
2. 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
3. Garcia-Mata R, Boulter E, Burridge K. The 'invisible hand': regulation of RHO GTPases by RHOGDIs. Nat Rev Mol Cell Biol 2011; 12:493-504; PMID:21779026; http://dx.doi.org/10.1038/nrm3153
4. Riou P, Kjær S, Garg R, Purkiss A, George R, Cain RJ, Bineva G, Reymond N, McColl B, Thompson AJ, et al. 14-3-3 proteins interact with a hybrid prenyl-phosphorylation motif to inhibit G proteins. Cell 2013; 153:640-53; PMID:23622247; http://dx.doi.org/10.1016/j.cell.2013.03.044
5. Hall A. Rho family GTPases. Biochem Soc Trans 2012; 40:1378-82; PMID:23176484; http://dx.doi.org/10.1042/BST20120103
6. Ridley AJ, Paterson HF, Johnston CL, Diekmann D, Hall A. The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. Cell 1992; 70:401-10; PMID:1643658; http://dx.doi.org/10.1016/0092-8674(92)90164-8
7. Ridley AJ, Hall A. The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. Cell 1992; 70:389-99; PMID:1643657; http://dx.doi.org/10.1016/0092-8674(92)90163-7
8. Nobes CD, Hall A. Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell 1995; 81:53-62; PMID:7536630; http://dx.doi.org/10.1016/0092-8674(95)90370-4
9. Eden S, Rohatgi R, Podtelejnikov AV, Mann M, Kirschner MW. Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck. Nature 2002; 418:790-3; PMID:12181570; http://dx.doi.org/10.1038/nature00859

10. Chen Z, Borek D, Padrick SB, Gomez TS, Metlagel Z, Ismail AM, Umetani J, Billadeau DD, Otwinowski Z, Rosen MK. Structure and control of the actin regulatory WAVE complex. Nature 2010; 468:533-8; PMID:21107423; http://dx.doi.org/10.1038/nature09623
11. Katoh H, Negishi M. RhoG activates Rac1 by direct interaction with the Dock180-binding protein Elmo. Nature 2003; 424:461-4; PMID:12879077; http://dx.doi.org/10.1038/nature01817
12. Rohatgi R, Ma L, Miki H, Lopez M, Kirchhausen T, Takenawa T, Kirschner MW. The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. Cell 1999; 97:221-31; PMID:10219243; http://dx.doi.org/10.1016/S0092-8674(00)80732-1
13. Tang H, Li A, Bi J, Veltman DM, Zech T, Spence HJ, Yu X, Timpson P, Insall RH, Frame MC, et al. Loss of Scar/WAVE complex promotes N-WASP- and FAK-dependent invasion. Curr Biol 2013; 23:107-17; PMID:23273897; http://dx.doi.org/10.1016/j.cub.2012.11.059
14. Dang I, Gorelik R, Sousa-Blin C, Derivery E, Guérin C, Linkner J, Nemethova M, Dumortier JG, Giger FA, Chipysheva TA, et al. Inhibitory signalling to the Arp2/3 complex steers cell migration. Nature 2013; 503:281-4; PMID:24132237
15. Narumiya S, Tanji M, Ishizaki T. Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion. Cancer Metastasis Rev 2009; 28:65-76; PMID:19160018; http://dx.doi.org/10.1007/s10555-008-9170-7
16. Pertz O, Hodgson L, Klemke RL, Hahn KM. Spatiotemporal dynamics of RhoA activity in migrating cells. Nature 2006; 440:1069-72; PMID:16547516; http://dx.doi.org/10.1038/nature04665
17. Machacek M, Hodgson L, Welch C, Elliott H, Pertz O, Nalbant P, Abell A, Johnson GL, Hahn KM, Danuser G. Coordination of Rho GTPase activities during cell protrusion. Nature 2009; 461:99-103; PMID:19693013; http://dx.doi.org/10.1038/nature08242
18. Raftopoulou M, Hall A. Cell migration: Rho GTPases lead the way. Dev Biol 2004; 265:23-32; PMID:14697350; http://dx.doi.org/10.1016/j.ydbio.2003.06.003
19. Vega FM, Fruhwirth G, Ng T, Ridley AJ. RhoA and RhoC have distinct roles in migration and invasion by acting through different targets. J Cell Biol 2011; 193:655-65; PMID:21576392; http://dx.doi.org/10.1083/jcb.201011038
