ANTICANCER RESEARCH *31*: 3645-3658 (2011)

Review

Rho Kinase Proteins—Pleiotropic Modulators of Cell Survival and Apoptosis

CATHARINE A. STREET[1] and BRAD A. BRYAN[2]

[1]*Ghosh Science and Technology Center, Department of Biology, Worcester State University, Worcester, MA, U.S.A.;*
[2]*Center of Excellence in Cancer Research, Department of Biomedical Sciences, Paul L. Foster School of Medicine, Texas Tech University Health Sciences Center, El Paso, TX, U.S.A.*

Abstract. *Rho kinase (ROCK) proteins are Rho-GTPase activated serine/threonine kinases that function as modulators of actin-myosin cytoskeletal dynamics via regulation of Lin11, Isl-1 & Mec-3 domain (LIM) kinase, myosin light chain (MLC), and MLC phosphatase. A strong correlation between cytoskeletal rearrangements and tumor cell invasion, metastasis, and deregulated microenvironment interaction has been reported in the literature, and the utilization of pharmacological inhibitors of ROCK signaling for the treatment of cancer is actively being pursued by a number of pharmaceutical companies. Indeed, in many preclinical models ROCK inhibitors have shown remarkable efficacy in reducing tumor growth and metastasis. Interestingly, ROCK signaling has been shown to be either pro-apoptotic or pro-survival in a cell type and context dependent manner, though the molecular mechanisms controlling ROCK-mediated cell fate decisions are unknown. This review summarizes the many pleiotropic roles of ROCK signaling in survival and apoptosis, and suggests that controlled modulation of ROCK activity in tumor cells has the potential to significantly affect tumor survival and patient outcome.*

ROCK Proteins

Rho associated protein kinases (ROCK, also known as Rho kinase) belong to a family of serine/threonine kinases modulated by interactions with Rho GTPases to serve as key regulators of actin cytoskeletal dynamics, and therefore control cell migration and motility (1). Specifically, ROCK proteins promote the formation of stress fibers and focal adhesions (Figure 1), but have also been implicated in diverse processes such as cell junction integrity and cell cycle control (2). ROCK activity is responsible for stabilization of actin microfilaments as well as promoting cellular contraction and cell substratum contact. ROCK stimulates actin polymerization *via* an inhibitory phosphorylation of the actin severing LIM kinase (Figure 2). ROCK promotes cellular contraction and attachment *via* an activating phosphorylation of myosin light chain (MLC) to increase myosin ATPase activity, and an inhibitory phosphorylation of MLC phosphatase leading to increased activation of MLC (Figure 3). Additionally, numerous other downstream targets of ROCK proteins have been identified including, but not limited to, intermediate filaments, ezrin/radixin/moesin (ERM) family proteins, collapsing response mediator protein 2 (CRMP2), calponin and adducin.

Two paralogs of ROCK have been identified in mammals (ROCK1 and ROCK2). These proteins were originally isolated as RhoA-GTP interacting proteins, and share 65% overall identity and 92% identity in their kinase domains (1). ROCK1 and ROCK2 are widely expressed from C. elegans to mammals and demonstrate both overlapping and unique tissue expression patterns and signaling functions within the cell. ROCK1 and ROCK2 knockout mice show distinct phenotypes, suggesting these proteins perform, at least to some degree, non-overlapping roles during development. ROCK1 knockout mice exhibit failure of eyelid and ventral body wall closure resulting in lethality soon after birth (3), while ROCK2 knockout mice exhibit embryonic lethality due to intrauterine growth retardation and placental dysfunction (4). The generation of heterozygote ROCK1 and ROCK2 mice leads to viable, fertile litters with no obvious phenotypic abnormalities, however a detailed examination of

Correspondence to: Brad A. Bryan, Ph.D., Texas Tech University Health Sciences Center, Paul Foster School of Medicine, Center of Excellence in Cancer Research, 5001 El Paso Blvd., El Paso, TX 79905, U.S.A. Tel: +1 9157835235, Fax: +1 9157835222, e-mail: brad.bryan@ttuhsc.edu

Key Words: ROCK, Rho kinase, ROCK signaling, ROCK pathways, review.

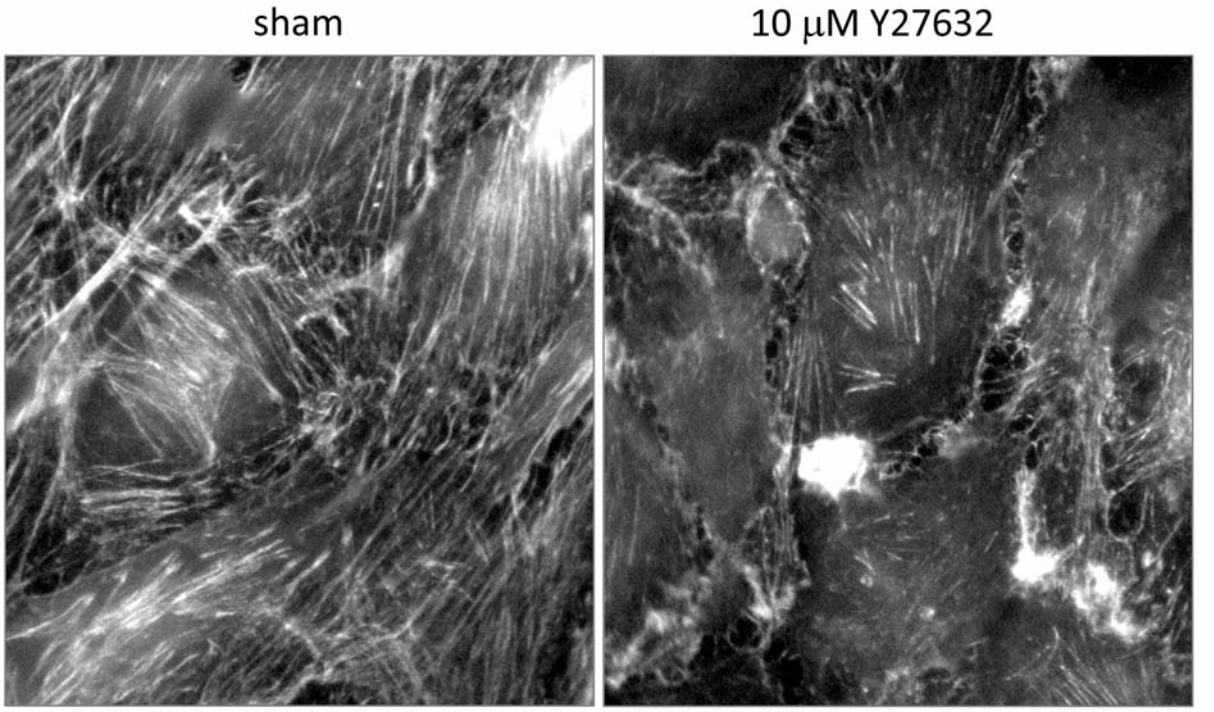

Figure 1. *ROCK activity in actin polymerization. MS1 endothelial cells were sham treated or treated with 10 μM of the ROCK1 and 2 pharmacological inhibitor Y27632. Cells were then stained with FITC-labelled phalloidin, which specifically binds to polymerized actin microfilaments. Disruption of total ROCK activity results in a dramatic reduction in the quantity of polymerized actin.*

ROCK1(+/–) mice revealed reduced neointima formation following carotid artery ligation correlating with decreased vascular smooth muscle cell proliferation and survival, decreased levels of proinflammatory adhesion molecule expression, and decreased leukocyte infiltration (5). Moreover, ROCK1(+/–) mice exhibit increased resistance to perivascular fibrosis, accompanied by decreased expression of tissue growth factor-beta, connective tissue growth factor and type III collagen (6). ROCK2(+/–), but not ROCK1(+/–), mice demonstrate no obvious cardiac phenotype, however they display decreased platelet endothelial cell adhesion molecule staining of endothelial cells in the lung, suggesting that ROCK2 plays a strong role in capillary development (7).

Deregulation of Rho/ROCK signaling has been reported across diverse tumors types. For instance, Rho-signaling proteins are elevated in, and contribute to the metastatic behavior of a variety of tumors (8-12). Several preclinical and clinical studies have utilized inhibitors of Rho/ROCK signaling for anticancer therapeutics in prostate, lung, melanoma, glioblastoma and many other tumor types with remarkable success (13-17). Many of the positive outcomes claimed from targeting Rho/ROCK signaling have been attributed to a reduction in invasion/metastatic potential of the cancer cells; however a wealth of findings have demonstrated that ROCK proteins are key modulators of cell survival and apoptosis, suggesting that cell viability may also be affected by ROCK inhibition.

