

Rock the Rock of Atherosclerosis

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Abstract

The Rho-Associated Coiled-Coil Containing Protein Kinase (ROCK1 and ROCK2) were one of the downstream effectors of the small GTPase Rho. The Rho/ROCK pathway plays an important role in mediating multiple cellular processes, including endothelial dysfunction, the proliferation and migration of smooth muscle cells, foam cell formation, and arterial stiffness and aging, all of which are involved in the pathogenesis of atherosclerosis. Vascular cells (including endothelial cells, smooth muscle cells, and macrophages) undergo pathophysiological changes through the ROCK signaling pathway and ROCK inhibitors are being developed as effective therapeutic agents for atherosclerotic cardiovascular diseases. However, it is not entirely clear how ROCK isoforms are regulated, and how both isoforms contribute to the pathogenesis of atherosclerosis. A recent article from Liao's laboratory demonstrated that deletion of the ROCK2 allele in BM-derived cells attenuates plaque formation in cholesterol-fed LDLr^{-/-} mice. Mechanistically, ROCK2 deletion decreases foam cell formation (the hallmark of atherosclerosis) by facilitating Reverse Cholesterol Transport (RCT) in macrophages, through peroxisome proliferator-activated receptor- γ /liver X receptor- α /ATP-binding cassette transporter A1 pathway. This study provides further mechanistic insight into the therapeutic benefits of ROCK2 inhibition in preventing the development of atherosclerosis.

Keywords: Rho-Associated Coiled-Coil Containing Protein Kinase (ROCK); Foam cell; Reverse cholesterol transport

Introduction

Atherosclerosis is a chronic inflammatory disease, and also characterized by both the innate and the adaptive immune responses [1]. Lipid (cholesterol) retention is a classic hypothesis of atherogenesis. In human body, the cholesterol homeostasis is finely tuned by the balance between lipid uptake and efflux by macrophages. The rapid and unregulated uptake of oxidized LDL (oxLDL) by scavenger receptors (such as scavenger receptor-A (SR-A), CD36, and lectin-like oxLDL receptor-1 (LOX-1)) contributes to monocyte-derived foam cells in atherosclerotic lesions [2]. Macrophages are also able to transport excessive cholesterol in peripheral tissues by cholesterol efflux or reverse cholesterol transport (RCT) pathways. This process is mediated by multiple cholesterol exporters such as the ATP-binding cassette transporters A1 (ABCA1), ABCG1, ABCG4, and SR- BI [3]. Targeting lipid uptake and/or cholesterol efflux represents an effective therapeutic strategy to influence the development and progression of atherosclerotic lesions. The Rho-Associated Coiled-Coil Containing Protein Kinase (ROCK) isoforms, ROCK1 and ROCK2, are protein serine/threonine kinases of 160 kDa, and are downstream effectors of the small GTPase Rho [4]. ROCK1 mRNA was ubiquitously expressed except in the brain and muscle, whereas ROCK2 mRNA was expressed abundantly in the brain, muscle, heart, lung and placenta [4]. Indeed, ROCK is up-regulated by inflammatory stimuli, such as angiotensin II and interleukin-1 β , lipopolysaccharide (LPS) and oxLDL [3,5]. More recently, there are several excellent reviews [5-8] addressing the role of Rho/ROCK pathway in mediating multiple cellular functions and the use of ROCK inhibitors in cardiovascular diseases. ROCK has been shown to be up-regulated in inflammatory atherosclerotic lesions and administration of a RhoA/ROCK inhibitor (Y-27632, 30 mg/kg/d, 9 weeks) significantly decreased early atherosclerotic lesion formation [9]. However, the roles of ROCK in more advanced stages of atherosclerosis (i.e., plaque rupture) as well as ROCK isoforms in the progression of atherosclerosis were not addressed in this study.

A previous study from Liao's laboratory showed that macrophage ROCK1 is involved in the pathogenesis for atherosclerosis [10]. Deletion of ROCK1 in bone marrow-derived cells is atheroprotective.

