[Frontiers in Bioscience 13, 759-776, January 1, 2008]

Role of Rho GTPases in breast cancer

Yong Tang[1], Lola Olufemi[2], Man-Tzu Wang[1], Daotai Nie[1]

[1]Department of Medical Microbiology, Immunology, and Cell Biology, Southern Illinois University School of Medicine and SimmonsCooper Cancer Institute, Springfield, IL 62702, USA, [2]Department of Molecular Biology and Biochemistry, Southern Illinois University School of Medicine, Carbondale, IL 62901

TABLE OF CONTENTS

1. ABSTRACT

Small GTPase Rho signaling pathways regulate the growth, motility, invasion and metastasis of breast cancer cells. Aberrant Rho signaling, as results from alterations in the levels of Rho GTPase proteins, the status of activation, and the abundance of effector proteins, is found in breast cancers. Alterations of Rho signaling particularly impact the cytoskeleton, whose organization and reorganization underpin the motility of breast cancer cells during the invasive growth and metastasis of breast cancer. Progress is being made to elucidate the underlying mechanisms by which Rho GTPases activate the downstream signaling effectors. Further investigations are required for development of novel tumor therapeutic strategies targeting the Rho GTPase signaling pathways to treat breast cancer.

2. INTRODUCTION

2.1. Cell migration and tumor metastasis

In metazoan organisms, migration of cells, either as individuals or as groups, is essential for morphogenesis during embryonic development (1). In the adult, it is crucial for tissue integrity maintenance and immune surveillance, in which leukocytes migrate from the lymph or blood circulation into the surrounding tissues to destroy exogenous microorganisms as well as infected somatic cells. Cell migration also contributes to several severe human diseases, including tumor formation and metastasis, atherosclerosis, osteoporosis, mental retardation and chronic inflammatory diseases, such as rheumatoid arthritis. In cancer, metastases is the principal cause of death for most cancer patients, accounting for approximately 90% of all cancer related deaths (2-4).

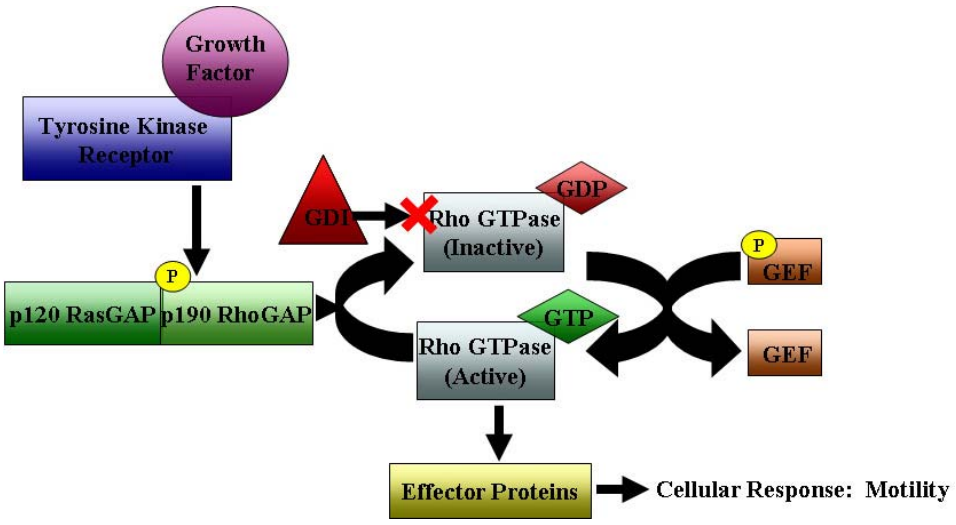

Figure 1. Regulation of Rho family proteins. GEFs, guanine nucleotide exchange factors; GAPs, GTPase-activating proteins; GDIs, guanine nucleotide dissociation inhibitors.

For dissemination and metastasis to occur, tumor cells must invade the tissue surrounding the primary tumor, intravasate into the lymphatic system or blood supply system, extravasate and attach to target tissue at the distant site, and then form macrometastases (5). Acquisition of cell motility has been implicated in the spreading of cancer cells (6) and is essential for metastasis (7). Since cancer mortality has been mainly associated with metastatic disease rather than with the primary tumor, better understanding of the factors leading to elevated motility of tumor cells is of vital importance for development of effective therapeutic approaches to inhibit invasion and metastasis of cancer cells.

Cell migration is a coordinated multi-step process involving directed protrusion of the cell membrane, adhesion of the cell membrane to surrounding substrates, contraction of the cell body, and detachment of the cell at the trailing edge (8). The notion that Rho family GTPases could regulate cell migration originates from their ability to mediate the formation of specific actin containing structures (9, 10). Subsequent findings, which show the Rho family to regulate focal adhesion complex assembly, cell polarity, vesicle trafficking and gene transcription, reinforce its important role relevant to cell migration. Overexpression and hyperactivation of Rho GTPases (particularly overexpression of RhoA) and their corresponding signaling components are found in breast cancer (11, 12). Therefore, Rho GTPases are involved in breast cancer metastasis, proliferation and progression which contribute substantially to patient morbidity and mortality.

Herein, we first make a brief summarization of the general established conceptual framework of the Rho GTPases family, including their family members, their active-inactive regulators, and their biochemical and biological functions. Next, we focus on how Rho proteins and their signaling partners contribute to cell migration and address the alterations in GTPase signaling in breast cancer and their roles in metastasis and growth of breast cancer. Finally therapeutic approaches targeting Rho GTPases are discussed as potential treatment for metastasis of cancer in general and breast cancer in particular.

2.2. Rho subfamilies and their regulation

Rho was first identified in 1985 due to its homology to Ras (Rho stands for Ras homologous) from the sea slug, *Aplysia Californica* (13). Later it was reported that Rho GTPases constitute a distinct subgroup within the superfamily of Ras-related small GTPases and are found in all eukaryotic cells. Five and seven Rho family members have been found in *Drosophila melanogaster* and *Caenorhabditis elegans*, respectively, most of which have been implicated in cell migration and morphogenesis. Plant-specific Rho subfamily, the Rop proteins, has also been reported (14). So far, twenty-two mammalian Rho family proteins have been identified. Of these proteins, RhoA/B, Rac1/2 and Cdc42 are the most extensively studied for their significant implications in regulating the organization of the actin filament system for the morphogenesis and movement of mammalian cells.

Like all members of the Ras superfamily, Rho GTPases function as molecular switches, cycling between an active GTP-bound conformation and an inactive GDP-bound conformation (Figure 1). In the GTP-bound active form, they interact with downstream target proteins to trigger cellular response events. The switch between active GTP-bound conformation to inactive GDP-bound conformation is regulated by the opposing effects of guanine nucleotide exchange factors (GEFs), which promote the active GTP-bound state by facilitating the exchange of GDP by GTP, and by the GTPase-activating proteins (GAPs), which inactivate the Rho GTPases by promoting GTP hydrolysis. In addition, Rho proteins are also regulated by guanine nucleotide dissociation inhibitors (GDIs), which can inhibit both the exchange of GTP and the hydrolysis of bound GTP (15). In most cases, Rho proteins are posttranslationally modified at their C-termini

by prenylation at a conserved cysteine, which is required for the attachment of Rho proteins to cell membranes (16).

2.2.1. GEFs

As regulators of Rho GTPases, GEFs for Rho-like GTPases belong to a protein family sharing a Dbl-homology (DH) domain whose prototype is Dbl oncogene product (17). Originally discovered by its capability to induce focal complex formation and tumorigenicity when expressed in NIH-3T3 cells, Dbl proteins represent the GDP/GTP exchange protein (GEP) activity on all the Rho GTPases family members, including RhoA and Rac1 as well as Cdc42 (18). In addition to DH domain, many of GEFs of Rho GTPases possess other domains that are commonly found in signaling molecules, such as Src homology 3 (SH3) domain and a diacylglycerol-binding zinc butterfly motif, suggesting their involvement in other cellular regulation events (17). A case in point here is the Rho GTPases GEF named Vav, whose prototype is the product of proto-oncogene Vav. Vav was first identified by a mutation that enables it to transform fibroblasts (19, 20). Vav contains a domain similar to the protooncogene Dbl. In addition, Vav contains a pleckstrin homology domain, a single SH2 domain, and two SH3 domains, which suggest that Vav can interact with several components of signal transduction events (20). Moreover, biochemical and genetic analyses in yeast have shown that the tyrosine phosphorylated form of Vav could promote Rac1 and other Rho-family proteins to the active GTP-bound active conformation (21).

2.2.2. GAPs

The first GAP protein specific to the Rho family was purified based on its biochemical interaction with recombinant Rho. This protein, named p50Rho–GAP, possesses GAP activity toward Rho, Cdc42, and Rac *in vitro* (22, 23). Since then, additional proteins that exhibit GAP activity toward Rho GTPases have been identified, all of which share a GAP domain that bears no significant resemblance to Ras GAP. In addition to accelerating the hydrolysis of GTP Rho, GAPs may function as an effector of the Rho proteins to mediate other downstream functions in mammalian systems, such as cytoskeletal rearrangements (24).

2.2.3. GDIs

Rho GDI was first characterized by its association with the GDP-bound form of the Rho family members, such as RhoA, Cdc42 and Rac to inhibit the dissociation of GDP (25, 26). Further studies demonstrated that Rho GDI is also associated weakly with the GTP-bound form of Rho, Cdc42, and Rac (27, 28). This weak interaction leads to inhibitory effects on the intrinsic and GAP-stimulated GTPase activity of the Rho GTPases. Thus, Rho GDI functions as a molecule capable of blocking the GTP-GDP binding cycling of GTPase through two approaches: at the GDP/GTP exchange step and/or at the GTP hydrolytic step. In addition, a significant role of Rho GDIs during Rho GTPases circulation lies in regulation of the translocation of the Rho GTPases between membranes and the cytoplasm. In resting cells, the Rho proteins exist as a complex with Rho GDIs in the cytosol to inhibit their GTP/GDP exchange ratio. During cellular activation events, Rho proteins are released from the GDI and translocated to the membranes (29).

3. UPSTREAM SIGNALING PATHWAYS OF Rho GTPases

Rho family proteins are involved in translating the cellular signals sent from plasma membrane receptors into the assembly and organization of the actin cytoskeleton. In fibroblasts, diverse extracellular stimuli have been shown to result in Rho GTPase activation. Addition of lysophosphatidic acid (LPA) to quiescent fibroblasts induces the assembly of focal adhesions and actin stress fibers, which can be blocked with C3 transferase, a bacterial coenzyme that ribosylates ADP and inactivates Rho proteins (30). A Rac-regulated signaling pathway linking growth factor receptors to the polymerization of actin at the plasma membrane has also been established. Growth factors, such as platelet-derived growth factor (PDGF), insulin, and bombesin, stimulate polymerization of actin at the plasma membrane to induce lamellipodia formation and membrane ruffling, which can be inhibited by the dominant-negative mutant of Rac, RacN17 (31, 32). Moreover, the activation of Cdc42 by bradykinin promotes the formation of peripheral actin microspikes and filopodia, with the subsequent formation of lamellipodia, which can be inhibited by a dominant-negative mutant of Cdc42, Cdc42N17 (33). Since the bradykinin, LPA, and bombesin receptors belong to the seven-transmembrane-domain heterotrimeric G protein coupled receptor family, the trimeric G proteins are likely to play a role in the activation of the respective GTPases.

Several lines of evidence suggest the involvement of phosphoinositide 3 kinase (PI3 kinase) in PDGF and insulin induced lamellipodia formation and membrane ruffling. PDGF could stimulate an increase in GTP-Rac by enhancing GEF activity in a PI3 kinase activation dependent manner (34). Furthermore, treatment of fibroblasts with PI3 kinase inhibitor wortmannin inhibits Rho and Rac mediated membrane ruffling induced by PDGF, epidermal growth factor (EGF), and insulin or insulin-like growth factor 1 (IGF-1) (32, 35, 36). These data suggest that PI3 kinase acts on the upstream of Rac to induce membrane ruffling when exposed to extracellular growth stimuli.

