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Review

What tangled webs they weave: Rho-GTPase control of angiogenesis

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Abstract. The members of the Rho family of small GTPases are involved in an array of cellular processes, including regulation of the actin cytoskeleton, cell polarity, microtubule dynamics, membrane transport, and transcription factor activity. Recent findings have implicated the Rho-proteins as key regulators of angiogenesis, modulating a diversity of cellular processes, including vascular permeability, extracellular matrix remodeling, migration, proliferation, morphogenesis, and survival.

Keywords. Angiogenesis, RhoA, Rac1, Cdc42, endothelial cell.

Introduction

Angiogenesis, in a number of physiological and aberrant conditions, coincides with vascular permeability increases that allow extravasation of plasma proteins, which lay down a provisional scaffold for migrating endothelial cells (ECs). Subsequent to increases in EC permeability, degradation of the extracellular matrix (ECM) by matrix-metalloproteases (MMPs) relieves pericyte-EC contacts and liberates ECM-sequestered growth factors. ECs then proliferate and migrate to their perivascular destination, where they undergo a complex morphogenesis to assemble as lumen-bearing cords with branching structure (Fig. 1). These processes are largely controlled by the angiogenic factor VEGF and its specific binding to the integral membrane tyrosine receptor kinases VEGFR-1 and VEGFR-2. VEGF stimulation of the VEGF receptors leads to activation of a number of downstream signaling cascades, including the MAPK, PI3K, and PLCγ pathways, which ultimately manage the multifaceted processes that occur during angiogenesis [1]. Significant research is directed toward examining the complex intracellular signal transduction pathways downstream of VEGF during angiogenesis, and in recent years a growing number of studies have implicated the Rho-family of small GTPases as essential downstream effectors of VEGF signaling in the angiogenic process.

The Rho-family of small GTPases as downstream effectors of VEGF

The Rho-proteins control an incredibly diverse array of cellular processes, including cytoskeletal dynamics, cell polarity, membrane transport, gene expression, cell proliferation, apoptosis, and transcription factor activity [2], in both normal and aberrant cellular

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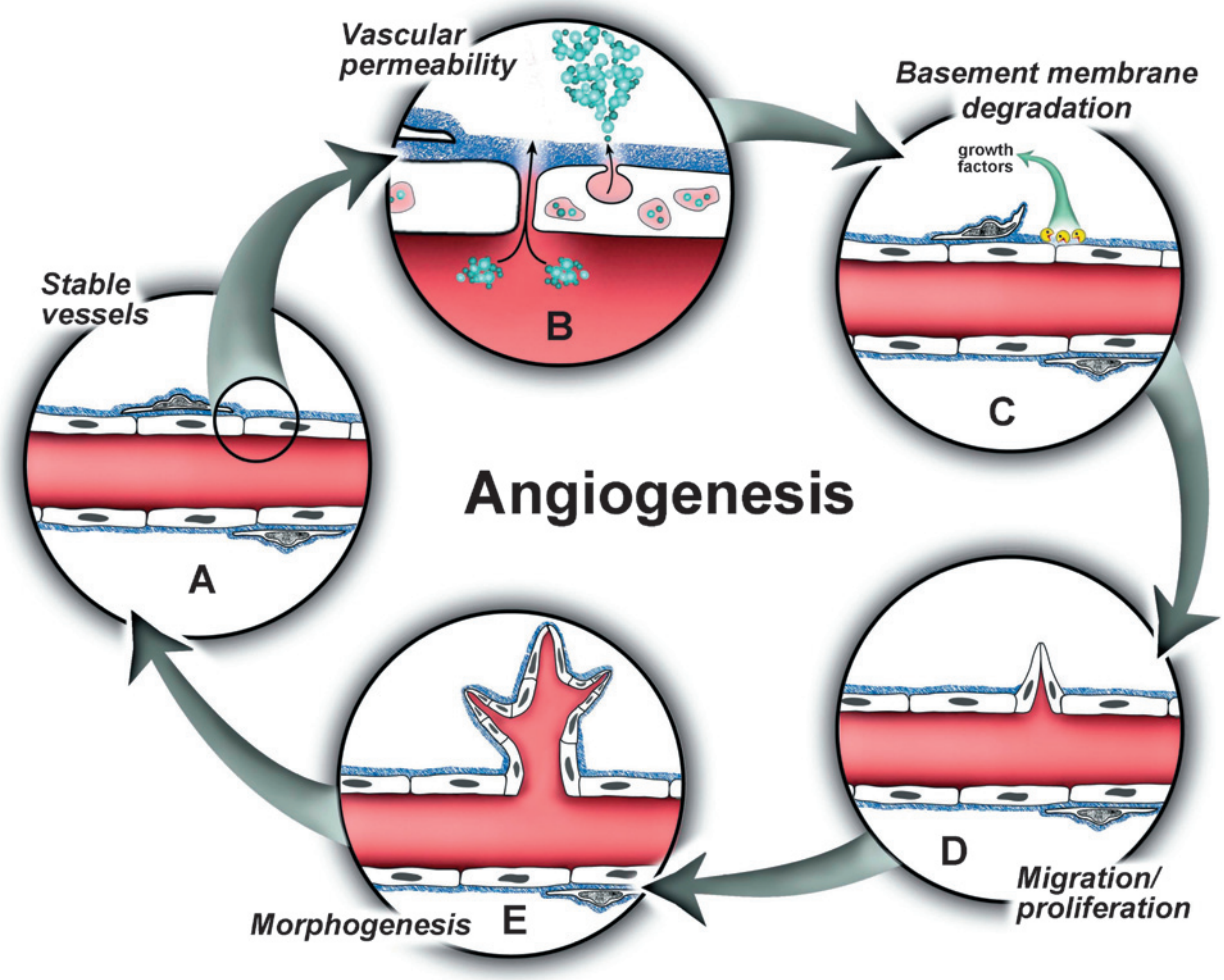

Figure 1. The angiogenic cascade. During the process of angiogenesis, stable vessels (*a*) undergo a vascular permeability increase (this has been demonstrated only under certain conditions – see text), which allows extravasation of plasma proteins (*b*). Degradation of the ECM by MMPs relieves pericyte-EC contacts and liberates ECM-sequestered growth factors (*c*). ECs then proliferate and migrate to their final destination (*d*) and assemble as lumen-bearing cords (*e*). ECM, extracellular matrix; MMPs, matrix-metalloproteases; EC, endothelial cell.

processes. The processes include, but certainly are not limited to, neuronal outgrowth and pathfinding [3], skeletal myogenesis [4–5], tumorigenesis [6], and inflammation [7]. The Rho-proteins – whose most studied members are RhoA, Rac1, and Cdc42 – are molecular switches cycling between an inactive guanosine diphosphate (GDP)-bound and an active guanosine triphosphate (GTP)-bound state. Control of this cyclic activation/inactivation process is modulated by three known classes of regulatory proteins: GTPase-activating proteins (GAPs), guanine nucleotide dissociation inhibitors (GDIs), and guanine nucleotide exchange factors (GEFs) [8–10]. GAPs negatively regulate Rho GTPase activation by promoting their inactive GDP-bound state, while GDIs modulate RhoA activity by sequestration of Rho proteins in the GDP or GTP-bound state. Activation of the Rho family is directly controlled by GEFs, which stimulate the exchange of GDP for GTP. These three classes of regulatory molecules work immediately upstream of Rho proteins to provide a molecular link between Rho-protein activation status and cell-surface receptors for various cytokines, growth factors, adhesion molecules, and G-protein-coupled receptors. An increasing number of downstream effectors mediating the cellular effects of the Rho-protein signaling cascades are well reported in the literature [11–13]. The most extensively studied RhoA effectors are the serine/threonine Rho-associated kinases (ROCKs), which exist in a cytoplasmically-localized autoinhibited state that is disrupted and transported to the plasma membrane upon association with active RhoA [14–17]. The Rho associated kinases are best known for their regulation

Table 1. Rho-GTPase roles during angiogenesis.

	RhoA	Rac1	Cdc42
EC barrier function	induces loss of barrier function in response to inflammatory mediators	latent restoration of barrier function	latent restoration of barrier function
Basement membrane degradation	increases MMP expression + secretion to promote ECM degradation	increases MMP expression + secretion to promote ECM degradation.; latent increase in TIMP expression + activity to inhibit ECM degradation	increases MMP expression + secretion to promote ECM degradation; latent increase in TIMP expression + activity to inhibit ECM degradation
Migration	promotes contraction at rear of cell	promotes lamellipodia at leading edge of cell	promotes filopodia at leading edge of cell
Proliferation	promotes G1/S transition	promotes G1/S transition	promotes G1/S transition
Morphogenesis	regulates capillary branching	promotes vacuole/lumen formation; regulates capillary branching	promotes vacuole/lumen formation; regulates capillary branching
Capillary Survival	pro- or anti-apoptotic depending on environmental cues	pro- or anti-apoptotic depending on environmental cues	pro- or anti-apoptotic depending on environmental cues

of actomyosin contractility via a direct phosphorylation of myosin light chain (MLC) and phosphorylation and inactivation of the myosin-binding subunit of myosin phosphatase that is responsible for dephosphorylation of MLC [14]. Moreover, ROCK has been reported to phosphorylate LIM-kinase, which subsequently phosphorylates the actin-regulatory protein cofilin to contribute to Rho-induced reorganization of the actin cytoskeleton [18]. The most-studied effectors of both Rac1 and Cdc42 are the p21-activated kinase (PAK) family of serine/threonine kinases. PAK proteins contain an N-terminal autoinhibitory region, and association with GTP-bound Rac or Cdc42 disrupts the autoinhibition, resulting in autophosphorylation and membrane localization of PAK, and leads to phosphorylation of LIM-kinase and its phosphorylation of cofilin [19–20].

