What is VEGF and what is its function in relation to angiogenesis?
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Introduction
The vasculature of the human body is essential for supplying oxygen and nutrients to tissues. The molecular basis of the formation of new blood vessels, called angiogenesis, has been studied extensively. A major regulator of angiogenesis is Vascular Endothelial Growth Factor (VEGF) and its receptors, the Vascular Endothelial Growth Factor Receptors (VEGFR’s).

The human body produces five forms of VEGF (VEGF-A, VEGF-B, VEGF-C, VEGF-D and the placenta growth factor, PLGF) and three forms of VEGFR’s (VEGFR-1, VEGFR-2 and VEGFR-3). VEGF was first known as vascular permeability factor (VPF) which was secreted by tumor cells.\(^1\)

The interaction of VEGF with its receptors is complex and responsible for angiogenesis and lymphangiogenesis in embryo’s, during development, and in adults during wound healing. VEGF is also responsible for the tumor angiogenesis, therefore anti-VEGF has been proposed, and approved, as an anti-cancer therapy.\(^2\)

In this paper we will review biological properties of VEGF. We will first discuss the structures of VEGF and the VEGFR’s. We will also take a look at how angiogenesis works and what function VEGF signaling has in this process. Next we will review the VEGF-VEGFR signaling pathways and which components are involved in it. Furthermore we will take a look at the last step of the angiogenesis process; the maturation of the blood vessels.

In this paper we will discuss mainly VEGF, and VEGFR’s, in the context of angiogenesis. We will not review its function in relation to lymphangiogenesis.
VEGF General

VEGF is part of the platelet-derived growth factor (PDGF) super family. It was first discovered and isolated in 1989 from the conditioned medium of the AtT-20 pituitary cell line. VEGF was regarded as a mitogen which was specific for VEGFR. VEGF was found mostly on endothelial cells. There are five known types of VEGF in humans. VEGF-A is the most important for angiogenesis and binds to both the VEGF receptor 1 (VEGFR-1) and VEGFR2. VEGF-B only binds to VEGFR-1 and has a variety of functions under stressed conditions. VEGF-C and D bind to VEGFR-3 which plays a role in lymphangiogenesis. The placenta growth factor (PLGF) is also a form of VEGF. There are also two forms of VEGF known, which do not exist in the human genome. VEGF-E (Orf-VEGF) is found in some viruses. svVEGF is found in certain snake venom. In this paper VEGF-A is mainly discussed.

When VEGF-A binds to its receptor (VEGFR-1 and VEGFR-2) the complex gathers two co-receptors, heparin/heparansulfate proteoglycan (HSPGs) and neuropilin-1 (NRP-1), which respectively modulate or enhance the signaling. All VEGF forms have a common structure of eight cysteine residues at fixed positions in a VEGF homology domain. These eight cysteine residues, also known as the cysteine knot motif, are also present in the PDGF family. Six of the eight cysteine residues form three intramolecular disulfide bonds. The remaining two cysteine residues form an intermolecular bond with the opposite homodimer of VEGF. VEGF-A, commonly named VEGF, is the best studied form of VEGF. It plays a major role in angiogenesis, physiological and pathophysiological. VEGF-A is an antiparallel homodimer, which has two receptor binding sites at each end of the structure. The VEGF-A gene is situated at the 6p21.3 chromosome and contains 8 exons and 7 introns, its coding region spans 14 kilobases (kb). VEGF-A plays a crucial role in angiogenesis and vasculogenesis. A VEGF-A homozygote knock-out mouse as well as a VEGF-A heterozygote knock-out mouse have a lethal embryotic phenotype due to not-completed blood vessel formation. VEGF-A can be spliced into six different isoforms. VEGF-A121, VEGF-A145, VEGF-A165, VEGF-A189, VEGF-A206, VEGFxx06. The numbers in the name represent the amount of amino acids that are present in the respective isoform. In mice all isoforms are 1 amino acid shorter than in human. The exons 1-5 are present in all forms of VEGF-A, the splicing occurs in exon 6 and 7. All peptides have a basic stretch near the carboxyl terminus, except for VEGF-A121. VEGF-A121, the shortest of the isoforms, lacks exons 6 and 7. Exons 6 and 7 encode for the binding domains of heparin/heparansulfate proteoglycan (HSPGs) and neuropilin-1 (NRP-1), which is a membrane protein in neuronal cells and co-receptor for VEGF-A. VEGF-A121 is not present on the cell surface or in the extracellular matrix (ECM) in high amounts, due to the lack of binding to heparin and neuropilin. It is therefore highly diffusible and acts as a chemoattractant and mitogen on endothelial cells during angiogenesis.

