Sangdun Choi
Editor

Encyclopedia of Signaling Molecules

Second Edition

With 1893 Figures and 247 Tables

Springer

Editor
Sangdun Choi
Department of Molecular Science and Technology
Ajou University
Suwon, Korea

ISBN 978-3-319-67198-7 ISBN 978-3-319-67199-4 (eBook)
ISBN 978-3-319-67200-7 (print and electronic bundle)
https://doi.org/10.1007/978-3-319-67199-4

Library of Congress Control Number: 2017951593

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

VEGF

M. Luisa Iruela-Arispe
Department of Molecular, Cell and Developmental Biology, Molecular Biology Institute – UCLA, Los Angeles, CA, USA

Synonyms

MVCD1; Vascular endothelial growth factor; Vascular endothelial growth factor A; Vascular permeability factor; VEGFA; VEGF-A; VPF

Historical Background

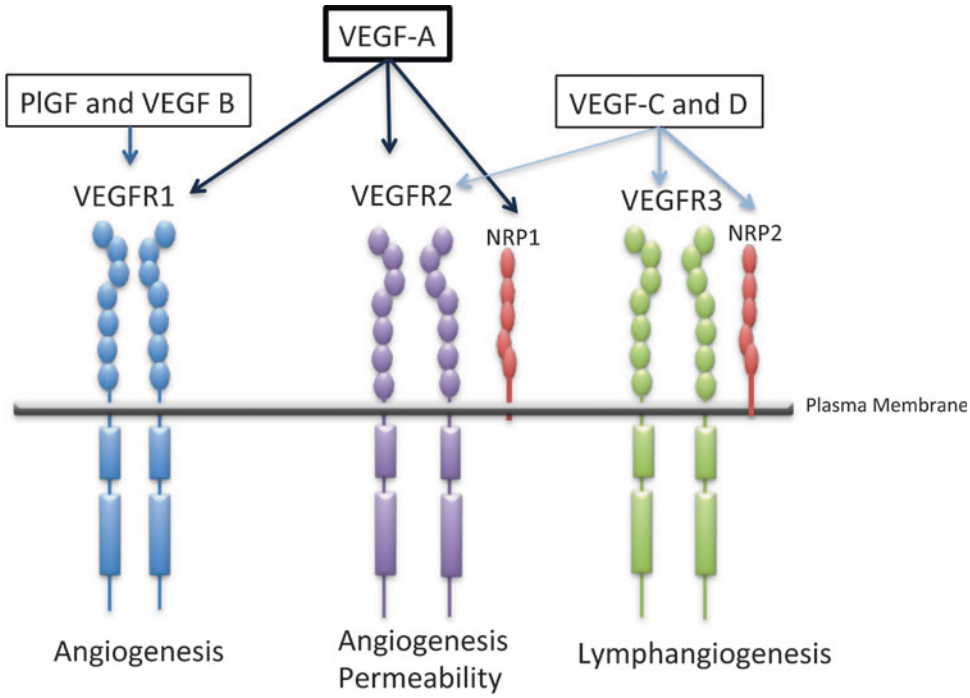
Vascular Endothelial Growth Factor (VEGF) was first identified as a molecule secreted by tumor cells that promoted breakdown of endothelial barrier and induced vascular permeability (Senger et al. 1983). Subsequent purification of the protein and amino acid sequence revealed that this molecule was identical to a cDNA that coded for a protein with mitogenic properties when applied to endothelial cells (Leung et al. 1989; Senger et al. 1990). It became clear that both molecules were identical with functional attributes that included induction of proliferation, migration, and disruption of barrier function on endothelial cells (Connolly et al. 1989; Dvorak 2006). It was quickly determined that VEGF exerted its effects through binding to two cell-surface receptor tyrosine kinases expressed by endothelial cells. Therefore, VEGF signaling was established as a paracrine ligand-receptor pathway where the ligand (VEGF) is secreted by a variety of cells, including tumor cells, and the receptors are expressed in the endothelium with broad consequences to the vasculature.

Subsequent gene-targeting studies in mice revealed that VEGF is a key coordinator of angiogenesis and that absence of half of the amount of VEGF is insufficient to support vascular development, as even heterozygous animals die at mid-gestation (Ferrara et al. 1996; Carmeliet et al. 1996). These findings together with the fact that VEGF was secreted by tumor cells and it promoted blood vessel formation in tumors, attracted vast interest to the emerging angiogenesis field. Major impetus was placed in understand function, unravel molecular mechanisms, and explore potential therapeutic targeting of VEGF. Rapidly, it became clear that VEGF belongs to a family of five growth factors (VEGF-A, B, C, D, and PlGF) responsible for the morphogenesis of blood and lymphatic vessels. In 2004, the first antibody to block VEGF-A function was approved by the Food and Drug Administration (FDA) to treat colon cancer (Ferrara et al. 2004).