An Overview of Apoptosis

Apoptosis is a controlled form of cell death that involves cell shrinkage, membrane blebbing, cellular disintegration, chromosome condensation, and subsequent removal of the apoptotic fragments by phagocytosis (1). This process is initiated by activation of caspase cystein proteases which cleave a large number of downstream protein targets to induce orderly morphological and biochemical changes within the cell, involving reorganization of actin microfilaments, microtubules, and intermediate filaments. The initial stages of apoptosis involve partial detachment of the cell from the extracellular matrix (ECM) due to caspase-mediated cleavage of focal adhesion kinase (FAK) as well as other structural proteins linking actin to focal adhesions (18). Following focal adhesion disassembly at the cell periphery, extensive cellular retraction occurs due to a loss of stress fibers and reorganization of actin microfilaments to form short fibers that bundle together and increase the tensile strength of the cell. As a consequence of retraction, cells undergoing apoptosis round up and reassemble new focal adhesion complexes ventral to the retracted cell body. Moreover, the formation of dynamic membrane protrusions

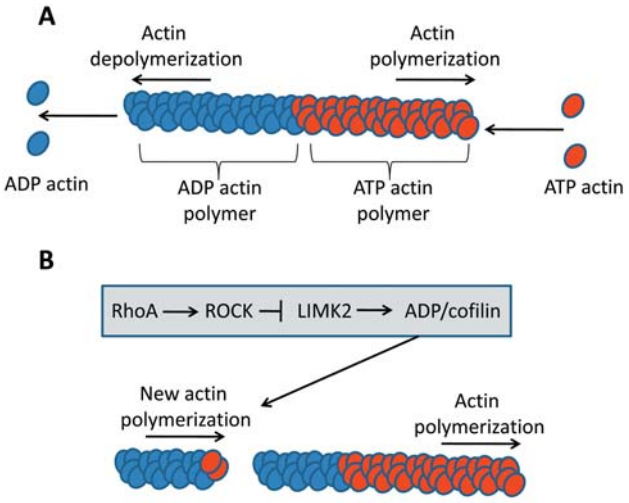

Figure 2. *ROCK control of actin polymerization. A: Individual subunits of ATP-bound globular actin (G-actin) assemble into long filamentous polymers (F-actin), creating a double helix structure. Hydrolysis of the ATP destabilizes the polymer, causing dissolution of F-actin polymers into G-actin monomers. B: ROCK stimulates stabilization of actin polymerization via an inhibitory phosphorylation of Lin11, Isl1, Mec3 (LIM) domain kinase (LIMK), which when active promotes ADP/cofilin-mediated actin severing.*

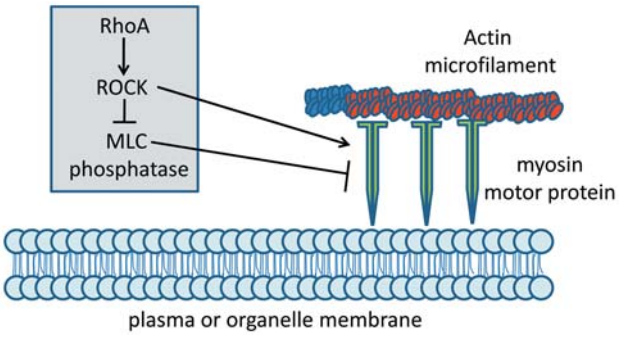

Figure 3. *ROCK control of cellular contractility. Actin filaments in association with myosin motor proteins control cellular movement, cell division and other biological processes across all cell types. ROCK promotes cellular contraction and attachment via an activating phosphorylation of myosin light chain (MLC) to increase myosin ATPase activity, and an inhibitory phosphorylation of MLC phosphatase leading to increased activation of MLC.*

called blebs is driven by modulation of actin-myosin activity, creating hydrodynamic forces during contraction to induce collapse at points of structural weakness within the cell (19, 20). Occurring concomitant with this process is caspase-8 mediated activation of deoxyriboneuclease, which catalyzes internucleosomal DNA cleavage (21). Finally, apoptotic bodies of varying size and composition are produced in an actin/myosin dependent manner and are phagocytized by nearby cells and scavenging immune cells, to be ultimately degraded by lysosomal enzymes (22).

Apoptosis can be activated in the cell by two major processes: the intrinsic and extrinsic apoptotic pathways. The extrinsic apoptotic pathway responds to secreted death ligands (such as apoptosis stimulating fragment [Fas] ligand, tumor necrosis factor [TNF] alpha and tumor necrosis factor alpha related apoptosis inducing ligand [TRAIL]) that bind specifically to transmembrane death receptors (such as TNF-R, Fas and TRAIL-R) in the target cell, initiating a signal for apoptosis. Death ligand activation of these receptors induces the formation of a death-inducing signaling complex (DISC) composed of the death ligand, a trimeric death receptor and death domain containing adaptor proteins which trigger cleavage of caspases into their active form (23). This process leads to further rounds of caspase cleavage and activation, resulting in cellular apoptosis.

The intrinsic apoptotic pathway is initiated as a p53 induced cascade in response to DNA damage, defective cell

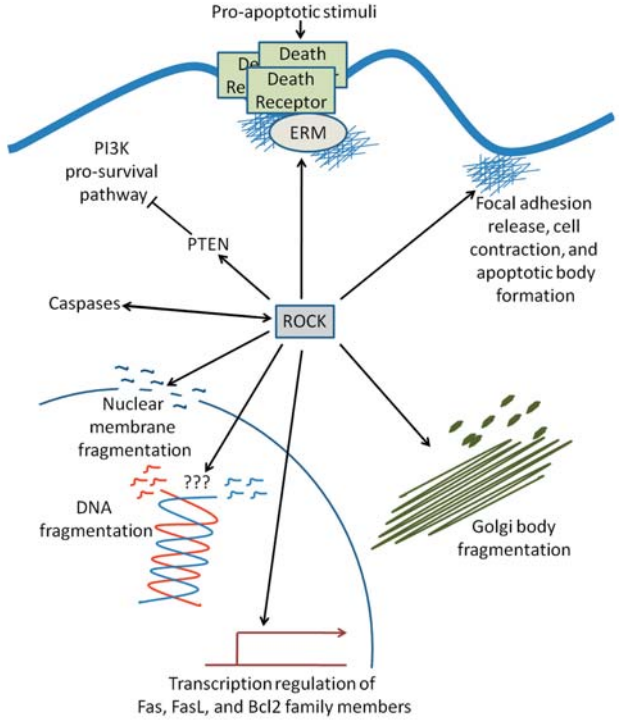

Figure 4. *Essential role of ROCK in apoptosis. ROCK proteins are activated by caspase cleavage and promote the cleavage of procaspases into their active caspase forms. ROCK activity is necessary for multiple aspects of both intrinsic and extrinsic apoptosis including death receptor activation via ezrin, radixin, and moesin (ERM) proteins, apoptotic bleb and body formation, nuclear and organelle fragmentation, and DNA fragmentation. Moreover, ROCK phosphorylates and inhibits phosphatase and tensin homology (PTEN), thus blocking the pro-survival phosphoinositide 3-kinase (PI3K) pathway.*

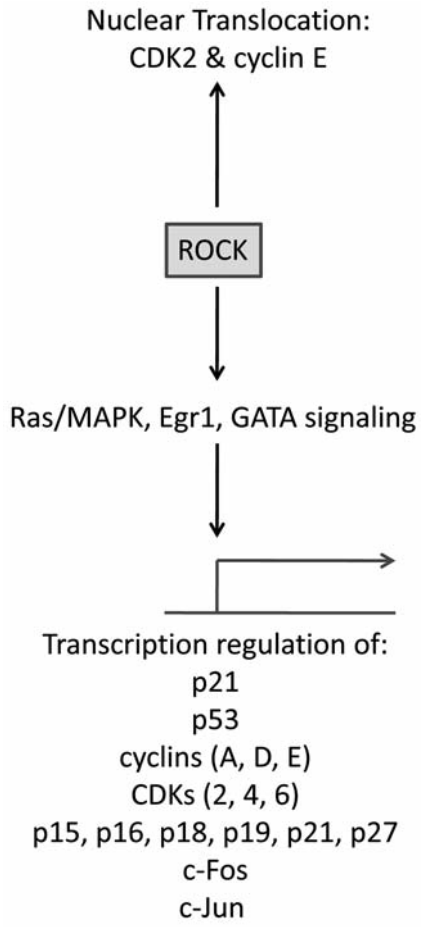

Figure 5. *Role of ROCK activity in cell survival. ROCK activity is necessary for progression from the G_1 to S-phase of the cell cycle by controlling the expression of cyclins, cyclin dependent kinases (CDKs), and numerous other cell cycle regulators. Additionally, ROCK activity has been shown to promote CDK2 and cyclin E translocation into the nucleus.*

cycle progression, or other severe cell stresses. This pathway is regulated by the fine balance of B-cell CLL/lymphoma 2 (Bcl2) family proteins within the cell (24). The Bcl2 proteins are apoptotic regulatory proteins that modulate mitochondrial membrane permeability, with some members being pro apoptotic and others anti-apoptotic. Under normal conditions, the anti-apoptotic Bcl2 proteins (such as Bcl2, Bcl-xl, BclW, bifunctional Bcl2 family protein 1 [Bfl1], myeloid leukemia cell differentiation protein 1 [Mcl1], Bcl2 related protein A1 [A1], and Bcl2 homologue of ovary [Boo]) maintain mitochondrial integrity by counteracting the activation and function of pro-apoptotic Bcl2 family members (such as Bcl2 associated X protein [Bax], Bcl2 homologous antagonist killer [Bak], BclX5, Bcl2 associated death promoter [Bad], BH3 interacting domain death agonist [Bid], Blc2 interacting killer [Bik], and hara-kiri [Hrk]) whose role is to induce mitochondrial damage. When pro-apoptotic Bcl2 proteins are activated, cytochrome-c is released from the mitochondria where it binds with apoptotic protease activating factor 1 (Apaf-1), forming the apoptosome. The activation of initiator caspases by the apoptosome begins a cascade of cleavage events ultimately leading to cellular apoptosis.

Role of ROCK Proteins in Apoptosis/Survival

Both disassembly and excessive crosslinking of the actin microfilament cytoskeletal architecture has been extensively demonstrated to induce apoptosis in numerous cell types (25-28) through modulation of signaling components such as Bcl2 activation (29), death receptor activation (30, 31), caspase activation (32), and p53 signaling (33). Moreover, an intimate association exists between cytoskeletal dynamics, the extracellular microenvironment, cell-to-cell adhesions and cell-to-substratum adhesions, where alterations in any of these components could be detrimental to the survival of the cell (34). ROCK protein signaling reportedly acts in either a pro- or anti-apoptotic fashion depending on cell type, cell context and microenvironment. For instance, ROCK proteins are essential for multiple aspects of both the intrinsic and extrinsic apoptotic processes, including regulation of cytoskeletal-mediated cell contraction and membrane blebbing, nuclear membrane disintegration, modulation of Bcl2-family member and caspase expression/activation and phagocytosis of the fragmented apoptotic bodies (discussed extensively below, Figure 4). In contrast, ROCK signaling exhibited pro-survival roles in a number of experimental studies (Figure 5) (14, 15, 35-40). Though a wealth of data exists to suggest both pro- and anti-survival roles for ROCK proteins, the molecular mechanisms that modulate these pleitropic roles are largely unknown.

