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Novel aspects of the roles of Rac1 GTPase in the cardiovascular system

Naoki Sawada¹, Yuxin Li², and James K. Liao²

Naoki Sawada: nsawada.gcoe@tmd.ac.jp; Yuxin Li: yxli@rics.bwh.harvard.edu; James K. Liao: jliao@rics.bwh.harvard.edu

¹Global Center of Excellence Program, International Research Center for Molecular Science in Tooth and Bone Diseases, Tokyo Medical and Dental University, 24th floor, Research Building II, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan

²Vascular Medicine Research, Brigham & Women's Hospital, 65 Landsdowne Street, Room 275, Cambridge, Massachusetts 02139, USA

Abstract

Rac1 GTPase is an established master regulator of cell motility through cortical actin reorganization and of reactive oxygen species generation through regulation of NADPH oxidase activity. Numerous molecular and cellular studies have implicated Rac1 in various cardiovascular pathologies: vascular smooth muscle proliferation, cardiomyocyte hypertrophy, and endothelial cell shape change. The physiological relevance of these in vitro findings, however, is just beginning to be reassessed with the newly developed, conditional mouse mutagenesis technology. Conditional gene targeting has also revealed unexpected, cell type-specific roles of Rac1. The aim of this review is to summarize the recent advance made in Rac1 research in the cardiovascular system, with special focus on its novel roles in the regulation of endothelial function, angiogenesis, and endothelium-mediated neuroprotection.

Keywords

Rac1; ROS; NADPH oxidase; actin cytoskeleton; cardiac hypertrophy; heart failure; atrial fibrillation; eNOS; angiogenesis; endothelium-derived neurotrophic activity

Introduction

The Rho family of small GTPases has been increasingly implicated in cardiovascular pathophysiology. Numerous molecular and cellular studies have identified the key role for Rac1 GTPase, a Rho family member, in the regulation of vascular smooth muscle cell (VSMC) proliferation and migration, cardiomyocyte hypertrophy, and the flow-induced realignment of endothelial cells (ECs) [1–4]. These effects of Rac1 on cardiovascular cells are primarily mediated through the regulation of re-organization of the actin cytoskeleton, and the activity

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Corresponding author: James K. Liao, Vascular Medicine Research, Brigham & Women's Hospital, 65 Landsdowne Street, Room 275, Cambridge, Massachusetts 02139, USA, Tel: 1-617-768-8424, Fax: 1-617-768-8425, jliao@rics.bwh.harvard.edu.

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of the enzyme complex NADPH oxidase to control superoxide formation [5]. Beyond these classical functions, however, it is now becoming clear that long-term effects exerted by Rac1 pivotally involve regulation of gene expression.

Recent progress in mouse mutagenesis has enabled conditional deletion of Rac1 gene in mouse cardiovascular cells in vivo [6–10]. Furthermore, the development of the Rac1 specific inhibitor, NSC23766, has fueled the investigation of the roles of Rac1 [11]. The technical advance made with these new tools to specifically inhibit Rac1 function in vivo has substantially refined our knowledge about the roles of Rac1 GTPase in cardiac and vascular pathophysiology. In this article, we will briefly overview the recent progress in the research on Rac1 functions revealed in cardiovascular system, with special emphasis on its emerging roles in cardiac remodeling and endothelial function.

