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Targeting Rho-associated coiled-coil forming protein kinase (ROCK) in cardiovascular fibrosis and stiffening

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Abstract

Introduction: Pathological cardiac fibrosis, through excessive extracellular matrix protein deposition from fibroblasts and pro-fibrotic immune responses and vascular stiffening is associated with most forms of cardiovascular disease. Pathological cardiac fibrosis and stiffening can lead to heart failure and arrhythmias and vascular stiffening may lead to hypertension. ROCK, a serine/threonine kinase downstream of the Rho-family of GTPases, may regulate many pro-fibrotic and pro-stiffening signaling pathways in numerous cell types.

Areas covered: This article outlines the molecular mechanisms by which ROCK in fibroblasts, T helper cells, endothelial cells, vascular smooth muscle cells, and macrophages mediate fibrosis and stiffening. We speculate on how ROCK could be targeted to inhibit cardiovascular fibrosis and stiffening.

Expert opinion: Critical gaps in knowledge must be addressed if ROCK inhibitors are to be used in the clinic. Numerous studies indicate that each ROCK isoform may play differential roles in regulating fibrosis and may have opposing roles in specific tissues. Future work needs to highlight the isoform- and tissue-specific contributions of ROCK in fibrosis, and how isoform-specific ROCK inhibitors in murine models and in clinical trials affect the pathophysiology of cardiac fibrosis and stiffening. This could progress knowledge regarding new treatments for heart failure, arrhythmias and hypertension and the repair processes after myocardial infarction.

Keywords

Rho; ROCK; fibrosis; heart failure; vascular stiffness; arrhythmias; myocardial infarction

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1. INTRODUCTION

Myocardial fibrosis and vascular stiffening underlie a broad spectrum of cardiovascular diseases.[1] In the myocardium, pathological remodeling caused by the deposition of extracellular matrix (ECM) proteins by cardiac fibroblasts may lead to changes in the cardiac interstitium. These changes often either cause or accompany myocardium-related cardiovascular disease, including heart failure, diastolic dysfunction, and cardiac arrhythmias. In the vasculature, endothelial and smooth muscle cells modulate arterial stiffness and myocardial fibrosis through the production of cytokines, growth factors, and other enzymes, as well as further deposition of ECM proteins. Parallel to these processes, the innate and adaptive immune responses participate to induce cardiac fibrosis and arterial stiffening in the myocardium and vasculature, respectively.

The Rho-associated coiled-coil forming protein kinases (ROCKs) are serine/threonine kinases directly activated by RhoA, a protein belonging to the Rho family of small GTPases. The RhoA/ROCK pathway has received a great deal of attention by researchers due to its central role in a multitude of cellular and pathogenic processes. ROCK was first identified as an important target of RhoA[2] and a regulator of the actin cytoskeleton in 1995 and 1996 through inhibitory phosphorylation at Thr696 and Thr853 of the myosin binding subunit (MBS) of myosin light chain (MLC) phosphatase (now more commonly referred to as the myosin phosphatase binding subunit 1 [MYPT1]),[3] thereby leading to MLC phosphorylation and actomyosin stress fiber formation. Following papers suggested that these actions affected vascular tone, cell migration, gene expression, inflammation, and oxidative stress.[4] As cell migration, inflammation, and oxidative stress play important roles in the pathogenesis of fibrosis, it is likely that ROCK could also affect fibrosis through these cellular mechanisms.

Due to the pathophysiological significance of fibrosis in the cardiovascular system, identifying the fibrotic contributions of important molecular pathways, including Rho/ROCK, may yield new therapies that can decrease cardiovascular mortality and morbidity. Such findings could be beneficial as cardiovascular diseases related to fibrosis are major public health burdens. For instance, heart failure alone afflicts 26 million people worldwide with an estimated economic burden of over \$100 billion a year.[5] Moreover, a variety of fibrosis-related cardiovascular diseases, including heart failure with preserved ejection fraction (HFpEF), remain without effective therapeutic interventions. In this review on the role of ROCK in cardiovascular fibrosis and stiffening, we will begin by summarizing the structure and general functions of ROCK and then discussing the role of ROCK in the molecular mechanisms of fibrosis and stiffening. In addition, we will provide an overview of how ROCK contributes to fibrosis and stiffening in a diverse array of cell types involved in cardiovascular disease. We conclude by considering current and future developments of ROCK inhibitors as therapeutic agents for cardiovascular fibrosis and stiffening.

2. STRUCTURE AND FUNCTIONS OF ROCK

The structure of ROCK, and its two isoforms ROCK1 and ROCK2, are well characterized (Figure 1). ROCK1 and ROCK2 are highly homologous, sharing 92% of the amino acids in

the kinase domain, 55% in the coiled-coil region, and 65% in the overall amino acid sequence.[4] The C-terminus of ROCK contains an autoinhibitory region, composed of a Pleckstrin-homology domain and a Rho-binding domain. Binding of lipids, particularly phosphatidylinositol (3,4,5)-trisphosphate (PIP₃), to the Pleckstrin-homology domain and interaction of the active form of Rho with the Rho-binding domain induces the open and catalytically-active conformation of ROCK (Figure 1). The open conformation may also be induced by cleavage of the C-terminus by caspase-3 or granzyme B. Moreover, the N-terminal kinase domain and the coiled-coil domain may mediate dimerization of the open form of ROCK, linking each kinase domain together. It is likely that dimerization of ROCK is necessary for some of its downstream functions. While the primary mechanism of activation for both isoforms of ROCK is through RhoA, other proteins are also implicated in the activation of ROCK. For instance, ROCK appears to be activated by other members of the Rho family, including RhoB and RhoC,[2] as well as polo-like kinase 1.[6] A range of other proteins, however, may inhibit ROCK, including the GTPases RhoE (Rnd3),[7] Gem and Rad[8], and the chaperone protein Morgana/chp-1.[9] It is likely that there are additional, yet undiscovered, pathways involved in the regulation of ROCK activity.

