Rac1 is a member of the Rho family of GTPases, which includes Rho, Rac, and cdc42 subfamilies. On activation by guanine nucleotide exchange factors, the Rho family GTPases exchange a GDP molecule for a GTP and act as molecular switches to transduce signals in response to various extracellular stimuli. Apart from their characteristic role in the regulation of cytoskeletal rearrangement and cell adhesion, the Rho family proteins have been found to regulate polarization, endocytosis, intracellular trafficking, cell cycle progression, differentiation, and, gene transcription. Rac family GTPases play a role in endothelial permeability, polarization, leukocyte adhesion, and production of reactive oxygen species, including the superoxide anion (O$_2^-$). More specifically, activation of endothelial cells with growth factors such as vascular endothelial growth factor or by fluid shear stress has been shown to activate Rac1, which, in turn, promotes cytoskeletal rearrangement and changes in cell motility. Endothelial activation by growth factors or shear stress also results in the activation of NADPH oxidases (NOXs) and endothelial NO synthase (eNOS), which are responsible for the production of O$_2^-$ and NO, respectively. Because the modulation of endothelial NOXs by Rac1 has been well demonstrated, it is believed that Rac1 influences endothelial function, as measured by NO bioavailability, by enhancing the production of O$_2^-$ and not by direct modulation of eNOS. This assumes mutually exclusive mechanisms for the production of O$_2^-$ and NO and that a decreased bioavailability of NO on Rac1 activation is a result of O$_2^-$-dependent peroxynitrite production. Any association with or direct link between Rac1 activation and regulation of eNOS, therefore, has remained unexplored despite the observation that Rac1 and eNOS are both required for endothelial motility, proliferation, survival, and angiogenesis.

The normally functioning endothelium maintains vascular homeostasis. It is intrinsically anti-thrombotic, anti-inflammatory, and responsive to vasodilators. The dysfunctional endothelium loses these protective properties and, as such, negatively participates in vascular pathology. Endothelial dysfunction has been demonstrated in the metabolic syndrome, dyslipidemic states, types 1 and 2 diabetes mellitus, and hypertension and is associated with cardiovascular events, most notably of a coronary nature. It is thought to be the earliest stage in vascular pathological development. A critical and master regulator that maintains the protective features of normal endothelial function is NO. Thus, endothelial dysfunction is commonly considered a defect in NO production and/or bioavailability. This has led to extensive work, performed in many laboratories, dissecting the complex mechanisms that control eNOS expression and activity. Apart from the coenzymes, cosubstrates and cofactors required for normal eNOS function, many other eNOS-interacting proteins have been shown to modulate eNOS function under various conditions. These include several G protein–coupled receptors, porin, dynamin-2, caveolin-1, NOS-interacting protein, and heat shock protein-90. Phosphorylation on several critical serine/threonine residues (with defined downstream kinases), myristoylation, and intracellular translocation have also been described to alter eNOS function. However, a functional relationship between Rac family GTPases and eNOS has only begun to emerge. With the discovery that statins, hydroxymethylglutaryl-coenzyme A reductase inhibitors, can increase NO production by effects on RhoA, and can reduce NOX-dependent O$_2^-$ production via Rac1, a potential link has been proposed between Rac family GTPases and eNOS function. A recent report by Selvakumar et al demonstrating that Rac1 both interacts with eNOS and regulates eNOS activity has further corroborated the hypothesis that Rac1 may directly modulate eNOS function.

In an article published in this issue of Circulation Research, Sawada et al provide significant new insights on the functional link between activated Rac1 and the modulation of eNOS function in the endothelium. Using an endothelial-specific Rac1 haploinsufficient (EC-Rac1$^{+/}$) mouse and Rac1–modulated cell lines, the authors demonstrate that Rac1 is required for the maintenance of eNOS mRNA expression and stabilization, eNOS enzymatic activity, and NO production in the endothelium (see Figure). The attenuation of Rac1 expression resulted in impaired endothelium-dependent vasodilation, mild hypertension, and retarded angiogenesis, all consistent with an NO-deficient state. Aortic rings from EC-Rac1$^{+/}$ mice demonstrated an elevated phenylephrine-induced contraction and attenuated acetylcholine-induced relaxation. Decreased blood flow recovery to hindlimb ischemia was seen in the EC-Rac1$^{+/}$ mice. Moreover, aortic explants from EC-Rac1$^{+/}$ mice implanted into Matrigel also showed impaired capillary sprouting, rescued either by exogenous addition of the eNOS substrate l-arginine or by the NO donor S-nitrosothioglutathione. The use of a Rac1 pharmacological inhibitor or dominant negative Rac1 expression in endothelial cell lines displayed that active Rac1 regulates eNOS promoter activity but not the phosphorylation of either eNOS or its upstream kinase, Akt. Dominant negative p21-regulated kinase (PAK) expression blocked...
Rac-induced eNOS promoter induction, suggesting that PAK is the Rac1 effector driving, at the least, Rac-induced enhanced eNOS transcription. Finally, the association of PAK with the cationic transporter, CAT1, responsible for the import of eNOS substrates was demonstrated. Taken together, the report by Sawada et al.\textsuperscript{19} suggests that Rac1 is intimately involved in the regulation of eNOS at multiple levels including transcription, posttranscriptional modification, and posttranslational function, which together contribute to 1 mechanism whereby the small G protein affects endothelial function.

