Cellular Composition of Tumors

**Carcinomas:** *Epithelial cell-derived.*

**Stroma:**
- Fibroblasts, Myofibroblasts, Fibrocytes
- Inflammatory/Immune Cells
  - Lymphocytes
  - T-Cells, Dendritic cells
  - NK cells
  - Neutrophils
  - Monocytes/Macrophages
  - Mast cells
- Vascular Cells
  - Endothelial cells
  - Endothelial Precursor Cells
  - Pericytes/Smooth Muscle Cells

**Sarcomas:** *Mesenchymal cell-derived*

**Stroma:** All the above!
Figure 13.14  The Biology of Cancer (© Garland Science 2007)
Moses Judah Folkman, 1933-2008
“Father of Angiogenesis”
ANGIOGENESIS

• Prominent during embryogenesis, development and growth
• Virtually absent in adults
• Prominent in ovulation, menstrual cycle and placental formation
• Critical in wound repair and granulation tissue formation
• Prominent in chronic inflammation and fibrosis
• Critical in solid tumor growth and development
Granulation Tissue
Tumour Angiogenesis
Figure 22–23. Molecular Biology of the Cell, 4th Edition.
ANGIOGENESIS

• Target Cells:
  – Endothelial Cells and Pericytes/Smooth Muscle Cells of Capillaries and Small Venules

• Processes Involved:
  – Disruption of Blood Vessel Continuity
  – Activation/De-Repression of Endothelial Cells
  – Degradation of Basement Membrane
  – Cell Migration
  – Cell Proliferation
  – Lumen Formation
  – Reformation of Basement Membrane
  – Cell Maturation
  – Capillary Loop Formation
This endothelial cell will generate a new capillary branch. Pseudopodial process guides the development of the capillary sprout as it grows into the surrounding connective tissue. Capillary sprout hollows out to form tube.

Figure 22–25. Molecular Biology of the Cell, 4th Edition.
ANGIOGENIC FACTORS

- **Fibroblast Growth Factors (FGFs)**
  - Two major forms:
    - FGF-1 (aFGF) and FGF-2 (bFGF)
    - M.Wt. 17kDa
    - Bind strongly to HEPARIN
    - No SECRETORY SIGNAL SEQUENCE
    - Found in BASEMENT MEMBRANES
  - **Questions**:
    - How are FGFs mobilized in Angiogenesis?
    - Are FGFs an autocrine control factor for endothelial cells?
Vascular Endothelial Growth Factor (VEGF)
Vascular Permeability Factor

- Dimeric, heparin binding protein
- Potent regulator of vascular permeability
  - 50,000 X more potent than histamine
- Secreted by a wide range of tumor cells
  - carcinomas, sarcomas, glioblastomas, monocytic leukemia cells
- Secreted by macrophages
- VEGF = VPF
  - One gene, eight alternatively spliced exons
  - In human, four molecular species (121, 165, 189, 206 aa)
  - In mouse, three molecular species (121, 165, 189 aa)
- Hypoxia is a major regulator of expression via activation of HIF1.
- Adenosine signaling is also major regulator of VEGF expression.
<table>
<thead>
<tr>
<th>LIGAND</th>
<th>RECEPTOR</th>
<th>FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF</td>
<td>VEGFR-2 (KDR, Flk-1)</td>
<td>Endothelial Mitogen</td>
</tr>
<tr>
<td></td>
<td>VEGFR-1 (Flt-1)</td>
<td></td>
</tr>
<tr>
<td>Angiopoietin-1</td>
<td>TIE-2</td>
<td>Recruitment of accessory cells (SMCs, pericytes)</td>
</tr>
<tr>
<td>Angiopoietin-2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(acts as antagonist)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>?</td>
<td>TIE-1</td>
<td>Endothelial cell-cell Interactions</td>
</tr>
<tr>
<td>Ephrin-B2 (Arterial)</td>
<td>Eph-B4 (Venous)</td>
<td>Differentiation of arterial vs venous microvasculature</td>
</tr>
<tr>
<td>Neuropilins</td>
<td>VEGF-R2</td>
<td>Patterning?</td>
</tr>
</tbody>
</table>
VEGF and VEGF RECEPTORS

• VEGF
  – Essential for both vasculogenesis and angiogenesis
  – Knockouts:
    • Die at 8.5-9.5 days in utero.
    • Delayed differentiation of endothelial cells.
    • Impairment of both angiogenesis and vasculogenesis.

