Vasculogenesis, angiogenesis and lymphangiogenesis: Signaling pathways

Erhard Hofer

Department of Vascular Biology and Thrombosis Research
Center for Biomolecular Medicine and Pharmacology
Medical University of Vienna
Vasculogenesis, angiogenesis and lymphangiogenesis: Signaling pathways

Vasculogenesis - angiogenesis - arteriogenesis
vasculogenesis - development
sprouting angiogenesis
arteriogenesis
lymphangiogenesis

Important factors and receptors
lessons from ko mice
hypoxia - HIF - VEGF
EC-specific ligands + receptors
downstream signals - genes - proteins

Angiogenesis and diseases
tumor angiogenesis
other diseases with excess or deficiency in angiogenesis
Literature:

Books:

Pg. 1279-1283

Endothelial Biomedicine
W. Aird, ed.
Cambridge University Press, 2007

Pg. 556-585

Tumor Angiogenesis - Basic mechanisms and Cancer Therapy,
D. Marme, N. Fusenig, ed.
Springer Verlag 2008

Angiogenesis - From basic science to clinical application
N. Ferrara, ed.
CRC Press, Taylor&Francis Group, 2007
Nature Insight Angiogenesis

Angiogenesis Focus, Nature Med 9, June 2003
Peter Carmeliet, Angiogenesis in Health and Disease
Napoleone Ferrara et al., The biology of VEGF and its receptors
Rakesh K. Jain, Molecular regulation of vessel maturation
Shanin Rafii and David Lyden, Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration
Christopher W. Pugh and Peter J. Ratcliffe, Regulation of angiogenesis by hypoxia: role of the HIF system

Angiogenesis, Nature Reviews Cancer 3, June 2003
Gabriele Bergers and Laura E. Benjamin, Tumorigenesis and the angiogenic switch

Carmeliet, Angiogenesis in life, disease and medicine
Coultas, Endothelial cells and VEGF in vascular development
Alitalo, Lymphangiogenesis in development and human disease
Greenberg, From angiogenesis to neuropathology
Garino, Retinal angiogenesis in development and disease
Ferrara, Angiogenesis as a therapeutic target


Stem cells and vascular progenitor cells:

*Angiogenesis Focus, Nature Med 9, June 2003*
Shanin Rafii and David Lyden, Therapeutic stem and progenitor cell

*Nature Insight Angiogenesis, Vol. 438, pg. 931-974, December 2005*
L. Coultas et al., Endothelial cells and VEGF in vascular development

*Nature Insight Cardiovascular Disease, Vol. 451, 903, February 2008*
V.F.M. Segers and R.T. Lee, Stem-cell therapy for cardiac disease

*Nature Insight Regenerative Medicine, Vol. 453, 7193, May 2008*
R. Passier et al., Stem-cell-based therapy and lessons from the heart


2010-2011:


Pdf for download:

http://mailbox.univie.ac.at/erhard.hofer
Student point, Vorlesungsunterlagen

erhard.hofer@meduniwien.ac.at
1- Vasculogenesis - Angiogenesis - Arteriogenesis - Lymphangiogenesis

**Vasculogenesis**
Formation of blood vessels by differentiation from (hem)angioblasts

**Sprouting angiogenesis**
Sprouting of cells from mature endothelial cells of the vessel wall

**Arteriogenesis**
growth of large arteries from pre-existing small vessels/capillaries

**Lymphangiogenesis**
Formation of the lymphatic vasculature
Vasculogenesis

Development from mesoderm

Connective tissue

Blood cells

Blood vessels

Muscle

Etc.
Vasculogenesis

Formation of vessels by differentiation of cells from angioblasts (e.g. in the yolk sac of the embryo)

Is differentiation and proliferation of endothelial cells in a non-vascularized tissue

