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Angiogenesis versus arteriogenesis: neuropilin 1 modulation of VEGF signaling

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Abstract

In development and disease, vascular endothelial growth factor (VEGF) regulates the expansion of the vascular tree. In response to hypoxia, VEGF promotes new capillary formation through the process of angiogenesis by inducing endothelial cell sprouting, proliferation, and migration. Wound healing, tissue regeneration, and tumor growth depend on angiogenesis for adequate nutrient and oxygen delivery. Under different conditions, VEGF promotes arterial growth, modulates lumen expansion, and induces collateral vessel formation, events collectively referred to as arteriogenesis. Induction of arteriogenesis after cardiac or cerebral arterial occlusion can reduce ischemia and improve disease outcome. Endothelial VEGF receptor 2 (VEGFR2) signaling governs both processes. However, modulation of downstream VEGF signaling effectors, such as extracellular-signal-regulated kinase (ERK) activation, differs in order to achieve angiogenic versus arteriogenic outcomes. Recent reports show that neuropilin 1 (NRP1), a VEGF receptor, can instill VEGF signaling outcomes that specifically regulate either angiogenesis or arteriogenesis. Here, we discuss how NRP1 functions as a VEGFR2 co-receptor in angiogenesis and a modulator of VEGFR2 trafficking in arteriogenesis. The unique role played by neuropilin in different endothelial processes makes it an exciting therapeutic target to specifically enhance angiogenesis or arteriogenesis in disease settings.

Introduction

VEGF signaling pathways drive the development of the vascular system by regulating three distinct processes: vasculogenesis, angiogenesis, and arteriogenesis. Embryonic vascular development is initiated by vasculogenesis when VEGF induces differentiation of endothelial progenitors to endothelial cells that then form a primitive vascular network. This network is then remodeled and expanded through the process of angiogenesis, where endothelial cells respond to VEGF-A by sprouting, proliferating, and migrating to form new lumenized vessels. Concurrently, arteriogenesis ensures the formation of arteries through VEGF-induced arterial fate specification, lumen expansion, and endothelial cell proliferation. Proper coordination of angiogenesis and arteriogenesis results in a hierarchal vascular network consisting of arteries, capillaries, and veins that provides a means to effectively deliver oxygen and nutrients throughout the body [1]. Disruption of either one of these processes can impair growth and potentially lead to embryonic lethality.

In the developed vascular system, angiogenesis and arteriogenesis continue to play unique yet equally important roles in both health and disease. In situations such as wound healing and cancer, hypoxic tissues secrete VEGF to induce angiogenesis, thereby expanding the capillary network and increasing nutrient and oxygen supply [1,2]. Tumor angiogenesis promotes cancer progression by increasing tumor growth and providing a conduit for metastasis[3]. Thus, anti-angiogenic therapies are now a common component of cancer therapies for multiple tumor types.

Diseases characterized by vascular occlusion, such as coronary or cerebral artery occlusions, may benefit from

therapies that promote arteriogenesis to expand pre-existing collateral anastomosis and induce new arterial growth. When sufficiently developed, collateral vessels function as biological bypasses; artery-to-artery communications bypass the capillary bed to provide blood flow to tissues served by an occluded artery. VEGF signaling is critical for this process, promoting collateral growth and *de novo* arteriogenesis [4]. Humans and mice with a higher number of nascent collateral vessels display decreased ischemia and improved recovery after occlusion of a major arterial branch [5]. In contrast, therapies that promote angiogenesis after ischemia have had limited success in treating advanced arterial occlusive diseases [6]. Understanding the molecular mechanisms that distinguish endothelial function in arteriogenesis from angiogenesis is of great interest in order to develop targeted and more effective therapies for diseases characterized by inadequate blood flow.

The VEGF signaling pathway regulates endothelial cell function in both angiogenesis and arteriogenesis. However, differences in the growth factor-induced signaling between these two processes remain incompletely understood. A number of studies have suggested that VEGF-driven activation of ERK1/2 signaling is critical for arterial fate specification in both developmental and adult arteriogenesis [7–11]. Investigations of the molecular details of VEGF activation of ERK have revealed a critical role played by the VEGFR, NRP1.