VEGF145 is an isoform which lacks exons 6b and 7. VEGF145 occurs in intermediary form, because it is secreted. However a significant part remains on the cell surface and in the ECM. VEGF145 binds to VEGFR1 and 2, HSPG and NRP2. VEGF-A165, which is missing exon 6a and 6b, is the most important form of VEGF-A. It will bind to all receptors. VEGF-A165 affinity for VEGFR-1 is a 10-fold higher than its affinity for VEGFR-2. Meas et al. reported that VEGF-A164 is essential and sufficient for angiogenesis, because VEGF-A164 transgenic mice in a VEGF-A-null genetic background are alive and healthy. These mice only expressed VEGF-A164.

All the exons are present in VEGF-A189 and VEGF-A206. VEGF-A189 has an insertion of 24 highly enriched amino acids. VEGF-A206 has an additional insertion of 17 amino acids. These extra amino acids make that these molecules are not secreted but are bound to HSPGs and NRP-1. Therefore VEGF-A189 is mostly localized on the cell surface and in the ECM. The expression of VEGF-A189 is usually low while VEGF-A206 is only expressed in embryonic tissue.
All together these six isoforms are all forms of VEGF-A. These isoforms will activate both VEGFR-1 and VEGFR-2, both of which promote angiogenesis, cell migration and vascular permeability.\(^\text{18}\) 

**VEGF\(^{xxx}\)**

There is a variant of VEGF-A, which is called VEGF\(^{xxx}\). The xxx is standing for one of the isoforms of VEGF-A.\(^\text{19}\) VEGF\(^{xxx}\) is lacking 6 amino acids at the C-terminal end of the molecule. In receptor-binding studies, it binds to VEGFR2 with the same affinity as VEGFxx, but it will not fully activate a downstream response or phosphorylate the receptor. VEGF\(^{xxx}\) is a physiological competitor of VEGF-A, in other words an antagonist of VEGF-A.\(^\text{20-22}\) To give an example, VEGF\(_{165}\text{b}\) is the antagonist for VEGF\(_{165}\).

**VEGF-B**

VEGF-B encodes 188 amino acids and consist 8 exons and 6 introns. VEGF-B is located on chromosome 11q13. It is also known as VEGF-related factor (VRF). VEGF-B is highly expressed in skeletal muscle, myocardium and brown fat. VEGF-B only activates VEGFR-1. VEGF-B has two isoforms: VEGF-B\(_{165}\) and VEGF-B\(_{186}\). The promoting region of VEGF-B is the same as that of VEGF-A, both promoting areas are associated with CpG islands and contain binding sites for Sp1 and AP2. The difference between VEGF-A and B is that VEGF-B promoter region has Egr-1 sites, not hypoxia-inducible factor-1 and AP-1 sites like VEGF-A. Therefore, hypoxia, a stimuli which regulates VEGF-A, will not stimulate VEGF-B expression.\(^\text{21,22}\) VEGF-B is one of the least studied VEGF's, probably because its neutral activity under normal conditions. However, VEGF-B seems to have certain protective qualities. VEGF-B has been associated with the down regulation of genes, through VEGFR-1, which are involved in apoptosis in the retina and the brain.\(^\text{25}\)

**VEGF-R**

VEGF-R's are members of the class V of the receptor tyrosine kinase (RTK) superfamily. The RTK superfamily exists of multiple classes. PDGF receptors (PDGFR) are also members of this family (class III). VEGF-R's are tyrosine kinase receptors with an extracellular part to bind to VEGF. This domain is 750 amino-acid long and organized in seven immunoglobulin-like folds. The Ig-domain 2 is the ligand-binding site of the VEGFR-1 and the Ig-domain 2 and 3 are the binding domain of the VEGFR-2. After this extracellular part, there is a transmembrane part, followed by a juxta-membrane domain, which is mostly regulatory. Then there are two kinase domains with, in between a 70 amino acid long kinase insert and at the end of the tail there is a C terminus.