• VEGF-R2 (Flk-1, KDR):
  – Restricted to endothelial cells and their embryonic precursors.
  – Knockouts:
    • Die in utero between 8.5 and 9.5 days.
    • Yolk sac blood islands do not form.
    • No organized blood vessels in embryo yolk sac.
    • Required for hemangioblast to endothelial cell differentiation

• VEGF-R1 (Flt-1):
  – Restricted to endothelial cells and their embryonic precursors.
  – Knockouts:
    • Die in utero at mid-somite stage (Day 9).
  – Essential for organization of embryonic vasculature (EC cell-cell or cell-matrix interaction).
  – Not essential for endothelial cell differentiation.
Endothelial Cell-Specific Tyrosine Kinase Receptors

• TIE-1:
  – Knockouts:
    • Form a primitive vasculature
    • Fail to develop structural integrity of vascular endothelial cells.
    • Develop edema and localized hemorrhage.
    • Die in utero at 9-10 days.
    • Ligand unknown

• TIE-2:
  – Knockouts:
    • Fail to recruit smooth muscle cells and pericytes precursors to primitive vasculature.
    • Poorly developed pericardium in heart.
    • Die in utero at 9-10 days.
  – Important in angiogenesis for vascular network formation.
  – Ligand is ANGIOPOIETIN-1 (Ang-1) – EC chemoattractant.
    • Knockouts of Ang-1 have phenotype similar to Tie-2 knockouts.
  – Mutation of TIE-2 in patients (Arg - Tryp):
    • Venous malformations.
    • Develop vein-like structures deficient in non-endothelial cells. Mainly lack smooth muscle cells and pericytes).
    • Angiopoietin-2 (Ang-2): Competitive inhibitor of Ang-1.
Endothelial Cell - Pericyte Interactions

Figure 22–24. Molecular Biology of the Cell, 4th Edition.
Endothelial Cell - Pericyte Interactions

- Recruitment of pericytes involves Tie-2, PDGF and Angiopoietin.

- Pericytes are associated with the microvasculature, lying outside the endothelium, but within the basement membrane
- Pericytes extend long processes from their cell body, making contact with several endothelial cells
- Interaction is suppressive, resulting in quiescence of endothelial cells
- Activation of latent TGF-β to active TGF-β occurs at sites of pericyte-endothelial cell contact
Figure 13.6d  The Biology of Cancer (© Garland Science 2007)
**ARTERIES vs VEINS**

*Role of Receptor Tyrosine Kinases of the EPHRIN-B / EphB Family*

Eph Family: Has at least 14 members

Ephrins: Ligands for Eph family proteins;
- At least 8 family members
- 2 classes: A and B

Ephrin-B ligands are trans-membrane proteins, that bind preferentially to receptors of the Eph-B sub-class. Ephrin-A ligands are GPI-linked membrane proteins.

These molecules are NOT SOLUBLE mediators. They are membrane-bound, and activate cognate receptor on partner cells by cell-cell interactions. Signaling is RECIPROCAL, i.e. forward and reverse.

**Ephrin-B2 is ARTERIAL. Eph-B4 is Venous**
Primary Capillary Plexus
(Formation blocked in VEGF/VEGFR2 KOs)

Angiogenic Remodeling
(Interdigitation, Differential Vessel Growth, Branching, Sprouting, etc.)

Maturing Vascular Network
(Remodelling Perturbed in Ephrin-B2 & Ang1/Tie2 KOs)

Arterial Endothelial Cells (ephrin-B2*)
Venous Endothelial Cells (EphB4*)

site of ephrinB2/EphB4 interaction
THE BALANCE HYPOTHESIS FOR THE ANGIOGENIC SWITCH

Activators:
- VEGF
- aFGF
- bFGF
- IL-8

Inhibitors:
- Thrombospondin
- TGFβ
- Angiostatin
- Endostatin
- TIMP
- IL-12
- γIP10
Model for Tumor Suppressor Gene Control of Angiogenesis

Sup\(^+\)  

Sup\(^-\)
Tumor Suppressor Gene

Inhibitor of Angiogenesis

Inhibitor of Angiogenesis

Inhibitor of Angiogenesis

Inhibitor of Angiogenesis
**Thrombospondin**

**Fig. 1.** Cartoon of the 180-kDa subunit of the homotrimeric human TSP molecule. Binding sites for individual monoclonal antibodies raised to TSP are indicated above the diagram; specific domains are below. The portion of the TSP molecule containing homology to the N-terminal amino acids of gp140 and shared antigenic sites is indicated by the bar. Numbers 1 and 5 mark the positions of the peptides used to make TSP anti-peptide serum. EGF, epidermal growth factor.
Li-Fraumeni Fibroblasts and Regulation of Angiogenesis by p53

- LF patients show a greatly increased susceptibility to tumor development.
- Patients have 1 defective p53 allele and 1 wild-type allele.
- LF fibroblasts in culture lose remaining wt p53 allele over several generations.

