FORUM REVIEW ARTICLE

Rho/Rho-Associated Coiled-Coil Forming Kinase Pathway as Therapeutic Targets for Statins in Atherosclerosis

Naoki Sawada^{1,2} and James K. Liao²

Abstract

Significance: The 3-hydroxy-methylglutaryl coenzyme A reductase inhibitors or statins are important therapeutic agents for lowering serum cholesterol levels. However, recent studies suggest that statins may exert atheroprotective effects beyond cholesterol lowering. These so-called “pleiotropic effects” include effects of statins on vascular and inflammatory cells. Thus, it is important to understand whether other signaling pathways that are involved in atherosclerosis could be targets of statins, and if so, whether individuals with “overactivity” of these pathways could benefit from statin therapy, regardless of serum cholesterol level. **Recent Advances:** Statins inhibit the synthesis of isoprenoids, which are important for the function of the Rho/Rho-associated coiled-coil containing kinase (ROCK) pathway. Indeed, recent studies suggest that inhibition of the Rho/ROCK pathway by statins could lead to improved endothelial function and decreased vascular inflammation and atherosclerosis. Thus, the Rho/ROCK pathway has emerged as an important target of statin therapy for reducing atherosclerosis and possibly cardiovascular disease. **Critical Issues:** Because atherosclerosis is both a lipid and an inflammatory disease, it is important to understand how inhibition of Rho/ROCK pathway could contribute to statins’ antiatherosclerotic effects. **Future Directions:** The role of ROCKs (ROCK1 and ROCK2) in endothelial, smooth muscle, and inflammatory cells needs to be determined in the context of atherogenesis. This could lead to the development of specific ROCK1 or ROCK2 inhibitors, which could have greater therapeutic benefits with less toxicity. Also, clinical trials will need to be performed to determine whether inhibition of ROCKs, with and without statins, could lead to further reduction in atherosclerosis and cardiovascular disease. *Antioxid. Redox Signal.* 20, 1251–1267.

Introduction

ATHEROSCLEROSIS IS A complex pathophysiological process characterized by progressive inflammation, lipid accumulation, and arterial wall fibrosis, underlying the development of various vascular occlusive conditions, such as coronary artery disease (CAD) (134). The process of atherosclerosis is typically initiated with endothelial dysfunction in the vessel wall leading to the endothelial activation and recruitment of proinflammatory cells. The ensuing local inflammation then promotes leukocyte chemotaxis and adhesion, and the recruitment of activated platelets to the damaged endothelium. This leads to increased permeability of blood vessels for lipid components in the plasma (84).

Lipid-rich monocytes then accumulate in the arterial intima and differentiate into macrophage-derived foam cells (96, 97). After the accumulation of additional inflammatory cell subsets and extracellular lipids, these early plaques, also known as fatty streaks, progress into mature atherosclerotic plaques. By secreting cytokines and growth factors these plaque cells stimulate their own growth, resulting in further deposition of extracellular matrix components and progression of plaques and stenosis. The thinning of the fibrous cap, with possible consecutive plaque erosion, is caused by matrix-degrading proteases and cytokines secreted by the plaque cells (12).

The 3-hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, are potent inhibitors of

¹GCOE Program and Department of Molecular Endocrinology and Metabolism, Tokyo Medical and Dental University, Tokyo, Japan.

²Section of Cardiology, Department of Medicine, University of Chicago, Chicago, Illinois.

cholesterol biosynthesis. They have emerged as the leading lipid lowering agents and are established in the primary and secondary prevention of CAD. Because serum cholesterol level is strongly associated with coronary heart disease, it has been generally assumed that cholesterol reduction by statins is the predominant, if not the only mechanism, underlying their beneficial effects. Data from a meta-analysis of lipid lowering trials suggest lipid modification alone accounts for the clinical benefits associated with statin therapy (20, 136, 148, 168a). Indeed, the slope of the relationship between cholesterol reduction and mortality risk reduction was the same for statins and nonstatins. However, this type of meta-analysis does not take into account the differences in terms of the length of the individual trials with respect to cardiovascular benefits. Some of the nonstatin lipid lowering trials, such as the Lipid Research Clinic-Coronary Primary Prevention Trial using the bile acid resin, cholestyramine (168a), or the Program on the Surgical Control of the Hyperlipidemias using partial ileal bypass surgery (20), reported benefits after 7.4 and 9.7 years, respectively; whereas most of the statin trials showed benefits at earlier time points, within 5 years. These results suggest that the beneficial effects of statins occur more rapidly and may not be entirely dependent on cholesterol reduction. Thus, in contrast to the original rationale of the biological effect of statins, it is becoming increasingly apparent that the overall benefits observed with statins are not mediated solely by their lipid-lowering properties (103, 123, 174, 175), but also through effects apparently independent of cholesterol lowering, known as "pleiotropic" effects.

Statin Pharmacology and Isoprenylated Proteins

Statins were initially identified as secondary metabolites of fungi (6). One of the first natural inhibitors of HMG-CoA reductase, ML-236B, was isolated as a metabolite from cultures of *Penicillium citrinum* and was shown to be an extremely potent competitive inhibitor of HMG-CoA reductase (7). Thus, statins inhibit HMG-CoA reductase through binding to the enzyme's active site and block the substrate-product transition state of the enzyme (65). Each of the statins is unique in its tissue permeability and pharmacokinetics. Although all statins can enter hepatic cells through either active or passive transport, hydrophilic statins, such as pravastatin and rosuvastatin are less likely to enter nonhepatic cells, while lipophilic statins, for example, atorvastatin and simvastatin are more likely to enter hepatic and nonhepatic cells through passive diffusion. This difference in tissue permeability and metabolism may account for some of the differential pleiotropic effects among the statins (44).

A second mechanism by which statins exert extrahepatic effects is their ability to prevent the synthesis of other important isoprenoid intermediates of the cholesterol biosynthetic pathway, such as farnesylpyrophosphate (FPP) and geranyl-geranylpyrophosphate (GGPP) that are downstream from L-mevalonic acid (46) (Fig. 1). These intermediates serve as important lipid attachments for the post-translational modification of variety of proteins, including the β subunit of heterotrimeric G-proteins, Heme-a, nuclear lamins, and small GTP-binding protein Ras, and Ras-like proteins, such as Rho, Rab, Rac, Ral, or Rap (170). Thus, protein isoprenylation

FIG. 1. Cholesterol biosynthesis pathway and isoprenoids as modulators of Ras and Rho small GTP-binding proteins. Statins inhibit HMG-CoA reductase activity leading to a decrease in isoprenylation of small GTP-binding proteins. Ras-family proteins require FPP, while Rho-family proteins (such as RhoA, Rac1, and Cdc42) require GGPP for their anchoring to the cell membrane, where the small GTPases become activated by GTP loading and transduce signals to their effectors. FPP, farnesylpyrophosphate; GAP, GTPase-activating protein; GDI, GDP dissociation inhibitor; GEF, guanine nucleotide exchange factor; GGPP, geranyl-geranylpyrophosphate; HMG-CoA, 3-hydroxy-methylglutaryl coenzyme A.

permits the covalent attachment, subcellular localization, and intracellular trafficking of membrane-associated proteins. Members of the Ras and Rho GTPase family are major substrates for post-translational modification by prenylation (50, 170). By virtue of inhibiting isoprenoid synthesis, statins inhibit both Ras and Rho functions in hepatic and peripheral tissues.

Each member of the Rho family serves specific functions in terms of cell shape, motility, secretion, and proliferation, although overlapping functions between the members could be observed in overexpressed systems. The activation of Rho in Swiss 3T3 fibroblasts by extracellular ligands, such as platelet-derived lysophosphatidic acid, leads to myosin light chain (MLC) phosphorylation and formation of focal adhesion complexes (49, 50, 170). Indeed, Rho and its effector Rho-kinase (Rho-associated coiled-coil forming kinase [ROCK]) increase the sensitivity of vascular smooth muscle to calcium in hypertension (169) and coronary spasm (74). In contrast, activation of Rac leads to the formation of lamellipodia, membrane ruffles, and oxidative stress, while activation of Cdc42 induces actin-rich surface protrusions called filopodia. Rho is a major target of geranylgeranylation, and inhibition of Rho and ROCK by statins is likely a key mechanism mediating some of the pleiotropic effects of statins on the vascular wall (85, 168). This concept is strongly supported by numerous basic and clinical studies, as described below in this review, showing that inhibition of Rho or ROCK *in vitro* and *in vivo* lead to prominent cardiovascular protective effects that mimic those of statins. These comprise protection against abnormal vascular tone, endothelial dysfunction, inflammation, oxidative stress, and vascular remodeling (116, 155).

For example, early studies showed that statins can inhibit cell proliferation through an isoprenoid-dependent way (67). In fibroblasts, G1 cell cycle arrest induced by lovastatin is reversed by the addition of mevalonate or GGPP (173). Inhibition of platelet-derived growth factor (PDGF)-induced DNA synthesis in vascular smooth muscle cells (VSMCs) by statins is reversed by isoprenoid, but not cholesterol (89, 180). Because the small GTP-binding proteins, Ras and Rho, require posttranslational modification for membrane localization and activity and are implicated in cell-cycle regulation, they are likely targets for the direct antiproliferative vascular effects of statins. Ras can promote cell cycle progression *via* activation of the mitogen-activated protein kinase pathway, whereas Rho causes cellular proliferation through destabilization of the cyclin-dependent kinase inhibitor, p27Kip1 (55, 62). It appears that inhibition of Rho, but not Ras, may be the predominant effect of statins on VSMC proliferation as the inhibition is reversed by GGPP, but not by FPP or low density lipoprotein (LDL) cholesterol (89). Indeed, direct inhibition of Rho by *Clostridium botulinum* C3 transferase or by dominant-negative Rho mutant increases p27Kip1 and inhibits SMC proliferation after PDGF stimulation. In addition, recent studies showed that atorvastatin and simvastatin inhibit angiotensin II-induced connective tissue growth factor production, the pivotal mediator of vascular fibrosis, which was reversed by mevalonate and GGPP. This effect was again mimicked by inhibition of Rho or ROCK (135). Atorvastatin prevented KCl-induced upregulation of *myocardin* and its downstream targets *SM α -actin* and *SM22*, the contractile proteins in VSMCs (92). Again this effect was reversed by mevalonate or GGPP, but not FPP, and mimicked by the ROCK inhibitor Y-27632. Consistent with this, contractility of aortic

rings in response to either KCl or phenylephrine was reduced in mice treated with atorvastatin.

Thus, by inhibiting mevalonate synthesis, statins prevent membrane targeting of Rho and its subsequent activation of ROCK (185). Indeed, a recent report demonstrated that high-dose atorvastatin rapidly attenuates ROCK activity in circulating leucocytes within 2 weeks of treatment, independent of cholesterol reduction in patients with atherosclerosis. This is the first demonstration of ROCK inhibition by statins in humans (113). Although to what extent the pleiotropic effects of statin therapy are attributable to Rho/ROCK inhibition still remains undefined, it is becoming increasingly clear that Rho/ROCK is an important therapeutic target for vascular diseases like atherosclerosis and various nonvascular diseases. Indeed, several pharmaceutical companies are already actively engaged in the development of ROCK inhibitors as the next generation of therapeutic agents for cardiovascular disease because evidence from animal studies suggests the potential involvement of ROCK in systemic and pulmonary hypertension, vascular inflammation, atherosclerosis and stroke (183). In this review, we discuss the mechanisms of pleiotropic effects of statins, with special emphasis on the roles of Rho proteins and ROCK as a therapeutic target of statins in vascular diseases and atherosclerosis.