Like other RTKs, VEGFRs signaling is initiated by a covalent binding of a ligand by the extracellular domain.

**VEGF-R-1**

VEGF-like tyrosine kinase 1(Flt1), is a 180 kDA glycoprotein which is required in normal blood vessel formation. Knock-out of the VEGF-R-1 gene is lethal in mice due to disorganization of the vasculature and an excess of endothelial cells.\(^\text{26}\) There is a splice variant of VEGF-R-1, sVEGF-R-1, which is lacking the transmembrane domain and the intracellular kinase domains. Although this variant does not have any signaling possibilities, there is a normal vasculature.\(^\text{27}\) VEGF-A has a high affinity for VEGF-R-1. VEGF-R-1 downstream kinase activity is weak when compared with VEGF-R-2. These are all points that indicate that VEGF-R-1 function is to anchor VEGF to the membrane.\(^\text{28}\) The current opinion is that VEGF-R-1 is a ‘trap’ VEGFR. VEGF-R-1 kinase activity is not essential for vessel development but plays a more important role in pathological angiogenesis and in wound healing, by potentiating VEGF-R-2 signaling.\(^\text{29}\)
**VEGFR-2**

VEGFR-2, also known as KDR and FLK1, is the major receptor for VEGF-A in angiogenesis. VEGFR-2 induces the proliferation, survival, sprouting and migration of endothelial cells and also increases the endothelial permeability. VEGFR-2 is a 200-kDa glycol protein. It has 19 tyrosine residues present in the intracellular domain. The most important tyrosine residue is Tyr1175 due to activation of many pathways through PLCγ-1. The PLCγ-1 pathway, activated by Tyr1175 plays an important role in angiogenesis from VEGFR-2. This is interesting because most TKRs are activating the Ras pathway or PI3K pathway.

After activation of VEGFR-2 by VEGF-A, VEGFR-2 is endocytosed via a clathrin-mediated pathway and is then targeted for degeneration or recycling. Degeneration occurs in the Rab7 endosome. Recycling is controlled by APPL 1 and 2 and occurs through the Rab5/Rab4/Rab11 pathway. Depending on the ligand, the VEGFR-2 endosome is following one of these two pathways. VEGF-A165 will direct the endosome to the recycling pathway. VEGF-A165b will direct the endosome to degeneration.
VEGF & Angiogenesis

Angiogenesis, the process

In this paragraph the mechanism of angiogenesis will be described from a broader perspective. Angiogenesis depends on endothelial cells, which form the blood vessels. Endothelial cells have the capability to change their numbers and the way they are arranged to the local needs. Without endothelial cells angiogenesis would not be possible.

Arteries and veins are the largest blood vessels in the human body. They are made up out of three different layers. From the lumen to the outside these layers are the tunica intima (including the endothelium), the tunica media (which contains smooth muscles and elastin) and the tunica externa (which contains collagen fibers). The most important layer is the tunica intima, containing the endothelium, which is a thin sheet of endothelial cells. The endothelium is separated from the surrounding outer layers by a basal lamina. The amount of layers and the thickness of these layers vary a lot, depending on the function and thickness of the blood vessel. The endothelium however is always present. In the smallest blood vessels, the capillaries, the blood vessel wall contains just endothelial cells and some pericytes.

To understand the process of angiogenesis it is important to understand the endothelial cells. Endothelial cells originate from the mesoderm, or middle layer, of the embryo. At specific sites they emerge from precursor cells which are also giving rise to blood cells. The early endothelial cells undergo a process called vasculogenensis, which creates essentially the first elements of the first blood vessel. The process includes migration, proliferation and differentiation of the endothelial cells. From this point on the blood vessel system will be created from this first vessel through a process called angiogenesis.