Both ROCK isoforms mediate changes in cytoskeletal dynamics through the phosphorylation of numerous downstream actin cytoskeleton-mediating proteins. In particular, from an intense period of research conducted predominantly in the late 1990s and early 2000s, it was found that ROCK phosphorylates LIM kinase-1 and -2, adducin, ezrin/radixin/moesin (ERM) proteins, formin homology domain protein 1 (FHOD1), MLC, and MYPT1.[10] Furthermore, ROCK can mediate the polymerization of G-actin to F-actin through the action of LIM kinase-1 on cofilin.[11] These interactions lead to force generation and morphological changes, contributing to a broad range of important actin-myosin mediated processes, including cell adhesion, polarity, motility, cytokinesis, and phagocytosis. Both ROCK isoforms also appear to play important roles in gene expression, proliferation, and apoptosis, largely mediated by the kinase's effects on the proteins involved in stress fiber formation. As examples, ROCK regulates the cell cycle regulatory proteins cyclin A via LIM kinase-2 and also regulates cyclin D via Ras and the mitogen-activated protein kinase.[12] In addition, through its effects on MLC, ROCK mediates apoptotic processes such as membrane blebbing, cellular fragmentation, and nuclear disintegration.[13] Consequently, ROCK plays a central role across a broad spectrum of fundamental cellular functions, often attributed to its actions on actomyosin function.

Despite similarities in the kinase domain, ROCK1 and ROCK2 may play different cellular functions. Indeed, ROCK2 and ROCK1 may have different subcellular localizations. ROCK2 appears to have a predominantly cytosolic localization but also accumulates in the cleavage furrow, nucleus, intermediate filaments, and centrosomes.[13] ROCK1 also accumulates in the cytosol, but may be less likely to be distributed at the plasma membrane compared with ROCK2.[13] Likewise, ROCK1 and ROCK2 are differentially expressed: ROCK1 is ubiquitously expressed in all tissues, although less in brain and skeletal muscle, while ROCK2 is more abundantly expressed than ROCK1 in the brain, muscle, heart, lung, and placenta.[14] ROCK1 also may play a more important pro-fibrotic role than ROCK2 across a wide variety of tissues, particularly in the heart,[15–19] as evidenced by global knockout and haploinsufficient ROCK1 mice. A recent study published in 2017, however,

indicates that ROCK2 deletion in cardiac fibroblasts leads to decreased cardiac hypertrophy and fibrosis,[20] suggesting that each ROCK isoform may possess varying pro-fibrotic functions depending on cell type. Indeed, a study published a year later suggested that ROCK1 and ROCK2 in cardiomyocytes may in fact play opposing roles in cardiac hypertrophy and fibrosis.[19] Consequently, while an emerging body of research may suggest that both ROCK1 and ROCK2 play important roles in cardiac fibrosis, the role of each ROCK isoform and their knockout/knockdown phenotypes in murine models of diastolic dysfunction, pressure overload, or heart failure remain to be fully characterized.

Intriguingly, upstream negative regulators of ROCK, including the GTPases RhoE and Rad, appear to also mediate cardiac fibrosis and heart failure. One study suggests that RhoE haploinsufficient mice develop hyperactivation of ROCK activity, myocardial apoptosis, and heart failure, a phenotype that was partially attenuated by administration of fasudil, a well-established ROCK inhibitor, or through deletion of ROCK1.[21] Moreover, Rad knockout in mice led to severe cardiac fibrosis through upregulated expression of connective tissue growth factor (CTGF).[22] Therefore, while research into the Rho subfamily and its related GTP-binding proteins has often focused on RhoA and ROCK itself, regulators of ROCK may also be potential therapeutic targets to ameliorate cardiac fibrosis and vascular stiffening. Further research into these proteins may help clarify whether these negative regulators of ROCK affect cardiovascular fibrosis or stiffening through ROCK-dependent or -independent mechanisms.

3. ROCK-MEDIATED PATHWAYS IN FIBROBLASTS

ROCK appears to mediate many of the cellular responses of fibroblasts and plays an important role in the pathological inflammatory environment that persists after injury. Knowledge of these pathways are central towards developing strategies that target ROCK in myocardial fibrosis and vascular stiffening. Hence, in this section of the review, we explore pathways through which ROCK may modulate fibroblast activation and differentiation.

3.1 MRTF/SRF signaling

Serum response factor (SRF), belonging to the MADS box-containing family of transcription factors, is critically involved in fibroblast differentiation, proliferation, and activation.[23] SRF interacts with cofactors from the myocardin-related transcription factor (MRTF) and ternary complex factor (TCF) families, thus coupling growth factor signaling to gene transcription.[24] In particular, MRTF-A and MRTF-B recruitment is crucial for SRF signaling: of the >3100 SRF binding sites identified through chromatin immunoprecipitation sequencing (ChIP-seq), >2600 exhibited MRTF-dependent SRF binding.[24] Indeed, numerous MRTF-SRF-binding sites are located in the promoter regions governing fibroblast activation and inhibition of MRTF/SRF prevents scar tissue formation.[25] The MRTF/SRF pathway is regulated by actin dynamics.[20] For example, G-actin binds to MRTF-A and MRTF-B, sequestering the transcription factor in the cytoplasm (Figure 2). Polymerization of G-actin to F-actin liberates the MRTFs, allowing it to enter the nucleus to associate with SRF and to activate target pro-fibrotic target genes.

Since the polymerization of G-actin into F-actin is regulated by ROCK through the ROCK-LiM kinase-cofilin pathway,[11, 26] and RhoA was first identified as a regulator of transcriptional activity of SRFs in 1995,[27] ROCK may play a central role in controlling the MRTF-SRF pathway. This appears to be the case, as Esnault et al. (2014) have shown that RhoA/ROCK signaling through actin drives SRF binding and MRTF recruitment.[24] Numerous studies have also shown that pro-fibrotic and MRTF/SRF-dependent transcription in the myocardium, including expression of CTGF,[28] differentiation of fibroblasts into myofibroblasts,[29] activation of myofibroblasts,[30] and ECM stiffening and production, [31] are regulated by ROCK. In consequence, some studies have suggested that MRTF-mediated upregulation of pro-fibrotic target genes are broadly regulated by activation of RhoA and ROCK.[32] Indeed, the use of ROCK inhibitors prevented the nuclear accumulation of MRTF-A across a wide variety of pro-fibrotic activators (e.g. lysophosphatidic acid [LPA] or transforming growth factor- β [TGF- β]).[28, 30]

Pro-fibrotic activators, such as LPA, were first found to activate RhoA and ROCK through G-protein coupled receptor (GPCRs) in 1999 by Kranenburg et al.[33] Subsequent studies throughout the early 2000s identified numerous RhoGEFs that mediate this mechanism, including p115-RhoGEF, PSD-95/Disc-large/ZO-1 homology-RhoGEF, leukemia-associated RhoGEF and lymphoid blast crisis-RhoGEF.[34] Intriguingly, ROCK has also been shown to be activated downstream of Gq through a pathway involving phospholipase C, Ca²⁺, and protein kinase C, although this induction of RhoA activation was through thrombin and not LPA.[35] Nonetheless, as LPA also activates Gq pro-fibrotic pathways, it is possible that LPA can activate ROCK through alternative GPCR signaling pathways to induce MRTF/SRF-mediated fibrosis.