VEGF-A Structure

VEGF-A is a homodimeric glycoprotein of 22–43 kDa (depending on the spliced form). The receptor-binding domain is located in the amino-terminal end, while the carboxy-terminus of the protein is involved in establishing connections with the extracellular matrix. In a given endothelial cell, VEGF-A binds to two receptor tyrosine kinases: VEGFR1 and VEGFR2; and depending on the isoform, it can also bind to neuropilin1 (NRP1). Because the preponderance of expression of these receptors is in endothelial cells, the large majority of the responses to VEGF relate to vascular effects. These responses include stimulation of endothelial cell proliferation and migration, as well as endothelial barrier disruption with consequent vascular permeability. However, low levels of these receptors are also found in neurons, hematopoietic cells, and tumor cells. Thus, VEGF effects on cell types other than the endothelium have been also reported. For example, VEGF-A has been shown to have neurodevelopmental, neurotrophic, and neuroprotective roles.

The human VEGFA gene is composed of eight exons and seven introns. However, due to extensive alternatively splicing, several isoforms have been identified and classified by the number of amino acids present in each form as: VEGF121, VEGF145, VEGF165, VEGF189, and VEGF206. Amongst all these isoforms, VEGF165 is the most common, potent, and abundant. The main difference between the isoforms relates to their interaction with the extracellular matrix, located in the



VEGF, Fig. 1 VEGF signaling axis. The interaction between the five different VEGF growth factors and receptors is not random. As noted, PlGF and VEGFB only bind to the tyrosine kinase VEGFR1. In contrast, VEGF-A binds to VEGFR1, VEGFR2, and NRP1. Finally, VEGF-C and D bind to VEGFR3 and NRP2 showing weak interactions with VEGFR2

carboxy-terminal end of the protein and their ability to bind to neuropilins (VEGF121 and 145 are unable to interact with neuropilins). The relevance of these isoforms has been studied extensively, and it is clear that their association with extracellular matrix proteins modulates the input received by receptors. Furthermore, the spatial distribution of the different VEGF isoforms in the extracellular space is also critical for the balance between vessel branching and growth. Importantly, in addition to splicing, the interaction of VEGF with the matrix is also regulated by intramolecular cleavage of VEGF by extracellular proteases including plasmin and matrix metalloproteinases (Lee et al. 2005).

VEGF: A Diversity of Genes

There are five genes with extreme similarity to the originally discovered VEGF gene (VEGF-A). Thus, the VEGF family includes: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PlGF) (Sullivan and Brekken 2010). Their respective protein products bind to specific receptors that include both tyrosine kinases: VEGFR1 and VEGFR2 and nontyrosine kinase receptors that belong to the neuropilin family: NRP1 and NRP2 (Fig. 1).

VEGF-B and PlGF are known to only bind to VEGFR1. VEGF-B does not affect developmental angiogenesis, but it appears to regulate cardiac muscle function and the coronary vasculature. PlGF has been shown to induce angiogenesis and inflammation.

The other two VEGF family members: C and D bind strongly to VEGFR3 with very weak affinity to VEGFR2. Consequently, they play minor roles in postnatal angiogenesis and some restricted roles in developmental angiogenesis, but they are critical regulators of lymphangiogenesis, as VEGFR3 is highly

expressed in lymphatic endothelial cells (Zheng et al. 2014).

There are also family members not encoded by mammals: VEGF-E and VEGF-F. VEGF-E encodes a viral gene from a parapoxvirus that infects goats, sheep, and at times humans inducing local vascular growth at the site of infection. VEGF-E has been shown to exclusively bind and activate VEGFR2. VEGF-F was identified in the venom of some snakes.

VEGF-A Signaling

VEGF binding induces dimerization of VEGFR1 and VEGFR2 promoting their phosphorylation and downstream signaling effects (Simons et al. 2016). VEGFR2 is considered the primary receptor in endothelial cell activation, and responsible for the initiation of proliferative and migratory activities. VEGF-A has a higher affinity for VEGFR1 than for VEGFR2, yet its tyrosine kinase activity is weaker. These attributes fit a model in which VEGFR1 functions as a decoy receptor, sequestering VEGF, and thus, reducing the amplitude of the response driven by VEGFR2. However, VEGFR1 also has additional functions on its own right, and it has been shown to promote migration and invasion in certain settings. VEGFR1 can be alternatively spliced to generate a soluble form of the receptor that lacks transmembrane and intracellular domains. The resulting isoform called s-FLT1 inhibits VEGF signaling by sequestering free VEGF, and thus, sFLT1 acts as an inhibitor of angiogenesis.

