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Rho kinase signaling and cardiac physiology

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Abstract

Rho kinases (ROCKs) are the first discovered RhoA effectors that are now widely known for their effects on actin organization. Recent studies have shown that ROCKs play important roles in cardiac physiology. Abnormal activation of ROCKs participate in multiple cardiovascular pathological processes, including cardiac hypertrophy, apoptosis, fibrosis, systemic hypertension, and pulmonary hypertension. ROCK inhibitors, fasudil and statins, have shown beneficial cardiovascular effects in many animal studies, clinical trials, and applications. Here, we mainly discuss the current understanding of the physiological roles of Rho kinase signaling in the heart, and briefly summarize the roles of ROCKs in cardiac-related vascular dysfunctions. We will also discuss the clinical application of ROCK inhibitors.

Introduction

The Rho-associated coiled-coil containing kinases (ROCKs) are members of the AGC serine/threonine kinase family [1]. Originally identified as downstream effectors of RhoA, ROCKs facilitate RhoA-induced stress fiber formation and focal adhesion assembly [2]. It has been proven that RhoA/ROCK signaling is closely involved in regulating cell morphology, growth, migration, and apoptosis [3]. Many studies show that ROCK signaling has different functions in different types of cells in the heart and vessels, which contribute to various aspects of cardiovascular diseases. Proteomics data also found that many proteins in cardiomyocytes are ROCK substrates [4**]. In addition, beneficial cardiovascular effects of ROCK inhibitors have been observed in multiple animal studies and clinical trials. In this review, we will discuss the current understanding of the physiological roles of Rho kinase signaling in the cardiovascular system and associated diseases, functional differences of ROCK1 and ROCK2, and the clinical application of ROCK inhibitors.

Structure and expression of ROCKs

ROCKs are expressed in both invertebrates and vertebrates. Two isoforms of ROCKs encoded by two different genes are identified, ROCK1 and ROCK2 [5]. ROCKs consist of a kinase domain at the amino terminal, followed by a coiled-coil region containing the Rho-

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binding domain (RBD), and a Pleckstrin homology (PH) domain containing a cysteine-rich domain (CRD). ROCK1 and ROCK2 are highly homologous with 65% identity in their amino acid sequences and 94% identity in their kinase domains [6, 7].

ROCK1 and ROCK2 are ubiquitously expressed in different tissues. ROCK1 mRNA is highly expressed in the lungs, liver, spleen, kidneys, and testis; whereas ROCK2 mRNA is preferentially expressed in the brain and heart [3, 5]. ROCKs are predominantly distributed in the cytoplasm, while activated ROCKs translocate to the plasma membrane. A recent study showed that ROCK1, but not ROCK2, concentrates at the zonula adherens (ZA) to maintain tissue integrity and morphogenesis by protecting RhoA activity in junctions [8*]. ROCK1 has also been found to preferentially localize to actomyosin filament bundles, while ROCK2 localizes to membrane protrusions to differentially regulate dendritic spine morphology during development [9**].

Regulation of ROCKs

There is an auto-inhibitory mechanism to regulate the activity of ROCKs. In the inactive form, the carboxyl terminal of ROCKs binds to its kinase domain and forms a “closed” inhibitory loop [10]. GTP-bound Rho proteins, such as RhoA and RhoC, activate ROCKs through interacting with the Rho binding domain (RBD) of ROCKs to alter the intramolecular “closed” conformation of ROCKs [11, 12]. Besides Rho proteins, lipid messengers, especially arachidonic acid (AA), also interact with the negative regulatory region of ROCKs to disrupt the inhibitory conformation and promote the activity of ROCKs [13]. ROCKs can also be activated by cleavage and removal of the inhibitory carboxyl terminal. In the context of apoptosis, ROCK1 and ROCK2 are cleaved by caspase-3 and granzyme B, respectively, resulting in constitutively active kinase fragments of ROCKs [14–16].

Although Rho GTPases generally promote the activity of ROCKs, some Rho proteins function as negative regulators of ROCKs. RhoE, a special small G protein due to its deficiency of GTPase activity, for example, directly interacts with the N-terminal region of ROCK1 to inhibit ROCK1 activity. By recruiting p190RhoGAP, RhoE can facilitate RhoA into the GDP-bound inactive status. RhoE also prevents RhoA binding to the RBD region of ROCK1 and therefore impedes RhoA-induced ROCK1 activation [17, 18*]. In contrast, protein kinase PDK1 was recently reported to antagonize RhoE’s inhibition on ROCK1. PDK1 directly competes with RhoE for binding to ROCK1 at the plasma membrane, and PDK1 is required for ROCK-dependent actomyosin contraction [19]. Two other Rho proteins, Gem and Rad, were shown to directly interact with the coiled-coil region to inhibit ROCK1 and ROCK2 activity, respectively [20]. Whether ROCKs are regulated at the transcriptional level is unclear. A recent study showed that thrombin is able to increase ROCK2 expression and activation in endothelial cells [21].

