VASCULAR ENDOTHELIAL GROWTH FACTOR-B IN PHYSIOLOGY AND DISEASE

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Bry M, Kivelä R, Leppänen V-M, Alitalo K. Vascular Endothelial Growth Factor-B in Physiology and Disease. Physiol Rev 94: 779–794, 2014; doi:10.1152/physrev.00028.2013.—Vascular endothelial growth factor-B (VEGF-B), discovered over 15 years ago, has long been seen as one of the more ambiguous members of the VEGF family. VEGF-B is produced as two isoforms: one that binds strongly to heparan sulfate in the pericellular matrix and a soluble form that can acquire binding via proteolytic processing. Both forms of VEGF-B bind to VEGF-receptor 1 (VEGFR-1) and the neuropilin-1 (NRP-1) coreceptor, which are expressed mainly in blood vascular endothelial cells. VEGF-B-deficient mice and rats are viable without any overt phenotype, and the ability of VEGF-B to induce angiogenesis in most tissues is weak. This has been a puzzle, as the related placenta growth factor (PlGF) binds to the same receptors and induces angiogenesis and arteriogenesis in a variety of tissues. However, it seems that VEGF-B is a vascular growth factor that is more tissue specific and can have trophic and metabolic effects, and its binding to VEGFR-1 shows subtle but important differences compared with that of PlGF. VEGF-B has the potential to induce coronary vessel growth and cardiac hypertrophy, which can protect the heart from ischemic damage as well as heart failure. In addition, VEGF-B is abundantly expressed in tissues with highly active energy metabolism, where it could support significant metabolic functions. VEGF-B also has a role in neuroprotection, but unlike other members of the VEGF family, it does not have a clear role in tumor progression. Here we review what is hitherto known about the functions of this growth factor in physiology and disease.

I. INTRODUCTION

The blood circulatory system is the first organ system to develop, starting in the third week of development in the human embryo when passive diffusion of nutrients and waste products becomes insufficient for development (118). Members of the vascular endothelial growth factor (VEGF) family are major regulators of blood and lymphatic vessel development. Vasculogenesis, referring to the initial formation of the primitive vascular plexus, begins when hemangioblast progenitors of mesodermal origin migrate and differentiate to form primary blood islands, from which both hematopoietic and endothelial cells are formed (114). The primitive vascular plexus is subsequently remodeled into a network consisting of arteries, veins, and capillaries of different sizes, and the vessels are stabilized by recruited mural cells, such as pericytes and smooth muscle cells. Angiogenesis, the process of blood vessel formation from preexisting vessels, occurs through either sprouting or intussusception (splitting) in development and in various disease processes (reviewed in Ref. 30; see also Ref. 113).

VEGF, the archetypal angiogenic growth factor, was first identified as a permeability-inducing factor secreted by tumor cells (121) and later as a growth factor for vascular endothelial cells (ECs) (46, 86). Other members of the VEGF family were subsequently characterized, but VEGF-B has remained one of the more ambiguous growth factors, as efforts to discover angiogenic effects for it in vivo have given mainly negative results. The aim of this review is to summarize what is currently known about VEGF-B to endeavor to elucidate the main functions for this growth factor in physiology and disease. We first briefly review what is known about the other members of the VEGF family and their receptors before concentrating on VEGF-B.

II. THE VASCULAR ENDOTHELIAL GROWTH FACTOR FAMILY

The VEGF family consists of five secreted dimeric glycoprotein growth factors in mammals: VEGF (or VEGF-A), VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PlGF) (for reviews, see Refs. 64, 125). As members of the platelet-derived growth factor (PDGF)/VEGF superfamily, VEGFs contain a VEGF/PDGF homology domain with eight conserved cysteine residues involved in inter- and intramolecular disulfide bond formation. The VEGF ligands bind with differing specificities to three mainly endothelial transmembrane tyrosine kinase receptors, VEGFR-1/fms-like...
tyrosine kinase 1 (Flt1), VEGFR-2/human kinase insert domain receptor (KDR)/mouse fetal liver kinase 1 (Flk1), and VEGFR-3/fms-like tyrosine kinase 4 (Flt4) (for recent reviews, see Refs. 77, 123) (FIGURE 1A). Neuropilins (NRP)-1 and NRP-2, which transduce semaphorin signals mainly in neuronal cells, also provide coreceptor functions for the various VEGFRs in endothelial cells (reviewed in Ref. 106).

VEGF is essential for vasculature development, as mice lacking even a single VEGF allele die during embryogenesis as a result of impaired angiogenesis and blood island formation (23, 45). VEGF binds to VEGFR-1 and VEGFR-2 (35, 111), as well as to NRP-1 and NRP-2 (55, 130).