Intriguingly, ROCK itself may be upregulated downstream of matrix stiffening.[36] Consequently, in a feed-forward loop, increased ECM stiffening may increase ROCK activity, which in turn enhances fibroblast differentiation, myofibroblast activation, and further ECM production and stiffening.

3.2 YAP/TAZ/TEAD signaling

Yes-associated protein (YAP) and transcriptional coactivator with a PDZ-binding domain (TAZ) are key downstream transcriptional coactivators of the Hippo kinase cascade[37] and are important mediators of fibroblast activation and fibrosis.[38] In the canonical Hippo signaling pathway, the serine/threonine kinases MST1 and MST2, orthologs of the *Drosophila* Hippo kinase, interact with and phosphorylate the proteins SAV1, MOB1A/B, and LATS1/2 to activate LATS1/2 kinases (Figure 3). The activated enzyme complex directly phosphorylates YAP and TAZ on multiple serine residues via interaction with 14-3-3 (Figure 3). After phosphorylation, YAP and TAZ are retained in the cytoplasm and are primed for future proteasomal degradation. When the Hippo kinase cascade is inhibited, however, the unphosphorylated YAP and TAZ translocate into the nucleus to bind with transcription factors, of which the most studied are the TEA domain family members 1–4 (TEAD1–4). Importantly, TEAD, together with YAP and TAZ, mediate the upregulation of important pro-fibrotic target genes, including CTGF,[39] plasminogen activator inhibitor-1 (PAI-1),[40] and TGF- β .[38]

An important aspect of the Hippo pathway is its role as a transducer of alterations in cell structure, including changes in cell shape, cell-cell interactions, mechanotransduction, and matrix stiffness. Indeed, numerous studies, primarily from the early 2010s, have shown that this is where ROCK plays a role in regulating YAP/TAZ signaling.[41] For instance, studies have shown that disruptions in F-actin stress fibers (e.g. through ROCK inhibition) prevents nuclear TAZ/YAP translocation (Figure 3).[42] Likewise, F-actin capping or cleavage proteins, such as cofilin, retain TAZ/YAP in the cytoplasm and the inactivation of these factors (as mediated by ROCK) induces YAP/TAZ-mediated transcription.[43] The regulation of YAP/TAZ signaling by ROCK is also evidenced in studies using inhibitors of ROCK, such as Y-27632, which reduce nuclear YAP translocation and inhibit YAP/TAZ-mediated fibroblast differentiation[41, 42] and myofibroblast activation.[44] Regulation of YAP/TAZ signaling through RhoA and ROCK, in a similar manner to MRTF/SRF signaling, may also occur through $G\alpha_{12/13}$. Yu et al. (2012) showed that overexpression of active $G\alpha_{12/13}$ led to YAP dephosphorylation while knockdown of $G\alpha_{12/13}$ blocked LPA-mediated YAP dephosphorylation.[45] Moreover, use of dominant negative mutants of RhoA blocked the LPA-mediated YAP dephosphorylation while constitutively active mutants of RhoA resulted in YAP dephosphorylation.[45] Hence, ROCK may act as an inhibitor of LATS1/2 kinase activity downstream of $G\alpha_{12/13}$. While the precise mechanisms mediating ROCK's regulation of LATS1/2 remains undetermined, a recently published paper by Ibar et al. (2018) suggested that LIMD1, a member of the Ajuba family proteins known to associate with LATS kinases, is required for Rho-mediated regulation of LATS1/2 and YAP.[46]

While ROCK itself may regulate cellular cytodynamics to influence YAP/TAZ signaling, it is possible that changes in cellular actomyosin dynamics themselves may regulate ROCK in a feed forward loop or affect YAP/TAZ signaling through mechanisms independent of ROCK.[36, 42] Indeed, it may be difficult to divorce the effect of changes in myosin-actin dynamics and ROCK function on YAP/TAZ signaling from the effect of each on the other. One study suggests that in human glioblastoma cells, YAP dephosphorylation and activation was abrogated by RhoA inhibition but not affected by the use of Y-27632.[32] Moreover, the same study suggested that YAP dephosphorylation could be achieved through the use of cytochalasin D, an inhibitor of actin polymerization.[32] Consequently, while most studies appear to indicate that the Rho/ROCK signaling pathway is a robust regulator of YAP/TAZ/TEAD signaling and, in turn, its pro-fibrotic target genes, conflicting reports of ROCK on YAP phosphorylation may indicate that further mechanistic studies are necessary to clarify how ROCK governs LATS kinase activity and subsequent YAP phosphorylation.

3.3 TGF- β signaling

The cytokine TGF- β is an important mediator of all aspects of cardiovascular fibrosis.[47] TGF- β is composed of three isoforms (TGF- β 1, TGF- β 2, and TGF- β 3) and all three can bind to TGF- β receptor 2 (TGFR2) as homodimers to recruit and activate TGF- β receptor 1. In the canonical signaling pathway, the binding of TGF- β to its receptors leads to the phosphorylation and activation of Smad2 and Smad3, which complex with Smad4 to translocate to the nucleus. The Smad3 portion of the enzyme complex binds directly with promoters to induce transcription of well-characterized pro-fibrotic agents, including

collagen I,[48] tissue inhibitor of matrix metalloproteinases (TIMPs),[49] α -smooth muscle actin (α -SMA),[50] and PAI-1.[51]

ROCK is involved in both the canonical TGF- β signaling pathway and in its non-canonical pathways. ROCK's role in the canonical TGF- β pathway was first identified by Kamaraju et al. (2005).[52] In the canonical pathway, transfection of dominant negative RhoA or the use of ROCK inhibitors prevented the phosphorylation of Smad2 and Smad3 on its TGF- β -regulated linker region,[52] inhibited Smad nuclear translocation, and decreased Smad-binding element promoter activity.[53, 54] Moreover, use of Y-27632 and statins, well-characterized ROCK inhibitors, have been shown to inhibit Smad2 expression and TGF- β -mediated synthesis of type I collagen mRNA in fibroblasts and epithelial cells.[55, 56] Consequently, ROCK may be an important regulator of TGF- β -mediated SMAD signaling.