Rho kinase and cardiac hypertrophy

Cardiac hypertrophy is an adaptive response of myocardium to various stresses [22]. Rho kinase signaling is closely involved in the development of cardiac hypertrophy [23]. A

recent study found that ROCK activity is significantly increased in hypertensive patients with left ventricular hypertrophy compared with hypertensive patients without left ventricular hypertrophy [24]. ROCK inhibitors, such as fasudil, effectively suppress angiotensin II (Ang II) induced hypertrophy [25, 26]. On the contrary, activated ROCK signaling by leptin promotes cardiac hypertrophy [27].

Studies from ROCK1 and ROCK2 genetically modified mouse models distinguished the different role of ROCK1 and ROCK2 in the development of cardiac hypertrophy. Cardiac specific ROCK2 knockout mice showed decreased hypertrophy, fibrosis, and apoptosis in the left ventricle [28], while ROCK1 null mice only showed reduced cardiac fibrosis and apoptosis [29], suggesting that ROCK2 is responsible for cardiac hypertrophy while ROCK1 is only necessary for cardiac fibrosis and cardiomyocyte apoptosis. Mechanistically, ROCK2 activates serum response factor (SRF) and extracellular signal-regulated kinase (ERK) by reducing the expression of four-and-a-half Lim-only protein 2 (FHL2). Activation of SRF and ERK then induces cardiac fetal gene expression, which contributes to cardiac hypertrophy [28]. In addition, a recent study demonstrated that the activity of formin homology 2 domain containing 3 (FHOD3), a pro-hypertrophic protein in cardiomyocytes, is also regulated by ROCK2 [30**]. ROCK2 not only increases FHOD3 expression but also promotes FHOD3 activity by directly phosphorylating FHOD3 at both 1412S and 1416T.

Besides the pro-hypertrophic role of ROCK2 in the left ventricle, a recent study showed that ROCK2 is also essential in the development of right ventricular hypertrophy and dysfunction. The expression of ROCK2 was found to be increased in the right ventricle of the pulmonary artery constriction mouse model, which contributes to right ventricular hypertrophy. Additionally, cardiac-specific overexpression of dominant-negative Rho-kinase protected mouse hearts against pressure overload induced right ventricular hypertrophy and dysfunction [31]. Together, these studies demonstrate that ROCK2 is an important mediator in the development of both left and right ventricular hypertrophy and cardiac dysfunction.

Rho kinase and cardiac apoptosis

Rho kinase plays an important role in the process of cell apoptosis [32]. Upon activation of apoptotic signaling, ROCK1 is cleaved by caspase-3 and its inhibitory C-terminus is removed, resulting in constitutive kinase activity [14]. Meanwhile, ROCK2 is cleaved by granzyme B to generate the active ROCK2 fragment [15]. Active ROCK1 and ROCK2 then continuously phosphorylate myosin light chain (MLC), which causes apoptotic membrane blebbing. The pro-apoptotic role of ROCKs was also demonstrated in the heart [16, 28, 33*]. Clinical studies found that excessive ROCK activity predicts worsened outcomes in myocardial infarction patients [34**]. We previously observed the accumulation of the constitutively active ROCK1 isoform along with increased apoptotic activities in human failing hearts. The increased protein levels of the truncated ROCK1 return to the normal control heart levels along with the attenuated myocardial apoptosis after left ventricular assist device treatment [16]. Further experiments reveal that PTEN-AKT signaling contributes to ROCK1-mediated apoptosis [16]. ROCK2 also has a pro-apoptotic role in the heart. Decreased cardiac apoptosis is detected in the cardiac-specific ROCK2 knockout mice [28]. Furthermore, ROCK inhibitors have been proven to have anti-apoptotic effects in

myocardium and significantly improve the impaired cardiac function after pressure-overload or ischemic injury challenges [35–38]. Our lab previously found that when the endogenous ROCK1 inhibitor RhoE was deficient in mice, ROCK1 activity was increased in the hearts in response to TAC-induced pressure-overload. RhoE deficiency led to increased cardiac apoptosis, which can be partially rescued by genetic deletion of ROCK1 [39].

Rho kinase and fibrotic cardiomyopathy

Cardiac fibrosis is a deleterious pathological change associated with nearly all forms of cardiovascular diseases [40]. ROCKs positively regulate cardiac fibrosis through promotion of pro-fibrotic gene expression in fibroblasts. We previously found that transgenic mice overexpressing the cleaved ROCK1 active fragment spontaneously developed marked cardiac fibrosis. AngII stimuli further exacerbated the fibrotic phenotype, while treatment with ROCK inhibitor, fasudil, attenuated the fibrosis in the myocardium, suggesting ROCK1 as a driver of cardiac fibrosis. In this study, the transgenic mouse hearts also displayed activated TGF β 1 signaling and NF- κ B signaling along with increased expression of a subset of cytokines [41], indicating the crosstalk between Rho kinase signaling and other pro-fibrotic signaling during the development of cardiac fibrosis. In other studies, ROCK1^{+/-} mice, ROCK1^{-/-} mice, and ROCK2^{-/-} mice all showed decreased cardiac fibrosis in response to AngII or trans-aortic constriction (TAC) [28, 29, 42]. Treatment with ROCK inhibitors significantly ameliorates the process of fibrotic cardiomyopathy and adverse cardiac remodeling. Indeed, some recently discovered drugs exert their cardio-protective effects possibly through inhibition of ROCK-mediated cardiac fibrosis [43*–45].