Leads to formation of a primitive tubular network

Has to undergo angiogenic remodeling to stable vascular system
Angiogenesis - Vasculogenesis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIF1α</strong></td>
<td>embryonic lethal E11</td>
</tr>
<tr>
<td><strong>VEGFR1</strong></td>
<td>embryonic lethal E8.5-9.5, overgrowth of endothelial vasculature</td>
</tr>
<tr>
<td><strong>VEGFR1 TK del</strong></td>
<td>healthy</td>
</tr>
<tr>
<td><strong>VEGFR2</strong></td>
<td>embryonic lethal E8.5, impaired hematopoietic and endothelial vasculature</td>
</tr>
<tr>
<td><strong>VEGFR3</strong></td>
<td>90% lethal soon after E9.5, reduced and disorganized EC</td>
</tr>
<tr>
<td><strong>VEGF-A</strong></td>
<td>heterozygous lethal E9.5, impaired vasculogenesis</td>
</tr>
<tr>
<td><strong>VEGF-B</strong></td>
<td>viable with smaller heart, dysfunctional coronary vasculature</td>
</tr>
<tr>
<td><strong>VEGF-C</strong></td>
<td>embryonic lethal E15.5, failure in lymphangiogenesis</td>
</tr>
<tr>
<td><strong>Notch1</strong></td>
<td>embryonic lethal E11, failure of vascular remodeling</td>
</tr>
<tr>
<td><strong>Jag1</strong></td>
<td>embryonic lethal E9.5-11.5, failure of vascular remodeling</td>
</tr>
<tr>
<td><strong>Tie-1</strong></td>
<td>embryonic lethal E13.5, loss of vascular integrity</td>
</tr>
<tr>
<td><strong>Tie-2</strong></td>
<td>embryonic lethal E9.5</td>
</tr>
<tr>
<td><strong>Ang-1</strong></td>
<td>phenocopies Tie-2</td>
</tr>
<tr>
<td><strong>Ang-2</strong></td>
<td>viable, but vascular defects</td>
</tr>
</tbody>
</table>
Hemangioblast → Angioblast → EC

Figure 22–35. Molecular Biology of the Cell, 4th Edition.
Mobilization of endothelial, lymphatic and hematopoietic progenitor cells

Figure 2 Molecular switches involved in the mobilization and recruitment of endothelial, lymphatic and hematopoietic stem and progenitor cells. Vascular trauma results in the plasma elevation of angiogenic factors, including VEGF-A and PLGF, that activate MMP-9. Activation of MMP-9 results in increased bioavailability of the stem cell–active cytokine, soluble Kit ligand (sKitL), enhancing the cycling and proliferation of hibernating VEGFR1+ c-Kit+ HSCs, VEGFR3+ lymphatic and c-Kit+ VEGFR2+ vascular progenitors. Increased cycling of the stem cells results in localization of precursors to the vascular zone, setting the stage for mobilization to the peripheral circulation and homing to the neoangiogenic site. Co-mobilization of proangiogenic VEGFR1+ hematopoietic stem and progenitor cells may facilitate functional incorporation of VEGFR2+ EPCs into neovessels. In addition, Syk+ and SLP-76+ hematopoietic cells may convey signals for the separation of newly formed blood and lymphatic vessels. mKitL, membrane Kit ligand.
Structure of vessels and capillaries

**Small artery:** Monocellular layer of endothelial cells

**Capillary:** endothelial cell, basal lamina, pericytes
Life time of endothelial cells:

months (lung, liver) to years (brain, muscle)

Slow repair and renewal of vascular wall

New vessel formation:

Embryo, growth
In uterus, during menstruation cycle
Wound repair
Mouse cornea: wounding induces angiogenesis, chemotactic response to angiogenic factors.
Angiogenesis:
Sprouting of cells from mature endothelial cells of the vessel wall

- secretion of proteases, resolution of basal lamina, migration towards chemotactic gradient, proliferation, tube formation
- VEGF is factor largely specific for endothelial cells, bFGF can also induce, not specific for EC

Diagram:
- red blood cell
- endothelial cell
- capillary lumen
- pseudopodial processes guide the development of the capillary sprout as it grows into the surrounding tissue
- capillary sprout hollows out to form tube
- stem
- tip cell
- this endothelial cell will generate a new capillary branch
capillaries sprouting in the retina of an embryonic mouse
capillary lumen opening up behind the tip cell (red dye injected)
Sprouting towards chemotactic gradient: VEGF

HIGH O₂ → LOW HIF

tissue cells

LOW O₂ → HIGH HIF

capillary sprout

secreted VEGF

small blood vessel

(A)

(B)
Hypoxia - HIF - VEGF
every cell must be within 50 to 100 µm of a capillary

HIF: hypoxia inducible factor
VEGF: vascular endothelial growth factor
**VEGF-gene:**
Regulated by HIF, HIF is continuously produced, ubiquitinylated, degraded in proteasome, therefore low concentration;

