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BDNF: A Newly Described Mediator of Angiogenesis

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Abstract

Recent studies indicate that, in addition to its neurotrophic actions, brain derived neurotrophic factor (BDNF) promotes endothelial cell survival and induces neoangiogenesis in ischemic tissues. Unlike many vascular growth factors that act on many vascular beds, BDNF activity is relatively restricted to central arteries and cardiac skeletal muscle, skin and vasculature. Studies of newly described biological mediators that act on large vessel and microvascular beds in these organs will help us to better understand organ-specific vascular development as well as to develop novel therapeutic strategies to improve the condition of patients with cardiac and peripheral vascular disease. In this review we summarize dual pro-angiogenic actions of BDNF, which through local activation of TrkB receptor expressed on a sub-population of endothelial cells (ECs) and additionally by recruitment of bone marrow-derived cells, contribute to neoangiogenesis.

Importance of endothelial cells in angiogenesis.

The development of a mature vascular bed is a complex and dynamic process, characterized by the coordinated proliferation and recruitment of endothelial and smooth muscle cells and subsequent patterning into capillaries, arteries and veins. It is controlled by locally produced growth factors, adhesion molecules and proteinases (Carmeliet 2000) that establish the primary vascular plexus consisting of endothelial cells. Subsequently, accessory cells such as pericytes and smooth muscle cells are recruited to sculpt the vasculature, by pruning and remodeling. During adulthood, the maintenance of blood vessels is further promoted by a combination of growth factors, cell:matrix interactions and shear stress forces. The molecular mechanisms that are utilized during development to sculpt the immature vascular bed may be utilized during the adaptive neovascularization of the heart that occurs as a consequence of cardiac ischemia.

Endothelial cells, comprising the inner layer of cells of the vasculature, are the main regulators of vascular homeostasis. They play important roles in the physiology and maintenance of vascular integrity and tone, as well as in pathological situations such as inflammation and tumor angiogenesis. Although endothelial cells exhibit many common morphological characteristics, they display significant heterogeneity in different organs, to promote organ-specific functions (Garlanda and Dejana 1997). While coronary arteries nourish the myocardium, the highly specialized endothelial cells in the bone marrow sinusoids are intimately involved in the homing of hematopoietic progenitors and play an important role in maturation, proliferation and trafficking of those cells to the blood circulation. These characteristics may be endowed by organ-specific growth factors secreted by parenchymal cells in a paracrine manner.

The healthy endothelium is both anti-coagulant and anti-thrombotic, by expressing or secreting numerous proteins that regulate blood coagulation and platelet activation (Robson et al.

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1997, Urbich et al. 2002). Thus, endothelial cells are prime pharmacological targets to modify susceptibility to cardiovascular disease. Under normal physiological conditions, the vasculature is relatively quiescent, owing to the overexpression of molecules that inhibit angiogenesis. However, during embryonic development, vessel formation and maturation is a very dynamic process, involving growth factors that widely modulate endothelial proliferation and migration, such as Vascular Endothelial Growth Factor (VEGF), as well as less well characterized factors that exert organ-specific effects (BDNF, see below, in the heart). Although VEGF is the major angiogenic protein that directs vessel network formation in early to mid-gestation, its angiogenic actions in adulthood are more limited to the pathogenesis of disease, such as tumor growth, rheumatoid arthritis, wound healing and inflammation. Indeed, clinical trials evaluating angiogenic actions of VEGF upon delivery of recombinant protein, naked plasmid DNA or adenoviral vectors either by direct myocardial injection or by catheter based methods to ischemic tissues of human patients have demonstrated only limited or no success (Isner et al. 2001; Epstein et al. 2001). Thus, identifying organ specific growth factors that direct vessel growth and stabilization during embryogenesis may provide important candidate molecules that may have greater efficacy in promoting angiogenesis in ischemic human tissues.

Biology of neurotrophins and their receptors.