To date, ROCKs are found to promote fibrosis via fibroblasts through regulation of the MRTF-SRF pathway and YAP/TAZ-TEAD pathway. Activation of ROCK drives actin polymerization, which induces the nuclear translocation of myocardin-related transcription factor A and B (MRTF-A and MRTF-B). MRTF-A and MRTF-B then trans-activate SRF-dependent transcription of the profibrotic genes, such as connective tissue growth factor (CTGF) and matrix metalloproteinases (MMPs) [46]. ROCK-mediated actin polymerization also promotes the translocation of YAP and TAZ into the nucleus, where YAP and TAZ bind to and activate the transcription factor TEAD, which then up-regulates profibrotic gene transcription [47, 48]. Finally, our lab found that ROCK1 may work together with TGF β 1 signaling and NF- κ B signaling to mediate the development of cardiac fibrosis [41].

Rho kinase in vasospasm and right ventricular hypertrophy

Vascular homeostasis is quite important for normal heart function; irregular vascular conditions, such as systemic hypertension, pulmonary hypertension, and atherosclerosis, can impair the myocardium and induce deleterious cardiac remodeling. Rho kinase is closely associated with vascular homeostasis. Indeed, ROCK inhibitors exert their beneficial cardiovascular effects partially through controlling vascular homeostasis [49**, 50].

Rho kinase is recognized as the major regulator of vasoconstriction. Studies showed that ROCK activity is increased in the development of pulmonary hypertension and vasospasm [51–53]. RhoA/ROCK activation increases the activity of myosin light chain (MLC) in the

vascular smooth muscle cells and induces vascular contraction [54]. ROCK also inhibits the expression of endothelial nitric oxide synthase (eNOS) in endothelial cells and therefore reduces the production of nitric oxide (NO). Due to the lower level of NO secreted from endothelium, the vascular smooth muscle further contracts and the vascular tone further increases, resulting in increased systemic blood pressure and pulmonary hypertension. ROCK inhibitors have shown to be an effective treatment for cerebral vasospasm and pulmonary hypertension patients. Recently, a mechanistic study showed a novel interplay between ROCK and phosphatase and tension homolog (PTEN) in hypoxic pulmonary vasoconstriction [55**]. They found that ROCK mediates the conjoint translocation of PTEN and transient receptor canonical 6 (TRPC6) into caveolae, which allows calcium influx into pulmonary artery smooth muscle cells and induces vasoconstriction. ROCKs also contribute to vasospasm. ROCKs expression and activity were found to be increased at sites of coronary artery spasm, indicating the contribution of Rho kinase signaling to the onset of coronary artery contraction and vasospasm. Indeed, intracoronary administration of fasudil and hydroxyfasudil effectively reduces the rate of coronary spasm in a porcine model [56, 57].

Together, these observations have demonstrated that ROCKs contribute to different types of cardiomyopathy, which cause cardiac dysfunction and finally may result in heart failure (HF) [58]. Here we summarized the role of ROCKs in the development of HF. Various stimuli and stresses selectively activate ROCK1 or ROCK2, which mediates downstream targets and induces pathological changes, such as cardiomyocyte hypertrophy, cardiomyocyte apoptosis, cardiac fibrosis, and pulmonary hypertension. Animal studies showed that deletion of ROCKs attenuate the development of HF and improve the cardiac functions [28, 29, 31, 42]. ROCK inhibitors have also been proven to protect hearts against heart failure in both animal studies and clinical trials [25, 26, 35, 36, 49, 59] (Figure 1).

Rho kinase and ARVC

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a type of nonischemic cardiomyopathy characterized by myocardial atrophy, fibrofatty replacement, cardiac fibrosis, and ultimate thinning of the right ventricular wall [60]. Mutations in genes coding for desmosome complex are the main causes for ARVC. Interestingly, a recent study showed that ROCK inhibition associates with the pathogenesis of ARVC during cardiac development in the mouse [61**]. They found that mice with cardiac-specific overexpression of dominant-negative Rho kinase spontaneously develop an ARVC phenotype. They also observed up-regulation of noncanonical Wnt signaling and PPAR γ , and down-regulation of Wnt/ β -catenin signaling in the transgenic mouse hearts, indicating the possible crosstalk between Rho kinase signaling and noncanonical Wnt and canonical Wnt/ β -catenin signaling in the development of ARVC.

Clinical application of ROCK inhibitors in cardiovascular diseases

To date, fasudil and its derivatives are the only ROCK inhibitors approved for clinical use [62, 63]. Fasudil initially was described as an intracellular calcium antagonist and then was found to function through specific inhibition of ROCK activity. Fasudil has been shown

effective for the treatment of cerebral vasospasm and pulmonary hypertension, and only minimal and acceptable side effects have been observed. Moreover, many animal studies and small clinical trials have shown that fasudil treatment is beneficial in several other cardiovascular diseases, including systemic hypertension, pulmonary hypertension, coronary artery bypass grafting, acute coronary artery spasm, acute ischemic stroke, and myocardial infarction [50, 64, 65*–69]. Recently, a clinical study showed that fasudil treatment improves the in-hospital mortality rate of patients with right ventricular heart failure in severe pulmonary hypertension [49]. Another study also showed that fasudil improves the diastolic function in patients with type 2 diabetes with preserved left ventricular fraction [70], indicating the potential therapeutic application of ROCK inhibitors in heart failure patients.

