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Review

Signaling networks of Rho GTPases in cell motility

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ABSTRACT

The last decades have witnessed an exponential increase in our knowledge of Rho GTPase signaling network which further highlighted the cross talk between these proteins and the complexity of their signaling pathways. In this review, we summarize the upstream and downstream players from Rho GTPases that are mainly involved in actin polymerization leading to cell motility and potentially playing a role in cancer cell metastasis.

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1. Introduction

Cell motility is an essential cellular process involved in numerous physiological events including embryogenesis, wound healing, inflammation and tissue regeneration. It is a central process for cancer cell invasion and metastasis. Cell migration usually occurs in response to a chemoattractant or a growth factor present around the cell, a process known as chemotaxis. A great effort in research has been directed towards understanding the molecular basis of cell motility in an attempt to find novel therapeutic targets that would inhibit tumor growth and metastasis [1]. Once a signal is detected, a migrating cell enters the cell motility cycle in an amoeboid-like manner. This starts with determining the direction of motion towards the chemoattractant. Then the cell extends a protrusion, towards the direction of motion, by initiating the polymerization of new actin filaments [2]. The actin-rich protrusion then needs to be stabilized by the formation of adhesions to the cell substratum. This provides anchorage to the cell to transmit a mechanical force used to pull its cell body forward towards the direction of motion. Simultaneously, the adhesion structures at the rear end of the cell are disassembled and the cell retracts its tail and moves forward [3,4,2]. Motility is largely dependent on localized actin polymerization at the leading

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edge of lamellipodia [4–6]. Actin filaments are the dominant structures in the lamellipodia and are intrinsically polarized with a slow growing or pointed end decorated with myosin and the fast growing end or the barbed end. Actin monomer addition at the barbed end is highly favored. At the fast-growing end, ATP-actin monomers bind with a rate constant that is 10-fold higher than the rate of monomer binding at the pointed end. Barbed ends are oriented outwards with respect to cell surface. The rate limiting steps for actin polymerization in vivo are the availability of free barbed ends and the availability of free actin monomers for filament elongation [7]. Three mechanisms lead to the generation of free barbed ends and these consist of uncapping, severing and de novo nucleation [8,9]. Cofilin generates free barbed ends through the severing of actin filaments, leading to actin nucleation [10]. Severing involves the cooperative activity of two cofilin molecules that bind directly to the actin filament, introducing a tilt of approximately 12° that twists the filament and results in its breakage [11]. The need for severing to elongate actin filaments might seem counterintuitive, but severing is needed to expose free barbed ends since most filaments in resting cells are capped. Cofilin was found to localize to the extreme leading edge in fibroblasts and carcinoma cells [1] where actin polymerization occurs [12–14]. In carcinoma cells, the kinetics of cofilin activation coincides with the kinetics of barbed end formation, and the microinjection of a function-blocking antibody against cofilin inhibited barbed end production in the nucleation zone [15,13]. Cofilin-dependent actin polymerization during cell motility is pH-dependent. Cofilin activity is also regulated through phosphorylation at Ser-3 by LIM-kinases (LIMK1 and LIMK2). Cofilin is positively regulated through dephosphorylation by the activity of PP2A and the *Slingshot* family of phosphatases [16–18]. In carcinoma cells, however, over-expressing S3A-cofilin mutant fails to stimulate the generation of barbed ends in resting cells [19]. In addition, in response to EGF stimulation, the increase in cofilin severing activity coincided with an increase in the levels of phosphorylated cofilin in the cells [20]. This indicates that, in carcinoma cells, dephosphorylation is not the main regulatory mechanism leading to the activation of cofilin in response to EGF stimulation. In addition to the regulation by pH and LIMK, cofilin is kept inactive in resting cells through binding to $PI(4,5)P_2$ at the cell membrane [21]. This inhibition through binding is proposed to be alleviated through the hydrolysis of PIP2 by PLCγ. Indeed, high resolution fluorescence microscopy staining for PIP2 and cofilin localization revealed that inhibition of PLCγ blocked the dissociation of cofilin from PIP2 in the membrane. Also the inhibition of PLCγ led to the inhibition of cofilin severing activity and cofilin-dependent barbed ends production in carcinoma cells [22,23]. These studies indicate that PLCγ is the main regulator of cofilin activity in carcinoma cells [16]. De novo nucleation is the third mechanism through which barbed ends can be generated and this is mediated by the Arp2/3 complex [24,9]. Arp2/3 complex is a stable assembly of seven highly conserved subunits, of which two are actin-related proteins (Arp2 and Arp3) that provide a template for actin nucleation. Electron microscopic analysis revealed that actin filaments at the leading edge are highly branched with a predominant 70° angle. This configuration contributes to the mechanical protrusive power needed in the lamellipodia. Arp2/3 nucleates actin by creating daughter filaments at an angular branch of 70° from pre-existing mother filaments, and Arp2/3 is localized at the pointed end of the Y junctions formed by the mother and the daughter filaments [12]. Arp2/3 is activated by the WASP/SCAR/WAVE family of scaffold proteins, which are key regulators of actin polymerization that are activated by Rho GTPases [25].

