FEBS Letters 580 (2006) 2879–2887

Minireview

Biology of vascular endothelial growth factors

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Received 14 February 2006; revised 27 March 2006; accepted 31 March 2006

Available online 17 April 2006

Edited by Horst Feldmann

Abstract **Angiogenesis is the process by which new blood vessels are formed from existing vessels. The vascular endothelial growth factors (VEGFs) are considered as key molecules in the process of angiogenesis. The VEGF family currently includes VEGF-A, -B, -C, -D, -E, -F and placenta growth factor (PlGF), that bind in a distinct pattern to three structurally related receptor tyrosine kinases, denoted VEGF receptor-1, -2, and -3. VEGF-C and VEGF-D also play a crucial role in the process of lymphangiogenesis. Here, we review the biology of VEGFs and evaluate their role in pathological angiogenesis and lymphangiogenesis.**

Keywords: VEGF; Angiogenesis; Signaling; Diseases

1. Introduction

Vascular endothelial growth factor (VEGF) is a specific mitogen for vascular endothelial cells (EC). VEGF family consists of seven members – VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F and placental growth factor (PlGF). They share a common structure of eight characteristically spaced cysteine residues in a VEGF homology domain. These members have different physical and biological properties and act through specific tyrosine kinase receptors – VEGFR-1, VEGFR-2 and VEGFR-3. Neuropilin-1 (Nrp-1) and Nrp-2 are receptors for semaphorins, but they also bind to some members of the VEGF family. VEGF/VEGF-receptor system is a key component in the complex process of angiogenesis that also includes many other stimulators, inhibitors and angiogenic modulators. VEGFR-3 and its ligands VEGF-C and VEGF-D are important regulators of lymphangiogenesis, while PlGF has been associated with arteriogenesis. VEGFs are crucial in embryonic development and in other physiological and pathological conditions, including wound healing, rheumatoid arthritis, ocular neovascularization, tumor progression, endometriosis and cardiovascular diseases.

2. VEGF-A

VEGF-A is a key molecule in induction of angiogenesis and vasculogenesis it causes proliferation, sprouting, migration and tube formation of ECs [1]. VEGF-A gene is located at chromosome 6p21.3 and is encoded by 8 exons separated by 7 introns. VEGF induces angiogenesis in a variety of physiological and pathological conditions including embryogenesis, corpus luteum formation, tumor growth, wound healing, and compensatory angiogenesis in the heart.

VEGF-A mediates its responses primarily by activating VEGFR-1 and VEGFR-2 but it also binds to Nrp-1 and Nrp-2 [2]. Overexpression of VEGF-A produces a pronounced strong angiogenic response in different tissues. However resulting vessels are often large, dilated and leaky [3–5].

VEGF-A was initially described as a vascular permeability factor secreted by carcinoma cell lines that enhanced permeability in skin blood vessels and also stimulated the production of ascites [6]. Molecular mechanisms by which VEGF-A induces these effects are not well characterized. It has been postulated that VEGF-A increases permeability by binding to VEGFR-2 and thereafter activating guanylyl cyclase and cGMP via a nitric oxide dependent pathway. Increased cGMP levels probably enhance endothelial permeability by increasing the vesico-vascular organelles, fenestrations and transcellular gaps [7]. VEGF-A mediated extravasation of fluid and plasma proteins, including fibrin might contribute to enhanced migration of ECs in extracellular matrix [8]. VEGF-A also causes vasodilatation by induction of endothelial nitric oxide synthase (eNOS) and increasing nitric oxide production [9].

VEGF-A promotes EC survival by inducing the expression of anti-apoptotic proteins Bcl-2 and A1 in the ECs. This action of VEGF-A might be related to the activation of phosphatidylinositol-3 kinase and Bcl-2 pathways [10]. Most of the studies on VEGF-A have primarily focused on their action on ECs. However, the actions of VEGF-A on other cell types have also been described. VEGF-A is mitogenic for retinal pigment epithelial cells and Schwann cells. VEGF-A also has a neuroprotective effect on hypoxic motor neurons, and is a modifier of amyotrophic lateral sclerosis [11]. Role of VEGF-A in vascular smooth muscle cell proliferation and migration has also been reported [12]. VEGF-A is also reported to have hematopoietic effects. It induces colony formation by mature subsets of granulocyte-macrophage progenitor cells and regulates hematopoietic stem cell survival by an internal autocrine loop mechanism [13] and promotes monocyte chemotaxis [14]. It also exerts procoagulant activity via its ability to stimulate the production of the potent initiator of coagulation tissue factor in ECs and monocytes [14].