Outside of the canonical TGF- β signaling pathway, numerous studies have shown ROCK activity is increased in response to TGF- β stimulation, which may mediate the expression of pro-fibrotic genes.[57] Indeed, the increase in ROCK activity in response to TGF- β may cause polymerization of F-actin to drive MRTF/SRF-dependent myofibroblast activation and differentiation.[58] Intriguingly, the activation of latent TGF- β by the α v β 6 integrin via the effect of thrombin on protease activated receptor 1 is ROCK-dependent.[58] These studies are supported by reports indicating that use of TGF- β /Smad inhibitors can downregulate RhoA and ROCK1 expression, suggestive of crosstalk between the two pathways.[56] Thus, ROCK may regulate the canonical TGF- β pathway and also mediate non-canonical TGF- β pathways both in the activation of and downstream of TGF- β .

4. OTHER CELLULAR TARGETS OF ROCK IN CARDIOVASCULAR FIBROSIS

4.1 T helper cells

Inflammation is an important etiology of fibrosis. Prolonged or acute tissue damage and chronic diseases in the cardiovascular system (e.g. hypertension) result in activation of immune cells that in turn release cytokines that induce the activation and differentiation of fibroblasts. In this section of the review, we cover how ROCK may mediate pro-fibrotic cytokine release from CD4⁺ T helper (T_h) cells, including from T_h2, T_h1, and T_h17 cells.

T_h cells are important mediators of the adaptive immune response and produce distinct repertoires of cytokines that are critical for stimulating tissue repair and fibrosis. For instance: T_h2 releases three pro-fibrotic cytokines, IL-4, IL-5, and IL-13; T_h1 releases anti-fibrotic IFN- γ and IL-12; T_h17 releases pro-fibrotic IL-17. ROCK appears to mediate the expression or release of many of these cytokines. For example, in ROCK1 haplo-insufficient mice, IL-13 and IL-5 were significantly decreased[59] while the use of Y-27632, a potent pan ROCK inhibitor, attenuated expression or promoter activity of IL-4, IL-6, and IL-13.[60] Furthermore, use of Y-27632 appeared to increase expression of IFN- γ [61] and IL-12.[62] Finally, administration of ROCK inhibitors Y-27632 and fasudil in mice[63] and the ROCK2-specific inhibitor KD025 in humans[64] decreased production of IL-17. As each of these inflammatory cytokines has been linked with cardiac fibrosis,[65–67] ROCK likely

plays an additional role in the regulation of cardiac fibrosis via inhibition of anti-fibrotic or upregulation of pro-fibrotic cytokines. However, despite the importance of ROCK in the production or upregulation of a broad array of cytokines, the precise molecular mechanisms by which ROCK performs these actions remain to be determined and remains a subject of active research.

ROCK may also mediate inflammatory cytokine pathways through regulation of JAK/STAT signaling. Binding of cytokines from the interleukin (IL-1 through IL-31) or interferon (e.g. IFN- γ) families to their cognate receptors leads to receptor aggregation and recruitment of two JAK molecules in approximate proximity. This results in trans-phosphorylation of JAK, augmenting their kinase activities to phosphorylate tyrosine residues on their cognate receptors. These phosphorylated tyrosine residues can subsequently serve as docking sites for numerous signaling and adaptor proteins, among which the most studied are the STAT proteins. The JAK-receptor complex can phosphorylate tyrosine residues on STAT, leading to homo/heterodimerization of two STAT proteins and consequent translocation to the nucleus to activate pro-inflammatory or pro-fibrotic genes. Several recent papers have highlighted a potential crosstalk between the Rho/ROCK and JAK/STAT pathways, where ROCK appears to modulate phosphorylation of various JAK and STAT proteins. For example, ROCK1 may be able to directly phosphorylate JAK2 in certain cell types, leading to phosphorylation and activation of STAT3 and FOXO1.[68] Interestingly, in T cells, ROCK2 has been linked to dephosphorylation of STAT3 but phosphorylation of STAT5 to regulate cytokine production.[64] It remains to be seen, however, how exactly ROCK increases phosphorylation of JAK and STAT (e.g. if ROCK modulates STAT phosphorylation directly or through regulation of JAK) and how cross-talk between Rho/ROCK and JAK/STAT may mediate cardiovascular fibrosis.

4.2 Endothelial cells

The vascular endothelium is an important endocrine organ that controls vascular tone through the release of vasoconstrictive or vasodilating factors. Moreover, the endothelium is a major mediator of inflammation, angiogenesis, and fibrosis through the upregulation of cell adhesion molecules and secretion of a variety of growth factors, including vascular endothelial growth factors (VEGFs) and fibroblast growth factors (FGFs). Principal to the modulation of these endothelial functions is the synthesis and release of endothelial-derived nitric oxide (NO). Indeed, endothelial dysfunction is characterized by reduced NO bioavailability. In the myocardium, decreased NO production by coronary endothelial cells has been linked with vascular stiffness,[69] ventricular remodeling,[70] and myocardial fibrosis [71, 72]. Moreover, studies have shown that inhibition of endothelial nitric oxide synthase (eNOS) induces and enhances cardiac fibrosis through stromal-derived-factor-1/C-X-C chemokine receptor type 4-mediated upregulation of ECM production in cardiac fibroblasts and can lead to apoptosis of both cardiac fibroblasts and cardiomyocytes.[71] In the vasculature, arterial contraction is regulated by nitric oxide through the direct effect of NO on vascular smooth muscle cells (VSMCs). Thus, endothelial dysfunction may lead to cardiac fibrosis and vessel stiffening through depressed NO production.

ROCK was first reported as a negative regulator of eNOS and endothelial NO production in 2001 and 2002 by several groups.[73–75] Follow-up studies indicated that the use of ROCK inhibitors, such as Y-27632 and fasudil, dominant negative mutants of ROCK, and ROCK knockout mice increase eNOS mRNA half-life and expression.[76] Aside from transcriptional regulation of eNOS, ROCK may directly inhibit phosphorylation of eNOS through inhibition of the PI3K/Akt signaling pathway, thus altering eNOS activity.[77] The Rho/ROCK pathway and the PI3K/Akt pathway may intersect at PTEN, a phosphatase that dephosphorylates phosphoinositide (PIP) substrates.[77] PTEN acts in opposition to PI3K-mediated Akt signaling by dephosphorylating PIP 3,4,5-triphosphate (PIP3) to generate PIP 4,5-bisphosphate (PIP2), consequently inhibiting the ability of Akt to associate with the plasma membrane through its Pleckstrin-homology domain. Moreover, ROCK phosphorylation of PTEN appears to be necessary for the phospholipid phosphatase activity of PTEN.[78] Consequently, ROCK may regulate eNOS at the level of expression and activity, thus governing an important aspect of endothelial function-mediated fibrosis.