Ubiquitinylation dependent on Hippel-Lindau tumor suppressor (part of an E3 ubiquitin-ligase complex)

HIF1α is modified by a prolyl hydroxylase, then better interaction with vHL protein, high turnover; Hydroxylase is regulated by O2
FIH: factor inhibiting HIF (HIF asparaginyl hydroxylase)

![Mechanism of the 2-oxoglutarate-dependent-oxygenase FIH](image)

Figure 6 | Mechanism of the 2-oxoglutarate-dependent-oxygenase FIH. The figure shows an outline of the mechanism of the 2-oxoglutarate-dependent-oxygenase FIH (factor inhibiting HIF (hypoxia-inducible factor)), which is a HIF asparaginyl hydroxylase. Fe²⁺ is bound to the enzyme through the “facial triad” of two histidyl residues and one aspartyl residue, and the remaining three coordination sites are occupied by two to three labile water molecules. Studies with FIH and other 2-oxoglutarate-dependent oxygenases indicate that, in most cases, the sequential binding of 2-oxoglutarate and then the protein substrate (HIFα subunit) to the active site occurs. Binding of the latter displaces a water molecule from Fe²⁺, which leaves a vacant coordination site. Together, these processes allow the productive binding of triplet-state molecular oxygen. Subsequent oxidative decarboxylation of 2-oxoglutarate generates carbon dioxide, succinate and a ferryl (Fe⁵⁺=O) species. The latter is responsible for hydroxylating the β-position of Asn803 in the CAD (carboxy-terminal activation domain) of HIFα subunits. Release of the hydroxylated product probably precedes that of succinate. The point at which carbon dioxide leaves the active site is unclear. Coloured text is used in the figure to highlight the changes that occur at each step.
Factors and receptors

Endothelium-specific factors:
- VEGF family: 5 factors
- Angiopoietin family: 4 factors
- Ephrin family: at least 1 factor

Non EC-specific factors:
- bFGF
- PDGF
- TGF-β
VEGF/VEGFR family

VEGF/VEGFR:
VEGF-A: initiation of vasculogenesis and sprouting angiogenesis,
Immature vessels,
Vascular permeability factor,
Haploid insufficiency in k.o. mice,

PIGF: remodeling of adult vessels
VEGF-B: heart vascularization?
VEGF-C: lymphatic vessels
VEGF-D: lymphatic vessels?

VEGFR-2: growth and permeability
VEGFR-1: negative role?, decoy receptor,
synergism with VEGFR-2 in tumor angiogenesis
VEGFR-3: lymphatic vessels
Docking of proteins via SH2 (Src-homology) Domains
binds P-Tyr and neighbouring amino acids
Was originally described for the intracellular Tyr-Kinase c-Src
(Onkogen of Rous Sarcoma Virus)

3 important
Signaling cascades can be induced

- Ras
- PLC-γ
  (Phospholipase C-γ)
- PI3-Kinase
  (Phosphoinositol 3-Kinase)
Grb-2 Adaptor: SH2- Domain

SOS is a Ras-GEF (guanine nucleotide exchange factor)

Ras: GTP-binding Protein
(Onkogen detected in Rat Sarcoma)
Ras activates MAP-Kinase Pathway

1- MAPKKK
2- MAPKK
3- MAPK

MAPK: Mitogen-activated Kinase

(there are three MAP-Kinase cascades: MEK/ERK P38 JNK)
PKB, PDK:
(PDK: PI-dependent kinase)
Ser/Thr kinases

PI-3 Kinase Pathway and Survival

PHOSPHORYLATION OF BAD

INHIBITION OF APOPTOSIS

Figure 15–60. Molecular Biology of the Cell, 4th Edition.
PLC-γ signaling pathway

activated PLC-γ

PKC
phosphoryliert viele Substrate,
kann MAP Kinase Signalweg induzieren,
→ Genregulation

Ca++
Calmodulin/
Calcineurin
NFAT- Trankriptionsfaktor

Figure 15–36. Molecular Biology of the Cell, 4th Edition.
Ca$^{++}$ signaling pathway/gene regulation

the phosphatase calcineurin dephosphorylates NFAT

NFAT translocates into the nucleus

NFAT = transcription factor (nuclear factor activated T cell)
Differential signaling by tyr kinase receptors

EC “specific” factors/receptors:

<table>
<thead>
<tr>
<th>VEGFR1</th>
<th>VEGF-A, PLGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGFR2</td>
<td>VEGF-A</td>
</tr>
<tr>
<td>VEGFR3</td>
<td>VEGF-A</td>
</tr>
<tr>
<td>TIE1</td>
<td>VEGF-C</td>
</tr>
<tr>
<td>TIE2</td>
<td>?</td>
</tr>
</tbody>
</table>

- P38, src (vascular leakage?)
- TSAd (migration)
- PI-3 kinase (survival)
- PLC-γ
  - gene regulation
  - proliferation
  - vasculogenesis
  - angiogenesis

Sakurai et al. PNAS 2005
VEGF-A
VEGFR2
PLC-γ
Ca++
PKC/
calcineurin/
MAPK/NFAT
cooperative gene regulation

other signals

NAB2

EGF
HER1
Ras
MAPK
other signals
gene regulation

VEGFR2 vs. EGFR signaling
82 of the most strongly VEGF-regulated genes (over 5-fold) compared to EGF and IL-1 induction
About 60 genes reproducibly induced by VEGF over 3-fold

VEGF-induced genes overlap to a large degree with IL1-induced genes (50-60 %)

20 % of genes are preferentially induced by VEGF
Signaling by receptors of endothelial cells

Hofer E., Schweighofer B. Signaling transduction induced in endothelial cells by growth factor receptors involved in angiogenesis. Thrombosis ang haemostasis 2007
The VEGF cluster:
Part of the VEGF cluster genes is upregulated also by bFGF

Real time RT-PCR
### HLX induced genes (microarray)

<table>
<thead>
<tr>
<th>UniGene ID</th>
<th>Entrez Gene</th>
<th>Gene Symbol</th>
<th>Gene Title</th>
<th>4h</th>
<th>8h</th>
<th>16h</th>
<th>32h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hs.585457</td>
<td>219699</td>
<td><strong>UNC5B</strong></td>
<td>unc-5 homolog B (C. elegans)</td>
<td>1.41</td>
<td>2.23</td>
<td>5.71</td>
<td>7.14</td>
</tr>
<tr>
<td>Hs.432329</td>
<td>5361</td>
<td><strong>PLXNA1</strong></td>
<td>plexin A1</td>
<td>1.15</td>
<td>2.96</td>
<td>4.61</td>
<td>8.38</td>
</tr>
<tr>
<td>Hs.132781</td>
<td>9466</td>
<td><strong>IL27RA</strong></td>
<td>interleukin 27 receptor, alpha</td>
<td>0.91</td>
<td>1.86</td>
<td>4.18</td>
<td>5.53</td>
</tr>
<tr>
<td>Hs.9315</td>
<td>56944</td>
<td>OLFML3</td>
<td>olfactomedin-like 3 sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3G</td>
<td>1.09</td>
<td>1.72</td>
<td>4.62</td>
<td>23.68</td>
</tr>
<tr>
<td>Hs.59729</td>
<td>56920</td>
<td><strong>SEMA3G</strong></td>
<td></td>
<td>1.27</td>
<td>2.28</td>
<td>2.88</td>
<td>4.89</td>
</tr>
</tbody>
</table>

### Secreted protein

### Cytoplasmic (Signaling) protein

<table>
<thead>
<tr>
<th>UniGene ID</th>
<th>Entrez Gene</th>
<th>Gene Symbol</th>
<th>Gene Title</th>
<th>4h</th>
<th>8h</th>
<th>16h</th>
<th>32h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hs.417050</td>
<td>8900</td>
<td>CCNA1</td>
<td>cyclin A1</td>
<td>0.78</td>
<td>2.15</td>
<td>3.79</td>
<td>2.96</td>
</tr>
<tr>
<td>Hs.2128</td>
<td>1847</td>
<td>DUSP5</td>
<td>dual specificity phosphatase 5 serpin peptidase inhibitor, clade B (ovalbumin), member 1</td>
<td>1.07</td>
<td>2.38</td>
<td>3.76</td>
<td>3.54</td>
</tr>
<tr>
<td>Hs.381167</td>
<td>1992</td>
<td>SERPINB1</td>
<td></td>
<td>0.93</td>
<td>1.33</td>
<td>3.15</td>
<td>4.25</td>
</tr>
</tbody>
</table>