In addition to functioning as a mitogen for ECs, VEGF also regulates endothelial cell survival (6, 15, 16). Downstream signaling by VEGFR-2, following VEGF binding, receptor dimerization, and autophosphorylation of several tyrosine kinase residues, involves numerous pathways, including the phospholipase C (PLC)-γ/protein kinase C (PKC) pathway, resulting in the activation of the c-Raf-MEK-MAP kinase cascade and cell proliferation (58, 135, 144). Activation of the PI3K-Akt signal transduction pathway stimulates cell survival and migration and eNOS activation (54). Administration of VEGF results in robust angiogenesis in various tissues (reviewed in Ref. 44), but VEGF is also a potent inducer of vascular permeability and inflammation (98), which has hampered its application to therapeutic angiogenesis.

VEGF and VEGFR-1 are upregulated in hypoxia via hypoxia inducible factor (HIF)-1α-mediated transcription (50, 110, 119). Nutrient and oxygen deprivation also induce the potent metabolic regulator PGC-1α (peroxisome proliferator-γ coactivator-1α), which can induce VEGF (and VEGF-B) independently of HIF-1α in skeletal muscle (8, 18), highlighting the close coordination of blood supply and regulation of tissue metabolism.

PlGF was isolated from a human placental cDNA library shortly after the discovery of VEGF (for a review, see Ref. 38). PlGF exists as four isoforms in humans, but only one (PlGF-2) in mice. PlGF is expressed abundantly in the placenta, and at lower levels in for example the heart, lungs, and skeletal muscle (for reviews, see Refs. 34, 38). PlGF is very similar to VEGF-B in many respects, but their effects on angiogenesis and arteriogenesis seem to differ considerably. PlGF binds to heparan sulfate and to the same receptors as VEGF-B, namely, VEGFR-1 and NRP-1 (95, 104). PlGF gene-targeted mice are viable, but their angiogenesis and arteriogenesis are impaired in ischemia, inflammation, and wound healing, as well as in the hypoxic brain (24, 51). PlGF deficiency was shown to impair adipose tissue growth at least in part by inhibiting angiogenesis (90). Unlike

![FIGURE 1](https://physrev.physiology.org/)

**FIGURE 1.** The VEGF family, receptor interactions, and structure of the VEGF-B gene. A: specific binding of VEGFs to their endothelial receptors. The dashed line indicates proteolytic processing that is required before VEGF-C and human VEGF-D can bind to VEGFR-2. Proteolysis activates VEGF-C and VEGF-D. Recent data show that the collagen- and calcium-binding EGF domains 1 (CBBE1) protein promotes pro-VEGF-C binding to VEGFR-3 followed by proteolytic cleavage by a disintegrin and metalloprotease with thrombospondin motifs-3 (ADAMTS3), resulting in the mature form of VEGF-C and increased VEGFR-3 signaling (69a). B: schematic structure of the Vegfb gene and its alternatively spliced transcripts. Shown are exons (numbered) and introns with the alternative splice acceptor (SA) sites that produce the VEGF-B167 and VEGF-B186 isoforms, where exon 6A is lacking from VEGF-B167 mRNA. The arrowhead indicates the site of proteolytic processing of VEGF-B186. Red, sequence encoding the VEGF homology domain.
VEGF-B, PlGF is able to stimulate angiogenesis and collateral vessel growth in the ischemic heart and limb with similar efficiency as VEGF (92). PlGF also increases vessel permeability (20, 99) and inflammation (103, 120). However, in a recent report, intracranial PlGF administration using an adenovirus-associated virus (AAV) vector strongly stimulated angiogenesis and arteriogenesis in the brain without significant inflammation or edema (52). PlGF has been reported to stimulate the migration and survival of endothelial cells (4, 153) and the proliferation of smooth muscle cells (13).

It has been reported in numerous studies that PlGF can make heterodimers with VEGF, and some results have suggested that it regulates intermolecular crosstalk between VEGFR-1 and VEGFR-2, in part via VEGF/PlGF heterodimer formation (21, 104). However, PlGF also seems to be capable of inducing unique signals downstream of VEGFR-1 compared with VEGF and stronger VEGFR-1 tyrosine phosphorylation than VEGF-B (7, 9, 82). PlGF may mediate most of its arteriogenic effects via recruitment of growth factor-secreting monocytes that express VEGFR-1 (108).

Much remains to be learned about the mechanisms of PlGF-induced vascularization. Interestingly, in the mouse heart, PlGF was recently shown to induce myocardial angiogenesis and cardiac hypertrophy through a nitric oxide (NO)-dependent mechanism via the Akt/mTORC1 pathway (69). However, in another recent study, PlGF only secondarily supported pressure overload-induced cardiac hypertrophy through a paracrine mechanism via endothelial cells and fibroblasts to induce capillary growth and fibroblast proliferation (3).