The neurotrophin family consists of four structurally related protein members; Nerve Growth Factor (NGF), brain-derived neurotrophic factor (BDNF), Neurotrophin-3 (NT-3) and Neurotrophin-4 (NT-4) representing gene duplications of a common ancestral gene (Hallbook et al. 2006). All neurotrophins are synthesized as precursors (proneurotrophins) that are proteolytically cleaved to yield the C-terminal mature protein (Lee et al. 2001). Neurotrophins mediate their effects via two distinct classes of receptor: the tropomyosin-related kinase family of receptors (Trks) that display specificity for neurotrophin family members (Huang and Reichardt 2003), and p75^{NTR}, which binds all neurotrophins, including proneurotrophins (Hempstead 2002, Frade and Barde 1998). Activation of Trk receptors can lead to cell proliferation, survival, or chemotaxis, biological outcomes that are, in large part, cell-type-specific. Activation of p75^{NTR} can mediate two distinct responses. When co-expressed with Trks, p75^{NTR} enhances the affinity and specificity of neurotrophin binding to Trks, to promote survival signaling. Activation of p75^{NTR} by proneurotrophins, however, can initiate apoptosis when p75^{NTR} is co-expressed with sortilin, a member of the VPS10 family of receptors (Nykjaer et al. 2004).

Although neurotrophins are best characterized as trophic factors for neurons during development and adulthood, including regulation of synaptic plasticity and growth cone guidance (Bibel and Barde 2000, Kaplan and Miller 2000, Lu et al. 2005) numerous reports have uncovered critical roles for neurotrophins and their receptors on non-neuronal cells, such as endothelial cells, smooth muscle cells, immune cells, and epithelial cells in different organs, (Nemoto et al. 1998, Botchkarev et al. 1999, Coppola et al. 2004). Interestingly, p75^{NTR} is increased following vascular injury, and its expression correlates both temporally and spatially with apoptosis of neointimal smooth muscle cells (Kraemer 2002). Moreover, both NGF and BDNF are upregulated in vascular injury. These results suggest that neurotrophins and their receptors may play roles in vascular pathologies.

Recently, our group has shown that BDNF plays a critical role in regulating both vascular development and response to injury. Unlike VEGF-A, which activates receptors (VEGFR1 and VEGFR2) expressed on most endothelial cell populations and is critical for early stages of vascular development, BDNF is expressed in an organ-specific manner in perinatal and adult vasculature (Donovan et al. 2000). Endothelial cells lining arteries and capillaries of the heart and skeletal muscle express BDNF and TrkB, first detectable in late gestation and persisting

at high levels into adulthood. Mice deficient in BDNF ($\text{BDNF}^{-/-}$) exhibit impaired survival of TrkB-expressing endothelial cells in intramyocardial arteries and capillaries in the late gestational and early postnatal period, although the embryonic vasculature of the heart forms and can remodel into arteries, capillaries and veins (Donovan et al. 2000). Vascular hemorrhage in neonatal $\text{BDNF}^{-/-}$ mice is restricted to cardiac vessels, likely reflecting the localized expression of BDNF and TrkB by mid-gestational capillaries and arterioles in cardiac and skeletal muscle. BDNF deficiency results in a reduction in endothelial cell-cell contacts and in endothelial cell apoptosis leading to hypocontractility of the heart and perinatal lethality. Conversely, BDNF overexpression in the mid-gestational mouse heart results in an increase in capillary density, establishing the essential role of BDNF in modulating cardiac microvascular endothelial cells during development (Donovan et al. 2000). Recent studies have confirmed that BDNF mediates these effects during development by activating TrkB (Wagner et al. 2005). BDNF-induced TrkB activation of PI3-kinase and Akt may mediate endothelial survival although the signaling pathways that promote directed migration remain to be clarified.

p75^{NTR} is also expressed by vascular smooth muscle cells during development, but its biological actions are less clear. Examination of one p75^{NTR} gene targeted animal (exon IV) (von Schack et al. 2001), which subsequently has been found to aberrantly express a truncated p75^{NTR} receptor isoform (Paul et al. 2004), noted defects in vascular smooth muscle cell ensheathment of aorta, with blood cell leakage that contributes to late gestational lethality. Furthermore, p75^{NTR} is up-regulated in vascular smooth muscle cells in models of vascular injury, where it initiates apoptosis and neointimal lesion regression (Kraemer 2002). These results suggest that p75^{NTR} may play a role in the recruitment of pericytes or smooth muscle cells, although the mechanisms and ligands that mediate these effects are unclear.

Given the critical role of BDNF in perinatal vessel stabilization and continued expression of TrkB in the adult vasculature we have investigated direct angiogenic actions of exogenous delivery of BDNF in normal and ischemic conditions in the adult mouse (Kermani et al. 2005). In a femoral artery ligation model, BDNF protein is significantly induced in ischemic, as compared to non-ischemic tissue. Local overexpression of BDNF promotes blood flow recovery and capillary density comparable to that elicited by VEGF. Importantly, these effects appear to be mediated by TrkB, as the effects of exogenous BDNF are attenuated in haplodeficient animals ($\text{TrkB}^{+/-}$), as assessed by blood flow and capillary density.