The Rho-family of small GTPases as downstream effectors of VEGF

While the MAPK, PI3K, and PLCγ pathways are reported to be the major downstream effectors in VEGF signaling, multiple reports have suggested that the Rho-family of small GTPases plays an essential role in transmitting signals downstream of VEGF. With the use of bacterial toxins and chemical agents known to inhibit Rho-proteins, as well as adenoviral overexpression of constitutively active (ca) and dominant negative (dn) Rho-proteins, it has been reported that Rho signaling is essential for *in vivo* angiogenesis and *in vitro* capillary formation in a VEGF-dependent manner [21–23]. Indeed, the Rho-proteins appear to be direct downstream targets of VEGF-mediated EC signaling whereby VEGF stimulation leads to a dramatically rapid activation of RhoA, Rac1, and Cdc42 within several minutes of stimulation (generally 1–5 min varying by report), resulting in their increased GTP-bound state and enhanced membrane localization [24]. This very rapid activation of the Rho-proteins then returns to basal levels within 5–30 min (varying by report), suggesting that these proteins may be important during the very early stages of VEGF-mediated angiogenesis. These studies suggest that Rho-proteins and their downstream effectors could function as key mediators of VEGF-induced capillary formation. While only a handful of molecular details exist as to how the Rho-proteins control the complex multi-step processes involved in capillary formation, this manuscript examines what is known of the major members of Rho signaling in capillary formation by reviewing the present knowledge of these proteins' role in EC function and tube formation, and, for areas that are largely unknown, extrapolating potential roles in ECs from findings in non-EC systems. It is interesting that a large amount of literature suggests that the Rho-pathway may impinge on nearly every aspect of the angiogenic process (Table 1).

Vascular permeability

Enhanced vascular permeability is associated with pathological neovascularization, however a topic of debate remains as to whether this process occurs during development. In response to inflammatory mediators, EC permeability is controlled by numerous intracellular signaling pathways activated by molecules such as VEGF, thrombin, and sphingosine 1-phosphate, all leading to a highly coordinated rever-

sible loss of junctional integrity mediated by alterations in adherens and tight junctions [25]. This process allows recoil of adjacent cells, creating space between bordering cells to facilitate the flux of fluid and macromolecules across the endothelium. Inhibition of Rho-ROCK signaling with the ROCK-selective inhibitor Y-27632 leads to ablation of cell permeability in ECs by inhibiting the ROCK-dependent formation of transcellular gaps, vesiculo-vacuolar organelles, and fenestrations involving alterations in myosin-light-chain phosphorylation and actin stress fiber formation [26–27], suggesting that Rho signaling is partially responsible for destabilizing adherins and tight junctions, thereby increasing EC permeability (Fig. 2). Activation of RhoA during thrombin-induced EC barrier loss occurs in part via p115RhoGEF [28] and GEFH1 [29] activation and PKC-mediated inactivation of the RhoA-inhibitor RhoGDI [30], all of which are heavily implicated in regulating junctional assembly and EC permeability. In many cell types, including ECs, Rac1 and Cdc42 appear in most instances to regulate barrier function antagonistically to RhoA by stabilizing the integrity of junctional complexes, thereby inhibiting loss of EC barrier function (Fig. 2). Indeed, overexpression of caRac1 has been shown to stabilize adherins junctions, whereas overexpression of dnRac1 dissolves this complex [31]. In ECs with stabilized junctions, Rac1 and Cdc42 are highly localized at cell-cell contacts, and are inactivated and rapidly dissociated from these complexes during permeability increases [30, 32–33]. Interestingly, a transient loss of EC barrier function is latently restored via a p190-RhoGAP-mediated inactivation of RhoA [34] and subsequent activation of Rac1/Cdc42/PAK signaling by Tiam-GEF, which stabilizes cadherin/catenin/actin complexes and promotes stability [35–39]. This cyclic coordination of activation/inactivation suggests that timely regulation of Rho-protein activity is balanced by inhibitory and stimulatory proteins, and interference with this balance may dramatically shift EC permeability. Thus far, the activation status of RhoA, Rac1, and Cdc42 following VEGF stimulation have only been examined for a short time following treatment (30 min or less); however, restoration of barrier function is known to occur within several hours following VEGF treatment. Given that Rac1 and Cdc42 appear to inhibit endothelial permeability in ECs, future studies examine the activation status and subcellular localization of these proteins throughout the cycle of VEGF-induced barrier loss and subsequent barrier restoration.

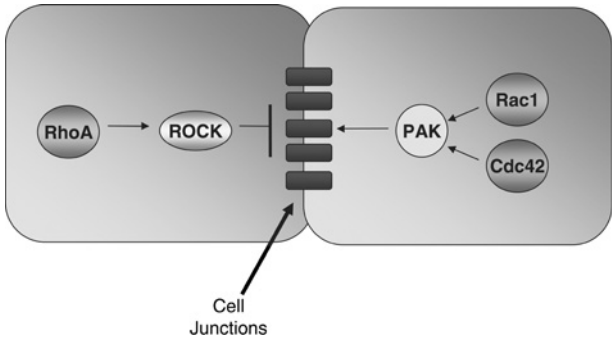

Figure 2. Rho-protein modulation of endothelial permeability. Treatment of ECs with molecules such as VEGF, thrombin, and sphingosine 1-phosphate leads to a highly coordinated reversible loss of junctional integrity between cells mediated by the rapid activation of RhoA signaling to the Rho-kinase (ROCK)-dependent formation of trancellular gaps, vesiculo-vacuolar organelles, and fenestrations. Barrier function is restored and maintained by the latent activation of Rac1, Cdc42, and their common downstream effector, PAK, which stabilizes the integrity of junctional complexes. VEGF, vascular endothelial growth factor; PAK, p21-activated kinase.

ECM remodeling

Degradation of the basement membrane and remodeling of the ECM allows EC-pericyte detachment and EC invasion into the surrounding tissue by providing organizational cues in the absence of cell-cell contact. Additionally, the ECM controls the EC cytoskeleton in an integrin-dependent manner to orchestrate a process by which proliferating ECs organize into multicellular tubes containing functional lumens. The Rho-proteins have been implicated in ECM degradation via modulation of MMP expression/secretion largely in non-EC cell types [40–41]. Indeed, MMP synthesis is known to be oxidant dependent [42] and is triggered by activation of redox-sensitive transcription factors such as NFκB and AP-1 [43–44], both of which are known downstream effectors of Rho signaling [45–46]. In keratinocytes, MMP expression depends on two distinct pathways involving Rac1/Cdc42 control of p38 activation and RhoA-mediated JNK stimulation [40, 47]. Rac1 has also been shown to inhibit the activity of MMPs by promoting TIMP (tissue inhibitors of MMPs) expression and activity in renal carcinoma cells via a reactive oxygen species-dependent activation of MAPK and AP1 [48], suggesting that in addition to promoting MMP expression, Rac1 induces a feedback loop responsible for repressing MMP activity. Analogous mechanisms of Rho-protein-mediated control of MMP and TIMP do exist in ECs (Fig. 3). Indeed, ectopic expression of caRhoA in ECs induces an enhancement of MMP-9 transcription, followed by an increase in MMP subcellular localization to apical vesicles and advancing lamellipodia, where it colocalizes with RhoA [49].

This finding corroborates data from a report demonstrating that TIMP overexpression in ECs significantly reduces the invasive phenotype of ectopic caRhoA, suggesting ECM reorganization, which is dependent on MMP expression and activity, is a critical mechanism for the RhoA-induced EC invasion. While multiple studies suggest that Rac1 and Cdc42 are important mediators of MMP and TIMP expression/activity in non-ECs, it is unknown whether such mechanisms exist in ECs. However, it has been reported that TIMP-2 overexpression in human microvascular ECs leads to migratory inhibition via disassembly of the paxillin-Crk-DOCK180 complex and subsequent inactivation of Rac1 [50], suggesting MMP degradation of the extracellular matrix is essential for Rac1-mediated cell migration. Furthermore, VEGF is known to upregulate MMPs as well as inhibit TIMPs [51–52]. Given that the Rho-proteins function as downstream mediators of this cytokine, it is surprising that no reports to date have linked Rho-proteins as downstream effectors of VEGF-mediated MMP expression. It would be interesting and likely promising to investigate the role of Rho-proteins in this VEGF-mediated process.