<table>
<thead>
<tr>
<th>EARLY PASSAGE</th>
<th>LATE PASSAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>p53</td>
</tr>
<tr>
<td>Low</td>
<td>Angiogenic Activity</td>
</tr>
<tr>
<td>High</td>
<td>TSP-1</td>
</tr>
<tr>
<td>Low</td>
<td>VEGF</td>
</tr>
</tbody>
</table>

Transfected p53 T^0^-sensitive mutant: WT at 32°C, Mutant at 38°C.

<table>
<thead>
<tr>
<th></th>
<th>TSP-1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>38°C.</td>
<td>Low</td>
<td></td>
</tr>
<tr>
<td>32°C.</td>
<td>High</td>
<td></td>
</tr>
</tbody>
</table>
Angiogenesis Inhibitors:

- **ANGIOSTATIN:**
  - Fragment of Plasminogen.
  - Produced by Primary Tumor Mass
  - Present in the Circulation
  - Suppresses Growth of Metastases
  - Removal of Primary Tumor de-suppresses Growth of metastases

- **ENDOSTATIN:**
  - Fragment of Type XVIII collagen
<table>
<thead>
<tr>
<th>Name</th>
<th>Status</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Endogenous inhibitors of angiogenesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endostatin</td>
<td>in clinical trial</td>
<td>scattered responses</td>
</tr>
<tr>
<td>Interferons-α and -β</td>
<td>effective in treating hemangioblastomas</td>
<td>Kaposi’s sarcomas; limited efficacy against most other types of tumors</td>
</tr>
<tr>
<td>B. Agents that block VEGF and VEGF-R signaling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avastin anti-VEGF MoAb</td>
<td>in clinical trial</td>
<td>delayed progression 1–3 months in lung, 3–4 months in colon</td>
</tr>
<tr>
<td>SU5416 inhibitor of VEGF-R2 (Flk-1)</td>
<td>trial abandoned</td>
<td>severe vascular toxicities</td>
</tr>
<tr>
<td>ZD6474 inhibitor of VEGF-R2</td>
<td>under clinical test</td>
<td></td>
</tr>
<tr>
<td>CPS47, 632 inhibitor of VEGF-R2</td>
<td>in trial</td>
<td></td>
</tr>
<tr>
<td>C. Miscellaneous other drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalidomide</td>
<td>in trial</td>
<td>inhibits bFGF- and VEGF-dependent angiogenesis</td>
</tr>
<tr>
<td>Squalamine sterol from shark liver</td>
<td>in trial</td>
<td>strong anti-angiogenic activity</td>
</tr>
<tr>
<td>Celecoxib anti-inflammatory drug</td>
<td>in trial</td>
<td>multiple anti-neoplastic effects</td>
</tr>
<tr>
<td>ZD6126</td>
<td>in trial</td>
<td>antagonist of tubulin in endothelial cell</td>
</tr>
<tr>
<td>Fumagillin and TNP-470</td>
<td>in trial; slowed tumor growth</td>
<td>cytoskeleton</td>
</tr>
<tr>
<td></td>
<td></td>
<td>antagonist of methionine aminopeptidase in endothelial cells</td>
</tr>
<tr>
<td>D. Inhibitors of ECM breakdown—MMP inhibitors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marimastat</td>
<td>in clinical trial</td>
<td>no delay of tumor progression</td>
</tr>
<tr>
<td>Prinomastat</td>
<td>in clinical trial</td>
<td>no slowing of tumor progression</td>
</tr>
<tr>
<td>BMS275291</td>
<td>in clinical trial</td>
<td></td>
</tr>
<tr>
<td>BAY12-9566</td>
<td>in clinical trial</td>
<td></td>
</tr>
<tr>
<td>Neovastat (shark cartilage MMPi)</td>
<td>in clinical trial</td>
<td></td>
</tr>
</tbody>
</table>

