Balancing Acts

VEGF-B and Blood Vessel Homeostasis
Modifying the Th17/Treg Axis for Therapy
Immune Deficiency and Autoimmunity in Lupus
Complicated life, complicated VEGF-B

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No other member of the VEGF (vascular endothelial growth factor) family has been as mysterious as VEGF-B. Notwithstanding its name, VEGF-B can hardly be regarded as a growth factor because growth occurs fairly normally in VEGF-B deficient mice. Moreover, VEGF-B is barely angiogenic under most conditions, although it was expected to be an angiogenic factor for a long time. Under certain conditions, VEGF-B has been shown to be involved in blood vessel growth. Under other conditions, however, VEGF-B can act to inhibit tumor growth and angiogenesis. Given these contradictory findings, the biological function of VEGF-B appears enigmatic. In this review, we summarize recent advances in VEGF-B biology and discuss its multifaceted roles, the underlying mechanisms, and the potential therapeutic implications.

VEGF-B: a multifunctional safeguarding molecule?
If the biological function of a molecule is to safeguard a normal condition, it would be a remarkable challenge to try to understand the biological function of that molecule under normal conditions, when there is no need of its action. One might be misled to conclude that this molecule is redundant and therefore unimportant. Furthermore, to fulfill its function of safeguarding a normal condition, this molecule needs to act in opposite ways under different conditions, thus displaying a functional ambiguity. For example, under degenerative conditions when cells are dying, this molecule can act as a survival factor to rescue the cells from death. By contrast, in the presence of high levels of potent growth factors, this molecule may act as a growth-inhibiting factor to prevent overgrowth. Thus, due to these seemingly controversial actions, comprehending the biological function of this molecule would be very difficult. In this review, we summarize recent advances in VEGF-B biology, and discuss how VEGF-B, a mysterious member of the vascular endothelial growth factor (VEGF) family, may be one such multifunctional safeguarding molecule.

VEGF-B is a close relative of VEGF-A
VEGF-B was discovered in 1996 as a VEGF-A homolog [1,2]. The amino acid sequence of the VEGF homology domain of VEGF-B is approximately 47% and 37% identical to those of VEGF165 and PIGF (placenta growth factor), respectively [3–8] (Figure 1a). VEGF-B is abundantly expressed in most tissues and organs [1,9,10] and is produced as a secreted homodimer. Owing to alternative splicing, the VEGF-B gene gives rise to two isoforms,

Glossary

**Age-related macular degeneration (AMD):** an aging-associated disease that causes central vision loss due to photoreceptor damages in the retina. AMD is the leading cause of blindness in the aged population. There are two types of AMD, wet and dry. Wet AMD (neovascular or exudative AMD) is an advanced condition, in which overgrowth of blood vessels from the choroid invade into the retina, interfering with central vision. In dry AMD, light sensitive retinal neurons (rod and cone photoreceptors) are damaged because of retinal pigment epithelial cell atrophy, leading to blurred vision.

**Amyotrophic lateral sclerosis (ALS):** also called Lou Gehrig’s disease is an invariably fatal, adult-onset neurodegenerative disorder characterized by rapid and progressive loss of motoneurons in the primary motor cortex, corticospinal tracts, brainstem and spinal cord, leading to muscular paralysis and eventually death.

**B3H-only proteins:** the Bcl-2 homology 3 (B3H)-only protein family members contain only the B3H domains of the four BH domains of the Bcl-2 family proteins and are essential inducers of apoptosis. The B3H-only proteins trigger the apoptotic pathway by inactivating the survival activity of the Bcl-2 family and by activating the pro-apoptotic activities of the Bax/Bak-like proteins. The B3H-only protein family includes Bad, Bim/Bod/Bcl2i11, Bid, Bik/Bik/Nsk, Noxa, Puma/Bbc3, Bmf, SNIP3, SNIP3L/Nix, Bcl-Gs, Apol-1, Mule and Egl-1.

**Blood-brain barrier (BBB):** an anatomical-physiologic feature of the brain consisting of walls of capillaries and special tight junctions around them, as well as thick basement membranes and astrocytic glial end-feet. The BBB separates the parenchyma of the central nervous system from circulating blood and extracellular fluid in the brain. It restricts the diffusion of microscopic objects and large or hydrophilic molecules into the cerebrospinal fluid, while allowing the diffusion of small hydrophobic molecules, such as O2, hormones and CO2.