ROCK has also been shown to be involved in the endothelial-to-mesenchymal transition (EndMT), which is a form of epithelial-to-mesenchymal transition that primarily occurs during embryonic development of the myocardium and is hypothesized to play a role in the progression of cardiac fibrosis.[79] For instance, the regulation of the cytoskeleton through Rho/ROCK signaling is a necessary component of cytoskeletal rearrangements and migration that allows the cell to delaminate from the endothelial layer to induce EndMT.[80–82] In these experiments, it appears that ROCK inhibitors prevent EndMT while upregulation of ROCK increases EndMT. Moreover, important signaling pathways orchestrating this arrangement in cell structure include the TGF- β signaling pathways, such as the ones involving ROCK.[79] Additional pathways include the transcription of Snail, Slug, and Twist, which are vital genes in EndMT,[79] the downstream effects of which also appear to be mediated by ROCK.[83] It is important to note that while a few papers have suggested EndMT is involved in cardiac fibrosis in adult animals, other studies have suggested that de novo EndMT plays but a minor role in adult cardiac fibrosis.[84] While ROCK plays an important role in EndMT, further studies are necessary to clarify the role of EndMT in cardiac fibrosis and how ROCK may modulate EndMT-induced cardiac fibrosis.

4.3 Vascular smooth muscle cells

ROCK may regulate arterial stiffness through VSMC-ECM interactions, VSMC plasticity, and vascular tone. In particular, mechanical homeostasis between VSMCs and ECM proteins is a crucial element of arterial stiffness.[85] Cellular-ECM interactions involve collagen and elastin proteins in the ECM, adhesion proteins, and crucially, integrins which link at focal adhesion (FA) sites and the accompanying integrin linker proteins that associate with the actin-myosin cytoskeleton and GPCRs.[86] FAs are involved in inside-out or outside-in signaling, defined as the transmission of internal cell contractile forces outside the cell or the transmission of external mechanical forces to cause intracellular changes, respectively. For instance, the binding of linker proteins, such as talin, to the β -cytoplasmic tail of the integrin alters the conformation of the integrin in a form that allows strong binding with ECM proteins.[86] In contrast, binding of talin to actin filaments, such as G-actin, via vinculin promotes nascent FA complexes[86] in a process that appears to be mediated by ROCK's

effects on FA turnover.[87] Depending on the distribution of ECM proteins and the clustering of integrins and recruitment of additional adhesion proteins to the FA, signal transduction through ROCK may alter actin polymerization and MLC phosphorylation to induce changes in arterial stiffness.[86, 88] Thus, ROCK plays a vital role in VSMC-ECM interactions and arterial stiffness through the formation of FAs and stress fiber formation.

VSMC plasticity refers to the ability of smooth muscle cells to switch from a quiescent, contractile phenotype to a migratory, proliferative phenotype that accompanies ECM remodeling and arterial stiffness.[86] Protein markers for the contractile phenotype include α -SMA, myosin heavy chain, calponin, h-caldesmon, desmin, and smoothelin.[86] Along with increased expression of contractile molecular markers, studies have indicated that there is an increase in the number caveolae and their associated proteins, caveolin-1 and caveolin-2, in the contractile phenotype.[89] Intriguingly, RhoA and ROCK possess a conserved caveolin-binding domain that can putatively interact with the caveolin-scaffolding domain of caveolin-1.[90, 91] Indeed, ROCK appears to phosphorylate caveolin-1 to regulate FA dynamics.[87] Consequently, caveolin may link smooth muscle plasticity, VSMC-EC interactions, and the Rho/ROCK pathway to regulate arterial stiffness. A variety of other mechanisms governing VSMC phenotype switching may also be mediated by ROCK, either through the direct actions of ROCK on MRTF/SRF-mediated transcription of VSMC contractile genes or through other ROCK-mediated pathways (such as Smad and PTEN/PI3K/AKT).[92]

Vascular tone is the intrinsic level of vessel vasoconstriction and compliance and is modulated by the position of the VSMC within the cellular milieu, its interactions with ECM proteins, and through signaling pathways involved with VSMC constriction.[86] ROCK plays a direct role in controlling vascular tone through its actions on cellular contractility, as previously detailed in this review. For instance, ROCK phosphorylates MLC, phosphoinhibits MYPT1 of myosin light chain phosphatase, and controls eNOS expression and activity in order to regulate vasoconstriction.

4.4 Macrophages

Macrophages play central roles in wound healing and fibrosis. As examples, macrophages produce matrix metalloproteinases, cytokines, growth factors, and other inflammatory mediators that contribute to the initial cellular response after injury. Indeed, depletion of macrophages early in the inflammatory cascade can blunt the inflammatory response to injury, leading to reduced scarring and fewer myofibroblasts.[93] In contrast, removal of certain populations of macrophages can also result in an opposing response, such as less efficient repair and more fibrosis.[93] Consequently, intense research over the past decade into macrophages has focused on identifying the various macrophage populations that regulate the stages of cellular response to injury. Two major macrophage phenotypes are broadly identified as M1 (pro-inflammatory) and M2 (pro-regeneration and pro-fibrotic). The study of ROCK regulation of macrophage polarity is recent: Zandi et al. (2015) suggests that ROCK2 inhibition in a murine model or through ROCK pharmacological inhibition may decrease M2-like macrophages and upregulate M1 markers.[94] Liu et al. (2013) suggests the opposite: that ROCK inhibition with fasudil may instead shift macrophages to the M2

phenotype.[95] Lee et al. (2018) supports the later: that nicorandil, a drug able to activate protein kinase G (PKG) to phosphoinhibit RhoA and ROCK, or fasudil can polarize macrophages to M2, leading to an attenuation of myofibroblast-mediated cardiac fibrosis. [96] Despite these reports, the molecular role in which ROCK plays in macrophage polarization remains unclear, but may be due to ROCK-mediated regulation of JAK/STAT signaling[68, 97] or ROCK's actions on IRF4,[63] a well characterized effector in M2 polarization.[98]