Statins, originally used for reducing serum cholesterol levels, have been found to have beneficial cardiovascular effects in clinical trials [71–73]. Statins are known to improve the endothelial function, increase nitric oxide bioavailability, enhance the stability of atherosclerotic plaques, inhibit antioxidant properties, and ameliorate inflammatory responses. Mechanistically, statins were found to inhibit the isoprenylation step of RhoA, leading to reduction of RhoA activation and subsequent ROCK activation [74]. Thus, the so-called “pleiotropic” effects from statins may actually be mediated by RhoA/ROCK inhibition.

The small GTPase RhoE is an endogenous negative regulator for ROCK1. Over the course of a decade, we have uncovered significant roles of RhoE in intracellular Ca^{2+} homeostasis regulation in the heart [75*], apoptotic cardiomyopathy [39], and cardiac angiogenesis in response to chronic pressure overload [76*]. We have observed downregulation of RhoE in heart failure patients [76]. Thus, pharmaceutical up-regulation of RhoE has potential translational significance. Small GTPases and Rho signaling have been demonstrated as promising therapeutic options. Successful examples include the pleiotropic effects of statins, their Rho signaling inhibition as a form of therapy in cardiovascular diseases [77, 78], and discovery of small GTPase Ral and Rac1 inhibitors (small molecules) based on protein structure analyses [79, 80]. A similar strategy can be useful for the identification of RhoE agonists, small molecules mimicking the RhoE protein structure.

Conclusions and Future Directions

There is growing evidence that Rho kinase signaling plays important physiological roles in the regulation of the morphology and function of the cardiovascular system. Abnormal activation of Rho kinase signaling is closely associated with major cardiovascular diseases such as systemic hypertension, pulmonary hypertension, vasospasm, and adverse cardiac remodeling. However, current knowledge gaps in ROCK signaling still require more research studies. One such mystery is how different types of stimuli are transduced to activate ROCKs. Though mechanical signaling-mediated activation of ROCKs has been well elucidated, further studies are still in need to investigate the non-mechanical mechanism-mediated ROCK activation. Moreover, the redundant and non-redundant roles of ROCK1 and ROCK2 in the cardiovascular system have not yet been clearly defined to date. It will be necessary to study functions of each isoform in both cardiomyocytes and non-cardiomyocyte

cell types, such as cardiac fibroblasts, inflammatory cells, and vascular cells. Genetic mouse models with conditional silencing of each isoform would undoubtedly expand our understanding of the selective functions of different ROCK isoforms in different cell types.

The beneficial effects of fasudil and statins in clinical trials have delivered strong confidence that inhibition of ROCKs is a promising therapeutic strategy in the treatment of cardiovascular diseases. However, the short- and long-term safety issues in the application of ROCK inhibitors require further investigation. Also, there is an urgent need to develop isoform-selective ROCK inhibitors for the treatment of different types of cardiovascular diseases. Pharmaceutical targeting of the endogenous ROCK1 inhibitor RhoE may provide an alternative to ROCK inhibitory drugs. Finally, the development of sensitive, convenient, and reliable technologies allowing clinical assessment of ROCK activities remains challenging, which limits the clinical usage of ROCK inhibitors. It is therefore important to identify new biomarkers for the diagnosis and assessment of Rho kinase signaling disorders.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

** of outstanding interest

1. Riento K, Ridley AJ. Rocks: multifunctional kinases in cell behaviour. *Nat Rev Mol Cell Biol.* 2003; 4(6):446–56. [PubMed: 12778124]
2. Amano M, et al. Formation of actin stress fibers and focal adhesions enhanced by Rho-kinase. *Science.* 1997; 275(5304):1308–11. [PubMed: 9036856]
3. Schofield AV, Bernard O. Rho-associated coiled-coil kinase (ROCK) signaling and disease. *Crit Rev Biochem Mol Biol.* 2013; 48(4):301–16. [PubMed: 23601011]
4. Yura Y, et al. Focused Proteomics Revealed a Novel Rho-kinase Signaling Pathway in the Heart. *Cell Struct Funct.* 2016; 41(2):105–20. ** This study used a novel kinase-interaction substrate screening (KISS) method to exhaustively screen for potential ROCK substrates and about 330 proteins were recognized. The authors further focused on cardiac-substrates, and demonstrated CARP/Ankrd1 as a novel ROCK substrate. Non-phosphorylated form of CARP is found to be related to cardiac hypertrophy. [PubMed: 27334702]
5. Nakagawa O, et al. ROCK-I and ROCK-II, two isoforms of Rho-associated coiled-coil forming protein serine/threonine kinase in mice. *FEBS Lett.* 1996; 392(2):189–93. [PubMed: 8772201]
6. Tu D, et al. Crystal structure of a coiled-coil domain from human ROCK I. *PLoS One.* 2011; 6(3):e18080. [PubMed: 21445309]
7. Julian L, Olson MF. Rho-associated coiled-coil containing kinases (ROCK): structure, regulation, and functions. *Small GTPases.* 2014; 5:e29846. [PubMed: 25010901]
8. Priya R, et al. Feedback regulation through myosin II confers robustness on RhoA signalling at E-cadherin junctions. *Nat Cell Biol.* 2015; 17(10):1282–93. * This study well demonstrated how the major players of Rho kinase signaling, ROCK1, RhoA and Rnd3, work together to regulate actomyosin at the epithelial zonula adherens (ZA). The authors found RhoA zone is required for ZA formation, while Rnd3 antagonizes RhoA activity. ROCK1 is recruited to the ZA to protect RhoA