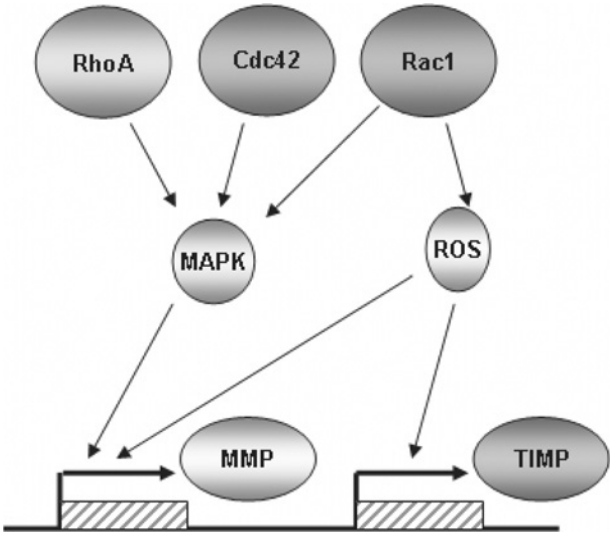

Figure 3. Extracellular matrix remodeling of ECs by Rho-protein mediated signaling. MMP-mediated degradation of the ECM surrounding ECs permits endothelial-pericyte detachment, release of ECM-bound growth factors, and EC invasion into the surrounding area. The Rho-proteins play an essential part in this process by increasing the transcriptional expression of MMPs through mitogen-activated protein kinase (MAPK) signaling and generation of reactive oxygen species (ROS). Based on findings in non-ECs, Rac1 has been shown to upregulate transcription of the MMP inhibitor tissue inhibitor of MMPs (TIMP) through ROS production, thus activating a feedback loop which suppresses MMP activity.

Migration

During EC migration, the cytoskeleton generates a protrusive force at the leading edge of the cell simultaneous with adhesion release at the rear of the cell. This process proceeds by the coordinated spatial activation of each GTPase, whereby Cdc42 activation promotes the formation of fine, actin-rich protrusions called filopodia in the direction of the pro-migratory signal, followed by localized Rac1-dependent actin polymerization at the cell periphery. This results in forward movement of the cell, and Rho-induced actomyosin-based contractility inducing cytoskeletal contraction toward the rear of the cell reviewed in depth by [53]. Given the enormous amount of literature supporting a role for Rho-proteins in cell morphology, migration, and polarity, it is not at all surprising to find multiple reports indicating that inhibition of Rho-signaling disrupts EC migration via interruptions in cytoskeletal remodeling [54–57] (Fig. 4). Indeed, VEGF-mediated EC migration impinges on the coordination of Rho-protein dependent actin polymerizing and depolymerizing processes. For instance, VEGF treatment activates the Rac1/Cdc42 effector, neural Wiskott-Aldrich syndrome protein (N-WASP), to promote actin nucleation [58]. Moreover, VEGF stimulation leads to the rapid phosphorylation and inactivation of the actin depolymerization factor cofilin, and its upstream regulator LIM-kinase (LIMK) [58]. Some very interesting recent findings, primarily using non-ECs, demonstrates the mechanisms dictating the very precise orchestration of spatial activation/inhibition of the Rho-proteins within a migrating cell that leads to this coordinate actin polymerization and depolymerization [59–60]. For instance, in migrating fibroblasts and epithelial cells, RhoA activity is high both at the contractile tail and at the leading edge, whereas Rac1 and Cdc42 activities are high primarily at the leading edge [61]. Moreover, in cells stimulated with epidermal growth factor (EGF), the activities of Rac1 and Cdc42 are reported to transiently elevate diffusely around the plasma membrane, while RhoA activity decreased near the plasma membrane. A handful of key studies in non-ECs have examined the molecular mechanisms underlying this spatiotemporal activity of the Rho-GTPases in migrating and protruding cells, and have demonstrated localized Smurf1-mediated proteasomal degradation of RhoA at the leading edge of the cell, followed by receptor-stimulated activation of Rac1- and Cdc42-signaling cascades at cellular protusions that coordinately lead to cellular migration [59–60, 62]. As in non-ECs, temporal and spatial modulation of Rho-proteins appears to be involved in EC migration [63]. In unstimulated ECs, RhoA and Rac1 are

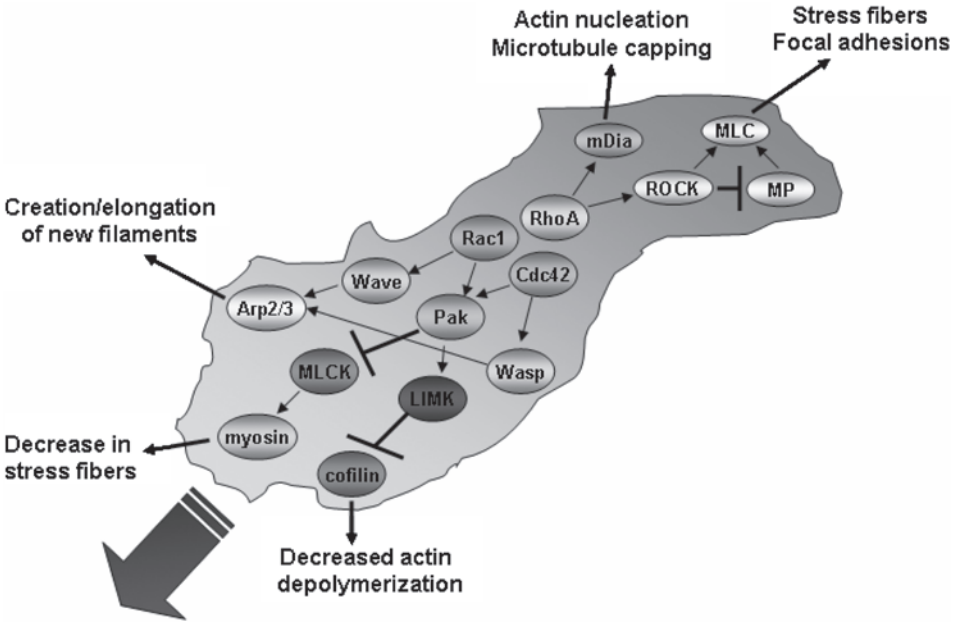

Figure 4. Rho-protein control of endothelial migration. Relief from pericyte-EC contacts and degradation of the ECM allows ECs to migrate to their perivascular destination. Given the depth of literature illustrating the essential role of the Rho-proteins in cell migration, significantly more is known about this aspect of angiogenesis than any other. At the leading edge of the cell, Cdc42 and Rac1 promote cell motility by stimulating actin polymerization in the direction of movement to change membrane shape and extend the cytoplasm forward. Rac1 and Cdc42, through their respective downstream effectors WAVE and WASP, orchestrate the proper spatio-temporal activation of the Arp2/3 complex to initiate the growth of a branching network of actin filaments that generate a protrusive force at the leading edge of the cell. Additionally, Rac1 and Cdc42 prevent cell spreading and actin depolymerization through Pak-mediated inhibition of myosin-light-chain kinase (MLCK), thus preventing its phosphorylation of the regulatory myosin light chain (Myosin), and by activation of LIM-kinase (LIMK), which subsequently inhibits cofilin-induced actin depolymerization. At the trailing edge of the cell, two major RhoA target molecules, Rho ROCK and mDia, assist in promoting tail detachment. ROCK induces stress fiber formation and retraction/formation of focal adhesions through precise spatial control of MLC by direct phosphorylation and inhibitory phosphorylation of myosin phosphatase (MP). mDia triggers the nucleation and polymerization of unbranched actin filaments and microtubule capping.

largely found in the cytosolic region of the cell. However, angiopoietin stimulation leads to greatly enhanced perinuclear RhoA localization and its association with stress fibers, while activated Rac1 becomes partially colocalized with F-actin at the leading edge of the cell and at cell-cell contacts. Ultimately, this coordinated control of Rho-proteins at discreet cellular regions is believed to modulate local areas of cytoskeletal rearrangements leading to EC migration and cell-matrix/cell-to-cell contacts, both of which are necessary for capillary formation. While many of these studies examine migration in a 2D context, many cells display differing morphologies when migrating in 3D matrices versus the 2D tissue culture systems that are typically used in standard lab experiments. For instance, bovine aortic ECs grown in 3D matrices form cylindrical branching pseudopodia, yet on 2D substrata they form wide flat lamellae [64]. While the mechanism controlling this phenomenon is unknown in ECs, analogous mechanisms could be derived from tumor cells whereby during 3D invasion assays of cancer cell lines and *in vivo* tumor cell migration assays, the inhibition of Smurf1, and therefore the stabilization of RhoA, induces a mesenchymal-amoeboid-like transition that is associated with a more invasive phenotype [65]. Further experiments, perhaps using fluorescence resonance energy transfer analysis on growth factor-stimulated EC or activation/inhibition of Rho-protein signaling in 2D verses 3D cultures, will help elucidate how the Rho-proteins precisely control EC migration during the process of capillary formation.