20. Melendez J, Stengel K, Zhou X, Chauhan BK, Debidda M, Andreassen P, Lang RA, Zheng Y. RhoA GTPase is dispensable for actomyosin regulation but is essential for mitosis in primary mouse embryonic fibroblasts. J Biol Chem 2011; 286:15132-7; PMID:21454503; http://dx.doi.org/10.1074/jbc.C111.229336
21. Adamson P, Paterson HF, Hall A. Intracellular localization of the P21rho proteins. J Cell Biol 1992; 119:617-27; PMID:1383236; http://dx.doi.org/10.1083/jcb.119.3.617
22. Gampel A, Mellor H. Small interfering RNAs as a tool to assign Rho GTPase exchange-factor function in vivo. Biochem J 2002; 366:393-8; PMID:12113653; http://dx.doi.org/10.1042/BJ20020844
23. Gampel A, Parker PJ, Mellor H. Regulation of epidermal growth factor receptor traffic by the small GTPase rhoB. Curr Biol 1999; 9:955-8; PMID:10508588; http://dx.doi.org/10.1016/S0960-9822(99)80422-9
24. Rodriguez PL, Sahay S, Olabisi OO, Whitehead IP. ROCK I-mediated activation of NFkappaB by RhoB. Cell Signal 2007; 19:2361-9; PMID:17728102; http://dx.doi.org/10.1016/j.cellsig.2007.07.021
25. Alfano D, Ragno P, Stoppelli MP, Ridley AJ. RhoB regulates uPAR signalling. J Cell Sci 2012; 125:2369-80; PMID:22366462; http://dx.doi.org/10.1242/jcs.091579
26. Smith HW, Marra P, Marshall CJ. uPAR promotes formation of the p130Cas-Crk complex to activate Rac through DOCK180. J Cell Biol 2008; 182:777-90; PMID:18725541; http://dx.doi.org/10.1083/jcb.200712050
27. Friedl P, Wolf K. Plasticity of cell migration: a multiscale tuning model. J Cell Biol 2010; 188:11-9; PMID:19951899; http://dx.doi.org/10.1083/jcb.200909003
28. Sahai E. Mechanisms of cancer cell invasion. Curr Opin Genet Dev 2005; 15:87-96; PMID:15661538; http://dx.doi.org/10.1016/j.gde.2004.12.002
29. Sanz-Moreno V, Gadea G, Ahn J, Paterson H, Marra P, Pinner S, Sahai E, Marshall CJ. Rac activation and inactivation control plasticity of tumor cell movement. Cell 2008; 135:510-23; PMID:18984162; http://dx.doi.org/10.1016/j.cell.2008.09.043
30. Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng J, Nakano T, Okawa K, et al. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). Science 1996; 273:245-8; PMID:8662509; http://dx.doi.org/10.1126/science.273.5272.245
31. Wilkinson S, Paterson HF, Marshall CJ. Cdc42-MRCK and Rho-ROCK signalling cooperate in myosin phosphorylation and cell invasion. Nat Cell Biol 2005; 7:255-61; PMID:15723050; http://dx.doi.org/10.1038/ncb1230
32. Gadea G, Sanz-Moreno V, Self A, Godi A, Marshall CJ. DOCK10-mediated Cdc42 activation is necessary for amoeboid invasion of melanoma cells. Curr Biol 2008; 18:1456-65; PMID:18835169; http://dx.doi.org/10.1016/j.cub.2008.08.053
33. Gaggioli C, Hooper S, Hidalgo-Carcedo C, Grosse R, Marshall JF, Harrington K, Sahai E. Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. Nat Cell Biol 2007; 9:1392-400; PMID:18037882; http://dx.doi.org/10.1038/ncb1658
34. Jaiswal M, Fansa EK, Dvorsky R, Ahmadian MR. New insight into the molecular switch mechanism of human Rho family proteins: shifting a paradigm. Biol Chem 2013; 394:89-95; PMID:23096567; http://dx.doi.org/10.1515/hsz-2012-0207
35. Pellegrin S, Mellor H. The Rho family GTPase Rif induces filopodia through mDia2. Curr Biol 2005; 15:129-33; PMID:15668168; http://dx.doi.org/10.1016/j.cub.2005.01.011
