

VEGF in Biological Control

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Abstract Vascular endothelial growth factor A (VEGF-A) belongs to a family of heparin binding growth factors that include VEGF-B, VEGF-C, VEGF-D, and placental-like growth factor (PLGF). First discovered for its ability to regulate vascular endothelial cell permeability, VEGF is a well-known angiogenic factor that is important for vascular development and maintenance in all mammalian organs. The development of molecular tools and pharmacological agents to selectively inhibit VEGF function and block angiogenesis and/or vascular permeability has led to great promise in the treatment of various cancers, macular degeneration, and wound healing. However, VEGF is also important in animals for the regulation of angiogenesis, stem cell and monocyte/macrophage recruitment, maintenance of kidney and lung barrier functions and neuroprotection. In addition to its role in regulating endothelial cell proliferation, migration, and cell survival, VEGF receptors are also located on many non-endothelial cells and act through autocrine pathways to regulate cell survival and function. The following review will discuss the role of VEGF in physiological angiogenesis as well as its role in non-angiogenic processes that take place in adult organs. *J. Cell. Biochem.* 102: 1358–1367, 2007. © 2007 Wiley-Liss, Inc.

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Vascular endothelial growth factor (VEGF) is a multifunctional molecule with several important biological activities that depend on both the stage of development and physiological function of the organ in which it is expressed. VEGF was first discovered as a permeability factor secreted by carcinoma cell lines and found to enhance the accumulation of ascites fluid in tumors. It was thus appropriately named, vascular permeability factor (VPF) [Senger et al., 1983]. Shortly thereafter, VEGF was also isolated as an angiogenic factor that displayed the ability to stimulate endothelial cells to proliferate, migrate, and survive in a serum poor environment [Leung et al., 1989]. The importance of this growth factor was further established in transgenic mouse gene deletion models which demonstrated that deletion of a single *Vegf*

allele or receptor gene allele, VEGFR-1 (Flt-1) or VEGFR-2 (KDR/Flk-1), results in embryonic lethality stemming from abnormal vascular development [Fong et al., 1995; Shalaby et al., 1995; Carmeliet et al., 1996; Ferrara et al., 1996]. The rapid expansion of VEGF-dependent capillary networks during organ development has led to many important insights into the functional role of this angiogenic factor in the regulation of vasculogenesis, the differentiation and organization of endothelial progenitor cells into a primary capillary plexus, and angiogenesis, the formation of new capillaries from preexisting vessels [reviewed in Ferrara et al., 2003]. While the critical importance of VEGF for development is well established, Gerber et al. [1999] using both VEGF gene deletion and inhibition of VEGF receptor signaling approaches, reported that VEGF was required for early postnatal development but that once a mature, stable vascular system had been formed capillaries became less dependent on continued VEGF expression. This finding, along with the Folkman hypothesis that neovascularization triggers increased tumor growth and metastasis [Folkman et al., 1971], has led to many promising anti-angiogenic reagents that are currently being developed to treat solid tumors. However, while pharmaceutical agents, which block VEGF signaling pathways, have great potential for treating pathological

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angiogenesis associated with cancer, macular degeneration, and aberrant tissue repair such as psoriasis, VEGF has many beneficial functions involved in regulating physiological angiogenesis associated with exercise and metabolism, ovarian follicular development and function, and wound healing. In addition VEGF plays important roles in several non-angiogenesis-related cellular functions including control of vascular permeability and maintenance and protection of both vascular endothelial and non-endothelial cells in mature mammals. This review will highlight some of the recent studies investigating VEGF-dependent biological control of adult organ-specific homeostasis.

