Targeting Rho in Cardiovascular Disease

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What began as molecular switches linking cell surface receptors to the reorganization of the actin cytoskeleton has now emerged as an important mediator of cardiovascular disease. The low-molecular-weight GTPases of the Rho family have appeared with increasing frequency in the cardiovascular literature. This interest stems from two seemingly opposite disciplines. From a basic science perspective, increasing evidence suggests a central role of Rho-dependent actin cytoskeleton in mediating changes in cell shape, contractility, and motility. However, how these actin cytoskeletal effects of Rho translate into cardiovascular pathophysiology is not entirely evident. From a clinical perspective, large prospective trials with 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors or statins suggest that these agents may have beneficial effects in cardiovascular disease in addition to their cholesterol-lowering effects. The realization that statins also inhibit isoprenoid synthesis, which is required for the posttranslational modification of Rho, has shifted the focus from a lipid-dependent effect of statins to their direct effects on Rho in the vasculature.

Several important pieces of the puzzle, which will bridge the biological functions of Rho with the clinical benefits of statins, are still missing. Foremost, what is the relationship between Rho and cardiovascular disease? In this issue of Circulation Research, Hernández-Perera et al provide additional evidence that Rho GTPases may play an important role in mediating vascular disease. They show that Rho is required for basal expression of preproendothelin-1 in vascular endothelial cells and that statins inhibit preproendothelin-1 expression by blocking Rho geranylgeranylation. The clinical relevance of these findings is underscored by the fact that preproendothelin-1 gives rise to endothelin-1, a potent vasoconstrictor and mitogen that regulates vascular tone and remodeling. Therefore, these findings fill in some of the missing pieces of the puzzle by linking the inhibition of Rho with the cholesterol-independent effects of statins. However, it is not known whether the actin cytoskeleton is involved in the regulation of preproendothelin-1 as it is in the case of endothelial nitric oxide synthase (eNOS) and tissue-type plasminogen activator. Interestingly, in contrast to eNOS, in which Rho regulates gene expression by altering mRNA stability, the effects of Rho on preproendothelin-1 seem to be transcriptional.

The Rho GTPases are members of the Ras superfamily of small GTP-binding proteins. They consist of at least 14 distinct proteins ranging from 20 to 24 kDa, which can be additionally subdivided into Rho, Rac, and Cdc42. Rho GTPases are major substrates for posttranslational modification by isoprenylation, and isoprenylation targets Rho GTPases to the membrane. Similar to the α subunit of heterotrimeric G proteins, Rho proteins cycle between the active GTP-bound and the inactive GDP-bound states. Activators of Rho include growth factors, cytokines, integrins, and G protein–coupled receptor ligands or hormones such as bradykinin or lysosphosphatidic acid. A key step in the activation of Rho is the attachment of geranylgeranial, an isoprenoid intermediate of the cholesterol biosynthesis pathway (see Figure). This posttranslational lipid modification is necessary for the translocation of inactive Rho from the cytosol to the membrane. Therefore, statins which block geranylgeranial synthesis, or geranylgeranyl transferase inhibitors which prevent the attachment of geranylgeranial to Rho, inhibit Rho membrane translocation and activity. Indeed, evidence suggests that inhibition of Rho isoprenylation mediates many of the cholesterol-independent effects of statins not only in vascular wall cells but also in leukocytes and bone.

Each member of the Rho family serves specific functions for cell shape, motility, secretion, and proliferation, although overlapping functions between the members could be observed in overexpressed systems. The activation of Rho in Swiss 3T3 fibroblasts by extracellular ligands, such as platelet-derived lysosphosphatidic acid, leads to myosin light chain phosphorylation and formation of focal adhesion complexes. Indeed, Rho-associated protein kinase increases the sensitivity of vascular smooth muscle to calcium in hypertension and coronary spasm. In contrast, activation of Rac leads to the formation of lamellipodia and membrane ruffles, whereas activation of Cdc42 induces actin-rich surface protrusions called filopodia. These distinct but complementary functions of Rho family members also extend to their effects on cell signaling. When cells undergo reorganization of their actin cytoskeleton in response to extracellular signals such as growth factors or during cell movement and mitosis, they alter the three-dimensional colocalization of intracellular proteins. Thus, changes in Rho-induced actin cytoskeleton can affect intracellular transport, membrane trafficking,
Inhibition of Rho by statins. Statins inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and block the synthesis of isoprenoids and cholesterol. The isoprenoid geranylgeraniol (GG) is an important lipid attachment for Rho, which permits the subsequent membrane translocation and activation of Rho. PP indicates pyrophosphate.

ing, mRNA stability, and gene transcription. Therefore, it is not too surprising to find that the heart and vasculature respond to mechanical forces by changes in cell shape and gene expression. In this respect, Rho-induced changes in the actin cytoskeleton and gene expression are interrelated.

Clinical trials with statins have led to increased understanding of the role of Rho in cardiovascular disease. For example, in cardiac myocytes, RhoA and Rac1 have been shown to mediate hypertrophy, myofibrillogenesis, and the expression of fetal genes such as atrial natriuretic factor. Therefore, it is interesting to speculate whether some of the beneficial effects of statin treatment in hypertension and heart failure may be attributable to inhibition of Rho proteins in the heart. In vascular smooth muscle cells, Rho promotes cell-cycle progression and proliferation, which are central events in the pathogenesis of vascular lesions, including postangioplasty restenosis, transplant arteriosclerosis, and vein graft occlusion. The molecular mechanism is attributable, in part, to Rho-induced posttranslational destabilization of the cyclin-dependent kinase inhibitor p27kip1. Indeed, statins, which effectively decrease the incidence of transplant-associated arteriopathy, attenuate smooth muscle cell proliferation through inhibition of RhoA geranylgeranylation. Recent studies also suggest that statins may exert additional antiinflammatory and antioxidative effects on the vascular wall. In certain cell types, Rho mediates the activation of the proinflammatory transcription factor nuclear factor–κB in response to cytokines. Furthermore, Rho proteins may be involved in mediating increases in oxidative stress. A major source of oxidants in vascular wall cells is the NAD(P)H oxidase. The Rho family member Rac1 is a regulatory component of the NAD(P)H oxidase in several cell types, including neutrophils and vascular wall cells. Indeed, inhibition of Rac1 isoprenylation by statins inhibits the release of reactive oxygen species in endothelial cells. Finally, Rho plays an important role in regulating endothelial function and gene expression, as illustrated in the present study by Hernández-Perera et al. Besides upregulating preproendothelin-1 expression, RhoA negatively regulates the production of endothelium-derived nitric oxide via Rho-induced changes in the endothelial actin cytoskeleton. Indeed, direct inhibition of Rho by Clostridium botulinum C3 transferase or disruption of the endothelial actin cytoskeleton by cytchalasin D leads to increases in aortic eNOS expression and activity in mice.

In summary, Rho seems to play an important role in cardiovascular disease, and inhibition of Rho may account for some of the cholesterol-independent pleiotropic effects of statins. However, additional studies are needed to understand exactly how Rho is activated, what its downstream targets are, and how it regulates cellular functions under pathophysiological conditions. Given the therapeutic implications of statin therapy, targeting Rho through inhibiting its geranylgeranylation or blocking its downstream effector Rho kinase may indeed yield some clinical benefits. However, it is too early to tell whether targeting Rho alone will produce favorable outcomes. The beneficial effects may be offset by the adverse effects of Rho inhibition, because Rho is critically involved in many important cellular functions. As the present study by Hernández-Perera et al reminds us, there is still much to be learned about how Rho is regulated and what it regulates.

References


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