TUMOR ANGIOGENESIS

Presented by : Fatemeh Zare Shahneh
Under Supervision of Dr. Behzad Baradaran

Tabriz University of Medical Science
Department of immunology
May 2013
Overview of vessel formation

- Angiogenesis and vasculogenesis
- Important factors and receptors
- VEGF receptor signaling
- Tumor angiogenesis
- Anti-angiogenesis therapies
Structure of vessels and capillaries

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

- loose connective tissue
- smooth muscle
- elastic lamina (elastin fibers)
- basal lamina
- endothelial lining

**Capillary:** endothelial cell, basal lamina, pericytes

- basal lamina
- nucleus of endothelial cell
- lumen of capillary
- lumen of artery

100 μm

10 μm

2 μm

1 μm
Vasculogenesis

Formation of vessels by differentiation of cells from angioblasts in the yolk sac of the embryo

Leads to formation of a primitive tubular network

undergo angiogenic remodeling to stable vascular system
At the time of Judah Folkman’s death 1,200,000 people were treated with anti-angiogenesis drugs.
The Hallmarks of Cancer

Tissue Invasion and Metastasis

Tumour acquired capabilities
- Evading apoptosis
- Limitless replicative potential
- Self-sufficiency in growth signals
- Insensitivity to anti-growth signals
- Sustained angiogenesis

Cell-autonomous capabilities

Environmental capacity
What is angiogenesis?

- The process of forming new blood vessels from pre-existing ones
- This process rarely occurs because the cells in the blood vessels rarely divide
  - During the creation of a child's circulatory system
  - In the menstrual cycle of females
  - In tissues during wound healing
- There are normally high amounts of inhibitor molecules and low amounts of activator molecules present in blood vessels which is why the cells do not divide often
Angiogenesis is a key step in new cell and tissue growth

- Tumors induce angiogenesis to obtain oxygen and nutrients
- 4 major steps of endothelial cells in angiogenesis
  1. Breaking through of basal lamina that envelopes existing blood vessels
  2. Migration towards source signal
  3. Proliferation
  4. Formation of tubes
- A number of pro-angiogenic growth factors, including VEGF, affect angiogenesis
<table>
<thead>
<tr>
<th>stimulus</th>
<th>examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental conditions</td>
<td>Low oxygen (hypoxia)</td>
</tr>
<tr>
<td>Increased activity of genes that promote cancer growth (oncogenes)</td>
<td>Ras, C-SRC, BCR-Abl</td>
</tr>
<tr>
<td>Decreased activity of genes that inhibit tumor growth (tumor suppressors)</td>
<td>P53, von Hippel-Lindau gene (VHL)</td>
</tr>
<tr>
<td>Activation of other cellular receptors/pathways in tumor cells</td>
<td>Epidermal growth factor receptor (EGFR, also called HER1 and ErbB1), HER2</td>
</tr>
</tbody>
</table>
Sprouting towards chemotactic gradient: VEGF

(A) HIGH O₂ → LOW HIF → tissue cells

(B) LOW O₂ → HIGH HIF → capillary sprout, secreted VEGF
Hypoxia - HIF - VEGF
every cell must be within 50 to 100 mm of a capillary

HIF: hypoxia inducible factor
VEGF: vascular endothelial growth factor
Angiogenesis - Basement Membrane Breakdown

Angiogenic Stimulus (VEGF)

Smooth Muscle Cells

Basement Membrane

Endothelium

Proteases
Angiogenesis - Endothelial Cell Migration

- VEGF
- Smooth Muscle Cells
- Basement Membrane
- Endothelium
- Nascent Vascular Sprouts
Angiogenesis - Endothelial Cell Proliferation

- VEGF
- Sprout Elongation
- Smooth Muscle Cells
- Basement Membrane
- Endothelium
Angiogenesis - Capillary Morphogenesis

VEGF

New Lumen Formation

Smooth Muscle Cells

Basement Membrane

Endothelium
Angiogenesis - Vascular Maturation

VEGF

Negative Feedback

Endothelial cell-cell junctions

Smooth Muscle Cells

Basement Membrane

Endothelium
• **Induction**
  ▫ Vasodilation and increased permeability of preexisting vessels
  ▫ Activated endothelial cells release proteases to degrade matrix
  ▫ Endothelial cells proliferate and migrate
  ▫ Proliferating cells adhere to one another