Unexpectedly, exogenous BDNF delivery to the ischemic hindlimb also promoted recruitment of myeloid cells (CD11b^{+}) and hematopoietic precursor (Sca-1^{+}) cells, which express the TrkB receptor (Kermani et al. 2005). These studies suggest that BDNF can induce mobilization and recruitment of a subpopulation of myeloid cells, which home to sites of vascular injury and may contribute to vessel formation or vessel stabilization. Thus, BDNF may exert its angiogenic effects by two distinct mechanisms; BDNF can activate local TrkB expressing endothelial cells to promote assembly of neo-vessels locally, and in addition can act as a direct chemotactic factor of bone-marrow-derived cells.

Is BDNF the only neurotrophin with effects in the vasculature? Studies examining the angiogenic actions of NGF demonstrated that exogenous administration of recombinant mature NGF in an ischemic rodent hindlimb model induced a marked increase of arteriole length density (Turrini et al. 2002). Recent studies by Dolle et al. (2005) indicate that NGF may induce endothelial cell migration and proliferation. Delivery of mature NGF protein induces a potent angiogenic response in the chorioallantoic membrane of the developing chick embryo (Cantarella et al. 2002) and stimulates angiogenesis and arteriogenesis, accelerating hemodynamic recovery in a mouse hindlimb model (Emanueli et al. 2002). NT-3 also exerts effects on cardiovascular development. Despite NT-3 expression in the walls of large arteries,

NT-3^{-/-} mice exhibit atrial and ventricular septal defects and valvular defects including pulmonic stenosis (Tessarolo et al. 1997) rather than hemorrhage of cardiac or great vessels. These results suggest that NT-3 exerts actions primarily upon TrkC expressing cardiac myocytes during embryonic development (Lin et al. 2000) (Table I).

Together, these studies suggest that neurotrophins play important and diverse roles in vessel survival and stabilization in late embryogenesis and contribute to new blood vessel formation following vascular injury in the adult.

Conclusion and future direction.

Angiogenesis therapy, to induce vascular regeneration, is one of the most ambitious but promising strategies to improve the outcome of patients with cardiovascular disease. In the past decade, substantial progress has been made in understanding angiogenic mechanisms, and the molecular factors that regulate differentiation of endothelial cells during embryogenesis in different vascular beds are being uncovered. This heterogeneity of endothelial cells in different organs has prompted investigation of new molecular players and local regulatory mechanisms which may provide new organ-specific tools for promoting neoangiogenesis.

Tissue regeneration that follows ischemia requires the coordination of several pathways: tissue parenchyma regeneration, nerve repair and neovascularization. The complexity of each of these pathways is further increased by simultaneous or sequential interactions that likely involve multiple growth factors. Delivery of circulating hematopoietic progenitor cells and peripheral blood-derived endothelial progenitor cells has been proposed to stimulate postnatal neoangiogenesis. Because of their unique plasticity, hematopoietic progenitor cells can differentiate in both vascular and non-vascular elements. In addition, recent work has demonstrated that VEGF is able to promote not only angiogenesis but also induce a neuroprotective effect (Storkebaum et al. 2004). These observations suggest that nature may utilize a single growth factor to target different cells in regenerating tissues. In this regard, BDNF may be a relevant growth factor to both promote neurogenesis, angiogenesis and the recruitment of circulating cells, and thereby potentially induce regeneration of ischemic tissue. In addition, the target organ specificity of BDNF, which activates TrkB receptor present primarily in the vasculature of cardiac and skeletal muscle, may limit potential deleterious effects in other vascular beds. Thus, investigation of growth factors such as neurotrophins may lead to new therapeutic strategies for cardiovascular diseases.

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Table I

Ligand/Receptor	Angiogenesis: In vitro assay	Angiogenesis: In vivo assay	References
NGF/TrkA BDNF/TrkB	HUVEC proliferation EC survival	CAM Ischemic hindlimb/Matrigel	Cantarella et al. 2002 Turrini et al. 2002; Emanuelli et al. 2002; Kermani et al. 2005
NT-4/TrkB NT-3/TrkC VEGF	ND ND HUVEC Proliferation and survival	ND ND CAM, Ischemic hindlimb, coronary ischemia, corneal pocket	Review by Carmeliet 2000