Another upstream signaling pathway of Rho family proteins is the tyrosine phosphorylation of the exchange factor Vav, which is crucial for its ability to activate members of the Rho family (21, 37). As described previously, Vav is guanine nucleotide exchange factor for Rho, Rac, and CDC42 family. When exposed to Lck, a member of the Src family, Vav increases the GDP/GTP exchange activity. Furthermore, coexpression of Lck with Vav enhances the Vav-transforming activity and its capability to induce Jun N-terminal kinase (JNK) activation. The mechanism by which LPA activates Rho appears also attributable to the involvement of tyrosin

Table 1. Roles of characterized Rho protein

Rho Protein	Function
RhoA	• Promotes the rearrangement of cell cytoskeleton • Initiates the assembly of focal adhesion • Induces the contraction of cell body • Stimulates the movement of the rear of the cell to induce forward migration of cell body and nucleus • Promotes the detachment of the rear of the cell from the ecm
Rac1	• Promotes actin-mediated formation of the lamellipodia • Stimulates the attachment of lamellipodia to ecm at the front of the cell body • Allows for new adhesion contacts to ecm
Cdc42	• Required for cell polarization • Promotes directionality of cell movement • Plays role in the rate of cell migration, by enhancing rac-mediated membrane extension • Causes the formation of filopodia

kinase because the LPA-induced stress fiber formation can be abolished by tyrphostin, a tyrosine kinase inhibitor (38, 39).

In summary, extracellular stimuli, such as LPA, PDGF, epidermal growth factor (EGF), and insulin, trigger Rho-family GTPases mediated assembly and organization of the actin cytoskeleton in a PI3 kinase dependent manner. Tyrosine phosphorylation is also involved in the activation of Rho. Further elucidation of upstream signaling molecules will provide a more thorough insight into the signaling pathways that lead to the activation of the Rho-like GTPases in response to extracellular stimuli.

4. BIOCHEMICAL AND BIOLOGICAL FUNCTIONS OF RHO GTPASES IN CELL MIGRATION

Eukaryotic cellular morphology and motility are underlain by the organization and reorganization of cytoskeleton. Cell migration and axonal path finding during development, immune cell patrolling in normal adults, phagocytosis, as well as pathological processes, such as metastasis, all rely on coordinated regulation of the actin network to produce directed cell movement. The actin cytoskeleton is part of the cytoskeleton (the internal framework of a cell) composed of actin filaments and many specialized actin-binding proteins (39, 40). Although actin filaments are created by the simple polymerization of actin monomers, regulation of the dynamics of the filament network requires participation of many interacting proteins and is regulated by numerous upstream signaling pathways. This regulation of actin polymerization, for the most part, is orchestrated by Rho GTPases.

Separate members of Rho GTPases proteins regulate the organization of the actin cytoskeleton in their own manners. Rho activation in fibroblasts results in the assembly of contractile actin/myosin filaments, the formation of stress fibers, and the clustering of integrins involved in the formation of focal adhesion complexes. Activation of Rac facilitates actin polymerization at the cell periphery to generate protrusive actin-rich lamellipodia and membrane ruffling. Activation of Cdc42 results in actin polymerization to form peripheral actin microspikes and filopodia. In addition, cellular signaling cross-talk between the members of the Rho GTPases proteins has been observed. For example, Cdc42 is a potent activator of Rac, leading to observations that filopodial extensions are usually accompanied with lamellipodial protrusions (30, 31, 33, 38) (Table 1).

4.1.1. Rho and cytoskeletion reorganization

A number of proteins have been identified as targets of Rho by the yeast two-hybrid selection system and affinity chromatography purification. These targets include Rho-kinase/ROK/ROCK, myosin-binding subunit (MBS) of myosin phosphatase, protein kinase N (PKN)/PRK1, p140mDia, rhophilin, rhotekin and citron and so on. Most of them are involved in Rho mediated cytoskeletal rearrangements (Figure 2).

The involvement of Rho in cytoskeleton reorganization is firstly implicated by the observations that the stress fibers and focal adhesion complexes were induced in fibroblasts with ectopic expression of an activated mutant of Rho, RhoV14 (30). The discoveries and characterization of numerous proteins that bind Rho in a GTP-dependent manner have shed light on the molecular mechanisms by which Rho affects the cytoskeleton (Figure 2). Among these proteins, serine/threonine kinase, ROK alpha (also known as Rho kinase) and its close relative (52% homology), p160Rho kinase (also known as ROCKII or ROKβ) have been extensively studied to date (41-45). The kinase activity of p160Rho kinase is enhanced (though not by much) after binding to Rho-GTP. Clues to their functions in the reorganization of actin filaments and in myosin contractility come from two lines of evidence. The expression of full-length ROKα and its amino-terminal portion induces stress fibers and focal adhesion complexes formation (41), which is abolished by the expression of a kinase-dead mutant of the protein (42). Furthermore, two substrates for the p160Rho kinase have been identified, the myosin-binding subunit (MBS) of myosin light-chain (MLC) phosphatase and myosin light chain itself (Figure 3). It was subsequently shown that phosphorylation of MBS downregulates MLC phosphatase activity, resulting in an accumulation of the phosphorylated form of MLC (46). Phosphorylation of MLC induces a conformational change in myosin, thereby increasing its binding to actin filaments to drive the formation of stress fibers and focal adhesions (47, 48). Together, phosphorylation of these two substrates would be expected to lead to an increase in myosin light chain phosphorylation, myosin filament assembly, F-actin bundling, and stress fiber formation.

Both Rho and Rac have been shown to stimulate the synthesis of phosphatidylinositol 4,5-bisphosphate (PIP2), the product of phosphatidylinositol 4-phosphate 5-kinase (PIP 5-kinase)(49, 50). The observation that PIP2 can

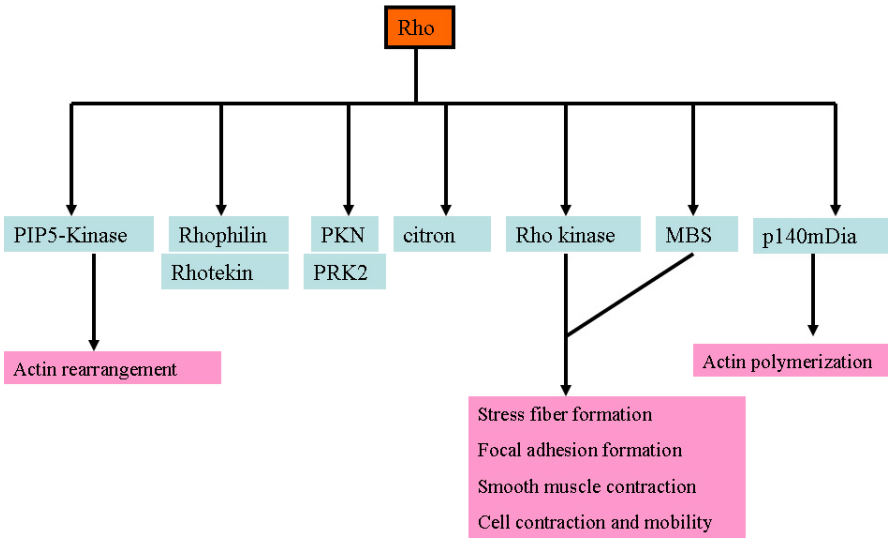

Figure 2. Mammalian targets of Rho. PIP, Phosphatidylinositol 4,5-bisphosphate; PKN, protein kinase N.

regulate the interactions of a number of actin-binding proteins, including profilin, α-actinin, gelsolin, and p39CapZ *in vitro* (51), led to the hypothesis that Rho-stimulated PIP2 synthesis may induce actin rearrangements. A case in point here is the protein vinculin. Vinculin is a membrane-cytoskeletal protein in focal adhesion plaques that is involved in linkage of integrin adhesion molecules to the actin cytoskeleton. More specifically, the amino-terminus of vinculin binds to talin which, in turn, binds to β-integrins, and the carboxy-terminus binds to actin, phospholipids and paxillin forming homodimers. The binding of vinculin to talin and actin is regulated by PIP2 and inhibited by acidic phospholipids (52). Furthermore, injection of anti-PIP2 antibodies into fibroblasts inhibits LPA-induced formation of stress fiber and focal adhesions, suggesting a crucial role for PIP2 in focal adhesion and stress fiber assembly (53).

Recently, a downstream effector of the Rho protein, p140mDia, was shown to selectively interact with mammalian Rho in a GTP-dependent manner and also interact with profilin (54). The NH2-terminal portion of p140mDia ensures that p140mDia binds selectively to the GTP-bound form of Rho. p140mDia also contains repetitive polyproline stretches that can bind profilin. Thus, p140mDia provides a direct molecular linkage between Rho and profilin. RhoA, p140mDia and profilin are colocalized in the spreading lamellae of cultured fibroblasts and also can be recruited around phagocytic cups induced by fibronectin (FN)-coated beads. Overexpression of p140mDia in COS cells enhances actin filament assembly, while colocalization of RhoA, p140mDia and profilin is abolished after Rho is inactivated by microinjection of botulinum C3 exoenzyme. Based on these findings, a model was proposed in which Rho regulates actin polymerization by targeting profiling to a specific site beneath the plasma membrane via p140mDia, which results in a locally increased profilin concentration (54, 55). The functions and subcellular localization of the three molecules suggest a role for p140mDia in mediating the effects of Rho on actin reorganization beneath the dynamic plasma membranes in mammalian cells.

In addition to Rho kinase, MBS, and p140mDia, other mammalian proteins have been identified as potential Rho targets (Figure 2). PKN is the first identified serine/threonine protein kinase that can bind to and be activated by a small GTPase Rho. It is a protein kinase that has a catalytic domain homologous to protein kinase C (PKC) family members and a unique regulatory region containing antiparallel coiled-coil (ACC) domains previously called the "CZ region" (charged amino acid and Leu-zipper-like sequence) or "HR1" (56). There are at least three different isoforms of PKN (PKN-α /PAK-1/PRK-1, PKN-β, and PRK2/PAK-2/ PKN-γ) in mammals, each of which shows different enzymological properties, tissue distributions, and varied functions. GTP-bound Rho interacts with the first leucine zipper-like motif in the NH2-terminal portion and activates the catalytic activity of PKN. The expression of a kinase-deficient form of PRK2 disrupts actin stress fibers, implicating PRK2 in actin cytoskeleton reorganization.

Whereas rophilin and Rhotekin share homology with both MBS and PKN/PRK1 in their Rho binding domain (57, 58), overall domain structure of citron is similar in sequence to Rho-kinase. However, citron lacks a kinase domain (59). Citron-kinase is localized at the cleavage furrow and mid-body during cytokinesis. Citron is implicated in cytokinesis (60). So far, however, there is still no well established evidence to clarify the role of Rho-mediated cytoskeletal rearrangements in cytokinesis.

4.1.2. RAC, CDC42 and cytoskeleton reorganization

In fibroblasts, Rac has been shown to be a crucial regulator in the reorganization of the actin cytoskeleton in growth factor-induced membrane ruffling (31). Later, a role for yet another member of the Rho subfamily, Cdc42, in

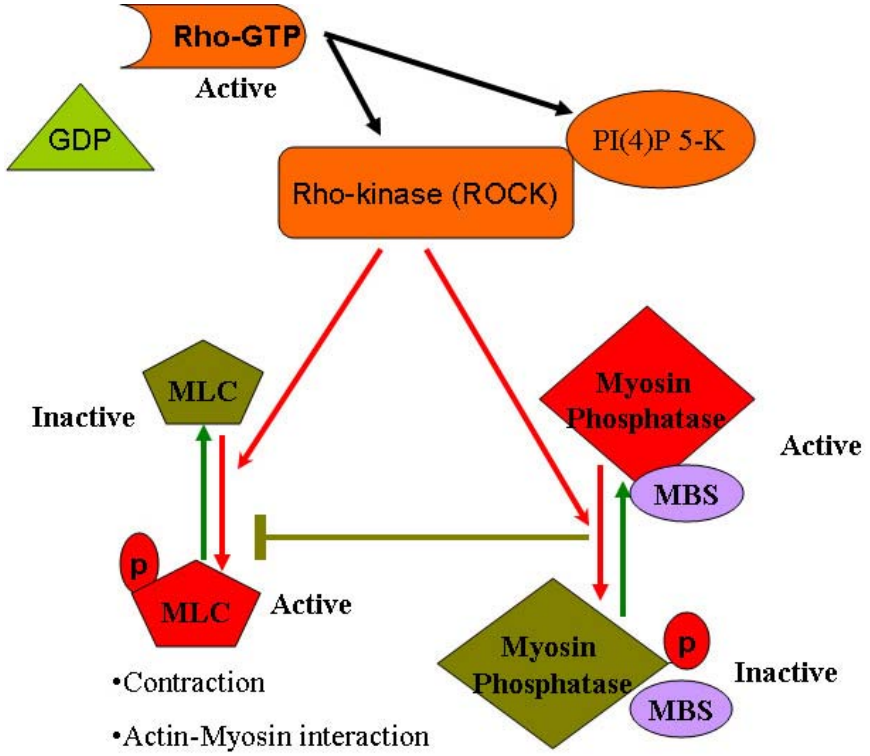

Figure 3. Model for regulation of MLC (myosin light chain) phosphorylation by Rho, Rho-kinase, and myosin phosphatase. cat, Catalytic subunit; MBS, myosinbinding subunit.