Proliferation

Formation of branching capillary networks requires that, in response to mitogenic factors, local spatial differentials of cell proliferation be established. While signaling cascades such as the Ras/MAPK pathway are well known to regulate cell cycle progression in ECs, modulation of the cytoskeleton by the Rho-proteins is also central to this process. Indeed, pharmacological disruption of cytoskeletal integrity has been shown to inhibit early mitogenic signaling leading to cell cycle arrest in G1 [66], suggesting that modulation of the actin cytoskeleton is necessary for progression through G1 and entry into S phase. The

requirement for Rho-protein function in the proliferation of numerous cell types has been revealed by studies utilizing selective inhibitors and dn/caRho-protein mutants whereby Rho/ROCK signaling utilizes multiple independent mechanisms to alter the levels of cell cycle regulatory proteins such as cyclin D1 and p21(Cip) elevation via Ras and MAPK signaling, cyclin A elevation via LIM-kinase signaling, and p27(Kip1) reduction via an unknown mechanism [67] (Fig. 5). Additionally, Rac1 and Cdc42 enhance cyclin D1 via MAPK signaling [68], increase cyclin A expression via a synergistic mechanism involving Ras [69], and activate the proteasomal degradation of p21(Cip) via an integrin-induced pathway [70]. Indeed, Rho-protein activity is reportedly essential for EC cell cycle progression in ways largely similar to those found in non-Ecs – stimulation of p27 degradation, Rb hyperphosphorylation, ERK activation, and cyclin-D1 expression [71–73], suggesting a major role in controlling the G1/S transition.

However, some controversy exists regarding the role of Rac1 in EC proliferation. VEGFR2 signaling has been demonstrated to induce EC proliferation partially via a Rac1-mediated activation of the NADPH oxidase complex which generates reactive oxygen species and leads to multiple effects, including EC migration, proliferation, and post-natal angiogenesis [74]; however, one study suggests the anti-proliferative effect of VEGFR1 in ECs is dependent on PI3K modulation of VEGFR1/Rac1/Cdc42 signaling to mediate intracellular calcium mobilization [75]. It is possible there exists dual regulation of Rac1 by VEGF receptors, leading to opposing cellular outcomes dependent on differential signaling; however, the VEGFR1/Rac1 report is complicated by the fact that the authors utilize an EGF-stimulated EGF-VEGFR1 chimera to activate Rho-protein signaling, likely exhibiting different kinetics than VEGFR1, and possibly manifesting in differing cellular outcomes. More studies will be necessary to shed light on this issue.

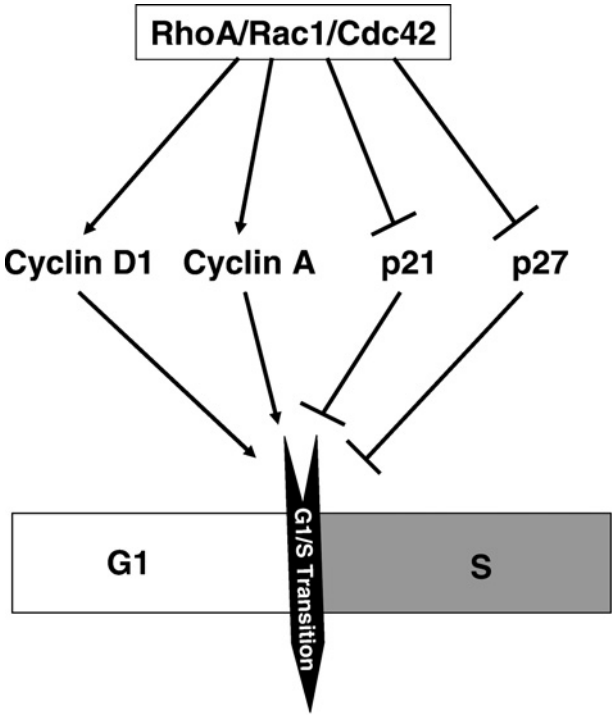

Figure 5. Endothelial proliferation is controlled by the Rho-GTPases. Capillary formation depends in part on the formation of new ECs, which undergo proliferation in response to cellular mitogens. While several reports suggest the Rho-proteins are essential in many parts of the cell cycle and during cytokinesis, in ECs they have only been extensively studied with regard to progression through the G1 phase. RhoA, Rac1, and Cdc42 have been shown to promote the G1/S transition via upregulation of cyclin D1 and cyclin A expression and inhibition of the cell cycle inhibitors p21 and p27.

Morphogenesis

Arguably the most complex and least-understood process in angiogenesis is vascular morphogenesis, which involves two separate processes: branching of endothelial cell cords into vascular networks and lumen formation [76–77]. Data from multiple laboratories suggest a critical role for integrin-ECM interactions, cytoskeletal reorganization, and MMPs in regulating capillary network formation, indicating the involvement of Rho-protein control of the cytoskeleton in this process. Indeed, increased expression of RhoA or Cdc42 results in enhanced branching and sprouting of vessels; however, little effect is seen with overexpression of Rac1 [78]. Moreover, disruption of Pak localization specifically blocks the formation of multicellular networks using *in vitro* tube-forming assays and chick chorioallantoic membrane assays [79]. The formation of complex, branched microvascular beds likely depends on the hemodynamic, hormonal and metabolic responses in the local microenvironment that may either promote or inhibit further angiogenesis. Indeed, morphogenesis in many tissue types including ECs is believed to be influenced by local alterations in Rho-protein-mediated cellular isometric tension [80]. For instance, loss of cytoskeletal tension in embryonic mouse lung rudiments by RhoA inhibition results in significant disruption of capillary networks, while increasing cell tension through RhoA activation increases capillary elongation [81]. These observations suggest that changes in cytoskeletal tension mediated by Rho signaling play an important role in the establishment of the spatial differentials in cell growth and ECM remodeling that drive angiogenesis. In addition to the

modulation of cytoskeletal architecture, Rho-protein-mediated MMP expression could hypothetically play a role in capillary morphogenesis. For instance, MMP-mediated ECM degradation is essential for EC morphogenesis in 3D gels [82–83], and TIMP-2 overexpression completely blocks morphogenesis [84]. Moreover, MMP-2 and MMP-9 knockout mice have revealed the essential roles for these proteins in angiogenesis *in vivo* [85–86]. While unknown to date, it is likely that capillary network remodeling is dependent on Rho-mediated MMP expression and activation given that Rho-proteins have been shown to modulate MMPs in ECs (see above).

EC intracellular vacuoles have been observed both *in vivo* and *in vitro* by many investigators as early as the 1930's [87]. While still a matter of debate, mounting *in vitro* evidence suggests that endocytotic vesicle assembly and coalescence may control lumen formation [88–90], and the Rho-proteins may be central in this process (Fig. 6). *Clostridium difficile* toxin B (which blocks all three Rho GTPases) completely inhibits EC lumen formation in 3D matrices, whereas C3 transferase, a selective inhibitor of Rho, does not [91], directly implicating Rac1 and Cdc42 in lumen assembly during *in vitro* capillary formation. In this study, both vacuole and lumen formation were initiated in ECs overexpressing dnRac1, but later collapsed, indicating a role for Rac1 during later stages of vessel development. In a number of cell types, including ECs, a matrix-integrin-cytoskeletal signaling axis controls Rac1 and Cdc42 subcellular localization, where they modulate actin reorganization during pinocytosis and phagocytosis [89, 92–94] through signaling pathways involving WASP, whose deficiency in Wiscott-Aldrich syndrome patients results in defective phagocytosis [95], and PAK, which localizes to phagocytic cups and macropinosomes [96–97]. Specifically, Rac1 is concentrated throughout the phagocytic cup, and upon closure, Rac1 transiently redistributes to the actin-poor region of the phagosomal membrane [98], indicating that coordinated regulation of Rac1 occurs during phagocytosis and endocytic vesicle formation. Though the majority of studies suggest that Rac1 and Cdc42 are the primary GTPases involved in lumen formation, one study has reported that inhibition of RhoA/ROCK signaling appears to disrupt EC vacuole and lumen formation [71], suggesting that under some conditions RhoA potentially plays a still unidentified role in lumen formation. While EC lumen formation in a 3-D matrix offers insights into the physiological mechanisms of capillary morphogenesis, the validity of these assays must be mirrored by experiments performed *in vivo*, and due to the technical difficulties associated with imaging dynamic EC lumen formation within living animals, few studies have been able to address this issue. However, using high-resolution time-lapse two-photon imaging of transgenic zebrafish, one study corroborates the reported *in vitro* data by demonstrating that Cdc42 localizes *in vivo* to highly dynamic vacuoles that fuse together to create a lumenal space encompassing most of the volume of the cell [90]. Though *in vivo* examination of EC lumen formation has thus far proven technically difficult, novel imaging techniques will greatly enhance our understanding of the molecular mechanisms controlling this process.