Neuropilins are a two-member family of non-tyrosine kinase receptors that bind VEGFs and semaphorins through two distinct extracellular domains. In particular, NRP1 is enriched in arterial endothelial cells and binds both VEGF-A_{165} and semaphorin-3A (SEMA3A), whereas NRP2 is expressed predominantly by lymphatics [12–14]. The short cytoplasmic domain of NRP1 contains a C-terminal PDZ-binding domain required for interactions with synectin, a cytoplasmic PDZ protein, and likely other PDZ-binding partners [15]. Mice homozygous null for NRP1 die *in utero* and display neuronal and cardiovascular defects [16,17]. Mice expressing mutant NRP1 that can bind VEGF, but not semaphorin, survive until birth, demonstrating that NRP1 regulation of VEGF signaling is critical for vascular function and thus embryonic viability [18]. More recently, studies of a mouse line carrying a knock-in of NRP1 missing its cytoplasmic domain revealed that these animals live but have a profound arteriogenic defect [10,19]. In contrast, mice carrying a knock-in of NRP1 with a mutated VEGF-A-binding site have predominantly perinatal angiogenic defects and display mildly impaired arteriogenesis [20,21]. These discoveries imply that NRP1 accounts for differential regulation of angiogenic versus arteriogenic signaling by VEGF. The different roles played by NRP1 make it an exciting target for developing therapeutics to target diseases that specifically require either capillary or arterial vessel regrowth.

Here, we review the function of NRP1 regulation of VEGFR2 signaling in both angiogenesis and arteriogenesis. We will first give background on the VEGF signaling pathway. We will then discuss how NRP1 functions as a VEGF co-receptor in angiogenesis and as a mediator of VEGFR2 trafficking in arteriogenesis. We propose that NRP1 functions as a VEGF signaling modulator that imparts unique endothelial cell function required for either arteriogenesis or angiogenesis.

VEGF signaling

VEGFs induce endothelial cell differentiation, proliferation, migration, and survival while promoting vascular permeability. The VEGF protein family consists of VEGF-A, -B, -C, and -D [22]; placenta growth factor (PlGF) [23]; VEGF-E produced by *orf* viruses [24]; and a group of snake venom-derived VEGF-Fs [25–27]. VEGFs mediate their effects on endothelial cells by activating cell surface VEGFR tyrosine kinases, VEGFR-1, -2, and -3 [28]. VEGFRs selectively bind certain VEGF ligands. For example, VEGF-A exclusively binds VEGFR1 and VEGFR2, whereas VEGF-C exclusively binds VEGFR2 and VEGFR3. Generally speaking, VEGFR3 activation by VEGF-C regulates the lymphatic vasculature, whereas VEGF-A activation of VEGFR2 is the main regulator of blood endothelial cell function [29,30]. Mice heterozygous for VEGF-A or homozygous null for VEGFR2 die embryonically and display severe vascular defects, demonstrating the critical role played by VEGFs in vascular development [31–33]. Furthermore, mice carrying a single amino acid mutation that prevents VEGFR2-induced activation of ERK1/2 die *in utero* because of vascular defects [34].

In line with VEGFR2 being a master regulator of endothelial cell function, activation of VEGFR2 initiates multiple signaling pathways that regulate endothelial proliferation, migration, adhesion, lumenization, and survival [28]. Activation of the phosphatidylinositol 3-kinase/Akt signaling pathway promotes endothelial cell survival by inhibiting apoptosis, whereas activation of Src-FAK signaling mediates endothelial cell migration and vascular permeability [35,36]. Phosphorylation of VEGFR2 also activates the Raf-MEK-ERK signaling cascade whereby ERK1/2 phosphorylation induces endothelial cell proliferation, network formation, and increased vessel lumen size [9,37,38]. In mice, newly formed collateral vessels express elevated levels of phosphorylated ERK1/2 after vascular occlusion, and decreased ERK signaling has

been associated with impaired arteriogenesis [9,11,39]. Thus, ERK activation in response to VEGFR2 signaling is hypothesized to be a critical component of arteriogenesis regulation.

