Ischemic disease is the leading cause of morbidity and mortality in the United States, accounting for almost 50% of overall mortality,\textsuperscript{1} and endothelial dysfunction is a key pathophysiological process that underlies both myocardial and peripheral ischemia. The prevalence of peripheral arterial disease is 12% in the United States, where 150,000 patients undergo lower-limb amputations every year.\textsuperscript{2–4} The overall prognosis after amputation is guarded at best, given a perioperative mortality rate of 5% to 20% and 2-year follow-up mortality rate of 40%.\textsuperscript{5} For those patients who have advanced ischemic cardiac or peripheral vascular diseases and are not suitable for currently available treatment options, such as endovascular intervention or surgical reconstruction, biological revascularization has emerged as a new therapeutic option.\textsuperscript{5}

Vasculogenesis and angiogenesis are the mechanisms responsible for the development of the new blood vessels (neovascularization). Angiogenesis refers to the formation of capillaries from preexisting vessels in the embryo and adult organism, whereas vasculogenesis is the development of new blood vessels via differentiation of endothelial progenitor cells or angioblasts in situ.\textsuperscript{6,7} Vascular endothelial growth factor (VEGF) family members and their receptors are major mediators of the regulatory machinery that governs these processes both during development and in pathological conditions.\textsuperscript{8} VEGF (or VEGF-A, VEGF-1), a primary regulator of angiogenesis and vasculogenesis,\textsuperscript{9} is a hypoxia-inducible endothelial cell (EC) mitogen,\textsuperscript{10} which stimulates EC migration and vessel permeability and promotes EC proliferation and survival of newly formed vessels.\textsuperscript{11} The family of VEGF-related molecules has recently grown and currently consists of five members: VEGF-A, VEGF-B, VEGF-C (VEGF-2), VEGF-D, and placental growth factor (PLGF).\textsuperscript{12} The viral homologues, collectively called VEGF-E, are encoded by different strains of the Orf virus. Whereas the function of VEGF-A and VEGF-C/D are well known for their angiogenic and lymphangiogenic activity, respectively, the function of VEGF-B has received less attention until recently.\textsuperscript{13}

The therapeutic implications of modulating angiogenic growth factor activity were first suggested by the pioneering work of Folkman three decades ago.\textsuperscript{14} His laboratory’s work documented the extent to which tumor development was dependent on neovascularization and suggested that this might involve angiogenic growth factors that could theoretically be blocked, starving the tumor and halting its growth. Recent clinical trial data have suggested that this hypothesis was correct.\textsuperscript{15}

The reverse strategy, augmenting neovascularization with the use of angiogenic cytokines, has been considered a potential means of improving perfusion in ischemic diseases. This approach, termed therapeutic angiogenesis,\textsuperscript{16} has been established by investigations using angiogenic growth factors such as VEGF-A, VEGF-C, fibroblast growth factors (FGFs), and hepatocyte growth factor (HGF), administered as recombinant protein or gene therapy, to expedite and/or augment collateral artery development in animal models of myocardial and hindlimb ischemia. More recent data suggest that the basis for native and therapeutic neovascularization is not restricted to angiogenesis but includes postnatal vasculogenesis as well.\textsuperscript{7}

Multiple pilot clinical trials performed in patients with myocardial ischemia and peripheral arterial obstructive diseases have yielded promising results, including the use of adenovirus-mediated gene transfer of VEGF\textsubscript{121} in patients with critical limb ischemia,\textsuperscript{17} recombinant FGF-2 in patients with coronary artery\textsuperscript{18} and peripheral vascular disease,\textsuperscript{19} Ad5-FGF-4 in patients with stable angina pectoris,\textsuperscript{20} and VEGF-1\textsubscript{21,22} and VEGF-2\textsubscript{23,24} naked DNA transfer in critical limb and myocardial ischemia.

In this issue of Circulation Research, Silvestre et al\textsuperscript{25} elucidate the potential of VEGF-B for therapeutic angiogenesis. This study discloses the role of the only remaining unproven member of the human VEGF family in the regulation of vascular growth in vivo. The data indicate that VEGF-B, via ligation and phosphorylation of VEGFR-1 can activate the Akt-eNOS pathway and thus lead to augmentation of angiogenesis (Figure). The role of VEGFR-1 is particularly intriguing given the role of that receptor as a mediator of stem-cell recruitment and mobilization from the bone marrow.