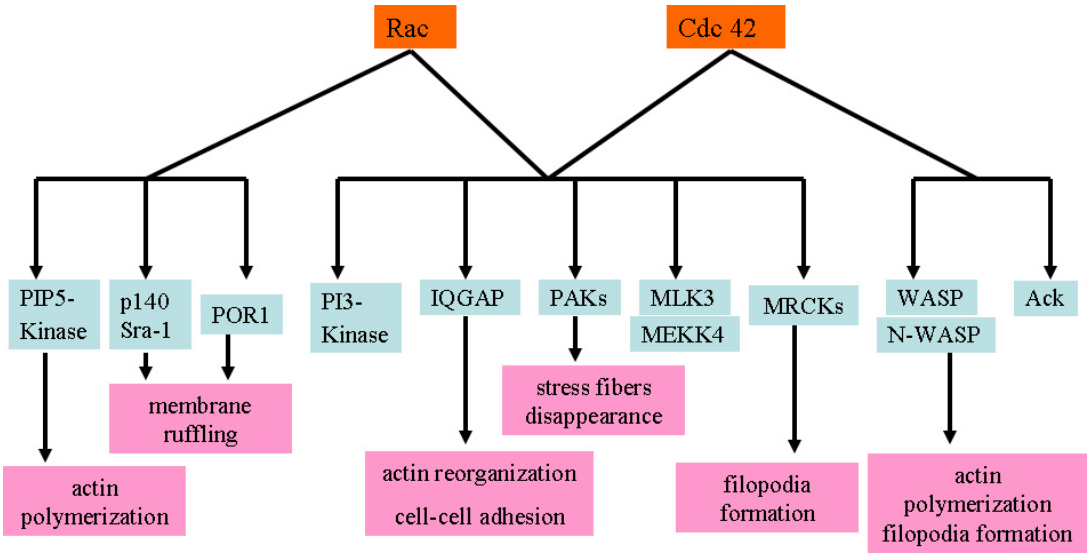

Figure 4. Mammalian targets of Rac and Cdc42. PIP, Phosphatidylinositol 4,5-bisphosphate; PI, phosphatidylinositol; PAK, p21-activated kinases.

actin remodeling was established. Both Rac and Cdc42 have also been shown to induce the assembly of multimolecular focal adhesion complexes (FACs) at the plasma membrane of fibroblasts (38). These focal complexes display morphologies like punctate spots of vinculin and phosphotyrosine around the leading edge of the lamellipodia and at the tips of filopodia, which are distinct from the Rho regulated focal adhesions, in spite of the similarity in their protein components. To date, several potential targets of Cdc42 and Rac have been identified, including p21-activated kinases (PAKs), WASP/N-WASP, IQGAP1, MRCK, Por1, p140Sra-1, and Posh (Figure 4),

which help to elucidate the molecular basis for the involvement of Rac and Cdc42 in cytoskeleton organization.

Although Rac and Cdc42 each have seven or eight known target proteins, some are common to both (Figure 4). For example, the first Rac target identified, p65PAK, also interacts with Cdc42. To date, at least three isoforms of p21-activated kinase (PAK1–3) have been identified (61-65), all of which bind Rac and Cdc42 in a GTP-dependent manner. PAKs comprise an NH2-terminal regulatory domain and a COOH-terminal catalytic domain (61). GTPase binding disrupts an inhibitory interaction between the PAK kinase domain and the PAK auto-inhibitory domain, which results in PAK phosphorylation and activation (66, 67). In addition, all PAK proteins identified to date share a conserved domain of approximately 20 residues that is responsible for interaction with Rac or Cdc42 and is referred to as CRIB (Cdc42/Rac interactive binding) site. Several other mammalian proteins of potential targets for Cdc42 and Rac, like MSE55, MLK2/3, and WASP, share this motif (68).

PAKs have been shown to be the key components of pathways that regulate cell morphology, including formation of lamellipodia and disassembly of stress fibers and focal adhesions in response to stimuli that activate Rac and Cdc42 and promote cell migration (69, 70). Overexpression of constitutively activated mutant PAK1 triggers dissolution of actin stress fiber and focal adhesions, accompanied by increased cell polarization, membrane protrusions and cell motility (71-73). PAKs are also upstream elements in the JNK and p38 kinase pathways that control gene expression (74). The N terminus of PAK1 contains multiple proline-rich sites that bind to Src homology (SH) 3 domain-containing proteins, including Pix, a GEF for Rac (75), adaptor proteins NCK (76) and Grb2 (77). The binding of PIX and NCK to PAK seems required for the localization of PAK to focal adhesions.

Despite the fact that Rac and Cdc42 trigger morphologically distinct lamellipodia and filopodia at the plasma membrane, the formation of both protrusions rely on the Arp2/3 complex to initiate peripheral actin polymerization. Arp2/3 complex is a heptameric, actin-nucleation machine associating with the sides and perhaps the ends of existing actin filaments to initiate new branched filaments formation (78). Both Rac and Cdc42 activate Arp2/3 indirectly through members of the Wiskott-Aldrich syndrome protein (WASP) family. N-WASP, or the closely related hemopoietic-specific WASP, binds to Cdc42 in a GTP-dependent manner. The binding generates an intra-molecular, auto-inhibitory interaction and exposes a C-terminal Arp2/3 binding/activation site, which contributes to Cdc42 mediated cytoskeletal rearrangements and actin clustering (79). Activation of Arp2/3 by Rac is mediated by WASP family Verprolin-homologous proteins (WAVE), which although structurally related to N-WASP, do not interact directly with the GTPase (80, 81).

Another protein with a potential role in cytoskeletal organization is IQGAP (82-84). The IQGAP1 gene was originally isolated as a member of the Ras GAP family (85). At least two isoforms of IQGAP have been identified, IQGAP1 and IQGAP2. Both isoforms directly interact with GTP-bound Cdc42 and Rac, but not with the GDP- bound forms. IQGAPs contain some interesting motifs found in signaling molecules, such as a WW domain, an SH3-binding domain, and a calmodulin-binding domain. However unlike PAKs and WASP, IQGAPs do not contain a CRIB site. IQGAP interacts with both Rac and Cdc42 and localizes to membrane ruffles. In addition, both IQGAP1 and IQGAP2 have been shown to interact directly with actin filaments. It is not known if they play any role in filopodia formation, but there are some data linking them to the assembly of actin filaments during cytokinesis (86). In addition to its role in actin filament organization, IQGAP1 appears to play a pivotal role in cell-cell adhesion through the cadherin-catenins pathway (87).

As described previously, cytoskeleton rearrangement is potentially linked to phospholipid metabolism. In fact, both Rac and Rho bind to PIP5 kinases and recruit them to the plasma membrane (88), while ADP-ribosylation factor 6 (Arf6) acts downstream of Rac to activate PIP5 kinases (89). It is likely that PIP5 kinase may be the responsible kinase mediating Rac-stimulated PIP2 generation. Although a number of potential targets or effectors of Rho family members have been identified and characterized, there is still not sufficient evidence for the physiological relevance of these proteins in Rho-mediated cytoskeletal remodeling, which is likely to be a focus of attention in the future.

4.1.3. Other functions of Rho family of gtpases

Rho GTPases have been reported to regulate various other cell functions, including membrane trafficking, transcriptional activation, cell growth control and development in mammals and lower eukaryotes (9, 10). Because this review focuses on the role of these GTPases proteins on cytoskeletons rearrangement and cell adhesions involved in cell migration, the above functions regulated by the Rho family GTPases are not described here.

4.2. Rho gtpases in cell migration

Cell migration can be mechanistically divided into separate steps: cell polarization, lamellipodium protrusion and adhesion formation, followed by cell body contraction, and tail detachment (8)(Figure 5).

The initiation of cell migration is polarization and generation of protrusions in the direction of migration. These protrusions can be large, broad lamellipodia or spike-like filopodia, which are usually driven by actin polymerization and stabilized by adhering to the ECM or adjacent cells via transmembrane receptors linked to the actin cytoskeleton. This in itself is not sufficient, however, because cell contractility is required to allow the body and rear of the cell to follow the extending front (90). All aspects of cellular motility and invasion, including cellular polarity, cytoskeletal organization, and transduction of signals from the outside environment involved in adhesion formation are controlled through the interplays between the Rho-GTPases. Herein, we will discuss the evidence linking

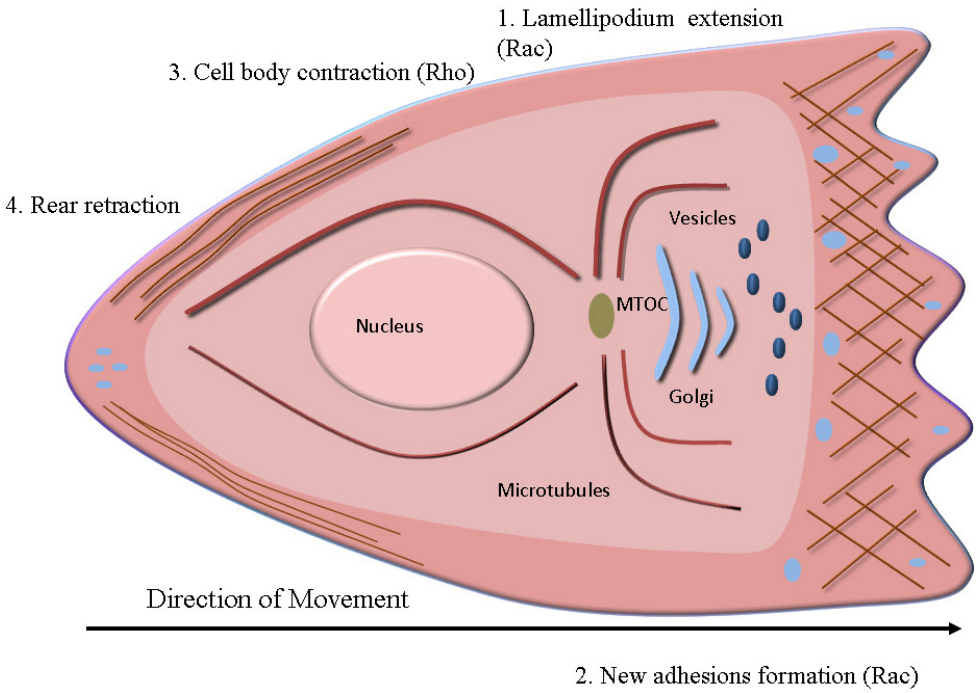

Figure 5. A model for cell migratory process. MTOC, microtubule-organizing center.

specific Rho proteins to each of these steps and the ways in which they contribute to cell movement.

4.2.1. Cell polarization: a keystone of cell migration

The ability of a cell to move depends on an asymmetrical organization of intracellular activities, which means the molecular processes at the front and the back of a moving cell must be different. Establishing and maintaining cell polarity in response to extracellular stimuli appear to be mediated by a set of interlinked positive feedback loops involving Rho family GTPases, PI3Ks, integrins, microtubules, and vesicular transport.

Among the Rho family GTPases involved in cell polarity regulation, Cdc42 is a master regulator in eukaryotic organisms ranging from yeast to humans. Genetic analysis of budding yeast provides the first piece of evidence for the involvement of Cdc42 in cell polarity. During the cell cycle, yeast cells adopt alternative states of polarized growth, ranging from tightly focused apical growth to non-focused isotropic growth. In the absence of Cdc42, *Saccharomyces cerevisiae* fail to establish focused apical growth and, as a consequence, cells expand isotropically (91). One way in which Cdc42 influences polarity is to direct where lamellipodia form (92). Cdc42 can also regulate cell polarity by directing the localization of the microtubule-organizing center (MTOC) and Golgi apparatus in front of the nucleus, oriented toward the cell migration direction. In this way, the direction of MTOC orientation at the front of leading edge may contribute to cell migration by facilitating delivery of Golgi derived vesicles to the leading edge and microtubule growth into the lamellipodium at the front (93). The effects of Cdc42 on MTOC position seem to be mainly exerted through its downstream effecters, such as kinase PAK1. PAK1 itself can mediate Cdc42 activation and the positive feedback loops between Cdc42 and PAK1 ensure the maintenance of high Cdc42 activity at the leading edge (94).

As targets of Rac, PIP3 and phosphatidylinositol(3,4) bisphosphate (PI(3,4)P2) are key signaling molecules that contribute to Cdc42 accumulation at the front of moving cells. This mediation involves both localized accumulation and activation of PI3Ks, which produce PIP3/PI(3,4)P2, and phosphatase PTEN, which removes them. To be more specific, PI3Ks rapidly accumulate at the leading edge of cells, whereas PTEN becomes restricted to the sides and the rear (95, 96). Altered PI3K or PTEN activity significantly compromises the ability of the cell to move directionally up a chemoattractant gradient. Together these results indicate that there exist positive feedback loops between Cdc42, PI3K products, and PTEN that coordinate together to ensure the initiation and maintenance of the polarity of migrating cells.