Capillary survival

Angiogenesis is dependent on the balance between the promotion of capillary formation by pro-angiogenic factors and capillary regression in response to lack of survival factors or the presence of inhibitors. The regulation of microvascular survival impacts both developmental remodeling of the vasculature, microvascular pathologies, and, importantly, chemotherapeutic de-stabilization of tumor vasculature. Support for a Rho signaling role in cell survival comes primarily from studies in non-ECs, whereby inhibition of Rho signaling manifests in cell apoptosis via alterations in cell adhesion and induction in p53 and other pro-apoptotic proteins [99–102]. In contrast, some studies have suggested that Rho signaling induces apoptosis via Rac1-mediated Fas-ligand up-regulation, RhoA/Rac1-mediated ceramide upregulation, and Cdc42-mediated JNK signaling – all leading to caspase cleavage and activation [103–108]. These contradictory results are likely due to variations in experimental conditions ranging from the method of Rho-protein activation/inhibition, serum conditions, and cell-type dependency. However, based on the majority of reported literature, the Rho pathway appears to promote survival in EC through mechanisms similar to those found in non-EC cells. Indeed, inhibition of RhoA signaling in human umbilical vein epithelial cells increases pro-apoptotic caspase and Bid activation, and decreases antiapoptotic Bcl-2 and Mcl-1 [107, 109], demonstrating that Rho-A promotes survival in ECs. It is worth mentioning that RhoB knockout in mice leads to apoptosis of newly formed retinal vasculature as a result of Akt nuclear exclusion and degradation [110]. Additionally, depletion of RhoB expression/activity with antisense technology, dominant negative mutants, or specific pharmaceuticals in neonatal rats and primary EC cultures is associated with apoptosis in the sprouting endothelial cells of newly forming vessels. These findings in ECs are in contrast to reports demonstrating that steady-state levels of cyclin B1 and

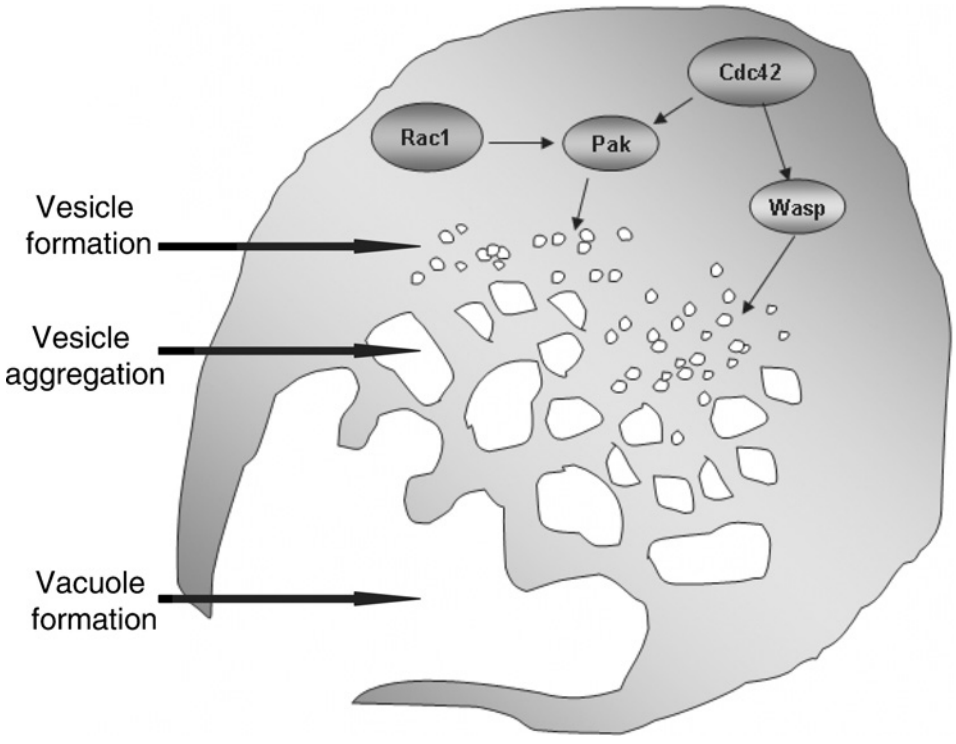

Figure 6. Rho-protein regulation of lumen formation. Mounting evidence suggests that endocytotic vesicle assembly and coalescence may control lumen formation; however, the mechanisms by which this occurs are largely unknown. In ECs, Rac1 and Cdc42 localize to EC vacuoles, and these proteins, as well as their downstream effectors Pak and Wasp, have been shown to be essential for lumen formation.

Cdk1 were suppressed in a RhoB-dependent manner in cells fated to undergo apoptosis [111–112], suggesting that the Rho-proteins may mediate survival or apoptosis in a cell type and/or context-dependent manner. Indeed, while the Rho-proteins appear to promote survival in actively proliferating monolayers of ECs, few studies have examined the survival role of Rho-proteins specifically during the process of capillary formation. As dual-survival roles in non-ECs are reported for the Rho-proteins, it is possible these proteins perform similar functions as the capillary bed forms, stabilizes, and regresses, responding to alternative signaling crosstalk at differing stages of capillary development. A recent report indicates that inhibition of RhoA/ROCK signaling in an EC/fibroblast co-culture system immediately prior to the point of vessel regression results in enhanced vessel stability [113], suggesting that, in contrast to other findings in ECs, Rho-proteins may reduce stability of pre-formed vessels in a context-dependent manner. Little has been reported regarding the survival/apoptotic role of Rac1 and Cdc42 in ECs grown either in proliferative monolayers or during the process of capillary formation. Reactive oxygen species, which are generated by Rac1-mediated activation of NADPH oxidase, are conversely implicated as both mediators of tumor necrosis factor (TNF)-induced apoptosis and in the promotion of cell growth and survival. It is reported that inhibition of Rac1 in HUVEC blocks TNF-induced intracellular bursts of reactive oxygen species, and leads to activation of NFkB, increases in caspase-3 activity, and augments the EC susceptibility to TNF-induced apoptosis [114], suggesting that Rac1-dependent reactive oxygen species generation leads to protection against TNF-induced cell death. Future studies should define the temporal pro- and anti-survival roles of the Rho-proteins using unstable EC monoculture and stabilized EC/fibroblast coculture systems, and examine differing signaling pathways that control Rho-protein function in capillary survival.

Conclusion

Given the necessity of the Rho-proteins in cellular processes such as permeability, ECM remodeling, migration, proliferation, morphogenesis, and survival, it is not surprising that these proteins play essential roles in many angiogenic processes. Significant amounts of research have led to the observation that Rho-proteins play important roles in capillary formation; however, future studies should examine Rho-protein activation in a stage-specific manner during the angiogenic process. These findings would likely

lead to a complex and coordinated interplay of Rho-protein activation/inactivation in a defined spatial and temporal manner. While this review examines the major members of the Rho-proteins, this family of proteins consists of over 20 members, with a multitude of activators and repressors. One study suggests unique roles for the less-known Rho-proteins in EC cytoskeletal reorganization, migration, and morphogenesis [115]; however, further assessment of these proteins during angiogenesis will provide a better understanding of the spatial and temporal control of capillary formation from its onset to completion. Considering the central role Rho-proteins play in mediating the angiogenic cascade, it is imperative that we further study this signaling pathway, as it may serve as an excellent target for therapeutic treatment of diseases of aberrant angiogenesis.