**Choroidal neovascularization (CNV):** is a common pathologic condition in the wet form of age-related macular degeneration (AMD). In CNV, choroidal blood vessels grow and invade into the retina, leading to deterioration of central vision or blindness, when uncontrolled. CNV is often associated with defects in the Bruch’s membrane, the innermost layer of the choroid. CNV is also linked to a high level of VEGF expression.

**Fatty acid transport proteins (FATPs):** are 70–80 kDa integral membrane proteins with an extracellular N-terminal and a cytosolic C-terminal domain. The human FATPs compromise a family of six highly homologous proteins that are expressed in many different tissues and promote the uptake of long chain fatty acid. FATPs play important roles in metabolic and inflammatory pathways.

**Heparan sulfate proteoglycans (HSPGs):** are present in all animal tissues. HSPGs consist of a core protein with one or more glycosaminoglycan chains attached to cell surface or extracellular matrix proteins. HSPGs bind to different extracellular proteins and are important regulators of the tissue distribution of extracellular signaling molecules. HSPGs play important roles in a wide variety of biological processes, including angiogenesis. The major cell membrane HSPGs are the transmembrane syndecans and the glycosylphosphatidylinositol anchored glypicans.

**Neuropilin-1 (NRP-1):** is a 130 kDa transmembrane protein that lacks an intrinsic catalytic activity. NRP-1 is a co-receptor for VEGF-A, VEGF-B, PI GF and Class III semaphorins. NRP-1 plays key roles in neuronal axon guidance and modulation of developing nervous and vascular systems. Genetic deletion of Nrp-1 in mice led to embryonic lethality with abnormal vascular development, and aberrant trigeminal and spinal nerve morphology.

**N’-Methyl-o-aspartic acid or N’-methyl-o-aspartate (NMDA):** is an amino acid derivative that acts as a specific agonist of the NMDA receptor. It mimics the action of glutamate, the neurotransmitter that normally binds to the NMDA receptor. NMDA is an excitotoxin that has applications in behavioral neuroscience research.

**Parkinson’s disease (PD):** is a neurodegenerative disorder of the brain that is characterized by the age-related progressive loss of dopaminergic neurotransmission in the basal ganglia, a region in the mid-brain, leading to tremors and difficulties of walking, movement, and coordination. PD can also cause other symptoms, such as sensory and sleep difficulties, alterations in mood, cognition, behavior or thought.

**Pigment epithelium-derived factor (PEDF):** is a 50 kDa secreted glycoprotein that belongs to the serpin (serine protease inhibitors) family. PEDF is known as...
VEGF-B167 and VEGF-B186 (Figure 1b), which form 42 kDa and 60 kDa homodimers, respectively. VEGF-B167 has a heparin-binding domain at its carboxyl terminus (Figure 1a, b), and upon secretion VEGF-B167 binds to cell-surface heparan sulfate proteoglycans (HSPGs) (see Glossary) (Figure 1b). VEGF-B186 does not contain the heparin-binding domain and is therefore more diffusible. Similar to VEGF-A, VEGF-B binds to vascular endothelial growth factor receptor 1 (VEGFR-1) and neuropilin 1 (NRP-1) [11,12] (Figure 2). Because of its high sequence homology and similar receptor binding pattern to VEGF-A, VEGF-B was initially believed to also be an angiogenic factor [13]. However, studies on its angiogenic activities have thus far led to controversial results [14]. Compared with the other VEGF family members (Figures 1 and 2), VEGF-B remains the least studied.

VEGF-B appears to be inert under normal conditions

One unique functional aspect of VEGF-B is that it appears to have no obvious function under normal conditions. Loss-of-function studies by gene deletion showed that Vegf-b deficient mice are largely healthy and can live a fairly standard life under normal conditions [15–18] (Table 1). By contrast, Vegf-a or Vegf-c gene deletion causes embryonic lethality in mice [19–21]. Unlike PIGF, the deficiency of which impairs pathological angiogenesis in various tissues and organs [22,23], Vegf-b deficiency does not affect pathological angiogenesis in many organs studied, such as wounded skin, hypoxic lung or limb [24]. Thus far, one report suggested a role for VEGF-B in inflammatory angiogenesis in an arthritis mouse model [25]. In contrast with Vegf-a and Plgf, Vegf-b gene deletion does not affect blood vessel remodeling in pulmonary hypertension [18].