ROCK Protein Regulation of Apoptosis

Essential roles of ROCK proteins in apoptosis. ROCK proteins are direct targets of caspase activity, whereby caspase 2 and 3 cleavage of ROCK proteins occurs in early apoptosis, thus removing the ROCK autoinhibitory C-terminal domain. This results in constitutive kinase activity of ROCK and its subsequent regulation of actin-myosin cellular contraction (41-47). Of note, ROCK cleavage also occurs early in apoptosis in a caspase-independent manner during endothelial release of microparticles and during invasion of colorectal cancer cells (48, 49). Granzyme-B has been shown to directly cleave ROCK2 in a caspase-independent manner, leading to cytotoxic lymphocyte granule-induced apoptosis (46). Whether *via* a caspase dependent or independent route, ROCK cleavage is an

essential step for apoptosis given that pharmacological inhibition of its kinase activity effectively abrogates apoptosis in a number of cell types.

In blebbing cells, caspase-cleaved ROCK-mediated phosphorylation of MLC is increased, thereby inducing contraction of cortical actin within the cell (50 19, 44, 45). Indeed, transfection of cells with either a truncated (constitutively activated) ROCK1 gene or overexpression of a wild type ROCK2 gene is sufficient to induce MLC-mediated membrane blebbing independently of apoptotic stimuli (44, 51). Studies using cytoskeletal or ROCK inhibitors have identified multiple stages in apoptotic blebbing. For instance, caspase independent blebbing (zeiosis) occurs immediately after cytochrome c release from the mitochondria into the cytoplasm, whereby surface swellings at the active edges of cells form small blebs that dynamically extend and retract (52). This early phase of apoptotic blebbing, which occurs at the point where adherent cells begin to retract away from their neighbors and partially detach from the substratum (53), is critically dependent on ROCK/MLC cytoskeletal signaling (52). Late phase blebbing leads to morphologically distinct blebs that are relatively stable, fewer in number than those seen during early blebbing, exhibit an absence of visible organelles, and contain a distinct layer of endoplasmic reticulum which envelops chromatin. Formation of these late blebs is efficiently blocked with Latrunculin A (an actin microfilament inhibitor), Blebbistatin (an inhibitor of myosin II), or Nocodazole (a microtubule inhibitor); however pharmacological inhibition of ROCK proteins only partially prevents the formation of late blebs (52).

In hypertrophic cardiomyocytes, Rho/ROCK signaling is necessary for apoptotic DNA fragmentation *via* activation of p53 and Bax (54). Conversely, inhibition of RhoA or ROCK protein signaling in heptatic stellate cells increases DNA fragmentation and condensation of nuclear chromatin (55). These limited data suggest that a more thorough examination is necessary before any consensus can be made regarding the role of ROCK proteins in apoptotic DNA fragmentation. Apoptotic nuclear disintegration, an actin microfilament-dependent and microtubule-independent process, requires ROCK modulation of the actin-myosin contractile force coupled with a ROCK-independent caspase-mediated degradation of nuclear lamin proteins (56). In addition to regulating nuclear disintegration, ROCK signaling is necessary for Golgi organelle fragmentation in apoptotic adrenal medulla pheochromocytoma cells (57). In this model, overexpression of constitutively active ROCK proteins induces Golgi fragmentation even in the absence of apoptotic stimuli. Moreover, ROCK proteins regulate protein traffic to and from cellular organelles during the apoptotic cascade. For instance, when myeloid leukemia cells become apoptotic, activated extracellular signal regulated kinase (ERK) is unable to translocate into the nuclei. Pharmacological inhibition of ROCK signaling is not only capable of rescuing these cells from apoptosis, but successfully restores the nuclear translocation of activated ERK (58). Furthermore, in apoptotic myeloid leukemia and fibroblast cells, caspase-independent ROCK signaling leads to nuclear exclusion of C1/C2 heterogenous nuclear ribonucleoproteins (hnRNPs), which play important roles in the packaging of nascent transcripts, alternative splicing and translational regulation (59). ROCK-mediated control of protein localization is well documented, as modulation of actin polymerization by ROCK has been shown to regulate nuclear localization of serum response factor (SRF) and sex determining region Y-box 9 (Sox9) during non-apoptotic conditions (60, 61). ROCK control of subcellular protein localization could potentially be a commonplace mechanism by which rapidly changing cytoskeletal dynamics during apoptosis alters cellular function.

Apoptotic body formation is driven by actin-myosin contraction initiated by caspase-mediated activating cleavage of ROCK1. In fibroblast and B-lymphoma cells this process is prevented by pharmacological inhibition or small interfering RNA (siRNA) knockdown of ROCK1, but not by inhibition of ROCK2 (57, 62). Moreover, ROCK activation is necessary for efficient phagocytosis of fragmented apoptotic bodies, and has been demonstrated to control the expression of N-acetylglucosamine (GlcNAc), a carbohydrate that serves as a major phagocytic marker (57, 63).

ROCK control of extrinsic apoptosis. The extrinsic apoptotic receptor Fas is linked to the actin cytoskeleton *via* an interaction with ezrin, radixin and moesin (ERM) proteins, whose function is to connect transmembrane proteins to the cytoskeleton (64, 65). The disruption of actin cytoskeleton dynamics or down-regulation of either ezrin or moesin inhibits extrinsic apoptotic signaling by blocking Fas aggregation and redistribution of Fas into lipid rafts, and by preventing association of flavin adenine dinucleotide (Fad) associated protein with death domain (FADD) with its procaspases (31, 64, 66). These data suggest that ligand-mediated activation of the extrinsic apoptotic pathway initiates a cytoskeleton driven clustering of the activated death receptor with its downstream death domain proteins and their associated caspases. This process is dependent on ROCK signaling as pharmacological inhibition or siRNA downregulation of ROCK proteins blocks clustering of FAS proteins to lipid rafts, inhibits ROCK-mediated phosphorylation of ezrin and disrupts procaspase 8 and 10 association with FAS and FADD (64, 66-68). A similar ROCK-driven cytoskeletal regulation has been demonstrated for extrinsic apoptotic induction following ligand driven Fas receptor clustering (69, 70). In addition to modulating death receptor activity, ROCK signaling controls the expression

levels of several extrinsic apoptotic regulators. Pharmacological inhibition of ROCK signaling results in a decrease in Fas, FasL and TRAIL expression during androgen induced apoptotic regression of prostate cancer cells and following cisplatin cytotoxicity in neuroblastoma cells (71-73). In contrast, ROCK inhibition reportedly enhances FasL expression in melanoma tumors (35).

ROCK control of intrinsic apoptosis. ROCK proteins perform a key role in cell cycle inhibition and impinge on the p53-driven intrinsic apoptotic cascade at multiple points from initial activation to output. However, the ROCK-mediated regulation of cell cycle and intrinsic apoptotic regulators seems to function in a cell type and context specific manner as conflicting results have been reported throughout the literature. Moreover, unlike that seen for ROCK regulation of the extrinsic apoptotic pathway, few consistent mechanisms have been proposed as to how ROCK proteins control intrinsic apoptotic regulation. ROCK inhibition has been shown to increase phosphorylation of p53 in neuronal cells, suggesting that ROCK signaling promotes murine double minute oncogene 2 (Mdm2)-mediated ubiquitination and degradation of p53 (74, 75). In contrast, fasudil treatment following nephropathy leads to decreased p53 expression, suggesting the opposite (76). No direct physical association has been reported in the literature between ROCK and p53, indicating that ROCK mediated regulation of p53 levels is likely modulated through indirect signaling crosstalk. For instance, ROCK activity has been shown to regulate phosphoinositol-3-kinase (PI3K)/Ak transforming (AKT) signaling (a negative regulator of p53 stability) through ROCK-dependent assembly of focal adhesions (77). A large proteomic screen demonstrated that ROCK2 physically associates and is activated by the serine/threonine kinase Polo-like kinase (Plk1) (78, 79), an important regulator of mitotic events such as centrosome maturation, mitotic entry, spindle formation, sister chromatid cohesion, and cytokinesis. This interaction could modulate p53 status given that Plk1 is a strong inhibitor of p53 function through a direct physical interaction between the two proteins (80). Moreover, Plk1 induces an inhibitory phosphorylation on the Sumo E3 ligase topoisomerase I-binding protein (Topors) leading to inhibition of p53 sumyolation and its subsequent ubiquitination and degradation (81). Another possibility that deserves further study involves ROCK/LIMK mediated regulation of tubulin-dynein motor protein transport into the nucleus. p53 has been shown to localize to cellular microtubules, and transport of p53 into the nucleus following DNA damage is tubulin-dynein motor protein depedent (82-84). ROCK activity has repeatedly been demonstrated to control microtubule activity in a number of systems ranging from cell protrusions to tubulin-dynein vesicular trafficking (85-87), but whether p53 nuclear localization is regulated *via* this ROCK/LIMK/motor protein process has yet to be determined.

In a number of studies, ROCK signaling reportedly controlled Bcl-2 family member gene expression in favor of apoptosis (54, 73, 88-90) and modulates activation of multiple caspases (54, 64, 67, 77, 88, 91). ROCK modulation of Bcl2 expression may occur *via* the PI3K mediated pathway (discussed below) or through c-jun N-terminal kinase (JNK) activation. The JNK pathway is primarily activated by cytokines or exposure to various environmental stresses and plays an important role in regulating stress-induced apoptosis by triggering cytochrome c release from the mitochondria through modulation of Bcl2 and Bcl-xl activity (92). It has been demonstrated that ROCK1 directly phosphorylates JNK-interacting protein (JIP)-3, a scaffolding protein responsible for recruitment and activation of JNK protein, leading to subsequent triggering of apoptosis (93). This process can be prevented by sequestration of ROCK1 into stress granules, thus blocking ROCK1 interaction with JIP3 and protecting cells from apoptosis (94).