VEGF binding to VEGFR2 stimulates RAS/RAF/MEK/ERK and PI3 Kinase/AKT/mTOR pathways that are responsible for promoting cell proliferation and survival. Another branch of the signaling cascade results in activation of PLC-gamma which regulates ERK with critical roles in adult arteriogenesis. Activation of SRC downstream VEGFR2 promotes changes in small GTPase, activation of the cytoskeleton, changes in cell shape, cell migration, and endothelial polarity. Signaling outputs by VEGFR2 are highly regulated through a myriad of interactions with coreceptors, particularly neuropilins, but also with proteoglycans, integrins, and protein tyrosine phosphatases.

In addition to promote angiogenesis, VEGF signaling also controls vascular permeability, which is defined as an increase of leakage of serum from blood. The effects of VEGF on permeability rely on the activation of VEGFR2 and Src and additional intermediary molecules, such as T cell-specific adaptor (TSAd), which is responsible for regulating VEGF-induced rearrangement of junctional complexes (Simons et al. 2016).

Another important effect of VEGF signaling is to control the inflammatory status of endothelial cells, by increasing the expression of E-selectin, vascular cell adhesion molecule-1 (VCAM1), and intracellular adhesion molecule 1 (ICAM-1). All of these molecules are involved in recruitment and extravasation of leukocytes from the blood stream.

VEGF-A in Development, Physiology, and Pathology

VEGF-A is essential for embryonic development, as inactivation of the VEGF-A gene is incompatible with life. In fact, deletion of a single VEGF-A allele (VEGF-A +/−) results in embryonic lethality due to immature vascular development and associated cardiac insufficiency (Ferrara et al. 1996; Carmeliet et al. 1996). Lethality resulting from loss of a single allele of a gene is rare in mammals, and therefore, the fact that heterozygous mice are unable to live reveals a strict relationship between VEGF dosage and morphogenesis of the vasculature. In fact, VEGF levels are tightly controlled by transcriptional and posttranscriptional mechanisms.

Additional studies using cell-specific deletion of VEGF showed essential requirements for this growth factor in organogenesis and function of a broad number of organs and tissues. While the relationship between organ expansion and nutrients is intuitive, endothelial cells provide more than the means to organize tubes that deliver oxygen and nutrients. There is increasing support to the notion that endothelial cells also offer critical

signals: angiocrine molecules, that are required for the differentiation of cells and for the 3-D architecture of organs (Rafii et al. 2016). Thus, by impacting endothelial cell survival and proliferation, VEGF is a fundamental growth factor in organogenesis.

Experiments blocking VEGF signaling with pharmacological inhibitors and also cell-specific genetic inactivation experiments uncovered the importance of this growth factor in endothelial homeostasis and vascular stability. Treatment of mice with small molecular inhibitors of VEGFRs significantly reduces vascular density and promotes proteinuria (Baffert et al. 2006). The findings highlighted a requirement for VEGF in the maintenance of an adequate volume/size of a vascular network that is unique to each organ. VEGF also regulates the stability of the glomerular capillary network in the kidney and its role in filtration of blood. In endothelial cells, endogenously produced VEGF is critical to actively ensure survival, as deletion of VEGF specifically in the endothelium result in spotty apoptosis of cells within the vascular tree, thrombotic effects, and microinfarcts (Lee et al. 2007).

Hypoxia, through hypoxia-inducible factor 1 alpha (HIF-1alpha) is a potent stimulator of VEGF which subsequently promotes vascular growth to mitigate oxygen deficiencies. This is a frequent mechanism employed, time and again, to induce vascular expansion (angiogenesis) in adult tissues and needed during tissue expansion (muscle growth upon prolonged exercise, adipose or other tissue growth, and repair of endometrium postmenses).

Traumatic injury of several tissues, particularly injury of the central nervous system is associated with acute upregulation of VEGF and VEGF receptors. VEGF expression correlates with time of vascular repair, but there is significant evidence to support that VEGF is also neuroprotective through the activation of VEGF receptors (low levels) in neurons and glia.

VEGF-A is not only a driver of angiogenesis during development but also in disease. VEGF is highly expressed in tumors and in this setting, drives abnormal vessel formation. High VEGF concentrations seem to correlate with poor disease-free and overall survival in cancer patients.