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0014-5793/$32.00
doi:10.1016/j.febslet.2006.03.087

At least six different isoforms, of VEGF-A polypeptides of different sizes (121, 145, 165, 183, 189 and 206 amino acid residues) are known to exist. These isoforms have distinct but overlapping functions in angiogenesis due to their differential binding to heparan sulphate and neuropilins [1,15]. Through alternative mRNA splicing, the VEGF-A isoforms differ by the presence or absence of sequences encoded by exons 6 and 7. VEGF-A121 does not bind to heparin or extracellular matrix while VEGF-A165 has moderate heparin binding ability. VEGF-A145 also contains a heparin binding domain and elements that enable the binding of VEGF-A145 to the extracellular matrix. VEGF-A189 and VEGF-A206 bind heparin more strongly and are sequestered in the extracellular matrix and at the cell surface and these two isoforms are probably less active than either VEGF-A121 or VEGF-A165 in vivo. The three secreted VEGF-A splice forms are VEGF-A121, VEGF-A145, and VEGF-A165 while the VEGF-A183, VEGF-A189 and VEGF-A206 are the matrix bound forms. Most VEGF-producing cells express VEGF-A121, VEGF-A165, VEGF-A183 and VEGF-A189, but VEGF-A 145 and VEGF-A206 seem to be restricted to cells of placental origin [1,15].

Homozygous VEGF-A knockout mice die at E8–E9 and mice lacking even a single VEGF-A allele die at E11–E12 indicating that VEGF-A expression at appropriate level is essential during embryogenesis. Knockout studies in mice have also suggested that the VEGF165 is probably the major isoform that brings about the VEGF-A actions [1,15].

VEGF-A mRNA expression is induced by hypoxia. It is now recognized that hypoxia-inducible factor-1α [HIF-1α] is a key mediator of the hypoxic responses. In response to hypoxia, HIF-1α binds to specific enhancer elements, resulting in increased gene transcription. Hypoxia induces binding of HIF-1α to the Hypoxia responsive element (HRE) in the VEGF-A gene promoter region, which in turn increases VEGF-A transcription. A role of von Hippel–Lindau (VHL) tumor suppressor gene in HIF-1α dependent hypoxic responses has also been described [15,16]. Mutations in the VHL gene are associated with increased angiogenesis, and tumors with VHL mutation display increased VEGF-A expression. While hypoxia is important for VEGF-A regulation, other pathways including growth factors, inflammatory cytokines and hormones also up-regulate VEGF-A mRNA expression [17].

3. PlGF

Placental growth factor (PlGF) is a member of the VEGF family which was first identified in placenta but is also known to be present in heart and lungs. Human PlGF gene has been mapped to chromosome 14q24. PlGF-coding sequence is encoded by seven exons spanning an 800-kb-long DNA interval. Four isoforms – PlGF-1, PlGF-2, PlGF-3 and PlGF-4, have been described [18]. PlGF-1 and PlGF-3 are non-heparin binding diffusible isoforms PlGF-2 and PlGF-4 have heparin binding domains. PlGFs mediate their effects through VEGFR-1 [19]. PlGF-2 is also able to bind Nrp-1 and NrP-2 due to the insertion of 21 basic amino acids at the carboxy terminus, while both PlGF-1 and PlGF-3 lack this amino acid insert.

Results available from in vitro studies on the angiogenic role of PlGF are inconsistent. In some studies PlGF binding to VEGFR-1 failed to produce EC growth and angiogenesis [20], while other studies show that PlGF/VEGFR-1 signaling promotes EC viability and angiogenesis [20]. In placenta and in PlGF-1 expressing tumors increased PlGF levels inhibit EC growth [21]. PlGF has direct effects on ECs, both by inducing its own signaling and by amplifying VEGF-driven angiogenesis [22]. PlGF-2 overexpression results in the production of significant angiogenesis in different tissues [21,23]. Various mechanisms by which PlGFs can enhance angiogenesis include (a) intracellular signal transduction through VEGFRs; (b) Increasing the fraction of VEGF-A available to activate VEGFR-2 by displacing VEGF-A from the 'VEGFR-1 sink', [22]; (c) Activation of VEGFR-1 by PlGFs results in intermolecular transphosphorylation of VEGFR-2 that could increase VEGF-A mediated angiogenesis [22]; (d) PlGF/VEGF-A heterodimer formation, which could act through VEGFR-1/VEGFR-2 [22]. Our recent results show that PlGF-2 overexpression in perivascular tissue increased VEGF-A165 and VEGF-A121 levels and produces significant angiogenesis (Fig. 1). These blood vessels were tortuous and well perfused. PlGF-2 mediated angiogenesis was effectively blocked by soluble VEGFR-1 and VEGFR-2 receptors. This data suggests that angiogenic responses to PlGF-2 are also indirectly mediated through VEGFR-2 [20]. The proposed role of PlGF in the process of arteriogenesis [24] is significant and holds promise for the treatment of ischemic diseases.