The process of angiogenesis is the same in adults as in embryos. A new vessel grows from an existing vessel as a capillary sprout. When a pro-angiogenic signal, like VEGF, reaches endothelial cells (EC) of the vessels they are activating their angiogenic process. This starts with cells breaking out of the vessel wall and the basal lamina needs to degenerate. After that the EC's start to proliferate, and adjust their phenotype and functions, coordinated in a ‘teamwork’ behavior. The EC’s at the beginning of the sprout are called tip cells. These tip cells are characterized by their long filopodia and their position. Tip cells move in the direction of the VEGF gradient until it reaches another sprout.

Behind the tip cells there are endothelial stalk cells, which proliferate and creating a lumen. Besides the functional differences, there are also many differences in gene expression. These differences arise through two signaling pathways: the VEGF pathway and the Notch pathway.

Notch pathway

The notch pathway is an intercellular signal mechanism that is involved in many processes to determine the fate of certain cells. The decision between tip or stalk cell is the same. In vertebrate there are four notch receptors, and five notch transmembrane ligands. The most important for tip/stalk cell determination are Dll4, notch and jagged1. Using a synthetic Jagged1 ligand peptide to activate the notch signaling, Hellstorm et al showed that the notch signaling pathway is crucial for formation of tip and stalk cells. When the notch pathway was activated, using jagged1, it reduced the formation of the filopodia, which implicated that there were less tip cells. A more recent study...
showed that the intracellular domain of these transmembrane ligands is processed in a γ-secretase-dependent manner and that the intracellular domains accumulate to the nucleus\(^39\). In summary, a tip cell is producing less Notch than its neighboring cells, while stalk cells express less DII4. Meaning, a tip cell is producing more DII4, and a stalk cell more Notch. High DII4 expression of the tip cell stimulates a high Notch signaling in the neighboring stalk cell\(^40\).

**VEGF pathway**

This is not the only way a tip cell is determined, this whole process is initiated by VEGF-A and VEGFR-2 expression. VEGF-A and hypoxia increases the expression of DII4, which in turn stimulate the Notch activity in the neighboring cell\(^37\). How this process exactly works is not clear at this point, what is known is that it involves the PI3K pathway and transcription factors FoxC1 and 2.\(^41\) So VEGF-A is working ‘upstream’ of DII4. On the other hand, notch also controls VEGF signaling. Notch works as a negative feedback on VEGF. Notch up-regulates the ‘trap’ VEGFR-1 receptor\(^42\), but down regulates the ‘signaling’ VEGFR-2 receptor\(^43\). This process will ensure that the stalk cells are less sensitive for the VEGF gradient, because they have a much weaker kinase activity of it, through the VEGFR-1. The VEGFR-2, which has a signaling function, is down regulated on stalk cells.

The notch and VEGF pathway are dependent of each other. Under normal conditions there are some cells which are producing more VEGFR-2 and DII4. This difference is determent through the notch-pathway. These cells also have long filopodia to ‘sense’ whether VEGF-A is released. When there is, these cells will be the tip cells because of the (extra) up-regulation of DII4 and VEGFR-2, through VEGF-A. The process is self-enhancing.

**Activation of VEGF**

Activation of VEGF starts in the target cell, which has low-oxygen levels, not in an endothelial cell. During growth or wound healing new cell emerge, which may experience hypoxia due to the fact that they are too far away from arteries. Hypoxia induces the activation of the Hypoxia Induced Factor-1(HIF-1) in the target cells. HIF-1 is, besides VEGF, a key element in the process of angiogenesis. HIF-1 is a heterodimer protein complex, containing HIF-1α and HIF-1β, an aryl hydrocarbon-receptor nuclear translocator. Under normal conditions, HIF-1β is stable. HIF-1 however, binds to the von Hippel-Lindau tumor suppressor protein (VHL). This leads to ubiquitination of HIF-1α, which is then rapidly degraded. Under hypoxia conditions HIF-1α, is more stable and accumulates in to the nucleus and binds to HIF-1β. This complex is then active and starts transcription of different factors including VEGF.\(^ {44}\)

