

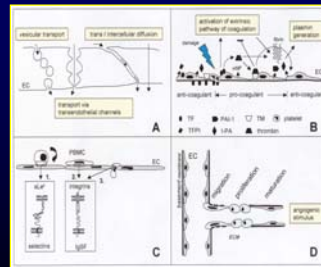
# Angiogenesis and tumor growth

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## 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)

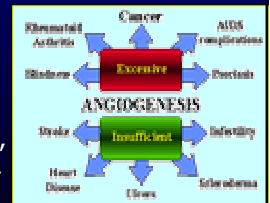
Griffioen & Molema, 2000, Pharmacol.Rev. 52:237.

## Neovasculature formation

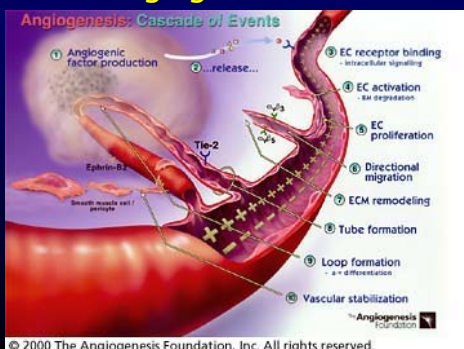
- Vasculogenesis - formation of blood vessels from stem cells or by sprouting/intussusception during embryogenesis.
- Angiogenesis - formation of capillaries from pre-existing vasculature.

## 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.



## The angiogenesis cascade



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## 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
- Midkine
- Placental growth factor (PlGF)
- Platelet-derived endothelial cell growth factor (PD-ECGF)
- Platelet-derived growth factor-BB (PDGF-BB)
- Pleiotrophin (PTN)
- Proliferin
- Transforming growth factor-alpha (TGF-alpha)
- Transforming growth factor-beta (TGF-beta)
- Tumor necrosis factor-alpha (TNF-alpha)
- Vascular endothelial growth factor (VEGF)

## Angiogenesis inhibition

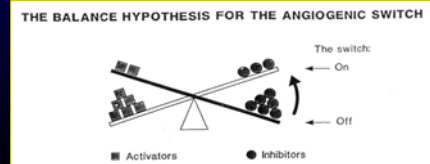
### The molecular players

**Angiostatin (plasminogen fragment)**  
**Antiangiogenic antithrombin III (aaATIII)**  
**Bactericidal permeability increasing protein (BPI)**  
 Canstatin  
 Cartilage-derived inhibitor (CDI)  
 CD59 complement fragment  
**Endostatin (collagen XVIII fragment)**  
 Fibronectin fragment  
 Gro-beta  
 Heparinases  
 Heparin hexasaccharide fragment  
 Human chorionic gonadotropin (hCG)  
**Interferon alpha/beta/gamma**  
 Interferon inducible protein (IP-10)  
**Interleukin-12 (IL-12)**  
 Kringle 5 (plasminogen fragment)  
 Metalloproteinase inhibitors (TIMPs)  
 2-Methoxyestradiol (2-ME)

Pigment epithelial-derived factor (PEDF)  
 Plasminogen activator inhibitor  
**Platelet factor-4 (PF4)**  
 Prolactin 16kD fragment  
 Proliferin-related protein  
 Restin  
 Retinoids  
 Tetrahydrocortisol-S  
**Thrombospondin-1**  
**Transforming growth factor-beta**  
 Tumistatin  
 Vasculostatin  
 Vasostatin (calreticulin fragment)

## 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.



Initial recognition of angiogenesis being therapeutically interesting began in the oncological arena.

Folkman et al. (1971) N.Engl.J.Med 285:1182

hypothesis:

- tumors are most vulnerable at the level of their blood supply
- angiogenesis inhibition would attenuate tumor growth

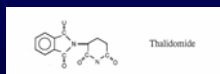
The discovery of the first specific angiogenesis inhibitors in the early '90s, resulted in a rapidly expanding research field.

## 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.
- Avalanche of effect; a lot of tumor cells depend on one endothelial cell.

## Angiogenesis 1

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



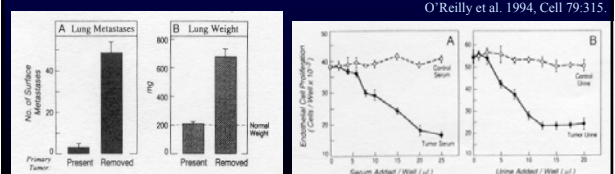
- A few years later the drug was identified as being teratogenic, causing stunted limb growth in humans
- 1994: Thalidomide is an angiogenesis inhibitor**

## Discovery of Angiostatin

Some tumors develop metastasis after removal of the primary tumor.

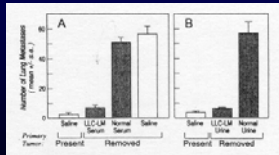
A model: Lewis Lung Carcinoma in mice.

O'Reilly et al. 1994, Cell 79:315.



## Discovery of angiostatin

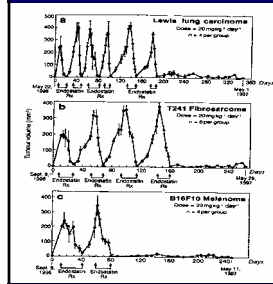
The active factor was a cleavage fragment of plasminogen called angiostatin.



O'Reilly et al. 1994, Cell 79:315.

## 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

## Strategies for inhibition of angiogenesis

### (2.) Inhibition of endothelial cell migration

- blockade of adhesion molecules e.g.  $\alpha v\beta 3$ -integrin, interferon- $\alpha$ .
- successful in hemangiomas and giant cell tumors.
- Phase I-II clinical studies.

## Strategies for inhibition of angiogenesis

### (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.

## Strategies for inhibition of angiogenesis

### (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,  $\alpha v\beta 3$ -integrin, CD44, endoglin/CD105, combretastatin).
- preclinical testing