Role of Rac1 in cardiac hypertrophy and heart failure

Previous studies with the isolated cardiomyocyte cell model and transgenic mice overexpressing Rac1 in the myocardium suggested relevance of cardiomyocyte Rac1 in the development of cardiac hypertrophy [1]. To conclusively elucidate the role of Rac1 in the development of cardiac hypertrophy, Satoh and colleagues generated a mouse model of tamoxifen-inducible, cardiomyocyte-specific excision of the Rac1 gene [6]. Cardiac-specific deletion of Rac1 markedly alleviated hypertrophic response of the heart to angiotensin II (Ang II) infusion. The anti-hypertrophic effect of Rac1 deletion was associated with reduction in reactive oxygen species (ROS), and the decreased activities of NADPH oxidase, apoptosis-regulated signaling kinase-1, and transcription factor NF- κ B. Another study used adenovirus-mediated gene transfer of dominant negative Rac1 to inhibit Rac1 activity in the myocardium and showed that Ang II-induced cardiac hypertrophy is mediated by Rac1 through activation of protein kinase Akt [12]. Custodis and colleagues showed, with the use of a mouse thoracic aorta constriction model, that Rac1 mediation of cardiac hypertrophy and activation of NADPH oxidase requires Rac1 binding to Rho guanine dissociation inhibitor α [13].

Chronic hypertrophic response is considered maladaptive and predisposes individuals to congestive heart failure (CHF). The underlying cellular mechanism of CHF is the progressive apoptotic cell death of cardiomyocytes. Recent studies demonstrated that Rac1 promotes cardiomyocyte apoptosis in mouse models of doxorubicin-induced cardiac toxicity [14] and diabetic cardiomyopathy [10]. In line with these observations, Rac1 has been shown to be responsible for the increased expression of TNF- α , the prominent pro-apoptotic cytokine, in the male hearts compared to female hearts in endotoxemia induced by lipopolysaccharide (LPS) administration [15]. Collectively, these data indicate that Rac1 plays an essential role in the progression of both cardiac hypertrophy and heart failure.

Role of Rac1 in atrial fibrillation

Atrial fibrillation (AF) is the most common arrhythmia in human subjects, yet the molecular mechanism underlying the pathogenesis of AF is incompletely understood. The augmented Rac1 activity and ROS production reported in porcine atrial tissues undergoing induced AF presaged a role for Rac1 in the pathogenesis of AF [16]. Adam and colleagues used transgenic mice with cardiac-specific overexpression of constitutively active Rac1 and examined the occurrence of AF in the Tg mice [17]. At 16 months of age, 75% of the Tg mice, but no control mice, manifested AF, suggesting the important role of Rac1 in the development and progression of AF. In human atrial tissues from AF patients, Ang II and phosphorylated signal transducer and activator of transcription (STAT) 3 were found to be elevated [18]. In fact, in cultured atrial myocytes and fibroblasts, Ang II provoked phosphorylation of STAT3 in a Rac1-dependent manner. This was associated with direct binding of Rac1 with STAT3, representing

a potential molecular mechanism leading to AF. Rac1-STAT3 association has been also proposed as a mechanism for PDGF-induced proliferation of VSMCs [19].

Role of Rac1 in endothelial function

ECs play a pivotal role in the maintenance of vascular homeostasis through synthesis of various vasoactive substances and polypeptides. Nitric oxide (NO), the most prominent among these factors, is constitutively synthesized by endothelial NO synthase (eNOS). NO causes vasorelaxation and inhibits VSMC proliferation and migration, platelet aggregation, and leucocyte adhesion to endothelium. Multiple stimuli and signaling pathways have been shown to regulate eNOS activity. To investigate the impact of Rac1 on endothelial function, we recently developed endothelial Rac1 haploinsufficient mice (EC-Rac1^{+/-}) by crossbreeding Tie2 promoter-driven Cre recombinase Tg mice with conditional Rac1 allele knock-in mice [8]. EC-Rac1^{-/-} mice were embryonic lethal due to the failure in vascular development (see below). Compared to wild-type (WT) mice, EC-Rac1^{+/-} mice showed decreased expression and activity of eNOS, and reduced incorporation of extracellular L-arginine, the eNOS substrate, through the membrane cationic amino acid transporter, CAT-1. The reduced NO synthesis from EC-Rac1^{+/-} vasculature was correlated with the decrease in endothelium-dependent vasorelaxation, and caused mild hypertension. Our data demonstrated the required role for Rac1 in the regulation of eNOS activity.