- against Rnd3. The complexity of the RhoA/ROCK1/Rnd3 triangle requires more studies. [PubMed: 26368311]
9. Newell-Litwa KA, et al. ROCK1 and 2 differentially regulate actomyosin organization to drive cell and synaptic polarity. *J Cell Biol.* 2015; 210(2):225–42. ** The subcellular localization of ROCK1 and ROCK2 is important for their functions. This paper showed the different subcellular localizations of ROCK1 and ROCK2, and found that these two isoforms differentially regulate distinct RhoA downstream pathways to drive cell and synaptic polarity. [PubMed: 26169356]
 10. Amano M, et al. The COOH terminus of Rho-kinase negatively regulates rho-kinase activity. *J Biol Chem.* 1999; 274(45):32418–24. [PubMed: 10542285]
 11. Dvorsky R, et al. Structural insights into the interaction of ROCK1 with the switch regions of RhoA. *J Biol Chem.* 2004; 279(8):7098–104. [PubMed: 14660612]
 12. Matsui T, et al. Rho-associated kinase, a novel serine/threonine kinase, as a putative target for small GTP binding protein Rho. *EMBO J.* 1996; 15(9):2208–16. [PubMed: 8641286]
 13. Araki S, et al. Arachidonic acid-induced Ca²⁺ sensitization of smooth muscle contraction through activation of Rho-kinase. *Pflugers Arch.* 2001; 441(5):596–603. [PubMed: 11294240]
 14. Sebbagh M, et al. Caspase-3-mediated cleavage of ROCK I induces MLC phosphorylation and apoptotic membrane blebbing. *Nat Cell Biol.* 2001; 3(4):346–52. [PubMed: 11283607]
 15. Sebbagh M, et al. Direct cleavage of ROCK II by granzyme B induces target cell membrane blebbing in a caspase-independent manner. *J Exp Med.* 2005; 201(3):465–71. [PubMed: 15699075]
 16. Chang J, et al. 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.* 2006; 103(39):14495–500. [PubMed: 16983089]
 17. Riento K, et al. RhoE binds to ROCK I and inhibits downstream signaling. *Mol Cell Biol.* 2003; 23(12):4219–29. [PubMed: 12773565]
 - 18*. Jie W, et al. Pathophysiological Functions of Rnd3/RhoE. *Comprehensive Physiology.* 2016; 6:169–186.
 19. Pinner S, Sahai E. PDK1 regulates cancer cell motility by antagonising inhibition of ROCK1 by RhoE. *Nat Cell Biol.* 2008; 10(2):127–37. [PubMed: 18204440]
 20. Ward Y, et al. The GTP binding proteins Gem and Rad are negative regulators of the Rho-Rho kinase pathway. *J Cell Biol.* 2002; 157(2):291–302. [PubMed: 11956230]
 21. Sapet C, et al. Thrombin-induced endothelial microparticle generation: identification of a novel pathway involving ROCK-II activation by caspase-2. *Blood.* 2006; 108(6):1868–76. [PubMed: 16720831]
 22. Schiattarella GG, Hill JA. Inhibition of hypertrophy is a good therapeutic strategy in ventricular pressure overload. *Circulation.* 2015; 131(16):1435–47. [PubMed: 25901069]
 23. Shimizu T, Liao JK. Rho Kinases and Cardiac Remodeling. *Circ J.* 2016; 80(7):1491–8. [PubMed: 27251065]
 24. Gabrielli L, et al. Increased rho-kinase activity in hypertensive patients with left ventricular hypertrophy. *Am J Hypertens.* 2014; 27(6):838–45. [PubMed: 24363278]
 25. Higashi M, et al. 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.* 2003; 93(8):767–75. [PubMed: 14500337]
 26. Wang YX, et al. Inhibition of Rho-kinase by fasudil attenuated angiotensin II-induced cardiac hypertrophy in apolipoprotein E deficient mice. *Eur J Pharmacol.* 2005; 512(2–3):215–22. [PubMed: 15840407]
 27. Rajapurohitam V, et al. Leptin-induced cardiomyocyte hypertrophy reveals both calcium-dependent and calcium-independent/RhoA-dependent calcineurin activation and NFAT nuclear translocation. *Cell Signal.* 2012; 24(12):2283–90. [PubMed: 22917533]
 28. Okamoto R, et al. FHL2 prevents cardiac hypertrophy in mice with cardiac-specific deletion of ROCK2. *FASEB J.* 2013; 27(4):1439–49. [PubMed: 23271052]
 29. Zhang YM, et al. Targeted deletion of ROCK1 protects the heart against pressure overload by inhibiting reactive fibrosis. *FASEB J.* 2006; 20(7):916–25. [PubMed: 16675849]