### Transcription factor

<table>
<thead>
<tr>
<th>UniGene ID</th>
<th>Entrez Gene</th>
<th>Gene Symbol</th>
<th>Gene Title</th>
<th>4h</th>
<th>8h</th>
<th>16h</th>
<th>32h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hs.360174</td>
<td>6591</td>
<td>SNAI2</td>
<td>snail homolog 2 (Drosophila)</td>
<td>1.58</td>
<td>3.87</td>
<td>4.51</td>
<td>6.91</td>
</tr>
<tr>
<td>Hs.525704</td>
<td>3725</td>
<td>JUN</td>
<td>Jun oncogene</td>
<td>1.48</td>
<td>1.51</td>
<td>4.06</td>
<td>3.14</td>
</tr>
<tr>
<td>Hs.250666</td>
<td>3280</td>
<td>HES1</td>
<td>hairy and enhancer of split 1, (Drosophila)</td>
<td>2.13</td>
<td>3.63</td>
<td>3.49</td>
<td>4.37</td>
</tr>
<tr>
<td>Hs.659681</td>
<td>54880</td>
<td>BCOR</td>
<td>BCL6 co-repressor</td>
<td>2.01</td>
<td>2.54</td>
<td>3.45</td>
<td>5.37</td>
</tr>
</tbody>
</table>
Guidance molecules in endothelial tip cell attraction and repulsion

Eichmann A, Curr Opin Neurobiol. 2005

Carmeliet P, Nature. 2005
Guidance cues regulating angiogenesis at capillary tip

Hypoxia

Endothelial tip cell: VEGFR-2, VEGFR-3, UNC5B, PDGFB, Dll4
Endothelial stalk cell: VEGFR-2, VEGFR-3; Robo4
Pericyte

Larrivée, B. et al, Circ Res 2009
Hypoxic gradient - regulates vessel guidance


Carmeliet, P. and Jain, R. Nature 2000
Angiopoietins und Tie Receptors:

- Ang1: remodeling and maturation
  - Quiescence and stability
  - Resistance to permeability,
  - Supports interaction with other cells and matrix,
  - Vessel size (VEGF number of vessels),
  - Repair of damaged vessels

- Ang2: natural antagonist,
  - Overexpression similar Ang-1 k.o. oder Tie-2 k.o.,
  - Destabilization signal for initiation of vascular remodeling
  - Either regression or increased VEGF sensitivity
  - Ang2 is induced in tumors

- Ang3: ?
- Ang4: ?

- Tie2: binds Ang1-4

- Tie1: ?
Ephrine und Eph-Rezeptors:

Largest family of growth factor receptors, Relevant for vascular system:
Ephrin B2/ Eph B4 : remodeling and maturation
Different for early arterial (Ephrin B2) and venous vessels (EphB4),
Hypothesis: role for fusion of arterial/ venous vessels
(b) Primary plexus → Juvenile vascular system → Mature vascular system

(c) Arterial endothelial cell interacts with Ephrin B2 and Eph B4 leading to venous endothelial cell
A growing sprout is composed of tip cell (green), stalk cells (red), and phalanx cells (blue). Each cell type is characterized by a unique molecular signature, resulting in a differential response to VEGF. Tip cells exhibit a migratory response to VEGF and show an upregulation of Dll4, VEGFR3, and VEGFR2. Stalk cells undergo proliferation and show upregulation of Notch1 and Jagged1. VEGF signaling in phalanx cells leads to a survival response mediated by increased levels of VE-cadherin and VEGFR1. VEGF, vascular endothelial growth factor.
Control of angiogenic sprouting

Four families of guidance cues and receptors

Adams, R. and Eichmann, A.
Cold Spring Harb Perspect Biol
2010
Fig. 1. Binding profile of semaphorins. A, members of the SEMA3 family bind to neuropilin with varying affinities and then complex with plexin. Whereas SEMA3A binds to NRP1-plexins A1 to A4, SEMA3F and SEMA3G bind to NRP2-plexins A1 to A4. SEMA3B, SEMA3C, and SEMA3D bind to both NRP1-plexin and NRP2-plexin.