This was due, in part, to decreased chemotaxis, cholesterol uptake, and foam cell formation in ROCK1-deficient macrophages [10]. However, it remains to be determined whether and how ROCK2 is involved in atherosclerosis. In a recent issue of *Circulation*, Zhou et al. [3] shed light on how the ROCK2 cell signaling pathway may serve as a promising anti-atherosclerosis target, owing to its role in peroxisome proliferator-activated receptor- γ mediated reverse cholesterol transport (RCT), which alleviates foam cell formation. Over the last decade, cumulative evidence suggests that Rho/ROCK pathway is involved in many steps of the atherosclerotic process, including arterial stiffness and aging [11], endothelial dysfunction, migration and proliferation of smooth muscle cells, foam cell formation, and plaque destabilization (Figure 1). In this editorial, we aim to integrate current understanding of the pathophysiological role of ROCK in atherosclerosis.

ROCK and foam cell formation

The transformation of macrophages into foam cells represents a key cellular event in the development of atherosclerosis. Lipid accumulation and atherosclerotic lesions were reduced in atherosclerosis-prone LDLr^{-/-} mice, whose bone marrows have been replaced with bone marrows derived from ROCK1^{-/-} mice [10]. *In vitro*, ROCK1-deficient macrophages showed reduced uptake of fluorescent-labeled acetyl-LDL (AcLDL), which was not altered in ROCK2-deficient macrophages. Oil-red O staining analysis of the *en face* aorta and aortic sinus revealed that ROCK2^{-/-} Bone Marrow Transplantation (BMT) and ROCK2^{+/-} BMT mice developed substantially fewer atherosclerotic lesions than

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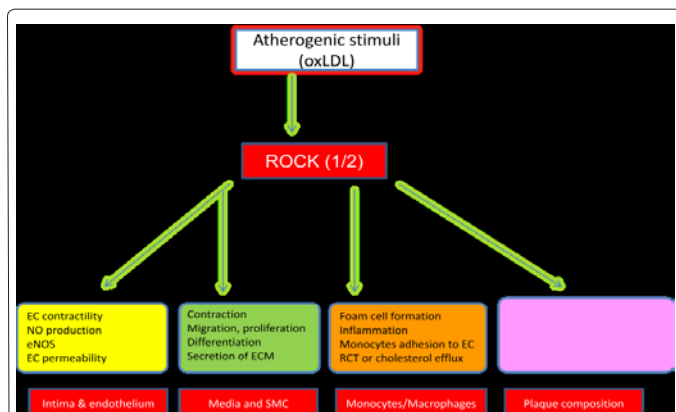


Figure 1: Schematic representation of ROCK in atherosclerosis. ROCK1 and ROCK2 are stimulated by atherogenic stimuli, such as oxLDL. ROCK activation leads to several cellular processes in vascular cells (endothelial cells, smooth muscle cell, and macrophages). The various pathophysiological changes include endothelial dysfunction, migration and angiogenesis, nitric oxide (NO) production contraction, migration, proliferation and differentiation in SMCs, vascular remodeling, neointimal formation and angiogenesis in adventitia. ROCK inhibitors (Y-27632, fasudil and statins) attenuate atherosclerosis by inhibiting ROCK1 and ROCK2.
 oxLDL: Oxidized LDL; LOX-1: Lectin-like oxLDL receptor-1; EC: Endothelial Cells; NO: Nitric Oxide; eNOS: endothelial NO Synthase; SMC: Smooth Muscle Cell; ECM: Extracellular Matrix; RCT: Reverse Cholesterol Transport; TIMP: Tissue Inhibitor of Metalloproteinases.

