H-RAS Controls Phenotypic Profiles of Vascular Smooth Muscle Cells and the Pathogenesis of Vascular Proliferative Disorders

Kenneth S. Ramos

Vascular smooth muscle (VSMC) migration, proliferation, and hypertrophy following physical, chemical, or biological injury are key events in the onset and progression of vascular proliferative disorders, such as atherosclerosis, postangioplasty stenosis, venous bypass graft failure, and transplantation. Under normal conditions, VSMCs exist in a quiescent state of growth that supports contractile function and structural and functional integrity of the vascular wall. Vascular injury disrupts critical cell–cell and cell–matrix interactions, leading to activation of VSMCs. The activated VSMC phenotype is characterized by gradual loss of differentiated characteristics, increased response to chemotactic agents, and uncontrolled proliferation. Not surprisingly, the quest for molecular triggers of VSMC proliferation led investigators to studies of cell cycle control proteins and the quest for molecular triggers of VSMC proliferation led investigators to studies of cell cycle control proteins and mitogenic signaling within the vascular wall. Of note are seminal studies examining the signaling cascades of platelet-derived growth factor and various protooncogenes, including myb, myc, and H-ras. H-RAS quickly emerged as a critical convergence point in VSMC mitogenic signaling and was identified as a putative target for pharmacological intervention in vascular proliferative disorders.

H- and K-ras were first identified as viral oncogenes in Harvey and Kirsten rat sarcomas, respectively. Subsequent studies identified a third member, N-ras, as a protooncogene present in various tumor cell lines. Studies of the cellular viral ras oncogene counterparts led to the identification of ras protooncogenes as TATA-less, GC-rich genes that encode for 21-kDa proteins with homology to the α subunit of G proteins. p21RAS binds guanine nucleotides and possesses intrinsic GTPase activity, and mutations at positions 12, 13, and 61 are associated with constitutive activation and neoplastic transformation. RAS proteins play central roles in morphological transformation, immortalization, anchorage independence, tumorigenicity, and metastases. Their biologic activity requires posttranslational modifications at the carboxy terminus and membrane association. Several modifications have been documented, most significant of which are farnesylation, proteolysis, and methylation of its CAAX sequence by isoprenylcysteine carboxyl methyltransferase. RAS activation is a central node in the regulation of mitogenic signaling from the cell membrane to the nucleus in VSMCs. Key elements of the complex biochemical interactions regulated by RAS in mammalian cells are summarized in the Figure. Signaling begins with the binding of growth factors to the extracellular domain of tyrosine kinase receptors, such as platelet-derived growth factor. These receptors become autophosphorylated at their tyrosine residues and associate with the adaptor protein GRB2 on their intracellular domain, which in turn recruits SOS to the membrane to induce RAS GTP binding and activation. Active RAS phosphorylates RAF, which in turn activates serine/threonine phosphorylation cascades. Extensive crosstalk exists between the extracellular signal-regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK) signaling cascades, with ERK predominantly involved in cell growth and differentiation, p38 in cytokine production and apoptosis, and JNK in survival, apoptosis, and inflammation.

A role for RAS in the regulation of VSMC proliferation was first proposed in the early 1990s using a variety of in vitro and in vivo models of cellular proliferation. Although H-RAS is constitutively expressed in VSMCs, mitogen-dependent induction of H-ras mRNA and protein is seen during early cell cycle progression in synchronized cell populations. Peak expression is seen in mid- to late G1 before initiation of S phase DNA synthesis and susceptible to physiological interference by cAMP. H-RAS mediates VSMC proliferation in cellular models of vascular injury and stable expression of constitutively active H-RAS induces morphological changes and mitogenesis and increased ERK2 expression and phosphorylation. Highly relevant to establishing a role for H-RAS in the regulation of VSMC proliferation in vivo are studies showing that local delivery of H-ras dominant negative mutant plasmids and adenovirus-mediated transfer of dominant negative H-ras or prevention of posttranslational modification by local delivery of a RAS farnesyl transferase inhibitor all result in inhibition of neointimal thickness.