Structure, Distribution, and Function of ROCKs

ROCKs are protein serine/threonine kinases of 160 kDa and are downstream effectors of the small GTPase Rho (127) (Fig. 2). They were initially characterized by their ability to mediate the formation of RhoA-induced stress fibers and focal adhesions through increasing the phosphorylation of MLC (21). ROCKs consist of an amino-terminal kinase domain, followed by a mid-coiled-coil-forming region containing a Rho-binding domain (RBD), and a carboxy-terminal cysteine-rich domain located within the pleckstrin homology (PH) motif. The two isoforms ROCK1 and ROCK2 share a 65% homology in their amino acid sequence and 92% homology in their kinase domains (127). The carboxy-terminal regions of ROCKs, which contain the PH domain and the RBD, serve as an autoregulatory inhibitor of the amino-terminal kinase domain (9). The interaction of GTP-bound RhoA to the RBD of ROCKs increases ROCK activity through repression of the carboxy-terminal RBD-PH domains on the amino-terminal kinase domain, leading to an active "open" kinase conformation. This open conformation can also be formed by the binding of arachidonic acid to the PH domain or by cleavage of the carboxy terminus by caspase-3 (28, 34, 145). Interestingly, ROCKs can also be activated independently of RhoA through amino-terminal transphosphorylation caused by protein oligomerization. Other small GTP-binding proteins, such as Gem and Rad specifically regulate either ROCK1 or ROCK2-mediated cell rounding and neurite retraction (176). Although further studies are needed to uncover the precise mechanism, these results indicate that ROCK1 and ROCK2 may have different physiological roles in cellular function.

ROCKs are important regulators of cellular apoptosis, growth, metabolism and migration *via* control of the actin cytoskeletal assembly and cell contraction. Stimulation of tyrosine kinase and G protein-coupled receptors recruits and activates Rho guanine nucleotide exchange factors (GEFs), leading to activation of RhoA. ROCKs are pivotal

FIG. 2. Structure of ROCKs and their mode of activation. (A) Structure of ROCK isoforms. Both isoforms (ROCK1 and ROCK2) consist of an N-terminal kinase domain followed by a coiled-coil forming region containing a RBD and a CRD located within the PH domain. The isoforms share overall 65% homology in amino acid sequence and 92% homology in their kinase domains. (B) In the inactive form, the PH domain and RBD domain bind to the amino terminus of the enzyme, forming an auto-inhibitory loop. Upon binding of RBD to active GTP-bound RhoA, the configuration becomes open, leading to the active kinase state of ROCK. CRD, carboxy-terminal cysteine-rich domain; PH, pleckstrin homology; RBD, Rho-binding domain; ROCK, Rho-associated coiled-coil forming kinase.

downstream effectors of RhoA in regulating the actin cytoskeleton by phosphorylation and inhibition of myosin phosphatase, which increases MLC phosphorylation and cellular contraction. Indeed, ROCKs have been shown to phosphorylate various substrates mediating actin cytoskeletal remodeling and focal adhesions, such as adducin, ezrin, radixin, moesin, profilin, vimentin, and LIM-kinase (Table 1). By affecting tight and adherent junctions through actin cytoskeletal contractions, ROCKs can also regulate macrophage phagocytic activity and endothelial cell permeability.

Despite having similar kinase domains, ROCK1 and ROCK2 might serve different functions and could have different downstream targets. Although ROCK1 and ROCK2 are

ubiquitously expressed in mouse tissues from early embryonic development to adulthood, mRNA encoding ROCK2 is highly expressed in cardiac muscle and vascular tissues, which indicates that ROCK2 might have a specialized role in these cell types (127). By contrast, ROCK1 is more abundantly expressed in immunological cells and has been shown to colocalize with centrosomes (25). Nevertheless, even in cells that contain both ROCK1 and ROCK2, recent findings suggest specific functions for each isoform. Indeed, there is evidence that ROCK1 expression (instead of ROCK2) is upregulated upon macrophage adhesion (37). At the same time, phagocytic uptake of fibronectin-coated beads is downregulated in ROCK2-depleted cells, but not in ROCK1-depleted cells (181).

TABLE 1. RHO-ASSOCIATED COILED-COIL FORMING KINASE TARGETS

Targets	Effect	References
MYPT1	Inhibition of MLCP activity	(75)
MLC	Stimulation of actin-myosin ATPase activity	(10)
CPI-17	Inhibition of MLCP activity	(78)
Calponin	Inhibition of calponin binding to actin	(71)
Adducin	Enhanced adducin/F-actin assembly	(39)
ERM proteins (ezrin, radixin, moesin)	Disruption of head-to-tail association. Enhanced F-actin assembly	(122)
LIM-kinases 1 and 2	Stimulation of kinase activity	(119, 165)
RhoE	Increased RhoE stability	(128)
ZIPK	Activation of ZIPK activity	(48)
Profilin	Inhibition of Profilin binding to actin	(147)
Vimentin	Enhanced filament disassembly	(47)
PTEN	Stimulation of phosphatase activity	(94)
IRS-1	Inhibition (Ser307) or activation (Ser632/635) of insulin signaling	(14, 33, 41, 98)

CPI-17, C-kinase-activated protein phosphatase-1 (PP1) inhibitor; ERM, ezrin-radixin-moesin; IRS, insulin receptor substrate; LIM kinase, LIM (Lin11, Isl-1 and Mec-3) domain kinase; MLC, myosin light chain; MLCP, myosin phosphatase; MYPT1, myosin phosphatase target subunit 1; PTEN, phosphatase and tensin homolog deleted from chromosome 10; ZIPK, zipper-interacting protein kinase.

These findings emphasize distinct functions for ROCK1 and ROCK2. Unfortunately, pharmacological inhibitors of ROCKs, such as Y27632 and fasudil/hydroxyfasudil (HA1077), which target their ATP-dependent kinase domains, inhibit ROCK1 and ROCK2 at equimolar concentrations. Furthermore, at higher concentrations, Y27632 can also inhibit protein kinase C-related kinase-2, protein kinase N, and citron kinase, whereas fasudil can inhibit protein kinase A and protein kinase C (PKC) (158). It is therefore, difficult to ascribe specific function of ROCKs based upon studies with these ROCK inhibitors because they are nonselective for ROCK isoforms and can nonspecifically inhibit other protein kinases. Further studies, including gene targeting or silencing will be necessary to unveil the precise mechanism(s) by which ROCK1 and ROCK2 regulate cell function.

Rho/ROCK Pathway and Crosstalk with Nitric Oxide in Vascular Homeostasis and Diseases

There is an intimate functional coordination between endothelial cells and VSMCs that contributes to the local homeostasis of vascular walls, and deterioration of this crosstalk underlies many aspects of vascular diseases, including vasospasm and atherosclerosis (Fig. 3). Endothelial-derived nitric oxide (NO) diffuses into the adjacent VSMCs of the blood vessels, activates soluble guanylate-cyclase, stimulates the formation of cGMP, and subsequently activates cGMP-dependent protein kinase (cGK). Stimulation of cGMP/cGK cascade causes relaxation of VSMCs and also inhibits VSMC proliferation, migration and fibrotic gene expression (60). The negative regulation of these VSMC functions by cGMP/cGK pathway has been known to be counteracted by Rho/ROCK pathway.

Importantly, recent evidence suggests that there is extensive crosstalk between NO/cGMP/cGK and RhoA/ROCK signaling underlying their antagonistic actions in VSMCs. There is compelling evidence that the NO/cGK pathway leads to an inhibition of RhoA/ROCK signaling. The NO-

donor sodium nitroprussid and constitutively active cGK are able to inhibit the phenylephrine-induced translocation of RhoA from the cytosolic fraction to the membrane in rat aorta; thus, abolishing subsequent ROCK activation (139). Furthermore, NO-mediated vasorelaxation has been demonstrated to be, in part, mediated by inactivation of RhoA/ROCK signaling in rat aorta (26). Chronic and acute inhibition of NO synthase (NOS) increases α 2-adrenoreceptor-stimulated activation of RhoA and ROCK (22), and promotes vascular inflammation and remodeling through angiotensin II-mediated augmentation of Rho/ROCK activity (73). cGK type I haploinsufficient mice show exaggerated peri-vascular fibrosis in the heart in response to systemic angiotensin II infusion (141). The inhibitory effect of cGMP signaling on Rho/ROCK pathway is at least, in part, exerted through direct phosphorylation by cGK type I of RhoA at serine 188 close to its c-terminus, which results in increased binding of GTP-bound RhoA to Rho GDP-dissociation inhibitor that sequesters RhoA into cytosol (139, 143). Transgenic overexpression of brain-natriuretic peptide, another cGMP agonist, confers profound inhibition of coronary artery remodeling and peri-vascular fibrosis in angiotensin II-infused mice, and this effect was abolished by VSMC-specific expression of mutant RhoA resistant to phosphorylation by cGK (141). Together these lines of evidence highlight the capability of NO/cGMP signaling to inhibit the RhoA/ROCK cascade leading to vasorelaxation and inhibition of vascular inflammation and remodeling.

Impaired endothelial function and decreased NO-production has been implicated in the etiology of hypertension and atherosclerosis. Decreased expression and activity of endothelial NO synthase (eNOS) in vascular walls is found in various cardiovascular disease models like spontaneously hypertensive rats (27) and patients with atherosclerosis, and eNOS-deficient mice have an elevated blood pressure (61) and are more prone to develop atherosclerosis (73). As detailed in the following sections, Rho/ROCK cascade in endothelium reciprocally influences NO signaling by downregulating eNOS expression and activity. Thus, it is conceivable that, in diseased and dysfunctional endothelium, aberrant augmentation of Rho/ROCK activity leads to decrease in endothelial-derived NO bioavailability that gives rise to activation of VSMC Rho/ROCK pathway leading to vascular hypercontraction (152), excessive vascular inflammation, and remodeling in atherosclerosis.

Taken together, increased NO production in endothelium will lead to an inhibition of the Rho/ROCK cascade in VSMCs; thus, protecting against the enhanced contractile and proliferative effects of Rho/ROCK signaling. On the other hand, stimulated Rho/ROCK activity in endothelium appears to diminish NO production; thus, even amplifying the increase in contractility and vascular remodeling induced by VSMCs Rho/ROCK signaling. cGMP agonists like NO donors; therefore, could be effective inhibitors of Rho/ROCK signaling in addition to statins and ROCK inhibitors. However, in inflammatory states like atherosclerosis and diabetes, lessened efficacy of NO donors is frequently encountered, known as NO tolerance, due, in part, to excessive oxidative stress, including peroxynitrite formation (109) and probably to overactivated Rho/ROCK activity in vascular tissues. Hence, for the treatment of atherosclerosis, direct inhibition of Rho/ROCK pathway

FIG. 3. Rho/ROCK crosstalk with NO in the vascular wall. Endothelium-derived NO diffuses into VSMCs, stimulates synthesis of cGMP and leads to the activation of cGK. cGK blocks Rho/ROCK signaling by phosphorylating GTP and GDP-bound RhoA at Ser188, which increases Rho binding to Rho GDI and results in the sequestration of RhoA into the cytosol. On the other hand, Rho/ROCK in endothelial cells inhibits eNOS expression and activity leading to endothelial dysfunction that underlies vascular diseases, such as atherosclerosis. cGK, cGMP-dependent protein kinase; cGMP, cyclic GMP; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; VSMCs, vascular smooth muscle cells.

with statins and ROCK inhibitors would be a more reasonable and effective approach.

Rho/ROCK Pathway and Endothelial Cells: Effects of Statins

The endothelium forms the inner lining of blood vessels and represents a metabolically active system. In addition to its function as a barrier, the endothelium senses and responds to environmental factors and serves as an important autocrine and paracrine organ that regulates the contractile state of blood vessels, blood cell trafficking, hemostatic balance, and other cellular compositions (4). Endothelial dysfunction, as defined by decreased bioavailability of endothelium-derived NO, is one of the earliest manifestations of atherosclerosis (163, 178). An important characteristic of endothelial dysfunction is the impaired synthesis, release, and activity of eNOS, as well as a decrease in NO bioavailability through inactivation by increased reactive oxygen species (ROS) production (8, 95). The antiatherogenic effects of eNOS include enhancement of vascular relaxation (63) and inhibition of platelet aggregation (125), VSMC proliferation (42), and leukocyte-endothelial interactions (43).

