Migration of smooth muscle cells (SMCs) into the developing neointima is a pivotal step during atherosclerosis development in native vessels as well as in restenosis after vascular intervention. Before migration can occur, a switch of the SMC phenotype from the quiescent to the secretory phenotype and production of extracellular matrix are required (1–3).

Previously, Blindt et al. (1) analyzed differences in gene expression pattern between the quiescent and the invasive SMC phenotype in vitro. They identified 9 clusters of differentially regulated genes—the most important being cluster 3 containing 17 of the 47 deregulated genes, among them genes that are involved in cell communication, matrix organization, and cell adhesion and migration. One of them was the angio-associated migratory cell protein (AAMP) gene. It was first identified as a member of the immunoglobulin superfamily present in endothelial cells of all human tissues and shown to mediate cell–cell and cell–substrate interactions (4).

In this issue of the Journal, Vogt et al. (5) demonstrated an important functional role of AAMP for migration of SMC in vitro and for the development of neointimal hyperplasia in vivo. The AAMP overexpression resulted in increased migration, whereas antibody- or silencing ribonucleic acid (RNA)—mediated inhibition of AAMP decreased SMC migration. Intraperitoneal administration of an anti-AAMP antibody reduced neointimal SMC density and neointima formation in apolipoprotein E–deficient mice. Their data show for the first time that AAMP inhibition offers a unique way of downstream targeting cytosolic RhoA pathways resulting in inhibition of SMC migration.

Studies in experimental models demonstrated that mechanical stretch and interaction with extracellular matrix leads to AAMP-mediated activation of RhoA. Activated RhoA then interacts with its effector rho-kinase (ROCK), generating the driving force for SMCs to migrate and to divide, leading to restenosis and atherosclerosis (Fig. 1). Direct inhibition of ROCK by fasudil and Y-27632 inhibited vascular SMC migration (6). Similarly, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors or statins blocked RhoA pathways (7). However, it remains to be determined whether statins do this by impairing RhoA translocation and consequent activation of RhoA or by binding to ROCK (Fig. 1). In addition, activation of RhoA pathways induced endothelial cell migration (8) and endothelial progenitor cell differentiation (9,10). Furthermore, RhoA/ROCK activation in endothelial cells decreased endothelial nitric oxide (NO) synthase (eNOS) expression by reducing eNOS messenger RNA stability. Consequently, ROCK inhibitors or statins upregulated eNOS expression (11,12) (Fig. 1). In aggregate, animal studies suggested that RhoA pathways might contribute to several cardiovascular pathologies, including arterial and pulmonary hypertension (13–15), myocardial hypertrophy and remodeling (16), coronary artery spasm (17), atherosclerosis (18), and restenosis (19,20).

Recent data obtained in patients, although sparse, support in part ROCK’s involvement in these conditions. Fasudil attenuated coronary vasoconstrictor responses to acetylcholine in patients with angina (21) and ameliorated myocardial ischemia in patients having coronary microvascular spasm as well (22). It also prolonged symptom-limited exercise duration of patients with stable angina more than placebo without affecting heart rate or blood pressure and was well-tolerated (23). Fasudil improved endothelium-dependent vasodilation and decreased vascular resistance in patients with coronary heart disease as well (24,25). Indeed, oral fasudil during 1 month was shown in an elegant study to significantly improve endothelium-dependent brachial artery dilatation in coronary artery disease patients (n = 13) but not in healthy subjects (n = 16). Because no effect of fasudil on endothelium-independent vasodilatation was observed in either groups these findings suggest that ROCK inhibition at least in part restores decreased NO availability associated with atherosclerosis (25). To our knowledge, proof of the effect of RhoA/ROCK inhibition on angioplasty- or stent-associated vascular SMC migration and proliferation in patients in vivo is lacking to date. However, inhibition of the RhoA/ROCK pathway does inhibit pulsatile stretch-induced proliferation of human saphenous vein SMCs (26), indicating that clinical studies with inhibitors of this
signal transduction system for the prevention of vein graft disease or restenosis should be considered.

One important limitation of fasudil and Y-27632 is that they are not specific for ROCK. They inhibit several other serine-threonine protein kinases, such as protein kinase A and C (27), which are also involved in several signaling pathways in the cardiovascular system, particularly in the regulation of myocardial contraction, hypertrophy, and remodeling (28). Therefore, the effects of currently available ROCK inhibitors might at least in part be due to blockage of other than RhoA pathways. However, there are no assays to evaluate how effective these agents are for inhibiting these specific pathways. The structure of ROCK dimers has been determined, in search of the mechanism for selectivity of ROCK. This structural analysis showed that subtle differences exist between the ROCK- and protein kinase A-bound conformations of the inhibitors. These structural differences are expected to determine the relative selectivity of these compounds. In in vitro binding studies, hydroxyfasudil—a metabolite of fasudil—was indeed selective for ROCK over protein kinase A (29). Its higher selectivity remains, however, to be confirmed in vivo.

The current study shows that blocking AAMP offers a possibility to downstream inhibition of RhoA-dependent pathways and therefore the development of restenosis and atherosclerosis. In addition, it offers the advantage of a well-defined target allowing more specific inhibition with high-affinity antibodies, silencing RNA, or synthetic inhibitors (30). The AAMP blockage might also improve endothelial function and increase NO availability, therefore enhancing its inhibitory effect on atherosclerosis and restenosis development. Some caution, however, is warranted. Indeed, AAMP also regulates the motility of endothelial cells (8) and is involved in endothelial progenitor cell differentiation (9). Thus, blocking AAMP in endothelial (progenitor) cells might impair re-endothelialization, leading to delayed arterial healing and promoting thrombosis (31), and therefore prevent adequate prevention of restenosis. Finally, further studies on the role of AAMP in regulating monocyte infiltration into the subendothelial space are needed. Indeed, ROCK inhibitors were shown to inhibit atherosclerosis by preventing macrophage migration (32) and inflammation (33).
In conclusion, this study is important because it draws our attention to the functional role of AAMP as a regulator of SMC migration. Further studies of the effect of AAMP blockage on SMC migration and endothelial (progenitor) cell and macrophage migration and inflammation, which are important processes in the development of atherosclerosis and restenosis, are warranted.

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