36. Goh WI, Lim KB, Sudhaharan T, Sem KP, Bu W, Chou AM, Ahmed S. mDia1 and WAVE2 proteins interact directly with IRSp53 in filopodia and are involved in filopodium formation. J Biol Chem 2012; 287:4702-14; PMID:22179776; http://dx.doi.org/10.1074/jbc.M111.305102
37. Fan L, Pellegrin S, Scott A, Mellor H. The small GTPase Rif is an alternative trigger for the formation of actin stress fibers in epithelial cells. J Cell Sci 2010; 123:1247-52; PMID:20233848; http://dx.doi.org/10.1242/jcs.061754
38. Murphy C, Saffrich R, Grummt M, Gournier H, Rybin V, Rubino M, Auvinen P, Lütcke A, Parton RG, Zerial M. Endosome dynamics regulated by a Rho protein. Nature 1996; 384:427-32; PMID:8945468; http://dx.doi.org/10.1038/384427a0
39. Gasman S, Kalaidzidis Y, Zerial M. RhoD regulates endosome dynamics through Diaphanous-related Formin and Src tyrosine kinase. Nat Cell Biol 2003; 5:195-204; PMID:12577064; http://dx.doi.org/10.1038/ncb935
40. Tsubakimoto K, Matsumoto K, Abe H, Ishii J, Amano M, Kaibuchi K, Endo T. Small GTPase RhoD suppresses cell migration and cytokinesis. Oncogene 1999; 18:2431-40; PMID:10229194; http://dx.doi.org/10.1038/sj.onc.1202604
41. Gad AK, Nehru V, Ruusala A, Aspenström P. RhoD regulates cytoskeletal dynamics via the actin nucleation-promoting factor WASp homologue associated with actin Golgi membranes and microtubules. Mol Biol Cell 2012; 23:4807-19; PMID:23087206; http://dx.doi.org/10.1091/mbc.E12-07-0555
42. Nehru V, Almeida FN, Aspenström P. Interaction of RhoD and ZIP kinase modulates actin filament assembly and focal adhesion dynamics. Biochem Biophys Res Commun 2013; 433:163-9; PMID:23454120; http://dx.doi.org/10.1016/j.bbrc.2013.02.046
43. Abe T, Kato M, Miki H, Takenawa T, Endo T. Small GTPase Tc10 and its homologue RhoT induce N-WASP-mediated long process formation and neurite outgrowth. J Cell Sci 2003; 116:155-68; PMID:12456725; http://dx.doi.org/10.1242/jcs.00208
44. Kaur S, Leszczynska K, Abraham S, Scarcia M, Hiltbrunner S, Marshall CJ, Mavria G, Bicknell R, Heath VL. RhoJ/TCL regulates endothelial motility and tube formation and modulates actomyosin contractility and focal adhesion numbers. Arterioscler Thromb Vasc Biol 2011; 31:657-64; PMID:21148427; http://dx.doi.org/10.1161/ATVBAHA.110.216341
45. Hou A, Toh LX, Gan KH, Lee KJ, Manser E, Tong L. Rho GTPases and regulation of cell migration and polarization in human corneal epithelial cells. PLoS One 2013; 8:e77107; PMID:24130842; http://dx.doi.org/10.1371/journal.pone.0077107
46. Riou P, Villalonga P, Ridley AJ. Rnd proteins: multifunctional regulators of the cytoskeleton and cell cycle progression. Bioessays 2010; 32:986-92; PMID:20836090; http://dx.doi.org/10.1002/bies.201000060
47. Wennerberg K, Forget MA, Ellerbroek SM, Arthur WT, Burridge K, Settleman J, Der CJ, Hansen SH. Rnd proteins function as RhoA antagonists by activating p190 RhoGAP. Curr Biol 2003; 13:1106-15; PMID:12842009; http://dx.doi.org/10.1016/S0960-9822(03)00418-4
48. Hidalgo-Carcedo C, Hooper S, Chaudhry SI, Williamson P, Harrington K, Leitinger B, Sahai E. 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
49. Gottesbühren U, Garg R, Riou P, McColl B, Brayson D, Ridley AJ. Rnd3 induces stress fibres in endothelial cells through RhoB. Biol Open 2013; 2:210-6; PMID:23430146; http://dx.doi.org/10.1242/bio.20123574
50. Tanaka H, Katoh H, Negishi M. Pragmin, a novel effector of Rnd2 GTPase, stimulates RhoA activity. J Biol Chem 2006; 281:10355-64; PMID:16481321; http://dx.doi.org/10.1074/jbc.M511314200