VEGFR2 trafficking

In contrast to the standard view of receptor tyrosine kinase (RTK) signaling, which holds that RTKs activate second messenger pathways at the plasma cell membrane, VEGFR2 activation of ERK requires receptor internalization and trafficking [9,40–42]. Thus, VEGFR2 trafficking through different endosomal compartments adds another layer of regulation to VEGFR2 signaling [43,44]. VEGFR2 is internalized via clathrin-coated pits after ligand-induced receptor dimerization and autophosphorylation [40,45]. VEGFR2 is then shuttled between different vesicular compartments to eventually be recycled back to the plasma membrane for another round of signaling or sent to the lysosome for degradation. Rab GTPases, found on the surface of vesicles, interact with an array of adaptor proteins in order to direct vesicles to different cellular locations [46]. Specific types of Rab GTPases are associated with different vesicular compartments; thus Rab proteins can be used to identify the different routes of endosomal transport. After internalization, VEGFR2 is first localized to Rab5-positive early endosomes, which then recruit the adaptor protein EEA1 [47]. At this point, VEGFR2 can be either shuttled back to the plasma membrane for receptor recycling via Rab11-dependent transport or directed to the lysosome for protein degradation via Rab7-positive endosomes [48,49]. In the absence of VEGF stimulation, VEGFR2 is constitutively returned to the plasma membrane by Rab4-dependent fast-loop receptor recycling. In response to VEGF stimulation, VEGFR2 is returned to the plasma membrane via slow-loop recycling, which depends on Rab11-positive endosomes. The process of slow-loop recycling of VEGFR2 via Rab11-positive endosomes functions to prolong VEGFR2 signaling [47].

NRP1, a VEGR-2 co-receptor in angiogenesis

During angiogenesis, endothelial cells sprout from pre-existing vasculature to form new capillary networks. Highly migratory endothelial cells, termed tip cells, lead endothelial sprouts. Tip cells extend filopodia into the extracellular environment and are highly responsive to VEGF because of high levels of VEGFR2 expression [50]. Mice deficient in NRP1 display a reduced number of tip cells in the developing mouse hindbrain [51]. More recently, NRP1 was shown to be enriched in endothelial tip cells during angiogenesis in the embryonic hindbrain [52]. Accordingly, mice chimeric for endothelial-specific NRP1 knockout demonstrate that endothelial cells that maintain expression of NRP1 preferentially take the tip cell position during sprouting angiogenesis [52].

NRP1 contributes to the tip cell phenotype by regulating the VEGFR2 response to VEGF stimulation. In cultured endothelial cells, VEGF stimulation induces NRP1-VEGFR2 complex formation and the presence of NRP1 increases VEGFR2 signaling potency to promote VEGF-induced chemotactic and mitogenic activity [12,53,54]. In zebrafish, NRP1 knockdown causes severe vascular defects, including impaired development of the intersomitic vessels, a process dependent on sprouting angiogenesis [55]. Combined inhibition of NRP1 and VEGF-A in the zebrafish embryo demonstrated that NRP1 cooperates with the VEGF signaling pathway whereby NRP1 lowers the required concentration of VEGF needed to activate VEGFR2 and induce angiogenesis [55]. By increasing VEGFR2 activation in response to VEGF, NRP1 acts as an important regulator of angiogenesis in zebrafish.

Antibodies that block NRP1 binding specifically to VEGF impair endothelial cell sprouting, both in the mouse retina and in tumor models [56]. Assessment of mice expressing endothelial knock-in mutations of NRP1 that specifically inhibit VEGF-A_{165} binding demonstrates that VEGF-A binding to NRP1 is not required for embryonic angiogenesis but instead contributes to postnatal angiogenesis [20,21]. Mice expressing a knock-in of an NRP1 mutation that inhibits VEGF-A_{165} binding and reduces NRP1 expression are embryonically viable, but display an increased rate of postnatal mortality and impaired angiogenesis in the hindbrain and retina [21]. A second NRP1 VEGF-A_{165} binding-deficient transgenic mouse line that expresses normal levels of NRP1 displayed normal viability and only mildly impaired retinal angiogenesis as characterized by delayed vessel outgrowth [20]. In contrast to endothelial-specific NRP1 knockout mice, which display severely impaired angiogenesis in the hindbrain accompanied by reduced VEGFR2 expression and phosphorylation levels, expression of VEGF-A_{165} binding-deficient NRP1 results in normal hindbrain angiogenesis, normal VEGFR2 expression, and only mildly reduced VEGFR2 phosphorylation levels [20]. These findings suggest that NRP1 promotes angiogenesis independently of its VEGF-A_{165}-binding capability. Instead, it is hypothesized that, during angiogenesis, NRP1 functions as a VEGFR2 co-receptor to enhance VEGFR2 activation.