ROCK Protein Regulation of Cell Survival

Control of cell survival by Rock proteins. Inhibition of ROCK promotes survival following balloon surgery and stent implantation of the carotid artery (95, 96), in autologous vein grafts (97), in pulmonary hypertension (98-100), following renal damage (101-106), in vaculogenic erectile dysfunction (107-110) and in diabetic retinal microvasculopathy (111). While it is highly likely that the effects of ROCK inhibition on numerous disease models are multifactorial, few mechanisms have been proposed to explain such observations. The PI3K/Akt pathway plays a central role in promoting cell survival by regulation of the activity and expression of Bcl2 family members, forkhead box 0 (FoxO) transcription factors, and p53 stability (112). PI3K activation is countered by phosphatase and tensin homolog (PTEN), a phosphatase that dephosphorylates proteins and phosphoinositide substrates (113). Activation of ROCK proteins by caspase cleavage or oncogene overexpression induces a direct phosphorylation of PTEN by ROCK, leading to the increased phosphatase activity and enhanced protein stability of PTEN. Activated PTEN then directly counters the pro-survival PI3K/AKT pathway, suggesting that ROCK activation blocks cell survival (41, 114-116). In addition, the PI3K/Akt pathway promotes the nitric oxide-mediated survival of endothelial cells by stimulating the expression of endothelial nitric oxide synthase (eNOS), the enzyme that converts the amino acid arginine to nitric oxide (117). Thus, ROCK-mediated activation of PTEN leads to a subsequent decrease in nitric oxide (NO) production and reduced cell survival of endothelial cells (118, 119), however ROCK's regulation of NO-driven survival is reportedly

PI3K/Akt independent in some cells and involves activation of the PKC pathway (120-122).

ROCK signaling regulates chemotherapy resistance in several tumor cell types, and thus affects overall tumor resilience and survival. For instance, in multiple myeloma cells, ROCK-mediated attachment to the extracellular matrix is an essential component of cell adhesion-mediated drug resistance, a process whereby integrin interactions lead to up-regulation of anti-apoptotic Bcl2 family members and overexpression of multidrug resistant genes (123). Similarly, inhibition of ROCK activity leads to enhancement of cisplatin-induced cytotoxicity in lung carcinoma cells through a focal adhesion kinase-independent mechanism (124). Conversely, following cisplatin injury to a panel of cultured neuroblastoma cells, pharmacological inhibition of ROCK activity resulted in increased cell survival, rapid acquisition of a chemoresistant phenotype and enhanced *in vivo* tumor survival. The increased chemoresistance in ROCK-inhibited neuroblastoma cells was attributed primarily to enhanced DNA damage repair, with observable alterations in the expression of multidrug resistance genes, p53, p21, Bcl2 family members and death receptors and their ligands (73).

ROCK Protein Regulation of Proliferation

Control of cell cycle progression by ROCK proteins. siRNA or pharmacological inhibition of ROCK blocks the G_1/S transition in a number of cell types. Indeed, ROCK signaling promotes cell cycle progression into the S phase through a diverse array of downstream targets including upregulation of cyclin A/D1/D3 and cyclin dependent kinase (CDK) 2/4/6, nuclear translocation of CDK2 and cyclin E, and downregulation of the cell cycle inhibitors cyclin dependent kinase 4 inhibitor B (CDKN4B), CDKN2A, CDKN2C, CDKN2D (p21), CDKN1A, and CDKN1B (125-130) ROCK utilizes multiple downstream signaling cascades to modulate proliferation where it activates Ras/MAPK to regulate cyclin D and p21 expression, and, alternatively, LIM Kinase 2 to regulate cyclin A expression (128). Moreover, ROCK increases the expression of the F-box protein s-phase kinase-associated protein 2 (Skp2) which is required for the degradation of the cell cycle inhibitor p27(Kip1) (126, 128). Inhibition of ROCK signaling leads to cell cycle arrest in the G_1 phase, decreased JNK, extracellular signal-regulated kinase (ERK), Ephrin-related tyrosine kinase (ELK), early growth response protein 1 (Egr1), and globin transcription factor (GATA) transcription factor activation, decreased c-FBJ murine osteosarcoma viral oncogene homolog (c-fos), jun proto-oncogene (c-jun), FasL, and Bcl2 expression, and increased Bax expression (15, 35, 39, 96, 131-134). Alternatively, a handful of papers suggest ROCK activity is capable of blocking cell cycle progression under certain conditions. For instance, during phorbol ester-induced apoptosis in prostate cancer cells, increased expression of the cell cycle inhibitor p21 is dependent on ROCK-mediated regulation of cytoskeleton dynamics (135). Additionally, pharmacological inhibition of ROCK activity in human Wharton's jelly stem cells leads to downregulation of the pro-apoptotic Bax gene and the cell cycle regulators p21 and p53, as well as upregulation of the anti-apoptotic Bcl2 gene (89, 90), suggesting ROCK can inhibit cell cycle progression under certain conditions.

In addition to the requirement of early growth factor-mediated progression through the cell cycle, microenvironment-dependent changes in cell shape and cytoskeleton regulation modulate the G_1/S transition whereby the major mitogenic-responsive pathways such as Ras, Rho, and PI3K are regulated by integrin mediated cell adhesion to the ECM (136). Disrupted integrin signaling is responsible for the change from anchorage dependent to anchorage independent cell growth in tumor cells, demonstrating a strong linkage between the extracellular microenvironment, cell adhesion, cellular morphology, and cell survival. Fibronectin/integrin interactions have been shown to stimulate cell proliferation in a ROCK-dependent manner by suppression of p21 and stimulation of cyclin D1 mRNA expression levels (130, 137, 138). Moreover, the degree of cell spreading on the ECM is a potent modulator of cell proliferation (139), and density dependent growth control is regulated by cell-to-cell adhesions *via* cadherin-mediated activation of p21 and p27 (140-143) and reduction in the strength and stability of cell-ECM contacts (144). Interestingly, cells that are restricted from spreading, such as fully confluent monolayers, exhibit a shape-dependent failure to increase the expression of cyclin D1, down-regulate p27 and phosphorylate retinoblastoma protein in late G_1 (145, 146) and low ROCK activity (147). Cell spreading and mechanical stretch (mimicking non-confluent cell density) has been shown to activate RhoA and ROCK in smooth muscle cells, resulting in membrane association of RhoA, leading to ROCK-dependent hyperphosphorylation of Rb and enhanced proliferation (147-149). The loss of cadherin-mediated cell-to-cell contacts as seen in subconfluent cultures leads to the formation of a signaling complex composed of ROCK, novel protein kinase C (nPKC), and sarcoma proto-oncogene (Src) family kinases (SFKs), resulting in protein kinase D-dependent activation of the pro-proliferation nuclear factor kappa-B (NFkappaB) protein (37). These findings suggest that the extracellular microenvironment, particularly the effect of cell density, may affect the outcome of ROCK signaling in the control of cell fate. Therefore, simple differences in cell plating density may explain the numerous conflicting observations regarding the role of ROCK proteins in cell survival.

Implications for Cancer Therapy

Despite the obvious complexity and the ever growing number of publications linking ROCK as well as cytoskeletal regulation in the cellular decision between life and death, no sufficient comprehensive mechanism has been established which comes close to explaining the fundamental intricacies governing the pleiotropic roles of the ROCK proteins in cell survival. Despite this shortcoming, targeting of ROCK signaling in animal models of tumor progression has manifested outstanding results in many cases particularly with regard to tumor cell invasion and metastasis, suggesting that manipulation of this pathway could hold the key to pushing cancer cells just over the edge so that patients might gain an upper hand not afforded by chemotherapy or radiation alone. Perhaps the greatest challenge to researchers and clinicians is the dissection of these conflicting signaling roles, thereby learning which tumor types and what physiological conditions are appropriate for the proper manipulation of ROCK signaling.

Acknowledgements

Preparation of this manuscript was supported by a National Heart, Lung, and Blood Institute Award (HL098931) awarded to BAB.

References

1 Shi J and Wei L: Rho kinase in the regulation of cell death and survival. Arch Immunol Ther Exp (Warsz) *55*: 61-75, 2007.

2 Narumiya S, Tanji M and Ishizaki T: Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion. Cancer Metastasis Rev *28*: 65-76, 2009.

3 Shimizu Y, Thumkeo D, Keel J, Ishizaki T, Oshima H, Oshima M, Noda Y, Matsumura F, Taketo MM and Narumiya S: ROCK-I regulates closure of the eyelids and ventral body wall by inducing assembly of actomyosin bundles. J Cell Biol *168*: 941-953, 2005.

4 Thumkeo D, Keel J, Ishizaki T, Hirose M, Nonomura K, Oshima H, Oshima M, Taketo MM and Narumiya S: Targeted disruption of the mouse rho-associated kinase 2 gene results in intrauterine growth retardation and fetal death. Mol Cell Biol *23*: 5043-5055, 2003.

5 Noma K, Rikitake Y, Oyama N, Yan G, Alcaide P, Liu PY, Wang H, Ahl D, Sawada N, Okamoto R, Hiroi Y, Shimizu K, Luscinskas FW, Sun J and Liao JK: ROCK1 mediates leukocyte recruitment and neointima formation following vascular injury. J Clin Invest *118*: 1632-1644, 2008.

6 Rikitake Y, Oyama N, Wang CY, Noma K, Satoh M, Kim HH and Liao JK: Decreased perivascular fibrosis but not cardiac hypertrophy in ROCK1+/– haploinsufficient mice. Circulation *112*: 2959-2965, 2005.