B, SEMA3s signal through various plexins. All SEMA3s can signal through plexins A1 to A4 with the exception of SEMA3E, which binds directly to plexin D1 to mediate its effects. SEMA3E does not require neuropilins.
Arteriogenesis

Growth of arteries from pre-existing small capillaries

Macrophages
MCP-1
Network of lymphatic vessels (red) and capillaries (green):
Lymphatic vessels are larger, not supported by underlying mural cells
Growth of tumor vessels

1-Sprouting

2-Intussusceptive growth

3-Incorporation of BM-derived precursors

4-Cooption of existing vessels

5-Lymphangioigenesis
Rolle von VEGF und Ang2 bei der Tumorangiogenese, VEGF-Blockade vielversprechende Anti-Angiogenese Therapie

Concept 1: non-vascularized Tumor

Concept 2: many tumors “home in” onto vessels, occupy existing vessels, Vessel produces Ang2, first tumor regression, then VEGF production by tumor

Figure 3 Models of tumour angiogenesis. a, Model of avascular tumour initiation contrasted with b, tumour initiation involving host vessel co-option. An attempt is made to assign the indicated vascular growth factors to roles in the various indicated steps in tumour development, and to indicate their expression patterns.
Recruitment of capillaries by an implanted tumor

Figure 13.32a  *The Biology of Cancer* (© Garland Science 2007)
Chaotic organization of tumor-associated vasculature
Structure and function of tumor vessels:

Chaotic architecture and blood flow
Therefore hypoxic and acidic regions in tumor
Permeability strongly increased
fenestrae
enlarged junctions
No functional lymphatics inside the tumor
enlarged in surrounding,
increases metastasis
**Figure 1** Chaotic and mosaic vessels in tumours. 

- **a.** Cancer cell in the lining of a tumour vessel, referred to as a mosaic vessel. Cellular components of the vascular wall in a human colon carcinoma xenograft: cancer cells (green fluorescence, green fluorescent protein (GFP)), endothelial cells (red fluorescence, CD31/CD105 antibody detected with cyanine 5) and lectin fluorescence to mark perfused vessels. The width of the endothelial gap exposing cancer cells to the vessel lumen is about 20 µm.

(Adapted from ref. 22). 

- **b.** Quantification of mosaic vessels. In colon carcinoma ~15% of tumour vessels are mosaic in nature, and cancer cells occupy ~4% of the total vascular surface area. If each of these cells intravasate in 2 days, the tumour will shed about $10^6$ cells per day per gram of tumour. 

Mosaic vessels
Abnormal endothelium

(Adapted from ref. 22)
electron microscopy of the luminal surface of a blood vessel in a murine mammary tumour showing various abnormalities. Bar length represents 15 μm (from ref. 28). c, The abnormal endothelial cells that partition the lumen (arrowheads); d, multiple intercellular openings (arrows) of the order of 1–5 μm.
Tumor vessel is only partially overlaid by pericytes and SMC
The Rip-Tag model of islet tumor cell progression

Transgene: SV40 large and small T transcription driven by insulin promoter

Transcription in β-cells of islets of Langerhans

- < 5 weeks
  - 100% normal islets
- 5–7 weeks
  - ~50% hyperplastic islets
- 7–12 weeks
  - 10% angiogenic islets
- 12–14 weeks
  - 2–4% tumors
The angiogenic switch and recruitment of inflammatory cells

Figure 13.38b  *The Biology of Cancer* (© Garland Science 2007)
Angiogenesis-dependent diseases

excess
Cancer

Infantile hemangiomas

Autoimmune diseases, chronic inflammatory diseases:
Rheumatoid arthritis
Psoriasis