Selvakumar and colleagues demonstrated that transduction of constitutively active Rac1 to human aortic ECs caused increase in the activities of NOS and NADPH oxidase, while inhibition of Rac1 resulted in decrease of the activities of both enzymes [20]. In addition, the regulation of NOS activity by Rac1 involved direct association of Rac1 with eNOS. Kou et al. reported that epinephrine-induced phosphorylation of eNOS 1177 requires Rac1-mediated Akt activation, which again involves eNOS/Rac1 association [21]. Together, these studies suggest that Rac1 in endothelium plays a key role in the regulation of eNOS activity through multiple mechanisms encompassing gene expression, substrate availability, phosphorylation, and direct association with Rac1, of eNOS.

Role of Rac1 in endothelial permeability

Vascular endothelium constitutes a semi-permeable barrier that allows the fluid and solute exchange between the blood and interstitial space. The endothelial barrier property is tightly regulated, and pathological conditions, such as inflammation and ischemia, can cause barrier dysregulation leading to tissue edema. Endothelial permeability also plays a role in the regulation of angiogenesis, by depositing provisional matrices in extracellular space to promote EC migration and tubulogenesis. Accumulating evidence suggests that various agonists and extracellular cues require Rac1 to modulate EC barrier property (summarized in Table 1). The regulation of endothelial permeability by Rac1 likely involves both ROS and actin cytoskeleton [22]. Rac1 mobilizes cortical actin network and modulates phosphorylation and distribution of the intercellular junction proteins, occludin, VE-cadherin, and β -catenin, which are critical for the integrity of tight junction and adherence junction [7,23–30].

Role of Rac1 in angiogenesis and vascular development

Angiogenesis is an essential tissue response for tumor growth, ischemia, arteriosclerosis and embryonic development. Its process is dependent on cell proliferation, migration and capillary tube formation in ECs. Recent EC culture studies demonstrated essential roles for Rac1 in stretch-provoked proliferation [31]; directed migration toward the gradient of thrombin [32], sphingosine 1-phosphate (S1P) [33], VEGF [7,34,35], and Ephrin-A1 [36]; and tube formation in three-dimensional in-gel environment [37–39]. Rac1 has been also suggested to regulate the

functions of endothelial progenitor cells, which critically contribute to neovascularization [40].

Our recent study with EC-Rac1^{+/-} mice demonstrated that endothelial Rac1 is required for postnatal angiogenesis in ischemic mouse limbs [8]. In addition, endothelial Rac1 mediates angiogenesis, in part, through activation of eNOS. Consistent with our findings, Tan and colleagues found that the genetic deletion of Rac1 in mouse lung ECs caused reduced migration, tube formation, adhesion, and permeability compared to control cells [7]. They also demonstrated that EC-Rac1^{-/-} mouse embryos die around E9.5, due to the defective development of major vessels and complete lack of small branched vessels. Together, EC-specific Rac1 gene deletion in vivo uncovered the essential role of EC Rac1 in angiogenesis in both postnatal and developmental stages.

Role of Rac1 in ischemic stroke

Ischemic stroke is the third leading cause of mortality; yet the pathogenesis is still incompletely understood and therapeutic means are limited. Blood vessels play a critical role in the development and progression of stroke. The cerebral blood flow (CBF) in the penumbra determines the infarct size; In addition, the destruction of the blood-brain barrier (BBB) causes brain edema, which deteriorates ischemic neuronal cell death. The endothelial functions involved in the regulation of BBB properties include permeability, ROS production, cell-cell and cell-matrix adhesion, and cell survival. Although Rac1 has been shown to play a critical role in all of these endothelial functions, no studies had previously addressed the contribution of Rac1 to stroke pathophysiology. We recently explored the impact of endothelial Rac1 by exposing EC-Rac1^{+/-} mice to middle cerebral artery occlusion, a mouse model of transient focal brain ischemia [9]. EC-Rac1^{+/-} mice showed a 37% reduction in infarct size and a 63% reduction in edema volume compared to controls. By contrast, the absolute CBF reduction in the ischemic hemisphere at the end of ischemia was similar between the genotypes. This suggests that EC Rac1 haploinsufficiency conferred protection against neuronal death and edema formation independent of CBF.

