Endothelial cell functions

- Transport of molecules over the vessel wall
- Initiation of the clotting system
- Selection of the white blood cells forming the leukocyte infiltrate
- New blood vessel formation (angiogenesis)


Neovasculature formation

- Vasculogenesis - formation of blood vessels from stem cells or by sprouting/intussusception during embryogenesis.
- Angiogenesis - formation of capillaries from pre-existing vasculature.

Angiogenesis is pivotal in tissue growth and development

Angiogenesis plays a role in:

- Normal physiology: wound healing, female reproductive cycle, inflammation, (embryogenesis).
- Pathological disorders: cancer, arthritis, diabetes retinopathy, cardiovascular diseases, endometriosis, ischemia, psoriasis, ulcers, decubitus, adiposity.

Angiogenesis stimulation

The molecular players

- Angiogenin
- Angiopoietins (-1, -2 and -3)
- Del-1
- Fibroblast growth factors: acidic (aFGF) and basic (bFGF)
- Follistatin
- Granulocyte colony-stimulating factor (G-CSF)
- Hepatocyte growth factor (HGF) / scatter factor (SF)
- Interleukin-8 (IL-8)
- Leptin
- Mekin
- Plecaryosin growth factor (IGF)
- Platelet-derived endothelial cell growth factor (PD-ECGF)
- Pleiotrophin
- Plasminogen activator inhibitor
- Transforming growth factor-alpha (TGF-alpha)
- Transforming growth factor-beta (TGF-beta)
- Vascular endothelial growth factor (VEGF)

Angiogenesis inhibition

The molecular players

- Angiostatin (plasminogen fragment)
- Antiangiogenic antithrombin III (aaATIII)
- Bacterial permeability increasing protein (BPI)
- Canstatin
- Cardiac-derived inhibitor (CDI)
- CD59 complement fragment
- Endostatin (collagen XVIII fragment)
- Fibrinogen fragment
- Fibrinogen beta
- Heparinase
- Heparinase
- Human chorionic gonadotropin (HCG)
- Interferon alpha/beta/gamma
- Interferon inducible protein (IP-10)
- Interleukin-12 (IL-12)
- Kringla 5 (plasminogen fragment)
- Metalloproteinase inhibitors (TIMPs)
- 2-Methoxyestradiol (2-ME)
- Pigment epithelial-derived factor (PEDF)
- Piasenin/fibroblast growth factor
- Plasminogen activator inhibitor
- Prolactin 16K fragment
- Prostatin-related protein
- Resistin
- Retinoids
- Tetrahydrocortisol-1
- Thrombospondin-1
- Transforming growth factor-beta
- Vasculostatin
- Vasostratin
Angiogenesis is regulated by stimulators and inhibitors

- In quiescent/normal tissue the angiogenic switch is in balance or off.
- Angiogenesis can be achieved by increase in stimulators or decrease of inhibitors.

The Balance Hypothesis for the Angiogenic Switch

Why target endothelium rather than tumor cells?

- Endothelial cells are the first to encounter the blood.
- Therapy independent of tumor type.
- Genetically stable; no mutation into drug resistant variants.
- Avalange of effect; a lot of tumor cells depend on one endothelial cell.

Angiogenesis inhibition
The molecular players

- AVE 6271 (Avastin)
- A23187 (Gleevec)
- AGS2344 (Apoptin)
- Angiostatin (deer milk)
- Batimastat (BB-95)
- BHE 940
- BMS-25 (CM Maz broadcast)
- C219
- CT-365 (human endothelin)
- CT-299
- Endostatin (EndoMax)
- Growth factor kinase inhibitor (GFKI)
- Hepatitis C virus-derived peptides
- Heparin cell antiproliferant (Hepcidin)
- Interferon
- Linomide
- Matrix metalloproteinase inhibitors (MMP)
- Methotrexate (anti-metabolite inhibitor)
- Minocycline
- NGF inhibitors
  - Anti-angiostatin (AP-1)
  - Anti-VEGF (Avastin)
  - Si-L(A)
  - SBS-23 (Chugai)
- α4β1 integrin (Integrin)