2. Rho GTPases: General properties

Numerous intracellular proteins are involved in the regulation of the cell motility cycle. One of the most important familie of proteins regulating this process is the Rho family GTPases. Members of this family play a crucial role in the reorganization of the actin cytoskeleton [9,26]. Members of the Rho-family GTPases are small GTP-binding proteins (GTPases) that range between 20 and 40 KDa in size. The Ras gene was first discovered as the v-Ras oncogene of the Rous sarcoma virus around 1980 [27]. The Ras superfamily contains more than 130 members, which fall into the Ras, Rho, Arf/Sar1 and Rab/Ran-subfamilies [27,5]. The Rho gene was discovered as a homolog of the Ras gene in *Aplysia* in 1985. Rho family-GTPases include 23 members divided into six subgroups. Rho homologues, RhoA, RhoB and RhoC which were discovered in mammalian cells [28]. Other members of the Rho-subfamily were later identified, including human Cdc42 and Rac1 and Rac2, which were found to be distinct in function from the other Rho proteins but share significant homology in amino acid sequence [29,30,6]. These proteins play a vital role in cancer cell motility. All aspects of cellular motility and invasion including cellular polarity, cytoskeletal re-organization, and signal transduction pathways are controlled through the interplay between the Rho-GTPases [31,5]. Activated Rho proteins can bind effector proteins and modulate cell behavior and morphology. Rho GTPases are implicated in cell migration through their ability to organize and regulate actin-containing structures. Frequent studies have shown that the Rho family GTPases regulate cell motility in breast cancer through their ability to mediate the remodeling of actin cytoskeleton as well as translating cellular signals from plasma membrane receptors to regulate focal adhesion, cell polarity, vesicular trafficking and gene expression [5]. All Rho GTPases have a consensus amino acid sequence at the N-terminal half that is responsible for specific interaction with GDP and GTP molecules and for a GTPase activity that hydrolyzes bound GTP into GDP and P_i. Four important domains are implicated in the binding and hydrolysis of GTP (Fig. 1A) [32]. One of the most important domains is the effector or switch I domain, which is required for downstream functions of Rho GTPases. Rho proteins also have sequences at their COOH termini that undergo post-translational modifications with lipids, such as farnesyl, geranylgeranyl, palmitoyl and methyl moieties, and that are necessary for proper localization in the cell [33].

Rho GTPases switch between two conformations, a GDP-bound inactive state where they are sequestered and kept in the cytoplasm; and an active GTP-bound state [31,34]. Since Rho GTPases control many important signal transduction pathways, their activation is tightly regulated in the cell. The activity of Rho GTPases is regulated by nucleotide binding and by subcellular localization [35]. In response to a certain signal, replacing the bound GDP with a GTP molecule activates Rho GTPases. This induces a conformational change favoring the binding of the active Rho protein to downstream effectors. After activation of the effector, the GTP molecule is hydrolyzed to GDP rendering the GTPase back to its inactive form [36,5]. This constitutes a single cycle of activation/inactivation of Rho GTPases. Rho GTPases mediate the transduction of numerous intracellular signaling pathways affecting cell behavior and morphology. Thus, these critical proteins are implicated in many essential cellular processes including actin dynamics, gene transcription, cell cycle progression, cell adhesion, motility and invasion [37,5]. Rho GTPases act as molecular switches that relay extracellular signals and translate them into intracellular events through their downstream effectors [34]. Since Rho GTPases control many important signal transduction pathways, their activation is tightly regulated in the cell. The activity of Rho GTPases is regulated by nucleotide binding and by subcellular localization [38,34,35]. Rho GTPases are found in two forms, a GDP-bound inactive and a GTP-bound active form. An upstream signal stimulates the dissociation of GDP and the binding of GTP. This leads to the conformational change of the effector-binding region of the GTPase so that this region interacts with downstream effectors. The GTP-bound form is then converted to the GDP-bound form releasing the bound effector. This constitutes a single cycle of activation/inactivation of Rho GTPases (Fig. 2B) [38]. The switch between active GTP-bound and inactive GDP-bound state is regulated by Guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs) and Guanine-nucleotide dissociation inhibitors (GDIs).