Studies also support a role for NRP1 in regulating angiogenesis independently of VEGFR2. NRP1 has been shown to mediate endothelial cell adhesion, in part through interactions with $\alpha5\beta1$ integrins [57–59]. More recently, it was shown that NRP1 complexes with the non-RTK ABL1 to promote endothelial cell motility

by stimulating fibronectin-induced actin remodeling [60]. Accordingly, pharmacological inhibition of ABL1 by treatment with imatinib impairs angiogenesis, as demonstrated by reduced tip cell number and decreased vessel branching in the developing retina. Thus, endothelial NRP1 functions independently of VEGFR2 to provide an additional means of angiogenic modulation.

NRP1 in arteriogenesis and VEGFR2 trafficking

Unlike deletion of full-length NRP1, which results in both angiogenic and arteriogenic defects, transgenic mice expressing a truncated version of NRP1 lacking the cytoplasmic domain ($NRP1^{cyto}$) do not display embryonic, postnatal, or pathological angiogenic phenotypes [10,19]. Instead, loss of the NRP1 cytoplasmic domain specifically impairs arteriogenesis [10]. $NRP1^{cyto}$ mice display reduced arterial network size and complexity in the kidney, heart, and hindlimb. After femoral artery ligation, blood flow recovery, which depends on *de novo* arterial formation, is significantly impaired in $NRP1^{cyto}$ adult mice. Defective arteriogenesis in $NRP1^{cyto}$ mice was attributed to impaired VEGFR2 trafficking from Rab5-positive sorting endosomes to EEA1-positive endosomes, which resulted in reduced VEGFR2 phosphorylation and impaired ERK activation.

Trafficking of VEGFR2 from Rab5-positive endosomes to EEA1-positive endosomes requires the PDZ (PSD-95/Dlg/ZO-1) domain-containing adaptor protein synectin, also termed GAIP interacting protein, COOH-terminus (GIPC) and neuropilin-interacting protein (NIP) [61]. Synectin forms a complex with PDZ-binding proteins and the actin-based molecular motor myosin VI to promote inward trafficking of endosomes away from the plasma membrane. VEGFR2 lacks a PDZ-binding domain, so it cannot directly interact with synectin. Through its cytoplasmic domain, NRP1 can act as a bridge between VEGFR2 and synectin to allow for proper VEGFR2 trafficking. Thus, endothelial cells lacking either the NRP1 cytoplasmic domain or synectin display impaired VEGF2 endocytic trafficking, resulting in diminished ERK activation [9,10].

NRP1 has also been shown to regulate VEGFR2 recycling to the plasma membrane. Under normal conditions, VEGF-A stimulation causes VEGFR2 localization to Rab11-positive endosomes and subsequent receptor recycling to the plasma membrane. In the absence of NRP1, VEGFR2 is instead shuttled to Rab7-positive endosomes that deliver VEGFR2 to the lysosome for degradation [47]. The carboxyterminal SEA domain of NRP1 that mediates synectin binding was also shown to be required for VEGFR2 recycling via Rab11-positive endosomes.

Thus, NRP1 amplifies VEGFR2 signaling by ensuring proper VEGFR2 trafficking to allow for prolonged signal activation. The high levels of ERK activation required for arteriogenesis appear to be uniquely dependent on proper VEGFR2 trafficking. In this way, the NRP1 cytoplasmic domain is indispensable for arteriogenesis.

Conclusions

VEGFR2 signaling is required for endothelial cell differentiation, sprouting, and lumen expansion. Yet, how VEGFR2 is differentially regulated to contribute to these very different endothelial cell outcomes is only just beginning to be elucidated. Intracellular trafficking of VEGFR2 through different endosomal compartments is a relatively new field of study. The data reviewed here show that VEGFR2 trafficking critically regulates the cellular outcomes in response to VEGFR2 activation. In elucidating the role of NRP1 in endothelial cells, our understanding of endothelial cell biology has been expanded to show that VEGFR2 signaling can be differentially regulated to alter two distinct vascular processes: angiogenesis and arteriogenesis. Optimal VEGFR2 trafficking, dependent on NRP1, is dispensable for angiogenesis but required for arteriogenesis. This suggests that arteriogenesis requires prolonged VEGFR2 signaling, which is accomplished through proper endosomal trafficking, and involves high levels of ERK activation.