4.2.2. The protrusive machinery

Actin filaments are polarized with fast growing plus (+) or barbed ends and slow growing minus (-) or pointed ends. This inherent polarity is used to drive membrane protrusion. However, the organization of filaments depends on the types of protrusion: as for lamellipodia, actin filaments form a branching dendritic network, whereas as for filopodia they are organized into long parallel bundles (97). The distinct molecular attributes of lamellipodia and filopodia account for their capacity to perform distinct

functions. Biophysically, the dendritic organization of lamellipodia provides a tight brush-like structure that is able to push along a broad range of plasma membrane (98). Through localization of activated Arp2/3 complex the lamellipodium can grow in a particular direction. In contrast, filopodia are particularly well designed to serve as sensors to explore the local environment with parallel bundles.

Rac is required for lamellipodium extension induced by extracellular stimuli, such as growth factors, cytokines and extracellular matrix components (99, 100). Reports about cells derived from Rac1- and Rac2-null mice support a pivotal role for Rac in cell migration. Effects of constitutively active Rac1 on cell migration vary in terms of cell types, stimuli, expression levels and time courses of expression. For example, constitutively active Rac1 inhibits macrophage migration induced by growth factors, because the lamellipodia extend all around the cells and fail to polarize.

How does activated Rac coordinate lamellipodium extension? Several Rac target proteins are likely to be involved in this process. As shown in Figure 4, first, Rac stimulates new actin polymerization relying on the Arp2/3 complex, which binds to pre-existing filaments. Activation of Arp2/3 complex by Rac is carried out by its target IRSp53 (101). After activation by Rac, IRSp53 interacts through its SH3 domain with WAVE, which finally binds to and activates the Arp2/3 complex. IRSp53 can also bind to Cdc42 with a separate domain (102). Therefore, IRSp53 can serve as a direct link between Cdc42 and Rac, which sheds light on the observation that Cdc42 can induce Rac-involved lamellipodium formation. IRSp53 can also bind to a Rho target, Dia1 (103), which might underlie the capability of Rho to facilitate lamellipodium extension.

In addition to activating the Arp2/3 complex, Rac can promote the uncapping of actin filaments at the plasma membrane to facilitate actin polymerization. In platelets, Rac acts via a PIP5-kinase to generate PIP2, which then binds to capping proteins and removes them from the barbed ends of actin filaments for actin polymerization (104, 105).

Apart from facilitating actin polymerization, Rac has been reported to downregulate the rate of actin depolymerization. Through its downstream target PAKs, Rac is capable of stimulating the activity of LIM-kinase (106, 107). As well as inducing actin polymerization, Rac may affect the rate of actin depolymerization. LIM-kinase can catalyze the phosphorylation and inactivation of cofilin, an actin-regulatory protein that will promote F-actin depolymerization. This suggests that Rac would inhibit cofilin-induced depolymerization.

4.2.3. Formation and turnover of new cell-substrate adhesions

For migration to occur, a protrusion must form and attach to surrounding substrates ECM. Small focal complex structures can be observed at the leading edge and are localized in the lamellipodia of most migrating cells. Early studies reported that cells plated on ECM proteins rapidly developed extended filopodia and lamellipodia, indicative of Cdc42 and Rac activity, respectively (108). These protrusive structures contain focal complexes, which are localized clusters of integrins, and cytoskeletal and signaling proteins that are smaller than, but similar in composition to focal adhesions. These adhesions stabilize the lamellipodium by attaching to ECM (8). As cells spread, larger integrin-based focal adhesions and actin stress fibers form, indicating Rho activity. In fibroblasts, PAK is rapidly activated by cell adhesion to fibronectin. Since PAK is one of the main effectors of Rac, it is strongly suggested that integrins could trigger biochemical activation of Rac or Cdc42, or both.

Adhesion assembly in migrating cells begins with small-scale clustering, dependent on the multivalent nature of ECM to which the cell is adhering. The speed of cell migration is dependent on ECM composition, which is decisive for the relative activation levels of Rho, Rac and Cdc42 (109, 110). Therefore, the continuous formation of new interactions between ECM and integrins at the leading edge of cells help to maintain the level of active Rac there, which means there exists a positive feedback loop to allow cells to carry on migration(99, 111). In this way, the continuous crosstalk between integrins and Rac allows cells to respond to changing ECM composition.

The formation of focal complexes is an integral step for lamellipodia attachment to the ECM of most migrating cells. However, cells with a high level of integrin-mediated adhesion are typically either nonmigratory or move very slowly due to the intense strength of attachment to ECM, which is correlated with high levels of Rho activity (112). In addition to being involved in focal complex formation in lamellipodia, Rac can induce focal complex turnover directly through PAK (113). PAK interacts with a complex of exchange factors, such as paxillin, to localize to focal adhesion complexes. Paxillin binds to many proteins, such as vinculin and actopaxin, which are involved in inducing changes in the organization of the actin cytoskeleton during cell migration.

4.2.4. Cell body contraction

Cell body contraction is dependent on actomyosin contractility and the force transmitted to sites of adhesion derives from the interaction of myosin II with actin filaments that attach to these sites. Myosin II activity is regulated by myosin light-chain (MLC) phosphorylation. The phosphorylation of MLS results in an increased contractility and transmission of tension to sites of adhesion, which is either positively regulated by ROCKs or negatively regulated by MLC phosphatase, which in turn is phosphorylated and inhibited by ROCK. MLC phosphorylation is also regulated by MLC kinase (MLCK), which is regulated by intracellular calcium concentration as well as stimulated by the ERK MAPKs (Figure 3).

In addition to mediating MLC phosphorylation, Rho exerts other effects on the actin cytoskeleton organization that are related to cell migration (Figure 2). Cofilin is an

actin binding protein that is essential for the depolymerization of actin filaments. Either through ROCK or LIMK, Rho abolishes the actin-binding activity of cofilin by inducing cofilin phosphorylation, thus enhancing the polymerization of actin filaments (114). As described previously, another way for Rho to induce F-actin accumulation is via PIP 5-kinases. Rho enlists Dia proteins for the actin cytoskeleton organization. Through interacting with either Src kinases or IRSp53(103), Dia proteins induce actin polymerization and stress fibers formation (115).

4.2.5. Adhesion disassembly and retraction at the rear

At the rear of migrating cells, adhesions must disassemble to ensure the fulfillment of cell movement. In most cases, tail detachment is the rate-limiting step during cell migration (116). The mechanisms regulating tail detachment depend on the cell types and their corresponding adhesion strength to the extracellular matrix. In slowly moving cells, tail detachment depends on calpain, which is a member of calcium-dependent, non-lysosomal cysteine proteases family. Calpain has been implicated as a critical calcium-dependent regulator of the actin cytoskeleton and cell migration by degrading focal adhesion components at the rear of cells. Inhibition of calpain reduces cell migration rates and invasiveness (116, 117). On the other hand, in cells, such as fibroblasts that have relatively large focal adhesion complexes, Rho assumes a role to reduce adhesion and promote tail detachment. In fibroblasts, the tight rearmost adhesions often tether the cell strongly to ECM. Strong tension forces exerted across the cells on the rear adhesions is required to break the link between integrin and the actin cytoskeleton physically. Several lines of evidence support a role for myosin II and Rho in this event. *Dictyostelium* cells deficient in myosin II or its regulator PAK alpha show impaired retraction (118) and similar phenotypes can be observed in monocytes or neutrophils by blocking myosin II assembly with Rho or Rho kinase inhibitors (119, 120). FAK, Src, and the other regulators that can induce focal adhesion disassembly also contribute to tail detachment (121).

5. ALTERED RHO GTPASE SIGNALING PATHWAYS IN BREAST CANCER CELLS

Metastases, but not the primary tumor, are the cause of death in 90% of cancer patients because of the difficulties in detecting and eradicating numerous small secondary tumors disseminated throughout the body, particularly those with breast cancer. Metastases from the breast commonly occur in the sentinal and axillary lymph nodes, lungs, spinal cord, brain and bones (122). To successfully metastasize tumor cells must perform an ordered series of steps that constitute the metastatic cascade. This includes invading the tissue surrounding the primary tumor, intravasating into the lymphatic or the blood supply system, avoiding host immune defenses and surviving in circulation, extravasating and arresting at the distant site for final macrometastasis formation. Two key components in obtaining metastatic competence are the acquisition of a motile and invasive phenotype, both of which are mainly controlled by members of the Ras-superfamily of small GTP-binding proteins. In following part of this review, we will discuss evidence for altered Rho GTPase signaling events in breast cancer and their role in tumor development, metastasis, and invasiveness.

5.1. Overexpression of Rho proteins in human breast tumors

Approximately 30% of human tumors carry an identifiable specific Ras mutation, accounting for its protein level overexpression or constitutive activation (122), while only approximately 5% of breast tumors harbor an activating Ras (123). In contrast to Ras, no mutation in any of the Rho GTPases has been identified in breast or other human cancers. Rather, these GTPases, particularly RhoA and RhoC, are often either overexpressed or hyperactive in breast cancer tissue.

In vitro, overexpression of RhoA results in transformation of mouse fibroblasts (124), supporting the notion that increased Rho A is a crucial event for malignant transformation and development of human tumor cells. Comparing the expression of proteins of the Rho family (RhoA, Rac, Cdc42) in tumorigenic tissue and the corresponding normal tissue obtained from the same patient, it was found that Rho GTPases, in particular RhoA, are overexpressed in different types of human tumors, including colon, breast and lung tumor (2). The higher levels of RhoA protein are correlated with more advanced grades of breast carcinoma (2).

Increased RhoC expression was found in inflammatory breast cancer (IBC) (2) and in invasive ductal carcinomas (32%) (4), particularly those with distant metastasis. Inflammatory breast cancer is a phenotypically distinct form of locally breast cancer due to its high metastatic capability. In an attempt to identify specific genes responsible for the unique IBC phenotype, Merajver's laboratory identified RhoC GTPase as being overexpressed in the SUM149 IBC cell line. Further studies indicate that RhoC seems to be a marker of metastatic potential in breast cancer, since 47% of the invasive ductal carcinomas that develop metastases express RhoC, in contrast to 12% of the invasive carcinomas without metastases (4). Subsequently, RhoC overexpression has been found to be a potential prognostic marker for tumors with the ability and propensity to metastasize. These results are in concordance with previous data showing that forced expression of RhoC GTPase induces the malignant transformation of immortalized human mammary epithelial (HME) cells by generating an aggressive, highly motile, and invasive phenotype, while a dominant-negative Rho inhibits metastasis (125).

In addition to Rho, increased protein levels of Cdc42 (2, 3) and Rac (3) also occur in breast cancer. The reports that Rac1 is required for Ras-induced malignant transformation implicate the involvement of Rac1 in breast cancer (126). Increased Rac1 mRNA expression and elevated Rac1 GTPase protein were seen in malignant versus benign breast tissues, suggesting that constitutive activation of Rac1 signaling may be present in more

aggressive breast cancers (127). Endogenous, hyperactive Rac3 is present in highly proliferative human breast cancer cell lines such as MCF-7, T47D, and MDA-MB-435, and is associated with persistent kinase activity of two isoforms of the Rac effectors PAK and of c-Jun N-terminal kinase (JNK) (128). The level of interactions between Rho GTPase and downstream effector proteins is determined by the absolute levels of the GTP-bound GTPases. By p21 binding domain (PBD) affinity assay, Mira *et al.*, found that consistently active Rac3 GTPases lead to hyperactivity of its effector protein kinase PAK in human breast cancer-derived epithelial cell lines (129).

Collectively, these findings suggest that Rho proteins are overexpressed in breast cancer. In addition, it appears that the level of these proteins correlates directly to the advancement of breast cancer. If a breast cancer is highly metastatic, Rho protein expression is likely to be high.

5.2. Role of Rho GTPases regulatory proteins in breast metastasis

Dominant inhibitory approaches have demonstrated a role of Rho GTPases in focal contact formation, motility, primary tumor growth, and macro-metastasis. Each dominant inhibitory Rho protein shows a decrease in intravasation and metastasis, suggesting that Rho, Cdc42, and Rac1 play a role in the formation of breast metastasis (130). As mentioned previously, no mutations have been observed in Rho proteins in tumors. However, overexpression of the Rho GTPases has been seen in human tumors. The examination of Rho regulatory proteins may provide an explanation for these observations. For example, mutation or oncogene expression that disrupts the Rho regulatory proteins can cause dysfunctional regulation of Rho proteins in breast cancer cells, leading to cellular motility, invasion and metastasis (131).