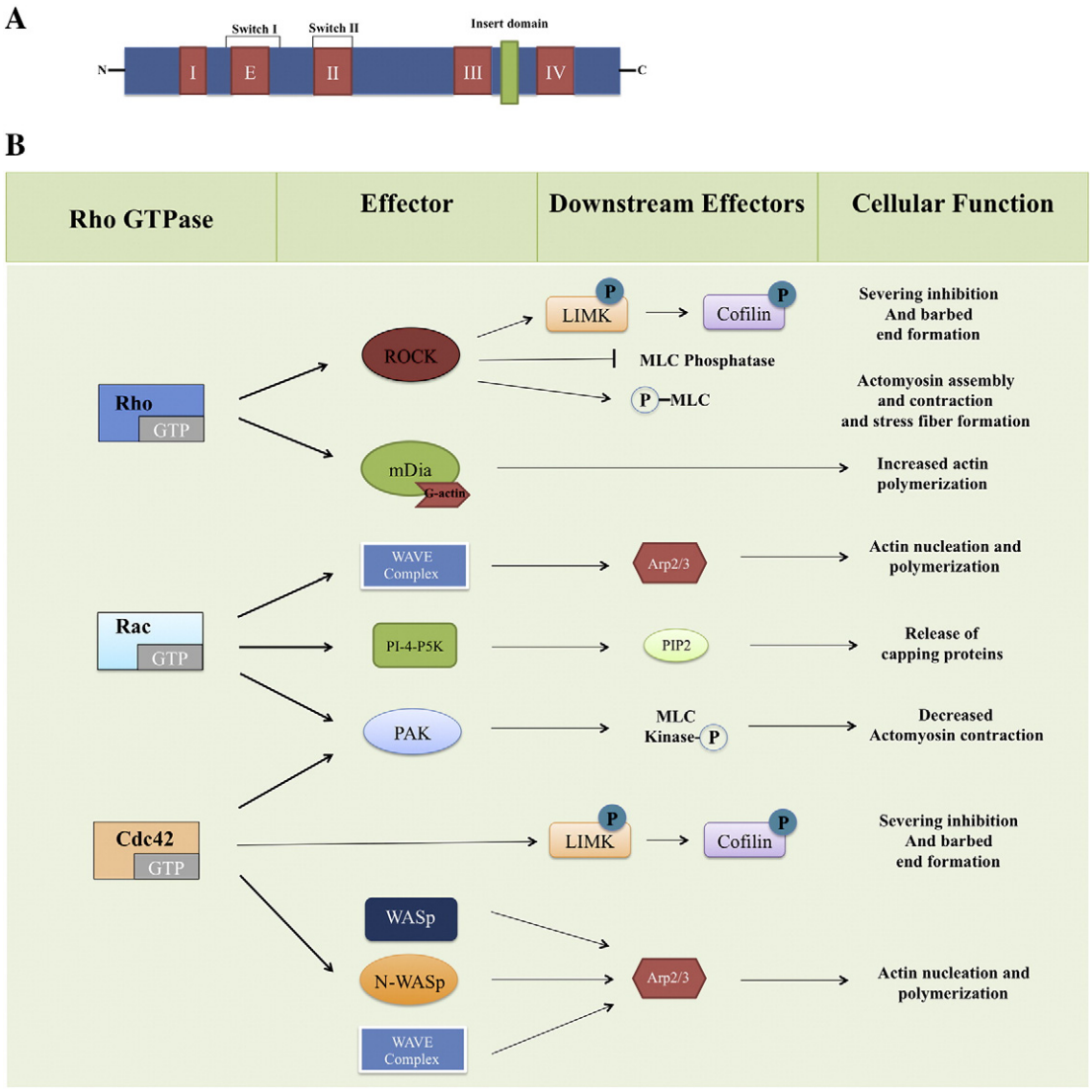

Fig. 1. Rho GTPases and downstream effectors. A) General structural domains of Rho GTPases include effector binding domains switch I and switch II, also important for GTP/GDP binding and interaction with upstream regulators. B) Downstream targets of Rho include the serine/threonine kinase ROCK which is mainly involved in the formation of stress fibers and focal adhesions. ROCK phosphorylates downstream myosin light chain (MLC) leading to actin–myosin contractility. At the same time, ROCK inhibits MLC dephosphorylation by inhibiting MLC phosphatases via their myosin binding subunit (MBS). The mammalian homolog of diaphanous (mDia) is another important Rho effector mediating actin nucleation. LIMK is also another downstream effector of Rho, which phosphorylates the actin severing protein cofilin inhibiting its severing activity and production of barbed ends. Downstream signaling of Cdc42 and Rac includes scaffold proteins belonging to the WASP/SCAR/WAVE family, key regulators of actin nucleation and polymerization. p21 activating kinase (PAK) is a common effector protein of both Rac and Cdc42. Active PAK phosphorylates MLCK thereby inactivating it and inhibiting MLC phosphorylation and contractility. PAK also phosphorylates and activates LIMK which potentially results in the phosphorylation of cofilin inhibiting its actin-severing function.