During angiogenesis, endothelial tip cells express exceptionally high levels of VEGFR2 and are exposed to high concentrations of VEGF-A. In such an environment, sustained VEGFR2 signaling may not be required for the cellular migration and network formation required for angiogenesis. In contrast, arteriogenesis involving luminal expansion and *de novo* arterial fate specification may occur in a setting with reduced VEGF-A, thus requiring NRP1-dependent mechanisms that amplify VEGFR2 signaling and prolong ERK activation, such as receptor recycling and receptor trafficking. Understanding additional levels of VEGFR2 signaling regulation, such as VEGFR2 trafficking, could help answer multiple outstanding questions in the field of vascular biology pertaining to tip cell selection and lumen formation. Clearly, proper VEGFR2 trafficking is an important component of collateral vessel formation. Expanding the field of VEGFR2 trafficking could shed light on this poorly understood vascular process.

Abbreviations

ERK, extracellular-signal-regulated kinase; NRP1, neuropilin 1; RTK, receptor tyrosine kinase; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

Disclosures

The authors declare that they have no disclosures.

References

1. Carmeliet P: **Angiogenesis in life, disease and medicine.** *Nature* 2005, **438:**932-6.
2. Shweiki D, Itin A, Soffer D, Keshet E: **Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis.** *Nature* 1992, **359:**843-5.
3. Folkman J: **Tumor angiogenesis: therapeutic implications.** *The New England journal of medicine* 1971, **285:**1182-6.
4. Lucitti JL, Mackey JK, Morrison JC, Haigh JJ, Adams RH, Faber JE: **Formation of the collateral circulation is regulated by vascular endothelial growth factor-A and a disintegrin and metalloprotease family members 10 and 17.** *Circulation research* 2012, **111:**1539-50.

   F1000Prime RECOMMENDED
5. Faber JE, Chilian WM, Deindl E, van Royen N, Simons M: **A brief etymology of the collateral circulation.** *Arteriosclerosis, thrombosis, and vascular biology* 2014, **34:**1854-9.
6. Grundmann S, Piek JJ, Pasterkamp G, Hoefer IE: **Arteriogenesis: basic mechanisms and therapeutic stimulation.** *European journal of clinical investigation* 2007, **37:**755-66.
7. Hong CC, Peterson QP, Hong J, Peterson RT: **Artery/vein specification is governed by opposing phosphatidylinositol-3 kinase and MAP kinase/ERK signaling.** *Current biology: CB* 2006, **16:**1366-72.

   F1000Prime RECOMMENDED
8. Chittenden TW, Claes F, Lanahan AA, Autiero M, Palac RT, Tkachenko EV, Elfenbein A, Ruiz de Almodovar, Carmen, Dedkov E, Tomanek R, Li W, Westmore M, Singh JP, Horowitz A, Mulligan-Kehoe MJ, Moodie KL, Zhuang ZW, Carmeliet P, Simons M: **Selective regulation of arterial branching morphogenesis by synectin.** *Developmental cell* 2006, **10:**783-95.
9. Lanahan AA, Hermans K, Claes F, Kerley-Hamilton JS, Zhuang ZW, Giordano FJ, Carmeliet P, Simons M: **VEGF receptor 2 endocytic trafficking regulates arterial morphogenesis.** *Developmental cell* 2010, **18:**713-24.
10. Lanahan A, Zhang X, Fantin A, Zhuang Z, Rivera-Molina F, Speichinger K, Prahst C, Zhang J, Wang Y, Davis G, Toomre D, Ruhrberg C, Simons M: **The neuropilin 1 cytoplasmic domain is required for VEGF-A-dependent arteriogenesis.** *Developmental cell* 2013, **25:**156-68.
11. Ren B, Deng Y, Mukhopadhyay A, Lanahan AA, Zhuang ZW, Moodie KL, Mulligan-Kehoe MJ, Byzova TV, Peterson RT, Simons M: **ERK1/2-Akt1 crosstalk regulates arteriogenesis in mice and zebrafish.** *The Journal of clinical investigation* 2010, **120:**1217-28.
12. Soker S, Takashima S, Miao HQ, Neufeld G, Klagsbrun M: **Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor.** *Cell* 1998, **92:**735-45.
13. Adams RH, Eichmann A: **Axon guidance molecules in vascular patterning.** *Cold Spring Harbor perspectives in biology* 2010, **2:**a001875.
14. Yuan L, Moyon D, Pardanaud L, Bréant C, Karkkainen MJ, Alitalo K, Eichmann A: **Abnormal lymphatic vessel development in neuropilin 2 mutant mice.** *Development (Cambridge, England)* 2002, **129:**4797-806.
15. Cai H, Reed RR: **Cloning and characterization of neuropilin-1-interacting protein: a PSD-95/Dlg/ZO-1 domain-containing protein that interacts with the cytoplasmic domain of neuropilin-1.** *The Journal of neuroscience: the official journal of the Society for Neuroscience* 1999, **19:**6519-27.
16. Kitsukawa T, Shimizu M, Sanbo M, Hirata T, Taniguchi M, Bekku Y, Yagi T, Fujisawa H: **Neuropilin-semaphorin III/D-mediated chemorepulsive signals play a crucial role in peripheral nerve projection in mice.** *Neuron* 1997, **19:**995-1005.
17. Kawasaki T, Kitsukawa T, Bekku Y, Matsuda Y, Sanbo M, Yagi T, Fujisawa H: **A requirement for neuropilin-1 in embryonic vessel formation.** *Development (Cambridge, England)* 1999, **126:**4895-902.
18. Gu C, Rodriguez ER, Reimert DV, Shu T, Fritzsch B, Richards LJ, Kolodkin AL, Ginty DD: **Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development.** *Developmental cell* 2003, **5:**45-57.