5.2.1. Rho GDP dissociation inhibitor (RhoGDI)

As previously mentioned, RhoGDI inhibits Rho proteins and consequently cellular motility. GDI inhibits Rho proteins by binding to the protein's prenylation group, preventing the localization of the GTPase to the inner plasma membrane via inhibiting the release of GDP. The introduction of RhoGDI to immortal fibroblasts has been shown to disrupt motility. However, the overexpression of RhoGDI in human keratinocytes leads to inhibition of cellular motility and the disturbance of the actin cytoskeleton (131). In addition, the overexpression of RhoGDI-α explicitly increases ERα and ERβ activation (132).

5.2.2. Rho GTPase activating protein (RhoGAPS)

Rho GTPase activating proteins (RhoGAPs) catalyze the hydrolysis of GTP to GDP, inactivating Rho proteins. p190RhoGAP is a well studied GAP protein that exists as two isoforms: p190-B and p190-A, a tumor suppressor gene. Previous results reveal that p190 inhibition leads to cytoskeleton reorganization. Introduction of p190 middle domain (MD) into motile cells disturbs heterodimer formation leading to inhibition of cell motility and migration, while the reintroduction of p120 into cells re-established directional cellular motility. The treatment of fibroblasts with sodium fluoride and LPA caused the inhibition of GAP activity while triggering GEF activity and amplifying Rho protein activation. Taken together, it appears that p190RhoGAP obstructs constitutive Rho activity (133).

p190RhoGAP and p120RasGAP have also been implicated in the development of inflammatory breast cancer. Amplification of EGF-R prompts the tyrosine phosphorylation of p190RhoGAP and p120RasGAP. This causes the association of p190 and p120, leading to the formation of a heterodimer which hinders the inactivation of Rho proteins, allowing for extended GTPase activation that leads to cellular invasion. Overexpression of both p190 and p120 has been seen in HME and SUM149 inflammatory breast cancer cells, consistent with aggressive mouse mammary tumors (131).

5.2.3. Rho guanine exchange factors (RhoGEFS)

Rho guanine exchange factors (RhoGEFs) activates Rho proteins by exchanging bound GDP for GTP, upon its phosphorylation. Once activated Rho proteins are able to activate downstream effectors that can impact the cellular motility. Activating mutations of RhoGEF can induce the constitutive activation of Rho proteins, suggesting a link between RhoGEF and the abnormal GTPase activity found in breast cancer. Proto-oncogene Dbl is the first identified RhoGEF. Mutations within Dbl can cause the constitutive activation of cellular transformation, possibly contributing to the metastatic phenotype (131).

5.3. Rho proteins in proliferation and progression of breast cancer cells

In addition to mediating breast cancer metastasis, Rho proteins can also promote breast cancer cell proliferation to facilitate their colonization. Several lines of evidence suggest an important role for Rho proteins in normal and cancerous cell growth, including G1 phase progression, mitogenesis, and Ras transformation (10, 134).

The transforming potential and cell growth stimulating activity of three GTPases correlate with elevated cyclin D1 transcription due to its promoter activation. The neu (c-erbB-2, HER-2) proto-oncogene encodes a receptor tyrosine kinase that is overexpressed in 20 to 30% of human breast tumors (135). ErbB-2 induced transformation of breast cells requires cyclin D1, which is induced through an E2F-dependent signaling pathway (136). In fact, Cyclin D1 is overexpressed in more than 50% of breast cancers. The mechanisms by which Rho GTPases induce cyclin D1 expression have been supported by several lines of evidence. Typically, cyclin D1 abundance is induced transcriptionally. Upon the withdrawal of growth factors, cyclin D1 undergoes rapid degradation via the ubiquitin-proteasome pathway (137). By a similar approach, the transcription regulation of cyclin D1 by Rac1 occurs via the transcription factors NF-κB and ATF-2, which directly bind to multiple sites in the cyclin D1 promoter for its activation (138, 139). Thus, activating mutants of Rac1 (RacLeu-61, RacVal-12) induced NF-κB

abundance and DNA-binding to the cyclin D1 NF-κB promoter, and expression of an NF-κB trans-dominant inhibitors inhibited Rac induction of cyclin D1. In addition, RhoA overexpression can also inhibit p21 (CIP1) expression (140), suggesting multiple cell cycle controllers are interfered with by Rho proteins.

5.4. Potential roles of rho gtpase effectors in breast cancer

Rho GTPases stimulate different downstream signaling pathways through a spectrum of effectors. The serine/threonine kinase PAKs, for example, are important downstream effectors of both Cdc42 and Rac, and have been shown to modulate dynamics of the actin and microtubule cytoskeletons to regulate cell migration. In some breast cancers, the protein levels and kinase activity of PAK1 are elevated (141). Increased PAK kinase activity and expression are correlated with enhanced motility and invasiveness of human breast cancer cell lines(142). Ectopic expression of constitutively activated PAK1 in non-metastatic MCF-7 breast carcinoma cells increases their motility (142). In addition to increased motility and invasiveness of breast cancer cells, enhanced PAK levels and PAK kinase activity can be also linked to a series of functions like DNA synthesis (129), anchorage-independent growth, and abnormal mitotic spindles (142). PAKs have been shown to physically interact with and directly phosphorylate Raf kinase, which binds to retinoblastoma protein (143), and interact with cyclin dependent kinases to upregulate cyclin D1 expression (144). Targeted overexpression of PAK1 in mice leads to mammary gland hyperplasia (145), while inhibiting PAK kinase activity in multiple human breast carcinoma cell lines decreases cell proliferation (129). Finally, it seems that PAK contributes greatly to the completion of the last stage of tumor progression, the formation of macrometastases. Once the tumor cells have reached a proper environment during metastatic cascades, the formation of large focal contacts could firmly attach the cell to the tissues and result in constitutive hyperactivation of PAK. Upon activation, PAK has been demonstrated to promote angiogenesis, resistance to apoptosis response, and cell proliferation and thereby promote tumor cell growth (146).

As a downstream effector of Rho, ROCK disrupts cell–cell contacts by blocking the formation of adherens junctions (AJs) to increase invasiveness of tumor cells, indicating that activation of the Rho–ROCK pathway may be an essential step during the transcellular migration of tumor cells (147). Cortactin, another downstream effector of Rho GTPases, is overexpressed in breast cancer and usually correlated with poor prognosis, presumably because of enhanced metastasis. Cortactin binds directly to the Arp2/3 complex and activates it to promote nucleation of actin filaments, an integral event in Rho GTPases regulated actin rearrangement (148). Thus, multiple Rho GTPase effectors have been implicated in the breast cancer phenotype.

5.5. Rho GTPase pathways as anti-cancer targets

Since Rho GTPases play a pivotal role in the development and progression of breast cancer, they have become targets for several anti-cancer treatments. So far, several drugs have been shown to abrogate or diminish Rho GTPases triggered signaling events. These drugs either target Rho GTPases themselves or directly inhibit their downstream effecters.

5.5.1. Farnesyltransferase inhibitors (FTIs) and statins

The activation of Rho proteins relies heavily on the post-translational modification of GTPase at the CAAX domain of the carboxyl terminus of the protein. C20 isoprenoid lipid moiety is attached to the C-terminus allowing for the localization of GTPase to the inner plasma membrane where it becomes activated by GAPs (149). Geranylgeranyl transferase I (GGTase I) and Farnesyltransferase (FTase) are responsible for catalyzing this modification. If the localization of Rho GTPases is prevented, then Rho activity could successfully be inhibited (150). This detail has made it an attractive target for several anti-cancer treatments.

Originally designed to prevent Ras attachment to inner cell membrane by abrogating Ras isoprenylation, farnesyltransferase inhibitors (FTIs) can exert similar effects on Rho protein modification. Treatment of tumor cells with a FTI leads to a decrease in farnesylated RhoB levels and a corresponding increase in geranylgeranylated RhoB levels, which promotes apoptosis as well as inhibits cell cycle transit selectively in malignant epithelial cells. FTI treatment of RhoC overexpressing HME or SUM149 IBC cells results in a marked reduction in their motility and invasiveness (151).

Inhibition of Rho GTPases can also be achieved by hindering the activity of hydroxymethylglutaryl coenzyme A reductase (HMG-CoA), a protein required for the cholesterol biosynthesis of farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) (152). Statins are effective 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Treatment of MDA-MB-231 breast cancer cells with cerivastatin obstructs the invasive phenotype of tumor cells, thus stimulating the disassembly of the actin cytoskeleton and focal adhesions (153). Cerivastatin was also shown to cause the delocalization of RhoA and decrease the activity of proteases associated with cell migration, further explaining the loss in cell motility and invasion observed in aggressive breast cancer cells (153). In MDA-MB-231 cells, an aggressive breast cancer cell line, cerivastatin induces inhibition of both cell proliferation and invasion by p21Waf1/Cip1-induced G1/S arrest (153). Atorvastatin also inhibits Rho geranylgeranylation; it represses Rho activation and reverses the metastatic phenotype of human melanoma cells *in vitro*. In addition, it inhibits *in vivo* metastasis, but not proliferation of melanoma cells that overexpress RhoC (154).

5.5.2. Strongylophorine-26

Strongylophorine-26 is an alternative anti-metastatic drug that also targets Rho activity (155). The anti-cancer treatment inhibits the invasive phenotype of breast tumor cells. Treatment of MDA-MB-231 breast cancer cells with Strongylophorine-26 inhibits tumor cell motility, suggesting that Strongylophorine-26 targets aspects of Rho-mediated motility. This treatment causes a

decrease in stress fiber formation, while causing an increase in the formation of focal adhesions and actin filaments that stabilize the cellular body. Strongylophorine-26 also induces the transient activation of Rho GTPases, possibly re-establishing more regulated Rho activity (155).

5.5.3. RHO siRNA

Another innovative approach to treatment of aggressive breast cancer is the use of siRNA against RhoA and RhoC. Previous studies demonstrate that the overexpression of RhoA contributes to the proliferative and invasive properties of human breast cancer (3, 156), suggesting that if RhoA synthesis is inhibited, breast metastasis can be prevented. In this approach, siRNA of anti-RhoA and anti-RhoC is chemically synthesized and used to obstruct the synthesis of the respective Rho GTPases (152). Pillé and colleagues transfected the siRNA of Rho into MDA-MB-231 breast cancer cells and demonstrated the inhibition of cell invasion and proliferation *in vitro* and the abolishment of tumor cell growth and angiogenesis *in vivo* (152). Therefore, siRNA may be employed in the future to inhibit the synthesis and activation of respective Rho GTPases.

5.5.4 Y-27632

Other promising approaches involve selective inhibition of certain downstream effecters of Rho GTPases' signaling pathways. A specific ROCK inhibitor, Y-27632, capable of blocking both Rho-mediated activation of actomyosin and invasive activity, can abolish Rho-mediated activation of actomyosin and invasive activity of MM1 hepatoma cells. The observation that the treatment of rats with Y-27632 for 11 days substantially inhibits tumor cell dissemination without apparent adverse side effects suggests Y-27632 as a valuable drug for breast tumor cell treatment (157).

5. CONCLUSION

It is apparent that Rho GTPases play a pivotal role in cell motility and invasion. The aberrant activity of Rho proteins in cancer can now be explained by the identification of several factors that influence Rho activity. Rho GTPases are found to be overexpressed in metastatic breast cancer cells. Manipulation of Rho GTPases' regulatory proteins and their effectors can also cause abnormal Rho activation. Abnormal Rho activation leads to the aberrant activity of transcription factors like NF-κB that can enhance the invasive phenotype of these tumor cells. Although several studies on Rho GTPases have provided a plethora of information, more studies are needed to examine the mechanisms that are driving Rho overexpression in breast cancer.