VEGF LIGAND-RECEPTOR INTERACTIONS

VEGF (or VEGF-A) is a ~23 kDa glycoprotein which belongs to a family of proteins that include VEGF-B, VEGF-C, VEGF-D, and placenta-like growth factor (PLGF). A review of VEGF receptors can be found in Olsson et al. [2006]. The predominant form, VEGF-A, is alternatively spliced into at least five different isoforms in humans VEGF₁₂₁, VEGF₁₄₅, VEGF₁₆₅, VEGF₁₈₉, and VEGF₂₀₆. The mouse VEGF-A isoforms have one less amino acid (VEGF₁₂₀, VEGF₁₆₄, etc.). It is thought that VEGF₁₂₀ is the most diffusible isoform due to the absence of a heparin-binding domain. VEGF₁₆₅ exists in both diffusible and matrix bound locations and larger isoforms remain localized within the cell or are stored in the extracellular matrix. However, this distribution may also depend on the organ-specific extracellular environment or localized presence of receptors and co-receptors, in particular heparin sulfate proteoglycans. It was recently shown that myoblast-delivered VEGF isoforms all display a limited ability to diffuse throughout skeletal muscle and comparable bioactivity. This is evident from the similar extent of hemangioma formations and presence of capillaries with a tortuous morphology irrespective of the presence of the heparin-binding domain [Springer et al., 2007]. In the extracellular matrix VEGF can be released or activated by plasmin, lactate [Kumar et al., 2007], or matrix metalloproteinase's (MMPs) that allow receptor binding to occur. In addition it has been reported that VEGF may also

be further post-translationally processed by MMP's leading to biologically active forms that signal either vessel dilation or the formation of neovascular sprouts in tumor models [Lee et al., 2005]. VEGF bioactivity is transmitted through the binding of specific receptors and co-receptors that are located on vascular and lymph-vessel endothelial cells as well as non-endothelial cell types including monocytes and macrophages, hematopoietic stem cells, epithelial cells, fibroblasts, smooth muscle cells, and myogenic precursor cells (satellite cells) [reviewed in Olsson et al., 2006]. Three types of VEGF receptors, [VEGFR-1 (flt-1), VEGFR-2 (KDR/flk-1), and VEGFR-3 (flt-4)], which contain tyrosine kinase activity, are activated by the binding of VEGF homo- or hetero dimers. Co-receptors, including heparin sulfate proteoglycans and neuropilins, lack tyrosine kinase activity but may modify the binding to tyrosine kinase containing VEGFRs or bind VEGF directly to signal a cellular response. VEGF-A, B, and PLGF bind to VEGFR-1 and transduce ligand dependent signals, possibly based on a ligand-specific pattern of receptor phosphorylation [Autiero et al., 2003], to regulate vascular development. Interestingly, it has been found that the tyrosine kinase domain of VEGFR-1 is not essential for embryonic development [Hiratsuka et al., 1998]. During embryogenesis VEGFR-1 acts as a "decoy" through its ability to bind VEGF-A and regulate the level of accessible VEGF-A available to bind to VEGFR-2 and initiate vessel formation. A soluble form of VEGFR-1 (sFlt-1) has also been found to be expressed through alternative transcription and act as a natural "VEGF-Trap" binding to VEGF-A and preventing angiogenesis. It has also been implicated as a contributing factor in the placental insufficiency condition known as preeclampsia [Maynard et al., 2003]. VEGFR-1 is located on monocytes, macrophages, and hematopoietic precursor cells which are directed to sites of inflammatory angiogenesis through the actions of VEGF-A and PLGF [Autiero et al., 2003]. The ligand for VEGFR-2 is VEGF-A and this interaction is important for angiogenesis, which occurs through the coordinate signaling of endothelial-cell proliferation, migration, and recruitment of endothelial cell progenitor cells (EPC). VEGF-A-VEGFR-2 receptor interactions have also been shown to provide pro-survival or anti-apoptotic signals in serum-starved conditions through

phosphatidylinositol 3'-kinase/Akt signaling [Gerber et al., 1998]. VEGF-C and VEGF-D bind to VEGFR-3 and are mainly involved in lymphangiogenesis. In addition the neuropilin co-receptors (Np-1 and Np-2) are increasingly being recognized for their ability to function in neuronal cell protection, axon growth, and guidance, as well as affecting VEGF signaling by modulating the distribution of VEGF in various tissues and their presence on and functional role in both vascular endothelial and non-endothelial target cells (i.e., podocytes) [Foster et al., 2003].