Increased expression of VEGF was also found to be a relevant factor in the pathogenesis of a myriad of ocular diseases and it is a common feature of psoriasis, rheumatoid arthritis, diabetes, bronchial asthma, and multiple inflammatory conditions.

Targeting VEGF

Since VEGF and their respective receptors play a central role in angiogenesis and are involved in so many pathological conditions, the concept of blocking the activity of these molecules for therapeutic purposes held a strong appeal. The initial impetus to pursue therapeutic avenues was cancer, as the idea of inhibiting angiogenesis as a means to starve tumors, was not only logical but had the potential to be applicable to most, if not all, tumors (Folkman 2007). Antibodies against VEGF-A were initially developed and commercialized by Genentech. Bevacizumab, a full-length humanized recombinant monoclonal IgG that binds and inhibits all VEGF isoforms was approved by the FDA for treatment of several solid tumors including colorectal, nonepithelial lung, breast, ovarian, renal, and glioblastomas (Ferrara et al. 2004). A similar drug, Ranibizumab (Lucentis) was also developed by Genentech as a Fab fragment of humanized monoclonal anti-VEGF-A antibody also recognizing all VEGFA isoforms. The drug was specifically designed for eye disease and it was approved for intraocular use in neovascular macular degeneration, macular edema, diabetic macular edema, and diabetic retinopathy (Cao et al. 2011; Sullivan and Brekken 2010). Another product that targets VEGF is Aflibercept, a fusion decoy protein of 115 kD that includes VEGF-binding domains of VEGFR1 and VEGFR2 fused to the Fc domain of human immunoglobulin G1. The drug, developed by Regeneron, binds all forms of VEGF-A but also PlGF-1 and PlFG-2 with high affinity. It was approved for colorectal metastatic

carcinoma and for ophthalmological pathologies (Cao et al. 2011; Sullivan and Brekken 2010).

Intracellular targets to interfere with VEGF were also developed. Pegaptanib (Macugen) is a 28base ribonucleic acid aptamer, covalently linked to two branched 20 kD polyethylene glycol moieties. The drug, developed by Pfizer binds to and blocks VEGF165 and it has been used in the treatment of wet macular degeneration (Cao et al. 2011).

In addition to blocking VEGF, targeting its receptors, particularly the tyrosine kinase receptors, has been an alternative therapeutic avenue. The two most popular drugs have been sorafenib and sunitinib and were both approved for treatment of renal and hepatocellular carcinoma. Although effective, these drugs are multikinase inhibitors and they block VEGFR2, as well as other receptor tyrosine kinases with multiple targeting overlap, something that can bring both advantages and disadvantages. For example, drugs such as rosiglitazone and pioglitazone developed as targets of the peroxisome proliferators-activated receptor gamma also inhibit VEGF signaling and are used clinically to control diabetes; in fact, rosiglitazone delayed the onset of diabetic retinopathy in type 2 diabetic patients.

There is little question that today anti-VEGF therapy is one of the most important advancements in suppressing progression and, in some cases, resolving age-related macular degeneration, diabetic retinopathy, and retinal vein occlusions. Anti-VEGF therapy has also been valuable in the management of retinopathy of prematurity and choroidal neovascularization. Given its success, it is not surprising that the use of anti-VEGF therapy in ophthalmological pathologies is increasing with fortunately very minor adverse effects noted. This reality contrasts the use of anti-VEGF therapy in cancer, where systemic, rather than local delivery is applied. Benefits are not as robust as in ocular disease and resistance of anti-angiogenic therapy is also associated with side effects due to systemic administration of the drugs. Bevacizumab has been associated with hypertension, proteinuria, thromboembolic events with strokes, and gastrointestinal perforations (Cao et al. 2011).

The tremendous success of anti-VEGF therapy in the eye contrasts the relatively poor performance of VEGF in cancer. The reasons for this discrepancy are not entirely clear, but acquire resistance, due to the versatility of the tumor microenvironment to adapt switching to utilize alternative proangiogenic factors are likely the cause (Bergers and Hanahan 2008). The use of biomarkers to discern between patients likely to respond to therapy has received attention and it is showing signs of success. For example, in metastatic nonsquamous non-small cell lung cancer, a serum proteomic signature was used to select patients likely to respond to a combination therapy of bevacizumab and erlotinib (EGFR inhibitor) (Akerley et al. 2012).