GEF proteins have a Dbl homology (DH) domain and other domains such as Src homology 3 (SH3) and pleckstrin homology domain (PH) (Fig. 2A). These regulators interact with phospholipids of the cell membrane and other proteins modulating the GDP–GTP exchange activity [5]. During nucleotide exchange, the initial dissociation of GDP from the inactive form of Rho GTPases is considered the rate-limiting step. This reaction is very slow and is stimulated by a guanine nucleotide exchange factors (GEFs). Thus, GEFs activate Rho GTPases by mediating the exchange of GDP to GTP [38,39]. Rho GTPases are negatively regulated by Rho GTPases activating proteins (GAPs). These proteins inhibit Rho GTPases by activating their intrinsic GTPase activity. This leads to the hydrolysis of the bound GTP into GDP converting Rho GTPases back to their inactive conformation [40]. In addition to activating GTP hydrolysis, GAPs may function as effectors of Rho GTPases to mediate other downstream effector functions [38,5]. Guanine nucleotide dissociation inhibitors (GDIs) block both the GDP/GTP exchange and the GTP hydrolysis. These proteins prevent the dissociation of GDP from the inactive Rho proteins and their interaction with downstream effectors. GDIs can also bind to the active GTP-bound form preventing their interaction with GAPs. Moreover, GDIs modulate the cycling of GTPases between the cell membrane and the cytoplasm. Since the activity of Rho GTPases crucially depends on their translocation to the cell membrane, GDIs are considered important regulators with the ability to sequester GTPases in the cytoplasm by masking their hydrophobic region/domains [41,42].

3. Signaling pathways upstream Rho GTPases

Rho family GTPases are activated in response to numerous extracellular stimuli captured by plasma membrane receptors. Hence, these proteins are involved in translating signals to regulate various cellular functions including cytoskeleton re-organization, cell–cell interaction, proliferation, cell adhesion, polarity, chemotaxis and many others [31,5]. Rho GTPase-activating protein GEFs contain the

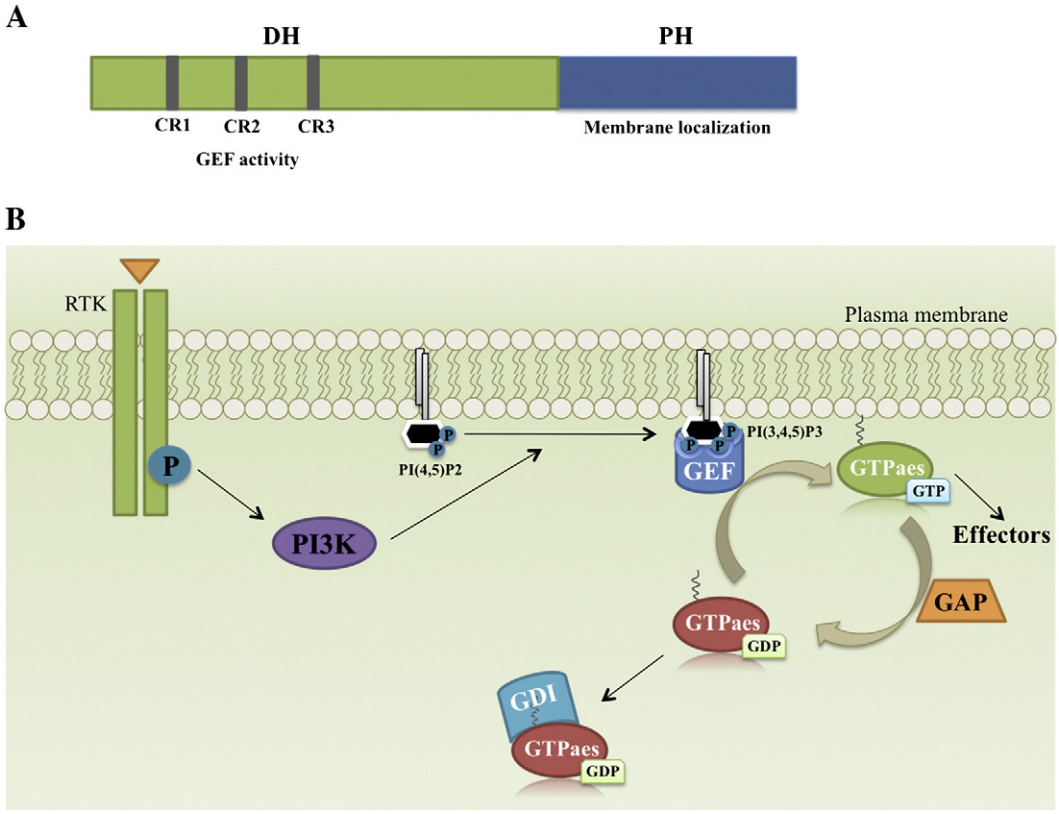

Fig. 2. Regulation of Rho GTPases. A) The GEF module consists of the DH–PH minimum structure. B) In response to external stimulus through receptor tyrosine kinases (RTKs), PI3K produces PIP3 which recruits Rho GEFs. This promotes the GTP nucleotide exchange. GAPs inactivate Rho GTPases by stimulating the intrinsic GTPase activity leading to the GDP-bound inactive form of Rho. Rho GTPases are kept inactive by being sequestered by GDIs in the cytosol.