DYNAMIC REGULATION OF VEGF AND CAPILLARITY IN SKELETAL MUSCLE

One physiological example of dynamic VEGF-dependent capillary regulation occurs in adult skeletal muscle. Changes in capillary number that occur in skeletal muscle with activity (exercise), or inactivity (disuse atrophy) are

biological processes of capillary formation and regression that are precisely controlled. The other well-studied example of adult physiological angiogenesis occurs in the ovary during follicular development of the corpus luteum during the ovarian cycle [Goede et al., 1998]. In skeletal muscle a close relationship exists between individual skeletal fiber types and the number of capillaries that perfuse each fiber [Smith et al., 1989]. This allows sufficient oxygen availability to meet myocyte metabolic demand, removal of cell debris and metabolic by products as well as providing a conduit for distributing hormones, growth factors, and cytokines. In mouse skeletal muscle it has been demonstrated that VEGF is necessary for maintaining capillary number in adult mouse skeletal muscle [Tang et al., 2004a] (Fig. 1). This was demonstrated using a Cre-LoxP strategy to deliver *cre* recombinase to a localized region of the gastrocnemius of mice that contain a floxed VEGF gene (*VEGFLoxP* mice [Gerber et al.,

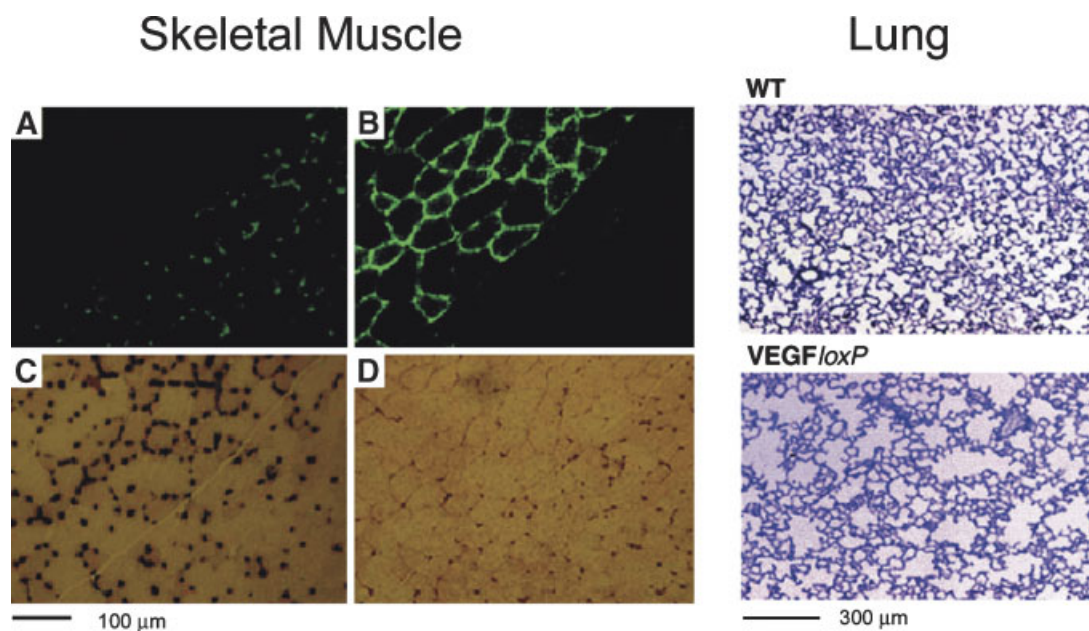


Fig. 1. Vascular endothelial growth factor (VEGF) gene deletion in skeletal muscle leads to capillary regression while in the lung it results in destruction of the alveolar-capillary barrier. The following micrographs demonstrate how VEGF gene deletion targeted to different organs in an adult mouse leads to divergent outcomes. In skeletal muscle (A–D), VEGF gene deletion was accomplished in a localized region by directly injecting the gastrocnemius muscle of *VEGFLoxP* mice with an adenovirus that expresses *cre* recombinase (5×10^9 AAV/*Cre* particles/muscle). Eight weeks later, infected fibers from *VEGFLoxP*(+/+) transgenic mice were identified by nuclear immunostaining of serial cross sections with a *Cre* recombinase antibody (green) (A) and revealed lower levels of VEGF at the myocyte periphery