In this issue of Circulation Research, Sedding et al present compelling evidence lending further support for the hypothesis that RAS functions as a primary regulator of VSMC proliferation and migration and that pharmacological strategies targeting RAS represent effective strategies to reduce neointima formation and uncontrolled VSMC proliferation following vascular injury. These findings are highly significant given the prominent role played by fibroproliferative—

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active deficits in the onset and progression of vascular disease. Following a detailed description of phenomenology related to the pharmacological response to 3-deazaadenosine (c3Ado) in terms of cell growth and migration, a series of molecular studies addressing the actions of the drug on RAS signaling were completed. C3Ado is a structural analog of adenosine that is deficient in its ability to interact with the adenosine receptor and instead inhibits $S$-adenosylhomocysteine hydrolase (SAH-hydrolase). c3Ado dose-dependently inhibits human coronary VSMC proliferation and migration in vitro and these effects are associated with increased expression of the cyclin-dependent kinase inhibitors p21war1/cip1 and p27kip1; decreased expression of G1/S cyclins A, B, D, and E; inhibition of retinoblastoma hyperphosphorylation; and reduced ERK1/2 and Akt phosphorylation. The ability of c3Ado to inhibit early cell cycle progression of VSMCs in response to mitogenic stimulation was found to be mediated by inhibition of RAS carboxyl methylation, membrane translocation, and activation (Figure). The specificity of these biochemical interactions was elegantly shown in molecular rescue experiments where a constitutively active RAS mutant was found to abrogate the effects of c3Ado on cell proliferation. After making a compelling case in vitro, the investigators went on to test their hypothesis in an in vivo model of mechanical injury to the femoral artery in mice. In these studies, oral C3Ado administration was found to prevent RAS activation, ERK1/2 and Akt phosphorylation, and neointima formation following dilation-induced vascular injury.

From a mechanistic perspective, c3Ado functions as a potent inhibitor of SAH-hydrolase, an enzyme that regulates cellular SAH levels by catalyzing its hydrolysis into adenosine and homocysteine. Elevated SAH levels following c3Ado administration leads to product inhibition of $S$-adenosylmethionine (SAM)-dependent methyltransferases. As a result of this inhibition, posttranslational methylation of CAAX sequence containing proteins, such as the RAS GTPases, is inhibited. As noted above, RAS methylation is among several key posttranslational modifications required for translocation and membrane association of RAS. Membrane association is in turn essential for activation of the RAS signaling pathway and, consequently, inhibition by c3Ado inhibits cell cycle progression and proliferation of VSMC. A significant challenge in the interpretation of the data summarized in this report is the degree to which other relevant actions of c3Ado including inhibition of thrombin-stimulated production of platelet-derived growth factor, expression of endothelial leukocyte adhesion molecule-1, production of cellular arachidonic acid and reactive oxygen species, inhibi-
bition of tumor necrosis factor-α production, and its modulation of endothelial adhesion molecules contribute to the vascular effects of this molecule. Further investigations are needed to determine the degree to which other CAAX GTPases are inhibited by c3Ado within the vasculature and the relative predominance of otherwise overlapping signaling cascades in the regulation of VSMC phenotypes. Finally, studies are needed to evaluate the bioavailability of c3Ado and its relative efficacy in humans. These issues notwithstanding, the absence of overt toxicity, as measured by the lack of apoptosis, necrosis, or otherwise toxic effects within the vascular wall, suggests that pharmacological inhibition of SAH-hydrolyase and cellular methyltransferase activities may represent safe approaches in the management of proliferative diseases of the vascular wall. Indeed, continued efforts to identify and characterize small molecules interfering with RAS activation may be fruitful in developing safer and more efficient therapeutic strategies for the prevention of vascular proliferative disorders.

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References


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