A. VEGFR-1

VEGFR-1 is composed of an extracellular ligand binding part, a transmembrane domain, intracellular tyrosine kinase (TK) domain, and carboxy-terminal region (126). The extracellular part consists of seven immunoglobulin-like (Ig) domains. VEGF and PlGF bind to the second and third Ig domains of VEGFR-1 and induce productive receptor dimers for autophosphorylation in the TK domain (35, 136). Mice lacking VEGFR-1 produce an excess of ECs and a disorganized vasculature and die in utero at E8.5–9 (48, 49). This suggests that at least during the development of the vascular system, VEGFR-1 is mainly a negative regulator of angiogenesis because of its high affinity for VEGF, as it can sequester VEGF away from actively signaling VEGFR-2 homodimers. Similarly, VEGFR-1 deletion in adult mice resulted in endothelial cell proliferation and increased vessel density in the lungs, heart, retina, brain, kidneys, and liver and protected against myocardial infarction (MI), at least in part via upregulation of VEGFR-2 signaling (63). This is consistent with the fact that mice engineered to express only a truncated form of VEGFR-1 lacking the TK domain responsible for signaling are viable and display no obvious vascular defects (62).

The tyrosine kinase activity of VEGFR-1 in cultured endothelial cells is weak, and its downstream signaling is poorly understood (reviewed in Ref. 124). On the other hand, in addition to ECs, VEGFR-1 is expressed at least in monocytes/macrophages, and activation of its TK domain is required for their activation and migration in response to ligand (11, 31). VEGFR-1 is also expressed in neuronal cells, with implications in glial cell development and survival (28, 79, 143). A soluble form of the VEGFR-1 extracellular domain (sVEGFR-1), which is able to neutralize VEGF (74), plays a role in regulating local guidance of emerging vessel sprouts (26), and it has been shown to be involved in the pathogenesis of preeclampsia (87, 105a, 112). Interestingly, VEGFR-1 is expressed also in the endothelium of coronary arteries in adult mouse and fetal human heart, whereas VEGFR-2 is not, suggesting that VEGFR-1 could play a role in coronary vessel development (70, 75, 105). VEGFR-1 mRNA was also detected in cultured neonatal rat cardiomyocytes; however, the expression level was three orders of magnitude lower than in cultured endothelial cells (149). VEGFR-1 can heterodimerize with VEGFR-2 and thus modify signaling through VEGFR-2 homodimers (32). It is currently not known if the expression pattern of VEGFR-1 is changed in pathological conditions in, e.g., the heart or adipose tissue.

B. Neuropilins

The neuropilins, NRP-1 and NRP-2, were originally identified as receptors for semaphorins that mediate repulsive signals during neuronal axon guidance (27, 78). NRP-1 and NRP-2 lack cytoplasmic enzyme activity, but they function as coreceptors, complexing with other transmembrane receptors such as the endothelial VEGFRs (reviewed in Refs. 106, 148). VEGF isoforms bind with differing specificities to NRP-1 and NRP-2. NRP-1, which binds also VEGF-B and PlGF (93, 95), is essential for the formation of the vasculature, as mice lacking NRP-1 develop vascular defects and die at E13.5 (73). In the heart, NRP-1 is expressed along with VEGFR-1 in coronary vessels, myocardial capillaries, and epicardial vessels (72, 105). Interestingly, NRP-1 is also expressed in the developing myocardium and endocardium in mouse embryos at E12.5 (93).

III. VEGF-B

A. Structure and Processing

VEGF-B (previously also known as VRF, VEGF-related factor), first discovered in 1996, has a structure very similar to that of VEGF, and mouse VEGF-B shares ~43% amino acid sequence identity with mouse VEGF164 (56, 101). The Vegfb gene is highly conserved in mammals with ~88% homology between the mouse and human growth factors at the amino acid sequence level (101). A primitive form of Vegfb with only 26% homology to human Vegfb may be
present in frogs, but Vegfb has not been identified in zebrafish (117). Vegfb consists of seven exons and generates two isoforms because of the existence of alternative splice acceptor sites in exon 6 (56, 102) (FIGURE 1B). VEGF-B167 has a highly basic heparin-binding carboxy terminus, whereas VEGF-B186 contains a hydrophobic carboxy terminus and is modified by O-glycosylation and proteolytic processing (102). VEGF-B167 thus binds tightly to heparan sulfate proteoglycans on the cell surface and in the extracellular matrix, whereas at least the uncleaved VEGF-B186 is freely diffusible. The molecular masses of homodimers of VEGF-B167 and VEGF-B186 are 42 and 60 kDa, respectively. Both isoforms are simultaneously expressed in various tissues and bind to VEGFR-1 (and sVEGFR-1) as well as to NRP-1, but not to the major mitogenic endothelial receptors VEGFR-2 and VEGFR-3 (93, 100). Proteolytic processing of VEGF-B186 is required for its binding to NRP-1 (93). In culture, VEGF-B is also able to form heterodimers with VEGF (101), but covalent heterodimers of this type have not been demonstrated in vivo.