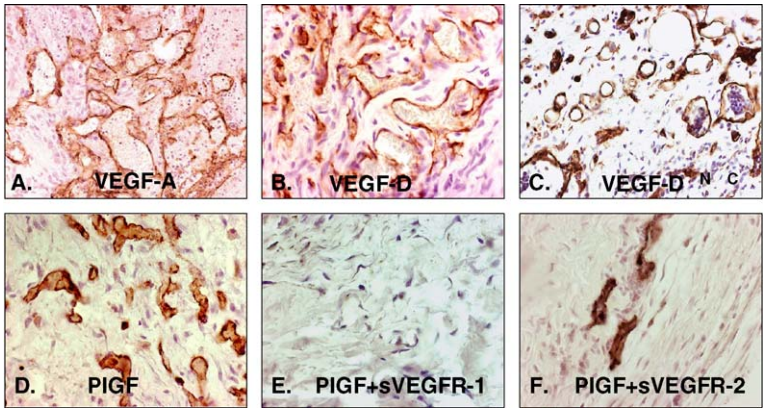

Fig. 1. Adenovirus mediated gene transfer of: (A) VEGF-A; (B) VEGF-D; (C) VEGF-DΔNΔC (proteolytically processed form of VEGF-D); and (D) PlGF to periadventital tissue resulted in formation of large tortuous blood vessels in the adventitia; (E) sVEGFR-1 gene transfer with PlGF blocks the PlGF mediated angiogenesis; (F) sVEGFR-2 gene transfer with PlGF also significantly reduces the PlGF mediated angiogenesis, thereby suggesting a role of VEGFR-1 and VEGFR-2 in PlGF mediated angiogenesis.

PlGF has a powerful chemotactic effect on monocytes and increased macrophage accumulation occurs after injection of PlGF protein [24] and adenovirus mediated PlGF gene transfer [20]. Increased infiltration of macrophages presumably contributes to the VEGF-A upregulation in the PlGF-2 transduced arteries [21].

PlGF expression is upregulated during a number of pathological conditions including non-small cell lung carcinoma (NSCLC), colorectal cancer and wound healing, while, PlGF levels are decreased in preeclampsia. A possible role of PlGF in atherosclerosis has also been described [25].

4. VEGF-B

The human VEGF-B gene spans about 4000 bp, contains eight exons and six introns, and is located on chromosome 11, band q13. The promoter region of VEGF-B is different to that of VEGF-A, and this might explain differences in regulation by physiological stimuli. While both promoters are associated with a CpG island and contain transcription factor binding sites for Sp1 and AP-2, the VEGF-B promoter contains Egr-1 sites, but lacks hypoxia-inducible factor-1 and AP-1 sites found in the VEGF-A promoter. Consequently, stimuli such as hypoxia which can induce VEGF-A expression do not appear to regulate levels of VEGF-B [26].

VEGF-B167 and VEGF-B186 are the two isoforms that are expressed in humans. The VEGF-B167 isoform is mainly expressed in the most tissues including skeletal muscles, myocardium and brown fat and accounts for more than 80% of the total VEGF-B transcripts. The VEGF-B186 isoform is expressed at lower levels and only in a limited number of tissues. VEGF-B is a ligand for VEGFR-1 and Nrp-1, and it can form heterodimers with VEGF-A [15,27]. Neither isoform binds VEGFR-2 or VEGFR-3. VEGF-B167 binds heparan sulfate proteoglycans and is mostly sequestered in the extracellular matrix while VEGF-B186 is freely diffusible.

The precise role of VEGF-B in vivo is not precisely known. Study with mice deficient in VEGF-B reported development of smaller hearts and impaired recovery after induced myocardial infarction suggesting that formation of coronary collaterals might be partly attributed to VEGF-B [28]. Also, VEGF-B has been reported to be weakly angiogenic after adenoviral delivery to periadventitial tissue or hindlimb skeletal muscle [3,4]. Reduced synovial angiogenesis in VEGF-B knockout arthritis models suggest a role of VEGF-B in inflammatory angiogenesis [29].

5. VEGF-C

The VEGF-C gene is located on chromosome 4q34. VEGF-C genes comprise over 40 kb pairs of genomic DNA and consist of seven exons. VEGF-C is produced as a precursor protein and is proteolytically activated in the extracellular space by proteases to generate a homodimeric protein with high affinity for both VEGFR- 2 and VEGFR-3. VEGF-C induces mitogenesis, migration and survival of ECs. VEGF-C is expressed in the heart, small intestine, placenta, ovary and the thyroid gland in adults. Developmental studies, knockout models and gene transfer experiments suggest that VEGF-C is primarily a lymphangiogenic growth factor and its lymphangiogenic effects are mediated by VEGFR-3 [30,31]. However, the increase in blood vascular permeability induced by VEGF-C is mediated by VEGFR-2 [31]. Disruption of the VEGF-C gene in mice demonstrates that the growth factor is indispensable in embryonic lymphangiogenesis [32]. VEGF-C is also involved in tumor and inflammation associated lymphangiogenesis. Examination of VEGF-C function in a number of assays has also shown an angiogenic activity, presumably via activation of VEGFR-2. VEGF-C gene transfer produced moderate angiogenesis in rabbit skeletal muscle [3] and perivascular tissue [4].

