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Signaling networks in focus

Rho family GTPases: Making it to the third dimension

Kristin M. Riching, Patricia J. Keely*

University of Wisconsin-Madison, United States

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ABSTRACT

The role of Rho family GTPases in controlling the actin cytoskeleton and thereby regulating cell migration has been well studied for cells migrating on 2D surfaces. *In vivo*, cell migration occurs within three-dimensional matrices and along aligned collagen fibers with rather different spatial requirements. Recently, a handful of studies coupled with new approaches have demonstrated that Rho GTPases have unique regulation and roles during cell migration within 3D matrices, along collagen fibers, and *in vivo*. Here we propose that migration on aligned matrices facilitates spatial organization of Rho family GTPases to restrict and stabilize protrusions in the principle direction of alignment, thereby maintaining persistent migration. The result is coordinated cell movement that ultimately leads to higher rates of metastasis *in vivo*.

Facts

- Cell migration requires precisely controlled signaling and cytoskeletal dynamic events at the leading and trailing edges to set up front-to-rear polarity.
- Rho GTPases are key regulators of cell migration, providing spatial and temporal control of the actin cytoskeleton, microtubules, and cell adhesions.
- Rho GTPase act as molecular switches that cycle between GDP-bound (off) and GTP-bound (on).
- Rho family GTPases are comprised of the Rho family, RhoA, B, and C, the Rac family, Rac1, 2, 3, RhoG, and Cdc42.
- Effectors of Rho GTPases regulate the actin cytoskeleton by affecting polymerization dynamics, branching and bundling, and contraction *via* actin–myosin interactions.
- For more information, see the Cell Migration Consortium Gateway: https://www.cellmigration.org/.

1. Introduction

Although cell migration has been studied for multiple decades, there is still a lack of understanding of how cell migration is regulated in the context of complex, three-dimensional matrices *in vivo*. There are several aspects of the *in vivo* extracellular matrix that differ from classic studies on 2D surfaces, and likely affect cell migration, including the greatly lower stiffness of many 3D matrices, as well as the composition, cross-linking, pore size, and topography. Recently, we demonstrated that collagen alignment accompanies tumor progression and facilitates local invasion (Provenzano et al., 2006, 2008). In breast cancer patient samples, the presence of aligned collagen fibers oriented radially to the tumor/stromal boundary is associated with poor prognosis (Conklin et al., 2011). The presence of aligned collagen facilitates invasion, creating a sort of "highway" that may serve to provide tumor cells with a means to escape a primary tumor and direct their migration to a nearby blood vessel. In this context, aligned collagen represents an aspect of cancer progression that requires further study, not only to better understand the mechanisms underlying the formation of aligned collagen fibers *in vivo*, but also to determine the molecular players involved in cell recognition of and migration along aligned fibers. Despite data demonstrating cell migration along collagen fibers *in vivo*, little is known of how cells recognize and track along aligned extracellular matrices. Here we consider signaling pathways that may control cell migration in 3D matrices and along aligned collagen fibers.

A wealth of literature investigating cell migration on 2D coated surfaces demonstrates a role for integrin-mediated activation of the Rho family of GTPases Rho, Rac, and Cdc42. The coordinated action of these GTPases controls the steps required for a cell to translocate, beginning with actin-induced protrusion generation, adhesion formation and maturation, and resulting in leading edge traction force generation and trailing edge detachment (Lauffenburger and

* Corresponding author at: University of Wisconsin-Madison, Department of Cell and Regenerative Biology, 4533 Wi Institute Medical Research, 1111 Highland Ave, Madison, WI 53705, United States. Tel.: +1 608 265 2398.
E-mail address: pjkeely@wisc.edu (P.J. Keely).

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Horwitz, 1996). While the role of these molecules in mediating cell migration on 2D surfaces has been well established, recent data suggests that these pathways are regulated somewhat differently in the context of 3D cell migration.

2. Functions

The Rho family members belong to the Ras superfamily of small GTPases, characterized by their ability to cycle between an inactive GDP and an active GTP-bound state. In the GTP-bound state, conformational changes allow binding of the GTPase to effectors and activation of downstream signaling pathways. For each GTPase, there are over a dozen possible effectors, and the regulation of these pathways is still under investigation. Among the effectors, several mediate the classic role for Rho GTPases as regulators of actin structure, first described in two classic papers by Ridley and Hall (Ridley and Hall, 1992; Ridley et al., 1992) and reviewed recently (Ridley, 2013). These initial observations demonstrated a role for Rac in membrane ruffling and protrusion, and a role for Rho in actin stress fiber formation. Since that time, thousands of papers have been published on the Rho family GTPases and their role in actin dynamics and cell migration. Effectors of Rho GTPases that are particularly relevant to cell migration have been reviewed in (Hanna and El-Sibai, 2013) and include WASP/Wave complex, PAK, PIP5K, ROCK, LIMK, and the formin family of proteins including mDia.