1 Ferrara, N., Gerber, H. P. and LeCouter, J. (2003) The biology of VEGF and its receptors. Nat. Med. 9, 669 – 676.
2 Jaffe, A. B. and Hall, A. (2005) Rho GTPases: biochemistry and biology. Annu. Rev. Cell. Dev. Biol. 21, 247 – 269.
3 Nikolic, M. (2002) The role of Rho GTPases and associated kinases in regulating neurite outgrowth. Int. J. Biochem. Cell. Biol. 34, 731 – 745.
4 Bryan, B. A., Mitchell, D. C., Zhao, L., Ma, W., Stafford, L. J., Teng, B. B. and Liu, M. (2005) Modulation of muscle regeneration, myogenesis, and adipogenesis by the Rho family guanine nucleotide exchange factor GEFT. Mol. Cell. Biol. 25, 11089 – 11101.
5 Bryan, B. A., Li, D., Wu, X. and Liu, M. (2005) The Rho family of small GTPases: crucial regulators of skeletal myogenesis. Cell. Mol. Life Sci. 62, 1547 – 1555.
6 Ridley, A .J. (2004) Rho proteins and cancer. Breast Cancer Res. Treat. 84, 13 – 19.
7 Ridley, A.J. (2001) Rho proteins, PI 3-kinases, and monocyte/macrophage motility. FEBS Lett. 498, 168 – 71.
8 Rossman, K. L., Der, C. J. and Sondek, J. (2005) GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. Nat. Rev. Mol. Cell. Biol. 6, 167 – 180.
9 Bernards, A. (2003) GAPs galore! A survey of putative Ras superfamily GTPase activating proteins in man and Drosophila. Biochim. Biophys. Acta 1603, 47 – 82.
10 DerMardirossian, C. and Bokoch, G. M. (2005) GDIs: central regulatory molecules in Rho GTPase activation. Trends Cell. Biol. 15, 356 – 363.
11 Zhao, Z. S. and Manser, E. (2005) PAK and other Rho-associated kinases –effectors with surprisingly diverse mechanisms of regulation. Biochem. J. 386, 201 – 214.
12 Wettschureck, N. and Offermanns, S. (2002) Rho/Rho-kinase mediated signaling in physiology and pathophysiology. J. Mol. Med. 80, 629 – 638.
13 Aspenstrom, P. (1999) Effectors for the Rho GTPases. Curr. Opin. Cell Biol. 11, 95 – 102.
14 Amano, M., Fukata, Y. and Kaibuchi, K. (2000) Regulation and functions of Rho-associated kinase. Exp. Cell. Res. 261, 44 – 51.
15 Sin, W. C., Chen, X. Q., Leung, T. and Lim, L. (1998) RhoA-binding kinase alpha translocation is facilitated by the collapse of the vimentin intermediate filament network. Mol. Cell. Biol. 18, 6325 – 6339.
16 Leung, T., Manser, E., Tan, L. and Lim, L. (1995) A novel serine/threonine kinase binding the Ras-related RhoA GTPase which translocates the kinase to peripheral membranes. J. Biol. Chem. 270, 29051 – 29054.
17 Matsui, T., Amano, M., Yamamoto, T., Chihara, K., Nakafuku, M., Ito, M., Nakaro, T., Okawa, K., Iwamatsu, A., and Kaibuchi, K. (1996) Rho-associated kinase, a novel serine/threonine kinase, as a putative target for small GTP binding protein Rho. EMBO J. 15, 2208 – 2216.
18 Maekawa, M., Ishizaki, T., Boku, S., Watanbe, N., Fujita, A., Iwamatsu, A., Obinata, T., Ohasi, K., Mizuno, K., and Nurumiya, S. (1999) Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase. Science 285, 895 – 898.
19 Lei, M., Lu, W., Meng, W., Parrini, M. C., Eck, M. J., Mayer, B. J. and Harrison, S. C. (2000) Structure of PAK1 in an autoinhibited conformation reveals a multistage activation switch. Cell 102, 387 – 397.
20 Zhao, Z. S., Manser, E., Chen, X. Q., Chong, C., Leung, T. and Lim, L. (1998) 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.
21 Park, H. J., Kong, D., Iruela-Arispe, L., Begley, U., Tang, D. and Galper, J. B. (2002) 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors interfere with angiogenesis by inhibiting the geranylgeranylation of RhoA. Circ. Res. 91, 143 – 150.
22 Hoang, M. V., Whelan, M. C. and Senger, D. R. (2004) Rho activity critically and selectively regulates endothelial cell organization during angiogenesis. Proc. Natl. Acad. Sci. USA 101, 1874 – 1879.
23 Connolly, J. O., Simpson, N., Hewlett, L. and Hall, A. (2002) Rac regulates endothelial morphogenesis and capillary assembly. Mol. Biol. Cell 13, 2474 – 2485.
24 van Nieuw Amerongen, G. P., Koolwijk, P., Versteilen, A. and van Hinsbergh, V. W. (2003) Involvement of RhoA/Rho kinase signaling in VEGF-induced endothelial cell migration and angiogenesis in vitro. Arterioscler. Thromb. Vasc. Biol. 23, 211 – 217.
25 Lum, H. and Malik, A. B. (1996) Mechanisms of increased endothelial permeability. Can. J. Physiol. Pharmacol. 74, 787 – 800.
26 Zeng, L., Xu, H., Chew, T. L., Eng, E., Sadeghi, M. M., Adler, S., Kanwar, Y. S. and Danesh, F. R. (2005) HMG CoA reductase inhibition modulates VEGF-induced endothelial cell hyperpermeability by preventing RhoA activation and myosin regulatory light chain phosphorylation. FASEB J. 19, 1845 – 1847.
27 Sun, H., Breslin, J. W., Zhu, J., Yuan, S. Y. and Wu, M. H. (2006) Rho and ROCK signaling in VEGF-induced microvascular endothelial hyperpermeability. Microcirculation 13, 237 – 247.
28 Holinstat, M., Mehta, D., Kozasa, T., Minshall, R. D. and Malik, A. B. (2003) Protein kinase Calpha-induced p115RhoGEF phosphorylation signals endothelial cytoskeletal rearrangement. J. Biol. Chem. 278, 28793 – 28798.
29 Birukova, A. A., Adyshev, D., Gorshkov, B., Bokoch, G. M., Birukov, K. G. and Verin, A. D. (2006) GEF-H1 is involved in agonist-induced human pulmonary endothelial barrier dysfunction. Am. J. Physiol. Lung. Cell. Mol. Physiol. 290, L540 – L548.
30 Mehta, D., Rahman, A. and Malik, A. B. (2001) Protein kinase C-alpha signals rho-guanine nucleotide dissociation inhibitor phosphorylation and rho activation and regulates the endothelial cell barrier function. J. Biol. Chem. 276, 22614 – 22620.
31 Kuroda, S., Fukata, M., Fujii, K., Nakamura, T., Izawa, I. and Kaibuchi, K. (1997) Regulation of cell-cell adhesion of MDCK cells by Cdc42 and Rac1 small GTPases. Biochem. Biophys. Res. Commun. 240, 430 – 435.
32 Vouret-Craviari, V., Grall, D. and Van Obberghen-Schilling, E. (2003) Modulation of Rho GTPase activity in endothelial cells by selective proteinase-activated receptor (PAR) agonists. J. Thromb. Haemost. 1, 1103 – 1111.

33 Qiao, J., Huang, F. and Lum, H. (2003) PKA inhibits RhoA activation: a protection mechanism against endothelial barrier dysfunction. Am. J. Physiol. Lung Cell. Mol. Physiol. 284, L972 – L980.

34 Holinstat, M., Knezevic, N., Broman, M., Samarel, A. M., Malik, A. B. and Mehta, D. (2006) Suppression of RhoA activity by focal adhesion kinase-induced activation of p190RhoGAP: role in regulation of endothelial permeability. J. Biol. Chem. 281, 2296 – 2305.

35 Singleton, P. A., Dudek, S. M., Chiang, E. T. and Garcia, J. G. (2005) Regulation of sphingosine 1-phosphate-induced endothelial cytoskeletal rearrangement and barrier enhancement by S1P1 receptor, PI3 kinase, Tiam1/Rac1, and alpha-actinin. FASEB J. 19, 1646 – 1656.

36 Seebach, J., Madler, H. J., Wojciak-Stothard, B. and Schnittler, H. J. (2005) Tyrosine phosphorylation and the small GTPase rac cross-talk in regulation of endothelial barrier function. Thromb. Haemost. 94, 620 – 629.

37 Waschke, J., Baumgartner, W., Adamson, R. H., Zeng, M., Aktories, K., Wilde, C., Curry, F. E., and Drenckhahn, D. (2004) Requirement of Rac activity for maintenance of capillary endothelial barrier properties. Am. J. Physiol. Heart Circ. Physiol. 286, H394 – H401.

38 Waschke, J., Burger, S., Curry, F. R., Drenckhahn, D. and Adamson, R. H. (2006) Activation of Rac-1 and Cdc42 stabilizes the microvascular endothelial barrier. Histochem. Cell Biol. 125, 397 – 406.

39 Kouklis, P., Konstantoulaki, M., Vogel, S., Broman, M. and Malik, A. B. (2004) Cdc42 regulates the restoration of endothelial barrier function. Circ. Res. 94, 159 – 166.

40 Turchi, L., Chassot, A. A., Bourget, I., Baldescchi, C., Ortonne, J. P., Meneguzzi, G., Lemichez, E. and Ponzio, G. (2003) Cross-talk between RhoGTPases and stress activated kinases for matrix metalloproteinase-9 induction in response to keratinocytes injury. J. Invest. Dermatol. 121, 1291 – 1300.

41 Turner, N. A., O'Regan, D. J., Ball, S. G. and Porter, K. E. (2005) Simvastatin inhibits MMP-9 secretion from human saphenous vein smooth muscle cells by inhibiting the RhoA/ROCK pathway and reducing MMP-9 mRNA levels. FASEB J. 19, 804 – 6.

42 Rajagopalan, S., Meng, X. P., Ramasamy, S., Harrison, D. G. and Galis, Z. S. (1996) Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. J. Clin. Invest. 98, 2572 – 2579.

43 Bond, M., Chase, A. J., Baker, A. H. and Newby, A. C. (2001) Inhibition of transcription factor NF-kappaB reduces matrix metalloproteinase-1, -3 and -9 production by vascular smooth muscle cells. Cardiovasc. Res. 50, 556 – 565.

44 Yoshida, M., Korfhagen, T. R. and Whitsett, J. A. (2001) Surfactant protein D regulates NF-kappa B and matrix metalloproteinase production in alveolar macrophages via oxidant-sensitive pathways. J. Immunol. 166, 7514 – 7519.

45 Marinissen, M. J., Chiariello, M., Tanos, T., Bernard, O., Narumiya, S. and Gutkind, J. S. (2004) The small GTP-binding protein RhoA regulates c-jun by a ROCK-JNK signaling axis. Mol. Cell. 14, 29 – 41.

46 Perona, R., Montaner, S., Saniger, L., Sanchez-Perez, I., Bravo, R. and Lacal, J. C. (1997) Activation of the nuclear factor-kappaB by Rho, CDC42, and Rac-1 proteins. Genes Dev. 11, 463 – 475.

47 Deroanne, C. F., Hamelryckx, D., Ho, T. T., Lambert, C. A., Catroux, P., Lapiere, C. M. and Nusgens, B. V. (2005) Cdc42 downregulates MMP-1 expression by inhibiting the ERK1/2 pathway. J. Cell Sci. 118, 1173 – 1183.