The HIF-complex binds to the VEGF gene promoter and recruits additional transcription factors. These transcription factors are CREB, P300 and STAT3. This starts the transcription of VEGF. After the process of angiogenesis is complete the target cell is receiving enough oxygen so the production of the HIF-complex decreases. This, in turn, decreases the expression of VEGF-A.\(^ {44}\)
VEGF Pathways

See figure 1

VEGFR-2
As mentioned above VEGFR-2 is a tyrosine kinase, which induces proliferation and sprouting of the ECs. This process is very complex. When VEGF-A binds to VEGFR-2, it will form a dimer. It will then be transferred into the cell through endocytosis. Meanwhile it will activate a series of downstream signaling pathways, which will be discussed here.

On binding VEGF-A, VEGFR-2 will form a dimer, which is further stabilized by low-affinity homotypic interactions between membrane Ig domains. This gives opportunity for trans/autophosphorylation of the intracellular kinase sites. The main phosphorylation sites of VEGFR-2 are Y951, which lies in the kinase insert, Y1054 and Y1059, which are lying in the second kinase domain, Y1175 and Y1214, which are lying in the C-terminal of the VEGFR-2.

The Y951 site is a binding site for T cell-specific adaptor molecule (TSAd). It is phosphorylated on VEGF-stimulation and is active during development and tumor-angiogenesis. The Y1175 site is the most important site due to its ability to be a binding site for several mediators, like PLCγ and SHB/SCK, which in turn activate the PI3K pathway.

Upon VEGF-A binding VEGFR-2 will initiate several pathways which lead to proliferation and migration of ECs. Furthermore increase it the permeability of blood vessels. These pathways will be discussed here.

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Description</th>
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<tbody>
<tr>
<td>ERK</td>
<td>Extracellular signal-regulated kinases</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein Kinase C</td>
</tr>
<tr>
<td>PKD</td>
<td>Protein Kinase D</td>
</tr>
<tr>
<td>HDAC</td>
<td>Histone deacetylases</td>
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<tr>
<td>HSP27</td>
<td>Heat Shock Protein 27</td>
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<tr>
<td>CREB</td>
<td>cAMP response element-binding protein</td>
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<tr>
<td>NCK</td>
<td>Non-catalytic region of tyrosine kinase adaptor protein</td>
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<tr>
<td>FYN</td>
<td>Proto-oncogene tyrosine-protein kinase Fyn</td>
</tr>
<tr>
<td>Src</td>
<td>Proto-oncogene tyrosine-protein kinase Src</td>
</tr>
<tr>
<td>TSAd</td>
<td>T Cell–Specific Adapter Protein</td>
</tr>
<tr>
<td>SHB</td>
<td>SH2 domain-containing adapter protein B</td>
</tr>
<tr>
<td>FAK</td>
<td>Focal-Adhesion Kinase</td>
</tr>
<tr>
<td>IQGAP1</td>
<td>IQ motif-containing GTPase activating protein 1</td>
</tr>
<tr>
<td>AKT</td>
<td>Protein Kinase B</td>
</tr>
<tr>
<td>Yes</td>
<td>Proto-oncogene tyrosine-protein kinase Yes</td>
</tr>
<tr>
<td>BAD</td>
<td>BLC-2 associated death promoter</td>
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Proliferation

Proliferation of the ECs is essential to growth a new blood vessel. VEGF-A initiates cell proliferation through activation of the RAS/RAF/ERK/MAPK pathway. Unlike other receptor tyrosine kinases, VEGFR-2 stimulates ERK via Y1175 dependent phosphorylation of PLCγ, which in turn activates PKC. PKC initiate three processes. First, PKC eventually activates p42/44 MAPK which leads to cell proliferation. Second, PKC increases the vascular permeability through eNOS. Third, PKC activates Protein Kinase D(PKD). PKD induces nuclear translocation of histone deacetylases 5 and 7(HDAC), which activates CREB, for gene transcription, and Heat Shock Protein 27(HSP27), for actine remodeling, which leads to migration.