(green) (B), a 67% loss of capillaries (capillary/fiber ratio) detected by alkaline phosphatase staining (purple) (C), and evidence of ongoing apoptosis detected by TUNEL staining (brown) (D) [Tang et al., 2004a “used with permission”]. However using the same AAV/*Cre* to target VEGF gene deletion in the lungs *VEGFLoxP* mice ($\sim 10^{10}$ AAV/*Cre* particle per lung) resulted in a caspase-3 dependent apoptosis and a heterogeneous destruction of alveolar septal wall cells. Insufficient lung VEGF resulted in enlarged airspaces resembling the parenchyma degradation observed in an emphysematous lung. WT—wild type mouse, *VEGFLoxP*—mice in which exon 3 of the VEGF gene is surrounded by loxP sites. WT and *VEGFLoxP* mice were both delivered the same dose of AAV/*Cre* [Tang et al., 2004b “used with permission”].

1999)). Furthermore, signals (hypoxia, inflammatory cytokines, reactive oxygen species, nitric oxide, and mechanical stimuli) generated during exercise lead to an increased expression of VEGF [Breen et al., 1996] and exercise training leads to a biphasic increase in VEGF expression with the second peak of expression correlating with capillary growth [Milkiewicz et al., 2005]. These data suggest a role for VEGF in both capillary maintenance and skeletal muscle angiogenesis. Capillary formation in skeletal muscle may take place via one of three mechanisms depending on the type of stimuli applied to the muscle. Increased blood flow or chronic vasodilatation has been shown to lead to intra-luminal splitting in which the existing capillary vessel is divided by the formation of an intercellular matrix structure [Brown and Hudlicka, 2003]. Alternatively, if a muscle is stimulated by increased stretch or chronic overload resulting in a change in sarcomere length, new capillaries form by abluminal sprouting which involves a breakdown of the basement membrane through the action of MMPs to allow a new vessel to proliferate, mature, and connect to the existing capillary network. Repeated bouts of exercise (training) or electrically stimulated muscle contraction over several weeks lead to an increase in capillary number through both mechanisms of intra-luminal splitting and neovascular sprouting [Brown and Hudlicka, 2003]. Thus, capillary number is adjusted to meet the metabolic demand of the muscle fibers, whether it be oxygen supply, a metabolic increase in adenosine or mechanical stretch that simulate new vessel growth until that demand or exercise-related stimuli is discontinued in a negative feedback loop that likely coordinates a balance of several pro- and anti-angiogenic factors [Adair et al., 1990]. In contrast, muscle subjected to an ischemic insult results in a dramatic and rapid increase in VEGF that occurs without an accompanying increase in angiogenesis. This imbalance between VEGF expression and capillarity may in part be due to the inhibition of blood flow-stimulated nitric oxide-dependent vasodilatation and endothelial VEGFR-2 expression or the absence of monocyte/macrophage recruitment [Heil et al., 2004]. Furthermore, these findings highlight the requirement for both the ligand and receptor to be available in order to achieve VEGF-dependent bioactivity and signaling of new

capillary formation [Milkiewicz et al., 2005, 2006].

ANGIOGENESIS IN INFLAMMATION, CANCER, WOUND HEALING, AND REPAIR

Unlike the controlled capillary formation and regression that occurs in physiological angiogenesis, *pathological* angiogenesis is often associated with abnormal capillary networks characterized by tortuous, malformed, highly permeable, and unstable vessels. These effects are likely to be of benefit in a temporary wound healing or reparative response as occurs in the skin or during bone repair as a way to mount an initial innate immune response and deliver neutrophils and macrophages to sites of injury. These inflammatory cell types participate in the removal of necrotic cells, express MMPs involved in tissue remodeling and initiate new collateral vessel formation [Carmeliet et al., 2001; Bussolati et al., 2004; Frantz et al., 2005]. This process of inflammatory angiogenesis is thought to rely on the coordinate expression of VEGFR-1 located on the cell surface of monocyte/macrophage and hematopoietic stem cells which are signaled through both VEGF-A and PLGF and recruited to the site of injury. Once recruited, these cells provide a source of both growth factors and proteases which function to modulate both capillary regression and neovascular formation [Carmeliet et al., 2001; Lobov et al., 2005]. In the absence of the correct balance of VEGF, PLGF, VEGFR-1, and -2 uncontrolled inflammatory angiogenesis leads to a more pathological state as may occur in psoriatic skin, rheumatoid arthritis, atherosclerosis, diabetic retinopathy, age-related muscular degeneration (AMD), retinopathy of prematurity and tumor progression [Ferrara et al., 2003]. This type of abnormal hyperpermeable capillary structure is also observed in many organ targeted transgenic mice or viral delivery models of VEGF over-expression in which even a relatively low level of increased VEGF expression leads to abnormal, leaky capillaries. This suggests that a localized threshold or critical level of VEGF within a cell microenvironment is responsible for inducing a vascular permeability response [Ozawa et al., 2004]. Factors implicated in down-regulation of leukocyte infiltration and vascular hyperpermeability associated with inflammatory angiogenesis include heme oxygenase 1 and Tie 2