30. Zhou Q, et al. Crucial Role of ROCK2-Mediated Phosphorylation and Upregulation of FHOD3 in the Pathogenesis of Angiotensin II-Induced Cardiac Hypertrophy. *Hypertension*. 2017; 69(6): 1070–1083. ** This study demonstrated a novel mechanism of how ROCK2 mediates cardiac hypertrophy. FHOD3, a pro-hypertrophic protein, was found to be increased in mouse hearts in response to AngII stimuli. Phosphorylation of FHOD3 by ROCK2 increased its activity and induced cardiomyocyte hypertrophy, which was abolished by inhibition of ROCK. [PubMed: 28438902]
31. Ikeda S, et al. Crucial role of rho-kinase in pressure overload-induced right ventricular hypertrophy and dysfunction in mice. *Arterioscler Thromb Vasc Biol*. 2014; 34(6):1260–71. [PubMed: 24675663]
32. Coleman ML, et al. Membrane blebbing during apoptosis results from caspase-mediated activation of ROCK I. *Nat Cell Biol*. 2001; 3(4):339–45. [PubMed: 11283606]
- 33*. Gao H, et al. Rho-Kinase inhibitor fasudil suppresses high glucose-induced H9c2 cell apoptosis through activation of autophagy. *Cardiovasc Ther*. 2016; 34(5):352–9. [PubMed: 27333569]
34. Li X, et al. Increased Rho kinase activity predicts worse cardiovascular outcome in ST-segment elevation myocardial infarction patients. *Cardiol J*. 2016; 23(4):456–64. ** This study compared the ROCK activity in STEMI patients with and without diabetes mellitus (DM). Patients with DM had higher risk of cardiovascular events along with increased ROCK1 activity. There was no difference of ROCK2 between the groups, indicating that ROCK1, but not ROCK2, maybe the major mediator during myocardial infarction in diabetes mellitus patients. [PubMed: 27320957]
35. Li Y, et al. Fasudil protects the heart against ischemia-reperfusion injury by attenuating endoplasmic reticulum stress and modulating SERCA activity: the differential role for PI3K/Akt and JAK2/STAT3 signaling pathways. *PLoS One*. 2012; 7(10):e48115. [PubMed: 23118936]
36. Wang N, et al. Fasudil hydrochloride hydrate, a Rho-kinase inhibitor, suppresses isoproterenol-induced heart failure in rats via JNK and ERK1/2 pathways. *J Cell Biochem*. 2011; 112(7):1920–9. [PubMed: 21433064]
37. Kan L, et al. Rho-Associated Kinase Inhibitor (Y-27632) Attenuates Doxorubicin-Induced Apoptosis of Human Cardiac Stem Cells. *PLoS One*. 2015; 10(12):e0144513. [PubMed: 26645568]
38. Patel P, et al. Inhibition of RhoA/Rho kinase by ibuprofen exerts cardioprotective effect on isoproterenol induced myocardial infarction in rats. *Eur J Pharmacol*. 2016; 791:91–98. [PubMed: 27565218]
39. Yue X, et al. Rnd3 haploinsufficient mice are predisposed to hemodynamic stress and develop apoptotic cardiomyopathy with heart failure. *Cell Death Dis*. 2014; 5:e1284. [PubMed: 24901055]
40. Travers JG, et al. Cardiac Fibrosis: The Fibroblast Awakens. *Circ Res*. 2016; 118(6):1021–40. [PubMed: 26987915]
41. Yang X, et al. Mechanism of fibrotic cardiomyopathy in mice expressing truncated Rho-associated coiled-coil protein kinase 1. *FASEB J*. 2012; 26(5):2105–16. [PubMed: 22278938]
42. Rikitake Y, et al. Decreased perivascular fibrosis but not cardiac hypertrophy in ROCK1+/- haploinsufficient mice. *Circulation*. 2005; 112(19):2959–65. [PubMed: 16260635]
43. Luo S, et al. ZYZ-168 alleviates cardiac fibrosis after myocardial infarction through inhibition of ERK1/2-dependent ROCK1 activation. *Sci Rep*. 2017; 7:43242. * This study discovered a novel cardio-protective conjugate of Leonurine, ZYZ-168, and demonstrated its role in myocardial infarction. ZYZ-168 was found to inhibit ERK1/2 activity and reduce ROCK1 expression. [PubMed: 28266583]
44. Lee TM, Lin SZ, Chang NC. Membrane ERalpha attenuates myocardial fibrosis via RhoA/ROCK-mediated actin remodeling in ovariectomized female infarcted rats. *J Mol Med (Berl)*. 2014; 92(1): 43–51. [PubMed: 24292258]
45. Cheng CI, et al. Cobalt chloride induces RhoA/ROCK activation and remodeling effect in H9c2 cardiomyoblasts: Involvement of PI3K/Akt and MAPK pathways. *Cell Signal*. 2017; 36:25–33. [PubMed: 28435089]
46. Sakai N, et al. LPA1-induced cytoskeleton reorganization drives fibrosis through CTGF-dependent fibroblast proliferation. *FASEB J*. 2013; 27(5):1830–46. [PubMed: 23322166]