VEGF-B\textsuperscript{26–28} is a secreted growth factor that has intimate sequence homology with VEGF-A. The VEGF-B gene, which is located on human chromosome 11q13, yields two polypeptide forms, VEGFB\textsubscript{167} and VEGFB\textsubscript{186} by alternative splicing. The coding sequence of the first five exons is incorporated into both splice forms. The amino acid sequence of VEGF-B\textsubscript{167} and VEGF\textsubscript{185} is \textasciitilde44% identical and their intermolecular disulfide bridging patterns are similar. Exon
Aase et al. claimed that these mice show a subtle cardiac recovery from experimentally induced myocardial ischemia, smaller hearts, dysfunctional coronary arteries, and impaired skeletal muscle, and smooth muscle cells of large vessels. Development and is widely distributed in cardiomyocytes, hypoxia. VEGF-B is expressed beginning early in fetal development and is widely distributed in cardiomyocytes, skeletal muscle, and smooth muscle cells of large vessels. In adult mice, VEGF-B mRNA is abundant in heart and kidney, where it overlaps with strong VEGF-A expression. The receptor specificities are only partially overlapping between VEGF-B and VEGF-A and, as indicated by the embryonic lethality of the VEGF-A knockout mouse, no other growth factors can compensate for the loss of even a single VEGF-A allele. Studies using VEGF-B knockout mice have yielded slightly conflicting results regarding the role of VEGF-B in angiogenesis and the development of cardiovascular system. In contrast to VEGF-A knockout mice, VEGF-B knockout mice are viable and fertile. However, while Bel-lomo et al. demonstrated that VEGF-B knockout mice have smaller hearts, dysfunctional coronary arteries, and impaired recovery from experimentally induced myocardial ischemia, Aase et al. claimed that these mice show a subtle cardiac phenotype and that VEGF-B is not required for proper development of the cardiovascular system either during development or for angiogenesis in adults. More complicated is the fact that VEGF-B can form stable heterodimers with VEGF-A and is generally coexpressed with VEGF-A. Although VEGF-B has been reported to behave as an endothelial cell mitogen, part of the mitogenic activity reported may be due to VEGF-B/VEGF-A heterodimers.

VEGF-A signals via three receptor tyrosine kinases, VEGFR-1 (Flt-1) and VEGFR-2 (Flk-1, KDR) and neuropilin-1. VEGF-B has strong intrinsic tyrosine kinase activity and is the major mediator of EC proliferation in response to VEGF-A. VEGF-B can bind to two of the VEGF-A receptors, VEGFR-1 and neuropilin-1, suggesting that it may regulate the bioavailability and/or activity of VEGF-A (Figure). VEGFR-1 also serves as a receptor for PLGF. VEGFR-1 undergoes weak tyrosine autophosphorylation in response to VEGF-A. Experiments evaluating the role of VEGFR-1 have provided intriguing but controversial data. Targeted gene inactivation of VEGFR-1 leads to embryonic death around embryonic day 8.5. ECs develop but do not form organized vessels. Excessive proliferation of angioblasts appears to be responsible for lethality in these mice, indicating that, during early development, VEGFR-1 can function as a negative regulator of VEGF-A action. This notion is supported by the fact that targeted mutation of the sole tyrosine kinase domain of VEGFR-1 does not result in lethality or any overt defect in vascular development. VEGFR-1 expression is upregulated by hypoxia via an HIF-1-dependent mechanism. An alternatively spliced, soluble form of VEGFR-1 (sFlt-1) is an inhibitor of VEGF-A.