WT BMT mice, but ROCK2 knockout in BM-derived cells did not affect plasma cholesterol levels [3]. Surprisingly, the loss of ROCK2 did not affect cholesterol uptake in macrophages as evidenced by no significant difference in the protein expression of SR-A and CD36, two main scavenger receptors mediating cholesterol uptake [3]. Although no differences were observed in the uptake of modified LDL, foam cell formation in the presence of apoAI was reduced in macrophages from ROCK2^{-/-} BMT mice compared with those from WT BMT mice. Further mechanistic studies indicate that the decrease in foam cell formation in ROCK2^{-/-} BMT mice is primarily due to enhanced apoAI-dependent cholesterol efflux (via PPAR-γ/LXR-α/ABCA-1 pathway), concurrent with reduced cholesterol ester formation (by increasing the mRNA expression of neutral cholesterol ester hydrolase -1) [3].

ROCK and endothelial dysfunction

The Rho/ROCK signaling pathway is involved in the regulation of endothelial barrier function, inflammation, and transendothelial leukocyte migration [7]. Clinical studies have demonstrated a correlation between ROCK activity and endothelial dysfunction in patients with Coronary Artery Disease (CAD) [12]. Furthermore, treatment with the ROCK inhibitor fasudil reduced the over-activation of ROCK in patients with atherosclerosis (but not in healthy individuals) and improved endothelium-dependent vasodilation as well as flow-mediated dilation (FMD), a surrogate marker for endothelial function. It has been suggested that the pleiotropic effects of lipid-lowering statins are mediated, at least in part, by their inhibitory effects on Rho and the resultant inhibition of ROCK [8]. However, it remains to be determined to what extent clinical concentrations of statins inhibit Rho/ROCK activity, thereby, confers the beneficial effects of statins. In endothelial cells, Rho/ROCK activation was reported to play a role in oxLDL-induced endothelial cell contractility [13]. Increased endothelial permeability is important in atherosclerosis because of its synergistic role in facilitating intimal accumulation of both LDL and leukocytes [11]. More recently, Liu et al. [14] observed that, in subjects

with stable atherosclerosis, there was no correlation between ROCK activity and changes in Low-Density Lipoprotein Cholesterol (LDL-C) among patients randomized to rosuvastatin treatment. However, there was a correlation between ROCK inhibition and change in FMD among patients with rosuvastatin therapy. They also found that rosuvastatin therapy, at clinical doses used for lipid lowering, inhibits leukocyte ROCK activity, and improves endothelial function in patients with atherosclerosis. These findings provide clinical evidence that statins are effective in improving endothelium dysfunction by a cholesterol-independent mechanism in patients with atherosclerosis.

Mechanistically, Rho/ROCK negatively regulates endothelial function at the level of endothelial nitric oxide Synthase (eNOS) expression and activity. ROCK activation decreases the expression of eNOS by reducing eNOS mRNA stability post-transcriptionally [15]. Inhibition of ROCK prevents hypoxia-induced down regulation of eNOS [16]. In human endothelial cells, ROCK negatively regulates the phosphorylation of eNOS through inhibition of protein kinase B/Akt [17]. Moreover, inhibition of ROCK leads to a rapid phosphorylation and activation of Akt via the Phosphatidylinositol 3-Kinase (PI3K) pathway, leading to increase NO production [18]. Another mechanism is the possible regulation of ROCK by LOX-1, which has been identified as a primary scavenger receptor for oxLDL uptake by endothelial cells. A number of studies on LOX-1 have implicated its role in multiple cardiovascular diseases including atherosclerosis. Mattaliano et al. [19] recently demonstrated that, ROCK2 dynamically interacts with LOX-1 in the presence of oxLDL. In addition, oxLDL treatment stimulated ROCK2 catalytic activity, and ROCK2 inhibition (either by Y27632 or fasudil) attenuated oxLDL induced CXCL2 level and IL-8 secretion. ROCK activity is required for activation of p50 and p65 subunits of NF-κB by oxLDL. This evidence suggests that ROCK2 could be activated on oxLDL stimulation of LOX-1. However, it remains to be determined whether ROCK2 mediates the effect of LOX-1-dependent other pro-atherogenic signaling pathways in atherogenesis.