5. ROLE OF ROCK IN FIBROSIS- OR STIFFENING-RELATED CARDIOVASCULAR DISEASES

5.1 Vascular stiffness and hypertension

The above signaling pathways and secretory molecules in endothelial cells and vascular smooth muscle cells suggest that ROCK plays a principal role in vascular stiffness. As a significant body of literature shows that vascular stiffness plays a causal or associative role in hypertension, for instance, in longitudinal analyses of patients (as measured by pulse wave velocity [PWV])[99] or in mouse models[100] that indicate arterial stiffening precedes hypertension, it is possible that ROCK also plays a pivotal role in hypertension. Indeed, numerous studies have connected ROCK with aortic stiffness and hypertension in the clinical setting and in murine models. For instance, Noma et al. (2007), by measuring carotid-femoral PWV in patients, linked ROCK activity with aortic stiffness and aging.[101] Moreover, a plethora of seminal studies on the effects of ROCK inhibition in mice have shown that inhibition of ROCK in mice, either through Y-27632 or fasudil, reduce blood pressure and attenuate endothelial dysfunction.[76, 102] The use of ROCK inhibitors in small clinical trials also reduce pulmonary hypertension.[103] Altogether, these lines of evidence suggest that ROCK is an important mediator of arterial stiffness and in the subsequent development of hypertension and that use of ROCK inhibitors in humans may be a potential therapeutic avenue for alleviating various forms of hypertension.

5.2 Heart Failure: HFpEF, HFrEF, and cardiomyopathies

Research into heart failure has led to widespread acknowledgement that myocardial fibrosis and cardiac hypertrophy play major roles in both HFpEF and heart failure with reduced ejection fraction (HFrEF) and various types of cardiomyopathies.[104] As with all forms of fibrosis, myocardial fibrosis is characterized histologically by excess deposition of fibrous tissue (e.g. collagen I and II) and can be induced by cardiomyocyte death or by mechanical, ischemic, or metabolic injuries to the myocardium.[104] Furthermore, accumulation of fibrosis in the myocardium can cause structural changes that lead to increased left ventricular end-diastolic pressure, impairment of left ventricular compliance and relaxation, reduced ejection fraction, and cardiomyocyte hypertrophy.[104] As diastolic dysfunction is a common etiology of HFpEF and cardiomyocyte hypertrophy and interstitial fibrosis are associated with hypertrophic and dilated cardiomyopathies, myocardial fibrosis plays an important role in mediating the development of HFpEF, HFrEF, and various cardiomyopathies.

Consequently, the importance of ROCK in heart failure has been largely explored through its role in cardiac fibrosis. Early studies using pan ROCK inhibitors such as Y-27632 and fasudil limited the progression of adverse cardiac remodeling and fibrosis after angiotensin II (AngII) and NG-nitro-L-arginine methyl ester (L-NAME, an inhibitor of nitric oxide synthase) treatment and after myocardial infarction.[70] Subsequent studies using ROCK1^{+/-} and ROCK1^{-/-} mice showed that ROCK downregulation decreases cardiac fibrosis, left ventricular dilation, and contractile dysfunction after treatment with AngII or after myocardial infarction.[15] More recent studies have focused on cell-specific and isoform-specific knockouts of ROCK to more precisely evaluate the role of ROCK in heart failure. Sunamura et al. (2018) suggested that deletion of ROCK1 in cardiomyocytes may decrease cardiac fibrosis, MMP activity, and ECM remodeling in response to pressure overload, albeit deletion of ROCK2 had no effect on cardiac fibrosis.[19] In contrast, another study suggested that global ROCK2^{+/-} and cardiomyocyte-specific ROCK2-deficient mice demonstrated less cardiomyocyte hypertrophy and apoptosis and cardiac fibrosis, which may be related to upregulation of four-and-a-half LIM-only protein 2 (FHL2) and FHL2-mediated inhibition of SRF and extracellular signal-regulated mitogen-activated protein kinase (ERK).[105] Shimizu et al. (2017) also suggested that ROCK2 may play a role in heart failure: ROCK2-specific deletion in fibroblasts attenuates cardiac hypertrophy, fibrosis, and diastolic dysfunction through inhibition of CTGF and FGF production.[20] Furthermore, Shi et al. (2019) reports that disruption of ROCK1 and ROCK2 in cardiomyocytes can promote autophagy to inhibit cardiac fibrotic remodeling, albeit ablation of only ROCK2 in cardiomyocytes led to *more* fibrosis.[18] Interestingly, the same study suggested that knocking out ROCK2 can lead to a compensatory increase in ROCK1 activity, which may explain these results. Altogether, these recent studies support earlier *in vitro* results that suggest ROCK1 and ROCK2 in fibroblasts and cardiomyocytes play central roles in cardiac fibrosis underlying heart failure and hypertrophic and dilated cardiomyopathies.

Additional *in vivo* evidence that ROCK-mediated fibrosis may be involved or be modulated to improve heart failure may come from pre-clinical and clinical trials of LCZ696, which is a drug composed of an angiotensin receptor blocker (valsartan) and a neprilysin inhibitor (sacubitril). In the PARADIM-HF trial published in 2014, LCZ696 was shown to offer an “overwhelming benefit” over standard therapy of care in patients with HFrEF and New York Heart Association Class II, III, and IV heart failure symptoms by a median follow-up of 27 months.[106] A follow-up pre-clinical trial attempting to elucidate the mechanisms of LCZ696 in 2019 suggested that valsartan/sacubitril can reduce pathological accumulation and activation of cardiac fibroblasts both *in vitro* and *in vivo* through protein kinase G-dependent inhibition of RhoA at Ser¹⁸⁸. [107] As the clinical efficacy of LCZ696 cannot be fully explained by a reduction in systemic blood pressure or increased atrial natriuretic peptide levels,[107] ROCK may play an important role in the major clinical benefits seen with LCZ696. Follow-up clinical studies, perhaps assaying for changes in ROCK activity in patients taking LCZ696, may provide more direct evidence for ROCK-mediated benefits in cardiovascular fibrosis in patients with HFrEF.

5.3 Cardiac Arrhythmias: Atrial fibrillation and ventricular tachycardia

Cardiomyocytes can propagate electrical signals to adjacent cells through connexin channels that connect the cytoplasm of cardiomyocytes.[108] Abrogation of this cell-to-cell contact, for instance, through cardiac remodeling or increased cardiac fibrosis, is arrhythmogenic and can result in atrial fibrillation (AF) and ventricular tachycardia (VT). This is supported by studies showing that in patients with hypertrophic cardiomyopathy, cardiac fibrosis as measured by late gadolinium enhancement cardiovascular magnetic resonance is an independent predictor for VT.[109] Moreover, increased cardiac fibrosis may limit the efficacy of catheter ablation of AF or VT.[110] Thus, therapies improving the effectiveness of AF or VT ablation or by lessening the presence of AF and VT may involve anti-fibrotic agents.