Initially, VEGFR-1 was not regarded as a receptor directly transmitting a mitogenic signal, but as a decoy receptor, which negatively regulates the activity of VEGF-A on the vascular endothelium, by inhibiting VEGF-A binding to VEGFR-2. Recently, the function of VEGFR-1 in postnatal life has been partly unveiled by another VEGFR-1–specific ligand, PLGF. The observed potentiation of the action of VEGF-A by PLGF could be explained, in part, by displacement of VEGF-A from VEGFR-1 binding. Not only the membrane-bound form of VEGFR-1, but also by sFlt-1, could play this decoy role. Recent studies have indicated a synergistic action between VEGF-A and PLGF in vivo, as evidenced by impaired tumorigenesis and vascular leakage in PLGF knockout mice. Moreover, PLGF can augment angiogenesis indirectly by increasing VEGF-A expression from fibroblasts in ischemic tissues. The downstream signaling of VEGFR-1 is also controversial. Gille et al. identified a repressor motif in the juxtamembrane region of VEGFR-1 that impairs phosphatidylinositol 3'-kinase (PI3K) activation in response to VEGF-A. Other studies as well as the present study indicated that VEGFR-1 is able to interact with various signal-transducing proteins and generate, under certain conditions, a mitogenic signal. VEGFR-1 has been shown to mediate hematopoiesis and recruitment of bone marrow–derived cells such as endothelial progenitors and monocytes. Through recruitment of monocytes via VEGFR-1, PLGF promotes collateral vessel growth in a model of myocardial ischemia. Therefore, the conflicting reports may be due, in part, to the fact that the functions and signaling properties of VEGFR-1 can be different depending on the developmental stage of the animal, the context (ischemia, tumor, etc), and tissue being studied. Recently, the role of neuropilin-1 in angiogenic regulation was suggested by the observation that it can act as an isoform-specific coreceptor for VEGF and PLGF. In addition to its neuronal expression, neuropilin-1 is also present in the developing embryo, in ECs of capillaries and blood vessels, and in mesenchymal cells surrounding the blood vessels, as well as in certain other non-neuronal tissues, including the endocardial cells of the embryonic heart. It has been proposed that neuropilin-1 enhances the binding of VEGF to VEGFR-2 and VEGF-mediated chemotaxis.

While Silvestre et al. propose a previously unrecognized role of VEGF-B in the promotion of angiogenesis in vivo, a number of critical questions remain unanswered. First, as
mentioned above, the angiogenic activity of VEGF-B administration in vivo, especially in ischemic tissue, may be complicated by the interaction with VEGF-A and VEGF-R2 since it is well known that both are upregulated in ischemia itself. The signal transduction studies were performed in the Matrigel model in which the regulation of other receptors, especially VEGFR-2, was not simultaneously evaluated. Accordingly, the possibility that VEGF-A–VEGFR-2 activity was involved cannot be ruled out. One possibility is that VEGF-B forms a heterodimer with VEGF-A and augments angiogenesis through VEGF-A. Other possibilities include a PLGF-like mechanism in which VEGF-B displaces VEGF-A from VEGFR-1, thereby indirectly increasing VEGF-A–mediated angiogenesis. Finally, and perhaps most interesting, ligation of VEGFR-1 may mobilize bone marrow–derived monocytes and progenitor cells and may contribute to neovascularization by augmenting vasculogenesis. The direct link of VEGFR-1 and Akt-eNOS pathway in augmentation of angiogenesis in ischemia needs to be clarified since this pathway is known to be involved in the survival pathway of ECs by inhibiting apoptosis.48,49 Further studies will be required to dissect the role of VEGF-B in the promotion of angiogenesis in vivo and the regulatory mechanisms involving VEGFR-1 phosphorylation and the relationship with the downstream PI3K–Akt–eNOS axis. In addition, although the authors demonstrate robust proangiogenic effects in both Matrigel and hindlimb ischemic models, more direct evidence of endothelial and smooth muscle proliferation, using cell proliferation markers such as bromodeoxyuridine and Ki67, would elucidate the temporal-spatial effects on EC proliferation in the context of new vessel formation. Finally, the role of neuropilin-1 in angiogenic regulation by VEGF-B still needs to be determined.

Notwithstanding these gaps in our understanding of the mechanism, a significant impact of VEGF-B on postnatal neovascularization is clearly elucidated by Silvestre et al.,25 thus adding another member of the VEGF family to the potential toolbox that future physicians may reach into, capitalizing on a natural mechanism for therapeutic neovascularization.

References


**Key Words:** vascular endothelial growth factor-B | vascular endothelial growth factor receptor-1 | VEGFR-1 | angiogenesis | Akt
All in the Family: VEGF-B Joins the Ranks of Proangiogenic Cytokines
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