Gain-of-function studies using VEGF-B transgenic mice also showed that VEGF-B displays no significant activities under normal conditions. In fact, VEGF-B is the only member of the VEGF family in which transgenic overexpression in different organs did not induce angiogenesis or lymphangiogenesis [26,27]. Indeed, transgenic expression of the other VEGF family members, such as VEGF-A [28–30], PIGF [31], VEGF-C [32], VEGF-D [33] or VEGF-E [34], all induced angiogenesis or lymphangiogenesis. By contrast, VEGF-B overexpression in cardiac myocytes under the α-myosin heavy chain promoter did not induce angiogenesis in the heart [26]. Instead, cardiac blood vessel density was even slightly decreased in the VEGF-B-overexpressing mouse hearts [26]. In addition, VEGF-B transgenic expression in vascular endothelial cells under the Tie2 promoter did not induce angiogenesis in different organs, including the liver, heart, and kidney [27]. VEGF-B transgenic expression in the skin under the keratin-14 promoter marginally potentiated but did not induce angiogenesis [26].

Studies using viral gene delivery also demonstrated the inert nature of VEGF-B as compared with the other VEGF family members; gene delivery of the other VEGF family members all led to abnormal vascular phenotypes. For example, adenoviral gene transfer of VEGF-A, VEGF-C or VEGF-D into rabbit hind limb skeletal muscles induced robust angiogenesis, vascular permeability, or lymphangiogenesis [35], whereas VEGF-B adenoviral gene transfer did not induce angiogenesis or lymphangiogenesis in the same model [35]. Similarly, adenoviral gene transfer of

Table 1. Phenotypes of VEGF-B deficient or transgenic mice

<table>
<thead>
<tr>
<th>Gene deletion or transgenic (Tg) expression</th>
<th>Phenotype</th>
<th>Refs</th>
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<tbody>
<tr>
<td>Vegf-b&lt;−&lt;−&gt;</td>
<td>Largely healthy, fertile, mild cardiac conduction defect</td>
<td>[15]</td>
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<tr>
<td></td>
<td>Largely healthy, fertile, mild vascular dysfunction</td>
<td>[16]</td>
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<td></td>
<td>No significant difference in pulmonary hypertension between Vegf-b&lt;−&lt;−&gt; and control mice</td>
<td>[18]</td>
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<tr>
<td></td>
<td>Reduction of arthritis and synovial inflammation</td>
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<td></td>
<td>Impaired development of hypoxic pulmonary hypertension</td>
<td>[25]</td>
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<tr>
<td></td>
<td>More severe motor neuron degeneration when intercrossed with mutant SOD1 mice</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>More retrograde degeneration of sensory neurons in a model of distal neuropathy</td>
<td>[54]</td>
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<tr>
<td></td>
<td>Impaired revascularization of ischemic myocardial, no effects on wounded skin, hypoxic lung, or ischemic limb</td>
<td>[69]</td>
</tr>
<tr>
<td>RPl1-Tag2-vegf-b&lt;−&lt;−&gt;</td>
<td>Larger tumor size</td>
<td>[57]</td>
</tr>
<tr>
<td>TIE2-VEGF-B167-Tg</td>
<td>Enhanced vessel density in Matrigel plug assay, elevated vessel sprouting in aortic explants, decreased circulating endothelial progenitor cells (EPCs)</td>
<td>[27]</td>
</tr>
<tr>
<td>TIE2-VEGF-B186-Tg</td>
<td>Increased vessel density in Matrigel plug assay, no effect on circulating EPCs</td>
<td>[27]</td>
</tr>
<tr>
<td>Adenoviral-hVEGF-B167</td>
<td>No effect on revascularization in ischemic limb</td>
<td>[24]</td>
</tr>
<tr>
<td>α-MHC-VEGF-B</td>
<td>Cardiac hypertrophy in myocardium, strong arteriogenesis in rat myocardium without inducing angiogenesis, vascular leak or inflammation</td>
<td>[70]</td>
</tr>
<tr>
<td>RPl1-Tag2-VEGF-B</td>
<td>Reduced tumor growth</td>
<td>[57]</td>
</tr>
<tr>
<td>Stop&lt;low&gt;VEGF-B186-Thy1.2&lt;term&gt;</td>
<td>Increased susceptibility to neuronal stress</td>
<td>[54]</td>
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VEGF-A or VEGF-D to rabbit carotid arteries induced strong adventitial angiogenesis, whereas VEGF-B adenoviral gene transfer failed to do so [36,37]. In addition, VEGF-B_{167} gene delivery to the mouse skin or ischemic limb did not induce blood vessel growth or other abnormalities [24].

