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Activate Rac to rescue new vessels

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Vascular endothelial growth factor (VEGF) is a major driver of physiologic and pathologic vascularization, but VEGF therapies generally fail to sustainably revascularize ischemic tissues. In this issue of Blood, Hoang and coworkers demonstrate that Rac1 may be the missing ingredient for achieving this goal.1

Vascular insufficiency limits recovery from acute traumatic injuries in soft tissues and is a major risk factor for limb loss in common chronic diseases of aging such as type 2 diabetes and peripheral vascular disease. Angiogenic growth factors such as VEGF and drugs that enhance proangiogenic signaling pathways downstream of these growth factors, such as nitric oxide donors, have been used in efforts to restore blood flow to such ischemic tissues.2,3 To date, these approaches have achieved only limited clinical success. However, studies of revascularization in mice are beginning to provide insights into how a durable restoration of tissue perfusion might be achieved.

Initial attempts using VEGF to induce growth of new blood vessels into ischemic tissues resulted in unstable vessels that regressed and did not develop into a stable mature vasculature. With an increasing understanding that formation of stable vascular beds during development requires recruitment of pericytes and hematopoietic cells as well as endothelial cells, we now realize that cooperation between several growth factors is needed to orchestrate durable tissue revascularization.2 For example, combining VEGF with angiopoietin-1 can elicit a stable vascular bed, whereas neither is effective if used alone. To understand how these factors work together, we need to determine which signaling pathways are activated by each angiogenic growth factor and, eventually, identify the minimal combination of signaling pathways that must be activated to induce a mature vascular bed without tipping the angiogenic balance too far and inducing hypervascular lesions such as keloids or hemangiomas (see figure).

Developmental studies of angiogenesis in transgenic mice have identified several such pathways. Selective deletion of the Rho GTPase Rac1 in endothelial cells disrupts embryonic neovascularization and leads to embryonic death.4 Adult mice lacking one copy of Rac1 in their endothelial cells exhibit impaired recovery from ligation of their femoral artery, a well-established model of fixed ischemic injury, suggesting that Rac1 signaling might be an important player in revascularization of human ischemic tissues.5 Furthermore, Rac1 is not generally necessary for tumor angiogenesis,6 suggesting that this pathway could be therapeutically activated without increasing the risk for promoting malignant vascular growth.

In this context, Hoang and coworkers used an in vivo angiogenesis model in mice to examine the potential synergism between VEGF and Rac1 to improve the structure and function of newly induced blood vessels. Delivering active Rac1 into Matrigel implants using a retroviral vector increased the number and diameter of perfused blood vessels induced by VEGF. Conversely, delivering a dominant-negative form of Rac1 decreased this vascular growth. Vessels induced by VEGF therapy alone and by VEGF expressed in tumors tend to be very leaky, but delivering Rac1 dramatically reduced this vessel defect. To study how Rac1 decreases vessel leakiness, Hoang and coworkers examined effects of active and dominant-negative Rac1 on endothelial cell-cell junctions and their actin cytoskeleton. Rac1 expression induced a reorganization of the actin cytoskeleton into cortical filaments adjacent to cell-cell junctions, and the cells formed more complete junctions with adjacent endothelial cells.

The bioactive lipid sphingosine 1-phosphate is also required for angiogenesis.7 The 2 receptors for this lipid that are expressed on endothelial cells, S1P1 and S1P3, are G protein–coupled receptors that activate Rac1. Sphingosine 1-phosphate stimulates angiogenesis via these receptors by increasing endothelial cell migration and decreasing vascular permeability.7 Hoang and coworkers now show that the S1P1 agonist SEW2871 facilitates VEGF-stimulated angiogenesis in vivo while decreasing neovessel leakiness.

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Because angiopoietin-1 can also normalize VEGF-induced neovessels, the authors examined whether Rac1 mediates this activity. They show that angiopoietin-1 indeed activates Rac1 and that a Rac1 inhibitor blocks the vascular normalizing activity of angiopoietin-1 in the presence of VEGF. Therefore, Rac1-mediated stabilization of cell-cell junctions appears to be a unifying theme for factors that cooperate with VEGF to induce stable mature neovascularization.

These interesting findings may lead to the development of new therapeutic approaches to restore perfusion of ischemic tissues. This effort could be facilitated if we better understood what effectors of Rac1 are critical to this activity and what conditions in acute and chronic ischemic injuries may limit this approach. Hoang and colleagues focus on the well-known effects of Rac1 on actin cytoskeletal dynamics. Another relevant activity of Rac1 for facilitating VEGF signaling involves activation of NADPH oxidases, which reversibly oxidize and inactivate protein tyrosine phosphatases. This prolongs VEGF signaling by preserving the activating phosphorylation of its receptor, which could account for the known trans-activation of VEGFR2 by sphingosine 1-phosphate. The role of Rac1 may extend beyond endothelial cells because similar mechanisms control the duration of signaling through other tyrosine kinase receptors such as platelet-derived growth factor, which drives the recruitment of pericytes that are critical for stabilization of new vessels. Proangiogenic effects of sphingosine 1-phosphate may also involve additional cell types. The S1P1 agonist used here, SEW2871, has been shown to modulate recruitment of neutrophils and macrophages into mouse kidney after an ischemia/reperfusion injury. Macrophages can also support angiogenic responses.

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REFERENCES


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