48 Engers, R., Springer, E., Michiels, F., Collard, J. G. and Gabbert, H. E. (2001) Rac affects invasion of human renal cell carcinomas by up-regulating tissue inhibitor of metalloproteinases (TIMP)-1 and TIMP-2 expression. J. Biol. Chem. 276, 41889 – 41897.

49 Abecassis, I., Olofsson, B., Schmid, M., Zalcman, G. and Karniguian, A. (2003) RhoA induces MMP-9 expression at CD44 lamellipodial focal complexes and promotes HMEC-1 cell invasion. Exp. Cell. Res. 291, 363 – 376.

50 Oh, J., Diaz, T., Wei, B., Chang, H., Noda, M. and Stetler-Stevenson, W. G. (2006) TIMP-2 upregulates RECK expression via dephosphorylation of paxillin tyrosine residues 31 and 118. Oncogene 25, 4230 – 4234.

51 Wang, H. and Keiser, J. A. (1998) Vascular endothelial growth factor upregulates the expression of matrix metalloproteinases in vascular smooth muscle cells: role of flt-1. Circ. Res. 83, 832 – 840.

52 Lamoreaux, W. J., Fitzgerald, M. E., Reiner, A., Hasty, K. A. and Charles, S. T. (1998) Vascular endothelial growth factor increases release of gelatinase A and decreases release of tissue inhibitor of metalloproteinases by microvascular endothelial cells in vitro. Microvasc. Res. 55, 29 – 42.

53 Raftopoulou, M. and Hall, A. (2004) Cell migration: Rho GTPases lead the way. Dev. Biol. 265, 23 – 32.

54 Paik, J. H., Chae, S., Lee, M. J., Thangada, S. and Hla, T. (2001) Sphingosine 1-phosphate-induced endothelial cell migration requires the expression of EDG-1 and EDG-3 receptors and Rho-dependent activation of alpha vbeta3- and beta1-containing integrins. J. Biol. Chem. 276, 11830 – 11837.

55 Panetti, T. S., Nowlen, J. and Mosher, D. F. (2000) Sphingosine-1-phosphate and lysophosphatidic acid stimulate endothelial cell migration. Arterioscler. Thromb. Vasc. Biol. 20, 1013 – 1019.

56 Okamoto, H., Yatomi, Y., Ohmori, T., Satoh, K., Matsumoto, Y. and Ozaki, Y. (2000) Sphingosine 1-phosphate stimulates G(i)- and Rho-mediated vascular endothelial cell spreading and migration. Thromb. Res. 99, 259 – 265.

57 Nakayama, M., Amano, M., Katsumi, A., Kaneko, T., Kawabata, S., Takefuji, M. and Kaibuchi, K. (2005) Rho-kinase and myosin II activities are required for cell type and environment specific migration. Genes Cells 10, 107 – 117.

58 Gong, C., Stoletov, K. V. and Terman, B. I. (2004) VEGF treatment induces signaling pathways that regulate both actin polymerization and depolymerization. Angiogenesis 7, 313 – 321.

59 Wang, H. R., Zhang, Y., Ozdamar, B., Ogunjimi, A. A., Alexandrova, E., Thomsen, G. H. and Wrana, J. L. (2003) Regulation of cell polarity and protrusion formation by targeting RhoA for degradation. Science 302, 1775 – 1779.

60 Bryan, B., Cai, Y., Wrighton, K., Wu, G., Feng, X. H. and Liu, M. (2005) Ubiquitination of RhoA by Smurf1 promotes neurite outgrowth. FEBS Lett. 579, 1015 – 1019.

61 Kurokawa, K. and Matsuda, M. (2005) Localized RhoA activation as a requirement for the induction of membrane ruffling. Mol. Biol. Cell. 16, 4294 – 4303.

62 Parrini, M. C., Matsuda, M. and de Gunzburg, J. (2005) Spatiotemporal regulation of the Pak1 kinase. Biochem. Soc. Trans. 33, 646 – 648.

63 Cascone, I., Giraudo, E., Caccavari, F., Napione, L., Bertotti, E., Collard, J. G., Serini, G. and Bussolino, F. (2003) Temporal and spatial modulation of Rho GTPases during in vitro formation of capillary vascular network. Adherens junctions and myosin light chain as targets of Rac1 and RhoA. J. Biol. Chem. 278, 50702 – 50713.

64 Martins, G. G., and Kolega, J. (2006) Endothelial cell protrusion and migration in three-dimensional collagen matrices. Cell Motil. Cytoskeleton 63, 101 – 115.

65 Sahai, E., Garcia-Medina, R., Pouyssegur, J., and Vial, E. (2007) Smurf1 regulates tumor cell plasticity and motility through degradation of RhoA leading to localized inhibition of contractility. J. Cell. Biol. 176, 35 – 42.

66 Huang, S. and Ingber, D. E. (2002) A discrete cell cycle checkpoint in late G(1) that is cytoskeleton-dependent and MAP kinase (Erk)-independent. Exp. Cell. Res. 275, 255 – 264.