This study adds substantially to the field in several ways. Sawada et al.\textsuperscript{19} are the first to examine the effects of Rac1 haploinsufficiency on endothelial dysfunction in vivo. Because global Rac1 gene deletion results in embryonic lethality,\textsuperscript{20} it has thus far been difficult to gauge how Rac1 deficiency would affect endothelial function. The endothelial-specific Rac1 haploinsufficient mouse model clearly demonstrates that attenuated Rac1 expression results in endothelial dysfunction as measured by impaired endothelium-dependent vasodilation and retarded angiogenesis. Secondly, this study is consistent with another recent report demonstrating that Rac1 functions as a common control element for NOS and NOX and coordinates signaling events involved in the generation of NO and O$_2^-$ in the endothelium.\textsuperscript{13} Thirdly, the finding that active Rac1 can modulate eNOS transcription and posttranscriptional and posttranslational events suggests that the effects of Rac1 on eNOS are multifaceted, critical in both the biosynthesis and bioavailability of NO and, therefore, in endothelial function. Additional novelty is provided by the finding that the function of CAT1, an L-arginine transporter, may be regulated by Rac1 in a PAK-dependent manner. To our knowledge, this is the first report suggesting CAT1 modulation by Rac1 in the endothelium and implies that Rac1 may affect endothelial function by other yet-undiscovered secondary mechanisms of action.

Based on its positive modulatory effect on eNOS levels and function, Sawada et al.\textsuperscript{19} speculate that Rac1 may be an important therapeutic target in the prevention of endothelial dysfunction. Although the data presented would certainly support this concept, the broad spectrum of cellular Rac1 effects, mediated by a variety of effector molecules, must be considered. Indeed, Rac activation has been traditionally thought to be an important component of many inflammatory responses, playing a role in leukocyte migration, adhesion, chemotaxis and, most notably, O$_2^-$ generation through the aforementioned NOX/NADPH induction/activation. Rac also plays a role in platelet activation, including signaling responses to thrombin and collagen.\textsuperscript{11} Rac has been linked to cardiomyocyte hypertrophy, which is, in part, O$_2^-$ dependent.\textsuperscript{21} This is among the major mechanisms by which angiotensin II exerts its pathological cardiovascular effects. Rac activation has also been linked to endothelial cell cytokine and O$_2^-$ release.\textsuperscript{22} Smooth muscle cell phenotypic changes, migration, and apoptosis can all be mediated by Rac.\textsuperscript{23,24} The guanine nucleotide exchange factors that regulate Rac by GTP cycling can be cell- and tissue-specific. Through unclear mechanisms, a particular guanine nucleotide exchange factor–activated Rac1 may preferentially stimulate 1 of the Rac effectors. For example, Rac1 that is GTP-loaded by Tiam1 (and thus Tiam1-bound) preferentially stimulates PAK.\textsuperscript{25} PAK is best known for its role in cytoskeletal reorganization, but it also plays a critical role in reactive oxygen species generation from membrane-localized NADPH oxidase.\textsuperscript{26} Sawada et al.\textsuperscript{19} demonstrated that the positive, potentially beneficial eNOS modulatory effects of Rac1 are PAK-dependent. However, they make the link to nuclear factor κB complex activation, which is a critical transcriptional activator not only of eNOS but also of many proinflammatory genes in endothelial cells, including those encoding adhesion molecules, chemokines, and procoagulants. Other downstream Rac effectors include c-Jun NH$_2$-terminal kinase and p38, activated either directly or through mitogen-activated protein kinase kinases. c-Jun NH$_2$-terminal kinase and p38 affect expression of many genes, a significant subset of which are proinflammatory. For example, some of the tumor necrosis factor-α–stimulated inflammatory responses, occurring through p38, are Rac1-dependent.\textsuperscript{27} Thus, although positive Rac1 targeting may be beneficial with regard to bioavailable NO release from the endothelium, there are numerous potentially negative consequences of such an action, both direct and indirect. This conundrum is underscored by the apparent pleiotropic effects of statins. Based on the isoprenylation inhibition exerted by statins, and the fact that the Rho family GTPTases gain function, in part, through isoprenylation and effective subcellular targeting, it has been suggested that this drug class can have significant antiinflammatory effects. Furthermore, the authors have previously demonstrated that a statin-induced reduction in Rho kinase (ROCK) function promotes eNOS mRNA stabilization and consequent enhanced NO production.\textsuperscript{28} If a reduction in Rac1 isoprenylation accordingly interferes with the ability of Rac1 to activate...
its effectors, the results presented in this report would suggest a significant reduction in eNOS levels and function.

In summary, the report by Sawada et al. provides new, exciting evidence of a mechanism by which a GTP-binding protein of the Rho family can regulate NO biosynthesis and positively influence endothelial function. It is the anticipation and hope that when cell- and stimulus-specific activation pathways are clarified, and the precise mechanism(s) by which Rac1 enhances eNOS levels and function are elucidated, these findings may well lead to highly selective therapeutic targeting. Until that time, it is important to recognize the breadth of Rac1-mediated effects on cardiovascular (and leukocyte) phenotype and function.

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Disclosures

None.

References


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