which are thought to both down-regulate inflammatory cell accumulation and enhance vessel permeability. At the same time they promote stable capillary formation associated with tissue repair [Hughes et al., 2003; Bussolati et al., 2004].

VEGF-DEPENDENT CAPILLARY REGRESSION

One area of active research is the identification of the phenotypes environmental signals, or cellular events that allow some capillary structures to lose their dependence on VEGF expression—that is, remain stable in the absence of continued VEGF expression. This has implications not only for the safety or side effects of using therapeutic agents to block VEGF function, but also for the potential for exploiting these differences to more directly target these agents to capillaries infiltrating tumor microenvironments. A recent study by Kamba et al. compared the extent of capillary regression in several organs of adult mice treated with a VEGF receptor tyrosine kinase inhibitor or soluble VEGF receptor, sVEGFR-1 or VEGF-Trap_{R1R2}, over a 1–3 week period. Several organs involved in the secretion of hormones and water and solute exchange showed VEGF-dependent capillary regression with the overall greatest regression of 68% observed in the thyroid [Kamba et al., 2006]. Additional organs, which responded to VEGF signaling blockade with a significant reduction in capillarity, included pancreatic islets, adrenal cortex, small intestinal villi, pituitary, epididymal adipose tissue and the trachea. Common features of these VEGF-dependent capillary beds were an endothelial cell layer containing numerous fenestrations and abundant expression of VEGFR-2 and VEGFR-3. However, these features do not appear to be a prerequisite for VEGF-dependent regression as the kidney did not respond to soluble VEGF receptors and also contains a well fenestrated-endothelium and abundant VEGFR-2 and VEGFR-3 expression. Furthermore, these regressed capillary structures were readily restored upon withdrawal of the VEGF blocking agents to the extent that the underlying basement membrane was preserved or did not become degraded by local extracellular matrix proteases [Kamba et al., 2006]. Another significant finding from this series of experiments is that tumor vasculature in spontaneous islet-cell

tumors of RIP-Tag2 transgenic mice and in mice with subcutaneously implanted Lewis lung carcinomas had the greatest reduction in capillary number in response to VEGF blockade compared to normal adult organs and the highest expression of endothelial VEGFR2 and VEGFR3. These studies suggest an alternative to the hypothesis that formation of stable, VEGF-independent capillary structures occurs upon the incorporation of pericytes around the newly formed vessel although this may still hold true during embryonic and neonatal capillary formation [Benjamin et al., 1998].