PKC is one of the most important downstream signaling proteins for VEGFR-2 because of its overall involvement in cellular processes crucial for angiogenesis.

Migration

Migration of the ECs is crucial for angiogenesis. ECs move to a concentration gradient of VEGF-A. Next to the PKC pathway mentioned above, there are some other ways to induce migration.

Another way to activate HSP27 for actine remodeling is through phosphorylated Y1214, which will recruit NCK (non-catalytic region of tyrosine kinase adaptor protein) and FYN. This complex will eventually activate p38MAPK which induce migration through HSP27.

Migration can also be induced through TSAd, which forms a complex with Src. This complex will also induce migration and actine-reorganization, but not proliferation.

Furthermore there is Y1175, which binds to SHB. This will then be phosphorylated by Src. SHB will then bind to phosphorylated Focal-Adhesion Kinase (FAK), which regulates migration and cell adhesion.

At last there is IQ motif-containing GTPase activating protein 1(IQGAP1), which binds to and activates RAC1 by inhibiting the GTPase activity. IQGAP1 knock down prevents VEGF-induced migration.

Migration can be activated through four processes. Migration is important for angiogenesis because sprouting goes towards the VEGF gradient, this would not be possible without migration.

Permeability

VEGF-A, first described as vascular permeability factor, regulates vessel permeability. Strangely the complete mechanism is until this day not clarified. What is known is that VEGF regulates the permeability through VEGFR-2. VEGFR-2 has two ways to induce permeability, both through the endothelial nitric oxygen synthase (eNOS) of nitric oxide (NO). eNOS can be activated either through the PLCγ pathway, or through AKT pathway. PLCγ causes a Ca+ influx, activating eNOS. AKT activates eNOS by phosphorylating it. The regulation of the permeability through VEGFR-2 is dependent on SRC en Yes. Src regulates the EC cell-cell contacts by phosphorylating VE-cadherin, an adherend junction protein. The permeability is regulated by endocytosis of VE-cadherin. This process is β-arrestin dependent. FAK, is also mediating the regulation of permeability.

Furthermore, a recent study showed that VEGF-A, but not PIGF, induces permeability through VEGFR-2. So, the process is ligand-dependent.

Survival

VEGF protects ECs against apoptosis, through the PI3K induced phosphorylation of AKT/PKB. AKT phosphorylates BLC-2 associated death promoter (BAD) and caspase 9. This inhibits their apoptotic function. VEGF also stimulates the expression of inhibitors of apoptosis (IAP), like XIAP and survivin.
They, in turn, inhibit caspase 3 and 7, which are associated with apoptosis.

**Co-receptors**
There are many different signaling pathways which are activated through VEGF-A binding to VEGFR-2. This is complicated by the fact that VEGFR-2 and its ligand acquire several co-receptors. These co-receptors are neuropilins, heparin sulfate, integrins and cadherins.

**Neuropilins**
Neuropilins (NRP) are transmembrane proteins with a small intracellular domain. NRP1 binds to exon 7 of VEGF-A, it will then enhance the p38MAPK signaling pathway of this VEGF-A/VEGFR-2 complex. NRP-1 interacts with VEGF-A, enhancing its signaling, this is very important for the tip cells. NRP-1 is mostly found, as co-receptor for VEGFR2 and VEGFR1, in arteries. NRP-2 is found in veins and lymph vessels.

**Heparin**
Heparin-sulfate proteoglycans (HSPGs) are proteins that contain covalently linked heparin sulfate chains that modulate activity of a large number of proteins. HSPGs modulate the VEGF signaling in different ways by binding not only VEGF but also its receptors and co-receptors like NRP1. All VEGF-A forms bind heparin-sulfate (HS) or heparin (H) except for VEGF-A121 and VEGFxxxb. Simultaneous binding of HS/H and another components of the ligand-receptor complex increases the stability. Tip cells of sprouting blood vessels migrate in the direction of the gradient, this gradient is shaped due to VEGF-A binding to HSPGs.