To investigate the mechanisms for the neuroprotection in EC-Rac1^{+/-} mice, we conducted a genome-wide microarray analysis to examine the differential gene expression between Rac1^{+/-} and Rac1^{+/+} ECs. Rac1^{+/-} ECs showed upregulation of gene clusters that contained three important categories relevant to neurovascular protection. These are stress response genes (*Gpx3* [glutathione peroxidase 3], *Cryab* [crystallin α B], *Hsp25* [heat shock protein 25], *Hsp70* [heat shock protein 70]); cell adhesion and extracellular matrix (ECM) genes (*Col2a1* [collagen 2a1], *Col8a1* [collagen 8a1], *FNI* [fibronectin 1], *Thbs2* [thrombospondin 2], *Spp1* [osteopontin], protocadherin 10, desmocollin 3, claudin 3); and growth factor genes. Consistent with the gene expression profiles, Rac1^{+/-} ECs showed reduced ROS production and apoptotic cell death in response to hypoxia/reoxygenation compared to Rac1^{+/+} ECs. Moreover, Rac1^{+/-} EC monolayer was more resistant to enzymatic dispersion than that of Rac1^{+/+} ECs.

The expression of genes encoding 13 growth factors was upregulated in Rac1^{+/-} ECs. Four of these growth factors (artemin, FGF-5, BMP-6, TGF- β) are endowed with potential neurotrophic capacity. Non-contiguous co-culture studies between neuronal cells and Rac1^{+/-} or Rac1^{+/+} ECs revealed the existence of paracrine neurotrophic activity elaborated by Rac1^{+/-} ECs. Subsequent studies using neutralizing antibodies determined that at least half of this activity is attributable to artemin. Collectively, these data suggest that endothelial Rac1 haploinsufficiency confers neuroprotection through coordinate upregulation of endothelial barrier-protective genes and humoral neurotrophic factors (Fig. 1).

Emerging heterogeneity of Rac1 signaling in the cardiovascular system

It is becoming increasingly clear that, depending on the types of cells and upstream stimuli, Rac1 exerts apparently opposing effects. These Janus-faced aspects of Rac1 functions have been best illustrated in endothelial cell signaling (Fig. 2)[41].

Traditionally, Rac1 activation has been thought to be an important component of many inflammatory responses. The pro-inflammatory effect of Rac1 most notably involves robust activation of NADPH oxidase-mediated superoxide production. Indeed, this is among the major mechanisms by which Ang II exerts its pathological cardiovascular effects, such as endothelial dysfunction and cardiac hypertrophy. Excessive formation of ROS by Rac1 has been also shown to induce apoptotic cell death in cardiomyocytes, ECs and other cell types. Until recently, it had been believed that the major mechanism whereby Rac1 influences NO bioavailability is through enhancement of superoxide production and not through direct modulation of eNOS. This assumes mutually exclusive mechanisms for the production of superoxide and NO and that a decreased bioavailability of NO on Rac1 activation by agonists like Ang II is a result of superoxide-dependent peroxynitrite formation. Our recent findings that Rac1 is required for the expression and activity of eNOS demonstrated a direct link between Rac1 and eNOS independent of ROS. It is most probable, therefore, that the combined effect of Rac1 on eNOS and NADPH oxidase alters NO bioavailability in either a positive or negative way, depending on the net effect (Fig. 2 A).