Age-related macular degeneration

Atherosclerosis

Deficiency:
Limb ischemia
Myocardial ischemia
Inhibition of angiogenesis

VEGF / VEGFR inhibitors

Erhard Hofer

Department of Vascular Biology and Thrombosis Research
Center for Biomolecular Medicine and Pharmacology
Medical University of Vienna
Figure 6 | Three general mechanisms of angiogenesis inhibitors currently approved by the FDA. Iressa blocks tumour expression of an angiogenic factor. Avastin blocks an angiogenic factor after its secretion from a tumour. Sutent blocks an endothelial cell receptor. VEGF, vascular endothelial growth factor.
<table>
<thead>
<tr>
<th>Ligand–receptor</th>
<th>Putative role in physiological angiogenesis</th>
<th>Implicated role in tumour angiogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF–VEGFR1 and VEGF–VEGFR2</td>
<td>Induce proliferation, sprouting and tube formation of endothelial cells; increase vascular permeability; suppress apoptosis for vessel stabilization; upregulate PDGFβ for mural cell recruitment</td>
<td>Recruitment of immune cells in suppressing anti-tumour immune response; recruitment of immune cells in promoting tumour growth and angiogenesis; promote sustained angiogenesis</td>
</tr>
<tr>
<td>VEGF-C–VEGFR3–NRP2</td>
<td>Lymphatic development</td>
<td>Lymphatic-mediated metastasis</td>
</tr>
<tr>
<td>Notch pathway</td>
<td>Negative feedback for VEGF-mediated vessel sprouting and participates in vessel fate determination (arterial compared with venous)</td>
<td>DLL4 expression upregulated in cancer and DLL4 inhibition in vivo result in non-productive vasculature and inhibits tumour growth</td>
</tr>
<tr>
<td>Ephrin-B2–EPHB4</td>
<td>Arterial compared with venous endothelial cell specialization determination; guides vessel branching</td>
<td>Expression and role in tumorigenesis may be cell type dependent</td>
</tr>
<tr>
<td>PDGF-BB–PDGF Rβ</td>
<td>Promotes migration, recruitment and proliferation of mural cells</td>
<td>Recruitment and survival of tumour- and tumour vasculature-associated stromal cells; mediates TGFβ-induced epithelial–mesenchymal transition</td>
</tr>
<tr>
<td>ANGPT1–TIE2</td>
<td>Facilitates EC–matrix and EC–mural cell interaction for vessel stabilization; suppresses EC apoptosis</td>
<td>Role in tumorigenesis may be dependent on cell type and ANGPT2 levels</td>
</tr>
<tr>
<td>ANGPT2–TIE2</td>
<td>Induces EC apoptosis in absence of VEGF; participates in lymphatic patterning</td>
<td>Recruitment of tumour-associated TEMs; promotes VEGF-mediated tumour neovascularization</td>
</tr>
<tr>
<td>TGFβ1–TGFβRII</td>
<td>Promotes ECM and protease production; promotes differentiation of fibroblasts to myofibroblasts and mesenchymal cells to mural cells</td>
<td>Promotes angiogenesis by inducing VEGF expression and pro-tumorigenic phenotypes of associated stroma cells</td>
</tr>
</tbody>
</table>

ANGPT, angiopoietin; DLL4, delta-like ligand 4; EC, endothelial cell; ECM, extracellular matrix; NRP2, Neurellin 2; PDGF, platelet-derived growth factor; TEMs, TIE2-expressing monocytes; TGF, transforming growth factor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.
<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Drug name or parent molecule</th>
<th>Mechanisms of action</th>
<th>Targeted cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FDA-approved anti-angiogenic therapeutics</strong></td>
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<tr>
<td>VEGF A-specific humanized antibody</td>
<td>Bevacizumab</td>
<td>Blocks antigen-receptor binding</td>
<td>Colon, lung, breast, glioblastoma and kidney</td>
</tr>
<tr>
<td>TKI targets VEGFR2, VEGFR3, Raf, PDGFR, KIT and RET</td>
<td>Sorafenib</td>
<td>Inhibits signalling of the VEGFR receptor tyrosine kinase</td>
<td>Kidney and liver</td>
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<tr>
<td>TKI targets VEGFR1–3, PDGFR, KIT and FLT3</td>
<td>Sunitinib</td>
<td>Inhibits signalling of the VEGFR receptor tyrosine kinase</td>
<td>Kidney and gastrointestinal stromal tumour</td>
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<td><strong>Endogenous angiogenic inhibitors</strong></td>
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<tr>
<td>Thrombospondin 1</td>
<td>NA</td>
<td>Binds CD36 and αvβ3 integrin in inhibiting several intracellular pathways</td>
<td>NA</td>
</tr>
<tr>
<td>Endostatin</td>
<td>Collagen XVIII</td>
<td>Binds to the α5β1 integrin receptor in inducing endothelial cell death</td>
<td>NA</td>
</tr>
<tr>
<td>Tumstatin</td>
<td>Collagen IV</td>
<td>Binds αvβ3 in attenuating endothelial cell proliferation</td>
<td>NA</td>
</tr>
<tr>
<td>Canstatin</td>
<td>Collagen IV</td>
<td>Binds αvβ3 or αvβ5 in inducing CD95-dependent apoptosis in endothelial cells</td>
<td>NA</td>
</tr>
<tr>
<td>Arrestin</td>
<td>Collagen IV</td>
<td>Binds to integrin α1β1 in inhibiting endothelial cell migration, proliferation and induces apoptosis</td>
<td>NA</td>
</tr>
</tbody>
</table>