   F1000Prime RECOMMENDED
19. Fantin A, Schwarz Q, Davidson K, Normando EM, Denti L, Ruhrberg C: **The cytoplasmic domain of neuropilin 1 is dispensable for angiogenesis, but promotes the spatial separation of retinal arteries and veins.** *Development (Cambridge, England)* 2011, **138:**4185-91.

   F1000Prime RECOMMENDED
20. Gelfand MV, Hagan N, Tata A, Oh W, Lacoste B, Kang K, Kopycinska J, Bischoff J, Wang J, Gu C: **Neuropilin-1 functions as a VEGFR2 co-receptor to guide developmental angiogenesis independent of ligand binding.** *eLife* 2014, **3:**e03720.
21. Fantin A, Herzog B, Mahmoud M, Yamaji M, Plein A, Denti L, Ruhrberg C, Zachary I: **Neuropilin 1 (NRP1) hypomorphism combined with defective VEGF-A binding reveals novel roles for NRP1 in developmental and pathological angiogenesis.** *Development (Cambridge, England)* 2014, **141:**556-62.
22. Ferrara N, Davis-Smyth T: **The biology of vascular endothelial growth factor.** *Endocrine reviews* 1997, **18:**4-25.
23. Maglione D, Guerriero V, Viglietto G, Delli-Bovi P, Persico MG: **Isolation of a human placenta cDNA coding for a protein related to the vascular permeability factor.** *Proceedings of the National Academy of Sciences of the United States of America* 1991, **88:**9267-71.
24. Ogawa S, Oku A, Sawano A, Yamaguchi S, Yazaki Y, Shibuya M: **A novel type of vascular endothelial growth factor, VEGF-E (NZ-7 VEGF), preferentially utilizes KDR/Flk-1 receptor and carries a potent mitotic activity without heparin-binding domain.** *The Journal of biological chemistry* 1998, **273:**31273-82.
25. Junqueira de Azevedo IL, Farsky SH, Oliveira ML, Ho PL: **Molecular cloning and expression of a functional snake venom vascular endothelium growth factor (VEGF) from the Bothrops insularis pit viper. A new member of the VEGF family of proteins.** *The Journal of biological chemistry* 2001, **276:**39836-42.
26. Takahashi H, Hattori S, Iwamatsu A, Takizawa H, Shibuya M: **A novel snake venom vascular endothelial growth factor (VEGF) predominantly induces vascular permeability through preferential signaling via VEGF receptor-1.** *The Journal of biological chemistry* 2004, **279:**46304-14.
27. Yamazaki Y, Takani K, Atoda H, Morita T: **Snake venom vascular endothelial growth factors (VEGFs) exhibit potent activity through their specific recognition of KDR (VEGF receptor 2).** *The Journal of biological chemistry* 2003, **278:**51985-8.
28. Koch S, Tugues S, Li X, Gualandi L, Claesson-Welsh L: **Signal transduction by vascular endothelial growth factor receptors.** *The Biochemical journal* 2011, **437:**169-83.
29. Eichmann A, Simons M: **VEGF signaling inside vascular endothelial cells and beyond.** *Current opinion in cell biology* 2012, **24:**188-93.
30. Adams RH, Alitalo K: **Molecular regulation of angiogenesis and lymphangiogenesis.** *Nature reviews. Molecular cell biology* 2007, **8:**464-78.
31. Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, Schuh AC: **Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice.** *Nature* 1995, **376:**62-6.

