ROCK the Rock of Atherosclerosis

Suowen Xu*

Cardiovascular and Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, USA

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 (PPAR-γ) mediated lipid efflux. 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-1β, 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 (PPAR-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 acetylated-LDL (Ac-LDL), 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 recipients of ROCK1-/- BMT or ROCK1+/-- BMT mice [10].

*Corresponding author: Suowen Xu, Ph.D. Cardiovascular and Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Building 10, Room 5N316, MSC 1590, Bethesda, MD 20892-1590, USA, Tel: 301-594-2892; E-mail: suow-xu@nih.gov

Received December 28, 2012; Accepted December 29, 2012; Published December 31, 2012

Citation: Xu S (2013) ROCK the Rock of Atherosclerosis. J Vasc Med Surg 1: e101. doi:10.4172/2329-6925.1000e101

Copyright: © 2013 Xu S. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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 [13]. It has been suggested that the plexietropic effects of lipid-lowering 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 increased 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 CXC12 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), angiogenesis [20]. ROCK is implicated in the regulation of SMC proliferation and migration. 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 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 matrix

\[ \text{Atherogenic stimuli (oxLDL)} \rightarrow \text{ROCK} \rightarrow \text{EC contractility} \]

\[ \text{ROCK inhibitors (Y27632, fasudil, statins)} \]
metalloproteinases (MMPs) activities (which degrade components of ECM in the fibrous cap), increased SMC 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: mice and saline treated control animals. However, the number of CD3-positive T lymphocytes per lesion area and expression of NF-κB p65 subunit was reduced by Y-27632 [9]. Furthermore, 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-fibrocol 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 plaque rupture will be obtained from the treatment of patients with acute coronary syndrome with specific ROCK inhibitors.

Suowen Xu receives a “New Investigator Award” from Ministry of Education of China.