- AM 0.9 (1DCF6c/2)
- Pexilin (i) (i254)
- PEN-148 (P1)
- TNFα binding protein (TNB-1)
- TPX
- Endostatin (EndoMax)
- TNP-470 (Thalidomide)
- Tac-1 (Thalidomide)
- Tanespimycin
- Dictyostelium (DD)
- Thalidomide (D-Coid)
- Tumor necrosis factor inhibitors (TNF-inhibitors)
- TNAPIC
- Tumor necrosis factor
- UFT (G5)
- ZD451 (AGSTN inhibitor (Integrin))

Angiogenesis 1

- Early 1960s
- Search for sleeping drug for pregnant women
- Thalidomide was selected for low or absent toxicity

- A few years later the drug was identified as being teratogenic, causing stunted limb growth in humans
- 1994: Thalidomide is an angiogenesis inhibitor

Thalidomide inhibits angiogenesis

Discovery of Angiostatin

Some tumors develop metastasis after removal of the primary tumor.
A model: Lewis Lung Carcinoma in mice.

Discovery of angiostatin
The active factor was a cleavage fragment of plasminogen called angiostatin.


Angiogenesis inhibition does not induce resistance
A subsequent discovery was endostatin. Endostatin brings tumors in dormant state. Endostatin treatment is efficacious in multiple rounds of treatment.

Strategies for inhibition of angiogenesis
(1.) Inhibition of endothelial cell proliferation
- TNP-470, angiostatin, endostatin, anginex
- most successful as yet in animal experiments
- Phase I-II clinical testing

(2.) Inhibition of endothelial cell migration
- blockade of adhesion molecules e.g. αvβ3-integrin, interferon-α.
- successful in hemangiomas and giant cell tumors.
- Phase I-II clinical studies.

(3.) Inhibition of matrix metalloproteinases (MMP-inhibitors)
- batimastat, marimastat, AG3340.
- Phase III clinical testing.
- initially very promising, several compounds have been retracted from clinical testing.

(4.) Inhibition of endothelial signaltransduction
- SU5416 inhibitor of VEGF-receptor signaling, CAI inhibitor of motility and metastasis by blocking of Ca-mobilization.
- phase III clinical testing
Strategies for inhibition of angiogenesis

(5.) Attenuation of tumor blood flow (vascular targeting)
- targeting of toxins or isotopes to tumor vasculature specific antigens (TNF, αvβ3-integrin, CD44, endoglin/CD105, combretastatin).
- preclinical testing

Assays and models

- Endothelial cell growth (proliferation / death), migration, differentiation.
- In vitro angiogenesis assays - sprouting / tube formation.
- In vivo angiogenesis assays - chick CAM, semi-natural matrix plugs, dorsal skin fold chamber.
- Microvessel density and architecture.

In vitro angiogenesis assay

Chorio-allantoid membrane (CAM) assay
The dorsal skin fold chamber

Staining of microvessels

Analysis of microvessel density and vessel architecture

An applied and preclinical study

Development of new angiogenesis inhibitors

- Approach: ‘de novo’ design of cytokine-like peptide that inhibit angiogenesis.
- Preparation of a library of PF4 related 33-mer peptides, β-sheet structured, stable and water-soluble.
- 3 out of 35 different peptides had anti-angiogenic properties.
- One peptide, βpep-25, inhibited angiogenesis better than PF4.

Design of a new angiogenesis inhibitor for the treatment of cancer.

- De novo design of a peptide library.
- Well structured, stable and water-soluble.
- Screen for peptides in HTP (high through put) assay
- One peptide inhibited angiogenesis better than all other known compounds.
- This peptide is named anginex.
**Anginex induces apoptosis in endothelial cells**

The peptide library

Anginex inhibits EC proliferation

Anginex inhibits angiogenesis in vitro

Anginex inhibits angiogenesis in vivo

Angiogenesis inhibition in mice
Anginex targets tumor vessels and inhibits tumor growth.