7 Bryan BA, Dennstedt E, Mitchell DC, Walshe TE, Noma K, Loureiro R, Saint-Geniez M, Campaigniac JP, Liao JK and D'Amore PA: RhoA/ROCK signaling is essential for multiple aspects of VEGF-mediated angiogenesis. FASEB J *24*: 3186-3195, 2010.

8 Kamai T, Arai K, Sumi S, Tsujii T, Honda M, Yamanishi T and Yoshida KI: The rho/rho-kinase pathway is involved in the progression of testicular germ cell tumour. BJU Int *89*: 449-453, 2002.

9 Kamai T, Tsujii T, Arai K, Takagi K, Asami H, Ito Y and Oshima H: Significant association of Rho/ROCK pathway with invasion and metastasis of bladder cancer. Clin Cancer Res *9*: 2632-2641, 2003.

10 Kaneko K, Satoh K, Masamune A, Satoh A and Shimosegawa T: Expression of ROCK-1 in human pancreatic cancer: its down-regulation by morpholino oligo antisense can reduce the migration of pancreatic cancer cells *in vitro*. Pancreas *24*: 251-257, 2002.

11 Kleer CG, van Golen KL, Zhang Y, Wu ZF, Rubin MA and Merajver SD: 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.

12 Zhou J, Zhao LQ, Xiong MM, Wang XQ, Yang GR, Qiu ZL, Wu M and Liu ZH: Gene expression profiles at different stages of human esophageal squamous cell carcinoma. World J Gastroenterol *9*: 9-15, 2003.

13 Somlyo AV, Bradshaw D, Ramos S, Murphy C, Myers CE and Somlyo AP: Rho-kinase inhibitor retards migration and *in vivo* dissemination of human prostate cancer cells. Biochem Biophys Res Commun *269*: 652-659, 2000.

14 Rattan R, Giri S, Singh AK and Singh I: Rho/ROCK pathway as a target of tumor therapy. J Neurosci Res *83*: 243-255, 2006.

15 Routhier A, Astuccio M, Lahey D, Monfredo N, Johnson A, Callahan W, Partington A, Fellows K, Ouellette L, Zhidro S, Goodrow C, Smith A, Sullivan K, Simone P, Le L, Vezuli B, Zohni M, West E, Gleason D and Bryan B: Pharmacological inhibition of Rho-kinase signaling with Y-27632 blocks melanoma tumor growth. Oncol Rep *23*: 861-867, 2010.

16 Spencer C, Montalvo J, McLaughlin SR and Bryan BA: Small molecule inhibition of cytoskeletal dynamics in melanoma tumors results in altered transcriptional expression patterns of key genes involved in tumor initiation and progression. Cancer Genomics Proteomics *8*: 77-85, 2011.

17 Amine A, Rivera S, Opolon P, Dekkal M, Biard DS, Bouamar H, Louache F, McKay MJ, Bourhis J, Deutsch E and Vozenin-Brotons MC: Novel anti-metastatic action of cidofovir mediated by inhibition of E6/E7, CXCR4 and Rho/ROCK signaling in HPV tumor cells. PLoS One *4*: e5018, 2009.

18 Levkau B, Herren B, Koyama H, Ross R and Raines EW: Caspase-mediated cleavage of focal adhesion kinase pp125FAK and disassembly of focal adhesions in human endothelial cell apoptosis. J Exp Med *187*: 579-586, 1998.

19 Coleman ML and Olson MF: Rho GTPase signalling pathways in the morphological changes associated with apoptosis. Cell Death Differ *9*: 493-504, 2002.

20 Ndozangue-Touriguine O, Hamelin J and Breard J: Cytoskeleton and apoptosis. Biochem Pharmacol *76*: 11-18, 2008.

21 Mukae N, Yokoyama H, Yokokura T, Sakoyama Y, Sakahira H and Nagata S: Identification and developmental expression of inhibitor of caspase-activated DNase (ICAD) in Drosophila melanogaster. J Biol Chem *275*: 21402-21408, 2000.

22 deCathelineau AM and Henson PM: The final step in programmed cell death: phagocytes carry apoptotic cells to the grave. Essays Biochem *39*: 105-117, 2003.

23 Duprez L, Wirawan E, Vanden Berghe T and Vandenabeele P: Major cell death pathways at a glance. Microbes Infect *11*: 1050-1062, 2009.

24 Rolland SG and Conradt B: New role of the BCL2 family of proteins in the regulation of mitochondrial dynamics. Curr Opin Cell Biol *22*: 852-858, 2010.

25 Hinoue A, Takigawa T, Miura T, Nishimura Y, Suzuki S and Shiota K: Disruption of actin cytoskeleton and anchorage-dependent cell spreading induces apoptotic death of mouse neural crest cells cultured *in vitro*. Anat Rec A Discov Mol Cell Evol Biol *282*: 130-137, 2005.

26 Cabado AG, Leira F, Vieytes MR, Vieites JM and Botana LM: Cytoskeletal disruption is the key factor that triggers apoptosis in okadaic acid-treated neuroblastoma cells. Arch Toxicol 78: 74-85, 2004.

27 Celeste Morley S, Sun GP and Bierer BE: Inhibition of actin polymerization enhances commitment to and execution of apoptosis induced by withdrawal of trophic support. J Cell Biochem *88*: 1066-1076, 2003.

28 White SR, Williams P, Wojcik KR, Sun S, Hiemstra PS, Rabe KF and Dorscheid DR: Initiation of apoptosis by actin cytoskeletal derangement in human airway epithelial cells. Am J Respir Cell Mol Biol *24*: 282-294, 2001.

29 Martin SS and Vuori K: Regulation of Bcl-2 proteins during anoikis and amorphosis. Biochim Biophys Acta *1692*: 145-157, 2004.

30 Bijian K, Takano T, Papillon J, Le Berre L, Michaud JL, Kennedy CR and Cybulsky AV: Actin cytoskeleton regulates extracellular matrix-dependent survival signals in glomerular epithelial cells. Am J Physiol Renal Physiol *289*: F1313-1323, 2005.

31 Gajate C and Mollinedo F: Cytoskeleton-mediated death receptor and ligand concentration in lipid rafts forms apoptosis-promoting clusters in cancer chemotherapy. J Biol Chem *280*: 11641-11647, 2005.

32 Yamazaki Y, Tsuruga M, Zhou D, Fujita Y, Shang X, Dang Y, Kawasaki K and Oka S: Cytoskeletal disruption accelerates caspase-3 activation and alters the intracellular membrane reorganization in DNA damage-induced apoptosis. Exp Cell Res *259*: 64-78, 2000.

33 Rubtsova SN, Kondratov RV, Kopnin PB, Chumakov PM, Kopnin BP and Vasiliev JM: Disruption of actin microfilaments by cytochalasin D leads to activation of p53. FEBS Lett *430*: 353-357, 1998.

34 Mammoto A, Mammoto T and Ingber DE: Rho signaling and mechanical control of vascular development. Curr Opin Hematol *15*: 228-234, 2008.

35 Sarrabayrouse G, Synaeve C, Leveque K, Favre G and Tilkin-Mariame AF: Statins stimulate *in vitro* membrane FasL expression and lymphocyte apoptosis through RhoA/ROCK pathway in murine melanoma cells. Neoplasia *9*: 1078-1090, 2007.

36 Moore M, Marroquin BA, Gugliotta W, Tse R and White SR: Rho kinase inhibition initiates apoptosis in human airway epithelial cells. Am J Respir Cell Mol Biol *30*: 379-387, 2004.

37 Cowell CF, Yan IK, Eiseler T, Leightner AC, Doppler H and Storz P: Loss of cell-cell contacts induces NF-kappaB *via* RhoA-mediated activation of protein kinase D1. J Cell Biochem *106*: 714-728, 2009.

38 Krijnen PA, Sipkens JA, Molling JW, Rauwerda JA, Stehouwer CD, Muller A, Paulus WJ, van Nieuw Amerongen GP, Hack CE, Verhoeven AJ, van Hinsbergh VW and Niessen HW: Inhibition of Rho-ROCK signaling induces apoptotic and non-apoptotic PS exposure in cardiomyocytes *via* inhibition of flippase. J Mol Cell Cardiol *49*: 781-790, 2010.

39 Shibata R, Kai H, Seki Y, Kusaba K, Takemiya K, Koga M, Jalalidin A, Tokuda K, Tahara N, Niiyama H, Nagata T, Kuwahara F and Imaizumi T: Rho-kinase inhibition reduces neointima formation after vascular injury by enhancing Bax expression and apoptosis. J Cardiovasc Pharmacol *42(Suppl 1)*: S43-47, 2003.

40 Svoboda KK, Moessner P, Field T and Acevedo J: ROCK inhibitor (Y27632) increases apoptosis and disrupts the actin cortical mat in embryonic avian corneal epithelium. Dev Dyn *229*: 579-590, 2004.

41 Chang J, Xie M, Shah VR, Schneider MD, Entman ML, Wei L and Schwartz RJ: Activation of Rho-associated coiled-coil protein kinase 1 (ROCK-1) by caspase-3 cleavage plays an essential role in cardiac myocyte apoptosis. Proc Natl Acad Sci USA *103*: 14495-14500, 2006.

42 Coudray AM, Louvet C, Kornprobst M, Raymond E, Andre T, Tournigand C, Faivre S, De Gramont A, Larsen AK and Gespach C: Increased anticancer activity of the thymidylate synthase inhibitor BGC9331 combined with the topoisomerase I inhibitor SN-38 in human colorectal and breast cancer cells: induction of apoptosis and ROCK cleavage through caspase-3-dependent and -independent mechanisms. Int J Oncol *27*: 553-561, 2005.

43 Ark M, Ozdemir A and Polat B: Ouabain-induced apoptosis and Rho kinase: a novel caspase-2 cleavage site and fragment of Rock-2. Apoptosis *15*: 1494-1506, 2010.