   F1000Prime RECOMMENDED
32. 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.

33. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, Declercq 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.

34. Sakurai Y, Ohgimoto K, Kataoka Y, Yoshida N, Shibuya M: **Essential role of Flk-1 (VEGF receptor 2) tyrosine residue 1173 in vasculogenesis in mice.** *Proceedings of the National Academy of Sciences of the United States of America* 2005, **102:**1076-81.

F1000Prime RECOMMENDED

35. Gerber HP, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V, Ferrara N: **Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation.** *The Journal of biological chemistry* 1998, **273:**30336-43.

36. Bazzoni G, Dejana E: **Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis.** *Physiological reviews* 2004, **84:**869-901.

37. Mavria G, Vercoulen Y, Yeo M, Paterson H, Karasarides M, Marais R, Bird D, Marshall CJ: **ERK-MAPK signaling opposes Rho-kinase to promote endothelial cell survival and sprouting during angiogenesis.** *Cancer cell* 2006, **9:**33-44.

F1000Prime RECOMMENDED

38. Wheeler-Jones C, Abu-Ghazaleh R, Cospedal R, Houliston RA, Martin J, Zachary I: **Vascular endothelial growth factor stimulates prostacyclin production and activation of cytosolic phospholipase A2 in endothelial cells via p42/p44 mitogen-activated protein kinase.** *FEBS letters* 1997, **420:**28-32.

39. Eitenmüller I, Volger O, Kluge A, Troidl K, Barancik M, Cai W, Heil M, Pipp F, Fischer S, Horrevoets, Anton J G, Schmitz-Rixen T, Schaper W: **The range of adaptation by collateral vessels after femoral artery occlusion.** *Circulation research* 2006, **99:**656-62.

40. Lampugnani MG, Orsenigo F, Gagliani MC, Tacchetti C, Dejana E: **Vascular endothelial cadherin controls VEGFR-2 internalization and signaling from intracellular compartments.** *The Journal of cell biology* 2006, **174:**593-604.

F1000Prime RECOMMENDED

41. Wang Y, Nakayama M, Pitulescu ME, Schmidt TS, Bochenek ML, Sakakibara A, Adams S, Davy A, Deutsch U, Lüthi U, Barberis A, Benjamin LE, Mäkinen T, Nobes CD, Adams RH: **Ephrin-B2 controls VEGF-induced angiogenesis and lymphangiogenesis.** *Nature* 2010, **465:**483-6.

F1000Prime RECOMMENDED

42. Sawamiphak S, Seidel S, Essmann CL, Wilkinson GA, Pitulescu ME, Acker T, Acker-Palmer A: **Ephrin-B2 regulates VEGFR2 function in developmental and tumour angiogenesis.** *Nature* 2010, **465:**487-91.

F1000Prime RECOMMENDED

43. Zhang X, Simons M: **Receptor tyrosine kinases endocytosis in endothelium: biology and signaling.** *Arteriosclerosis, thrombosis, and vascular biology* 2014, **34:**1831-7.

44. Horowitz A, Seerapu HR: **Regulation of VEGF signaling by membrane traffic.** *Cellular signalling* 2012, **24:**1810-20.

45. Dougher M, Terman BI: **Autophosphorylation of KDR in the kinase domain is required for maximal VEGF-stimulated kinase activity and receptor internalization.** *Oncogene* 1999, **18:**1619-27.

46. Zerial M, McBride H: **Rab proteins as membrane organizers.** *Nature reviews. Molecular cell biology* 2001, **2:**107-17.

47. Ballmer-Hofer K, Andersson AE, Ratcliffe LE, Berger P: **Neuropilin-1 promotes VEGFR-2 trafficking through Rab11 vesicles thereby specifying signal output.** *Blood* 2011, **118:**816-26.