Moreover, results from studies using VEGF-B protein showed that VEGF-B by itself has no obvious function under normal conditions. Sands et al. recently reported that whereas VEGF-A and PlGF promoted vascular endothelial cell proliferation and migration in cultured cells, VEGF-B displayed no such effect [38]. In vivo, injection of
VEGF-B₁₆₇ recombinant protein into adult mouse eyes at a dose effective for retinal neuron survival did not induce ocular angiogenesis or other abnormalities [39]. Furthermore, Poesen et al. showed that intracerebroventricular injection of VEGF-B₁₆₇ recombinant protein did not induce any blood vessel growth or blood–brain barrier defect in the brain [40].

VEGF-B₁₆₇ does not induce blood vessel permeability

Another functional uniqueness of VEGF-B₁₆₇ is that it does not induce blood vessel permeability (Figure 1b), unlike all of the other VEGF family members [22,35,41–43]. This observation has been shown by numerous studies from different laboratories using different models and approaches, such as Vegf-b deficient and transgenic mice, VEGF-B recombinant protein, and gene transfer [15,17,27]. Whereas intradermal injection of VEGF-A₁₆₅, VEGF-A₁₂₁, or VEGF-C in mice ears increased vascular permeability, injection of VEGF-B₁₆₇ had no such effect [44]. Injection of VEGF-B₁₆₇ recombinant protein into mouse brain or eye did not induce blood vessel permeability [39]. In preserved lung grafts, VEGF-A and VEGF-C, but not VEGF-B, increased vascular permeability [45]. Indeed, VEGF-B₁₆₇ or VEGF-B₁₈₆ overexpression in the lung by adenoviral gene transfer had no effect on blood vessel permeability [18]. Whereas delivery of adenoviruses expressing VEGF-A or VEGF-D into rabbit hind limb skeletal muscles induced vascular permeability, delivery of adenovirus encoding VEGF-B₁₆₇ failed to do so [35]. Interestingly, one recent study showed that intraocular injection of adeno-associated virus (AAV) encoding VEGF-B₁₈₆, but not VEGF-B₁₆₇, potentiated retinal vascular permeability [46]. Taken together, mounting data have shown that VEGF-B₁₆₇ is the only member of the VEGF family that does not induce blood vessel permeability.

VEGF-B is a potent survival factor

VEGF-B and its receptors are expressed by different types of vascular cells [10,24,47]. VEGF-B has been shown to be a survival factor for vascular endothelial cells (ECs), pericytes (PCs), and smooth muscle cells (SMCs) [14,47] (Figure 3a). Indeed, increased apoptosis was observed in VEGF-B deficient ECs and SMCs when the cells were challenged by oxidative stress or serum starvation [47]. In vivo, VEGF-B deficiency led to poorer blood vessel survival in the cornea after withdrawal of the implanted growth factors, fewer surviving hyaloid vessels in postnatal mouse eyes, and greater oxygen-induced retinal blood vessel degeneration in neonatal mice [47]. In addition, a recent investigation reported that in a laser injury-induced choroidal neovascularization model and an ischemia-induced retinal neovascularization model, subretinal injection of AAV-VEGF-B₁₆₇ or AAV-VEGF-B₁₈₆ augmented neovessel formation in the retinae and choroids [46] (Figure 3b). Moreover, VEGF-B is most abundantly expressed in the heart [1,9] and is important for cardiac blood vessel survival. In a cardiac ischemia mouse model, VEGF-B treatment increased cardiac blood vessel density.
in the ischemic myocardium, where blood vessels underwent severe degeneration [24,48] (Figure 3c). This observation was corroborated by another study demonstrating that in pigs and rabbits, VEGF-B_{186} gene transfer increased myocardium-specific blood vessel density [49]. Thus, both gain-of-function and loss-of-function analyses showed that VEGF-B is critically required for blood vessel survival under pathological conditions.