pleckstrin homology (PH) domain, a 100–120 amino acid module that has a high binding affinity to phosphoinositides and can affect the catalytic DH domain of GEFs [43]. Binding to PI(4,5)P2 of the plasma membrane favors the interaction between PH domain of GEFs and the catalytic DH domain inhibiting its activity (Fig. 2). PI3K phosphorylates PI(4,5)P2 producing PI(3,4,5)P3 that has high affinity to PH domain, thus releasing the DH domain and activating GEFs. In turn, GEFs can bind and activate Rho GTPases [39]. External stimuli such as LPA, PDGF, EGF and insulin have been shown to trigger the activation of Rho GTPases in a PI3K dependent manner (Fig. 2B). Treatment of fibroblasts with wortmannin, a PI3K inhibitor, inhibited Rho and Rac mediated membrane ruffling in response to EGF [44]. These data suggest that PI3K acts upstream of Rho GTPases to stimulate membrane ruffling in response to growth factors. A well-characterized nucleotide exchanger, Vav, is known to be activated by PI3K. Vav is phosphorylated on tyrosine residues in the N-terminal region by Src and Syk kinases leading to the activation of its catalytic activity [45]. An autoinhibitory constraint is imposed by its PH domain. However, when bound to PI(3,4,5)P3 produced by PI3K, the PH/DH interaction is weakened and this alleviates the inhibition. The PH domain of Son of svenless (Sos) also binds to PIP3 relieving intramolecular inhibition. Sos is a GEF for both Ras and Rac. It forms a complex with a number of adaptor proteins downstream of receptor tyrosine kinases [46,47]. Therefore, the activity of Rho GTPases is spatially regulated in many cellular functions following the subcellular localization of GEFs. PI3K activation plays an essential role in regulating the localization of GEFs through the production of PIP3 which binds to the PH domain of GEFs. Deletion of the PH domain in many GEFs results in the loss of in vitro activity, which can be restored by the addition of a CAAX motif that targets the protein to the plasma membrane [48]. In addition, many studies in mammary tumors have shown that PI3K acts downstream of Rac and Cdc42 [49]. These findings suggest that PI3K and Rho GTPases are involved in a positive feedback loop that stimulates lamellipodia formation during cell motility.

4. Activation of Rho GTPases by adhesion

Numerous signaling pathways involving Rho GTPases are activated downstream of the cell adhesion to the ECM [50]. In this respect, the activation of focal adhesion kinase (FAK) leads to the phosphorylation and activation of p130cas and paxillin [51,50]. Phosphorylated p130cas activates Rac by forming a complex with adaptor proteins Crk and DOCK180, which is a GEF for Rac [52,53]. Paxillin also forms a multi-component complex with the protein PKL and PIX, the latter being another Rac GEF. In addition to activating Rac, FAK also activates p190RhoGAP thus leading to the inhibition of RhoA. In many cells, during initial cell adhesion, FAK activity stimulates high Rac activity and low RhoA activity [54] [2].

5. Effectors of Rho GTPases in actin polymerization

The major function of Rho GTPases is to regulate actin polymerization required for a myriad of biological processes [38]. The effects of Rho, Rac and Cdc42 on actin were initially postulated using quiescent Swiss3T3 fibroblasts and dominant active or negative GTPase mutants. Addition of a stimulus, lysophosphatidic acid (LPA) induced the formation of contractile actin–myosin stress fibers and focal adhesions that associate with the tip of stress fibers. This process was blocked by C3 transferase, and implicated a role of Rho in stress fiber formation [6]. Growth factors, such as platelet-derived growth factor (PDGF), insulin or epidermal growth factor (EGF) induced the formation of protrusive structures, such as lamellipodia and membrane

ruffles and the dominant negative [Asn^{17}] Rac specifically inhibited this response [30]. Bradykinin induced the formation of microspikes and filopodia and the dominant negative [Asn^{17}] Cdc42 inhibited this response [55]. Based on these original studies, it was concluded that Rho, Rac and Cdc42 regulate the assembly of actin–myosin filaments, lamellipodia and filopodia, respectively [56]. Rho GTPase effectors, as previously mentioned, interact with the GTP-bound active form of Rho GTPases. Comparisons of the crystal structure of the GDP-bound form and the [Val^{14}]-Rho GTPase–GTP form (in Ras, RhoA, Cdc42 and Rac), revealed that the conformational differences between the GDP and the GTP-bound forms are restricted to two surface loops, named switch I (the effector domain) and switch II (Fig. 1A). The effector domain forms an extended β2-strand/loop structure. This extended structure makes it possible for different effectors and regulators to bind to different subdomains of the effector domain at the same time. Alternatively, binding to an effector at this domain might interfere with the binding of another effector/regulator at this domain (in another subdomain), providing a basis for the regulation of different processes downstream of Rho GTPases [57]. Numerous mutations have been introduced into the effector domain of Rho, Rac and Cdc42 and this prevented the binding of some target proteins. For example, the interaction of PAK (p21-activated kinase) with Cdc42/Rac could potentially be blocked or reduced by various mutations [58,59]. Effectors of Rho GTPases have multiple domains that play an additional role in regulating their activity. This includes a PH domain in N-WASP, WASP and ROCK, which promotes association with the membrane, where active Rho GTPases are present. [25]. WASP and PAK contain proline-rich SH3-binding motifs which bind to the adaptor Nck that in turn contains an SH2 domain and recruits these effectors to activated receptor tyrosine kinases. Many Rho GTPase effectors also contain coiled-coil regions (l which plays a role in protein–protein oligomerization [56].