Furthermore, it is still unresolved as to why some adult capillaries in addition to arterioles and post-capillary venules are not affected by an almost complete inhibition of VEGF and/or its receptors. While VEGF is essential for vasculogenesis and angiogenesis during the initial period of embryogenesis, recent genetic deletion of a single allele of delta-like ligand (DLL) 4 also prevents embryonic vasculogenesis and angiogenesis [Gale et al., 2004]. Furthermore, DLL4-dependent abnormalities appear to be limited to arterial capillaries, not capillaries on the venous side of the vascular system or post-capillary veins [Lobov et al., 2007]. These findings suggest that the arterial-venous (A-V) specialization of endothelial cells in capillaries and arterioles is predetermined during early embryogenesis. DLL4 is also a downstream target gene of VEGF and DLL4-Notch1 signaling leading to the coordinate expression of a select set of genes expressed in arterial endothelial cells including EphrinB2 [Lawson et al., 2001]. The DLL4-Notch1 signaling is also important in cell–cell communication between adjacent endothelial cells. DLL4 has been reported to be expressed at the tip of emerging vascular sprouts but also signals preceding endothelial cells to remain inactive or cease sprouting, thus directing the pattern of sprouting and branching within a capillary network [Lobov et al., 2007]. Thus, the fate of endothelial cells to specialize as arterial or venous in the capillary network is predetermined during development but may be modulated later on by factors such as an increase in mechanical stimuli during exercise or hypoxia that allows capillary structures containing arterial endothelial cells to differentiate into larger arterioles or arteries [White et al., 1998; Aranguren et al., 2007; Lobov et al., 2007]. A complexity of interactions between several pro-angiogenic

factors and anti-angiogenic factors are likely to regulate the survival of VEGF-independent microvasculature and subtle changes in the spatial and temporal relationships of these factors in a specific organ or tumor environment may effect the endothelial specialization, pattern and function of each capillary network.

PROTECTION OF SPECIALIZED BARRIERS IN THE KIDNEY AND LUNG

Two organs which have abundant VEGF expression in epithelial cells are the kidney and lung [Maharaj et al., 2006]. These organs are both comprised of highly specialized, regulated barriers. In the case of the kidney, VEGF is expressed by specialized epithelial cells called podocytes, which overlay glomerular blood vessels and function in the regulation of water and macromolecular solute exchange. The kidney endothelium is also highly fenestrated with abundant VEGFR-2 and VEGFR-3 expression but, as mentioned above, is fairly resistant to VEGF-dependent capillary regression by administration of soluble receptors [Kamba et al., 2006]. In the kidney, VEGF, in addition to signaling glomerular endothelial cells which express high levels of constitutively phosphorylated VEGFR-2 [Maharaj et al., 2006], regulates the survival of renal glomerular epithelial cells (podocytes) through an autocrine interaction with VEGFR-1 and neuropilin 1 (Np-1) but not VEGFR-2 [Foster et al., 2003]. The autocrine action of VEGF is essential for maintaining podocyte intracellular cytosolic calcium levels and a selective barrier to macromolecules [Foster et al., 2003]. Consequently dysregulated podocyte function may contribute to a loss in macromolecular selectivity, glomerular filtration, and proteinuria. For instance, the decreased survival in early postnatal mice treated with the soluble VEGF receptor chimeric protein, mFlt(1-3)-IgG, was accompanied by both kidney and liver failure [Gerber et al., 1999]. In adults, VEGF-dependent kidney malfunction (as evident by the presence of hypertension, proteinuria and glomerular endotheliosis) has been described in patients with preeclampsia characterized by high circulating sFLT-1 levels [Maynard et al., 2003]. Moreover, the clinical side effects reported after the use of the anti-VEGF therapy, bevacizumab (Avastin, Genentech), include hypertension and proteinuria [Zhu et al., 2007]. These findings collectively emphasize the importance of

VEGF in the homeostasis of adult kidney function.