In addition to its vascular survival effect, VEGF-B has been shown to be a potent survival factor for different types of neurons, including brain cortical neurons [39,50], retinal neurons [39], and motor neurons in the spinal cord [40]. In vitro, VEGF-B protein treatment increased the survival of cultured primary brain cortical neurons [39,50] and protected cultured primary motor neurons from apoptosis [40]. In vivo, VEGF-B treatment reduced stroke volume in a middle cerebral artery ligation-induced stroke model [39]. In the retina, VEGF-B treatment increased the survival of retinal ganglion cells in an optic nerve crush injury model and protected neurons in different nuclear layer in the retina in an NMDA-induced neuronal apoptosis model [39]. Moreover, loss-of-function assays showed that Vegf-b deficiency in mice led to more severe strokes in the experimental stroke model and exacerbated retinal ganglion cell death in the optic nerve crush injury model [39]. In addition, Vegf-b deficient mice developed a more severe form of motor neuron degeneration when intercrossed with the superoxide dismutase 1 (SOD1) mutant mice, and VEGF-B intracerebroventricular injection increased the survival of SOD1 mutant rats [40]. Thus, both in vitro and in vivo results have shown that VEGF-B is a critical survival factor for different types of neurons.

VEGF-B has been shown to promote cardiac myocyte survival. In an ischemia–reperfusion mouse model, transgenic mice expressing a cardiac-specific VEGF-B transgene under the α-myosin heavy chain promoter displayed a greater survival rate of cardiac myocytes after ischemia stress [26]. It is noteworthy that VEGF-B overexpression did not increase cardiac blood vessel density. Instead, cardiac blood vessel density slightly decreased in the VEGF-B-overexpressing mouse hearts [26]. Thus, the enhanced cardiac myocyte survival was most probably due to a direct survival effect of VEGF-B on cardiac myocytes [26]. Indeed, this observation was supported by another study showing that in a myocardial infarction mouse model, intravenous injection of adenovirus encoding human VEGF-B_{167} increased cardiomyocyte viability and improved left ventricular function [51].

At a molecular level, genome-wide gene profiling revealed that VEGF-B inhibits the expression of numerous apoptosis/cell death-inducing genes, particularly the BH3-only protein family members, which are essential inducers of apoptosis [39]. Indeed, VEGF-B treatment inhibited the expression of the BH3-only protein genes in vitro in cultured cells and in different disease models in vivo [39]. The survival effect of VEGF-B is mediated by VEGFR-1 and NRP-1, which are known to mediate cell survival [40,52–54]. In addition, another possible mechanism underlying the survival effect of VEGF-B might be related to its role in promoting energy metabolism, which is important for cell survival. Indeed, energy metabolism deficit is involved in various degenerative conditions and improvement of energy metabolism increases cell survival [55]. In a recent study, Hagberg et al. reported that VEGF-B plays important roles in energy metabolism by regulating fatty acid transportation through VEGFR-1 and neuropilin-1 [56]. Thus, apart from its antiapoptotic effect, VEGF-B may potentially promote cell survival by regulating bioenergetic pathways. Further studies are needed to verify this.

**Antigrowth and antiangiogenic effect of VEGF-B**

Perhaps the most unexpected and therefore surprising roles of VEGF-B are the recent findings that under certain conditions it can act to inhibit growth or angiogenesis.
Albrecht et al. showed that transgenic expression of VEGF-B under the insulin promoter in insulin-producing β cells inhibited tumor growth by approximately 40% in a pancreatic neuroendocrine tumorigenesis mouse model [57] (Figure 4a). This observation was corroborated by the finding that tumors implanted in Vegf-b deficient mice acquired tumor volumes approximately 1.8-fold larger than the tumors implanted in wild-type mice [57]. The authors did not observe increased β cell apoptosis, lipid accumulation or fatty acid transporter protein expression in the VEGF-B-overexpressing tumors. Thus, the changes in tumor growth were unlikely to be due to lipid accumulation or toxicity [57]. Although the cellular and molecular mechanisms underlying these surprising observations were not resolved, this study provided evidence of a growth-inhibiting effect of VEGF-B. Moreover, Hagberg et al. recently reported that adult Vegf-b deficient mice display a greater body weight, being approximately 15% heavier than wild-type mice [56] (Figure 4b). In this study, the greater body weight of the Vegf-b deficient mice was attributed to an impaired lipid uptake in the peripheral organs, resulting in a subsequent lipid accumulation in the white adipose tissues. Yet, one possibility that cannot be excluded could be that the greater body weight of the Vegf-b deficient mice might be a result of the lack of a growth-inhibiting activity of VEGF-B, as exemplified by the greater tumor volume in the Vegf-b deficient mice [57]. This possibility remains to be tested. In addition, VEGF-B was very recently reported to inhibit VEGF-A-induced migration of human pulmonary microvascular endothelial cells in a monolayer scratch assay [38] (Figure 4c). By contrast, the VEGF-B homolog PIGF did not display such an inhibitory effect. Instead, PIGF potentiated the migratory effect of VEGF-A [38], demonstrating a functional uniqueness and specificity of VEGF-B. Taken together, recent studies have pointed to a highly unexpected function of VEGF-B as an endogenous inhibitor of growth and angiogenesis.