Downstream targets of Rho include the serine/threonine kinase p160ROCK which is mainly involved in the formation of stress fibers and focal adhesions (Fig. 1B) [60,61]. ROCK is known to phosphorylate myosin light chain (MLC) leading to actin–myosin contractility. At the same time, ROCK inhibits MLC dephosphorylation by inhibiting MLC phosphatases via their myosin binding subunit (MBS) [62]. However, normal stress fiber formation also requires the activity the mammalian homolog of diaphanous (mDia), another important Rho effector mediating actin nucleation. LIMK is another downstream effector of Rho, which phosphorylates and inhibits the actin severing protein cofilin [63]. However, a recent study showed that RhoC in particular is responsible for the phosphorylation of cofilin in a spatiotemporal restriction in invadopodial protrusions [64]. Other Rho effectors include members of the ezrin–radixin–moesin (ERM) proteins. These proteins are found to associate with the cell plasma membrane where they mediate Rho-dependent actin cytoskeleton remodeling [5]. Downstream signaling of Cdc42 and Rac includes scaffold proteins belonging to the WASP/SCAR/WAVE family containing the VCA domain (Fig. 1B) [49,5]. These are key regulators of actin nucleation protein Arp2/3 complex that stimulates actin polymerization at the leading edge of the cell [37,24]. Some proteins are target effectors of both Rac and Cdc42. One of these proteins is the serine/threonine p21 activating kinase (PAK). PAK isoforms have an N-terminal regulatory domain and a C-terminal catalytic domain. Moreover, all PAK proteins share a common domain responsible for interaction with Rac and Cdc42, referred to as Cdc42/Rac interactive binding (CRIB) domain. Binding of active GTPase disrupts the autoinhibitory conformation of PAK and activates its catalytic domain by phosphorylation [65,59,54]. Active PAK phosphorylates MLCK thereby inactivating it and inhibiting MLC phosphorylation and contractility [66,67]. PAK is also targeted to adhesion complexes regulating focal adhesion turnover. In addition, PAK phosphorylates and activates LIMK [68]. LIMK is therefore activated through ROCK and PAK pathways downstream of Rho and Cdc42/Rac respectively (Fig. 1B). Moreover, in MTLn3 cells, Cdc42 has been shown to mediate the translocation of both N-WASP and WAVE2 to the leading edge in response to EGF stimulation [71].

6. Role in cell motility

In general, cell migration can be divided into separate successive steps: determining the direction of motion, cell polarization, lamellipodial protrusion, adhesion formation followed by cell body contraction and tail retraction (Fig. 3) [1]. The initial step during cell migration is the determination of direction of motion by generating membrane protrusions. These protrusions can be either spike-like filopodia or larger, broad lamellipodia [5]. Filopodia are classically regarded as sensors for chemotactic cues required for direction sensing during cell migration. These protrusions extend out from the cell detecting and transmitting any environmental changes [69]. Cdc42 regulates the formation of filopodia by initiating actin polymerization through the activation of N-WASP [44]. The lamellipodium is a meshwork of highly branched actin filaments at the cell edge [70]. The structure of the lamellipodia is known to be Rac-dependent involving a number of Rac downstream effectors such as severing proteins ADF/cofilin and actin binding proteins like the Arp2/3 complex that is responsible for actin nucleation and branching [18]. This is in addition to the activation of PIP-5 kinase that produces PIP2 inhibiting actin-capping proteins. However these effectors are also downstream of Cdc42 (Fig. 1B). Consequently, Cdc42 is regarded as a potential regulator that drives Rac-dependent lamellipodia [71]. In addition to its role in actin regulation, Cdc42 plays a crucial role in defining cell polarity with respect to the direction of motility, through the regulation of the microtubule cytoskeleton [32].