F1000Prime RECOMMENDED

48. Gampel A, Moss L, Jones MC, Brunton V, Norman JC, Mellor H: **VEGF regulates the mobilization of VEGFR2/KDR from an intracellular endothelial storage compartment.** *Blood* 2006, **108:**2624-31.

F1000Prime RECOMMENDED

49. Simons M: **An inside view: VEGF receptor trafficking and signaling.** *Physiology (Bethesda, Md.)* 2012, **27:**213-22.

50. Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, Betsholtz C: **VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia.** *The Journal of cell biology* 2003, **161:**1163-77.

F1000Prime RECOMMENDED

51. Gerhardt H, Ruhrberg C, Abramsson A, Fujisawa H, Shima D, Betsholtz C: **Neuropilin-1 is required for endothelial tip cell guidance in the developing central nervous system.** *Developmental dynamics: an official publication of the American Association of Anatomists* 2004, **231:**503-9.

52. Fantin A, Vieira JM, Plein A, Denti L, Fruttiger M, Pollard JW, Ruhrberg C: **NRP1 acts cell autonomously in endothelium to promote tip cell function during sprouting angiogenesis.** *Blood* 2013, **121:**2352-62.

F1000Prime RECOMMENDED

53. Soker S, Miao H, Nomi M, Takashima S, Klagsbrun M: **VEGF165 mediates formation of complexes containing VEGFR-2 and neuropilin-1 that enhance VEGF165-receptor binding.** *Journal of cellular biochemistry* 2002, **85:**357-68.

F1000Prime RECOMMENDED

54. Whitaker GB, Limberg BJ, Rosenbaum JS: **Vascular endothelial growth factor receptor-2 and neuropilin-1 form a receptor complex that is responsible for the differential signaling potency of VEGF(165) and VEGF(121).** *The Journal of biological chemistry* 2001, **276:**25520-31.

F1000Prime RECOMMENDED

55. Lee P, Goishi K, Davidson AJ, Mannix R, Zon L, Klagsbrun M: **Neuropilin-1 is required for vascular development and is a mediator of VEGF-dependent angiogenesis in zebrafish.** *Proceedings of the National Academy of Sciences of the United States of America* 2002, **99:**10470-5.

F1000Prime RECOMMENDED

56. Pan Q, Chanthery Y, Liang W, Stawicki S, Mak J, Rathore N, Tong RK, Kowalski J, Yee SF, Pacheco G, Ross S, Cheng Z, Le Couter J, Plowman G, Peale F, Koch AW, Wu Y, Bagri A, Tessier-Lavigne M, Watts RJ: **Blocking neuropilin-1 function has an additive effect with anti-VEGF to inhibit tumor growth.** *Cancer cell* 2007, **11:**53-67.

F1000Prime RECOMMENDED

57. Murga M, Fernandez-Capetillo O, Tosato G: **Neuropilin-1 regulates attachment in human endothelial cells independently of vascular endothelial growth factor receptor-2.** *Blood* 2005, **105:**1992-9.

58. Valdembri D, Caswell PT, Anderson KI, Schwarz JP, König I, Astanina E, Caccavari F, Norman JC, Humphries MJ, Bussolino F, Serini G:

Neuropilin-1/GIPC1 signaling regulates alpha5beta1 integrin traffic and function in endothelial cells. *PLoS biology* 2009, **7:**e25.

F1000Prime RECOMMENDED

59. Fukasawa M, Matsushita A, Korc M: **Neuropilin-1 interacts with integrin beta1 and modulates pancreatic cancer cell growth, survival and invasion.** *Cancer biology & therapy* 2007, **6:**1173-80.

F1000Prime RECOMMENDED

60. Raimondi C, Fantin A, Lampropoulou A, Denti L, Chikh A, Ruhrberg C: **Imatinib inhibits VEGF-independent angiogenesis by targeting neuropilin 1-dependent ABL1 activation in endothelial cells.** *The Journal of experimental medicine* 2014, **211:**1167-83.

F1000Prime RECOMMENDED

61. Naccache SN, Hasson T, Horowitz A: **Binding of internalized receptors to the PDZ domain of GIPC/synectin recruits myosin VI to endocytic vesicles.** *Proceedings of the National Academy of Sciences of the United States of America* 2006, **103:**12735-40.

F1000Prime RECOMMENDED