References
1. Libby P, Ridker PM, Hansson GK (2011) Progress and challenges in translating the biology of atherosclerosis. Nature 473: 317-325.
2. Xu S, Ogura S, Chen J, Little PJ, Moss J, et al. (2012) LOX-1 in atherosclerosis: biological functions and pharmacological modifiers. Cell Mol Life Sci DOI: 10.1007/s00018-012-1194-z.
3. Zhou Q, Mei Y, Shoji T, Han X, Kaminiski K, et al. (2012) ROCK-associated coiled-coil-containing kinase 2 deficiency in bone marrow-derived cells leads to increased cholesterol efflux and decreased atherosclerosis. Circulation 126: 2236-2247.
4. Nakagawa O, Fujisawa K, Ishizaki T, Saito Y, Nakao K, et al. (1996) ROCK-I and ROCK-II, two isoforms of ROCK-associated coiled-coil forming protein serine/threonine kinase in mice. FEBS Lett 392: 189-193.
5. Liao JK, Seto M, Noma K (2007) ROCK kinase (ROCK) inhibitors. J Cardiovasc Pharmacol 50: 17-24.
6. Zhou Q, Gensch C, Liao JK (2011) ROCK-associated coiled-coil forming kinases (ROCKs): potential targets for the treatment of atherosclerosis and vascular disease. Trends Pharmacol Sci 32: 167-173.
7. Zhou Q, Liao JK (2009) Rho kinase: an important mediator of atherosclerosis and cardiovascular disease. Curr Pharm Des 15: 3108-3115.
8. Shimokawa H, Rashid M (2007) Development of Rho-kinase inhibitors for cardiovascular medicine. Trends Pharmacol Sci 28: 296-302.
9. Mallat Z, Gojoa A, Sauzeau V, Brun V, Silvestre JS, et al. (2003) Rho-associated protein kinase contributes to early atherosclerotic lesion formation in mice. Circ Res 93: 884-888.
10. Wang HW, Liu PY, Oyama N, Rikitake Y, Kitamoto S, et al. (2008) Deficiency of ROCK1 in bone marrow-derived cells protects against atherosclerosis in LDLR-/− mice. FASEB J 22: 3561-3570.
11. Lauffenburger DA (2011) ROCK in a stiff place. Sci Transl Med 3: 112sf12.
12. Nohria A, Grumet ME, Rikitake Y, Noma K, Prsic A, et al. (2006) Rho kinase inhibition improves endothelial function in human subjects with coronary artery disease. Circ Res 99: 1426-1432.
13. Essler M, Retzer M, Bauer M, Heemskerk JW, Aepfelbacher M, et al. (1999) Mildly oxidized low density lipoprotein induces contraction of human endothelial cells through activation of Rho/Rho kinase and inhibition of myosin light chain kinase. J Biol Chem 274: 30361-30364.
14. Liu B, Zhang JY, Cao HM, Wang Q, Wang HB (2012) Effect of rosvustatin on ROCK activity, endothelial function, and inflammation in Asian patients with atherosclerosis. Intern Med 51: 1177-1182.
15. Laufs U, Lafa V, Plutzky J, Liao JK (1998) Upregulation of endothelial nitric oxide synthase by HMGCoA reductase inhibitors. Circulation 99: 1129-1135.
16. Laufs U, Lafa VL, Liao JK (1997) Inhibition of 3-hydroxy-3-methylglutaryl (HMG) CoA reductase blocks hypoxia-mediated down-regulation of endothelial nitric oxide synthase. J Biol Chem 272: 31725-31729.
17. Ming XF, Viswambharan H, Barandier C, Ruffleux J, Kalbuchi K, et al. (2002) Rho-GTPase/Rho kinase negatively regulates endothelial nitric oxide synthase phosphorylation through the inhibition of protein kinase B/Akt in human endothelial cells. Mol Cell Biol 22: 8467-8477.
18. Wolfrein S, Dendorfer A, Rikitake Y, Stalker TJ, Gong Y, et al. (2004) Inhibition of Rho-kinase leads to rapid activation of phosphatidylinositol 3-kinase/protein kinase Akt and cardiovascular protection. Arterioscler Thromb Vasc Biol 24: 1842-1847.
19. Mattaliano MD, Wooters J, Shih HH, Paulsen JE (2010) ROCK2 associates with lectin-like oxidized LDL receptor-1 and mediates oxidized LDL-induced IL-6 production. Am J Physiol Cell Physiol 298: C1180-3187.
20. Lonard D, Guerin P, Pecaud P (2006) Rho kinases in cardiovascular physiology and pathophysiology. Circ Res 98: 322-334.
21. Seasholtz TM, Majumdar M, Kaplan DD, Brown JH (1999) Rho and Rho kinase mediate thromb-in-stimulated vascular smooth muscle cell DNA synthesis and migration. Circ Res 84: 1186-1193.
22. Sauzeau V, Le Mellonncene E, Bertolglna J, Scallert E, Pecaud P, et al. (2001) Human ureotensin II-induced contraction and arterial smooth muscle cell proliferation are mediated by RhoA and ROCK. Circ Res 88: 1102-1104.
23. Noma K, Rikitake Y, Oyama N, Yan G, Alcaide P, et al. (2008) ROCK1 mediates leukocyte recruitment and neointima formation following vascular injury. J Clin Invest 118: 1632-1644.
24. Kataoka C, Egashira K, Inoue S, Takeo M, Ni W, et al. (2002) Important role of Rho-kinase in the pathogenesis of cardiovascular inflammation and remodeling induced by long-term blockade of nitric oxide synthesis in rats. Hypertension 39: 245-250.
25. Matsunoto A, Manthey HD, Marsh SA, Fassett RG, de Haan JB, et al. (2012) Effects of exercise training and Rho/ROCK inhibition on plaque in Apo(−/−) mice. Int J Cardiol.
26. Xu S, Little PJ, Fan T, Huang Y, Le K, et al. (2011) Tanshinirone II-A attenuates and stabilizes atherosclerotic plaques in apolipoprotein-E knockout mice fed a high cholesterd diet. Arch Biochem Biophys 515: 72-79.
27. Liu PY, Liu YW, Lin LJ, Chen JH, Liao JK (2009) Evidence for statin pleiotropy in humans: differential effects of simvastatin and ezetimibe on roh-associated coiled-coil containing protein kinase activity, endothelial function, and inflammation. Circulation 119: 131-138.