A few mouse models have suggested that ROCK inhibition may be cardioprotective against ventricular arrhythmias [111] and that ROCK may mediate atrial remodeling and fibrosis prior to AF.[112] However, it is doubtful that these anti-arrhythmogenic effects are mediated solely through the actions of ROCK on fibrosis. Ellawindy et al. (2015) reports that overexpression of a dominant-negative ROCK in the developing heart (SM22 α or α MHC-restricted) leads to spontaneous development of ventricular arrhythmias.[111] There was indeed increased fibrosis in both the left and right ventricles as early as postnatal day 3, which progressed to involve the entire right ventricle free wall in some areas of the ventricle by week 19.[111] Nonetheless, given that there were also desmosomal abnormalities, it is likely that arrhythmias through ROCK inhibition are not solely due to ROCK's role in reducing cardiac fibrosis, but also due to its role in regulating cell junctions.

5.4 Myocardial infarction

The pro-fibrotic response after myocardial infarction, such as the production of ECM proteins by activated fibroblasts after injury, is important in the pathophysiology of heart failure and arrhythmias.[113] The fibrosis induced post-myocardial infarction can be broadly classified into two types: (1) replacement fibrosis (i.e. scar tissue), which can may initially provide structural support to the injured myocardium but eventually may lead to maladaptive alterations to myocardial mechanics that increases the amount and phenotype of collagen fibers in areas remote from the initial infarction[114] and (2) reactive fibrosis, which refers to the increase in ECM proteins synthesized by fibroblasts at the site of injury and in the infarct border zone.[1]

Inhibition of ROCK through pharmacological inhibition or through mouse models has indicated that ROCK plays an important role in governing the extent of the infarct after acute myocardial injury. For instance, pre-treatment of mice with Y-27632 before coronary artery occlusion led to a large reduction in infarct size, attenuation of the deterioration in cardiac function, and a decrease in cardiomyocyte apoptosis.[115, 116] The benefits of ROCK inhibition after myocardial infarction appears to be partially mediated by upregulation of the PI3K/AKT/NO pathway[115] and leads to significantly lower cardiac fibrosis.[116] Consequently, the reduction of infarct size through ROCK inhibition may prevent maladaptive increases in collagen synthesis due to abnormalities in myocardial mechanics as well as decrease fibrosis proximal to the site of the injury itself.

6. CONCLUSION

ROCK is an important regulator of cardiovascular fibrosis through its effects on fibroblasts, T_h cells, endothelial cells, vascular smooth muscle cells, and macrophages (Figure 4). From its pro-fibrotic role in these cell types, ROCK may mediate fibrosis and stiffening underlying vascular stiffness, hypertension, heart failure, cardiac arrhythmias, and recovery after myocardial infarction. Further clinical studies, however, are needed to validate ROCK as a therapeutic target for cardiac fibrosis and vascular stiffening.

7. EXPERT OPINION

The diverse role of ROCK in mediating cardiovascular fibrosis identifies the kinase as an important therapeutic target in cardiovascular disease, suggesting the need for future clinical trials that determine the efficacy of ROCK inhibitors in limiting cardiac fibrosis or vascular stiffening in the cardiovascular system. In the absence of direct clinical trials on the effect of ROCK inhibitors on cardiac fibrosis, the benefits may perhaps be ascertained by statin clinical trials through the so-called “pleiotropic effects of statins.” Statins inhibit HMG-CoA reductase, preventing downstream synthesis of isoprenoids such as farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP). The isoprenylation, or addition of hydrophobic molecules such as FPP or GGPP, of small Rho GTPases (including RhoA) facilitates their localization, activation, and function. Consequently, statin trials may provide insight into RhoA/ROCK inhibition if some of the beneficial effects of statins are lipid-independent. Indeed, evidence of the benefit of ROCK inhibitors on cardiac fibrosis may come from statin trials on heart failure. For instance, in a large meta-analysis of both published and non-published data from statin trials, Preiss et al. suggested that statins reduce non-fatal heart failure hospitalization and heart failure death.[117] Moreover, this reduction was not associated with reduced risk of non-fatal myocardial infarction or LDL-C levels, suggesting that the beneficial effects of statins in heart failure occurs through a mechanism not related to LDL-C lowering. Furthermore, a few small clinical trials on the use of ROCK inhibitors in humans have supported findings from animal studies, describing a decrease in coronary vasoconstrictor responses to acetylcholine in patients with vasospastic angina[118], an improvement in endothelial dysfunction[119], and an improvement in diastolic dysfunction.[120] Despite these encouraging reports on the benefits of ROCK inhibition, more direct clinical studies on the role of ROCK in cardiovascular fibrosis are required to better characterize the potential benefits of ROCK as an anti-fibrotic therapeutic target in humans.

It is important to note that the majority of preclinical and clinical studies with ROCK inhibitors use isoform-non-specific inhibitors such as fasudil or Y-27632. Due to the importance of ROCK in cytoskeleton dynamics and its expansive roles in other signaling pathways, use of pan-ROCK inhibitors may lead to cellular toxicity and other adverse effects. Indeed, targeting proteins both upstream and downstream of ROCK, such as by LCZ696's targeting of PKG, or the abundance of phase II clinical trials that target IL-6 or IL-6 receptors to reduce fibrotic inflammation,[121] may provide simpler therapeutic modalities than targeting ROCK itself. In addition, an emerging body of literature suggests that ROCK1 and ROCK2 may play varying fibrotic roles dependent on cell type, and indeed,

may play opposing roles in certain tissues such as macrophages and cardiomyocytes.[18, 19, 94] Consequently, future studies may need to highlight the importance of each ROCK isoform in specific tissues, possibly through continued experiments on tissue- and isoform-specific knockout or haploinsufficient murine models. Moreover, in order to facilitate translation to the clinical setting, isoform-specific inhibitors should be developed and better characterized.