Adding to the complexity is the fact that there are multiple isoforms of these molecules. Thus, while Rho A, Rho B, and Rho C all share several effectors, they have different roles in cancer (Ridley, 2013). Much work is focused on understanding the unique effectors that account for these differing functions. For example, Rho C has been implicated in enhancing metastasis and promotes invasiveness through effects on an alternate formin, FMNL3 downstream of RhoC but not RhoA (Vega et al., 2011; Ridley, 2013). Moreover, the effectors themselves are complex. For example, Rho A works to limit cell invasiveness by restricting protrusions through ROCK1. However, the closely related Rho effector, ROCK 2, enhances protrusions through Rac.

The choice of which effector is bound by Rho isoforms is likely determined in part by location. In 2D migration, RhoA localizes to the rear of the cell where it is thought to promote rear retraction. At the front of the cell, Rho A is found at the border of the lamellipod and lamella, while RhoC is found at the leading edge (Zawistowski et al., 2013; Bravo-Cordero et al., 2014).

2.1. Rho GTPases in 2D directional migration

A wealth of knowledge exists about the spatial organization of Rho GTPase signaling pathways in 2D migration, making this knowledge base a springboard to understand regulation of cell migration in 3D. In 2D scratch wound assays, persistently protruding cells align their actin network relative to the axis of migration (Lim et al., 2010). Actin polymerization at the leading edge forces membrane deformation and expansion, which results in a growing protrusion. Protrusions are stabilized by adhesion to an underlying surface, which serve to anchor the contractile forces to allow forward translocation of the cell. The control of directional migration, in part through the regulation of actin, is likely dictated by the spatial and temporal activation of Rho GTPases.

Rac is localized to protrusion tips (Kraynov et al., 2000) and is known to increase actin polymerization by activating Arp2/3 and increasing actin network branching, as well as inhibiting actin-capping proteins. This initial protrusion of the membrane facilitates new contacts with the substratum at the protruding edge *via* integrin-mediated adhesions. The ability of active Rac to spatially and temporally control membrane protrusions has been demonstrated by the use of a photoactivatable protein switch coupled to a constitutively active Rac1. Wu, et al. showed that local photoactivation of Rac1 induced protrusions at the site of light irradiation within seconds. Additionally, repeated activations sustained cell polarity by maintaining extension and coordinated retraction of the rear, allowing persistent migration (Wu et al., 2009). These results demonstrate not only that Rac activation precisely controls the spatial and temporal localization of protrusions, but that consistent localization and activation of Rac1 facilitates persistent directed migration.

Cdc42 is also involved in the regulation of directional migration. It is well established that Cdc42 regulates actin and filopodial extension through mechanisms that are similar to Rac. Additional evidence suggests an additional role for Cdc42 in stabilizing protrusions through the involvement of microtubules. Cdc42 regulates the orientation of the microtubule-organizing centers and maintains microtubule plus end polymerization in the direction of a growing lamellipod (Etienne-Manneville and Hall, 2002). Microtubules regulate local Rac and Rho GTPase activation (Waterman-Storer et al., 1999; Wittmann and Waterman-Storer, 2001) and appear to determine the localization of nascent adhesions (Stehbens and Wittmann, 2012). In addition to potentially stabilizing a growing protrusion in a similar manner to actin, microtubules provide tracts for kinesin-based delivery of membrane vesicles, signaling proteins, and matrix altering proteases (Stehbens and Wittmann, 2012). Thus, the prolonged organization of microtubules also likely serves to more efficiently shuttle proteins involved in migration to the leading edge, thereby enhancing directional persistence.

Rho provides intracellular contractility through actin–myosin force generation. Both RhoA and RhoC play roles in the extension of membrane protrusions during migration on a 2D surface, with RhoC preceding protrusion formation (Pertz et al., 2006; Zawistowski et al., 2013). Rho A also regulates the contraction at the lateral and rear of the cell, further supporting forward migration.

Rho C has emerged as an important spatial regulator of protrusions and invadopodia in 3D matrices and *in vivo* (Bravo-Cordero et al., 2014).

Integrin engagement at the leading edge at nascent adhesions spatially activates RhoA, a mechanism dependent on c-Src and p190RhoGAP, but does not affect Rac1 or Cdc42. Following initial activation, RhoA is transiently suppressed *via* p190RhoGAP, allowing a subsequent cycle of Rac induced protrusion generation (Arthur and Burridge, 2001). The temporal regulation of Rho and Rac activity suggests they are mutually opposed, and highlights the importance of their precise timing to allow for efficient migration through coordinated protrusion and contraction cycles (Ridley, 2013).

2.2. Rho GTPases in 3D migration

Membrane protrusions are thought to be driven largely by the forward force of actin polymerization at the barbed end, which overcomes the tension of the membrane, or by the forward protrusion of membrane blebs due to contractility near the rear of the cell with a force sufficient to displace local collagen fibers (Wyckoff et al., 2006). In 2D migration, protrusions based on actin polymerization dominate, while in 3D environments cells make switches between actin-based protrusions and contraction-driven blebs. Accordingly, the spatial and temporal use of Rho GTPases appears to differ when cells are migrating within 3D matrices compared to on 2D substrata. When Rho activity is inhibited in cells cultured in 3D, the cells exhibit increased cytoskeletal remodeling that is dependent on cofilin, which leads to an increase in cellular protrusions. Unlike on 2D surfaces, where increased protrusions lead to faster cell migration (Arthur and Burridge, 2001),