Unlike VEGF, VEGF-B expression does not seem to be directly regulated by hypoxia (41), although hypoxia appeared to induce VEGF-B in the mouse retina in a recent report (128). VEGF-B has a wide tissue distribution in mice, being most abundant in tissues with high metabolic activity such as the myocardium, skeletal muscle, and vascular smooth muscle, as well as in brown adipose tissue, brain, kidney, and parietal cells of the stomach (1, 22, 80, 101). This suggests a role for VEGF-B in coordinating the cross-talk between angiogenesis and metabolism.

Analysis of VEGF-B mRNA expression in normal C57Bl/6J mouse tissues by quantitative RT-PCR confirmed that VEGF-B expression levels were the highest in the heart and skeletal muscle (FIGURE 2A) (see also Ref. 59). Slow-twitch metabolic muscles such as soleus and the red part of the gastrocnemius had higher levels of VEGF-B than fast-twitch glycolytic muscles, such as tibialis anterior and the white part of the gastrocnemius, further supporting the idea that VEGF-B is involved in aerobic energy metabolism. The levels of VEGF-B in brown adipose tissue were between those found in slow and fast muscles, while in visceral white adipose tissue the levels were lower, although still higher than in most other tissues. Staining of VEGF-B gene-targeted Vegfb−/−;Z/2 mouse hearts (14) for β-galactosidase shows strong VEGF-B expression in the cardiomyocytes (FIGURE 2B).

Analysis of VEGF-B mRNA expression in healthy human tissues using the Medisapiens database consisting of hundreds of microarrays of various human tissues (www.medisapiens.com) revealed the highest expression levels in the heart, adipose tissue, skeletal muscle, and blood vessels, which also expressed high levels of VEGFR-1 and NRP-1 (FIGURE 3, A AND B). The adipose tissue samples from humans mainly represent biopsies from subcutaneous depots, which may have different expression levels than visceral fat. Interestingly, VEGF-B and PlGF had very different expression patterns (FIGURE 3C). VEGF, which is required during embryonic development, showed the broadest expression across different tissues compared with VEGF-B and PlGF.

B. Differences Between VEGF-B and PlGF

Although VEGF-B and PlGF transduce their signals via VEGFR-1 and NRP-1, their effects on blood vessel growth, permeability, and tissue inflammation differ considerably (20). However, both growth factors are dispensable for normal embryonic development.

Like many other receptor tyrosine kinases, the VEGFRs are activated by ligand-induced dimerization, followed by tyrosine autophosphorylation of the intracellular kinase domain to generate downstream signaling (83). For ligand binding, the VEGFRs share a conserved interface in Ig domain (D) 2, while D3 provides additional interactions (29, 67, 84, 142). The membrane-proximal domains 4–7 (D4–7) facilitate dimerization through homotypic contacts formed between D4–5 and D7 (FIGURE 4A) (85, 116, 145). VEGFRs show a hinge-like rigid body motion in D2–3 (19, 84), suggesting that ligand-induced D2–3 reorientation initiates the cascade of structural rearrangements behind the homotypic interactions and receptor activation.

Crystal structure analysis shows that VEGF-B, VEGF, and PlGF interact with VEGFR-1 D2 in a similar manner (29, 67, 68, 142) (FIGURE 4B). VEGF and PlGF also require D3 of VEGFR-1 for high-affinity binding (29, 33). However, D3 does not provide additional affinity for VEGF-B binding to VEGFR-1 (7). The receptor specificity of VEGF ligands is determined by an amino-terminal alpha helix and three peptide loops. VEGF-B fails to efficiently induce productive VEGFR-1 dimerization and signaling as a result of the unique structure of VEGF-B loop 1 (L1; FIGURE 4, C AND D). Importantly, swapping L1 from PlGF to VEGF-B conferred the angiogenic properties of PlGF to the resulting chimera and vice versa. Since L1 in VEGFs interacts only with D3 (19, 84), the poor ability of VEGF-B to activate VEGFR-1 compared with PlGF can be explained by inadequate L1-D3 interactions. These results also suggest that, at least in endothelial cells, the effects of VEGF-B occur through inhibition of the interaction of other ligands with VEGFR-1 (see also FIGURE 6), thus inducing more efficient and spatially controlled interaction of VEGF with the highly mitogenic VEGFR-2. How VEGF-B and PlGF signaling interactions are regulated in macrophages, which also express VEGFR-1 and NRP-1 (25, 31), remains to be studied.