The breadth of pre-clinical reports on ROCK inhibition as a potential therapeutic strategy in cardiovascular disease has led to the development of a large number of ROCK inhibitors over the past three decades. The classic ROCK inhibitor for clinical use is fasudil, which has been approved in Japan and China since 1995 for treatment of cerebral vasospasm, a potentially fatal result of subarachnoid hemorrhage.[122] Despite only moderate specificity for ROCK (K_i of ~0.33 μ M towards ROCK2) and off-target inhibition of other kinases, including protein kinase A and other members of the AGC kinase family,[123] subsequent follow-up trials in thousands of patients have suggested that fasudil is relatively safe, notwithstanding adverse events such as changes in hepatic function and hypotension. [122, 124, 125] In the context of cardiac fibrosis and vascular stiffening, small clinical trials in the early to mid-2000s provided evidence that fasudil can improve symptoms and outcomes for patients with systemic hypertension[126] and chronic heart failure.[127] Moreover, a recent clinical trial published in 2015 suggested that fasudil can improve outcomes in patients with severe hypertension and right heart failure[128] while a trial in 2018 indicated that fasudil can improve diastolic function in HFpEF patients.[120] Both trials suggested that fasudil was well-tolerated by patients, although the long-term effects of fasudil administration is still unknown. Nonetheless, perhaps due to fasudil's lack of specificity for ROCK and the potential for ROCK isoform-specific inhibitors to offer greater therapeutic benefit and less side effects than fasudil, research over the last decade has also focused on the creation of hundreds of new ROCK inhibitors, which have been covered in exhaustive detail by Feng et al. (2015).[123] An important ROCK isoform-specific inhibitor is KD025, a specific ROCK2 inhibitor (IC₅₀ values of 60–110 nM for ROCK2 and >20 μ M for ROCK1) [64] undergoing phase II clinical trials for various immune-related diseases and for idiopathic pulmonary fibrosis. A phase I clinical trial using KD025 in a small number of patients suggested that there are few to no side effects from taking the drug.[64] However, the effect of many of these inhibitors, including KD025, in cardiac fibrosis and vascular stiffening remain largely unknown and remain firmly in the preclinical and discovery phase of research.

Another avenue of ROCK clinical research has been through measurements of the ratio of phosphorylated MYPT1 to total MYPT1, a direct downstream effector of ROCK, in peripheral blood leukocytes as an easily obtainable readout for ROCK activity. For example, patients with left ventricular hypertrophy [129] or congestive heart failure [130, 131] diseases associated with myocardial fibrosis, have elevated ROCK activity compared with corresponding controls. These studies may allow clinicians to use ROCK activity as a biomarker for prognostic outcomes or may allow future clinical trials to administer ROCK therapy to those with elevated ROCK activity. There are a few drawbacks to using ROCK activity from peripheral blood leukocytes. First, activation of ROCK in peripheral blood leukocytes may not necessarily correlate with activation of ROCK in cardiac fibroblasts.

Studies, however, have indicated that the ROCK activity of circulating leukocytes does indeed closely correlate with the ROCK activity of tissue extracted from the left ventricle and aortic wall.[132] Intriguingly, in the same study, fasudil also decreased the ROCK activity from all three protein types.[132] Second, the ROCK activity of peripheral blood leukocytes may be altered by inflammation or damage in other organs. Future studies may seek to validate the use of ROCK activity in peripheral blood leukocytes as a readout of cardiac (or systemic) ROCK activity, perhaps through biopsies in patients undergoing heart transplantation.

In conclusion, we believe that ROCK is an important regulator of cardiovascular fibrosis. Future studies characterizing the isoform- and tissue-specific roles of ROCK are required to fully validate the kinase as a clinical target to reduce cardiovascular fibrosis. Nonetheless, the last two decades of ROCK research has greatly informed our understanding of the pro-fibrotic cellular mechanisms underlying cardiovascular disease. Future clinical trials on the use of ROCK inhibitors to decrease cardiac fibrosis or improve vascular stiffening are required to fully assess ROCK as a therapeutic target.

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ARTICLE HIGHLIGHTS

- ROCK plays a key role in the regulation of fibrosis and stiffening in hypertension, heart failure, cardiomyopathies, arrhythmias, and recovery after myocardial infarction
- ROCK activates pro-fibrotic genes in fibroblasts and myofibroblasts through MRTF/SRF, YAP/TAZ/TEAD, and TGF- β signaling
- ROCK plays an important role in the release of both pro-fibrotic and anti-fibrotic cytokines from T_h cells and may also play a role in macrophage polarization
- ROCK regulates endothelial function, including NO production and EndMT, to cause cardiac fibrosis and stiffening
- ROCK governs VSMC-ECM dynamics, VSMC phenotype switching, and vascular tone
- Future clinical trials on the use of ROCK inhibitors to decrease cardiac fibrosis or improve vascular stiffening are required to fully assess ROCK as a therapeutic target.

Figure 1. Structure and activation of ROCK.

ROCK is composed of a N-terminal kinase domain followed by a coiled-coil region. The coiled-coil region of ROCK contains the Rho-binding domain (RBD) and is followed by a Pleckstrin-homology (Ph) domain and a C-terminal cysteine rich domain (CRD). The RBD and Ph domain associate independently with the N-terminal kinase domain for ROCK autoinhibition. The binding of RhoA to the RBD disrupts the autoinhibition of ROCK, causing its unfolding and activation. Cleavage of ROCK by caspase-3 and granzyme B can also activate ROCK independent of RhoA.

Figure 2. Involvement of ROCK in MRTF/SRF signaling.

The binding of G-actin to myocardin-related transcription factors (MRTFs) sequesters MRTFs in the cytosol. The polymerization of G-actin to F-actin liberates MRTFs to enter the nucleus and bind serum response factors (SRFs) to induce transcription of pro-fibrotic genes. A variety of upstream activators, including lysophosphatidic acid (LPA) and extracellular matrix (ECM) stiffening, increase Rho-kinase activity to enhance G-actin to F-actin polymerization, thus enhancing the translocation of MRTFs into the nucleus.

Figure 3. Involvement of ROCK in YAP/TAZ/TEAD signaling.

Yes-associated protein (YAP) and transcriptional coactivator with a PDZ-binding domain (TAZ) are retained in the cytoplasm by the phosphorylation of YAP and TAZ by large tumor suppressor (LATS) kinases, mediated by 14-3-3. The activation of ROCK by upstream G-proteins, including lysophosphatidic acid receptor-1 (LPA1), and by extracellular matrix (ECM) stiffening leads to the polymerization of actin stress fibers that increases YAP/TAZ nuclear translocation and transcription of pro-fibrotic genes. How ROCK regulate LATS

kinases and YAP/TAZ translocation is not well characterized, although may involve capping proteins such as cofilin.

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Figure 4. Targets of ROCK in cardiac fibrosis and stiffening.

The cellular targets of ROCK in fibrosis and stiffening include T helper cells, vascular smooth muscle cells, fibroblasts, endothelial cells, and macrophages. Subsequently, through the role of ROCK on these cellular targets, ROCK may mediate many cardiovascular diseases associated with pathological fibrosis and stiffening, including hypertension, heart failure, and arrhythmias.