Pathophysiological vascular smooth muscle cell (VSMC) migration is a critical component of atherosclerosis and contributes substantially to neointimal hyperplasia. Injury to the intimal layer of the vessel promotes the dedifferentiation of VSMCs in the tunica media from a quiescent contractile phenotype to a synthetic phenotype that proliferates and migrates, thus contributing to neointimal thickening. A better understanding of the signaling mechanisms that promote VSMC dedifferentiation, proliferation, and migration may lead to the identification of new pharmacological targets for atherosclerosis and other vascular diseases.

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Several signal transduction pathways regulate actin polymerization and cell contractility, both integral to VSMC migration. The process of cell migration is complex but requires fundamental processes that depend on tight regulation of the GTPases Rac and Rho (Figure). Migration initiates when a cell is exposed to a chemoattractant gradient and establishes polarity. This triggers the extension of the plasma membrane, called a lamellipodium, in the direction of eventual cell movement and establishes the front/leading edge of the cell. In vascular injury, a gradient is often established by the release of platelet-derived growth factor (PDGF), such that VSMCs migrate toward the lumen of the vessel. The formation of lamellipodia requires the polymerization and assembly of actin, which is regulated by the GTPase Rac. Rac stimulates actin polymerization via several mechanisms including nucleation of new actin filaments, extension of existing filaments, and activation of LIM kinase, which phosphorylates and inactivates the actin-capping protein cofilin, thus preventing actin depolymerization. Stabilization of the lamellipodia occurs through the formation of adhesive complexes within the translocation. As the cell migrates, these focal complexes cluster, activate, and bind to extracellular matrix components at the leading edge. Focal complexes then strengthen and grow into larger focal adhesions, which serve as points of traction over which the cell body moves. The precise mechanisms regulating the conversion of focal complexes to focal adhesions are unclear but require the activation of RhoA and the recruitment of signaling proteins, including the serine/threonine kinase p21-activated kinase. Stimulation of actin-myosin contractility by Rho and its downstream effector Rho-associated protein kinase leads to the bundling of actin fibers to generate stress fibers and the clustering of integrins to mature focal complexes into focal adhesions. To generate force required for forward progression of the cell, the GTPases cdc42 and Rho regulate contractile forces by influencing interactions with actin. Rho-associated protein kinase, activated by Rho-GTP, functions to phosphorylate and inactivate the myosin phosphatase-targeting subunit-1, allowing myosin light chains to remain in a contractile (phosphorylated) state. Finally, for a cell to make forward progression, it must release rear adhesions to allow for net forward displacement. Thus, activation of Rac and Rho plays a central role in regulating pathological VSMC migration. Identifying the guanine nucleotide exchange factors (GEF) that activate the Rac and Rho GTPases in VSMCs could provide unique therapeutic targets to block the pathophysiological consequences of VSMC migration.

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Wu et al describe the presence of the novel GEF for Rac1 and RhoA, Kalirin-9, in VSMCs. Kalirin was described previously in the central nervous system, where it regulates neuronal shape, growth, and plasticity, in part, through effects on the actin cytoskeleton. Kalirin is a unique protein in that it contains 2 GEF domains. The RhoGEF1 domain and RhoGEF2 domain activate Rac1 and RhoA, respectively. Wu et al demonstrated Kalirin-9 expression in the aorta, and that Kalirin expression was increased in a model of atherosclerosis, consistent with clinical findings. They also established that Kalirin-9 is expressed by multiple cell types found within the vascular wall, including endothelial cells, VSMCs, and macrophages. The authors set out to test whether Kalirin-9 functions in VSMCs as a dual Rho-GEF in vitro and in vivo. To test this in vitro, the authors compared VSMCs from wild-type mice with Kalm- mice, as well as VSMCs in which Kalirin is knocked down by RNAi. They demonstrated that the RhoGEF1 domain of Kalirin activates Rac1 downstream of PDGF receptor stimulation, as measured by increased phosphorylation of p21-activated kinase, which was blocked in Kalm- and siKalirin-treated VSMCs. PDGF-induced p21-activated kinase phosphorylation was also blocked in the presence of 1-(3-nitrophenyl)-1H-pyrrole-2,5-dione (NPPD), a selective RhoGEF1 domain inhibitor. These changes in Rac activation in NPPD, siKalirin, and Kalm- VSMCs translated to an 35% reduction in PDGF- or serum-induced migration. In contrast, Kalm- , siKalirin- , and NPPD-treated VSMCs exhibited no change in PDGF-induced RhoA activation, measured by unchanged levels of myosin phosphatase-targeting subunit-1 phosphorylation. Furthermore, Kalm- VSMCs exhibited reduced Rac activity, whereas Rho activity

**Editorial**

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remained unchanged. Wu et al\textsuperscript{16} convincingly demonstrated that in VSMCs, despite the presence of a GEF domain specific for Rac1 and a GEF domain specific for RhoA,\textsuperscript{17} Kalirin primarily mediates Rac1 activation via its RhoGEF1 domain, but does not alter RhoA activation, to promote VSMC migration.

Cheng et al\textsuperscript{19} recently demonstrated that Rac is required for the activation of Nox1. Additionally, it is established that receptor tyrosine kinase activation can lead to Rac activation in VSMCs, via an unknown GEF, and thus activation of the Nox1 enzyme.\textsuperscript{13,20} Furthermore, the involvement of Rac and Nox activity in mediating the formation of lamellipodia in VSMCs has recently become an area of active investigation.\textsuperscript{21–23} Kalirin-9 may represent the previously unidentified GEF that mediates Nox1 activity downstream of receptor tyrosine kinase activation.

The upregulation of Kalirin has been associated with atherosclerosis in several clinical studies.\textsuperscript{18,24,25} Wu et al\textsuperscript{16} established Kalirin as a signaling protein that specifically promotes Rac1 activation and contributes to VSMC migration downstream of PDGF in vitro. Importantly, the authors demonstrate that decreased Kalirin expression in vivo significantly reduced neointimal formation in a model of arterial endothelial denudation. Even though a causal role for Kalirin in human atherosclerosis remains to be tested, the very exciting findings presented in this article suggest that inhibition of Kalirin may represent a novel therapeutic strategy to block VSMC migration and, as a result, prevent atherosclerosis.

### Disclosures

None.

### References

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