7. REFERENCES

1. R Cotran, V Kumar, T Collins & S Robbins: Robbins Pathologic Basis of Disease. W.B. Saunders Company, PA (1999)
2. Fritz, G., Just, I., and Kaina, B: Rho GTPases are over-expressed in human tumors. *Int J Cancer*, 81, 682-687 (1999)
3. Fritz, G., Brachetti, C., Bahlmann, F., Schmidt, M., and Kaina, B: Rho GTPases in human breast tumours: expression and mutation analyses and correlation with clinical parameters. *Br J Cancer*, 87, 635-644 (2002)
4. Kleer, C. G., van Golen, K. L., Zhang, Y., Wu, Z. F., Rubin, M. A., and Merajver, S. D: Characterization of RhoC expression in benign and malignant breast disease: a potential new marker for small breast carcinomas with metastatic ability. *Am J Pathol*, 160, 579-584 (2002)
5. Fidler, I. J: Critical factors in the biology of human cancer metastasis: twenty-eighth G.H.A. *Clowes memorial award lecture. Cancer Res*, 50, 6130-6138 (1990)
6. Liotta, L. A., Wewer, U., Rao, N. C., Schiffmann, E., Stracke, M., Guirguis, R., Thorgeirsson, U., Muschel, R., and Sobel, M: Biochemical mechanisms of tumor invasion and metastasis. *Anticancer Drug Des*, *2*, 195-202 (1987)
7. Stracke, M. L., Aznavoorian, S. A., Beckner, M. E., Liotta, L. A., and Schiffmann, E: Cell motility, a principal requirement for metastasis. *Exs*, 59, 147-162 (1991)
8. Lauffenburger, D. A. and Horwitz, A. F: Cell migration: a physically integrated molecular process. *Cell*, 84, 359-369 (1996)
9. Hall, A: Rho GTPases and the actin cytoskeleton. *Science*, 279, 509-514 (1998)
10. Van Aelst, L. and D'Souza-Schorey, C: Rho GTPases and signaling networks. *Genes Dev*, 11, 2295-2322 (1997)
11. Peck, J., Douglas, G. t., Wu, C. H., and Burbelo, P. D: Human RhoGAP domain-containing proteins: structure, function and evolutionary relationships. *FEBS Lett*, 528, 27-34 (2002)
12. van Golen, K. L., Davies, S., Wu, Z. F., Wang, Y., Bucana, C. D., Root, H., Chandrasekharappa, S., Strawderman, M., Ethier, S. P., and Merajver, S. D: A novel putative low-affinity insulin-like growth factor-binding protein, LIBC (lost in inflammatory breast cancer), and RhoC GTPase correlate with the inflammatory breast cancer phenotype. *Clin Cancer Res*, 5, 2511-2519 (1999)
13. Madaule, P. and Axel, R: A novel ras-related gene family. *Cell*, 41, 31-40 (1985)
14. Winge, P., Brembu, T., Kristensen, R., and Bones, A. M: Genetic structure and evolution of RAC-GTPases in Arabidopsis thaliana. *Genetics*, 156, 1959-1971 (2000)
15. Olofsson, B: Rho guanine dissociation inhibitors: pivotal molecules in cellular signalling. *Cell Signal*, 11, 545-554 (1999)
16. Seabra, M. C: Membrane association and targeting of prenylated Ras-like GTPases. *Cell Signal*, 10, 167-172 (1998)
17. Cerione, R. A. and Zheng, Y: The Dbl family of oncogenes. *Curr Opin Cell Biol*, 8, 216-222 (1996)
18. Eva, A. and Aaronson, S. A: Isolation of a new human oncogene from a diffuse B-cell lymphoma. *Nature*, 316, 273-275 (1985)
19. Katzav, S., Martin-Zanca, D., and Barbacid, M: vav, a novel human oncogene derived from a locus ubiquitously expressed in hematopoietic cells. *Embo J*, 8, 2283-2290 (1989)
20. Collins, T. L., Deckert, M., and Altman, A: Views on Vav. *Immunol Today*, 18, 221-225 (1997)
21. Crespo, P., Schuebel, K. E., Ostrom, A. A., Gutkind, J. S., and Bustelo, X. R: Phosphotyrosine-dependent

activation of Rac-1 GDP/GTP exchange by the vav proto-oncogene product. *Nature*, 385, 169-172 (1997)
22. Hall, A: ras and GAP--who's controlling whom? *Cell*, 61, 921-923 (1990)
23. Lancaster, C. A., Taylor-Harris, P. M., Self, A. J., Brill, S., van Erp, H. E., and Hall, A: Characterization of rhoGAP. A GTPase-activating protein for rho-related small GTPases. *J Biol Chem*, 269, 1137-1142 (1994)
24. Kozma, R., Ahmed, S., Best, A., and Lim, L: The GTPase-activating protein n-chimaerin cooperates with Rac1 and Cdc42Hs to induce the formation of lamellipodia and filopodia. *Mol Cell Biol*, 16, 5069-5080 (1996)
25. Fukumoto, Y., Kaibuchi, K., Hori, Y., Fujioka, H., Araki, S., Ueda, T., Kikuchi, A., and Takai, Y: Molecular cloning and characterization of a novel type of regulatory protein (GDI) for the rho proteins, ras p21-like small GTP-binding proteins. *Oncogene*, 5, 1321-1328 (1990)
26. Abo, A., Pick, E., Hall, A., Totty, N., Teahan, C. G., and Segal, A. W: Activation of the NADPH oxidase involves the small GTP-binding protein p21rac1. *Nature*, 353, 668-670 (1991)
27. Hart, M. J., Maru, Y., Leonard, D., Witte, O. N., Evans, T., and Cerione, R. A: A GDP dissociation inhibitor that serves as a GTPase inhibitor for the Ras-like protein CDC42Hs. *Science*, 258, 812-815 (1992)
28. Chuang, T. H., Xu, X., Knaus, U. G., Hart, M. J., and Bokoch, G. M: GDP dissociation inhibitor prevents intrinsic and GTPase activating protein-stimulated GTP hydrolysis by the Rac GTP-binding protein. *J Biol Chem*, 268, 775-778 (1993)
29. Takai, Y., Sasaki, T., Tanaka, K., and Nakanishi, H: Rho as a regulator of the cytoskeleton. *Trends Biochem Sci*, 20, 227-231 (1995)
30. Ridley, A. J. and Hall, A: The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell*, 70, 389-399 (1992)
31. Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D., and Hall, A: The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cel*l, 70, 401-410 (1992)
32. Nobes, C. D., Hawkins, P., Stephens, L., and Hall, A: Activation of the small GTP-binding proteins rho and rac by growth factor receptors. *J Cell Sci*, 108 (Pt 1), 225-233 (1995)
33. Kozma, R., Ahmed, S., Best, A., and Lim, L: The Ras-related protein Cdc42Hs and bradykinin promote formation of peripheral actin microspikes and filopodia in Swiss 3T3 fibroblasts. *Mol Cell Biol*, 15, 1942-1952 (1995)
34. Hawkins, P. T., Eguinoa, A., Qiu, R. G., Stokoe, D., Cooke, F. T., Walters, R., Wennstrom, S., Claesson-Welsh, L., Evans, T., Symons, M., and *et al*: PDGF stimulates an increase in GTP-Rac via activation of phosphoinositide 3-kinase. *Curr Biol*, 5, 393-403 (1995).
35. Kotani, K., Yonezawa, K., Hara, K., Ueda, H., Kitamura, Y., Sakaue, H., Ando, A., Chavanieu, A., Calas, B., Grigorescu, F., and *et al*: Involvement of phosphoinositide 3-kinase in insulin- or IGF-1-induced membrane ruffling. *Embo J*, 13, 2313-2321 (1994)
36. Wennstrom, S., Hawkins, P., Cooke, F., Hara, K., Yonezawa, K., Kasuga, M., Jackson, T., Claesson-Welsh, L., and Stephens, L: Activation of phosphoinositide 3-kinase is required for PDGF-stimulated membrane ruffling. *Curr Biol*, 4, 385-393 (1994)
37. Han, J., Das, B., Wei, W., Van Aelst, L., Mosteller, R. D., Khosravi-Far, R., Westwick, J. K., Der, C. J., and Broek, D: Lck regulates Vav activation of members of the Rho family of GTPases. *Mol Cell Biol*, 17, 1346-1353 (1997)
38. Nobes, C. D. and Hall: A. Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell*, 81, 53-62 (1995)
39. Small, J. V: Lamellipodia architecture: actin filament turnover and the lateral flow of actin filaments during motility. *Semin Cell Biol*, 5, 157-163 (1994)
40. Zigmond, S. H: Signal transduction and actin filament organization. *Curr Opin Cell Biol*, 8, 66-73 (1996)
41. Leung, T., Manser, E., Tan, L., and Lim, L: A novel serine/threonine kinase binding the Ras-related RhoA GTPase which translocates the kinase to peripheral membranes. *J Biol Chem*, 270, 29051-29054 (1995)
42. Leung, T., Chen, X. Q., Manser, E., and Lim, L: The p160 RhoA-binding kinase ROK alpha is a member of a kinase family and is involved in the reorganization of the cytoskeleton. *Mol Cell Biol*, 16, 5313-5327 (1996)
43. Ishizaki, T., Maekawa, M., Fujisawa, K., Okawa, K., Iwamatsu, A., Fujita, A., Watanabe, N., Saito, Y., Kakizuka, A., Morii, N., and Narumiya, S: The small GTP-binding protein Rho binds to and activates a 160 kDa Ser/Thr protein kinase homologous to myotonic dystrophy kinase. *Embo J*, 15, 1885-1893 (1996)
44. Matsui, T., Amano, M., Yamamoto, T., Chihara, K., Nakafuku, M., Ito, M., Nakano, T., Okawa, K., Iwamatsu, A., and Kaibuchi, K: Rho-associated kinase, a novel serine/threonine kinase, as a putative target for small GTP binding protein Rho. *Embo J*, 15, 2208-2216 (1996)
45. Nakagawa, O., Fujisawa, K., Ishizaki, T., Saito, Y., Nakao, K., and Narumiya, S: ROCK-I and ROCK-II, two isoforms of Rho-associated coiled-coil forming protein serine/threonine kinase in mice. *FEBS Lett*, 392, 189-193 (1996)
46. Kimura, K., Ito, M., Amano, M., Chihara, K., Fukata, Y., Nakafuku, M., Yamamori, B., Feng, J., Nakano, T., Okawa, K., Iwamatsu, A., and Kaibuchi, K: Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science*, 273, 245-248 (1996)
47. Tan, J. L., Ravid, S., and Spudich, J. A: Control of nonmuscle myosins by phosphorylation. *Annu Rev Biochem*, 61, 721-759 (1992)
48. Chrzanowska-Wodnicka, M. and Burridge, K: Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. *J Cell Biol*, 133, 1403-1415 (1996)
49. Chong, L. D., Traynor-Kaplan, A., Bokoch, G. M., and Schwartz, M. A: The small GTP-binding protein Rho regulates a phosphatidylinositol 4-phosphate 5-kinase in mammalian cells. *Cell*, 79, 507-513 (1994)
50. Hartwig, J. H., Bokoch, G. M., Carpenter, C. L., Janmey, P. A., Taylor, L. A., Toker, A., and Stossel, T. P: Thrombin receptor ligation and activated Rac uncap actin filament barbed ends through phosphoinositide synthesis in permeabilized human platelets. *Cell*, 82, 643-653 (1995)