6. VEGF-D

VEGF-D is a secreted glycoprotein and is structurally 48% identical to VEGF-C. VEGF-D is expressed in many adult tissues including the vascular endothelium, heart, skeletal muscle, lung, and bowel. The human VEGF-D gene is 2.0 kb in size and is located on chromosome Xp22.31. The human VEGF-D is proteolytically processed in its N-terminal and C-terminal ends; the mature form binds to and activates VEGFR-2 and VEGFR-3 [32]. However the mouse VEGF-D binds only to VEGFR-3. VEGF-D has been shown to be responsible for proliferation of ECs, and it shows angiogenic properties in vitro and in vivo. Similar to VEGF-C, it also shows lymphangiogenic potential. The lack of a profound lymphatic vessel defect in VEGF-D deficient mice may reflect a subtle, redundant, or nonexistent role of this growth factor during embryonic development [33]. Nonetheless, VEGF-D may induce lymphatic vessel growth in adult life in response to pathological conditions.

VEGF-D has been proposed to have a role in tumor angiogenesis and lymphangiogenesis [34,35]. VEGF-D is able to induce strong angiogenesis in addition to lymphangiogenesis in the rabbit hindlimb muscles [7]. Adenovirus mediated gene transfer of VEGF-D and mature VEGF-D in the periadventitial space produces significant activation of angiogenesis (Fig. 1) and vascular smooth muscle cell (SMC) proliferation [4,12].

7. VEGF-E

VEGF-E was discovered in the genome of the parapoxvirus (Orf virus) that infects sheep, goats, and occasionally humans [36]. Infection by this virus causes proliferative skin lesions in which extensive capillary proliferation and dilation are prominent histological features. Several strains of the virus encode different VEGF-E variants, which bind specifically to VEGFR-2 and Nrp-1 and are able to stimulate EC mitogenesis and vascular permeability. Gene expression of VEGF-E induces a strong angiogenic response. Edematous lesions and hemorrhagic spots on the ear which were reported as side effects in VEGF-A transgenic mice were not detectable in VEGF-E transgenic mice [37,38].

8. VEGF-F

Recently a seventh member of the VEGF family, VEGF-F, was identified from snake (viper) venom. VEGF-F consists of

two VEGF-related proteins designated vammin (110 residues) and VR-1 (109 residues) that have a 50% primary structure identity with VEGF-A165 and bind selectively to VEGFR-2 [39]. VEGF-F contains a short C-terminal heparin-binding region and the C-terminal peptide of VEGF-F exhibits a specific blockage of VEGF-A165 activity both in vitro and in vivo [40].

9. VEGF receptors (VEGFR)

9.1. VEGFR-1

VEGFR-1 is expressed in ECs as well as pericytes, placental trophoblasts, osteoblasts, monocytes/macrophages, renal mesangial cells and also in some hematopoietic stem cells [41]. VEGFR-1 binds VEGF-A, VEGF-B and PlGF with high affinity (Fig. 2). VEGFR-1 knockout mice die at early stages of embryogenesis due to disorganization of blood vessels and overgrowth of EC. VEGFR-1 transmits only weak mitogenic signals in ECs, but it is known to form a heterodimer with VEGFR-2, that has strong signaling properties [42]. VEGFR-1 activation at least by PlGF can also promote angiogenesis, presumably through intracellular crosstalk with VEGFR-2 [22]. VEGFR-1 is associated with monocyte chemotaxis and in the recruitment and survival of bone marrow derived progenitor cells. VEGFR-1 expression is upregulated during angiogenesis and also by hypoxia, unlike that of VEGFR-2 and VEGFR-3. A soluble form of VEGFR-1 (sVEGFR-1), consisting of the extracellular domain of VEGFR-1, is able to inhibit VEGF action in humans and mice and has been linked to preeclampsia [43].

9.2. VEGFR-2

VEGFR-2 (kinase-insert domain receptor, KDR/fetal liver kinase, Flk-1) binds VEGF-A, VEGF-C and VEGF-D. VEGFR-2 is the primary receptor transmitting VEGF signals in ECs [38,41]. The VEGFR-2 signaling pathway is crucial in bringing about the effects of VEGFs including vasodilatation, endothelial cell migration and proliferation (Fig. 2). Besides endothelial cells, VEGFR-2 is also expressed by circulating endothelial progenitor cells, pancreatic duct cells, retinal progenitor cells and megakaryocytes [1]. VEGFR-2 may be associated with integrin-dependent migration of ECs, as it forms a complex with integrin $\alpha_V\beta_3$ upon binding VEGF-A [44].