**Integrins**
Integrins are transmembrane heterodimers that mediate cell-matrix adhesion by specific binding to the extracellular matrix, such as collagen and laminin. Integrins of the β3 subfamily binds to the extracellular domain of VEGFR-2. Integrin β3 regulate the VEGFR-2 activity. Mice which did not expressed these integrins showed increased VEGFR-2 activity and tumor angiogenesis. Integrins are the ‘controllers’ of VEGFR activity, during non-active periods or during active periods like wound healing.

**Cadherins**
The interaction between VE-cadheren and VEGFR-2 is regulated through β-catenin. The VE-cadherin-β-catenin complex participates in contact inhibition of VEGF signaling, at high cell density. When stimulated, VEGFR-2 associates with this complex, it will then be concentrated at the cell-cell contacts. Here it will be inactivated through junctional phosphatases, like DEP-1. At low cell density, VEGFR-2 will associate with αvβ3 instead of VE-cadherin-β-catenin. This will stimulate cell migration and proliferation of the EC.

**VEGFR-1**
VEGFR-1 is widely expressed throughout the body, but not essential for angiogenesis. However, VEGFR-1 knock-out mice embryo’s die at day 9 due to disorganized vasculature and an excess of EC progenitors. When the intracellular domain of VEGFR-1 is missing, the mice show a normal vasculature.

VEGFR-1 has several tyrosine phosphorylation sites, Tyr794, Tyr 1169, Tyr 1213, Tyr2142, Tyr1309, Tyr1327, Tyr1333. The site that will be phosphorylated is dependent on the ligand. For example,
PIGF, but not VEGF-A, will induce phosphorylation of Tyr1309. Tyr 795 and Tyr1169 are binding sites for PLCγ. Tyr1213 is a major binding site for SH2-containing proteins, like NCK and SHP2 (SH2-domain-containing protein tyrosine phosphatase 2).

Although these sites are identified, the precise role of VEGFR-1, apart from being a storage place for VEGF, has not been identified.

While some studies state that VEGFR-1 signaling is not essential for proliferation and migration, others imply that VEGFR1 is active in the migration of ECs through the Receptor for Activated C-Kinase 1 (RACK1) pathway. Differentiation and organization may also involve VEGFR-1 dependent PI3K/Akt pathway activation, through Tyr1213.

Furthermore, during blood vessel formation, absence of VEGR-1 increases the availability of ligand for VEGFR-2. The first step of the signal transduction, receptor tyrosine phosphorylation, is upregulated, due to the increased availability of these ligands. This up regulation leads to increase of signal transduction of all the pathways of VEGFR-2. However, another study showed that the VEGFR-1 receptor is crucial to the VEGF-notch signaling and determines which cell becomes the tip cell.

A recent study showed that, VEGF-A induces up-regulation of the soluble form of VEGFR-1. VEGF-A modulates the alternative splicing of VEGFR-1, through the VEGFR-2 C-MEK pathway. It however does not change the ‘normal’ VEGFR-1. This up regulation can be a sort of negative feedback of VEGF-A.

Furthermore, VEGFR-1 seems to have an important role in pathological angiogenesis. The signaling cascade of VEGFR-1 during the pathological angiogenesis remains to be clarified.

In this chapter we summed up the functions of VEGFR-2 and VEGFR-1. The key message of this chapter is that VEGFR-2 is the more signaling receptor, while VEGFR-1 is the more supporting VEGFR. The signaling of VEGFR-2 is well understood that of VEGFR-1 still needs to be investigated. More interesting is however the complex interaction between these two receptors.
Figure 1: The downstream signaling of VEGF and VEGFR
Blood vessel maturation

The stabilization and maturation of the new blood vessels are the late events of angiogenesis. Blood flow has a critical role in this process. Vessels with a high blood flow will widen while others with a low blood flow may degenerate. Several cellular and non-cellular components in the blood vessel regulate the maintenance of the vessel, like ECs, pericytes, smooth muscle cells, ECM, fibroblasts and glial cells. Blood vessels are composed of endothelial cells and mural cells. Mural cells can be divided into two cell types, pericytes and vascular smooth muscle cells. Pericytes are embedded within the basement membrane of the capillaries and share their basal membrane with the ECs. Pericyte adhesion to capillaries and wrapping of endothelial cell by surrounding pericytes are processes in blood vessel maturation and stabilization. There are two theories about the pericyte wrapping. First theory is that during the sprouting ECs direct the differentiation of mural precursor’s cells from surrounding tissue. Second theory is that mural cells become associated with ECs by migration along the new formed blood vessel.