51. Oinuma I, Kawada K, Tsukagoshi K, Negishi M. Rnd1 and Rnd3 targeting to lipid raft is required for p190 RhoGAP activation. Mol Biol Cell 2012; 23:1593-604; PMID:22357615; http://dx.doi.org/10.1091/mbc.E11-11-0900
52. Vega FM, Ridley AJ. Rho GTPases in cancer cell biology. FEBS Lett 2008; 582:2093-101; PMID:18460342; http://dx.doi.org/10.1016/j.febslet.2008.04.039
53. Li X, Bu X, Lu B, Avraham H, Flavell RA, Lim B. The hematopoiesis-specific GTP-binding protein RhoH is GTPase deficient and modulates activities of other Rho GTPases by an inhibitory function. Mol Cell Biol 2002; 22:1158-71; PMID:11809807; http://dx.doi.org/10.1128/MCB.22.4.1158-1171.2002

54. Gu Y, Jasti AC, Jansen M, Siefring JE. RhoH, a hematopoietic-specific Rho GTPase, regulates proliferation, survival, migration, and engraftment of hematopoietic progenitor cells. Blood 2005; 105:1467-75; PMID:15494435; http://dx.doi.org/10.1182/blood-2004-04-1604
55. Chae HD, Lee KE, Williams DA, Gu Y. Cross-talk between RhoH and Rac1 in regulation of actin cytoskeleton and chemotaxis of hematopoietic progenitor cells. Blood 2008; 111:2597-605; PMID:18089848; http://dx.doi.org/10.1182/blood-2007-06-093237
56. Saras J, Wollberg P, Aspenström P. Wrch1 is a GTPase-deficient Cdc42-like protein with unusual binding characteristics and cellular effects. Exp Cell Res 2004; 299:356-69; PMID:15350535; http://dx.doi.org/10.1016/j.yexcr.2004.05.029
57. Shutes A, Berzat AC, Cox AD, Der CJ. Atypical mechanism of regulation of the Wrch-1 Rho family small GTPase. Curr Biol 2004; 14:2052-6; PMID:15556869; http://dx.doi.org/10.1016/j.cub.2004.11.011
58. Shutes A, Berzat AC, Chenette EJ, Cox AD, Der CJ. Biochemical analyses of the Wrch atypical Rho family GTPases. Methods Enzymol 2006; 406:11-26; PMID:16472646; http://dx.doi.org/10.1016/S0076-6879(06)06002-2
59. Ory S, Brazier H, Blangy A. Identification of a bipartite focal adhesion localization signal in RhoU/Wrch-1, a Rho family GTPase that regulates cell adhesion and migration. Biol Cell 2007; 99:701-16; PMID:17620058; http://dx.doi.org/10.1042/BC20070058
60. Chuang YY, Valster A, Coniglio SJ, Backer JM, Symons M. The atypical Rho family GTPase Wrch-1 regulates focal adhesion formation and cell migration. J Cell Sci 2007; 120:1927-34; PMID:17504809; http://dx.doi.org/10.1242/jcs.03456
61. Sanz-Moreno V, Gaggioli C, Yeo M, Albrengues J, Wallberg F, Viros A, Hooper S, Mitter R, Féral CC, Cook M, et al. ROCK and JAK1 signaling cooperate to control actomyosin contractility in tumor cells and stroma. Cancer Cell 2011; 20:229-45; PMID:21840487; http://dx.doi.org/10.1016/j.ccr.2011.06.018
62. Fort P, Guémar L, Vignal E, Morin N, Notarnicola C, de Santa Barbara P, Faure S. Activity of the RhoU/Wrch1 GTPase is critical for cranial neural crest cell migration. Dev Biol 2011; 350:451-63; PMID:21156169; http://dx.doi.org/10.1016/j.ydbio.2010.12.011
63. Faure S, Fort P. Atypical RhoV and RhoU GTPases control development of the neural crest. Small GTPases 2011; 2:310-3; PMID:22545228; http://dx.doi.org/10.4161/sgtp.18086
64. Weisz Hubsman M, Volinsky N, Manser E, Yablonski D, Aronheim A. Autophosphorylation-dependent degradation of Pak1, triggered by the Rho-family GTPase, Chp. Biochem J 2007; 404:487-97; PMID:17355222; http://dx.doi.org/10.1042/BJ20061696