9.3. VEGFR-3

VEGFR-3 (fms-like tyrosine kinase 4, Flt4) binds VEGF-C and VEGF-D. VEGFR-3 is present on all endothelia during development but in the adult it becomes restricted to lymphatic ECs and certain fenestrated blood vascular ECs [45]. Knockout and developmental studies suggest that VEGFR-3 signaling is essential for development of blood vessels during embryonic stage but becomes redundant in mature vessels. However, VEGFR-3 is upregulated on ECs of vascular tu-

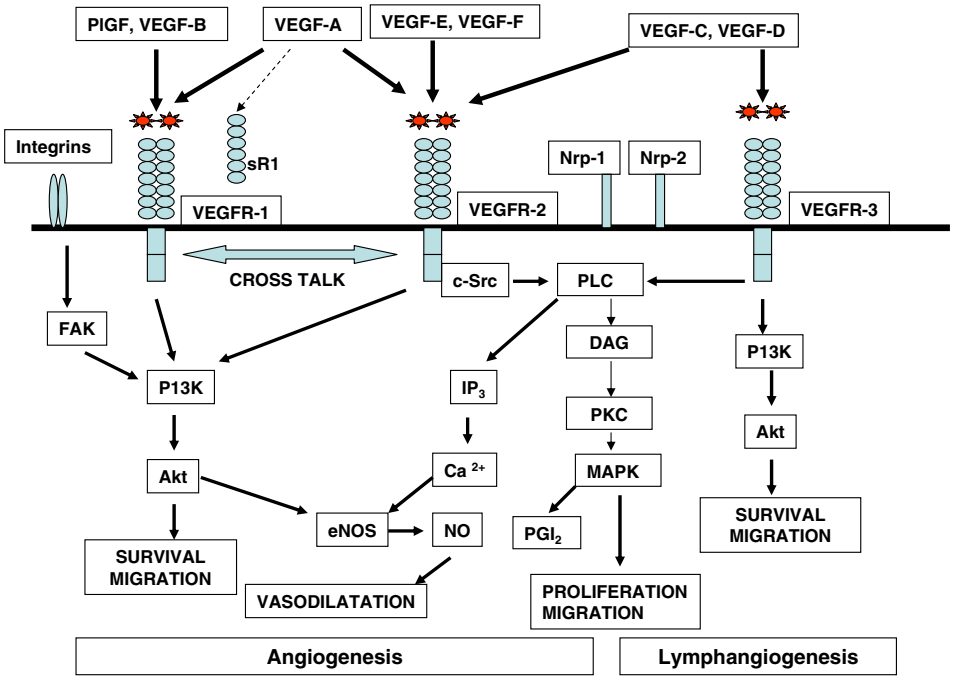

Fig. 2. Signaling pathway for vascular endothelial growth factors- The VEGF receptors have seven extracellular Ig-like domains. VEGFR-2 and VEGFR-3 are the main signaling receptors on ECs of blood and lymphatic vessels, respectively. PI3K/Akt, MAPK, Ca^{2+} and NO are key mediators of the blood vascular effects of VEGFR-2 signaling. PI3K/Akt pathway phosphorylate Bad, caspase 9 (apoptotic proteins) and eNOS, thereby increasing cell survival. Integrin mediated focal adhesion kinase (FAK) is a point of convergence between integrin and VEGF mediated survival and migration signaling. Signal mechanism for mitogenesis is through PCLγ. The biological role of VEGFR-1 is currently unclear but it can act as a negative modulator of angiogenesis and exists also as a soluble form. VEGFR-1 is associated with monocyte chemotaxis. VEGFR-1 activation at least by PlGF can also promote angiogenesis, perhaps through intracellular crosstalk with VEGFR-2. NRPs are co-receptors for VEGFs. Known ligands for NRP-1 and NRP-2 are VEGF165, PlGF-2, VEGF-B and VEGF-E; and VEGF145, VEGF165, PlGF-2 and VEGF-C, respectively. (MAPK, mitogen activated protein kinase; FAK, focal adhesion kinase; PLC, phospholipase C; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; IP_3-inositol 1,4,5-trisphosphate; Akt, anti-apoptotic kinase; DAG, diacylglycerol; eNOS, endothelial constitutive nitric oxide synthase; NO, nitric oxide; PGI2, prostacyclin).

mors. VEGFR-3 signaling pathway is crucial in the process of lymphangiogenesis (Fig. 2) Transgenic mice overexpressing a soluble VEGFR-3-Ig fusion protein in the skin lack dermal lymphatic vessels [46].