Several studies have demonstrated that statins improve endothelial function even before significant reduction in serum cholesterol levels has occurred (11, 118). These effects have been linked to the upregulation of eNOS by statins (31, 86) and are further supported by studies in normocholesterolemic animals, where statins protect against stroke, myocardial ischemia, and reperfusion injury through noncholesterol mechanisms (30, 91).

Statins can upregulate eNOS by several different mechanisms. First, statins increase the stability of eNOS mRNA through inhibition of the Rho/ROCK pathway, thereby leading to increased eNOS expression (86, 87). For example, RhoA/ROCK negatively regulates eNOS expression and activity as demonstrated by studies showing that direct inhibition of Rho by *C. botulinum* C3 transferase or overexpression of a dominant-negative mutant of RhoA increases eNOS ex-

pression (88, 168). Consistent with this, ROCK inhibitor hydroxyfasudil prevented hypoxia-induced downregulation of eNOS (168). The inhibitory effects of Rho and ROCK on eNOS expression are exerted through inhibition of eNOS mRNA stability and not that of eNOS transcription (Fig. 4). Although the effects of statins on Ras and Rho isoprenylation are reversed in the presence of FPP and GGP, respectively, the effects of statins on eNOS expression are reversed only by GGPP, but not by FPP or LDL cholesterol (88). These findings indicate that the Rho/ROCK pathway rather than the Ras pathway predominantly regulates eNOS expression. The 3' poly(A) tail is important in mRNA stability and translational efficiency. Recent evidence suggests that laminar shear stress and statins induce eNOS 3' poly(A) tail lengthening from less than 25 nt at basal condition to more than 75 nt (81, 177). eNOS mRNA polyadenylation was also increased after Rho inhibition and actin cytoskeletal disruption by cytochalasin D. This may account for at least part of the mechanism by which statins and Rho/ROCK inhibition increase eNOS mRNA stability, and thus, its expression.

A second important mechanism by which statins activate eNOS is mediated through the serine-threonine protein kinase Akt. Akt is activated through protein tyrosine kinase and G protein-coupled receptors and has been shown to be involved in mediating growth, metabolism, and survival through downstream targets, such as glycogen synthase kinase- β and Forkhead/FOXO transcription factors. Interestingly, eNOS is also a substrate of Akt and is phosphorylated at Ser 1177/1179 (29). Thus, statins have been shown to rapidly promote the activation of Akt in endothelial cells leading to eNOS phosphorylation and increased NO production. Because this process is inhibited by the phosphatidylinositol-3 kinase (PI3K) inhibitors, wortmannin and LY294002, these findings indicate that statins activate Akt by upregulating PI3K signaling (82). The statin-Akt pathway appears to be specific for endothelial cells, as activation of Akt by statins is not observed in cardiac or smooth muscle cells (82). Consistent with the statin induction of PI-3K/Akt/eNOS pathway, ROCK negatively regulates phosphorylation of eNOS through inhibition of

FIG. 4. Biological actions of ROCKs in the vasculature. In endothelial cells, Rho/ROCK decreases eNOS mRNA expression *via* reduction of its half-life, and also inhibits eNOS phosphorylation at Ser1177 through inactivation of Akt. In VSMCs, ROCK1 haploinsufficiency leads to decreased migration and reduced fibrotic gene expression. Pharmacological inhibition of ROCK prevents the development of atherosclerosis by inhibition of altered chemotaxis of macrophages and its transformation into foam cells. Genetic mouse models of ROCK1 or ROCK2 deletion revealed that ROCK1 promotes cholesterol uptake, while ROCK2 suppresses cholesterol efflux, in macrophages in the atherosclerotic lesion.

protein kinase B/Akt in human endothelial cells (105). Moreover, inhibition of ROCK leads to a rapid phosphorylation and activation of Akt *via* the PI3K, leading to increased NO production (179) (Fig. 4). The inhibitory effect of Rho/ROCK on PI-3K/Akt in endothelial cells is likely mediated by ROCK phosphorylation of phosphatase and tensin homologue, which increases its stability and phosphatase activity leading to the reduction of Akt phosphorylation (24, 94, 172).

Rho/ROCK Pathway and VSMC: Effects of Statins

VSMC contribute to vascular proliferative diseases, including postangioplasty restenosis, transplant arteriosclerosis and venous graft occlusion (16). Recent studies have shown that statins can attenuate cytokine-mediated VSMC proliferation in coronary artery smooth muscle cells and also inhibit pathological proliferation, such as that observed in transplant-associated arteriopathy (23, 79, 153). Because transplant-associated arteriopathy is more dependent on immunological mechanisms rather than lipid disorders, this finding further underscores the importance of statin pleiotropy. As mentioned above, simvastatin inhibits PDGF-induced DNA synthesis of VSMCs in a Rho-dependent fashion (89). Another study showed that atorvastatin inhibits serotonin-induced mitogenesis and migration through inhibition of GTP-RhoA formation in pulmonary artery smooth muscle cells (93). This effect is also reversed by GGPP, but not FPP.

Consistent with these earlier findings, ROCK has been implicated in the proliferation and migration of VSMCs (140, 142, 144) (Fig. 4). Furthermore, ROCK mediates angiotensin II-induced expression of monocyte chemoattractant protein-1 (MCP-1) (40) and plasminogen activator inhibitor-1 (PAI-1) (167). In addition, several *in-vivo* studies have identified ROCK activation in experimental models of vascular inflammation or injury (73, 107, 142). In L-NAME treated rats, ROCK inhibitors attenuate the inflammatory response and vascular remodeling (64, 73). In addition, ROCK activity is increased in the neointima after balloon-induced vascular injury, which is suppressed by ROCK inhibitors or gene transfer of a dominant-negative mutant of ROCK (32, 142). Most of these studies, however, utilized pharmacological inhibitors of the Rho kinase: fasudil or Y-27632, both of which are unable to distinguish between ROCK1 and ROCK2 isoforms.

Recently, several groups have successfully generated mutant mice with deletion of the ROCK1 and ROCK2 allele. While mice harboring homozygous deletion of ROCK1 or ROCK2 allele are embryonic and postnatal lethal, haploinsufficient mice are fertile, and phenotypically normal (129, 131, 154, 182). This allowed for the detection of differential roles, if any, of ROCK1 and ROCK2 in cardiovascular injury. VSMC proliferation was decreased in the neointima of ROCK1 + / - mice after carotid artery ligation (117). Interestingly, VSMC proliferation in response to serum or PDGF was not different between wild-type and ROCK1 + / - mouse-derived VSMCs, while the migration of VSMC in response to PDGF was substantially reduced in ROCK1 + / - mice, suggesting that ROCK1 may contribute to increase VSMC migration and survival after vascular injury.

Rho/ROCK Pathway and Myocardium

Pathological cardiac hypertrophy is defined as the increase in cardiomyocyte size, and thus, ventricular mass, by patho-

logical stimuli, such as hypertension, valvular insufficiency and stenosis, or ischemia associated with CAD. The pathological cardiac hypertrophy is characterized by fetal cardiac gene expression, interstitial fibrosis, myocyte loss by apoptosis or necrosis, and diastolic and/or systolic contractile dysfunction. This type of hypertrophy is an adaptive response and initially beneficial to overcome adverse hemodynamic load and maintain cardiac output (17, 54, 58). However, persistent stress progressively causes marked enlargement of heart chambers and contractile dysfunction, leading to decompensated congestive heart failure, (17, 54, 58). Studies using ROCK inhibitors, Y27632 and fasudil, have provided considerable evidence that RhoA/ROCK signaling plays a critical role in the development of cardiac hypertrophy and remodeling in various animal models (53, 57, 80, 106, 138). However, the relative contribution between ROCK 1 and 2 isoforms in mediating hypertrophic process has been undefined (Fig. 5).

Recent genetic studies using ROCK1 deficient (149, 150, 182) and haploinsufficient mice (131) have demonstrated a critical role for this isoform in pathological cardiac remodeling. Interestingly, partial or full ROCK1 deletion did not block the development of cardiomyocyte hypertrophy, but profoundly protected against structural and functional alterations related to pathological hypertrophic remodeling, including cardiac fibrosis, cardiomyocyte apoptosis, cardiac dilation and contractile dysfunction.

The finding that ROCK1 is dispensable for the development of cardiac hypertrophy envisaged the possibility that ROCK2 may be the isoform responsible for the anti-hypertrophic actions seen in pharmacological ROCK

FIG. 5. Role of Rho/ROCK in cardiac hypertrophy. Pharmacological studies using ROCK inhibitors have shown that Rho/ROCK pathway mediates pathological cardiac hypertrophy in response to angiotensin II infusion and thoracic aorta constriction. Recent genetic mouse models have revealed that ROCK2, but not ROCK1, mediates cardiomyocyte hypertrophy. ROCK2 activates SRF and ERK through inhibition of FHL2. Both ROCK1 and ROCK2 contribute to myocyte apoptosis and interstitial fibrosis in pathological cardiac hypertrophy. ERK, extracellular signal-regulated mitogen-activated protein kinase; FHL2, four-and-a-half LIM-only protein-2; SRF, serum response factor.

inhibition. To address this question, our research group recently generated mice with cardiomyocyte-specific ROCK2 deletion (120). Cardiac ROCK2 knockout mice, when challenged with angiotensin II infusion or thoracic aorta constriction, exhibited a substantially smaller increase in the heart weight, myocyte size, hypertrophy-related fetal gene expression, intraventricular fibrosis, cardiac apoptosis, and oxidative stress compared to control mice. Deletion of ROCK2 in cardiomyocytes led to increased expression of four-and-a-half LIM-only protein-2 (FHL2) and FHL2-mediated inhibition of serum response factor and extracellular signal-regulated mitogen-activated protein kinase. Knockdown of FHL2 in ROCK2-deficient cardiomyocytes restored their hypertrophic response to angiotensin II. These observations suggest that ROCK1 and ROCK2 have redundant and nonredundant functions in the development of pathological cardiac hypertrophy.

Rho/ROCK Pathway and Atherosclerosis

Accumulating evidence suggests that ROCK pathway is involved in many steps of the inflammatory atherosclerotic process. ROCK activation downregulates eNOS expression and inhibition of ROCK prevents hypoxia-induced downregulation of eNOS (159). In addition, it has been shown that lysophosphatidic acid (LPA)-induced endothelial hyperpermeability requires RhoA/ROCK activation (171). Long-term inhibition of ROCK induces regression of arteriosclerotic coronary lesions in a porcine model *in vivo* (157). Inhibition of ROCK with Y-27632 limits early atherosclerotic plaque development in mutant mice with LDL receptor deficiency and fed with a high-cholesterol diet. This was associated with a significant reduction in T-lymphocyte accumulation (102).

Another study analyzed the distribution and phosphorylation of target proteins of ROCK, including MLC and ezrin-radixin-moesin (ERM) proteins, in the apolipoprotein E-deficient mouse model of atherosclerosis. Results showed that treatment with the ROCK inhibitor Y-27632 inhibited ERM phosphorylation in the atherosclerotic plaques (126). Indeed, mutant mice with ROCK1-deficiency in bone-marrow-derived cells exhibit decreased atherosclerosis on a LDL-receptor-deficient background (175) (Fig. 4). This was due, in part, to decreased chemotaxis, cholesterol uptake, and foam-cell formation in ROCK1-deficient macrophages. Furthermore, our research group recently investigated the role of ROCK2 in atherogenesis. ROCK2-deficiency in bone-marrow-derived cells showed substantially reduced lipid accumulation and atherosclerotic lesions in the LDL-receptor-null mice. This was associated with decreased foam cell formation and increased cholesterol efflux in ROCK2-deficient macrophages, which are mediated, in part, through peroxisome proliferator-activated receptor- γ /liver X receptor/ATP-binding cassette transporter A1 pathway (186). These findings indicate that both ROCK1 and ROCK2 in macrophages play a crucial part in the development of atherosclerosis through distinct mechanisms, and suggest potential therapeutic benefits of inhibition of either ROCK1 or ROCK2, or both, in atherosclerotic vascular disease.