Mural cells presenting phenotypes between pericytes and vascular smooth muscle cells are associated with arterioles and venules. The marker profile of pericytes varies depending on the tissue of origin. The most common markers are alpha-smooth muscle actin and PDGFR (Platelet-derived growth factor Receptor). The interaction between Platelet-derived growth factor B (PDGF-B), secreted by ECs, and PDGFR-β, expressed on pericytes, is essential for mural cell recruitment and thus for maturation of the blood vessel. PDGF-B is secreted as a homodimer and act as an attractant for migrating pericytes. PDGF-B stimulates proliferation of vascular smooth muscle cells and induces mural cell fate in undifferentiated mesenchymal cells.

A second important system for blood vessel maturation is the angioptetin (Ang)/Tie system. The Ang family consists of four members (Ang 1 t/m 4) and two corresponding tyrosine kinase receptors (Tie 1 and 2). Ang-1 is agonistic and Ang-2 is antagonistic for Tie2 receptor. Ang-1/Tie2 signaling is required for maintaining the ‘rest’ state of ECs. Ang-1 binds to the Tie2 receptor on ECs and establishes pericyte-endothelial interactions by inhibition of proliferation of the ECs. Ang-2 acts as an antagonist of Ang-1/Tie2 signaling.

Both the PDGF system and the Ang/Tie system are involved in the maturation of blood vessels. In tumors there are often problems in these systems with the consequence that tumor vessel are not matured and leaky.
Conclusion
In this paper we reviewed the process of angiogenesis. How does it work, and what factors are involved.

First of all there are (any) cells which experience a lack of oxygen. There is no difference in whether this is in a growing tumor or in a wound which is healing. Within these cells HIF1α will become stable and will not be degraded, initiating VEGF-A production and secretion. This VEGF-A is picked up by certain endothelial cells which have a higher VEGF sensibility then their neighbors. This difference is originated from the notch signaling. The cells with the increased sensibility will become the tip cells. The others will become the stalk cells. When VEGF-A binds to VEGFR-2, it will activate several complex downstream pathways which increase the permeability of the blood vessel, but also increase proliferation and migration of the endothelial cells.

The tip cell will move in the direction of the VEGF gradient until it reaches another sprout or blood vessel. A new blood vessel is formed. The oxygen can now get to the cells which secreted the VEGF-A. Therefore there are normal oxygen levels in these cells and thereby no stable HIF1α, which means no further secretion of VEGF-A.

The last step in angiogenesis is the maturation of the blood vessels. This last step is carried out through two systems, the PDGF system and the Ang/Tie system. Both attracting pericytes and vascular muscle cell to the blood vessels.

In conclusion the complete process of angiogenesis is well understood. The VEGFR-2 pathways are also well understood in relation to angiogenesis. The VEGFR-1 pathways however are not. Many downstream processes are not clear yet. To further understand the function of VEGF and the process of angiogenesis, the interaction between VEGFR-1 and VEGFR-2 needs to be investigated.
References


28. Hiratsuka, S. et al. Membrane fixation of vascular endothelial growth factor receptor 1 ligand-binding domain is important for vasculogenesis and angiogenesis in mice. Molecular and


43. Williams, C. K., Li, J.-L., Murga, M., Harris, A. L. & Tosato, G. Up-regulation of the Notch ligand...


58. Gerhardt, H. et al. Neuropilin-1 is required for endothelial tip cell guidance in the developing


72. Saito, T. et al. VEGF-A induces its negative regulator, soluble form of VEGFR-1, by modulating