9.4. Neuropilins

The neuropilins, Nrp-1 and Nrp-2, have roles in immunology and neuronal development but they are also involved in angiogenesis [2]. Neuropilins bind class 3 semaphorins, which are secreted molecules that mediate repulsive signals during neuronal axon guidance. Nrp-1 also binds VEGF-A165, VEGF-B and PlGF while Nrp-2 binds VEGF-A165, VEGF-C and PlGF [2]. Nrp-1 acts as a co-receptor enhancing VEGF-VEGFR-2 interactions, forming complexes with VEGFR-1. Overexpression of Nrp-1 in chimeric mice leads to excessive formation of capillaries and blood vessels and hemorrhages in addition to cardiac malformations [47]. It is thought that Nrp-1 is required for cardiovascular development because it regulates VEGF-A165 levels. In chick embryos, endothelial Nrp-1 expression is mostly confined to arteries, whereas Nrp-2 primarily marks veins [48]. Nrp-2 is expressed also on lymphatic ECs, and mutated Nrp-2 induces abnormalities in the formation of small lymphatic vessels and lymphatic capillaries in mice [49].

10. VEGFs in cardiovascular pathology

10.1. Therapeutic angiogenesis for coronary artery disease and peripheral artery disease (see Table 1)

Atherosclerotic narrowing of blood vessels causes a deceased tissue perfusion and ischemia. Narrowing or occlusion of coronary arteries and large peripheral arteries due to atherosclerotic lesions could result in coronary artery disease (CAD) and peripheral artery disease (PAD) [50,51]. Formation of collateral vessels can improve perfusion in the ischemic tissues. VEGFs have been used to promote development of collateral blood vessels in clinical trials and animal models [3,51]. Improvement in exercise tolerance was reported after adenovirus mediated VEGF-A121 gene transfer to ischemic myocardium [51]. Animal experiments using intramuscular or intramyocardial injections of adenovirus encoding VEGF-A and VEGF-D, have shown high angiogenic efficacy [3,5]. VEGF-A and VEGF-D enlarge the preexisting capillaries in skeletal muscle and also enhance collateral growth. PlGF also induces angiogenesis and arteriogenesis in animal models of myocardial infarction and lower limb ischemia [23]. VEGF overexpression increases vascular permeability and may cause substantial tissue edema, pericardial effusion in the heart and angioma formation. Use of combination therapy using different growth factors like VEGF and angiopoietin might reduce the side effects [52].

10.2. Intimal hyperplasia

Neointima formation occurs following an acute injury to the blood vessels as seen after angioplasty, stent placement and in vein graft stenosis. Multiple factors tend to influence the neointima formation but vascular smooth muscle cell proliferation is perhaps the most important factor responsible for the development of restenosis [12]. VEGF-A, VEGF-D and PlGF can influence smooth muscle cell migration.

The role of VEGFs in intimal hyperplasia has remained controversial. VEGF expression was detected in vascular SMC after balloon injury which suggests that VEGFs may play a role in the development of restenotic lesions [53]. Increased neovascularization has also been observed at sites of intimal hyperplasia. A correlation between adventitial angiogenesis following VEGF gene transfer and intimal hyperplasia was seen in a collar model of neointima formation [12]. However, in a balloon denudation model of neointima formation, transfer of VEGF gene to vessel wall has been shown to decrease neointima formation [50,51]. VEGF stimulates endothelial regeneration in injured blood vessels. It has been hypothesized that the rapid regeneration of ECs results in secretion of substances like nitric oxide, C-type natriuretic peptide and prostacyclin-I_2, which have anti-proliferative effects on smooth muscle cells [51].

10.3. Atherosclerosis

Plaque angiogenesis may be associated with increased atherogenesis and unstable vulnerable plaques. These vulnerable plaques are more likely to rupture and cause sudden intra-arterial occlusion. An abrupt, coronary artery occlusion following a plaque rupture could result in fatal acute coronary syndrome. VEGFs are potent angiogenic factors that can affect plaque neovascularization and thereby influence atherosclerotic process. Use of VEGFs for therapeutic angiogenesis in CAD and PAD has been questioned because of the concerns that the VEGFs might enhance the atherosclerotic lesion formation. VEGF along with other growth factors and cytokines can initiate and/or accelerate atherosclerosis by influencing monocyte activation, adhesion, migration and enhancing vascular permeability [54]. VEGF-A and VEGF-D, are expressed in medial smooth muscle cells and in macrophages of human atherosclerotic lesions. A role of PlGF in macrophage infiltration and development of early atherosclerotic lesions has also been suggested [25]. The debate over the role of VEGFs in atherosclerosis continues with a recent study in an animal model showing that the increased systemic levels of VEGF-A, VEGF-B, VEGF-C, and VEGF-D have no effect on atherosclerotic lesions [55].