Findings from *in vitro* experiments and animal studies in the past years have provided significant evidence for the importance for ROCK as a potential target for the treatment of endothelial dysfunction and atherosclerosis. Indeed, numerous clinical studies have demonstrated a link between ROCK and endothelial dysfunction and metabolic syndromes in

humans (56, 99, 114, 115). In particular, our research team has demonstrated a correlation between elevated ROCK activity and impaired endothelial function in patients with CAD (112) and stable atherosclerosis (113). Furthermore, treatment with the ROCK inhibitor fasudil reduced the overactivity of ROCK in patients with atherosclerosis and improved endothelium-dependent vasodilation, as well as flow-mediated, endothelium-dependent dilation. Importantly, this finding was only present in patients with CAD, but not in healthy individuals where ROCK is presumably not "overactive". Most interestingly, endothelium-dependent vasodilation in healthy subjects tended to worsen with fasudil therapy compared with placebo. This finding might be explained by the fact that inhibition of ROCK in healthy individuals could lead to a negative-feedback loop with increased transcription of Rho. This would in turn lead to a compensatory increase in the downstream effects of Rho, including suppression of eNOS production. Also, ROCK inhibition in healthy individuals might lead to an excess of NO production, resulting in the formation of peroxynitrite, and this could lead to eNOS uncoupling and worsening endothelial function (36). These results suggest that some basal ROCK activity is probably required for the maintenance of vascular homeostasis and emphasizes the importance of selective ROCK inhibitors for use in the clinic.

Rho/ROCK Pathway in Diabetes and Diabetic Complications

Although statins are generally well tolerated and are believed to have minimal adverse effects, recent meta-analysis of clinical trials have revealed that long-term statin use is related to a small increased risk of type 2 diabetes mellitus (68, 124, 137). In view of the overwhelming benefit of statins in the reduction of cardiovascular events, it would be clear that the small absolute risk for development of diabetes is outweighed by the cardiovascular benefits in patients for whom statin therapy is recommended (68, 69).

ROCK has been demonstrated to interact and phosphorylate insulin receptor substrate-1 (IRS-1) and thereby modulate insulin signaling (Fig. 6). However, both *in vitro* and *in vivo* studies have yielded conflicting results about the effects of RhoA/ROCK/IRS pathway on insulin signaling. The majority of the reported studies favor a detrimental role of ROCK activation in insulin signaling. These studies suggest that ROCK phosphorylates IRS-1 at Ser307 and impairs activation of PI-3K in rat VSMCs, H9c2 rat cardiac myoblasts and C2C12 mouse myoblasts (14, 33, 98). In contrast, Furukawa *et al.* reported that ROCK-mediated phosphorylation of IRS-1 at Ser632/635 positively regulates insulin action by facilitating tyrosine phosphorylation of IRS-1 in 3T3-L1 adipocytes and L6 myotubes (41). Although it was consistently reported that ROCK activation increases serine phosphorylation levels of IRS-1 and IRS-2, the effects on insulin-stimulated tyrosine phosphorylation of IRS-1 and IRS-2 and on PI-3K activation appear to be complex and may be context dependent.

In vivo, whether treatment with ROCK inhibitors improves or impairs insulin signaling is also context dependent. In supporting a negative effect of ROCK activation on insulin signaling, in obese Zucker rats ROCK inhibition with fasudil improves insulin signaling and glucose tolerance (70). In supporting a positive effect of ROCK activation on insulin

FIG. 6. Role of Rho/ROCK in the etiology of insulin resistance. Experimental evidence in cultured cells and mice show conflicting results, between different models, for the effect of Rho/ROCK on insulin sensitivity. While ROCK phosphorylation of IRS-1 at Ser307 negatively impacts insulin signaling (left), ROCK phosphorylation of IRS-1 at Ser632/635 enhances insulin signaling (right). IRS-1, insulin receptor substrate 1.

signaling, acute administration of Y27632 causes insulin resistance by reducing insulin-mediated glucose uptake in skeletal muscle (41), and global ROCK1 deficiency in mice causes insulin resistance *in vivo*, in part, *via* reduced serine 632/635 phosphorylation of IRS-1 (90). This latter mechanism would explain the recently demonstrated risk of statin therapy for development of diabetes.

Obesity, characterized by hypertrophy, and inflammatory changes of adipose tissue, underlies the development of metabolic syndrome and predisposes individuals to diabetes. A recent report has demonstrated that mice fed a high-fat diet (HFD) showed increased ROCK activity in adipose tissue compared to mice fed normal chow (51). Treatment with fasudil attenuated weight gain and insulin resistance in HFD-fed mice. Transgenic overexpression of dominant-negative RhoA in adipocytes showed decreased ROCK activity in adipocytes, decreased HFD-induced weight gain, improved glucose metabolism compared to wild-type littermates. Furthermore, compared to HFD-fed wild-type littermates, DN-RhoA transgenic mice on a HFD showed decreased adipocyte hypertrophy, reduced macrophage recruitment to adipose tissue, and lower expression of mRNAs encoding tumor necrosis factor- α and increased expression of adiponectin. Consistent with this finding, another study showed that constitutive activation of RhoA in 3T3-L1 adipocytes led to augmented expression of PAI-1 and MCP-1, which was reversible by ROCK inhibitor Y-27632, providing evidence that Rho/ROCK pathway directly induces inflammatory cytokine expression in adipocytes (110). Thus, inhibition of Rho/ROCK signaling in adipocytes may provide a therapeutic strategy for inflammation of adipose tissue associated with obesity and metabolic syndrome (Fig. 7).

Paradoxically, it is of interest to note that, in the context of development and regeneration, Rho/ROCK pathway has been shown to confer a negative impact on adipogenesis. Mice with constitutive Rho/ROCK activation due to p190-B Rho GTPase-activating protein-deficiency displayed a defect in adipogenesis, with a predisposition toward myogenesis (161). Normal adipogenesis was restored by Y-27632 administration to these mice. Consistent with this, activation of Rho/ROCK by forced expression of Rho-specific GEF enhanced regeneration of in-

FIG. 7. Role of Rho/ROCK in adipocyte dysfunction and development. (Left) In the setting of obesity and metabolic syndrome, Rho/ROCK pathway is activated in adipose tissues, which promotes adipocyte hypertrophy, insulin resistance, upregulation of TNF- α and downregulation of adiponectin. (Right) In development and tissue regeneration, activated Rho/ROCK pathway positively regulates myogenesis, while it suppresses adipogenic differentiation. TNF- α , tumor necrosis factor.

jured skeletal muscles in mice, and myogenesis of C2C12 cells in culture, while it inhibited insulin-induced adipogenesis in 3T3L1 preadipocytes (19). In mouse embryonic fibroblasts, deletion of ROCK2, but not ROCK1, enhanced their adipogenic differentiation, implicating ROCK2 as a key negative regulator in the development of adipocytes (111).

Endothelial dysfunction is one of the earliest manifestations of diabetes and underlies cardiovascular complications that constitute the major cause of mortality and morbidity. Impaired endothelium-dependent relaxation is a consistent finding in blood vessels from diabetic animals and patients (121, 162). An increasing body of evidence intensifies the idea that the injured endothelium in diabetes is due, in part, to alterations in Rho/ROCK signaling (Fig. 8). Increased vascular permeability is also a major characteristic of diabetic

FIG. 8. Role of Rho/ROCK in diabetic vasculopathies. Experimental evidence for the role of ROCK in diabetic vascular complications. In endothelial cells, activation of the RAGE activates ROCK and mediates increased endothelial permeability of diabetes. HG promotes PAI-1 expression through ROCK and NF- κ B. Also, high glucose upregulates arginase expression *via* Rho/ROCK and leads to decreased bioavailability of NO in diabetic endothelium. In VSMCs, HG upregulates Rho/ROCK through activation of PDGFR- β . The activation of PDGFR- β /Rho/ROCK results in the aberrantly, activated migration of diabetic VSMCs. HG, high glucose; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; PAI-1, plasminogen activator inhibitor-1; PDGFR, platelet-derived growth factor receptor; RAGE, receptor for advanced glycation end product.

vasculopathy and, in diabetic condition, evidence indicates that advanced glycation end products (AGEs) activate RAGE, a major receptor for AGEs, and modulate various cell functions by multiple pathways, including ROCK signaling (45). RhoA and RAGE can unexpectedly form a complex called RhoA/RAGE, which has been suggested to induce ROCK activation, resulting in reorganization of the actin cytoskeleton, leading to endothelial cell hyperpermeability in diabetes (59). PAI-1 is a key regulator of fibrinolysis *via* inhibition of plasminogen activators, and high plasma PAI-1 levels correlate with the development of diabetic cardiovascular complications. Our research group demonstrated that hyperglycemia increases ROCK activity in endothelial cells in a PKC and ROS-dependent fashion, and activated ROCK mediates high glucose-induced PAI-1 expression (130). Another group reported a similar finding showing that high glucose activated RhoA and induced nuclear factor kappa-light-chain-enhancer of activated B cells promoter activity and PAI-1 expression in a ROCK-dependent manner (66). Interestingly, recent studies showed that diabetes causes ROCK-mediated increase in endothelial arginase activity, contributing, in part, to the impaired NO bioavailability in diabetes (132, 133).

Diabetic vasculopathy is also characterized by increased migratory propensity of VSMCs in atherosclerosis and vascular injury. A study has reported concomitant upregulation of platelet-derived growth factor receptor (PDGFR)-beta and RhoA expressions in cultured VSMCs treated with high glucose and in the aorta of diabetic rats (5). High glucose induction of RhoA was abolished by PDGFR inhibitor. High glucose-induced augmentation in VSMCs migratory capacity was also abolished by Y-27632 and a neutralizing antibody against PDGF. Thus, the upregulation of PDGFR-beta/Rho/ROCK pathway by high glucose mediates the aberrant migration of VSMCs in diabetes.

Thus, the involvement of ROCK in diabetes and its complications is becoming clear due to the increased amount of results showing upregulation of the ROCK pathway in diabetic models, both *in vivo* and *in vitro*. The possibility of using ROCK inhibitors to prevent progression of some diabetic problems has been suggested as a promising novel therapy. However, it is necessary to first clarify all the implications of ROCK signaling alterations during the development of diabetes before employing this therapy in these patients.

In a previous article by Liu *et al.*, a correlation between ROCK activity in circulating leucocytes and metabolic syndrome in the Taiwanese population has been demonstrated (99). The authors showed that increased levels of ROCK activity independently predicted the diagnosis of metabolic syndrome, and that in addition, higher ROCK activity was also associated with greater body mass index, waist circumference, greater high-sensitive C-reactive protein, as well as lower adiponectin levels. This suggests that ROCK activity in inflammatory cells could serve as a new surrogate marker for the diagnosis of metabolic syndrome. Also, increased ROCK activity in leucocytes may likely play a role in the development and progression of metabolic syndrome.

ROCK Inhibitors as New Therapeutics for Atherosclerosis and Cardiovascular Diseases

ROCK inhibitors, such as fasudil have been shown to prevent cerebral vasospasm after subarachnoid hemorrhage (160,

166). Similarly, animal studies showed that fasudil and Y-27632 could inhibit the development of atherosclerosis and arterial remodeling after vascular injury (183). ROCK activity is involved in the expression of PAI-1 mediated by hyperglycemia, indicating that ROCK could function as a key regulator of cardiovascular injury in patients with diabetes mellitus (130). The RhoA/ROCK pathway has been reported to be involved in angiogenesis (77), cerebral ischemia (151), erectile dysfunction (15), hypertension (169), myocardial hypertrophy, myocardial ischemia-reperfusion injury (13), neointima formation (142), pulmonary hypertension (3), and vascular remodeling (52). In addition, ROCK inhibitors have shown benefits in animal models of Alzheimer's disease, bronchial asthma, cancers, demyelinating diseases, glaucoma, and osteoporosis (108, 158, 159). Although the majority of the previous studies have shown that inhibition of both isoforms by ROCK inhibitors results in beneficial effects, whether the effects are mediated by inhibition of ROCK1, ROCK2, or both, remains to be determined.