C. Role in Angiogenesis

Although initial reports indicated that VEGF-B is able to stimulate EC growth in vitro (101), this may have been due
to heterodimer formation with endogenous VEGF, as the ability of recombinant VEGF-B to stimulate sprouting angiogenesis directly is poor in most tissues. VEGF-B did not stimulate vessel growth when delivered into muscle or peri-adventitial tissue via adenoviral vectors (17, 115), and transgenic overexpression of VEGF-B in the skin increased blood vessel density only minimally, although it increased capillary diameter to some extent (72). VEGF-B did not improve vascular growth in the ischemic limb in two studies (81, 88), although opposite results have also been published (127, 140). On the other hand, VEGF-B overexpressed in endothelial cells of transgenic mice was claimed to potentiate, rather than initiate, angiogenesis (97). Overexpression of VEGF-B was also shown to aggravate pathological retinal and choroidal neovascularization in mice (152) and was proposed to act as a survival factor for ECs, regulating the expression of vascular pro-survival genes via both NRP-1 and VEGFR-1 (150).

D. Effects of VEGF-B in the Heart

Mice deficient of VEGF-B are viable and fertile and display only mild phenotypes in the heart. This is manifested as an atrioventricular conduction abnormality characterized by a
prolonged PQ interval in one strain (2), or as a smaller heart size with slightly dysfunctional coronary vasculature and impaired recovery after myocardial ischemia in another (14). The latter mouse strain also showed some resistance to development of pulmonary hypertension and vascular remodeling during chronic hypoxia (141). VEGF-B-deficient rats had no obvious vascular or developmental defects (75). After experimental MI, larger infarct scars were detected in cardiac sections from the VEGF-B gene-targeted rats, although heart function was not significantly affected (75). Collectively, the results from VEGF-B gene-deleted mice and rats suggest a role for VEGF-B in the heart in pathological settings.

Interestingly, VEGF-B expression is spatially and temporally correlated with the commencement and progression of coronary endothelial growth in the heart, suggesting that it could play a role in coronary vessel development (14). However, no obvious defects have been reported in mouse embryos lacking VEGF-B. Importantly, however, antibodies against VEGF-B were found to inhibit coronary artery development in the quail embryo (138, 139).

An excess of VEGF-B activates the Akt/mTORC1 and Erk1/2 MAPK pathways in the heart (FIGURE 6) (75). Activation of Erk1/2 occurred at least in part through VEGFR-2 phosphorylation, an indirect result of VEGF-B binding to...
VEGFR-1, which increased the availability of VEGF for activation of VEGFR-2 (75). The Erk1/2 activation via VEGFR-2 is likely responsible for the coronary vessel growth and arterialization observed in the VEGF-B overexpressing hearts. Importantly, blocking VEGFR-2 signaling was able to reduce the vessel enlargement seen in VEGF-B overexpressing mice. On the other hand, in contrast to the hypertrophy induced by PlGF (69), blocking NO signaling did not affect the VEGF-B-induced cardiac hypertrophy (75). Importantly, the VEGF-B-induced hypertrophy remained physiological even in old rats, and did not advance into heart failure (75).

E. VEGF-B as a Therapeutic Factor in Cardiac Disease

Several studies have indicated a role for VEGF-B in coronary vasculature and in cardioprotection. VEGF-B levels decreased following experimentally induced MI in rats as well as in heart failure resulting from transverse aortic constriction (65, 151). Myocardial expression levels of VEGF-B were decreased also in human heart disease (75). Low VEGF-B plasma levels were found to accurately predict left ventricular dysfunction and remodeling after MI,
suggesting that VEGF-B could be used as a prognostic biomarker with stronger predictive value than troponin T (36, 37). Interestingly, the opposite was true for PlGF, as increased levels of PlGF predicted heart failure (36). However, to date, studies about the kinetics of VEGF-B after ischemic damage in the myocardium are lacking.