65. Ramos S, Khademi F, Somesh BP, Rivero F. Genomic organization and expression profile of the small GTPases of the RhoBTB family in human and mouse. Gene 2002; 298:147-57; PMID:12426103; http://dx.doi.org/10.1016/S0378-1119(02)00980-0
66. Aspenström P, Ruusala A, Pacholsky D. Taking Rho GTPases to the next level: the cellular functions of atypical Rho GTPases. Exp Cell Res 2007; 313:3673-9; PMID:17850788; http://dx.doi.org/10.1016/j.yexcr.2007.07.022
67. Dupont S, Morsut L, Aragona M, Enzo E, Giulitti S, Cordenonsi M, Zanconato F, Le Digabel J, Forcato M, Bicciato S, et al. Role of YAP/TAZ in mechanotransduction. Nature 2011; 474:179-83; PMID:21654799; http://dx.doi.org/10.1038/nature10137
68. Connelly JT, Gautrot JE, Trappmann B, Tan DW, Donati G, Huck WT, Watt FM. Actin and serum response factor transduce physical cues from the microenvironment to regulate epidermal stem cell fate decisions. Nat Cell Biol 2010; 12:711-8; PMID:20581838; http://dx.doi.org/10.1038/ncb2074
69. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell 2006; 126:677-89; PMID:16923388; http://dx.doi.org/10.1016/j.cell.2006.06.044
70. Maul TM, Chew DW, Nieponice A, Vorp DA. Mechanical stimuli differentially control stem cell behavior: morphology, proliferation, and differentiation. Biomech Model Mechanobiol 2011; 10:939-53; PMID:21253809; http://dx.doi.org/10.1007/s10237-010-0285-8
71. Pelham RJ Jr., Wang Yl. Cell locomotion and focal adhesions are regulated by substrate flexibility. Proc Natl Acad Sci U S A 1997; 94:13661-5; PMID:9391082; http://dx.doi.org/10.1073/pnas.94.25.13661
72. Yang B, Radel C, Hughes D, Kelemen S, Rizzo V. p190 RhoGTPase-activating protein links the β1 integrin/caveolin-1 mechanosignaling complex to RhoA and actin remodeling. Arterioscler Thromb Vasc Biol 2011; 31:376-83; PMID:21051664; http://dx.doi.org/10.1161/ATVBAHA.110.217794
73. Calvo F, Ege N, Grande-Garcia A, Hooper S, Jenkins RP, Chaudhry SI, Harrington K, Williamson P, Moeendarbary E, Charras G, et al. Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts. Nat Cell Biol 2013; 15:637-46; PMID:23708000; http://dx.doi.org/10.1038/ncb2756
74. Ehrlicher AJ, Nakamura F, Hartwig JH, Weitz DA, Stossel TP. Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A. Nature 2011; 478:260-3; PMID:21926999; http://dx.doi.org/10.1038/nature10430
75. Ohta Y, Hartwig JH, Stossel TP. FilGAP, a Rho- and ROCK-regulated GAP for Rac binds filamin A to control actin remodelling. Nat Cell Biol 2006; 8:803-14; PMID:16862148; http://dx.doi.org/10.1038/ncb1437
76. Shifrin Y, Arora PD, Ohta Y, Calderwood DA, McCulloch CA. The role of FilGAP-filamin A interactions in mechanoprotection. Mol Biol Cell 2009; 20:1269-79; PMID:19144823; http://dx.doi.org/10.1091/mbc.E08-08-0872
77. Wolf K, Alexander S, Schacht V, Coussens LM, von Andrian UH, van Rheenen J, Deryugina E, Friedl P. Collagen-based cell migration models in vitro and in vivo. Semin Cell Dev Biol 2009; 20:931-41; PMID:19682592; http://dx.doi.org/10.1016/j.semcdb.2009.08.005
78. Hung WC, Chen SH, Paul CD, Stroka KM, Lo YC, Yang JT, Konstantopoulos K. Distinct signaling mechanisms regulate migration in unconfined versus confined spaces. J Cell Biol 2013; 202:807-24; PMID:23979717; http://dx.doi.org/10.1083/jcb.201302132
79. Tozluoglu M, Tournier AL, Jenkins RP, Hooper S, Bates PA, Sahai E. Matrix geometry determines optimal cancer cell migration strategy and modulates response to interventions. Nat Cell Biol 2013; 15:751-62; PMID:23792690; http://dx.doi.org/10.1038/ncb2775