FDA, US Food and Drug administration; NA, not applicable; PDGF, platelet-derived growth factor; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor.
Inhibition of tumor angiogenesis

1-Bevacizumab

2-VEGF-trap

3-Pegaptinib
   (Macular degeneration)

5- SU11248
   Bay43-9006

(Combination with 5-fluorouracil for colorectal cancer)

6- downstream Signals?
Inhibition of angiogenesis

inhibition of VEGF and VEGF signaling
mAb-based
  Anti-VEGF- Avastin
  anti-VEGFR
VEGF trap
tyrosine kinase inhibitors

Tumor:
normalization of tumor vessels
combination therapy

Macular degeneration

Prospects for vascular tumor targeting
and gene therapy
Avastin
(Bevacizumab)
Genentech Inc.

Effects on ATCC tumor cell lines
Kabbinavar et al., 2005

5-fluorouracil
leucovorin
Figure 1 | Tumour vessels are structurally and functionally abnormal.

a | In healthy tissue, a regularly patterned and functioning vasculature is formed (upper panel), with a normal vessel wall and endothelium (lower panel). b | In established tumours, the vasculature (upper panel), as well as the endothelium and vessel wall (lower panel) exhibit structural and functional abnormalities, leading to regions of severe hypoxia (represented by blue shading), BM, basement membrane; EC, endothelial cell; IFP, interstitial fluid pressure.
Figure 2 | Molecular changes leading to abnormal vessels. Abnormal tumour vessel formation can result from excess production of pro-angiogenic factors acting on endothelial cells (ECs) (a) or from defective pericyte recruitment or maturation (b). Recent data show that polarization of tumour-associated macrophages (TAMs) to an M2-like phenotype can also promote abnormal vessel formation (c). Partial inactivation of the oxygen-sensor enzyme prolyl hydroxylase domain-containing protein 2 (PHD2) at low oxygen tension normalizes vessels in a negative feedback manner (d). ANG, angiopletin; sFLT1, soluble FMS-like tyrosine kinase 1; NO, nitric oxide; PDGFβ, β-type platelet-derived growth factor receptor; PI GF, placenta growth factor; RGS5, regulator of G-protein signalling 5; VE-cadherin, vascular endothelial cadherin (a junctional molecule); VEGF, vascular endothelial growth factor.
Figure 3 | Potential targets and strategies for vascular normalization. 

(a) Blockade of vascular endothelial growth factor (VEGF) leads to a transient window of vessel normalization.

(b) A schematic illustrating the changes of the vessel wall and endothelium of normalized vessels. Genetic strategies lead to sustained vessel normalization by inducing endothelial cell (EC) quiescence or vessel maturation.

(c) Restored IFP improves flow and drug delivery.

Genetic strategies include:
- Hypoxia inducible factor (HIF)
- VEGF receptor tyrosine kinase (VEGFR)
- Prolyl hydroxylase domain-containing protein 2 (PHD2)
- Rgs5
- Ang2

Table: Genetic strategies lead to sustained vessel normalization by inducing endothelial cell (EC) quiescence or vessel maturation.

<table>
<thead>
<tr>
<th>EC quiescence</th>
<th>Vessel maturation</th>
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<tbody>
<tr>
<td>PHD2&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Mature PCs&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 mature PCs&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Tight EC junctions&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vessel density&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Vessel density&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benign tumour&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Immune response&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Invasion&lt;sup&gt;+&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Metastasis&lt;sup&gt;+&lt;/sup&gt;</td>
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</tbody>
</table>

Legend:
- PCs: Pericytes
- BM: Basement membrane
- IFP: Intercellular fluid pressure
- M1-TAM: M1-like phenotype tumour associated macrophage
- Ang2: Angiopoietin 2
- PHD2: Prolyl hydroxylase domain-containing protein 2
- Rgs5: Regulator of G-protein signalling

VEGF blockade:
- Window of normalization is transient
- During this window, the vessels exhibit many normalization features shared with genetic approaches
- After the window, excessive vessel pruning may increase hypoxia and inflammation, resulting in increased invasion or metastasis
Gentherapien:

rAdenoviren
rRetroviren

Targeting of viruses to tumors, tumor endothelium

Targeting of liposomes to tumors, tumor endothelium

Oncolytic viruses

BM progenitor cells home to tumor vasculature