44 Sebbagh M, Renvoize C, Hamelin J, Riche N, Bertoglio J and Breard J: Caspase-3-mediated cleavage of ROCK I induces MLC phosphorylation and apoptotic membrane blebbing. Nat Cell Biol *3*: 346-352, 2001.

45 Coleman ML and Marshall CJ: A family outing: small GTPases cyclin' through G1. Nat Cell Biol *3*: E250-251, 2001.

46 Sebbagh M, Hamelin J, Bertoglio J, Solary E and Breard J: Direct cleavage of ROCK II by granzyme B induces target cell membrane blebbing in a caspase-independent manner. J Exp Med *201*: 465-471, 2005.

47 Ueda H, Morishita R, Itoh H, Narumiya S, Mikoshiba K, Kato K and Asano T: Galpha11 induces caspase-mediated proteolytic activation of Rho-associated kinase, ROCK-I, in HeLa cells. J Biol Chem *276*: 42527-42533, 2001.

48 Sapet C, Simoncini S, Loriod B, Puthier D, Sampol J, Nguyen C, Dignat-George F and Anfosso F: Thrombin-induced endothelial microparticle generation: identification of a novel pathway involving ROCK-II activation by caspase-2. Blood *108*: 1868-1876, 2006.

49 Ehrenschwender M, Siegmund D, Wicovsky A, Kracht M, Dittrich-Breiholz O, Spindler V, Waschke J, Kalthoff H, Trauzold A and Wajant H: Mutant PIK3CA licenses TRAIL and CD95L to induce non-apoptotic caspase-8-mediated ROCK activation. Cell Death Differ *17*: 1435-1447, 2010.

50 Mills JC, Stone NL, Erhardt J and Pittman RN: Apoptotic membrane blebbing is regulated by myosin light chain phosphorylation. J Cell Biol *140*: 627-636, 1998.

51 Song Y, Hoang BQ and Chang DD: ROCK-II-induced membrane blebbing and chromatin condensation require actin cytoskeleton. Exp Cell Res *278*: 45-52, 2002.

52 Lane JD, Allan VJ and Woodman PG: Active relocation of chromatin and endoplasmic reticulum into blebs in late apoptotic cells. J Cell Sci *118*: 4059-4071, 2005.
53 Mills JC, Stone NL and Pittman RN: Extranuclear apoptosis. The role of the cytoplasm in the execution phase. J Cell Biol *146*: 703-708, 1999.
54 Del Re DP, Miyamoto S and Brown JH: RhoA/Rho kinase up-regulate Bax to activate a mitochondrial death pathway and induce cardiomyocyte apoptosis. J Biol Chem *282*: 8069-8078, 2007.
55 Ikeda H, Nagashima K, Yanase M, Tomiya T, Arai M, Inoue Y, Tejima K, Nishikawa T, Omata M, Kimura S and Fujiwara K: Involvement of Rho/Rho kinase pathway in regulation of apoptosis in rat hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol *285*: G880-886, 2003.
56 Croft DR, Coleman ML, Li S, Robertson D, Sullivan T, Stewart CL and Olson MF: Actin-myosin-based contraction is responsible for apoptotic nuclear disintegration. J Cell Biol *168*: 245-255, 2005.
57 Orlando KA, Stone NL and Pittman RN: Rho kinase regulates fragmentation and phagocytosis of apoptotic cells. Exp Cell Res *312*: 5-15, 2006.
58 Lai JM, Wu S, Huang DY and Chang ZF: Cytosolic retention of phosphorylated extracellular signal-regulated kinase and a Rho-associated kinase-mediated signal impair expression of p21(Cip1/Waf1) in phorbol 12-myristate-13- acetate-induced apoptotic cells. Mol Cell Biol *22*: 7581-7592, 2002.
59 Lee HH, Chien CL, Liao HK, Chen YJ and Chang ZF: Nuclear efflux of heterogeneous nuclear ribonucleoprotein C1/C2 in apoptotic cells: a novel nuclear export dependent on Rho-associated kinase activation. J Cell Sci *117*: 5579-5589, 2004.
60 Liu HW, Halayko AJ, Fernandes DJ, Harmon GS, McCauley JA, Kocieniewski P, McConville J, Fu Y, Forsythe SM, Kogut P, Bellam S, Dowell M, Churchill J, Lesso H, Kassiri K, Mitchell RW, Hershenson MB, Camoretti-Mercado B and Solway J: The RhoA/Rho kinase pathway regulates nuclear localization of serum response factor. Am J Respir Cell Mol Biol *29*: 39-47, 2003.
61 Haudenschild DR, Chen J, Pang N, Lotz MK and D'Lima DD: Rho kinase-dependent activation of SOX9 in chondrocytes. Arthritis Rheum *62*: 191-200, 2010.
62 Parent N, Sane AT, Droin N and Bertrand R: Procaspase-2S inhibits procaspase-3 processing and activation, preventing ROCK-1-mediated apoptotic blebbing and body formation in human B lymphoma Namalwa cells. Apoptosis *10*: 313-322, 2005.
63 Orlando KA and Pittman RN: Rho kinase regulates phagocytosis, surface expression of GlcNAc, and Golgi fragmentation of apoptotic PC12 cells. Exp Cell Res *312*: 3298-3311, 2006.
64 Hebert M, Potin S, Sebbagh M, Bertoglio J, Breard J and Hamelin J: Rho-ROCK-dependent ezrin-radixin-moesin phosphorylation regulates Fas-mediated apoptosis in Jurkat cells. J Immunol *181*: 5963-5973, 2008.
65 Parlato S, Giammarioli AM, Logozzi M, Lozupone F, Matarrese P, Luciani F, Falchi M, Malorni W and Fais S: CD95 (APO-1/Fas) linkage to the actin cytoskeleton through ezrin in human T lymphocytes: a novel regulatory mechanism of the CD95 apoptotic pathway. EMBO J *19*: 5123-5134, 2000.
66 Rebillard A, Jouan-Lanhouet S, Jouan E, Legembre P, Pizon M, Sergent O, Gilot D, Tekpli X, Lagadic-Gossmann D and Dimanche-Boitrel MT: Cisplatin-induced apoptosis involves a Fas-ROCK-ezrin-dependent actin remodelling in human colon cancer cells. Eur J Cancer *46*: 1445-1455, 2010.
67 Lai JM, Hsieh CL and Chang ZF: Caspase activation during phorbol ester-induced apoptosis requires ROCK-dependent myosin-mediated contraction. J Cell Sci *116*: 3491-3501, 2003.
68 Connell LE and Helfman DM: Myosin light chain kinase plays a role in the regulation of epithelial cell survival. J Cell Sci *119*: 2269-2281, 2006.
69 Soderstrom TS, Nyberg SD and Eriksson JE: CD95 capping is ROCK-dependent and dispensable for apoptosis. J Cell Sci *118*: 2211-2223, 2005.
70 Hoogwater FJ, Nijkamp MW, Smakman N, Steller EJ, Emmink BL, Westendorp BF, Raats DA, Sprick MR, Schaefer U, Van Houdt WJ, De Bruijn MT, Schackmann RC, Derksen PW, Medema JP, Walczak H, Borel Rinkes IH and Kranenburg O: Oncogenic K-Ras turns death receptors into metastasis-promoting receptors in human and mouse colorectal cancer cells. Gastroenterology *138*: 2357-2367, 2010.
71 Papadopoulou N, Charalampopoulos I, Anagnostopoulou V, Konstantinidis G, Foller M, Gravanis A, Alevizopoulos K, Lang F and Stournaras C: Membrane androgen receptor activation triggers down-regulation of PI-3K/Akt/NF-kappaB activity and induces apoptotic responses *via* Bad, FasL and caspase-3 in DU145 prostate cancer cells. Mol Cancer *7*: 88, 2008.
72 Papadopoulou N, Charalampopoulos I, Alevizopoulos K, Gravanis A and Stournaras C: Rho/ROCK/actin signaling regulates membrane androgen receptor induced apoptosis in prostate cancer cells. Exp Cell Res *314*: 3162-3174, 2008.
73 Street CA, Routhier AA, Spencer C, Perkins AL, Masterjohn K, Hackathorn A, Montalvo J, Dennstedt EA and Bryan BA: Pharmacological inhibition of Rho-kinase (ROCK) signaling enhances cisplatin resistance in neuroblastoma cells. Int J Oncol *37*: 1297-1305, 2010.
74 Qin Q, Baudry M, Liao G, Noniyev A, Galeano J and Bi X: A novel function for p53: regulation of growth cone motility through interaction with Rho kinase. J Neurosci *29*: 5183-5192, 2009.
75 Qin Q, Liao G, Baudry M and Bi X: Cholesterol perturbation in mice results in p53 degradation and axonal pathology through p38 MAPK and Mdm2 activation. PLoS One *5*: e9999, 2010.
76 Park JW, Park CH, Kim IJ, Bae EH, Ma SK, Lee JU and Kim SW: Rho kinase inhibition by fasudil attenuates cyclosporine-induced kidney injury. J Pharmacol Exp Ther *338*: 271-279, 2011.
77 Del Re DP, Miyamoto S and Brown JH: Focal adhesion kinase as a RhoA-activable signaling scaffold mediating Akt activation and cardiomyocyte protection. J Biol Chem *283*: 35622-35629, 2008.
78 Lowery DM, Clauser KR, Hjerrild M, Lim D, Alexander J, Kishi K, Ong SE, Gammeltoft S, Carr SA and Yaffe MB: Proteomic screen defines the Polo-box domain interactome and identifies Rock2 as a Plk1 substrate. EMBO J *26*: 2262-2273, 2007.
79 Li J, Wang J, Jiao H, Liao J and Xu X: Cytokinesis and cancer: Polo loves ROCK'n' Rho(A). J Genet Genomics *37*: 159-172, 2010.
80 Ando K, Ozaki T, Yamamoto H, Furuya K, Hosoda M, Hayashi S, Fukuzawa M and Nakagawara A: Polo-like kinase 1 (Plk1) inhibits p53 function by physical interaction and phosphorylation. J Biol Chem *279*: 25549-25561, 2004.