VEGF-B does not seem to induce capillary angiogenesis in most studies, but mainly enlargement of myocardial capillaries and growth of coronary arteries (20, 72). In addition, VEGF-B counteracts apoptosis of cardiomyocytes and improves cardiac contractility in animal models of human heart disease (149). However, the responsible mechanisms and signaling between endothelial cells and cardiomyocytes in these settings are still largely unknown. Therapeutic vectors expressing VEGF-B have shown promise in several experimental models of myocardial ischemia or heart failure (TABLE 1). An overdose of VEGF-B_{186} via transient adenoviral delivery into the pig myocardium enlarged myocardial vessels after acute infarction, which was inhibited by administration of either soluble VEGFR-1 or soluble NRP-1, but not by blocking VEGFR-2 signaling or nitric oxide production (81). Adenoviral delivery of VEGF-B_{167} activated the PI3K/Akt pathway, enlarged capillaries and ameliorated angiotensin II-induced diastolic dysfunction in rats (122). Adenoviral delivery of VEGF-B_{186} was equally capable of enlarging myocardial capillaries in mice (66). However, AAV-mediated administration of VEGF-B_{167} preserved cardiac contractility and prevented cardiomyocyte apoptosis after experimental MI in rats without significant vascular effects (149). Similar results were achieved in dogs subjected to tachypacing-induced development of dilated cardiomyopathy, where AAV-VEGF-B administration delayed the progression towards heart failure (107). In these studies, it is important to note that adenoviral vectors mediate very high but transient levels of expression and induce a strong inflammatory response, whereas AAVs mediate steady, long-term expression without significant inflammation.

Transgenic overexpression of VEGF-B induced cardiac hypertrophy as well as capillary enlargement in both mice and rats (20, 72, 88). In rats, VEGF-B also caused a striking growth of the epicardial and subendocardial coronary vessels, which importantly occurred without increasing inflammation or vascular permeability, in contrast to other members of the VEGF family (FIGURE 5) (20). The coronary arterial growth induced by VEGF-B led to increased functional coronary reserve, protecting the heart from ischemic damage caused by coronary artery ligation (75). Systemic delivery of an AAV-VEGF-B vector to adult rats reproduced both the vascular and hypertrophic phenotypes, an important consideration for possible therapeutic strategies. However, the effects of VEGF-B overexpression required 2–3 wk before becoming apparent, so it is unlikely that VEGF-B induced vascular changes would be beneficial during acute myocardial infarction. However, VEGF-B could improve long-term recovery from ischemia and counteract the development of heart failure. Indeed, a VEGF-B_{186} adenovirus was shown to improve systolic function in progressive left ventricular hypertrophy caused by transverse aortic constriction in mice (65). Thus the above studies and others have implicated the heart as a specific target for VEGF-B.

### F. Metabolic Effects of VEGF-B

Interestingly, expression of VEGF-B and a nuclear-encoded mitochondrial gene and protein cluster set appear to be

| Table 1. Experimental models of VEGF-B therapy in the heart |
|----------------|-----------------|-------------------|
| **Vector/Transgene/Isform** | **Species** | **Effect** |
| Transgene (αMHC-VEGF-B_{167}) | Mouse | Cardiomyocyte hypertrophy; myocardial capillary enlargement; ischemia protection ex vivo (72) |
| Transgene (αMHC-genomic VEGF-B) | Rat | Cardiomyocyte hypertrophy; myocardial capillary enlargement; expansion of coronary arteries; no increased inflammation or vascular leakage (20); increased functional coronary reserve; ischemia protection in vivo (75) |
| Adenovirus (VEGF-B_{186}) | Pig | Enlargement of myocardial vessels after myocardial infarction (81) |
| Adenovirus (VEGF-B_{186}) | Mouse | Capillary enlargement (66) |
| Adenovirus (VEGF-B_{186}) | Rat | Capillary enlargement and improved cardiac function in angiotensin II-induced diastolic dysfunction (122) |
| Adenovirus or protein infusion (VEGF-B_{186}) | Mouse | Increased density of capillaries and arterioles in infarct zone (88) |
| Adenovirus (human VEGF-B_{186}) | Mouse | Increased capillary density and cardiomyocyte area in remote myocardium after ischemia (137) |
| AAV2-VEGF-B_{186} | Rat | Antiapoptotic effect on cardiomyocytes after myocardial infarction; cardiomyocyte hypertrophy; no significant vascular effects (149) |
| AAV9-VEGF-B_{186} | Dog | Delayed progression of heart failure during tachypacing-induced cardiomyopathy; antiapoptotic effect on cardiomyocytes (107) |
| AAV9-VEGF-B_{186} | Mouse | Improves systolic function following transverse aortic constriction; antiapoptotic effect on cardiomyocytes; capillary enlargement (65) |
coordinately regulated, which is not the case for the other VEGF family members (59, 96). VEGF-B expression in skeletal muscle is upregulated by the transcription factor PGC-1α (18, 134), as has been previously shown for VEGF (8). In addition, VEGF-B expression was induced in skeletal muscle by voluntary exercise in both mice and humans (18, 76). As PGC-1α is the major regulator of mitochondrial biogenesis and oxidative metabolism, these findings underline the close interplay between oxygen delivery by the vasculature and target tissue metabolism.