Despite robust animal data with ROCK inhibitors, to translate the therapeutic benefits of ROCK inhibitors to humans, clinical trials will need to be performed to document their benefits in patients. Currently, small clinical trials have shown some benefits of ROCK inhibitors in cardiovascular diseases. For example, treatment with fasudil leads to improvements of symptoms and outcomes in patients with systemic hypertension (101, 169), pulmonary hypertension (38), vasospastic angina (104), stable effort angina (156), stroke (151), and chronic heart failure (76). Indeed, many of the so-called "pleiotropic" effects of statins might be mediated by ROCK inhibition (184, 185). However, the extent of clinical benefits obtained by ROCK inhibition with statin therapy remains to be determined. Currently, fasudil is the only ROCK inhibitor approved for human use. Fasudil was approved in Japan and China for the prevention and treatment of cerebral vasospasm after surgery for subarachnoid hemorrhage. Although several adverse effects have been reported (such as abnormal hepatic function, intracranial hemorrhage, and hypotension), fasudil appears to be relatively safe in patients with hemorrhagic stroke (151). Therefore, fasudil could be one of the first promising ROCK inhibitors to be used for cardiovascular conditions, such as acute stroke and pulmonary artery hypertension. Interestingly, another ROCK inhibitor, SAR407899, has been shown to be eightfold more active than fasudil. In animal models, the antihypertensive effect of this novel ROCK inhibitor has been shown to be superior to that of fasudil and Y-27632 (100). Thus, SAR407899 might represent a novel potent ROCK inhibitor for the treatment of cardiovascular disease. Finally, an isoform-selective ROCK2 inhibitor, SLx-2119 (Surface Logix), appears to be 100-fold more selective towards ROCK2 than ROCK1, and could have more favorable safety profile than dual ROCK inhibitors. However, further clinical studies with this compound will need to be performed to determine its efficacy and safety in patients with cardiovascular disease. Because ROCK1 and ROCK2 both mediate various aspects of cardiovascular disease, selective ROCK isoform inhibition is unlikely to be advantageous in terms of efficacy, although such a strategy could prove to be safer than dual ROCK isoform inhibition.

Conclusion

Growing evidence from animal and clinical studies suggest the importance of ROCK in the pathogenesis of

cardiovascular disease. Indeed, many of the pleiotropic or cholesterol-independent effects of statins may be attributed to their ability to block isoprenoid synthesis and inhibit the Rho/ROCK pathway. Thus, targeting ROCK with specific inhibitors may be an important therapeutic strategy in the prevention and treatment of cardiovascular disease in patients with normal or low lipid levels. Further research will see how statins and ROCK inhibitors could differentiate or overlap as therapeutics for cardiovascular medicine. As ROCKs are involved in various aspects of vascular function and disease, understanding their role in the vascular wall may provide key insights into how the vasculature as a whole is regulated under normal and pathophysiological conditions. However, despite a growing number of reports demonstrating that ROCK activity is increased under a variety of pathologic conditions, little is known regarding the molecular mechanisms that contribute to increased ROCK activity or what the downstream targets for ROCK are, especially in the context of inflammatory and metabolic disorders, including atherosclerosis and diabetes. Also, a greater understanding of the physiological role of each ROCK isoform in the cardiovascular system and further clinical trials will be needed to determine whether selective or nonselective ROCK inhibitors could be clinically useful in treating patients with atherosclerosis and vascular disease.

Acknowledgments

Supported by grants from National Institutes of Health (HL052233, NS070001, and DK085006) to J.K.L. and a Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science (Japan) to N.S.

References

1. This reference has been deleted.
2. This reference has been deleted.
3. Abe K, Shimokawa H, Morikawa K, Uwatoku T, Oi K, Matsumoto Y, Hattori T, Nakashima Y, Kaibuchi K, Sueishi K, and Takeshi A. Long-term treatment with a Rho-kinase inhibitor improves monocrotaline-induced fatal pulmonary hypertension in rats. *Circ Res* 94: 385–393, 2004.
4. Aird WC. Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms. *Circ Res* 100: 158–173, 2007.
5. Akiyama N, Naruse K, Kobayashi Y, Nakamura N, Hamada Y, Nakashima E, Matsubara T, Oiso Y, and Nakamura J. High glucose-induced upregulation of Rho/Rho-kinase via platelet-derived growth factor receptor-beta increases migration of aortic smooth muscle cells. *J Mol Cell Cardiol* 45: 326–332, 2008.
6. Alberts AW. Discovery, biochemistry and biology of lovastatin. *Am J Cardiol* 62: 10J–15J, 1988.
7. Alberts AW, Chen J, Kuron G, Hunt V, Huff J, Hoffman C, Rothrock J, Lopez M, Joshua H, Harris E, Patchett A, Monaghan R, Currie S, Stapley E, Albers-Schonberg G, Hensens O, Hirshfield J, Hoogsteen K, Liesch J, and Springer J. Mevinolin: a highly potent competitive inhibitor of hydroxymethylglutaryl-coenzyme A reductase and a cholesterol-lowering agent. *Proc Natl Acad Sci U S A* 77: 3957–3961, 1980.
8. Alderson LM, Endemann G, Lindsey S, Pronczuk A, Hoover RL, and Hayes KC. LDL enhances monocyte adhesion to endothelial cells *in vitro*. *Am J Pathol* 123: 334–342, 1986.
9. Amano M, Chihara K, Nakamura N, Kaneko T, Matsuura Y, and Kaibuchi K. The COOH terminus of Rho-kinase negatively regulates rho-kinase activity. *J Biol Chem* 274: 32418–32424, 1999.
10. Amano M, Ito M, Kimura K, Fukata Y, Chihara K, Nakano T, Matsuura Y, and Kaibuchi K. Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). *J Biol Chem* 271: 20246–20249, 1996.
11. Anderson TJ, Meredith IT, Yeung AC, Frei B, Selwyn AP, and Ganz P. The effect of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. *N Engl J Med* 332: 488–493, 1995.
12. Aukrust P, Halvorsen B, Yndestad A, Ueland T, Oie E, Otterdal K, Gullestad L, and Damas JK. Chemokines and cardiovascular risk. *Arterioscler Thromb Vasc Biol* 28: 1909–1919, 2008.
13. Bao W, Hu E, Tao L, Boyce R, Mirabile R, Thudium DT, Ma XL, Willette RN, and Yue TL. Inhibition of Rho-kinase protects the heart against ischemia/reperfusion injury. *Cardiovasc Res* 61: 548–558, 2004.
14. Begum N, Sandu OA, Ito M, Lohmann SM, and Smolenski A. Active Rho kinase (ROK-alpha) associates with insulin receptor substrate-1 and inhibits insulin signaling in vascular smooth muscle cells. *J Biol Chem* 277: 6214–6222, 2002.
15. Bivalacqua TJ, Champion HC, Usta MF, Celtek 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 U S A* 101: 9121–9126, 2004.
16. Braun-Dullaeus RC, Mann MJ, and Dzau VJ. Cell cycle progression: new therapeutic target for vascular proliferative disease. *Circulation* 98: 82–89, 1998.
17. Brown JH, Del Re DP, and Sussman MA. The Rac and Rho hall of fame: a decade of hypertrophic signaling hits. *Circ Res* 98: 730–742, 2006.
18. This reference has been deleted.
19. Bryan BA, Mitchell DC, Zhao L, Ma W, Stafford LJ, Teng BB, and Liu M. Modulation of muscle regeneration, myogenesis, and adipogenesis by the Rho family guanine nucleotide exchange factor GEFT. *Mol Cell Biol* 25: 11089–11101, 2005.
20. Buchwald H, Varco RL, Matts JP, Long JM, Fitch LL, Campbell GS, Pearce MB, Yellin AE, Edmiston WA, Smink RD, Jr., et al. Effect of partial ileal bypass surgery on mortality and morbidity from coronary heart disease in patients with hypercholesterolemia. Report of the Program on the Surgical Control of the Hyperlipidemias (POSCH). *N Engl J Med* 323: 946–955, 1990.
21. BurrIDGE K and Wennerberg K. Rho and Rac take center stage. *Cell* 116: 167–179, 2004.
22. Carter RW, Begaye M, and Kanagy NL. Acute and chronic NOS inhibition enhances alpha(2)-adrenoreceptor-stimulated RhoA and Rho kinase in rat aorta. *Am J Physiol Heart Circ Physiol* 283: H1361–H1369, 2002.
23. Chandrasekar B, Mummidi S, Mahimainathan L, Patel DN, Bailey SR, Imam SZ, Greene WC, and Valente AJ. Interleukin-18-induced human coronary artery smooth muscle cell migration is dependent on NF-kappaB- and AP-1-mediated matrix metalloproteinase-9 expression and is inhibited by atorvastatin. *J Biol Chem* 281: 15099–15109, 2006.
24. 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 U S A* 103: 14495–14500, 2006.
25. Chevrier V, Piel M, Collomb N, Saoudi Y, Frank R, Paintrand M, Narumiya S, Bornens M, and Job D. The Rho-associated protein kinase p160ROCK is required for centrosome positioning. *J Cell Biol* 157: 807–817, 2002.
 26. Chitaley K and Webb RC. Nitric oxide induces dilation of rat aorta via inhibition of rho-kinase signaling. *Hypertension* 39: 438–442, 2002.
 27. Chou TC, Yen MH, Li CY, and Ding YA. Alterations of nitric oxide synthase expression with aging and hypertension in rats. *Hypertension* 31: 643–648, 1998.
 28. Coleman ML, Sahai EA, Yeo M, Bosch M, Dewar A, and Olson MF. Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK I. *Nat Cell Biol* 3: 339–345, 2001.
 29. Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, and Zeiher AM. Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 399: 601–605, 1999.
 30. Endres M, Laufs U, Huang Z, Nakamura T, Huang P, Moskowitz MA, and Liao JK. Stroke protection by 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A* 95: 8880–8885, 1998.
 31. Essig M, Nguyen G, Prie D, Escoubet B, Sraer JD, and Friedlander G. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors increase fibrinolytic activity in rat aortic endothelial cells. Role of geranylgeranylation and Rho proteins. *Circ Res* 83: 683–690, 1998.
 32. Eto Y, Shimokawa H, Hiroki J, Morishige K, Kandabashi T, Matsumoto Y, Amano M, Hoshijima M, Kaibuchi K, and Takeshita A. Gene transfer of dominant negative Rho kinase suppresses neointimal formation after balloon injury in pigs. *Am J Physiol Heart Circ Physiol* 278: H1744–H1750, 2000.
 33. Farah S, Agazie Y, Ohan N, Ngsee JK, and Liu XJ. A rho-associated protein kinase, ROKalpha, binds insulin receptor substrate-1 and modulates insulin signaling. *J Biol Chem* 273: 4740–4746, 1998.
 34. Feng J, Ito M, Ichikawa K, Isaka N, Nishikawa M, Hartshorne DJ, and Nakano T. Inhibitory phosphorylation site for Rho-associated kinase on smooth muscle myosin phosphatase. *J Biol Chem* 274: 37385–37390, 1999.
 35. This reference has been deleted.
 36. Forstermann U and Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation* 113: 1708–1714, 2006.
 37. Fox R, Nhan TQ, Law GL, Morris DR, Liles WC, and Schwartz SM. PSGL-1 and mTOR regulate translation of ROCK-1 and physiological functions of macrophages. *EMBO J* 26: 505–515, 2007.
 38. Fujita H, Fukumoto Y, Saji K, Sugimura K, Demachi J, Nawata J, and Shimokawa H. Acute vasodilator effects of inhaled fasudil, a specific Rho-kinase inhibitor, in patients with pulmonary arterial hypertension. *Heart Vessels* 25: 144–149, 2010.
 39. Fukata Y, Oshiro N, Kinoshita N, Kawano Y, Matsuoka Y, Bennett V, Matsuura Y, and Kaibuchi K. Phosphorylation of adducin by Rho-kinase plays a crucial role in cell motility. *J Cell Biol* 145: 347–361, 1999.
 40. Funakoshi Y, Ichiki T, Shimokawa H, Egashira K, Takeda K, Kaibuchi K, Takeya M, Yoshimura T, and Takeshita A. Rho-kinase mediates angiotensin II-induced monocyte chemoattractant protein-1 expression in rat vascular smooth muscle cells. *Hypertension* 38: 100–104, 2001.
 41. Furukawa N, Ongusaha P, Jahng WJ, Araki K, Choi CS, Kim HJ, Lee YH, Kaibuchi K, Kahn BB, Masuzaki H, Kim JK, Lee SW, and Kim YB. Role of Rho-kinase in regulation of insulin action and glucose homeostasis. *Cell Metab* 2: 119–129, 2005.
 42. Garg UC and Hassid A. Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *J Clin Invest* 83: 1774–1777, 1989.
 43. Gauthier TW, Scalia R, Murohara T, Guo JP, and Lefer AM. Nitric oxide protects against leukocyte-endothelium interactions in the early stages of hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 15: 1652–1659, 1995.
 44. Germershausen JI, Hunt VM, Bostedor RG, Bailey PJ, Karkas JD, and Alberts AW. Tissue selectivity of the cholesterol-lowering agents lovastatin, simvastatin and pravastatin in rats *in vivo*. *Biochem Biophys Res Commun* 158: 667–675, 1989.
 45. Goldin A, Beckman JA, Schmidt AM, and Creager MA. Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* 114: 597–605, 2006.
 46. Goldstein JL and Brown MS. Regulation of the mevalonate pathway. *Nature* 343: 425–430, 1990.
 47. Goto H, Kosako H, Tanabe K, Yanagida M, Sakurai M, Amano M, Kaibuchi K, and Inagaki M. Phosphorylation of vimentin by Rho-associated kinase at a unique amino-terminal site that is specifically phosphorylated during cytokinesis. *J Biol Chem* 273: 11728–11736, 1998.
 48. Hagerty L, Weitzel DH, Chambers J, Fortner CN, Brush MH, Loisele D, Hosoya H, and Haystead TA. ROCK1 phosphorylates and activates zipper-interacting protein kinase. *J Biol Chem* 282: 4884–4893, 2007.
 49. Hall A. Small GTP-binding proteins and the regulation of the actin cytoskeleton. *Annu Rev Cell Biol* 10: 31–54, 1994.
 50. Hall A. Rho GTPases and the actin cytoskeleton. *Science* 279: 509–514, 1998.
 51. Hara Y, Wakino S, Tanabe Y, Saito M, Tokuyama H, Washida N, Tatematsu S, Yoshioka K, Homma K, Hasegawa K, Minakuchi H, Fujimura K, Hosoya K, Hayashi K, Nakayama K, and Itoh H. Rho and Rho-kinase activity in adipocytes contributes to a vicious cycle in obesity that may involve mechanical stretch. *Sci Signal* 4: ra3, 2011.
 52. Hattori T, Shimokawa H, Higashi M, Hiroki J, Mukai Y, Kaibuchi K, and Takeshita A. Long-term treatment with a specific Rho-kinase inhibitor suppresses cardiac allograft vasculopathy in mice. *Circ Res* 94: 46–52, 2004.
 53. Hattori T, Shimokawa H, Higashi M, Hiroki J, Mukai Y, Tsutsui H, Kaibuchi K, and Takeshita A. Long-term inhibition of Rho-kinase suppresses left ventricular remodeling after myocardial infarction in mice. *Circulation* 109: 2234–2239, 2004.
 54. Heineke J and Molkentin JD. Regulation of cardiac hypertrophy by intracellular signalling pathways. *Nat Rev Mol Cell Biol* 7: 589–600, 2006.
 55. Hengst L and Reed SI. Translational control of p27Kip1 accumulation during the cell cycle. *Science* 271: 1861–1864, 1996.
 56. Hidaka T, Hata T, Soga J, Fujii Y, Idei N, Fujimura N, Kihara Y, Noma K, Liao JK, and Higashi Y. Increased leukocyte rho kinase (ROCK) activity and endothelial dysfunction in cigarette smokers. *Hypertens Res* 33: 354–359, 2010.
 57. Higashi M, Shimokawa H, Hattori T, Hiroki J, Mukai Y, Morikawa K, Ichiki T, Takahashi S, and Takeshita A.