81 Yang X, Li H, Zhou Z, Wang WH, Deng A, Andrisani O and Liu X: Plk1-mediated phosphorylation of Topors regulates p53 stability. J Biol Chem *284*: 18588-18592, 2009.
82 Giannakakou P, Sackett DL, Ward Y, Webster KR, Blagosklonny MV and Fojo T: p53 is associated with cellular microtubules and is transported to the nucleus by dynein. Nat Cell Biol *2*: 709-717, 2000.
83 Trostel SY, Sackett DL and Fojo T: Oligomerization of p53 precedes its association with dynein and nuclear accumulation. Cell Cycle *5*: 2253-2259, 2006.
84 Giannakakou P, Nakano M, Nicolaou KC, O'Brate A, Yu J, Blagosklonny MV, Greber UF and Fojo T: Enhanced microtubule-dependent trafficking and p53 nuclear accumulation by suppression of microtubule dynamics. Proc Natl Acad Sci USA *99*: 10855-10860, 2002.
85 Gorovoy M, Niu J, Bernard O, Profirovic J, Minshall R, Neamu R and Voyno-Yasenetskaya T: LIM kinase 1 coordinates microtubule stability and actin polymerization in human endothelial cells. J Biol Chem *280*: 26533-26542, 2005.
86 Frampton AR, Jr., Uchida H, von Einem J, Goins WF, Grandi P, Cohen JB, Osterrieder N and Glorioso JC: Equine herpesvirus type 1 (EHV-1) utilizes microtubules, dynein, and ROCK1 to productively infect cells. Vet Microbiol *141*: 12-21, 2010.
87 Dehmelt L, Nalbant P, Steffen W and Halpain S: A microtubule-based, dynein-dependent force induces local cell protrusions: implications for neurite initiation. Brain Cell Biol *35*: 39-56, 2006.
88 He H, Yim M, Liu KH, Cody SC, Shulkes A and Baldwin GS: Involvement of G proteins of the Rho family in the regulation of Bcl-2-like protein expression and caspase 3 activation by Gastrins. Cell Signal *20*: 83-93, 2008.
89 Gauthaman K, Fong CY, Subramanian A, Biswas A and Bongso A: ROCK inhibitor Y-27632 increases thaw-survival rates and preserves stemness and differentiation potential of human Wharton's jelly stem cells after cryopreservation. Stem Cell Rev *6*: 665-676, 2010.
90 Gauthaman K, Fong CY and Bongso A: Effect of ROCK inhibitor Y-27632 on normal and variant human embryonic stem cells (hESCs) *in vitro*: its benefits in hESC expansion. Stem Cell Rev *6*: 86-95, 2010.
91 Wang YX, Martin-McNulty B, da Cunha V, Vincelette J, Lu X, Feng Q, Halks-Miller M, Mahmoudi M, Schroeder M, Subramanyam B, Tseng JL, Deng GD, Schirm S, Johns A, Kauser K, Dole WP and Light DR: Fasudil, a Rho-kinase inhibitor, attenuates angiotensin II-induced abdominal aortic aneurysm in apolipoprotein E-deficient mice by inhibiting apoptosis and proteolysis. Circulation *111*: 2219-2226, 2005.
92 Fan M, Goodwin M, Vu T, Brantley-Finley C, Gaarde WA and Chambers TC: Vinblastine-induced phosphorylation of Bcl-2 and Bcl-XL is mediated by JNK and occurs in parallel with inactivation of the Raf-1/MEK/ERK cascade. J Biol Chem *275*: 29980-29985, 2000.
93 Ongusaha PP, Qi HH, Raj L, Kim YB, Aaronson SA, Davis RJ, Shi Y, Liao JK and Lee SW: Identification of ROCK1 as an upstream activator of the JIP-3 to JNK signaling axis in response to UVB damage. Sci Signal *1*: ra14, 2008.
94 Tsai NP and Wei LN: RhoA/ROCK1 signaling regulates stress granule formation and apoptosis. Cell Signal *22*: 668-675, 2010.
95 Shibata R, Kai H, Seki Y, Kato S, Morimatsu M, Kaibuchi K and Imaizumi T: Role of Rho-associated kinase in neointima formation after vascular injury. Circulation *103*: 284-289, 2001.
96 Matsumoto Y, Uwatoku T, Oi K, Abe K, Hattori T, Morishige K, Eto Y, Fukumoto Y, Nakamura K, Shibata Y, Matsuda T, Takeshita A and Shimokawa H: Long-term inhibition of Rho-kinase suppresses neointimal formation after stent implantation in porcine coronary arteries: involvement of multiple mechanisms. Arterioscler Thromb Vasc Biol *24*: 181-186, 2004.
97 Furuyama T, Komori K, Shimokawa H, Matsumoto Y, Uwatoku T, Hirano K and Maehara Y: Long-term inhibition of Rho kinase suppresses intimal thickening in autologous vein grafts in rabbits. J Vasc Surg *43*: 1249-1256, 2006.
98 Abe K, Shimokawa H, Morikawa K, Uwatoku T, Oi K, Matsumoto Y, Hattori T, Nakashima Y, Kaibuchi K, Sueishi K and Takeshit A: Long-term treatment with a Rho-kinase inhibitor improves monocrotaline-induced fatal pulmonary hypertension in rats. Circ Res *94*: 385-393, 2004.
99 Xu EZ, Kantores C, Ivanovska J, Engelberts D, Kavanagh BP, McNamara PJ and Jankov RP: Rescue treatment with a Rho-kinase inhibitor normalizes right ventricular function and reverses remodeling in juvenile rats with chronic pulmonary hypertension. Am J Physiol Heart Circ Physiol *299*: H1854-1864, 2010.
100 Ziino AJ, Ivanovska J, Belcastro R, Kantores C, Xu EZ, Lau M, McNamara PJ, Tanswell AK and Jankov RP: Effects of rho-kinase inhibition on pulmonary hypertension, lung growth, and structure in neonatal rats chronically exposed to hypoxia. Pediatr Res *67*: 177-182, 2010.
101 Koshikawa S, Nishikimi T, Inaba C, Akimoto K and Matsuoka H: Fasudil, a Rho-kinase inhibitor, reverses L-NAME exacerbated severe nephrosclerosis in spontaneously hypertensive rats. J Hypertens *26*: 1837-1848, 2008.
102 Nishikimi T, Akimoto K, Wang X, Mori Y, Tadokoro K, Ishikawa Y, Shimokawa H, Ono H and Matsuoka H: Fasudil, a Rho-kinase inhibitor, attenuates glomerulosclerosis in Dahl salt-sensitive rats. J Hypertens *22*: 1787-1796, 2004.
103 Ishikawa Y, Nishikimi T, Akimoto K, Ishimura K, Ono H and Matsuoka H: Long-term administration of rho-kinase inhibitor ameliorates renal damage in malignant hypertensive rats. Hypertension *47*: 1075-1083, 2006.
104 Nishikimi T, Koshikawa S, Ishikawa Y, Akimoto K, Inaba C, Ishimura K, Ono H and Matsuoka H: Inhibition of Rho-kinase attenuates nephrosclerosis and improves survival in salt-loaded spontaneously hypertensive stroke-prone rats. J Hypertens *25*: 1053-1063, 2007.
105 Kanda T, Wakino S, Hayashi K, Homma K, Ozawa Y and Saruta T: Effect of fasudil on Rho-kinase and nephropathy in subtotally nephrectomized spontaneously hypertensive rats. Kidney Int *64*: 2009-2019, 2003.
106 Gojo A, Utsunomiya K, Taniguchi K, Yokota T, Ishizawa S, Kanazawa Y, Kurata H and Tajima N: The Rho-kinase inhibitor, fasudil, attenuates diabetic nephropathy in streptozotocin-induced diabetic rats. Eur J Pharmacol *568*: 242-247, 2007.
107 Park K, Kim SW, Rhu KS and Paick JS: Chronic administration of an oral Rho kinase inhibitor prevents the development of vasculogenic erectile dysfunction in a rat model. J Sex Med *3*: 996-1003, 2006.

108 Wingard CJ, Moukdar F, Prasad RY, Cathey BL and Wilkinson L: Reversal of voltage-dependent erectile responses in the Zucker obese-diabetic rat by rosuvastatin-altered RhoA/Rho-kinase signaling. J Sex Med *6(Suppl 3)*: 269-278, 2009.

109 Wingard CJ, Johnson JA, Holmes A and Prikosh A: Improved erectile function after Rho-kinase inhibition in a rat castrate model of erectile dysfunction. Am J Physiol Regul Integr Comp Physiol *284*: R1572-1579, 2003.

110 Bivalacqua TJ, Champion HC, Usta MF, Cellek S, Chitaley K, Webb RC, Lewis RL, Mills TM, Hellstrom WJ and Kadowitz PJ: RhoA/Rho-kinase suppresses endothelial nitric oxide synthase in the penis: a mechanism for diabetes-associated erectile dysfunction. Proc Natl Acad Sci USA *101*: 9121-9126, 2004.

111 Arita R, Hata Y, Nakao S, Kita T, Miura M, Kawahara S, Zandi S, Almulki L, Tayyari F, Shimokawa H, Hafezi-Moghadam A and Ishibashi T: Rho kinase inhibition by fasudil ameliorates diabetes-induced microvascular damage. Diabetes *58*: 215-226, 2009.

112 Engelman JA, Luo J and Cantley LC: The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat Rev Genet *7*: 606-619, 2006.