• **Resolution**
  ▫ Differentiation and maturation of blood vessels
a Dormant

b Perivascular detachment and vessel dilation

b Onset of angiogenic sprouting

d Continuous sprouting; new vessel formation and maturation; recruitment of perivascular cells

Tumour vasculature

Legend:

- Normal cell
- Blood vessel with pericyte
- Cancer cell
- Apoptosing, necrotic cell
- Dividing cell
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-b
VEGF/VEGFR family

VEGF/VEGFR:
- VEGF-A: initiation of vasculogenesis and sprouting angiogenesis
- Immature vessels
- Vascular permeability factor

- 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
Angiopoietins and Tie Receptors:

Ang1: remodeling and maturation
Quiescence and stability
Resistance to permeability
Repair of damaged vessels

Ang2: natural antagonist
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: ?
Ephrins and Eph-Receptors:

Largest family of growth factor receptors, Relevant for vascular system: Ephrin B2/ Eph B4 : remodeling and maturation
Growth of tumor vessels

1-Sprouting

2-Intussusceptive growth

3-Incorporation of BM-derived precursors

4-Cooption of existing vessels

5-Lymphangiogenesis
Healthy Vessel
• Well organized
• Define arteries and veins
• Non–permeable
• Low pressure
• Appropriate expression marker

Tumor Vessel
• Disorganized
• Undefined arteries and veins
• Highly permeable
• High pressure
• Low expression of markers
• Sluggish blood flow
Tumor vessel is only partially overlaid by pericytes and SMC
**Inhibitors:**
Thrombospondin-1
*The statins:*
Angiostatin
Endostatin
Canstatin
Tumstatin

**Activators**
VEGFs
FGFs
PDGFB
EGF
LPA
ANGIOGENESIS

Excessive

Rheumatoid Arthritis
Blindness
Cancer
AIDS complications
Psoriasis

Insufficient

Stroke
Heart Disease
Ulcers
Infertility
Scleroderma
Activators of Angiogenesis

Angiogenin
Angiopoietin-1
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 (PIGF)
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)
Vascular Endothelial Growth Factor (VEGF)

- Best characterized angiogenic factor
- Secretion of proteases, migration and proliferation specifically on EC
- Survival factor
- Inhibiting apoptosis
- High levels of VEGF expression are associated with a poor prognosis
- Hypoxic tumor cells express large amounts of VEGF
Basic Fibroblast Growth Factor (bFGF)

- bFGF acts on a variety of cell types, including smooth muscle cells, pericytes and fibroblasts, hypoxic tumor cells as well as EC
- Potent EC chemoattractant and mitogen
- Upregulates expression of both PA and PAI by EC
- Increasing the potential for invasion of the ECM by EC
Transforming Growth Factor Beta (TGF-b)

- Act on, a variety of cell populations, such as EC, periendothelial support cells, fibroblasts and tumor cells
- Stimulates the expression of protease inhibitors in excess of proteases
- Generate of plasmin (activated EC secrete PA)
- Limiting the amount of plasmin generated (increased PAI expression)
- Inhibit EC migration and proliferation
- Play a role in vessel maturation (inducing EC to revert to the quiescent phenotype and synthesis of a new basement membrane)
Platelet-derived Growth Factor (PDGF)

- Expressed by activated EC
- Chemoattractant and mitogen for support cells
- Recruitment to nascent capillaries
- Induce differentiation of fibroblasts to a pericyte-like phenotype
- Blocking PDGF signalling during angiogenesis disrupts pericyte recruitment, resulting in leaky, immature vessels
Angiopoietins

- Ang-1 and Ang-2
- Key players in the angiogenic balance between quiescence and activation of the endothelium
- The angiopoietins are ligands for the EC-specific receptor tyrosine kinase, Tie-2
- Ang-1 is necessary for the maturation of newly formed vessels
- Ang-2 is a natural antagonist for Ang-1
- Ang-2 binds to the Tie-2 receptor, but does not activate it (blocking the normal effects of Ang-1)
Inhibitors of Angiogenesis