Summary

Vascular endothelial growth factor includes a family of five genes (VEGF-A, B, C, D, and PlGF) that code for key regulators of physiological angiogenesis and lymphangiogenesis VEGF-A is the most abundant factor, critical for vascular morphogenesis in development and postnatal life. The human VEGF-A gene is organized as eight exons separated by seven introns. Exons 6 and 7 can be spliced to generate a variety of isoforms with distinct affinity for extracellular matrix proteins and effects in blood vessel formation. Activity of VEGF-A is mediated by cell surface receptors that are predominantly expressed by endothelial cells. These receptors include two tyrosine kinases: VEGFR1 and VEGFR2 and two nonreceptor kinases Neuropilin-1 and 2. VEGFR2 is a major mediator of the mitogenic, migratory, and permeability effects triggered upon binding to VEGF-A, but the other receptors are critical in regulating responses and imposing nuances to the angiogenic outcome (size of branches, number of branches). Because of the impact of angiogenesis in disease progression, including cancer, a variety of antiangiogenic drugs, including anti-VEGF

antibodies have been developed and approved for clinical use. Despite their remarkable success in the treatment of ocular disease, in cancer, most patients are either intrinsically resistant or acquire resistance within months of treatment, resulting in a few months of extended overall survival as benefit of the therapy. Mechanisms of resistance to anti-VEGF have been identified, and understanding the complex interactions between tumor cells, endothelial, and the constituency of the tumor microenvironment will be important in effectively blocking neovascularization in cancer.

References

Akerley W, Boucher K, Rich N, Egbert L, Harker G, Bylund J, Van Duren T, Chakravarthy R. A phase II study of bevacizumab and erlotinib as initial treatment for metastatic non-squamous, non-small cell lung cancer with serum proteomic evaluation. Lung Cancer. 2012;79:307–11.

Baffert F, Le T, Sennino B, Thurston G, Kuo CJ, Hu-Lowe D, McDonald DM. Cellular changes in normal blood capillaries undergoing regression after inhibition of VEGF signaling. Am J Physiol Heart Circ Physiol. 2006;290:H547–59.

Bergers G, Hanahan D. Modes of resistance to anti-angiogenic therapy. Nat Rev Cancer. 2008;8:592–603.

Cao Y, Arbiser J, De Amato RJ, De Amore PA, Ingber DE, Kerbel R, Klagsbrun M, Lim S, Moses MA, Zetter B, Dvorak H, Langer R. Forty-year journey of angiogenesis translational research. Sci Transl Med. 2011;3:114rv3.

Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, Declrecq C, Pawling J, Moons L, Collen D, Risau W, Nagy A. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature. 1996;380:435–9.

Connolly DT, Heuvelman DM, Nelson R, Olander JV, Eppley BL, Delfino JJ, Siegel NR, Leimgruber RM, Feder J. Tumor vascular permeability factor stimulates endothelial cell growth and angiogenesis. J Clin Invest. 1989;84:1470–8.

Dvorak HF. Discovery of vascular permeability factor (VPF). Exp Cell Res. 2006;312:522–6.

Ferrara N, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. Nat Rev Drug Discov. 2004;3:391–400.

Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, Powell-Braxton L, Hillan KJ, Moore MW. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature. 1996;380:439–42.

Folkman J. Angiogenesis: an organizing principle for drug discovery? Nat Rev Drug Discov. 2007;6:273–86.

Lee S, Chen TT, Barber CL, Jordan MC, Murdock J, Desai S, Ferrara N, Nagy A, Roos KP, Iruela-Arispe ML. Autocrine VEGF signaling is required for vascular homeostasis. Cell. 2007;130:691–703.

Lee S, Jilani SM, Nikolova GV, Carpizo D, Iruela-Arispe ML. Processing of VEGF-A by matrix metalloproteinases regulates bioavailability and vascular patterning in tumors. J Cell Biol. 2005;169:681–91.

Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science. 1989;246:1306–9.

Rafii S, Butler JM, Ding BS. Angiocrine functions of organ-specific endothelial cells. Nature. 2016;529:316–25.

Senger DR, Connolly DT, Van De Water L, Feder J, Dvorak HF. Purification and NH-terminal amino acid sequence of guinea pig tumor-secreted vascular permeability factor. Cancer Res. 1990;50:1774–8.

Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science. 1983;219:983–5.

Simons M, Gordon E, Claesson-Welsh L. Mechanisms and regulation of endothelial VEGF receptor signaling. Nat Rev Mol Cell Biol. 2016;17:611–25.

Sullivan LA, Brekken RA. The VEGF family in cancer and antibody-based strategies for their inhibition. MAbs. 2010;2:165–75.

Zheng W, Aspelund A, Alitalo K. Lymphangiogenic factors, mechanisms, and applications. J Clin Invest. 2014;124:878–87.

VEGFA

► VEGF

VEGF-A

► VEGF

Very Late Activation Antigen 2 (VLA-2)

► Integrin α2 (ITGA2)