The molecular and cellular mechanisms underlying the antigrowth and antiangiogenic effect of VEGF-B remain to be defined. However, one possible mechanism may lie with VEGFR-1, a receptor for VEGF-B. VEGFR-1 is known to have dual functions in vascular development and homeostasis [58]. Apart from its survival effect under pathological conditions [40,52,54], VEGFR-1 can also act as a VEGF-A decoy receptor to suppress angiogenesis [59,60]. In vivo, Vegfr-1 deficiency in mice led to overgrowth and disorganization of blood vessels resulting in early embryonic lethality [61], demonstrating a negative role of VEGF-R1 in developmental angiogenesis. Indeed, overexpression of VEGFR-1 in vascular endothelial cells inhibited VEGFR-2 and ERK phosphorylation [59]. By contrast, reduced VEGFR-1 expression led to VEGF-A-dependent activation of VEGFR-2 and its downstream signaling pathways [59]. Because VEGFR-2 and the signaling pathways induced by it play key roles during angiogenesis, this study provided an important molecular basis for the antiangiogenic effect of VEGFR-1. Given the fact that VEGF-B binds to VEGFR-1, it is conceivable that VEGF-B may contribute to the VEGFR-1-mediated antigrowth and antiangiogenic effects.

Figure 4. Antigrowth and antiangiogenic effects of VEGF-B. (a) In a pancreatic neuroendocrine tumorigenesis mouse model, transgenic expression of VEGF-B (VEGF-B-Tg) under the insulin promoter in insulin-producing β cells inhibited tumor growth by approximately 40% compared with the wild-type (WT) tumors. Moreover, tumors implanted in Vegf-b deficient mice acquired 1.8-fold larger tumor volumes compared with those implanted in wild-type mice [57]. (b) Vegf-b deficient mice (VEGF-B−/−) displayed approximately 15% greater body weight than wild-type (WT) mice due to impaired fatty acid uptake [56]. (c) In a monolayer cell scratch assay, VEGF-B inhibited VEGF-A-induced migration of human pulmonary microvascular endothelial cells [38].
Therapeutic potential of VEGF-B

Owing to its potent antiapoptotic and survival effect and its unique nature of being inactive under normal conditions, VEGF-B appears to have valuable therapeutic potential for the treatment of neurodegenerative diseases and an attractive safety profile. Preclinical studies reported by different laboratories using various disease models, such as the stroke model [39,62], Parkinson’s disease (PD) model [63,64], and an amyotrophic lateral sclerosis model [40], have shown promising results. However, clinical studies on VEGF-B in human patients with neurodegenerative diseases are lacking. It is important to note that as compared with the other known neurotrophic factors, the survival effect of VEGF-B appears to be considerably potent. A single VEGF-B186 protein treatment at a dose as low as 3 μg per rat rescued dopaminergic neurons from death in a PD model [64]. Indeed, endogenous VEGF-B expression was upregulated in rat midbrains after rotenone challenge [63], and VEGF-B produced by astrocytes and motor neurons was believed to exert a neuroprotective function [40]. In addition to its neuroprotective effect, VEGF-B is also a survival factor for the vascular system (Figure 5a), which is a crucial component in most neurodegenerative disorders. Because the neural and vascular systems are closely interdependent on each other [65], the survival/protective effect of VEGF-B on one system will subsequently improve the status and function of the other.