- Long-term inhibition of Rho-kinase suppresses angiotensin II-induced cardiovascular hypertrophy in rats *in vivo*: effect on endothelial NAD(P)H oxidase system. *Circ Res* 93: 767–775, 2003.
58. Hill JA and Olson EN. Cardiac plasticity. *N Engl J Med* 358: 1370–1380, 2008.
 59. Hirose A, Tanikawa T, Mori H, Okada Y, and Tanaka Y. Advanced glycation end products increase endothelial permeability through the RAGE/Rho signaling pathway. *FEBS Lett* 584: 61–66, 2010.
 60. Hofmann F, Feil R, Kleppisch T, and Schlossmann J. Function of cGMP-dependent protein kinases as revealed by gene deletion. *Physiol Rev* 86: 1–23, 2006.
 61. Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, and Fishman MC. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. *Nature* 377: 239–242, 1995.
 62. Hughes DA. Control of signal transduction and morphogenesis by Ras. *Semin Cell Biol* 6: 89–94, 1995.
 63. Ignarro LJ, Buga GM, Wood KS, Byrns RE, and Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci U S A* 84: 9265–9269, 1987.
 64. Ikegaki I, Hattori T, Yamaguchi T, Sasaki Y, Satoh SI, Asano T, and Shimokawa H. Involvement of Rho-kinase in vascular remodeling caused by long-term inhibition of nitric oxide synthesis in rats. *Eur J Pharmacol* 427: 69–75, 2001.
 65. Istvan ES and Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science* 292: 1160–1164, 2001.
 66. Iwasaki H, Okamoto R, Kato S, Konishi K, Mizutani H, Yamada N, Isaka N, Nakano T, and Ito M. High glucose induces plasminogen activator inhibitor-1 expression through Rho/Rho-kinase-mediated NF-kappaB activation in bovine aortic endothelial cells. *Atherosclerosis* 196: 22–28, 2008.
 67. Jakobsiak M, Bruno S, Skierski JS, and Darzynkiewicz Z. Cell cycle-specific effects of lovastatin. *Proc Natl Acad Sci U S A* 88: 3628–3632, 1991.
 68. Jukema JW, Cannon CP, de Craen AJ, Westendorp RG, and Trompet S. The controversies of statin therapy: weighing the evidence. *J Am Coll Cardiol* 60: 875–881, 2012.
 69. Kamari Y, Bitzur R, Cohen H, Shaish A, and Harats D. Should all diabetic patients be treated with a statin? *Diabetes Care* 32 Suppl 2: S378–S383, 2009.
 70. Kanda T, Wakino S, Homma K, Yoshioka K, Tatematsu S, Hasegawa K, Takamatsu I, Sugano N, Hayashi K, and Saruta T. Rho-kinase as a molecular target for insulin resistance and hypertension. *FASEB J* 20: 169–171, 2006.
 71. Kaneko T, Amano M, Maeda A, Goto H, Takahashi K, Ito M, and Kaibuchi K. Identification of calponin as a novel substrate of Rho-kinase. *Biochem Biophys Res Commun* 273: 110–116, 2000.
 72. This reference has been deleted.
 73. Kataoka C, Egashira K, Inoue S, Takemoto M, Ni W, Koyanagi M, Kitamoto S, Usui M, Kaibuchi K, Shimokawa H, and Takeshita A. Important role of Rho-kinase in the pathogenesis of cardiovascular inflammation and remodeling induced by long-term blockade of nitric oxide synthesis in rats. *Hypertension* 39: 245–250, 2002.
 74. Katsumata N, Shimokawa H, Seto M, Kozai T, Yamawaki T, Kuwata K, Egashira K, Ikegaki I, Asano T, Sasaki Y, and Takeshita A. Enhanced myosin light chain phosphorylations as a central mechanism for coronary artery spasm in a swine model with interleukin-1beta. *Circulation* 96: 4357–4363, 1997.
 75. Kimura K, Ito M, Amano M, Chihara K, Fukata Y, Nakafuku M, Yamamori B, Feng J, Nakano T, Okawa K, Iwamatsu A, and Kaibuchi K. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science* 273: 245–248, 1996.
 76. Kishi T, Hirooka Y, Masumoto A, Ito K, Kimura Y, Inokuchi K, Tagawa T, Shimokawa H, Takeshita A, and Sunagawa K. Rho-kinase inhibitor improves increased vascular resistance and impaired vasodilation of the forearm in patients with heart failure. *Circulation* 111: 2741–2747, 2005.
 77. Kishore R, Qin G, Luedemann C, Bord E, Hanley A, Silver M, Gavin M, Yoon YS, Goukassian D, and Losordo DW. The cytoskeletal protein ezrin regulates EC proliferation and angiogenesis via TNF-alpha-induced transcriptional repression of cyclin A. *J Clin Invest* 115: 1785–1796, 2005.
 78. Kitazawa T, Eto M, Woodsome TP, and Brautigan DL. Agonists trigger G protein-mediated activation of the CPI-17 inhibitor phosphoprotein of myosin light chain phosphatase to enhance vascular smooth muscle contractility. *J Biol Chem* 275: 9897–9900, 2000.
 79. Kobashigawa JA, Katznelson S, Laks H, Johnson JA, Yeatman L, Wang XM, Chia D, Terasaki PI, Sabad A, Cogert GA, et al. Effect of pravastatin on outcomes after cardiac transplantation. *N Engl J Med* 333: 621–627, 1995.
 80. Kobayashi N, Horinaka S, Mita S, Nakano S, Honda T, Yoshida K, Kobayashi T, and Matsuoka H. Critical role of Rho-kinase pathway for cardiac performance and remodeling in failing rat hearts. *Cardiovasc Res* 55: 757–767, 2002.
 81. Kosmidou I, Moore JP, Weber M, and Searles CD. Statin treatment and 3' polyadenylation of eNOS mRNA. *Arterioscler Thromb Vasc Biol* 27: 2642–2649, 2007.
 82. Kureishi Y, Luo Z, Shiojima I, Bialik A, Fulton D, Lefer DJ, Sessa WC, and Walsh K. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. *Nat Med* 6: 1004–1010, 2000.
 83. This reference has been deleted.
 84. Langer HF and Gawaz M. Platelet-vessel wall interactions in atherosclerotic disease. *Thromb Haemost* 99: 480–486, 2008.
 85. Laufs U, Endres M, Stagliano N, Amin-Hanjani S, Chui DS, Yang SX, Simoncini T, Yamada M, Rabkin E, Allen PG, Huang PL, Bohm M, Schoen FJ, Moskowitz MA, and Liao JK. Neuroprotection mediated by changes in the endothelial actin cytoskeleton. *J Clin Invest* 106: 15–24, 2000.
 86. Laufs U, Fata VL, and Liao JK. Inhibition of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase blocks hypoxia-mediated down-regulation of endothelial nitric oxide synthase. *J Biol Chem* 272: 31725–31729, 1997.
 87. Laufs U, La Fata V, Plutzky J, and Liao JK. Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation* 97: 1129–1135, 1998.
 88. Laufs U and Liao JK. Post-transcriptional regulation of endothelial nitric oxide synthase mRNA stability by Rho GTPase. *J Biol Chem* 273: 24266–24271, 1998.
 89. Laufs U, Marra D, Node K, and Liao JK. 3-Hydroxy-3-methylglutaryl-CoA reductase inhibitors attenuate vascular smooth muscle proliferation by preventing rho GTPase-induced down-regulation of p27(Kip1). *J Biol Chem* 274: 21926–21931, 1999.