11. Tumor angiogenesis and lymphangiogenesis

Malignant neoplasms are characterized by uncontrolled cellular proliferation. Adequate blood supply and nutrients are required to sustain their growth. Tumor growth and metastasis are angiogenesis-dependent events. Role of VEGF in tumor angiogenesis, especially in lung, gastrointestinal, ovarian and breast cancers has been investigated and tumor angiogenesis has become a potential target for cancer therapy [56]. Bevacizumab (Avastin™), a neutralizing monoclonal antibody to VEGF, was the first antiangiogenic agent that was approved for the treatment of metastatic colorectal cancer. Since then it has also been experimentally used for treatment of other cancers (see Table 1).

VEGF-B and PlGF act through VEGFR-1 and their role in tumor angiogenesis has also been investigated. Increased PlGF expression has been associated with pathological angiogenesis [18]. PlGF expression is significantly increased in non-small cell lung carcinoma tissues and in certain brain tumors. VEGF-B presumably has a role in early tumor development

Table 1
Potential clinical applications of VEGFs and VEGF inhibitors

Disease	Therapeutic agent	Potential clinical use	Comments
CAD PAD	VEGF-A, VEGF-D, PlGF	Therapeutic angiogenesis	Neo-angiogenesis/arteriogenesis to restore blood supply to ischemic areas
Wound healing and bone healing	VEGF-A, VEGF-C, VEGF-D, PlGF	Therapeutic angiogenesis, lymphangiogenesis	Angiogenesis to hasten wound/bone healing. Restore lymphatic supply across incisional wounds.
Lymphedema	VEGF-C, VEGF-D	Therapeutic lymphangiogenesis	Improved lymphatic drainage
Restenosis – post angioplasty, stent	VEGF-A, VEGF-C, VEGF-D	Suppress intimal hyperplasia	Rapid endothelial regeneration
Vein graft	VEGF-D (Trinam™)	Improved vein graft survival	Increased adventitial angiogenesis
Tumor angiogenesis, lymphangiogenesis	VEGF inhibitors e.g. monoclonal antibody VEGF-A (Avastin™), soluble VEGF receptors (VEGF Trap)	Inhibit tumor angiogenesis, lymphangiogenesis	Can be used in conjunction with other therapies. Reduce tumor growth and metastasis
Rheumatoid arthritis	VEGF inhibitors	Reduce angiogenesis in pannus	Can be used in conjunction with other therapies to reduce inflammation and angiogenesis
Psoriasis	VEGF-A inhibitors	Decrease angiogenesis and inflammation	Can be used in conjunction with other therapies to reduce inflammation and angiogenesis
Ocular disorders – DR, AMD, ROP	VEGF-A inhibitors, VEGF trap, Macugen™	Suppress angiogenesis	These ocular diseases are characterized by abnormal angiogenesis

PAD, peripheral arterial disease; CAD, coronary artery disease; DR, diabetic retinopathy; AMD, age related macular degeneration; ROP, retinopathy of prematurity.

and in oral squamous cell carcinomas but there is a paucity of conclusive data to indicate a significant role of VEGF-B in tumor progression [27].

Lymphatic vasculature provides another route for tumor metastasis. Certain tumors like carcinomas of breast, lung and gastrointestinal tract have a propensity to metastasize through lymphatic vessels. The production of lymphangiogenic growth factors VEGF-C, VEGF-D and their receptor VEGFR-3 stimulate lymphatic growth in the region of the tumor, enabling cancer cells to gain access to the lymphatic vasculature [57]. VEGF-C and VEGF-D have been associated with tumor lymphangiogenesis and metastatic spread of tumor cells and a role of VEGF-A in peritumoral lymphangiogenesis and lymphatic metastasis has also been proposed.

12. Lymphedema

Impaired drainage results in the retention of lymphatic fluid in subcutaneous tissues. Lymphedemas can be classified as hereditary (primary) or acquired (secondary). VEGFR-3 is important for normal lymphatic vascular functions. In some patients with congenital hereditary lymphedema (Milroy disease), missense mutations in the TK domain of VEGFR-3 interferes with the signaling and results in lymphedema [58]. VEGF-C gene therapy in lymphedema animal model has shown promising results.

13. Role of VEGFs in wound healing

Revascularization of damaged tissue is an important component of wound healing. Inadequate or unregulated vessel growth could result in a delayed healing. VEGF is widely expressed during different phases of wound healing and its role is critical in this process. Several preclinical studies have been done to study VEGF as a potential therapeutic factor in wound healing [59]. The overexpression of VEGF using gene therapy techniques resulted in an increased vascular density in the wound and a more rapid closure of the wound, but there is a significantly greater deposition of granulation tissue. Adenovirus mediated VEGF-A165 gene transfer and adeno-associated virus mediated VEGF gene transfer has been used to promote flap survival and wound healing in animal models [59]. Adenoviral VEGF-C gene transfer at the edges of epigastric skin flaps in mice results in the formation of anastomoses between the lymphatic vessels of the skin flap and the surrounding lymphatic vasculature [60]. In skin, PlGF expression is upregulated during wound healing and PlGF-deficient mice show delayed wound closure, indicating that this factor promotes angiogenesis during skin repair [61].