51. Janmey, P. A: Phosphoinositides and calcium as regulators of cellular actin assembly and disassembly. *Annu Rev Physiol*, 56, 169-191 (1994)
52. Ezzell, R. M., Goldmann, W. H., Wang, N., Parasharama, N., and Ingber, D. E: Vinculin promotes cell spreading by mechanically coupling integrins to the cytoskeleton. *Exp Cell Res*, 231, 14-26 (1997)
53. Gilmore, A. P. and Burridge, K: Regulation of vinculin binding to talin and actin by phosphatidyl-inositol-4-5-bisphosphate. *Nature*, 381, 531-535 (1996)
54. Watanabe, N., Madaule, P., Reid, T., Ishizaki, T., Watanabe, G., Kakizuka, A., Saito, Y., Nakao, K., Jockusch, B. M., and Narumiya, S: p140mDia, a mammalian homolog of Drosophila diaphanous, is a target protein for Rho small GTPase and is a ligand for profilin. *Embo J*, 16, 3044-3056 (1997)
55. Narumiya, S., Ishizaki, T., and Watanabe, N: Rho effectors and reorganization of actin cytoskeleton. *FEBS Lett*, 410, 68-72(1997)
56. Mukai, H: The structure and function of PKN, a protein kinase having a catalytic domain homologous to that of PKC. *J Biochem (Tokyo)*, 133, 17-27 (2003)
57. Watanabe, G., Saito, Y., Madaule, P., Ishizaki, T., Fujisawa, K., Morii, N., Mukai, H., Ono, Y., Kakizuka, A., and Narumiya, S: Protein kinase N (PKN) and PKN-related protein rhophilin as targets of small GTPase Rho. *Science*, 271, 645-648 (1996)
58. Reid, T., Furuyashiki, T., Ishizaki, T., Watanabe, G., Watanabe, N., Fujisawa, K., Morii, N., Madaule, P., and Narumiya, S: Rhotekin, a new putative target for Rho bearing homology to a serine/threonine kinase, PKN, and rhophilin in the rho-binding domain. *J Biol Chem*, 271, 13556-13560 (1996)
59. Madaule, P., Furuyashiki, T., Reid, T., Ishizaki, T., Watanabe, G., Morii, N., and Narumiya, S: A novel partner for the GTP-bound forms of rho and rac. *FEBS Lett*, 377, 243-248 (1995)
60. Madaule, P., Eda, M., Watanabe, N., Fujisawa, K., Matsuoka, T., Bito, H., Ishizaki, T., and Narumiya, S: Role of citron kinase as a target of the small GTPase Rho in cytokinesis. *Nature*, 394, 491-494 (1998)
61. Manser, E., Leung, T., Salihuddin, H., Zhao, Z. S., and Lim, L: A brain serine/threonine protein kinase activated by Cdc42 and Rac1. *Nature*, 367, 40-46 (1994)
62. Manser, E., Chong, C., Zhao, Z. S., Leung, T., Michael, G., Hall, C., and Lim, L: Molecular cloning of a new member of the p21-Cdc42/Rac-activated kinase (PAK) family. *J Biol Chem*, 270, 25070-25078 (1995)
63. Knaus, U. G., Morris, S., Dong, H. J., Chernoff, J., and Bokoch, G. M: Regulation of human leukocyte p21-activated kinases through G protein--coupled receptors. *Science*, 269, 221-223 (1995)
64. Bagrodia, S., Taylor, S. J., Creasy, C. L., Chernoff, J., and Cerione, R. A: Identification of a mouse p21Cdc42/Rac activated kinase. *J Biol Chem*, 270, 22731-22737 (1995)
65. Martin, G. A., Bollag, G., McCormick, F., and Abo, A: A novel serine kinase activated by rac1/CDC42Hs-dependent autophosphorylation is related to PAK65 and STE20. *Embo J*, 14, 4385 (1995)
66. Zhao, Z. S., Manser, E., Chen, X. Q., Chong, C., Leung, T., and Lim, L: A conserved negative regulatory region in alphaPAK: inhibition of PAK kinases reveals their morphological roles downstream of Cdc42 and Rac1. *Mol Cell Biol*, 18, 2153-2163 (1998)
67. Zenke, F. T., King, C. C., Bohl, B. P., and Bokoch, G. M: Identification of a central phosphorylation site in p21-activated kinase regulating autoinhibition and kinase activity. *J Biol Chem*, 274, 32565-32573 (1999)
68. Burbelo, P. D., Drechsel, D., and Hall, A: A conserved binding motif defines numerous candidate target proteins for both Cdc42 and Rac GTPases. *J Biol Chem*, 270, 29071-29074 (1995)
69. Dharmawardhane, S., Sanders, L. C., Martin, S. S., Daniels, R. H., and Bokoch, G. M: Localization of p21-activated kinase 1 (PAK1) to pinocytic vesicles and cortical actin structures in stimulated cells. *J Cell Biol*, 138, 1265-1278 (1997)
70. Sells, M. A., Pfaff, A., and Chernoff, J: Temporal and spatial distribution of activated Pak1 in fibroblasts. *J Cell Biol*, 151, 1449-1458 (2000)
71. Manser, E., Huang, H. Y., Loo, T. H., Chen, X. Q., Dong, J. M., Leung, T., and Lim, L: Expression of constitutively active alpha-PAK reveals effects of the kinase on actin and focal complexes. *Mol Cell Biol*, 17, 1129-1143 (1997)
72. Sells, M. A., Knaus, U. G., Bagrodia, S., Ambrose, D. M., Bokoch, G. M., and Chernoff, J: Human p21-activated kinase (Pak1) regulates actin organization in mammalian cells. *Curr Biol*, 7, 202-210 (1997)
73. Frost, J. A., Khokhlatchev, A., Stippec, S., White, M. A., and Cobb, M. H: Differential effects of PAK1-activating mutations reveal activity-dependent and -independent effects on cytoskeletal regulation. *J Biol Chem*, 273, 28191-28198 (1998)
74. Bagrodia, S., Derijard, B., Davis, R. J., and Cerione, R. A: Cdc42 and PAK-mediated signaling leads to Jun kinase and p38 mitogen-activated protein kinase activation. *J Biol Chem*, 270, 27995-27998 (1995)
75. Manser, E., Loo, T. H., Koh, C. G., Zhao, Z. S., Chen, X. Q., Tan, L., Tan, I., Leung, T., and Lim, L: PAK kinases are directly coupled to the PIX family of nucleotide exchange factors. *Mol Cell*, 1, 183-192 (1998)
76. Bokoch, G. M., Wang, Y., Bohl, B. P., Sells, M. A., Quilliam, L. A., and Knaus, U. G: Interaction of the Nck adapter protein with p21-activated kinase (PAK1). *J Biol Chem*, 271: 25746-25749 (1996)
77. Puto, L. A., Pestonjamasp, K., King, C. C., and Bokoch, G. M: p21-activated kinase 1 (PAK1) interacts with the Grb2 adapter protein to couple to growth factor signaling. *J Biol Chem*, 278, 9388-9393 (2003)
78. Millard, T. H., Sharp, S. J., and Machesky, L. M: Signalling to actin assembly via the WASP (Wiskott-Aldrich syndrome protein)-family proteins and the Arp2/3 complex. *Biochem J*, 380, 1-17 (2004)
79. Symons, M., Derry, J. M., Karlak, B., Jiang, S., Lemahieu, V., McCormick, F., Francke, U., and Abo, A: Wiskott-Aldrich syndrome protein, a novel effector for the GTPase CDC42Hs, is implicated in actin polymerization. *Cell*, 84, 723-734 (1996)
80. Miki, H., Suetsugu, S., and Takenawa, T: WAVE, a novel WASP-family protein involved in actin reorganization induced by Rac. *Embo J*, 17, 6932-6941 (1998)

81. Eden, S., Rohatgi, R., Podtelejnikov, A. V., Mann, M., and Kirschner, M. W: Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck. *Nature*, 418, 790-793 (2002)
82. Brill, S., Li, S., Lyman, C. W., Church, D. M., Wasmuth, J. J., Weissbach, L., Bernards, A., and Snijders, A. J: The Ras GTPase-activating-protein-related human protein IQGAP2 harbors a potential actin binding domain and interacts with calmodulin and Rho family GTPases. *Mol Cell Biol*, 16, 4869-4878 (1996)
83. Kuroda, S., Fukata, M., Kobayashi, K., Nakafuku, M., Nomura, N., Iwamatsu, A., and Kaibuchi, K: Identification of IQGAP as a putative target for the small GTPases, Cdc42 and Rac1. *J Biol Chem*, 271, 23363-23367 (1996)
84. McCallum, S. J., Wu, W. J., and Cerione, R. A: Identification of a putative effector for Cdc42Hs with high sequence similarity to the RasGAP-related protein IQGAP1 and a Cdc42Hs binding partner with similarity to IQGAP2. *J Biol Chem*, 271, 21732-21737 (1996)
85. Nomura, N., Nagase, T., Miyajima, N., Sazuka, T., Tanaka, A., Sato, S., Seki, N., Kawarabayasi, Y., Ishikawa, K., and Tabata, S: Prediction of the coding sequences of unidentified human genes. II. The coding sequences of 40 new genes (KIAA0041-KIAA0080) deduced by analysis of cDNA clones from human cell line KG-1 (supplement). *DNA Res*, 1, 251-262 (1994)
86. Machesky, L. M: Cytokinesis: IQGAPs find a function. *Curr Biol*, 8, R202-205 (1998)87. Kuroda, S., Fukata, M., Nakagawa, M., Fujii, K., Nakamura, T., Ookubo, T., Izawa, I., Nagase, T., Nomura, N., Tani, H., Shoji, I., Matsuura, Y., Yonehara, S., and Kaibuchi, K: Role of IQGAP1, a target of the small GTPases Cdc42 and Rac1, in regulation of E-cadherin- mediated cell-cell adhesion. *Science*, 281, 832-835 (1998)
88. Chatah, N. E. and Abrams, C. S: G-protein-coupled receptor activation induces the membrane translocation and activation of phosphatidylinositol-4-phosphate 5-kinase I alpha by a Rac- and Rho-dependent pathway. *J Biol Chem*, 276, 34059-34065(2001)
89. Honda, A., Nogami, M., Yokozeki, T., Yamazaki, M., Nakamura, H., Watanabe, H., Kawamoto, K., Nakayama, K., Morris, A. J., Frohman, M. A., and Kanaho, Y: Phosphatidylinositol 4-phosphate 5-kinase alpha is a downstream effector of the small G protein ARF6 in membrane ruffle formation. *Cell*, 99, 521-532 (1999)
90. Ridley, A. J: Rho GTPases and cell migration. *J Cell Sci*, 114, 2713-2722 (2001)
91. Pruyne, D. and Bretscher, A: Polarization of cell growth in yeast. I. Establishment and maintenance of polarity states. *J Cell Sci*, 113 (Pt 3), 365-375 (2000)
92. Srinivasan, S., Wang, F., Glavas, S., Ott, A., Hofmann, F., Aktories, K., Kalman, D., and Bourne, H. R: Rac and Cdc42 play distinct roles in regulating PI(3,4,5)P3 and polarity during neutrophil chemotaxis. *J Cell Biol*, 160, 375-385 (2003)
93. Rodriguez, O. C., Schaefer, A. W., Mandato, C. A., Forscher, P., Bement, W. M., and Waterman-Storer, C. M: Conserved microtubule-actin interactions in cell movement and morphogenesis. *Nat Cell Biol*, 5, 599-609 (2003)
94. Li, Z., Hannigan, M., Mo, Z., Liu, B., Lu, W., Wu, Y., Smrcka, A. V., Wu, G., Li, L., Liu, M., Huang, C. K., and Wu, D: Directional sensing requires G beta gamma-mediated PAK1 and PIX alpha-dependent activation of Cdc42. *Cell*, 114, 215-227 (2003)
95. Devreotes, P. and Janetopoulos, C: Eukaryotic chemotaxis: distinctions between directional sensing and polarization. *J Biol Chem*, 278, 20445-20448 (2003)
96. Merlot, S. and Firtel, R. A: Leading the way: Directional sensing through phosphatidylinositol 3-kinase and other signaling pathways. *J Cell Sci*, 116, 3471-3478 (2003)
97. Welch, M. D. and Mullins, R. D. Cellular control of actin nucleation. *Annu Rev Cell Dev Biol*, 18, 247-288 (2002)
98. Pollard, T. D. and Borisy, G. G: Cellular motility driven by assembly and disassembly of actin filaments. *Cell*, 112, 453-465 (2003)
99. Allen, W. E., Zicha, D., Ridley, A. J., and Jones, G. E: A role for Cdc42 in macrophage chemotaxis. *J Cell Biol*, 141, 1147-1157 (1998)
100. Nobes, C. D. and Hall, A: Rho GTPases control polarity, protrusion, and adhesion during cell movement. *J Cell Biol*, 144, 1235-1244 (1999)
101. Miki, H., Yamaguchi, H., Suetsugu, S., and Takenawa, T: IRSp53 is an essential intermediate between Rac and WAVE in the regulation of membrane ruffling. *Nature*, 408: 732-735 (2000)
102. Govind, S., Kozma, R., Monfries, C., Lim, L., and Ahmed, S: Cdc42Hs facilitates cytoskeletal reorganization and neurite outgrowth by localizing the 58-kD insulin receptor substrate to filamentous actin. *J Cell Biol*, 152, 579-594 (2001)
103. Fujiwara, T., Mammoto, A., Kim, Y., and Takai, Y: Rho small G-protein-dependent binding of mDia to an Src homology 3 domain-containing IRSp53/BAIAP2. *Biochem Biophys Res Commun*, 271, 626-629 (2000)
104. Carpenter, C. L., Tolias, K. F., Van Vugt, A., and Hartwig, J: Lipid kinases are novel effectors of the GTPase Rac1. *Adv Enzyme Regul*, 39, 299-312 (1999)
105. Tolias, K. F., Hartwig, J. H., Ishihara, H., Shibasaki, Y., Cantley, L. C., and Carpenter, C. L: Type Ialpha phosphatidylinositol-4-phosphate 5-kinase mediates Rac-dependent actin assembly. *Curr Biol*, 10, 153-156 (2000)
106. Edwards, D. C., Sanders, L. C., Bokoch, G. M., and Gill, G. N: Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. *Nat Cell Biol*, 1, 253-259 (1999)
107. Stanyon, C. A. and Bernard, O: LIM-kinase1. *Int J Biochem Cell Biol*, 31, 389-394 (1999)
108. Burridge, K. and Chrzanowska-Wodnicka, M: Focal adhesions, contractility, and signaling. *Annu Rev Cell Dev Biol*, 12, 463-518 (1996)
109. Adams, J. C. and Schwartz, M. A: Stimulation of fascin spikes by thrombospondin-1 is mediated by the GTPases Rac and Cdc42. *J Cell Biol*, 150, 807-822 (2000)
110. Ridley, A: What initiates actin polymerization? *Genome Biol*, 1, REVIEWS102 (2000)
111. Bailly, M., Wyckoff, J., Bouzahzah, B., Hammerman, R., Sylvestre, V., Cammer, M., Pestell, R., and Segall, J. E: Epidermal growth factor receptor distribution during chemotactic responses. *Mol Biol Cell*, 11, 3873-3883 (2000)
112. Cox, E. A., Sastry, S. K., and Huttenlocher, A: Integrin-mediated adhesion regulates cell polarity and