Angiostatin (plasminogen fragment)
Cartilage-derived inhibitor (CDI)
CD59 complement fragment
Endostatin (collagen XVIII fragment)
Heparin hexasaccharide fragment
Interferon alpha/beta/gamma
Interleukin-12 (IL-12)
Kringle 5 (plasminogen fragment)
Metalloproteinase inhibitors (TIMPs)
2-Methoxyestradiol (2-ME)
Pigment epithelial-derived factor (PEDF)
Placental ribonuclease inhibitor
Plasminogen activator inhibitor
Platelet factor-4 (PF4)
Retinoids
Thrombospondin-1v Transforming growth factor-beta
Vasculostatin
Vasostatin (calreticulin fragment)
Antiangiogenesis Targets

• Neovasculature
  ▫ 1. Proteases that breakdown the ECM
  ▫ 2. Growth factors that stimulate endothelial cell proliferation
  ▫ 3. Integrins that allow adhesion of endothelial cells
  ▫ 4. Endothelial cell apoptosis

• Preexisting Vasculature
  ▫ 5. Various Vasculature Targeting Agents
Neovasculature: Inhibiting ECM Breakdown

- MMPs (metalloproteinases) are proteolytic enzymes that cleave the basement membrane
- Three domains: pro-peptide, catalytic domain, haemopexin-like c-terminal domain
MMP-Inhibiting Drugs

- **Marimastat (left)**
  - Binds to zinc ion
  - Very limited success due to toxicity factors and need for cytotoxic combination

- **Batimastat (right)**
  - 1,4 bidentate hydroxamic acid ligand that binds very tightly to the zinc ion in the catalytic (active) site
Neovasculature: Inhibiting cell growth

• Tumor cells are hypoxic, which induces HIF1 to signal over production of growth factors
• Target the growth factor
  ▫ VEGF, PDGF, bFGF, IL-8
• Target the growth factor receptor
Drugs Preventing Cell Proliferation

- **Suramin**—prevents bFGF and VEGF from binding to the active site of their receptors through competitive inhibition.

- **Avastin**—antibody that targets VEGF (binds to VEGF A to inhibit VEGFR1 and VEGFR2):
  - Enables normalization: reduced blood vessel permeability and interstitial pressure.

- **Angiostatin**—binds to HGF (hepatocyte growth factor); blocks endothelial cell surface ATP-synthase.
Integrins in the vasculature
Neovasculature: Inhibiting Cell Adhesion

• Integrin avb3
  ▫ Targets:
    • Antibodies against avb3 ligands
    • Integrin binding antagonists
    • siRNA
Integrin Antagonists

- Cilengitide
  - Avb3 antagonist
Neovasculature: Inducing apoptosis

• Target: Tumor Necrosis Factor--causes endothelial cell apoptosis in tumor cells

• Target: Down-regulating/blocking Bcl-2 interactions with pro-apoptotic proteins
  ▫ Endostatin
  ▫ Angiostatin
Neovasculature: Other Novel Agents

- **Celecoxib**: COX-2 (cyclooxygenase-2) Inhibitor
  - Common use: arthritis treatment (Celebrex)
  - Decrease vascular permeability
  - Decrease EC proliferation
  - Decrease EC migration
  - Decrease MMP production
  - Affect integrin pathway
Thalidomide

- Discontinued use: treat morning sickness
- FDA approved in 2006 for combination therapy with dexamethasone for treatment of multiple myeloma (cancer of plasma cells)
  - Block bFGF and VEGF
  - Inhibit COX-2
  - Interferes with Tumor Necrosis Factor-alpha
Potential for Antiangiogenesis

- Combination Therapy
  - Antiangiogenic + chemotherapeutic drug
  - Inhibit vascularization + cytotoxic agent
  - Avastin + PDGFR inhibitor
    - Avastin clinical dose = 5-10mg/kg
    - Dose limiting toxicity = 20mg/kg
    - Selection against Avastin
  - Thalidomide combinational therapy
# Known adverse effects of VEGF blockade

<table>
<thead>
<tr>
<th>Adverse effect</th>
<th>Possible mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding</td>
<td>Endothelial cell apoptosis; loss of integrity of the endothelial vessel lining</td>
</tr>
<tr>
<td>Thrombotic events</td>
<td>Increased platelet activation; exposure of the prothrombotic basement membrane to the circulating blood</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Inappropriate balance between arteries and veins; reduced levels of prostaglandin I-2 and nitric oxide</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>Podocyte dysfunction</td>
</tr>
<tr>
<td>Leukopenia, lymphopenia</td>
<td>Disturbance of hematopoiesis</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>Regression of capillaries around thyroid follicles</td>
</tr>
</tbody>
</table>
Avastin Loses Approval From F.D.A. to Treat Breast Cancer

By ANDREW POLLACK

The commissioner of the Food and Drug Administration on Friday revoked the approval of the drug Avastin as a treatment for breast cancer, ruling on an emotional issue that pitted the hopes of some desperate patients against the statistics of clinical trials.