The conceptual framework concerning the roles of ROS in cell signaling has been recently refined, proposing their dual functions [42]: While excessive ROS produced under pathological conditions such as inflammation are detrimental to cells, the physiological, low level of ROS critically serve as an important signal transducer in the local environment of cells. For the latter function, ROS occurrence and localization in cells is tightly regulated through their short half-lives and targeting of the NADPH oxidase to specific cell compartments. Furthermore, evidence suggests that ROS act directly on actin-regulatory molecules and hence participate in the cell machinery for locomotion. This likely enables ROS to serve as signaling mediators for directed EC movement and angiogenesis [22].

Contradictory actions of Rac1 signaling have been also found for its mediation of endothelial barrier functions in response to various upstream agonists or cues (Table 1). Pro-angiogenic ligands, such as VEGF, increase endothelial permeability through Rac1, while anti-inflammatory ligands, such as prostaglandins E2 and I2 also activate Rac1 and decrease endothelial permeability. A review by Tzima proposed one explanation for the apparently conflicting effects of Rac1 on the endothelial barrier [3]. IQGAP1, a Rac1 effector, stabilizes intercellular junctions and thereby decrease permeability. On the other hand, p21-activated kinase (PAK), another Rac1 effector, has been shown to exert opposite effects. Therefore, different extracellular signals may promote or inhibit endothelial barrier property by selectively coupling Rac1 to either IQGAP1 or PAK1 (Fig. 2 B). Gregg et al. proposed that the effector selection by Rac1 is determined by which guanine nucleotide exchange factor (GEF) activates Rac1 [4]. Since agonists likely use different GEFs to activate Rac1, this may provide one answer to the Janus-faced roles of Rac1 in the regulation of endothelial function and permeability.

Concluding remarks and clinical perspective

Given the growing importance of Rac1 in the cardiovascular system, recently developed Rac1 inhibitors like NSC23766 hold promise for therapeutic intervention of various cardiovascular diseases [11]. Indeed, successful inhibition of Rac1-dependent effects in vivo by NSC23766 has been demonstrated in mouse models of proteinuric kidney disease and hematopoietic stem

cell mobilization [11,43]. However, caution should be used when one considers therapeutic strategies using Rac1 inhibitors, since evidence is accumulating to add more complexity to the roles of Rac1 in cardiovascular cell functions. For example, as discussed above, Rac1 exerts opposite effects on endothelial barrier property in response to distinct extracellular signals. This could be also true for other cardiovascular effects, such as cardiac hypertrophy and angiogenesis. It is noteworthy that the specificity of the cellular effect of Rac1 is conceivably determined by coordinated actions between the upstream activators (ie, GEFs), Rac1, and the downstream effectors. Therefore, there is critical need to fully delineate cell- and stimulus-specific signaling axes encompassing Rac1, rather than Rac1 by itself, for highly selective therapeutic targeting in the future clinical application of Rac1 inhibitors.

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Figure 1.

Proposed model of neuroprotection through targeting endothelial Rac1. BM: Basement membrane; EC: endothelial cell; ECM: Extracellular matrix; HSP: Heat shock protein; ROS: Reactive oxygen species.

Adapted with permission from the reference [9].

Figure 2.

Context-dependent, Janus-faced functions of Rac1 in endothelial cell signaling.

Different signaling axes encompassing distinct sets of upstream stimuli, GEF, Rac1 and effector(s) exert opposite effects on endothelial function, angiogenesis, EC survival (A), and endothelial barrier function (B).

Table 1

Rac1-mediated change in endothelial permeability by extracellular stimuli.

Stimulants	Rac1 activity	Rac1-dependent change in endothelial permeability	Refs
VEGF	↑	↑	[7,23,25]
S1P	ND	↑	[7]
interleukin 8	↑	↑	[26]
platelet activating factor	↑	↑	[27]
ischemia/reperfusion	ND	↑	[9]
chronic hypoxia	↓	↑	[30]
thrombin	ND	↑	[24,44]
prostaglandin E2 or I2	↑	↓	[29]
laminar flow	↑	↓	[3,28]
S1P	↑	↓	[44]
oxidized phospholipid	↑	↓	[45]