membrane protrusion through the Rho family of GTPases. *Mol Biol Cell*, 12, 265-277 (2001)
113. Zhao, Z. S., Manser, E., Loo, T. H., and Lim, L: Coupling of PAK-interacting exchange factor PIX to GIT1 promotes focal complex disassembly. *Mol Cell Biol*, 20, 6354-6363 (2000)
114. Maekawa, M., Ishizaki, T., Boku, S., Watanabe, N., Fujita, A., Iwamatsu, A., Obinata, T., Ohashi, K., Mizuno, K., and Narumiya, S: Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. *Science*, 285, 895-898 (1999)
115. Tominaga, T., Sahai, E., Chardin, P., McCormick, F., Courtneidge, S. A., and Alberts, A. S: Diaphanous-related formins bridge Rho GTPase and Src tyrosine kinase signaling. *Mol Cell*, 5: 13-25 (2000)
116. Palecek, S. P., Huttenlocher, A., Horwitz, A. F., and Lauffenburger, D. A: Physical and biochemical regulation of integrin release during rear detachment of migrating cells. *J Cell Sci*, 111 (Pt 7), 929-940 (1998)
117. Glading, A., Chang, P., Lauffenburger, D. A., and Wells, A: Epidermal growth factor receptor activation of calpain is required for fibroblast motility and occurs via an ERK/MAP kinase signaling pathway. *J Biol Chem*, 275, 2390-2398 (2000)
118. Chung, C. Y., Potikyan, G., and Firtel, R. A: Control of cell polarity and chemotaxis by Akt/PKB and PI3 kinase through the regulation of PAKa. *Mol Cell*, 7, 937-947 (2001)
119. Worthylake, R. A. and Burridge, K: RhoA and ROCK promote migration by limiting membrane protrusions. *J Biol Chem*, 278, 13578-13584 (2003)
120. Xu, J., Wang, F., Van Keymeulen, A., Herzmark, P., Straight, A., Kelly, K., Takuwa, Y., Sugimoto, N., Mitchison, T., and Bourne, H. R: Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils. *Cell*, 114, 201-214 (2003)
121. Cox, E. A. and Huttenlocher, A: Regulation of integrin-mediated adhesion during cell migration. *Microsc Res Tech*, 43, 412-419 (1998)
122. Rodenhuis, S: ras and human tumors. *Semin Cancer Biol*, 3, 241-247 (1992)
123. Rochlitz, C. F., Scott, G. K., Dodson, J. M., Liu, E., Dollbaum, C., Smith, H. S., and Benz, C. C: Incidence of activating ras oncogene mutations associated with primary and metastatic human breast cancer. *Cancer Res*, 49, 357-360 (1989)
124. Avraham, H. and Weinberg, R. A: Characterization and expression of the human rhoH12 gene product. *Mol Cell Biol*, 9, 2058-2066 (1989)
125. Clark, E. A., Golub, T. R., Lander, E. S., and Hynes, R. O: Genomic analysis of metastasis reveals an essential role for RhoC. *Nature*, 406, 532-535 (2000)
126. Qiu, R. G., Chen, J., Kirn, D., McCormick, F., and Symons, M: An essential role for Rac in Ras transformation. *Nature*, 374, 457-459 (1995)
127. Schnelzer, A., Prechtel, D., Knaus, U., Dehne, K., Gerhard, M., Graeff, H., Harbeck, N., Schmitt, M., and Lengyel, E: Rac1 in human breast cancer: overexpression, mutation analysis, and characterization of a new isoform, Rac1b. *Oncogene*, 19, 3013-3020 (2000)
128. Stofega, M. R., Herrington, J., Billestrup, N., and Carter-Su, C: Mutation of the SHP-2 binding site in growth hormone (GH) receptor prolongs GH-promoted tyrosyl phosphorylation of GH receptor, JAK2, and STAT5B. *Mol Endocrinol*, 14, 1338-1350 (2000)
129. Mira, J. P., Benard, V., Groffen, J., Sanders, L. C., and Knaus, U. G: Endogenous, hyperactive Rac3 controls proliferation of breast cancer cells by a p21-activated kinase-dependent pathway. *Proc Natl Acad Sci U S A*, 97, 185-189 (2000)
130. Bouzahzah, B., Albanese, C., Ahmed, F., Pixley, F., Lisanti, M. P., Segall, J. D., Condeelis, J., Joyce, D., Minden, A., Der, C. J., Chan, A., Symons, M., and Pestell, R. G: Rho family GTPases regulate mammary epithelium cell growth and metastasis through distinguishable pathways. *Mol Med*, 7, 816-830 (2001)
131. Lin, M. and van Golen, K. L: Rho-regulatory proteins in breast cancer cell motility and invasion. *Breast Cancer Res Treat*, 84, 49-60 (2004)
132. Su, L. F., Knoblauch, R., and Garabedian, M. J: Rho GTPases as modulators of the estrogen receptor transcriptional response. *J Biol Chem*, 276, 3231-3237(2001)
133. Vincent, S. and Settleman, J: Inhibition of RhoGAP activity is sufficient for the induction of Rho-mediated actin reorganization. *Eur J Cell Biol*, 78,539-548 (1999)
134. Khosravi-Far, R., Solski, P. A., Clark, G. J., Kinch, M. S., and Der, C. J: Activation of Rac1, RhoA, and mitogen-activated protein kinases is required for Ras transformation. *Mol Cell Biol*, 15: 6443-6453 (1995)
135. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., and McGuire, W. L: Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*, 235, 177-182 (1987)
136. Lee, R. J., Albanese, C., Fu, M., D'Amico, M., Lin, B., Watanabe, G., Haines, G. K., 3rd, Siegel, P. M., Hung, M. C., Yarden, Y., Horowitz, J. M., Muller, W. J., and Pestell, R. G: Cyclin D1 is required for transformation by activated Neu and is induced through an E2F-dependent signaling pathway. *Mol Cell Biol*, 20, 672-683 (2000)
137. Diehl, J. A., Zindy, F., and Sherr, C. J: Inhibition of cyclin D1 phosphorylation on threonine-286 prevents its rapid degradation via the ubiquitin-proteasome pathway. *Genes Dev*, 11, 957-972 (1997)
138. Joyce, D., Bouzahzah, B., Fu, M., Albanese, C., D'Amico, M., Steer, J., Klein, J. U., Lee, R. J., Segall, J. E., Westwick, J. K., Der, C. J., and Pestell, R. G: Integration of Rac-dependent regulation of cyclin D1 transcription through a nuclear factor-kappaB-dependent pathway. *J Biol Chem*, 274, 25245-25249 (1999)
139. Guttridge, D. C., Albanese, C., Reuther, J. Y., Pestell, R. G., and Baldwin, A. S., Jr: NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1. *Mol Cell Biol*, 19, 5785-5799 (1999)
140. Liberto, M., Cobrinik, D., and Minden, A: Rho regulates p21(CIP1), cyclin D1, and checkpoint control in mammary epithelial cells. *Oncogene*, 21, 1590-1599 (2002)
141. Salh, B., Marotta, A., Wagey, R., Sayed, M., and Pelech, S: Dysregulation of phosphatidylinositol 3-kinase and downstream effectors in human breast cancer. *Int J Cancer*, 98, 148-154 (2002)
142. Vadlamudi, R. K., Adam, L., Wang, R. A., Mandal, M., Nguyen, D., Sahin, A., Chernoff, J., Hung, M. C., and

Kumar, R: Regulatable expression of p21-activated kinase-1 promotes anchorage-independent growth and abnormal organization of mitotic spindles in human epithelial breast cancer cells. *J Biol Chem*, 275, 36238-36244 (2000)
143. Wang, S., Ghosh, R. N., and Chellappan, S. P: Raf-1 physically interacts with Rb and regulates its function: a link between mitogenic signaling and cell cycle regulation. *Mol Cell Biol*, 18, 7487-7498 (1998)
144. Tjandra, H., Compton, J., and Kellogg, D: Control of mitotic events by the Cdc42 GTPase, the Clb2 cyclin and a member of the PAK kinase family. *Curr Biol*, 8, 991-1000 (1998)
145. Wang, R. A., Mazumdar, A., Vadlamudi, R. K., and Kumar, R: P21-activated kinase-1 phosphorylates and transactivates estrogen receptor-alpha and promotes hyperplasia in mammary epithelium. *Embo J*, 21, 5437-5447 (2002)
146. Bokoch, G. M: Biology of the p21-activated kinases. *Annu Rev Biochem*, 72, 743-781 (2003)
147. Sahai, E. and Marshall, C. J: ROCK and Dia have opposing effects on adherens junctions downstream of Rho. *Nat Cell Biol*, 4, 408-415 (2002)
148. Uruno, T., Liu, J., Zhang, P., Fan, Y., Egile, C., Li, R., Mueller, S. C., and Zhan, X: Activation of Arp2/3 complex-mediated actin polymerization by cortactin. *Nat Cell Biol*, 3, 259-266 (2001)
149. Mondal, M. S., Wang, Z., Seeds, A. M., and Rando, R. R: The specific binding of small molecule isoprenoids to rhoGDP dissociation inhibitor (rhoGDI). *Biochemistry*, 39, 406-412 (2000)
150. van Golen, K. L., Bao, L., DiVito, M. M., Wu, Z., Prendergast, G. C., and Merajver, S. D: Reversion of RhoC GTPase-induced inflammatory breast cancer phenotype by treatment with a farnesyl transferase inhibitor. *Mol Cancer Ther*, 1, 575-583 (2002)
151. Du, W. and Prendergast, G. C: Geranylgeranylated RhoB mediates suppression of human tumor cell growth by farnesyltransferase inhibitors. *Cancer Res*, 59, 5492-5496 (1999)
152. Pille, J. Y., Denoyelle, C., Varet, J., Bertrand, J. R., Soria, J., Opolon, P., Lu, H., Pritchard, L. L., Vannier, J. P., Malvy, C., Soria, C., and Li, H, Anti-RhoA and anti-RhoC siRNAs inhibit the proliferation and invasiveness of MDA-MB-231 breast cancer cells *in vitro* and *in vivo*. *Mol Ther*, 11, 267-274 (2005)
153. Denoyelle, C., Vasse, M., Korner, M., Mishal, Z., Ganne, F., Vannier, J. P., Soria, J., and Soria, C: Cerivastatin, an inhibitor of HMG-CoA reductase, inhibits the signaling pathways involved in the invasiveness and metastatic properties of highly invasive breast cancer cell lines: an *in vitro* study. *Carcinogenesis*, 22, 1139-1148 (2001)
154. Collisson, E. A., Kleer, C., Wu, M., De, A., Gambhir, S. S., Merajver, S. D., and Kolodney, M. S: Atorvastatin prevents RhoC isoprenylation, invasion, and metastasis in human melanoma cells. *Mol Cancer Ther*, 2, 941-948 (2003)
155. McHardy, L. M., Warabi, K., Andersen, R. J., Roskelley, C. D., and Roberge, M: Strongylophorine-26, a Rho-dependent inhibitor of tumor cell invasion that reduces actin stress fibers and induces nonpolarized lamellipodial extensions. *Mol Cancer Ther*, 4, 772-778 (2005)
156. Sahai, E. and Marshall, C. J: RHO-GTPases and cancer. *Nat Rev Cancer*, 2, 133-142 (2002)
157. Itoh, K., Yoshioka, K., Akedo, H., Uehata, M., Ishizaki, T., and Narumiya, S: An essential part for Rho-associated kinase in the transcellular invasion of tumor cells. *Nat Med*, 5, 221-225 (1999)

Abbreviations: GEF, guanine nucleotide exchange factor; GAP, GTPase-activating protein; GDI, guanine nucleotide dissociation inhibitor; JNK, Jun N-terminal kinase; MAP, mitogen-activated protein; ERK, extracellular regulated kinase; SRF, serum response factor; LPA, lysophosphatidic acid; PDGF, platelet-derived growth factor; PIP2, phosphatidylinositol 4,5-bisphosphate

Key Words: Rho GTPases, Rho, Rac, Cdc42, Actin, Cytoskeleton, Migration, Cell adhesion, Metastasis, Review

Send correspondence to: Dr Daotai Nie, Department of Medical Microbiology, Immunology, and Cell Biology, Southern Illinois University School of Medicine and SimmonsCooper Cancer Institute, Springfield, IL 62702, USA, Tel: 217-545-9702, Fax: 217-545-3227, dnie@siumed.edu

http://www.bioscience.org/current/vol13.htm