7. Adhesion formation

Extended protrusions of migrating cells need to be stabilized by adhering to the ECM. Cell adhesion to the ECM activates Rac and Cdc42, which is required for cell spreading [72]. It is therefore possible that there is continuous formation of new interactions between integrins and the ECM. The speed of cell migration depends on the composition of the substratum that dictates the relative levels of active Rho, Rac and Cdc42. Thus, a constant crosstalk between integrins and Rac is decisive for the cellular response to changing ECM composition. Rac stimulates the assembly of small punctate structures known as focal complexes that form behind the leading lamellipodium of the cell. However, these structures do not transmit adequate contractility during cell motility [73]. Rac induces focal complex formation directly, through activating PAK, which in turn interacts with a complex of the exchange factor PIX, paxillin and GIT family of proteins. Also, Rac can indirectly contribute to the formation of focal complexes through antagonizing Rho activation. As the cell moves in the direction of motion, focal complexes can either disassemble or mature into larger and more stabilized Rho-dependent structures known as focal adhesions [74,69]. Focal adhesions provide anchorage for the cell thus conferring mechanical strength needed for the cell to contract its cell body and slide along the ECM. Therefore, cell migration requires the focal complex/adhesion turn over regulated by the interplay between Rac and RhoA. Increasing Rho activation stabilizes focal adhesion attachments to the ECM, hence inhibiting cell motility [75,66,76].

8. Cell body contraction and tail retraction

In a migrating cell, adhesion to the ECM alone is not sufficient. Cell body contractility and tail retraction are needed for the completion of the cell motility cycle. Cell body contraction depends on actomyosin contractility, which is directly regulated by Rho. Rho acts via ROCK inducing contractility through the phosphorylation of MLC (Fig. 1B). This results in transmission of tension to the sites of adhesion. MLC is also regulated by MLC kinase (MLCK), thus it is likely that ROCK and MLCK act in concert to control cell contractility [5]. In addition to the ROCK pathway, RhoA is believed to negatively regulate cofilin leading to the inhibition of cell protrusions [77]. Thus, RhoA localizes

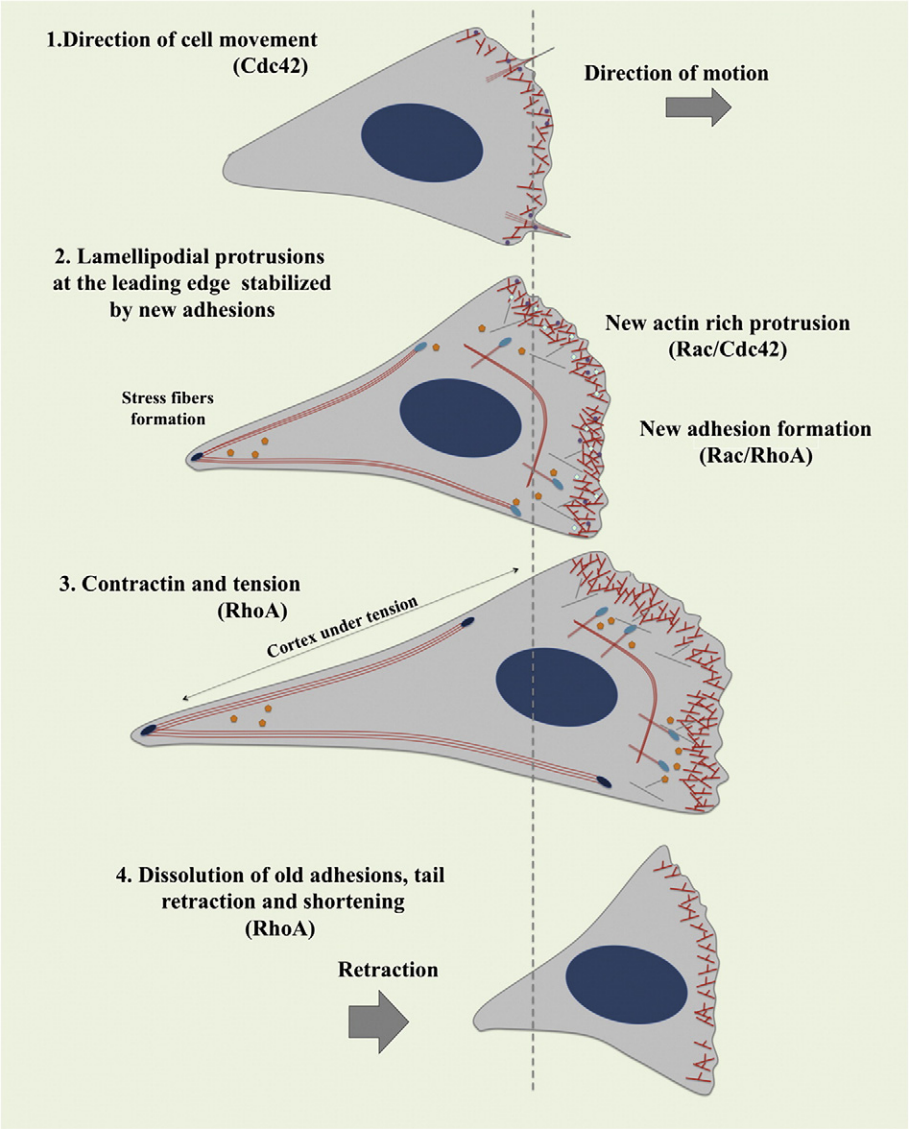

Fig. 3. Rho GTPases in the cell motility cycle. A migratory cell enters the cell motility cycle in response to a chemoattractant signal. Cdc42 determines the direction of motion. Rac induces the formation of actin-rich lamellopodial protrusion at the leading edge. New protrusion is stabilized by the formation of new adhesions to the underlying substratum, a process controlled mainly by Rac and RhoA [77]. Rho acts at the rear end leading to the formation of stress fibers and actin–myosin contractility providing tension for the cell to retract its tail and move forward.