47. Calvo F, et al. Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts. *Nat Cell Biol.* 2013; 15(6):637–46. [PubMed: 23708000]
48. Knipe RS, Tager AM, Liao JK. The Rho kinases: critical mediators of multiple profibrotic processes and rational targets for new therapies for pulmonary fibrosis. *Pharmacol Rev.* 2015; 67(1):103–17. [PubMed: 25395505]
49. Jiang R, et al. Intravenous fasudil improves in-hospital mortality of patients with right heart failure in severe pulmonary hypertension. *Hypertens Res.* 2015; 38(8):539–44. ** This clinical study evaluated the effects of ROCK inhibitors on patients with right heart failure in severe pulmonary hypertension. 209 patients were selected, and intravenous fasudil treatment group improved both in-hospital mortality and 30-day re-hospitalization. [PubMed: 25787034]
50. Fukumoto Y, et al. Double-blind, placebo-controlled clinical trial with a rho-kinase inhibitor in pulmonary arterial hypertension. *Circ J.* 2013; 77(10):2619–25. [PubMed: 23912836]
51. Sato M, et al. Involvement of Rho-kinase-mediated phosphorylation of myosin light chain in enhancement of cerebral vasospasm. *Circ Res.* 2000; 87(3):195–200. [PubMed: 10926869]
52. Barman SA, Zhu S, White RE. RhoA/Rho-kinase signaling: a therapeutic target in pulmonary hypertension. *Vasc Health Risk Manag.* 2009; 5:663–71. [PubMed: 19707285]
53. Thompson-Torgerson CS, et al. Rho kinase-mediated local cold-induced cutaneous vasoconstriction is augmented in aged human skin. *Am J Physiol Heart Circ Physiol.* 2007; 293(1):H30–6. [PubMed: 17416609]
54. Uehata M, et al. Calcium sensitization of smooth muscle mediated by a Rho-associated protein kinase in hypertension. *Nature.* 1997; 389(6654):990–4. [PubMed: 9353125]
55. Krauszman A, et al. Role of phosphatase and tensin homolog (PTEN) in hypoxic pulmonary vasoconstriction. *Cardiovasc Res.* 2017 ** This paper showed the interplay between ROCK and PTEN in hypoxic pulmonary vasoconstriction. Hypoxia promotes interaction of PTEN with TPRC to induce vasoconstriction; PTEN/TPRC translocate to caveolae, which is inhibited by ROCK inhibitor, indicating the involvement of ROCK1 in regulating PTEN-mediated vasoconstriction.
56. Shimokawa H, et al. Rho-kinase-mediated pathway induces enhanced myosin light chain phosphorylations in a swine model of coronary artery spasm. *Cardiovasc Res.* 1999; 43(4):1029–39. [PubMed: 10615430]
57. Katsumata N, et al. Enhanced myosin light chain phosphorylations as a central mechanism for coronary artery spasm in a swine model with interleukin-1beta. *Circulation.* 1997; 96(12):4357–63. [PubMed: 9416904]
58. Ziaeian B, Fonarow GC. Epidemiology and aetiology of heart failure. *Nat Rev Cardiol.* 2016; 13(6):368–78. [PubMed: 26935038]
59. Ahuja P, et al. Re-expression of proteins involved in cytokinesis during cardiac hypertrophy. *Exp Cell Res.* 2007; 313(6):1270–83. [PubMed: 17316608]
60. Corrado D, Link MS, Calkins H. Arrhythmogenic Right Ventricular Cardiomyopathy. *N Engl J Med.* 2017; 376(1):61–72. [PubMed: 28052233]
61. Ellawindy A, et al. Rho-Kinase Inhibition During Early Cardiac Development Causes Arrhythmogenic Right Ventricular Cardiomyopathy in Mice. *Arterioscler Thromb Vasc Biol.* 2015; 35(10):2172–84. ** This study demonstrated that the inhibition of ROCK is detrimental during cardiac development. ARVC phenotype was found in mice with overexpression of dominant-negative ROCK in smooth muscle. Noncanonical Wnt pathway was increased while Wnt/ β -catenin was decreased. This study revealed the essential role of ROCK in cardiac development, so long-term investigation of ROCK inhibitors safety is needed. [PubMed: 26315406]
62. Daggrell SA. Rho-kinase inhibitors show promise in pulmonary hypertension. *Expert Opin Investig Drugs.* 2005; 14(9):1157–9.
63. Shibuya M, Suzuki Y. Treatment of cerebral vasospasm by a protein kinase inhibitor AT 877. *No To Shinkei.* 1993; 45(9):819–24. [PubMed: 8217408]
64. Jiang X, et al. Acute hemodynamic response of infused fasudil in patients with pulmonary arterial hypertension: a randomized, controlled, crossover study. *Int J Cardiol.* 2014; 177(1):61–5. [PubMed: 25499341]