67 Croft, D. R. and Olson, M. F. (2006) The Rho GTPase effector ROCK regulates cyclin A, cyclin D1, and p27Kip1 levels by distinct mechanisms. Mol. Cell. Biol. 26, 4612 – 4627.
68 Page, K., Li, J. and Hershenson, M. B. (1999) Platelet-derived growth factor stimulation of mitogen-activated protein kinases and cyclin D1 promoter activity in cultured airway smooth-muscle cells. Role of Ras. Am. J. Respir. Cell. Mol. Biol. 20, 1294 – 1302.
69 Philips, A., Roux, P., Coulon, V., Bellanger, J. M., Vie, A., Vignais, M. L. and Blanchard, J. M. (2000) Differential effect of Rac and Cdc42 on p38 kinase activity and cell cycle progression of nonadherent primary mouse fibroblasts. J. Biol. Chem. 275, 5911 – 5917.
70 Bao, W., Thullberg, M., Zhang, H., Onischenko, A. and Stromblad, S. (2002) Cell attachment to the extracellular matrix induces proteasomal degradation of p21(CIP1) via Cdc42/Rac1 signaling. Mol. Cell. Biol. 22, 4587 – 4597.
71 Somlyo, A. V., Phelps, C., Dipierro, C., Eto, M., Read, P., Barrett, M., Gibson, J. J., Burnitz, M. C., Myers, C., and Somlyo, A. P. (2003) Rho kinase and matrix metalloproteinase inhibitors cooperate to inhibit angiogenesis and growth of human prostate cancer xenotransplants. FASEB J. 17, 223 – 234.
72 Pille, J. Y., Denoyelle, C., Varet, J., Bertrand, J. R., Soria, J., Opolon, P., Lu, H., Pritchard, L. L., Vannier, J. P., Malvy, C. et al. (2005) 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.
73 Singleton, P. A., Lingen, M. W., Fekete, M. J., Garcia, J. G. and Moss, J. (2006) Methylnaltrexone inhibits opiate and VEGF-induced angiogenesis: role of receptor transactivation. Microvasc. Res. 72, 3 – 11.
74 Yamaoka-Tojo, M., Ushio-Fukai, M., Hilenski, L., Dikalov, S. I., Chen, Y. E., Tojo, T., Fukai, T., Fujimoto, M., Patrushev, N. A., Wang, N. et al. (2004) IQGAP1, a novel vascular endothelial growth factor receptor binding protein, is involved in reactive oxygen species–dependent endothelial migration and proliferation. Circ. Res. 95, 276 – 283.
75 Zeng, H., Sanyal, S. and Mukhopadhyay, D. (2001) Tyrosine residues 951 and 1059 of vascular endothelial growth factor receptor-2 (KDR) are essential for vascular permeability factor/vascular endothelial growth factor-induced endothelium migration and proliferation, respectively. J. Biol. Chem. 276, 32714 – 32719.
76 Vernon, R. B., Angello, J. C., Iruela-Arispe, M. L., Lane, T. F. and Sage, E. H. (1992) Reorganization of basement membrane matrices by cellular traction promotes the formation of cellular networks in vitro. Lab. Invest. 66, 536 – 547.
77 Davis, G. E. and Camarillo, C. W. (1995) Regulation of endothelial cell morphogenesis by integrins, mechanical forces, and matrix guidance pathways. Exp. Cell. Res. 216, 113 – 123.
78 Davis, G. E., Bayless, K. J. and Mavila, A. (2002) Molecular basis of endothelial cell morphogenesis in three-dimensional extracellular matrices. Anat. Rec. 268, 252 – 275.
79 Kiosses, W. B., Hood, J., Yang, S., Gerritsen, M. E., Cheresh, D. A., Alderson, N. and Schwartz, M. A. (2002) A dominant-negative p65 PAK peptide inhibits angiogenesis. Circ. Res. 90, 697 – 702.
80 Moore, K. A., Huang, S., Kong, Y., Sunday, M. E. and Ingber, D. E. (2002) Control of embryonic lung branching morphogenesis by the Rho activator, cytotoxic necrotizing factor 1. J. Surg. Res. 104, 95 – 100.
81 Moore, K. A., Polte, T., Huang, S., Shi, B., Alsberg, E., Sunday, M. E. and Ingber, D. E. (2005) Control of basement membrane remodeling and epithelial branching morphogenesis in embryonic lung by Rho and cytoskeletal tension. Dev. Dyn. 232, 268 – 281.
82 Hiraoka, N., Allen, E., Apel, I. J., Gyetko, M. R. and Weiss, S. J. (1998) Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. Cell 95, 365 – 377.
83 Hotary, K., Allen, E., Punturieri, A., Yana, I. and Weiss, S. J. (2000) Regulation of cell invasion and morphogenesis in a three-dimensional type I collagen matrix by membrane-type matrix metalloproteinases 1, 2, and 3. J. Cell. Biol. 149, 1309 – 1323.
84 Davis, G. E., Black, S. M. and Bayless, K. J. (2000) Dual stimulation of Ras/mitogen-activated protein kinase and RhoA by cell adhesion to fibronectin supports growth factor-stimulated cell cycle progression. In Vitro Cell Dev. Biol. Anim. 36, 513 – 519.
85 Vu, T. H., Shipley, J. M., Bergers, G., Berger, J. E., Helms, J. A., Hanahan, D., Shapiro, S. D., Senior, R. M., and Werb, Z. (1998) MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. Cell 93, 411 – 422.
86 Bergers, G., McMahon, G., Vu, T. H., Itoh, T., Tamaki, K., Tanzawak, K., Thorpe, P., Itohara, S., Werb, Z., and Hanahan, D. (2000) Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat. Cell Biol. 2, 737 – 744.
87 Clark, E. (1939) Microscopic observations on the growth of blood capillaries in the living mammal. Am. J. Anat. 64, 251 – 301.
88 Davis, G. E. and Camarillo, C. W. (1996) An alpha 2 beta 1 integrin-dependent pinocytic mechanism involving intracellular vacuole formation and coalescence regulates capillary lumen and tube formation in three-dimensional collagen matrix. Exp. Cell. Res. 224, 39 – 51.
89 Davis, G. E. and Bayless, K. J. (2003) An integrin and Rho GTPase-dependent pinocytic vacuole mechanism controls capillary lumen formation in collagen and fibrin matrices. Microcirculation 10, 27 – 44.
90 Kamei, M., Saunders, W. B., Bayless, K. J., Dye, L., Davis, G. E. and Weinstein, B. M. (2006) Endothelial tubes assemble from intracellular vacuoles in vivo. Nature 442, 453 – 456.
91 Bayless, K. J. and Davis, G. E. (2002) Molecular basis of endothelial cell morphogenesis in three-dimensional extracellular matrices. J. Cell. Sci. 115, 1123 – 1136.
92 Caron, E. and Hall, A. (1998) Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases. Science 282, 1717 – 1721.
93 Chimini, G. and Chavrier, P. (2000) Function of Rho family proteins in actin dynamics during phagocytosis and engulfment. Nat. Cell. Biol. 2, E191 – E196.
94 Cox, D., Chang, P., Zhang, Q., Reddy, P. G., Bokoch, G. M. and Greenberg, S. (1997) Requirements for both Rac1 and Cdc42 in membrane ruffling and phagocytosis in leukocytes. J. Exp. Med. 186, 1487 – 1494.
95 Lorenzi, R., Brickell, P. M., Katz, D. R., Kinnon, C. and Thrasher, A. J. (2000) Wiskott-Aldrich syndrome protein is necessary for efficient IgG-mediated phagocytosis. Blood 95, 2943 – 2946.
96 Dharmawardhane, S., Schurmann, A., Sells, M. A., Chernoff, J., Schmid, S. L. and Bokoch, G. M. (2000) Regulation of macropinocytosis by p21-activated kinase-1. Mol. Biol. Cell. 11, 3341 – 3352.
97 Diakonova, M., Bokoch, G. and Swanson, J. A. (2002) Dynamics of cytoskeletal proteins during Fcgamma receptor-mediated phagocytosis in macrophages. Mol. Biol. Cell. 13, 402 – 411.
98 Hoppe, A. D. and Swanson, J. A. (2004) Cdc42, Rac1, and Rac2 display distinct patterns of activation during phagocytosis. Mol. Biol. Cell. 15, 3509 – 3519.
99 Giry, M., Popoff, M. R., von Eichel-Streiber, C. and Boquet, P. (1995) Transient expression of RhoA, -B, and -C GTPases in HeLa cells potentiates resistance to Clostridium difficile toxins A and B but not to Clostridium sordellii lethal toxin. Infect. Immun. 63, 4063 – 4071.
100 Fiorentini, C., Fabbri, A., Flatau, G., Donelli, G., Matarrese, P., Lemichez, E., Falzano, L. and Boquet, P. (1997) Escherichia coli cytotoxic necrotizing factor 1 (CNF1), a toxin that activates the Rho GTPase. J. Biol. Chem. 272, 19532 – 19537.

101 Henning, S. W., Galandrini, R., Hall, A. and Cantrell, D. A. (1997) The GTPase Rho has a critical regulatory role in thymus development. EMBO J. 16, 2397 – 2407.
102 Bobak, D., Moorman, J., Guanzon, A., Gilmer, L. and Hahn, C. (1997) Inactivation of the small GTPase Rho disrupts cellular attachment and induces adhesion-dependent and adhesion-independent apoptosis. Oncogene 15, 2179 – 2189.
103 Jimenez, B., Arends, M., Esteve, P., Perona, R., Sanchez, R., Ramon y Cajal, S., Wyllie, A. and Lacal, J. C. (1995) Induction of apoptosis in NIH3T3 cells after serum deprivation by overexpression of rho-p21, a GTPase protein of the ras superfamily. Oncogene 10, 811 – 816.
104 del Peso, L., Hernandez-Alcoceba, R., Embade, N., Carnero, A., Esteve, P., Paje, C. and Lacal, J. C. (1997) Rho proteins induce metastatic properties in vivo. Oncogene 15, 3047 – 3057.
105 Chuang, T. H., Hahn, K. M., Lee, J. D., Danley, D. E. and Bokoch, G. M. (1997) The small GTPase Cdc42 initiates an apoptotic signaling pathway in Jurkat T lymphocytes. Mol. Biol. Cell. 8, 1687 – 1698.
106 Embade, N., Valeron, P. F., Aznar, S., Lopez-Collazo, E. and Lacal, J. C. (2000) Apoptosis induced by Rac GTPase correlates with induction of FasL and ceramides production. Mol. Biol. Cell. 11, 4347 – 4358.
107 Hippenstiel, S., Schmeck, B., N'Guessan, P. D., Seybold, J., Krull, M., Preissner, K., Eichel-Streiber, C. V. and Suttorp, N. (2002) Rho protein inactivation induced apoptosis of cultured human endothelial cells. Am. J. Physiol. Lung Cell. Mol. Physiol. 283, L830 – L838.
108 Petrache, I., Crow, M. T., Neuss, M. and Garcia, J. G. (2003) Central involvement of Rho family GTPases in TNF-alpha-mediated bovine pulmonary endothelial cell apoptosis. Biochem. Biophys. Res. Commun. 306, 244 – 249.
109 Li, X., Liu, L., Tupper, J. C., Bannerman, D. D., Winn, R. K., Sebti, S. M., Hamilton, A. D. and Harlan, J. M. (2002) Inhibition of protein geranylgeranylation and RhoA/RhoA kinase pathway induces apoptosis in human endothelial cells. J. Biol. Chem. 277, 15309 – 15316.
110 Adini, I., Rabinovitz, I., Sun, J. F., Prendergast, G. C. and Benjamin, L. E. (2003) RhoB controls Akt trafficking and stage-specific survival of endothelial cells during vascular development. Genes Dev. 17, 2721 – 2732.
111 Kamasani, U., Huang, M., Duhadaway, J. B., Prochownik, E. V., Donover, P. S. and Prendergast, G. C. (2004) Cyclin B1 is a critical target of RhoB in the cell suicide program triggered by farnesyl transferase inhibition. Cancer Res. 64, 8389 – 8396.
112 Liu, A. and Prendergast, G. C. (2000) Geranylgeranylated RhoB is sufficient to mediate tissue-specific suppression of Akt kinase activity by farnesyltransferase inhibitors. FEBS Lett. 481, 205 – 208.
113 Mavria, G., Vercoulen, Y., Yeo, M., Paterson, H., Karasarides, M., Marais, R., Bird, D. and Marshall, C. J. (2006) ERK-MAPK signaling opposes Rho-kinase to promote endothelial cell survival and sprouting during angiogenesis. Cancer Cell 9, 33 – 44.
114 Deshpande, S. S., Angkeow, P., Huang, J., Ozaki, M. and Irani, K. (2000) Rac1 inhibits TNF-alpha-induced endothelial cell apoptosis: dual regulation by reactive oxygen species. FASEB J. 14, 1705 – 1714.
115 Aspenstrom, P., Fransson, A. and Saras, J. (2004) Rho GTPases have diverse effects on the organization of the actin filament system. Biochem. J. 377, 327 – 337.