In the lung VEGF is expressed at high levels by specialized epithelial cells in the gas exchanging regions of the lung, called alveolar type II cells and bronchial epithelial cells which are located in the parenchyma and small airways, respectively [Maharaj et al., 2006]. The specialized alveolar epithelial cell, known as a type II cell, is also the surfactant-producing cell, and VEGF delivery to mice with a deficiency in HIF-2 α , which have a phenotype similar to fetal respiratory distress syndrome (RDS), were found to have improved survival and restoration of surfactant homeostasis [Compernelle et al., 2002]. These two epithelial cell populations, bronchial airway and alveolar epithelial cells, although located on a continuum of the airway tree demonstrate a differential response to VEGF inhibition. In the trachea VEGF-expressing cells signal a fenestrated endothelium and inhibition of VEGF signaling leads to regression of the capillaries that supply the cellular components of the airway wall [Baffert et al., 2006]. Over-expression of VEGF in the upper airways, induced by inflammatory cytokines such IL-3, has also been hypothesized to increase vascular permeability and enhance respiratory antigen sensitization and T(H)2 inflammation in pathological settings such as asthma [Lee et al., 2004]. In contrast, loss of VEGF expression or receptor signaling in the parenchyma of the lung leads to caspase-3 dependent apoptosis of alveolar septal endothelial and epithelial cells and down regulation of VEGFR-2 leading to destruction of the alveolar wall and a heterogeneous enlargement of airspaces throughout the lung parenchyma [Kasahara et al., 2000; Tang et al., 2004b] (Fig. 1). These parenchymal changes in lung structure are very similar to the alveolar destruction that occurs in patients with emphysema and indeed biopsies taken from patients with COPD revealed a decrease in VEGF expression and enhanced apoptotic cell number [Kasahara et al., 2001]. Unlike the highly fenestrated endothelium of the water and solute exchanging kidney, the lung alveolar-capillary barrier is thought to consist of an endothelial layer that is devoid of fenestrations and allows rapid fluid clearance and lymphatic drainage which is essential to maintaining efficient gas exchange. Studies of VEGF in the lung suggest a main role for this epithelially expressed growth factor

as an anti-apoptotic, pro-survival factor for both endothelial and epithelial cells located throughout the vast alveolar-capillary barrier [Klekamp et al., 1999; Kasahara et al., 2001; Tang et al., 2004b]. It is also an important factor for the maintenance of surfactant homeostasis [Comperolle et al., 2002]. The lung represents an example in which VEGF-expressing cells within the same organ or airway may have specialized function in vascular permeability regulation or epithelial barrier repair. Thus cellular VEGF functions, which may depend on the differentiated cell type expressing VEGF and its location within the lung, provides a first line of defense against exposure to environmental pollutants, antigens, and infectious agents.

VEGF AND NEUROPROTECTION

In addition to a cellular role for VEGF in regulating proliferation, migration, permeability, and action as an anti-apoptotic molecule, the most recently ascribed function assigned to VEGF is that of a neuroprotective agent. The first clue that VEGF may play a role in neuronal cells came from the identification of the VEGF co-receptors, neuropilins, which bind both VEGF and several members of the semaphorin family of proteins involved in axon guidance [Olsson et al., 2006]. *In vitro*, VEGF has been reported to have a direct effect on cultured motor neurons protecting them from toxicity due to hypoxia, increased reactive oxygen species, serum deprivation, glutamate-induced excitotoxicity [Oosthuyse et al., 2001; Svensson et al., 2002] as well as a trophic factor for neuronal stem cells [Schanzer et al., 2004]. However, the discovery of VEGF as a critical factor in peripheral motor neuron function came from a study by Oosthuyse et al., [2001] that described symptoms of severe adult onset muscle weakness due to the degeneration of the peripheral motoneurons that stimulate skeletal muscle fibers in a transgenic mouse engineered with a deletion in the hypoxic response regions (HRE) of the VEGF promoter, referred to as VEGF^{δ/δ} mice. The VEGF^{δ/δ} mice, with impaired hypoxic transcriptional regulation of VEGF, were found to have many similarities to the symptoms observed in patients with amyotrophic lateral sclerosis (ALS), more commonly known as Lou Gehrig's disease. Careful analysis of the VEGF^{δ/δ} mice