90. Lee DH, Shi J, Jeoung NH, Kim MS, Zabolotny JM, Lee SW, White MF, Wei L, and Kim YB. Targeted disruption of ROCK1 causes insulin resistance *in vivo*. *J Biol Chem* 284: 11776–11780, 2009.
91. Lefer AM, Campbell B, Shin YK, Scalia R, Hayward R, and Lefer DJ. Simvastatin preserves the ischemic-reperfused myocardium in normocholesterolemic rat hearts. *Circulation* 100: 178–184, 1999.
92. Li J, Jiang J, Yin H, Wang L, Tian R, Li H, Wang Z, Li D, Wang Y, Gui Y, Walsh MP, and Zheng XL. Atorvastatin inhibits myocardin expression in vascular smooth muscle cells. *Hypertension* 60: 145–153, 2012.
93. Li M, Liu Y, Dutt P, Fanburg BL, and Toksoz D. Inhibition of serotonin-induced mitogenesis, migration, and ERK MAPK nuclear translocation in vascular smooth muscle cells by atorvastatin. *Am J Physiol Lung Cell Mol Physiol* 293: L463–L471, 2007.
94. 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.
95. Liao JK. Inhibition of Gi proteins by low density lipoprotein attenuates bradykinin-stimulated release of endothelial-derived nitric oxide. *J Biol Chem* 269: 12987–12992, 1994.
96. Libby P. Inflammation in atherosclerosis. *Nature* 420: 868–874, 2002.
97. Libby P, Ridker PM, and Maseri A. Inflammation and atherosclerosis. *Circulation* 105: 1135–1143, 2002.
98. Lim MJ, Choi KJ, Ding Y, Kim JH, Kim BS, Kim YH, Lee J, Choe W, Kang I, Ha J, Yoon KS, and Kim SS. RhoA/Rho kinase blocks muscle differentiation via serine phosphorylation of insulin receptor substrate-1 and -2. *Mol Endocrinol* 21: 2282–2293, 2007.
99. Liu PY, Chen JH, Lin LJ, and Liao JK. Increased Rho kinase activity in a Taiwanese population with metabolic syndrome. *J Am Coll Cardiol* 49: 1619–1624, 2007.
100. Lohn M, Plettenburg O, Ivashchenko Y, Kannt A, Hofmeister A, Kadereit D, Schaefer M, Linz W, Kohlmann M, Herbert JM, Janiak P, O'Connor SE, and Ruetten H. Pharmacological characterization of SAR407899, a novel rho-kinase inhibitor. *Hypertension* 54: 676–683, 2009.
101. Loirand G and Pacaud P. The role of Rho protein signaling in hypertension. *Nat Rev Cardiol* 7: 637–647, 2010.
102. Mallat Z, Gojova A, Sauzeau V, Brun V, Silvestre JS, Esposito B, Merval R, Groux H, Loirand G, and Tedgui A. Rho-associated protein kinase contributes to early atherosclerotic lesion formation in mice. *Circ Res* 93: 884–888, 2003.
103. Maron DJ, Fazio S, and Linton MF. Current perspectives on statins. *Circulation* 101: 207–213, 2000.
104. Masumoto A, Mohri M, Shimokawa H, Urakami L, Usui M, and Takeshita A. Suppression of coronary artery spasm by the Rho-kinase inhibitor fasudil in patients with vasospastic angina. *Circulation* 105: 1545–1547, 2002.
105. Ming XF, Viswambharan H, Barandier C, Ruffieux J, Kaibuchi K, Rusconi S, and Yang Z. Rho GTPase/Rho kinase negatively regulates endothelial nitric oxide synthase phosphorylation through the inhibition of protein kinase B/Akt in human endothelial cells. *Mol Cell Biol* 22: 8467–8477, 2002.
106. Miyamoto S, Del Re DP, Xiang SY, Zhao X, Florholmen G, and Brown JH. Revisited and revised: is RhoA always a villain in cardiac pathophysiology? *J Cardiovasc Transl Res* 3: 330–343, 2010.
107. Morishige K, Shimokawa H, Eto Y, Hoshijima M, Kaibuchi K, and Takeshita A. *In vivo* gene transfer of dominant-negative rho-kinase induces regression of coronary arteriosclerosis in pigs. *Ann N Y Acad Sci* 947: 407–411, 2001.
108. Mueller BK, Mack H, and Teusch N. Rho kinase, a promising drug target for neurological disorders. *Nat Rev Drug Discov* 4: 387–398, 2005.
109. Munzel T and Gori T. Nitrate therapy and nitrate tolerance in patients with coronary artery disease. *Curr Opin Pharmacol* 13: 251–259, 2013.
110. Nakayama Y, Komuro R, Yamamoto A, Miyata Y, Tanaka M, Matsuda M, Fukuhara A, and Shimomura I. RhoA induces expression of inflammatory cytokine in adipocytes. *Biochem Biophys Res Commun* 379: 288–292, 2009.
111. Noguchi M, Hosoda K, Fujikura J, Fujimoto M, Iwakura H, Tomita T, Ishii T, Arai N, Hirata M, Ebihara K, Masuzaki H, Itoh H, Narumiya S, and Nakao K. Genetic and pharmacological inhibition of Rho-associated kinase II enhances adipogenesis. *J Biol Chem* 282: 29574–29583, 2007.
112. Nohria A, Grunert ME, Rikitake Y, Noma K, Prsic A, Ganz P, Liao JK, and Creager MA. Rho kinase inhibition improves endothelial function in human subjects with coronary artery disease. *Circ Res* 99: 1426–1432, 2006.
113. Nohria A, Prsic A, Liu PY, Okamoto R, Creager MA, Selwyn A, Liao JK, and Ganz P. Statins inhibit Rho kinase activity in patients with atherosclerosis. *Atherosclerosis* 205: 517–521, 2009.
114. Noma K, Goto C, Nishioka K, Jitsuiki D, Umemura T, Ueda K, Kimura M, Nakagawa K, Oshima T, Chayama K, Yoshizumi M, Liao JK, and Higashi Y. Roles of rho-associated kinase and oxidative stress in the pathogenesis of aortic stiffness. *J Am Coll Cardiol* 49: 698–705, 2007.
115. Noma K, Higashi Y, Jitsuiki D, Hara K, Kimura M, Nakagawa K, Goto C, Oshima T, Yoshizumi M, and Chayama K. Smoking activates rho-kinase in smooth muscle cells of forearm vasculature in humans. *Hypertension* 41: 1102–1105, 2003.
116. Noma K, Oyama N, and Liao JK. Physiological role of ROCKs in the cardiovascular system. *Am J Physiol Cell Physiol* 290: C661–C668, 2006.
117. 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.
118. O'Driscoll G, Green D, and Taylor RR. Simvastatin, an HMG-coenzyme A reductase inhibitor, improves endothelial function within 1 month. *Circulation* 95: 1126–1131, 1997.
119. Ohashi K, Nagata K, Maekawa M, Ishizaki T, Narumiya S, and Mizuno K. Rho-associated kinase ROCK activates LIM-kinase 1 by phosphorylation at threonine 508 within the activation loop. *J Biol Chem* 275: 3577–3582, 2000.
120. Okamoto R, Li Y, Noma K, Hiroi Y, Liu PY, Taniguchi M, Ito M, and Liao JK. FHL2 prevents cardiac hypertrophy in mice with cardiac-specific deletion of ROCK2. *FASEB J* 27: 1439–1449, 2013.
121. Okon EB, Szado T, Laher I, McManus B, and van Breemen C. Augmented contractile response of vascular smooth muscle in a diabetic mouse model. *J Vasc Res* 40: 520–530, 2003.
122. Oshiro N, Fukata Y, and Kaibuchi K. Phosphorylation of moesin by rho-associated kinase (Rho-kinase) plays a crucial role in the formation of microvilli-like structures. *J Biol Chem* 273: 34663–34666, 1998.

123. Palinski W. New evidence for beneficial effects of statins unrelated to lipid lowering. *Arterioscler Thromb Vasc Biol* 21: 3–5, 2001.
124. Preiss D and Sattar N. Statins and the risk of new-onset diabetes: a review of recent evidence. *Curr Opin Lipidol* 22: 460–466, 2011.
125. Radomski MW, Rees DD, Dutra A, and Moncada S. S-nitroso-glutathione inhibits platelet activation *in vitro* and *in vivo*. *Br J Pharmacol* 107: 745–749, 1992.
126. Reikhter M, Chandrasekhar K, Gifford-Moore D, Huang XD, Rutherford P, Hanson J, and Kauffman R. Immunohistochemical analysis of target proteins of Rho-kinase in a mouse model of accelerated atherosclerosis. *Exp Clin Cardiol* 12: 169–174, 2007.
127. Riento K and Ridley AJ. Rocks: multifunctional kinases in cell behaviour. *Nat Rev Mol Cell Biol* 4: 446–456, 2003.
128. Riento K, Totty N, Villalonga P, Garg R, Guasch R, and Ridley AJ. RhoE function is regulated by ROCK I-mediated phosphorylation. *EMBO J* 24: 1170–1180, 2005.
129. Rikitake Y, Kim HH, Huang Z, Seto M, Yano K, Asano T, Moskowitz MA, and Liao JK. Inhibition of Rho kinase (ROCK) leads to increased cerebral blood flow and stroke protection. *Stroke* 36: 2251–2257, 2005.
130. Rikitake Y and Liao JK. Rho-kinase mediates hyperglycemia-induced plasminogen activator inhibitor-1 expression in vascular endothelial cells. *Circulation* 111: 3261–3268, 2005.
131. 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.
132. Romero MJ, Iddings JA, Platt DH, Ali MI, Cederbaum SD, Stepp DW, Caldwell RB, and Caldwell RW. Diabetes-induced vascular dysfunction involves arginase I. *Am J Physiol Heart Circ Physiol* 302: H159–H166, 2012.
133. Romero MJ, Platt DH, Tawfik HE, Labazi M, El-Remessy AB, Bartoli M, Caldwell RB, and Caldwell RW. Diabetes-induced coronary vascular dysfunction involves increased arginase activity. *Circ Res* 102: 95–102, 2008.
134. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 362: 801–809, 1993.
135. Ruperez M, Rodrigues-Diez R, Blanco-Colio LM, Sanchez-Lopez E, Rodriguez-Vita J, Esteban V, Carvajal G, Plaza JJ, Egido J, and Ruiz-Ortega M. HMG-CoA reductase inhibitors decrease angiotensin II-induced vascular fibrosis: role of RhoA/ROCK and MAPK pathways. *Hypertension* 50: 377–383, 2007.
136. Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JM, Wun CC, Davis BR, and Braunwald E. The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events Trial investigators. *N Engl J Med* 335: 1001–1009, 1996.
137. Sampson UK, Linton MF, and Fazio S. Are statins diabetogenic? *Curr Opin Cardiol* 26: 342–347, 2011.
138. Satoh S, Ueda Y, Koyanagi M, Kadokami T, Sugano M, Yoshikawa Y, and Makino N. Chronic inhibition of Rho kinase blunts the process of left ventricular hypertrophy leading to cardiac contractile dysfunction in hypertension-induced heart failure. *J Mol Cell Cardiol* 35: 59–70, 2003.
139. Sauzeau V, Le Jeune H, Cario-Toumaniantz C, Smolenski A, Lohmann SM, Bertoglio J, Chardin P, Pacaud P, and Loirand G. Cyclic GMP-dependent protein kinase signaling pathway inhibits RhoA-induced Ca²⁺ sensitization of contraction in vascular smooth muscle. *J Biol Chem* 275: 21722–21729, 2000.
140. Sauzeau V, Le Mellionnec E, Bertoglio J, Scalbert E, Pacaud P, and Loirand G. Human urotensin II-induced contraction and arterial smooth muscle cell proliferation are mediated by RhoA and Rho-kinase. *Circ Res* 88: 1102–1104, 2001.
141. Sawada N, Itoh H, Miyashita K, Tsujimoto H, Sone M, Yamahara K, Arany ZP, Hofmann F, and Nakao K. Cyclic GMP kinase and RhoA Ser188 phosphorylation integrate pro- and antifibrotic signals in blood vessels. *Mol Cell Biol* 29: 6018–6032, 2009.
142. Sawada N, Itoh H, Ueyama K, Yamashita J, Doi K, Chun TH, Inoue M, Masatsugu K, Saito T, Fukunaga Y, Sakaguchi S, Arai H, Ohno N, Komeda M, and Nakao K. Inhibition of rho-associated kinase results in suppression of neointimal formation of balloon-injured arteries. *Circulation* 101: 2030–2033, 2000.
143. Sawada N, Itoh H, Yamashita J, Doi K, Inoue M, Masatsugu K, Fukunaga Y, Sakaguchi S, Sone M, Yamahara K, Yurugi T, and Nakao K. cGMP-dependent protein kinase phosphorylates and inactivates RhoA. *Biochem Biophys Res Commun* 280: 798–805, 2001.
144. Seasholtz TM, Majumdar M, Kaplan DD, and Brown JH. Rho and Rho kinase mediate thrombin-stimulated vascular smooth muscle cell DNA synthesis and migration. *Circ Res* 84: 1186–1193, 1999.
145. 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.
146. This reference has been deleted.
147. Shao J, Welch WJ, Diprospero NA, and Diamond MI. Phosphorylation of profilin by ROCK1 regulates polyglutamine aggregation. *Mol Cell Biol* 28: 5196–5208, 2008.
148. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH, and Packard CJ. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N Engl J Med* 333: 1301–1307, 1995.
149. Shi J, Zhang YW, Summers LJ, Dorn GW, 2nd, and Wei L. Disruption of ROCK1 gene attenuates cardiac dilation and improves contractile function in pathological cardiac hypertrophy. *J Mol Cell Cardiol* 44: 551–560, 2008.
150. Shi J, Zhang YW, Yang Y, Zhang L, and Wei L. ROCK1 plays an essential role in the transition from cardiac hypertrophy to failure in mice. *J Mol Cell Cardiol* 49: 819–828, 2010.
151. Shibuya M, Hirai S, Seto M, Satoh S, and Ohtomo E. Effects of fasudil in acute ischemic stroke: results of a prospective placebo-controlled double-blind trial. *J Neurol Sci* 238: 31–39, 2005.
152. Shiga N, Hirano K, Hirano M, Nishimura J, Nawata H, and Kanaide H. Long-term inhibition of RhoA attenuates vascular contractility by enhancing endothelial NO production in an intact rabbit mesenteric artery. *Circ Res* 96: 1014–1021, 2005.
153. Shimizu K, Aikawa M, Takayama K, Libby P, and Mitchell RN. Direct anti-inflammatory mechanisms contribute to attenuation of experimental allograft arteriosclerosis by statins. *Circulation* 108: 2113–2120, 2003.