113 Carracedo A and Pandolfi PP: The PTEN-PI3K pathway: of feedbacks and cross-talks. Oncogene *27*: 5527-5541, 2008.

114 Man JH, Liang B, Gu YX, Zhou T, Li AL, Li T, Jin BF, Bai B, Zhang HY, Zhang WN, Li WH, Gong WL, Li HY and Zhang XM: Gankyrin plays an essential role in Ras-induced tumorigenesis through regulation of the RhoA/ROCK pathway in mammalian cells. J Clin Invest *120*: 2829-2841, 2010.

115 Li Z, Dong X, Wang Z, Liu W, Deng N, Ding Y, Tang L, Hla T, Zeng R, Li L and Wu D: Regulation of PTEN by Rho small GTPases. Nat Cell Biol *7*: 399-404, 2005.

116 Vemula S, Shi J, Hanneman P, Wei L and Kapur R: ROCK1 functions as a suppressor of inflammatory cell migration by regulating PTEN phosphorylation and stability. Blood *115*: 1785-1796, 2010.

117 Hisamoto K, Ohmichi M, Kanda Y, Adachi K, Nishio Y, Hayakawa J, Mabuchi S, Takahashi K, Tasaka K, Miyamoto Y, Taniguchi N and Murata Y: Induction of endothelial nitric-oxide synthase phosphorylation by the raloxifene analog LY117018 is differentially mediated by Akt and extracellular signal-regulated protein kinase in vascular endothelial cells. J Biol Chem *276*: 47642-47649, 2001.

118 Hamid SA, Bower HS and Baxter GF: Rho kinase activation plays a major role as a mediator of irreversible injury in reperfused myocardium. Am J Physiol Heart Circ Physiol *292*: H2598-2606, 2007.

119 Song P, Zhang M, Wang S, Xu J, Choi HC and Zou MH: Thromboxane A2 receptor activates a Rho-associated kinase/LKB1/PTEN pathway to attenuate endothelium insulin signaling. J Biol Chem *284*: 17120-17128, 2009.

120 Radisavljevic Z: Nitric oxide suppression triggers apoptosis through the FKHRL1 (FOXO3A)/ROCK kinase pathway in human breast carcinoma cells. Cancer *97*: 1358-1363, 2003.

121 Wang YZ and Feng ZQ: Induction of apoptosis by L-NMMA, *via* FKHRL1/ROCK pathway in human gastric cancer cells. Biomed Environ Sci *19*: 285-291, 2006.

122 Chandra S, Romero M, Shatanawi A, Alkilany A and Caldwell R: Oxidative species increase arginase activity in endothelial cells through RhoA/Rho Kinase pathway. Br J Pharmacol ahead of print, 2011.

123 Kobune M, Chiba H, Kato J, Kato K, Nakamura K, Kawano Y, Takada K, Takimoto R, Takayama T, Hamada H and Niitsu Y: Wnt3/RhoA/ROCK signaling pathway is involved in adhesion-mediated drug resistance of multiple myeloma in an autocrine mechanism. Mol Cancer Ther *6*: 1774-1784, 2007.

124 Igishi T, Mikami M, Murakami K, Matsumoto S, Shigeoka Y, Nakanishi H, Yasuda K, Gutkind JS, Hitsuda Y and Shimizu E: Enhancement of cisplatin-induced cytotoxicity by ROCK inhibitor through suppression of focal adhesion kinase-independent mechanism in lung carcinoma cells. Int J Oncol *23*: 1079-1085, 2003.

125 Chen J, Guerriero E, Lathrop K and SundarRaj N: Rho/ROCK signaling in regulation of corneal epithelial cell cycle progression. Invest Ophthalmol Vis Sci *49*: 175-183, 2008.

126 Mammoto A, Huang S, Moore K, Oh P and Ingber DE: Role of RhoA, mDia, and ROCK in cell shape-dependent control of the Skp2-p27kip1 pathway and the G1/S transition. J Biol Chem *279*: 26323-26330, 2004.

127 Zhao Z and Rivkees SA: Rho-associated kinases play an essential role in cardiac morphogenesis and cardiomyocyte proliferation. Dev Dyn *226*: 24-32, 2003.

128 Croft DR and Olson MF: The Rho GTPase effector ROCK regulates cyclin A, cyclin D1, and $p27^{Kip1}$ levels by distinct mechanisms. Mol Cell Biol *26*: 4612-4627, 2006.

129 Zhang S, Tang Q, Xu F, Xue Y, Zhen Z, Deng Y, Liu M, Chen J, Liu S, Qiu M, Liao Z, Li Z, Luo D, Shi F, Zheng Y and Bi F: RhoA regulates G1-S progression of gastric cancer cells by modulation of multiple INK4 family tumor suppressors. Mol Cancer Res *7*: 570-580, 2009.

130 Han S, Sidell N and Roman J: Fibronectin stimulates human lung carcinoma cell proliferation by suppressing p21 gene expression *via* signals involving Erk and Rho kinase. Cancer Lett *219*: 71-81, 2005.

131 Ma J, Liang S, Wang Z, Zhang L, Jiang J, Zheng J, Yu L, Zheng X, Wang R and Zhu D: ROCK pathway participates in the processes that 15-hydroxyeicosatetraenoic acid (15-HETE) mediated in the pulmonary vascular remodeling induced by hypoxia in rat. J Cell Physiol *222*: 82-94, 2010.

132 Ma J, Zhang L, Li S, Liu S, Ma C, Li W, Falck JR, Manthati VL, Reddy DS, Medhora M, Jacobs ER and Zhu D: 8,9-Epoxyeicosatrienoic acid analog protects pulmonary artery smooth muscle cells from apoptosis *via* ROCK pathway. Exp Cell Res *316*: 2340-2353, 2010.

133 Chen XY, Dun JN, Miao QF and Zhang YJ: Fasudil hydrochloride hydrate, a Rho-kinase inhibitor, suppresses 5-hydroxytryptamine-induced pulmonary artery smooth muscle cell proliferation *via* JNK and ERK1/2 pathway. Pharmacology *83*: 67-79, 2009.

134 Koyanagi M, Takahashi J, Arakawa Y, Doi D, Fukuda H, Hayashi H, Narumiya S and Hashimoto N: Inhibition of the Rho/ROCK pathway reduces apoptosis during transplantation of embryonic stem cell-derived neural precursors. J Neurosci Res *86*: 270-280, 2008.

135 Xiao L, Eto M and Kazanietz MG: ROCK mediates phorbol ester-induced apoptosis in prostate cancer cells *via* p21Cip1 up-regulation and JNK. J Biol Chem 284: 29365-29375, 2009.

136 Danen EH and Yamada KM: Fibronectin, integrins, and growth control. J Cell Physiol *189*: 1-13, 2001.

137 Danen EH, Sonneveld P, Sonnenberg A and Yamada KM: Dual stimulation of Ras/mitogen-activated protein kinase and RhoA

by cell adhesion to fibronectin supports growth factor-stimulated cell cycle progression. J Cell Biol *151*: 1413-1422, 2000.
138 Benoit YD, Lussier C, Ducharme PA, Sivret S, Schnapp LM, Basora N and Beaulieu JF: Integrin alpha8beta1 regulates adhesion, migration and proliferation of human intestinal crypt cells *via* a predominant RhoA/ROCK-dependent mechanism. Biol Cell *101*: 695-708, 2009.
139 Chen CS, Mrksich M, Huang S, Whitesides GM and Ingber DE: Geometric control of cell life and death. Science *276*: 1425-1428, 1997.
140 Huang ZY, Wu Y, Hedrick N and Gutmann DH: T-cadherin-mediated cell growth regulation involves G_2 phase arrest and requires p21(CIP1/WAF1) expression. Mol Cell Biol *23*: 566-578, 2003.
141 Levenberg S, Yarden A, Kam Z and Geiger B: p27 is involved in N-cadherin-mediated contact inhibition of cell growth and S-phase entry. Oncogene *18*: 869-876, 1999.
142 Mueller S, Cadenas E and Schonthal AH: p21WAF1 regulates anchorage-independent growth of HCT116 colon carcinoma cells *via* E-cadherin expression. Cancer Res *60*: 156-163, 2000.
143 Zhong Y, Lopez-Barcons L, Haigentz M Jr., Ling YH and Perez-Soler R: Exogenous expression of H-cadherin in CHO cells regulates contact inhibition of cell growth by inducing p21 expression. Int J Oncol *24*: 1573-1579, 2004.
144 Dudek SM and Garcia JG: Cytoskeletal regulation of pulmonary vascular permeability. J Appl Physiol *91*: 1487-1500, 2001.
145 Huang S, Chen CS and Ingber DE: Control of cyclin D1, p27(Kip1), and cell cycle progression in human capillary endothelial cells by cell shape and cytoskeletal tension. Mol Biol Cell *9*: 3179-3193, 1998.
146 Huang S and Ingber DE: A discrete cell cycle checkpoint in late G(1) that is cytoskeleton-dependent and MAP kinase (Erk)-independent. Exp Cell Res *275*: 255-264, 2002.
147 Bhadriraju K, Yang M, Alom Ruiz S, Pirone D, Tan J and Chen CS: Activation of ROCK by RhoA is regulated by cell adhesion, shape, and cytoskeletal tension. Exp Cell Res *313*: 3616-3623, 2007.
148 Kozai T, Eto M, Yang Z, Shimokawa H and Luscher TF: Statins prevent pulsatile stretch-induced proliferation of human saphenous vein smooth muscle cells *via* inhibition of Rho/Rho-kinase pathway. Cardiovasc Res *68*: 475-482, 2005.
149 Liu WF, Nelson CM, Tan JL and Chen CS: Cadherins, RhoA, and Rac1 are differentially required for stretch-mediated proliferation in endothelial *versus* smooth muscle cells. Circ Res *101*: e44-52, 2007.

Received August 30, 2011
Revised October 6, 2011
Accepted October 7, 2011