to the rear of the cell inhibiting protrusions during motility. Tail retraction is the final step of cell migration. At this point, adhesions must disassemble to ensure the completion of the cell motility cycle [78]. Although the mechanism of tail detachment depends on the cell type and the strength of adhesion to the ECM, it is known that the reduction in RhoA activity could potentially inhibit retraction through reduced actomyosin contractility [79,77]. Therefore, the continuous interplay between Rho GTPases govern all aspects of cell motility including cell polarity, cytoskeleton re-organization, adhesion formation, cell contraction and tail retraction (Fig. 3) [44,34].

9. Crosstalk between Rho GTPases

It was initially believed that Rho, Rac and Cdc42 play a defined role in regulating actin and adhesion dynamics during cell motility. However, this model is considered too simplistic due to crosstalk between the signaling pathways regulated by Rho GTPases [80,81]. It was shown that RhoA for instance, is not only restricted to the generation of contractile force at the rear end of the cell, but also coordinates with Rac and Cdc42 at the cell edge in regulating actin cytoskeleton [77]. Other studies have revealed an inverse relationship between Rho and Rac, where activation of Rac leads to the inactivation of Rho and vice versa [82]. This antagonism is explained in their antagonistic functions in cell adhesion. Other examples where one family member negatively affects the activity of the other is through stimulating a GAP, or positively activating another through stimulating a GEF [83]. Recent studies showed that distinct roles in migration and invasion could be implemented by different isoforms of Rho particularly RhoA and RhoC. This could be by acting through different targets [84].

10. Altered role in cancer

The acquisition of motile and invasive phenotypes is the key component in developing metastatic competence. Both of these processes are strictly regulated by members of the Rho family GTPases [31]. Studies have shown that constitutively activated Rho GTPases lead to the transformation of fibroblasts. In addition, genetic screening showed that Rho GTPases, particularly RhoA and RhoC, are found to be either

overexpressed or hyperactive in many tumors including breast cancer [31,5]. Moreover, overexpression of RhoC was associated with 32% of invasive breast cancer and invasive ductal carcinoma [26]. In fact, forced expression of Rho proteins induced malignant transformation of human mammary epithelial cells resulting in an aggressive and highly motile phenotype. In addition, expressing a dominant-negative form of Rho inhibited cellular motility [85]. Therefore, aberrant expression of Rho GTPases primarily contributes to cell transformation and tumor development, given their role in the regulation of actin polymerization and motility and their interaction with numerous signaling pathways in addition to regulating extracellular matrix remodeling [86,87]. Dominant inhibitory or activating approaches have been used to describe the role of Rho GTPases in primary tumor growth and metastasis. Regulatory proteins GEFs and GAPs can cause dysfunctional activation/inhibition of Rho GTPases affecting cellular motility, invasion and ultimately metastasis [26,82,86]. Moreover, as Rho GTPases are highly involved in promoting cellular transformation, many downstream effectors could be directly involved in tumor formation. For instance, direct inhibition of RhoA, through microinjection of C3T, or inhibiting its downstream effector ROCK using Y27632 leads to decreased motility and inhibition of focal complex maturation into focal adhesions [77]. There is also evidence that aberrant activation of Rho proteins can contribute to prolonged survival and prevent apoptosis. This is because of the ability of Rho GTPases to affect CDKs involved in regulating cell cycle progression promoting tumor initiation and growth [88]. Cyclin D1 is found to be overexpressed in 50% of breast cancers. Rho GTPases correlate to high expression of cyclin D1 through the activation of its promoter. Rac1 regulates a variety of signaling pathways implicated in malignant phenotype. Typically, Rac1 affects transcription of cyclin D1 through the activation of NF-κB and ATF-2 transcription factors that bind and activate cyclin D1 promoter. Thus, constitutively active forms of Rac1 stimulated transformation potential and cell proliferation through cyclin D1 overexpression. Moreover, the use of pharmacologic inhibitor NSC23766 selectively inhibits Rac1 and leads to the suppression of cell growth in breast cancer cell lines [89]. Furthermore, RhoA overexpression inhibits p21, a cyclin-dependent kinase inhibitor and an important tumor suppressor gene product [90]. Given their integration in various pathways involved in cancer, Rho GTPase and their regulators are considered important therapeutic targets through the inhibition of cancer cell proliferation, motility and invasion. Several drugs have been shown to abrogate Rho GTPase functions. These drugs could directly target Rho proteins such as farsenalytrasferase inhibitors (FTIs) and strongylophorine-26 or could act through inhibition of their downstream effectors such as ROCK inhibitor Y-27632 [91–93].

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