VEGF-B deletion was recently reported to lead to decreased expression of fatty acid transport proteins (FATPs) FATP-3 and FATP-4 in endothelial cells. This correlated with a decreased amount of lipid droplets in cardiomyocytes and skeletal muscle fibers, leading to increased white adipose tissue mass and body weight in old mice (59). Further analysis demonstrated that VEGF-B-deficient mice fed a high-fat diet or crossed with diabetic db/db mice have improved insulin sensitivity, glucose homeostasis, and blood lipid profiles (60). This was accompanied by reduced lipid storage in muscle, heart, and pancreas, but not in the liver, and increased weight gain on high-fat diet. VEGFR-1 and its tyrosine kinase activity as well as NRP-1 were needed for the VEGF-B-mediated effects on fatty acid transport across the endothelium (59). These results suggested that endothelial growth factors play a role in tissue lipid homeostasis and could provide a promising novel target for the treatment of metabolic and cardiovascular diseases.

The functional importance of angiogenesis and vascular endothelial regulation in adipose tissues has gained increased attention recently. Interestingly, both repression (91) and overexpression (40, 131) of VEGF were reported to protect against high-fat diet-induced obesity and/or insulin resistance. The expression of VEGF-B and FATPs 1–4 were increased in white adipose tissue of VEGF-deficient mice, via an unknown mechanism (91). These mice demonstrated lower body weight and resistance to high-fat diet-induced obesity, together with upregulation of brown adipose tissue-specific genes in white adipose tissue. In another study, the expression of VEGF-B and FATP-3 in brown adipose tissue was reduced after cold exposure, but mech-
anistic experiments were not conducted (12). VEGF-B was also shown to affect FATP-1 and FATP-4 levels in a neuroblastoma cell line (147).

In rats, however, we found no difference in fatty acid uptake between VEGF-B transgenic, gene-deleted, or wild-type hearts, although VEGF-B administration increased the levels of FATP-4 mRNA (75). In fact, fatty acid and triglyceride levels were actually reduced in the rat hearts overexpressing VEGF-B. On the other hand, VEGF-B overexpression led to an adjustment of cardiomyocyte metabolic pathways in favor of glucose oxidation and macromolecular biosynthesis. These metabolic changes may have contributed to the ischemia protection seen in this model, where cardiomyocyte mitochondria were protected against ischemia-reperfusion injury (75). Thus it is clear that the metabolic effects of VEGF-B are not as simple as has been previously suggested, and much remains to be learned about the specific role of VEGF-B in metabolic regulation.

In summary, there are several studies describing the role of vascular growth factors in adipose tissue homeostasis. Some studies have reported changes in VEGF-B expression by therapeutic drugs, such as rosiglitazone, which stimulates peroxisome proliferator-associated receptor-γ (53), but so far no study has investigated the role of VEGF-B in adipose tissue regulation. Based on the gene expression data from both mice and humans, VEGF-B and its receptors are highly expressed in both white and brown adipose tissue, suggesting that VEGF-B could have a function also in these tissues. An important question is whether VEGF-B acts in a paracrine manner via the endothelium, directly on the adipocytes, or also via VEGFR-1 expressing macrophages, which are important in adipose tissue regulation especially in pathological settings (94).

**G. VEGF-B-Induced Neuroprotection**

VEGF-B is also expressed in the central nervous system (1). Interestingly, VEGF-B-deficient mice showed impaired recovery from cerebral ischemic injury (132), and administration of VEGF-B stimulated neurogenesis in adult mice (133). Furthermore, VEGF-B186 protected primary motor neurons against degeneration in culture (109). Accordingly, mice lacking VEGF-B developed a more severe form of motor neuron degeneration when crossed with SOD1 mutant mice, a model of amyotrophic lateral sclerosis. Importantly, administration of VEGF-B186 into the ventricles of the brain prolonged the survival of mutant SOD1 rats, and the in vitro and in vivo effects of VEGF-B were dependent on the tyrosine kinase activity of its receptor VEGFR-1 in motor neurons.

VEGF-B was furthermore upregulated in an in vitro model of Parkinson’s disease induced by the neurotoxin rotenone, and the addition of exogenous VEGF-B167 improved neuronal survival (43). In a preclinical in vivo Parkinson’s disease model, exogenous VEGF-B186 showed neuroprotective effects, likely due to the partial protection of dopaminergic fibers in the striatum and partial rescue of the dopaminergic neurons in the caudal subregion of the substantia nigra (42). This neuroprotective effect was further investigated in a follow-up study, where VEGF-B expression was confirmed in the substantia nigra pars compacta of human patients with Parkinson’s disease (147).