ROCK and SMC proliferation and migration

The Rho/ROCK pathway is master regulator of Smooth Muscle Cell (SMC) contraction, migration, proliferation, differentiation, apoptosis and survival, secretion of extracellular matrix (ECM), angiotensin II-induced expression of monocyte chemoattractant protein-1 (MCP-1) and plasminogen activator inhibitor type-1 (PAI-1) [5,9,20-22]. The *in vivo* evidence of ROCK in vascular inflammation and remodeling has been demonstrated in several studies. ROCK activity is increased in the neointima following balloon-induced vascular injury, which is suppressed by ROCK inhibitors or gene transfer of a dominant-negative mutant of ROCK [5]. In consistence with this finding, Noma et al. [23] observed that SMC proliferation was decreased in the neointima of ROCK1^{+/-} mice following carotid artery ligation, compared with that of WT or ROCK2^{+/-} mice. Furthermore, in L-NAME (an eNOS inhibitor) treated rats, ROCK inhibitors attenuate the inflammatory response and vascular remodeling [24]. It is also reported that exercise also decreased neointimal area and luminal stenosis in ApoE knockout mice [25], further substantiating the pivotal role of ROCK in SMC proliferation and migration following vascular injury.

ROCK and plaque instability

The main cause of cardiovascular morbidity and mortality is the rupture of Vulnerable Atherosclerotic Plaques (VAP), so stabilizing vulnerable plaques is becoming of increasing importance [26]. VAP typically consists of enlarged necrotic cores, elevated Matrix Metalloproteinases (MMPs) activities (which degrade components

of ECM in the fibrous cap), increased smooth muscle and collagen content, decreased inflammatory cell infiltration. The relationship of ROCK and plaque instability remains incomplete, even controversial. The expression of macrophages, smooth muscle cells and collagen in the plaque did not differ between the Y-27632 treated and saline treated control animals. However, the number of CD3-positive T lymphocytes per lesion area and expression of NF- κ B p65 subunit was reduced by Y-27632 [9]. However, the amount of macrophages in ROCK2^{-/-} BMT and ROCK2^{+/-} BMT mice was reduced significantly, compared with WT BMT mice. Masson trichrome staining of the aortic sinus revealed that there was less collagen deposition in ROCK2^{-/-} BMT and ROCK2^{+/-} BMT mice compared with WT BMT mice. However, the content of smooth muscle cells within plaque lesions show no difference. The expression of pro-fibrotic transforming growth factor- β (TGF- β) in atherosclerotic lesions was also reduced. In contrast, one report demonstrated that exercise alone and fasudil treatment alone also showed similar effects on plaque composition, but increased both SMC and macrophage density [25], raising the plaque-stabilizing effect of ROCK inhibitors controversial. It still remains to be determined that whether ROCK inhibition will affect the turnover of ECM in the fibrous cap (by influencing MMPs). The direct proof of ROCK in plaque rupture will be obtained from the treatment of patients with acute coronary syndrome with specific ROCK inhibitors.

Concluding remarks and future perspective

Accumulating experimental and clinical evidence indicates that the Rho/ROCK signaling pathway is critically involved in the pathogenesis of atherosclerotic diseases and that inhibition of ROCK by ROCK inhibitors (such as fasudil, Y-27632 and lipid-lowering statins) are beneficial [6,8,27]. In terms of the fact that ROCK1 and ROCK2 mediate different cellular processes, more efforts will be made to develop more-specific and more-potent ROCK inhibitors. Therefore, inhibition of ROCK may represent an attractive therapeutic target in the prevention and management of atherosclerotic diseases. However, there are still many unknowns, such as whether ROCK inhibitors also function to block the oxidation of LDL (the initiating event in atherosclerosis) to exert their atheroprotective effects? And whether scavenger receptor LOX-1 mediated the effects of ROCK on atherogenesis? Further studies are required to understand the precise cellular and molecular mechanisms of ROCK isoforms in atherosclerosis.

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