revealed some very intriguing findings. A primary observation was that while VEGF is transcriptionally regulated by numerous hypoxia—dependent and independent transcription factors that recognized VEGF promoter elements, deletion of the HRE in these mice resulted in a 60% decrease in survival. The surviving mice grew at a slower rate and remained reduced in size. These mice were also found to display an inability to increase VEGF levels in response to hypoxia that was limited to neuronal tissue and did not include skeletal or cardiac muscle. Furthermore, the capillary number in skeletal muscle was not altered suggesting that skeletal muscle angiogenesis or oxygen availability was not a contributing factor to the observed muscle weakness [Oosthuyse et al., 2001]. What was reported was a muscle weakness primarily due to impaired motor neuron performance that progressed slowly and at 4 months of age showed signs of overall neurogenic muscle atrophy. *In vivo* VEGF is expressed in the spinal cord neurons and astrocytes which signal through VEGFR-2 and neuropilin-1 located on motor neurons and peripheral nerve axons [Oosthuyse et al., 2001]. The defects appear to stem from a loss of motor axons in the peripheral nerves of VEGF^{δ/δ} mice as sciatic and phrenic nerves progressively lost their large myelinated A γ axons that innervate muscle fibers. The underlying cause proposed by these investigators was a decrease in neural vascular perfusion leading to chronic ischemia in the motor neurons of the spinal cord [Oosthuyse et al., 2001]. This group of investigators went on to perform further studies to reveal a genetic link between individuals with haplotypes in the VEGF promoter and leader sequences, lower VEGF plasma levels and increased risk of ALS in three European populations [Lambrechts et al., 2003]. In addition they have shown that direct delivery of VEGF to the cerebrospinal fluid in a rat model of ALS due to mutation in the superoxide dismutase gene [SOD1(G93A)] was able to delay the onset of paralysis, improve motor performance and increase survival time by several weeks [Storkebaum et al., 2005]. This novel role for VEGF in maintaining neuronal cells and/or recruiting neural progenitor cells brings up many interesting questions concerning the interconnection between peripheral nerve input to skeletal muscles and neuronal stimulated fiber type composition

and vascular patterns that are dependent on exercise (activity) and/or VEGF expression. Likewise much is to be learned about the potential cross talk between neuronal and growth factor signals that regulate organ growth and development during embryogenesis and early postnatal development. Also of medical interest is cerebral VEGF-dependent edema thought to occur in response to acute hypoxic exposure [Xu and Severinghaus, 1998].

FUTURE DIRECTIONS

VEGF, first discovered over 20 years ago as a VPF secreted by tumors [Senger et al., 1983], has turned out to be an impressive and potent multifunctional growth factor essential for development, hematopoietic stem cell recruitment and the regulation of permeability, angiogenesis, and neurogenesis in both the normal functioning of many adult organs as well as being implicated as a contributing factor in many diverse disease states. Several areas of research show great promise for development into clinical benefits:

1. Understanding how VEGF-induced angiogenesis differs in controlled physiologic settings as opposed to the inflammatory angiogenesis associated with disease states.
2. The elucidation not only of the signals that stimulate new blood vessel formation but the precise and controlled mechanisms that lead to cessation of angiogenesis and in some cases capillary regression. Many clues to understanding the control of pro- and anti-angiogenic factors that regulate angiogenesis are currently being revealed from the study of organ development as well as physiologic angiogenesis in the ovary and exercise-induced capillary regulation in adults.
3. Deciphering the VEGF signaling pathways that regulate different functions: permeability, angiogenesis, cell survival, and neuroprotection, to enable tailoring of pharmaceutical agents to selectively block deleterious VEGF-induced cell functions while preserving stable, oxygen- and nutrient-conducting capillaries.
4. Further understanding and identification of the non-endothelial cells, monocytes, macrophages, mast cells, eosinophils, dendritic cells, magakaryocytes, lymphocytes, hematopoietic cells, type II cells, lens epithelia,

podocytes, and hepatocytes, that are directly regulated by the autocrine actions of VEGF to maintain organ-specific functions and signal nearby endothelial cells through a combination of receptor and co-receptor interactions.

5. The understanding of VEGF in adult physiological functions will come through the further development of methods to neutralize VEGF in vivo in animal models as well as the rapid and ongoing development of anti-VEGF therapies, and the natural "VEGF-trap" or sFLT implicated in the placental disease, preeclampsia.
6. Finally, the exciting novel discovery that VEGF is also a regulator of neuronal cell survival and function will lead to a rethinking of how VEGF may meet the metabolic demands of each tissue by regulating both neural input as well as delivery of oxygen and nutrients and how these pathways are interconnected.

Therapeutic strategies on the horizon include not only anti-angiogenic treatments, but also ways to regulate the spatial and temporal delivery of VEGF to treat a variety of diseases characterized by poor capillarity and/or neurogenesis, including stroke, coronary artery disease, peripheral muscle impairment associated with chronic obstructive pulmonary disease, wound healing, Alzheimer's disease and ALS.

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