The commissioner, Dr. Margaret A. Hamburg, said that clinical trials had shown that the drug was not helping breast cancer patients to live longer or to meaningfully control their tumors, but did expose them to potentially serious side effects like severe high blood pressure and hemorrhaging.

“Sometimes, despite the hopes of investigators, patients, industry and even the F.D.A. itself, the results of rigorous testing can be disappointing,” Dr. Hamburg told reporters Friday. “This is the case with Avastin when used for the treatment of metastatic breast cancer.”

Avastin will remain on the market as a treatment for other types of cancers, so doctors can use it off-label for breast cancer. But insurers might no longer pay for the drug, which would put it out of reach of many women because it costs about $88,000 a year.

Federal officials said on Friday that Medicare would still provide coverage for the drug’s use in breast cancer, though the government plans to “monitor the issue and evaluate coverage options.”

The drug’s manufacturer, Genentech, which fought long and hard to retain the approval, said it was “disappointed.” The decision could cost Genentech and its Swiss parent company, Roche, up to $1 billion in annual sales. Use of Avastin has already declined to 20 percent of American patients with newly diagnosed metastatic breast cancer, from 60 percent, according to Genentech.
In Vitro Assays

- Endothelial cells
  - HUVEC (human umbilical vein endothelial cell)
  - Artery, Vein
  - microvascular

- Endothelial cell proliferation/DNA synthesis
- Endothelial cell outgrowth (aortic ring, microvascular construct)
- Endothelial cell migration - chemotaxis (Boyden chamber)
- Endothelial cell tube formation
- Endothelial apoptosis
- Endothelial cell viability (trypan blue)
- Angiogenesis factor-transfected endothelial cell lines
- Magnetized microbeads on endothelial cells
Ex Vivo Assays

- CAM (chick chorioallantoic membrane)
- Vertical CAM with polymer gel

In Vivo Assays

- Transparent chamber - rabbit ear, hamster cheek, dorsal skin
- Matrix implants - polyvinyl foam implant, sponge implant
- Cornea micropocket – rabbit, rodent
- Vertebrate embryos (knock-outs)
- Ischemia
  - Ameroid constriction (heart) – pig, dog
  - Hindlimb ischemia – rabbit, rodent
- Tumor implants - mice
- Microvascular constructs
Regulation of vascular morphogenesis by Notch signaling

Cristina Roca and Ralf H. Adams
Vascular Development Laboratory, Cancer Research UK London Research Institute, London WC2A 3FX, United Kingdom

Modulation of VEGF signalling output by the Notch pathway

Arndt F. Siekmann, Laurence Covassin, and Nathan D. Lawson

Crosstalk Between Vascular Endothelial Growth Factor, Notch, and Transforming Growth Factor-2 in Vascular Morphogenesis
Matthew T. Holderfield and Christopher C. W. Hui

Notch signaling in vascular development and physiology

Thomas Gridley

TIE RECEPTORS: NEW MODULATORS OF ANGIOGENIC AND LYMPHANGIOGENIC RESPONSES

Nina Jones*, Kristina Iljin, Daniel J. Dumont* and Kari Alitalo*

MOLECULAR MECHANISMS OF TUBULOGENESIS

Brigid L. M. Hogan* and Peter A. Kolodziej*

Review Article
Tie receptors and their angiopoietin ligands are context-dependent regulators of vascular remodeling

Bjorn R. Olsen*

BLOOD VESSELS AND NERVES: COMMON SIGNALS, PATHWAYS AND DISEASES

The semaphorins: versatile regulators of tumour progression and tumour angiogenesis

Gera Neufeld and Ofra Kessler

Molecular regulation of angiogenesis and lymphangiogenesis

Ralf H. Adams* and Kari Alitalo*
Thanks so much for your attention