VEGF-B is expressed in the eye, and its expression is upregulated after pathological challenge in the retina [39]. A recent study showed that subretinal injection of adeno-associated viruses encoding VEGF-B157 or VEGF-B186 augmented ischemia- and laser injury-induced retinal and choroidal neovascularization, respectively [46]. Indeed, another study showed that targeted inhibition of VEGF-B by shRNA (short hairpin RNA) or neutralizing antibody suppressed choroidal and retinal neovascularization in mice [47]. Thus far, little evidence exists to support that VEGF-B is capable of inducing vascular cell proliferation or migration, two critical steps of angiogenesis. Therefore, the seeming ‘angiogenic’ activity of VEGF-B during ocular neovascularization is probably due to its potent survival effect on vascular and nonvascular cells (Figure 5a). Indeed, it is known that during ocular neovascularization, the neovessels are challenged by apoptosis in vascular endothelial cells, stromal pigment epithelial cells, and macrophages [66,67]. In agreement with this, prominent Fas and Fas ligand expression were detected during ocular neovascularization [66,67]. In addition, various endogenous antiangiogenic factors, such as the pigment epithelium derived factor (PEDF), exist during ocular neovascularization to suppress neovessel formation [68]. Therefore, even though VEGF-B has a minimal role during the initial phase of blood vessel growth [47], the vascular survival activity of VEGF-B, which protects the neovessels from apoptosis, may play a significant role in enhancing ocular neovascularization. Thus, targeted VEGF-B inhibition may also have therapeutic implications for the treatment of ocular neovascular diseases.

Several groups have shown that VEGF-B has a specific role in the revascularization of ischemic myocardium in different disease models of mice, pigs and rabbits [24,48,49,51]. However, it remains to be defined whether the vascular effect of VEGF-B in the ischemic myocardium is a result of a vascular survival effect, a bona fide angiogenic effect, or a combination of both. Importantly, apart from the survival effect of VEGF-B on blood vessels, VEGF-B may also have a direct protective effect on cardiac

![Figure 5. Multifaceted actions of VEGF-B under different conditions. (a) Left: under degenerative conditions, VEGF-B acts as a survival factor for different types of vascular cells to rescue the endangered blood vessels from degeneration by inhibiting the expression of numerous apoptotic genes, thereby maintaining normal blood vessel density and integrity. Middle: under normal conditions, VEGF-B displays no obvious function and appears to be inert. Right: under other specific conditions, as in the presence of high levels of potent angiogenic/growth factors, VEGF-B can act as an inhibitory factor to prevent excessive blood vessel or tissue growth, ensuring a balanced blood vessel density and tissue growth. (b) At least one of the biological functions of VEGF-B is to safeguard a normal condition of the vascular system. To fulfill this function, VEGF-B acts in opposite ways under different conditions, thus displaying a functional ambiguity. For example, under degenerative conditions when the cells are dying, VEGF-B acts as a survival factor to rescue the cells. Under other conditions, e.g., in the presence of high levels of potent growth factors, VEGF-B acts as a growth-inhibiting factor to prevent overgrowth. (c) Because of its multifaceted and context-dependent functions, VEGF-B safeguards a balanced blood vessel growth and integrity.](image-url)
myocytes [26]. Thus, based on these studies, VEGF-B may have a therapeutic potential in treating cardiac ischemic diseases.

Concluding remarks and future perspectives

After being neglected for many years, VEGF-B is beginning to receive some attention because of recent exciting advances in VEGF-B biology. These advances have led to the following major understandings. Firstly, compared with the other VEGF family members, VEGF-B has a unique and rare functional property with no significant activity under normal conditions. This functional uniqueness of VEGF-B confers a minimal side effect upon it and therefore an attractive safety profile as a potential therapeutic molecule. Secondly, the function of VEGF-B is multifaceted and context-dependent. It can act as a survival factor or an antigrowth/antiangiogenesis factor, depending on the specific circumstance (Figure 5a). Under degenerative conditions, VEGF-B functions as a survival factor to protect cells from apoptosis or death. However, under other conditions, such as during development or in certain pathologies, it can instead act as an antigrowth or antiangiogenic factor to prevent overgrowth of body mass, blood vessels, or tumors, among other tissues (Figure 5a). Thus, the multifaceted roles of VEGF-B safeguard the balance between blood vessel growth and degeneration to ensure a normal blood vessel density and integrity (Figure 5b, c). Finally, VEGF-B may have promising therapeutic values in treating degenerative conditions and certain types of neovascular diseases. Despite significant advances in VEGF-B biology, outstanding questions remain to be addressed, several of which are summarized in Box 1. One important direction of VEGF-B research in the future is to address the key question of how the functional switch of VEGF-B is regulated. Another important aspect of future VEGF-B research is to better define its therapeutic potential in treating human diseases. Investigations into these aspects may lead to even more exciting findings.

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