VEGF-B and VEGFR-1 are expressed in dorsal root ganglion neurons that innervate the hindlimb skin (39). Mice lacking VEGF-B or functional VEGFR-1 developed more retrograde degeneration of sensory neurons in a distal neuropathy model, whereas mice overexpressing VEGF-B186 or VEGFR-1 selectively in neurons were protected against the distal neuropathy. Exogenous VEGF-B186 was shown to be protective by directly affecting sensory neurons and not the surrounding vasculature (39). Inhibition of apoptosis was suggested as a possible mechanism for the VEGF-B-induced neuroprotection (89). VEGF-B treatment also rescued neurons from apoptosis in the retina and brain in mouse models of ocular neurodegenerative disorders and stroke, respectively.

The neuroprotective effect of VEGF-B seems to be directly mediated by VEGFR-1 expressed by the neuronal cells and not via improved angiogenesis (39, 109). As a possible protection mechanism, inhibition of apoptosis by VEGF-B has also been proposed in cardiac ischemia (149). However, further studies are needed to clarify this as a potential downstream mechanism of VEGF-B - VEGFR-1 signaling.

**H. VEGF-B in Cancer**

It has been proposed that VEGF-B could play a role early in tumor development at the stage of adenoma formation, and VEGF-B expression is upregulated in for example ovarian, colorectal, renal, and prostate cancer (57, 61). However, only one study has addressed the role of VEGF-B in tumor development using a genetic model (5). This study analyzed the effects of VEGF-B overexpression versus deficiency in the context of pancreatic endocrine adenocarcinoma in transgenic RIP1-Tag2 mice. Interestingly, ectopic expression of VEGF-B reduced tumor growth, whereas mice lacking VEGF-B presented with larger tumors. The mechanism of VEGF-B action, however, remained unsolved, but the tumor vasculature and inflammation, the usual suspects, were not affected by the presence or absence of VEGF-B.

The role of PlGF in tumor angiogenesis is also controversial (10, 47, 146). PlGF blocking antibodies were reported to inhibit the growth of medulloblastomas and tumors that express VEGFR-1 in tumor cells in addition to endothelial cells (129, 146). However, it is not known whether the expression levels of VEGF-B in the tumors affect the efficacy of anti-PlGF therapies.
IV. SUMMARY

VEGF-B still remains an enigmatic vascular growth factor, but recent studies have indicated that VEGF-B can regulate blood vessel and cardiomyocyte growth in the heart, tissue metabolism, and cell survival (FIGURE 6). In addition to its effects on endothelial cells, studies in the central nervous system provided evidence that VEGF-B can directly affect neurons expressing VEGFR-1. Overall, VEGF-B seems to be important for tissue protection, whether it be in the context of heart failure or neuron degeneration. The possible role of VEGF-B in coronary artery development is an open question, but since gene-deleted mice and rats are viable and fertile and born in normal Mendelian ratios, this has not been studied in detail.

Because VEGF-B is dispensable during embryonic development and even high VEGF-B expression levels induced by viral delivery do not induce adverse effects, such as inflammation or vascular leakage, VEGF-B seems to possess a wide therapeutic window, which would be important for its possible use in human diseases. The idea that a vascular growth factor could also regulate tissue metabolism may sound unexpected at first; however, it is logical that both oxygen delivery by blood vessels and oxygen usage by mitochondria would be coordinated to be able to match nutrient/oxygen supply and demand. The close interplay between angiogenesis and mitochondrial biogenesis and oxidative metabolism will likely be the focus of numerous studies in the near future, and VEGF-B is one potential candidate molecule in this crosstalk.

**FIGURE 6.** A schematic model of the main effects of VEGF-B overexpression in the heart. VEGF-B, expressed by cardiomyocytes, binds to VEGFR-1 expressed in vascular endothelial cells and NRP-1 expressed in both endothelial cells and cardiomyocytes. Elevated levels of VEGF-B can displace VEGF from VEGFR-1, therefore increasing VEGF availability for VEGFR-2, which can induce capillary growth and arteriogenesis via increased Erk1/2 signals (9, 63, 75). Endothelial-cardiomyocyte crosstalk, which employs paracrine factors (PF), matrix molecules, and small-molecular-weight components, is essential for the coordination of physiological heart adaptation to various stresses. The vascular endothelium is also central as a gateway for the transport of nutrients and oxygen to the cardiomyocytes. Shown in the lower part of the figure are some of the signaling pathways and downstream effects of VEGF-B overexpression in the heart, leading to increased fatty acid and protein synthesis, cardiomyocyte hypertrophy, and decreased apoptosis (75, 107, 122, 149). AMPK, AMP-activated protein kinase; FASN, fatty acid synthase; mTOR, mammalian target of rapamycin; S6K, ribosomal protein S6 kinase.
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DISCLOSURES

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