14. VEGF in rheumatoid arthritis (RA)

Angiogenesis constitutes an early event of synovial hyperplasia which presumably promotes the destruction of cartilage and bone in later stages of RA. The role of angiogenesis is intricate and varied in RA. Angiogenesis helps in supplying nutrients for hyperplastic synovium but also promotes persistence of synovial inflammation through the influx of inflammatory cells and by producing inflammatory mediators [62]. The neovascular network in RA is dysfunctional and the joint affected by rheumatoid arthritis is hypoxic. VEGF is upregulated by proinflammatory cytokines and by hypoxia in RA [63]. Anti-angiogenic strategies including bevacizumab (a neutralizing monoclonal antibody to VEGF) may have a potential therapeutic role in RA [64].

15. Role of VEGF in ocular disorders

15.1. Diabetic retinopathy

Diabetic retinopathy (DR) becomes clinically apparent several years after the onset of diabetes mellitus (DM). Non-proliferative diabetic retinopathy (NPDR) is characterized by increased vascular permeability, leading to edema, and lipoprotein accumulation (hard exudates) in the outer plexiform layer and small haemorrhages and microaneurysms in retina [65]. In later stages there is periretinal neovascularization which typifies the proliferative diabetic retinopathy (PDR). Increased VEGF-A expression has been described in NPDR in humans and elevated levels of VEGF-A have been found in the aqueous humor and vitreous of patients with PDR. VEGF receptors are also upregulated in DR [66]. Increased VEGF-A levels presumably result in vascular leakage and periretinal neovascularization in DR.

15.2. Age-related macular degeneration

Age-related macular degeneration (AMD) is the major cause of central vision loss in elderly. There are two forms of AMD, neovascular and non-neovascular. The non-neovascular form of AMD is more common. Visual loss in AMD occurs from photoreceptor damage due to development of choroidal neovascularization (CNV) and related manifestations such as subretinal hemorrhage, detachment of the retinal pigmentary epithelium (RPE), and fibrovascular disciform scarring [67]. Vitreous VEGF-A levels were found to be significantly higher in patients with AMD and CNV [66]. Although the exact mechanisms for development of CNV are poorly understood, tissue hypoxia and VEGF overexpression presumably play a key role in the development of CMV [66,67].

15.3. Retinopathy of prematurity (ROP)

ROP is characterized by the proliferation of the retinal blood vessels in premature babies who receive prolonged mechanical ventilation and are therefore exposed to high concentrations of oxygen. Role of VEGF has been clearly established in the pathogenesis of ROP [66,68]. Hyperoxia causes obliteration of developing retinal vessels. Once the infant returns to normoxic environment retina become hypoxic, resulting in VEGF upregulation and vascular proliferation [66,68]. VEGF is expressed by the astrocytes and the Müller cells which participate in the development of the superficial and deep vascular layers, respectively. Insulin like growth factor (IGF)-1 also plays an important role in development of ROP. It has been suggested that VEGF may not be able to stimulate vascular growth in absence of IGF-1 [66].

16. Psoriasis

Psoriasis is a chronic inflammatory skin disease characterized by epidermal hyperplasia, impaired epidermal differentiation, and accumulation of distinct leukocyte subpopulations. VEGF is strongly upregulated in psoriatic skin lesions [69]. Single nucleotide polymorphisms of the VEGF gene occur more frequently in patients with early onset psoriasis and these haplotypes may contribute to the elevated VEGF levels observed in these patients [70]. Thus VEGF plays an important role in the pathogenesis of psoriasis and that therapeutic blockade of the VEGF/VEGF-receptor system might represent a novel, pharmacogenomic approach for the future treatment of psoriasis.

17. Conclusions

Recent advancements in vascular research have enhanced our understanding of VEGF/VEGF-receptor biology. These molecules are crucial regulators of vasculogenesis, angiogenesis and lymphangiogenesis. Several, VEGF/VEGF-receptor inhibition strategies have emerged for treating the diseases associated with pathological angiogenesis. Similarly 'therapeutic angiogenesis' is an emerging treatment modality for ischemic disorders. Future advances in our understanding of biology of VEGFs are likely to identifying newer functions and potential therapeutic uses for these molecules.

Acknowledgements: This study was supported by grants from Finnish Academy, Finnish Foundation for Cardiovascular Research and European Union (QLK3-CT-2002-01955) and (LSHG-CT-2004-503573).

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