154. 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.
155. Shimokawa H. Rho-kinase as a novel therapeutic target in treatment of cardiovascular diseases. *J Cardiovasc Pharmacol* 39: 319–327, 2002.
156. Shimokawa H, Hiramori K, Iinuma H, Hosoda S, Kishida H, Osada H, Katagiri T, Yamauchi K, Yui Y, Minamino T, Nakashima M, and Kato K. Anti-anginal effect of fasudil, a Rho-kinase inhibitor, in patients with stable effort angina: a multicenter study. *J Cardiovasc Pharmacol* 40: 751–761, 2002.
157. Shimokawa H, Morishige K, Miyata K, Kandabashi T, Eto Y, Ikegaki I, Asano T, Kaibuchi K, and Takeshita A. Long-term inhibition of Rho-kinase induces a regression of arteriosclerotic coronary lesions in a porcine model *in vivo*. *Cardiovasc Res* 51: 169–177, 2001.
158. Shimokawa H and Rashid M. Development of Rho-kinase inhibitors for cardiovascular medicine. *Trends Pharmacol Sci* 28: 296–302, 2007.
159. Shimokawa H and Takeshita A. Rho-kinase is an important therapeutic target in cardiovascular medicine. *Arterioscler Thromb Vasc Biol* 25: 1767–1775, 2005.
160. Shirao S, Fujisawa H, Kudo A, Kurokawa T, Yoneda H, Kunitzugu I, Ogasawara K, Soma M, Kobayashi S, Ogawa A, and Suzuki M. Inhibitory effects of eicosapentaenoic acid on chronic cerebral vasospasm after subarachnoid hemorrhage: possible involvement of a sphingosylphosphorylcholine-rho-kinase pathway. *Cerebrovasc Dis* 26: 30–37, 2008.
161. Sordella R, Jiang W, Chen GC, Curto M, and Settleman J. Modulation of Rho GTPase signaling regulates a switch between adipogenesis and myogenesis. *Cell* 113: 147–158, 2003.
162. Sowers JR and Lester MA. Diabetes and cardiovascular disease. *Diabetes Care* 22 Suppl 3: C14–C20, 1999.
163. Steinberg D, Parthasarathy S, Carew TE, Khoo JC, and Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 320: 915–924, 1989.
164. This reference has been deleted.
165. Sumi T, Matsumoto K, and Nakamura T. Specific activation of LIM kinase 2 via phosphorylation of threonine 505 by ROCK, a Rho-dependent protein kinase. *J Biol Chem* 276: 670–676, 2001.
166. Suzuki Y, Shibuya M, Satoh S, Sugiyama H, Seto M, and Takakura K. Safety and efficacy of fasudil monotherapy and fasudil-ozagrel combination therapy in patients with subarachnoid hemorrhage: sub-analysis of the post-marketing surveillance study. *Neurol Med Chir (Tokyo)* 48: 241–247; discussion 247–248, 2008.
167. Takeda K, Ichiki T, Tokunou T, Iino N, Fujii S, Kitabatake A, Shimokawa H, and Takeshita A. Critical role of Rho-kinase and MEK/ERK pathways for angiotensin II-induced plasminogen activator inhibitor type-1 gene expression. *Arterioscler Thromb Vasc Biol* 21: 868–873, 2001.
168. Takemoto M, Sun J, Hiroki J, Shimokawa H, and Liao JK. Rho-kinase mediates hypoxia-induced downregulation of endothelial nitric oxide synthase. *Circulation* 106: 57–62, 2002.
- 168a. The Lipid Research Clinics Coronary Primary Prevention Trial Results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. *JAMA* 251: 365–374, 1984.
169. Uehata M, Ishizaki T, Satoh H, Ono T, Kawahara T, Morishita T, Tamakawa H, Yamagami K, Inui J, Maekawa M, and Narumiya S. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature* 389: 990–994, 1997.
170. Van Aelst L and D'Souza-Schorey C. Rho GTPases and signaling networks. *Genes Dev* 11: 2295–2322, 1997.
171. van Nieuw Amerongen GP, Vermeer MA, and van Hinsbergh VW. Role of RhoA and Rho kinase in lysophosphatidic acid-induced endothelial barrier dysfunction. *Arterioscler Thromb Vasc Biol* 20: E127–E133, 2000.
172. 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.
173. Vogt A, Qian Y, McGuire TF, Hamilton AD, and Sefti SM. Protein geranylgeranylation, not farnesylation, is required for the G1 to S phase transition in mouse fibroblasts. *Oncogene* 13: 1991–1999, 1996.
174. Wang CY, Liu PY, and Liao JK. Pleiotropic effects of statin therapy: molecular mechanisms and clinical results. *Trends Mol Med* 14: 37–44, 2008.
175. Wang HW, Liu PY, Oyama N, Rikitake Y, Kitamoto S, Gitlin J, Liao JK, and Boisvert WA. Deficiency of ROCK1 in bone marrow-derived cells protects against atherosclerosis in LDLR^{-/-} mice. *FASEB J* 22: 3561–3570, 2008.
176. Ward Y, Yap SF, Ravichandran V, Matsumura F, Ito M, Spinelli B, and Kelly K. The GTP binding proteins Gem and Rad are negative regulators of the Rho-Rho kinase pathway. *J Cell Biol* 157: 291–302, 2002.
177. Weber M, Hagedorn CH, Harrison DG, and Searles CD. Laminar shear stress and 3' polyadenylation of eNOS mRNA. *Circ Res* 96: 1161–1168, 2005.
178. Werns SW, Walton JA, Hsia HH, Nabel EG, Sanz ML, and Pitt B. Evidence of endothelial dysfunction in angiographically normal coronary arteries of patients with coronary artery disease. *Circulation* 79: 287–291, 1989.
179. Wolfrum S, Dendorfer A, Rikitake Y, Stalker TJ, Gong Y, Scalia R, Dominiak P, and Liao JK. Inhibition of Rho-kinase leads to rapid activation of phosphatidylinositol 3-kinase/protein kinase Akt and cardiovascular protection. *Arterioscler Thromb Vasc Biol* 24: 1842–1847, 2004.
180. Yang Z, Kozai T, van der Loo B, Viswambharan H, Lachat M, Turina MI, Malinski T, and Luscher TF. HMG-CoA reductase inhibition improves endothelial cell function and inhibits smooth muscle cell proliferation in human saphenous veins. *J Am Coll Cardiol* 36: 1691–1697, 2000.
181. Yoneda A, Multhaupt HA, and Couchman JR. The Rho kinases I and II regulate different aspects of myosin II activity. *J Cell Biol* 170: 443–453, 2005.
182. Zhang YM, Bo J, Taffet GE, Chang J, Shi J, Reddy AK, Michael LH, Schneider MD, Entman ML, Schwartz RJ, and Wei L. Targeted deletion of ROCK1 protects the heart against pressure overload by inhibiting reactive fibrosis. *FASEB J* 20: 916–925, 2006.
183. Zhou Q and Liao JK. Rho kinase: an important mediator of atherosclerosis and vascular disease. *Curr Pharm Des* 15: 3108–3115, 2009.
184. Zhou Q and Liao JK. Statins and cardiovascular diseases: from cholesterol lowering to pleiotropy. *Curr Pharm Des* 15: 467–478, 2009.

185. Zhou Q and Liao JK. Pleiotropic effects of statins. Basic research and clinical perspectives. *Circ J* 74: 818–826, 2010.
186. Zhou Q, Mei Y, Shoji T, Han X, Kaminski K, Oh GT, Onusaha PP, Zhang K, Schmitt H, Moser M, Bode C, and Liao JK. Rho-associated coiled-coil-containing kinase 2 deficiency in bone marrow-derived cells leads to increased cholesterol efflux and decreased atherosclerosis. *Circulation* 126: 2236–2247, 2012.

Address correspondence to:

Prof. James K. Liao
Section of Cardiology
Department of Medicine
University of Chicago
5841 South Maryland Avenue
MC 6080, Room B608
Chicago, IL 60637

E-mail: jlliao@medicine.bsd.uchicago.edu

Date of first submission to ARS Central, July 7, 2013; date of acceptance, August 6, 2013.

Abbreviations Used

AGE = advanced glycation end product
CAD = coronary artery disease
cGK = cGMP-dependent protein kinase
CPI-17 = C-kinase-activated protein phosphatase-1 (PP1) inhibitor
CRD = carboxy-terminal cysteine-rich domain
eNOS = endothelial nitric oxide synthase
ERK = extracellular signal-regulated mitogen-activated protein kinase

ERM = ezrin-radixin-moesin
FHL2 = four-and-a-half LIM-only protein-2
FPP = farnesylpyrophosphate
GAP = GTPase-activating protein
GDI = GDP dissociation inhibitor
GEF = guanine nucleotide exchange factor
GGPP = geranyl-geranylpyrophosphate
HFD = high-fat diet
HG = high glucose
HMG-CoA = 3-hydroxy-methylglutaryl coenzyme A
IRS = insulin receptor substrate
LIM kinase = LIM (Lin11, Isl-1 and Mec-3) domain kinase
MLC = myosin light chain
MLCP = myosin phosphatase
MYPT1 = myosin phosphatase target subunit 1
NF- κ B = nuclear factor kappa-light-chain-enhancer of activated B cells
NOS = nitric oxide synthase
PAI-1 = plasminogen activator inhibitor-1
PDGF = platelet-derived growth factor
PDGFR = platelet-derived growth factor receptor
PH = pleckstrin homology
PI3K = phosphatidylinositol-3 kinase
PKC = protein kinase C
PTEN = phosphatase and tensin homolog deleted from chromosome 10
RAGE = receptor for advanced glycation end product
RBD = Rho-binding domain
ROCK = Rho-associated coiled-coil forming kinase
SRF = serum response factor
TNF- α = tumor necrosis factor- α
VSMC = vascular smooth muscle cell
ZIPK = zipper-interacting protein kinase