65. Watanabe G, et al. Fasudil Is an Effective Graft Vasodilator for Gastroepiploic Artery in Coronary Artery Bypass Grafting. *Innovations (Phila)*. 2015; 10(4):268–72. discussion 272. * This clinical study demonstrated the beneficial effects of ROCK inhibitors in coronary artery bypass grafting (CABG). Intraluminal injection of fasudil significantly increased the graft free flow of the right gastroepiploic artery (RGEA). Fasudil treatment significantly increased RGEA diameter while the integrity of the multiple elastic lamellae was still maintained. [PubMed: 26360979]
66. Otsuka T, et al. Administration of the Rho-kinase inhibitor, fasudil, following nitroglycerin additionally dilates the site of coronary spasm in patients with vasospastic angina. *Coron Artery Dis*. 2008; 19(2):105–10. [PubMed: 18300747]
67. Fukumoto Y, et al. Anti-ischemic effects of fasudil, a specific Rho-kinase inhibitor, in patients with stable effort angina. *J Cardiovasc Pharmacol*. 2007; 49(3):117–21. [PubMed: 17414222]
68. Shibuya M, et al. Effects of fasudil in acute ischemic stroke: results of a prospective placebo-controlled double-blind trial. *J Neurol Sci*. 2005; 238(1–2):31–9. [PubMed: 16005902]
69. Mera C, et al. Mechanisms of favorable effects of Rho kinase inhibition on myocardial remodeling and systolic function after experimental myocardial infarction in the rat. *Ther Adv Cardiovasc Dis*. 2016; 10(1):4–20. [PubMed: 26490279]
70. Guo R, et al. Fasudil improves short-term echocardiographic parameters of diastolic function in patients with type 2 diabetes with preserved left ventricular ejection fraction: a pilot study. *Heart Vessels*. 2015; 30(1):89–97. [PubMed: 24390764]
71. Sacks FM, et al. 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*. 1996; 335(14):1001–9. [PubMed: 8801446]
72. Long-Term Intervention with Pravastatin in Ischaemic Disease Study, G. Prevention of cardiovascular events and death with pravastatin in patients with coronary heart disease and a broad range of initial cholesterol levels. *N Engl J Med*. 1998; 339(19):1349–57. [PubMed: 9841303]
73. Davignon J. Beneficial cardiovascular pleiotropic effects of statins. *Circulation*. 2004; 109(23 Suppl 1):III39–43. [PubMed: 15198965]
74. Rikitake Y, Liao JK. Rho GTPases, statins, and nitric oxide. *Circ Res*. 2005; 97(12):1232–5. [PubMed: 16339495]
- 75*. Yang X, et al. Genetic deletion of Rnd3/RhoE results in mouse heart calcium leakage through upregulation of protein kinase A signaling. *Circ Res*. 2015; 116(1):e1–e10. [PubMed: 25348166]
- 76*. Yue X, et al. Rnd3/RhoE Modulates Hypoxia-Inducible Factor 1alpha/Vascular Endothelial Growth Factor Signaling by Stabilizing Hypoxia-Inducible Factor 1alpha and Regulates Responsive Cardiac Angiogenesis. *Hypertension*. 2016; 67(3):597–605. [PubMed: 26781283]
77. Heart Protection Study Collaborative, G. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*. 2002; 360(9326):7–22. [PubMed: 12114036]
78. Pedersen TR. Pleiotropic effects of statins: evidence against benefits beyond LDL-cholesterol lowering. *Am J Cardiovasc Drugs*. 2010; 10(Suppl 1):10–7. [PubMed: 21391729]
79. Yan C, et al. Discovery and characterization of small molecules that target the GTPase Ral. *Nature*. 2014; 515(7527):443–7. [PubMed: 25219851]
80. Bid HK, et al. RAC1: an emerging therapeutic option for targeting cancer angiogenesis and metastasis. *Mol Cancer Ther*. 2013; 12(10):1925–34. [PubMed: 24072884]

Highlights

Rho kinase signaling plays important roles in the cardiovascular system.

Rho kinase signaling mediates cardiac hypertrophy, apoptosis and fibrosis.

Rho kinase signaling regulates vasocontraction and vasospasm.

ROCK inhibitors provide beneficial cardiovascular effects.

Figure 1. Role of Rho kinase signaling in heart failure

RhoA/ROCKs closely participate in the development of multiple pathological changes in heart failure. ROCK1 and ROCK2 are cleaved by caspase-3 and granzyme B, respectively, resulting in active ROCK1 and ROCK2 kinase fragments, which facilitate cardiomyocyte apoptosis. ROCK2 promotes cardiac hypertrophy through 1) increasing the expression of SRF-targeted cardiac fetal genes; and 2) increasing the expression and activity of pro-hypertrophic gene FHOD3. ROCKs also promote cardiac fibrosis through activating MRTF/SRF pathway and YAP/TAZ-TEAD pathway in myofibroblasts. In addition, ROCKs promote pulmonary hypertension through enhancing vasocontraction and inhibiting nitric oxide production. SRF: serum response factor; FHOD3: formin homology 2 domain containing 3; MRTF: myocardin-related transcription factor; YAP: yes-associated protein; TAZ: transcriptional coactivator with PDZ-binding motif; TEAD: transcriptional enhancer factor TEF; RV: right ventricle.