Table 13.4 *The Biology of Cancer* (© Garland Science 2007)
OXYGEN AND ANGIOGENESIS

• Oxygen Tension & Erythropoiesis
  ▪ Low ppO₂ stimulates erythropoiesis
  ▪ Low ppO₂ stimulates *erythropoietin production in kidney*

• Oxygen Tension & Angiogenesis:
  ▪ High ppO₂ inhibits angiogenesis
  ▪ Low ppO₂ stimulates angiogenesis

Relationship to ALTITUDE PHYSIOLOGY:
  – Increased capillary density in muscle at high altitudes. Effect only up to certain elevation.
  – FAILURE of WOUND REPAIR above 15,000ft.
    » (Himalayas Expeditions)

Hypoxia Inducible Factor (HIF1)
Hypoxia Response Element (HRE)
Hypoxia Inducible Factor (HIF)

Dimer of HIF1-α and HIF1-β (ARNT)

Unstable under Normoxia
Stabilized under hypoxia

Constitutively Expressed
Stable

Mammalian bHLH-PAS proteins

Class I
- AHR
- CLOCK
- HIF-1α
- HIF-2α (EPAS1/HLF/HRF/MOP2)
- HIF-3α
- NPAS1 (MOP5)
- NPAS2 (MOP4)
- SIM1
- SIM2

Class II
- ARNT (HIF-1β)
- ARNT2
- ARNT3 (BMAL1/MOP3)
Hypoxia Inducible Genes

- Erythropoietin (Epo)
- VEGFs
- Glycolytic Enzymes
  - Lactate Dehydrogenase (LDH)
  - Pyruvate Kinase M (PKMP)
  - Enolase 1
  - Phosphoglycerate Kinase 1 (PGK1)
  - Aldolase A (ALDA)
  - Phosphofructokinase (PFKL)
  - Glucose Transporter 1 (GLUT-1)
- Inducible Nitric Oxide Synthase (iNOS)
- Heme Oxygenase
- Ferritin Receptor and Ferritin
- Tyrosine Hydroxylase
O₂ Sensors

- HIF-1 Prolyl-Hydroxylases 1-3
- Factor Inhibiting HIF-1 (FIH1, Asn-hydroxylase)
Hypoxia

HIF-1α Phosphorylated, stabilized

Nucleus

HIF-1α ARNT
HRE

Transcription factors
AP-1
ETS
CREB
RNA stabilization-HuR

Oncogene signalling
SRC
RAS
Protein kinase C
PI3K

VEGF (Angiogenesis)
GLUT1 (Glucose transport)
LDH-A (Glycolytic pathway)
NOS
EPO (Erythropoiesis)

Hypoxia-regulated genes

Nature Reviews | Cancer
Macrophage in Wound - EM
LYMPHOCYTES

Growth Regulators

Lymphokines

Antigen Processing

EPITHELIUM

Angiogenic factors

Permeability factors

Chemo-attractants

MACROPHAGE

Tissue Factor

Chemo-attractants

Growth Factors

ENDOTHELIUM

PLATELET

FIBROBLASTS
Role of Hypoxia in Angiogenesis
Figure 13.27a The Biology of Cancer (© Garland Science 2007)
Figure 13.27b  *The Biology of Cancer* (© Garland Science 2007)
Adenosine (μM)

Adenosine

HIF-1α, HIF-1β

Glycolysis

Survival / apoptosis

VEGF
Ang2
NOS
PDGF-B

HRE

Angiogenesis

pH

Distance (μm)

pO₂ (mm Hg)

> 100 μm

O₂

2/26/2015
Adenosine and Adenosine Receptors

- Unstable and ubiquitous purine nucleoside produced by breakdown of ATP.
- Released in response to stressful stimuli. Binds receptors to modulate, and protect cells from the harmful consequences of stress.
- Adenosine receptors (A<sub>1</sub>R, A<sub>2a</sub>R, A<sub>2b</sub>R, A<sub>3</sub>R) are expressed on many cell types: they bind a wide spectrum of natural and synthetic agonists and antagonists.
- Activation of ARs on immune cells generally exhibits anti-inflammatory effects:
  - Inhibition of phagocytosis.
  - Decreased expression of inflammatory cytokines and chemokines, ROS and NO.
  - Increased expression of anti-inflammatory cytokines.
Reciprocal Regulation of TNFα and VEGF in Macrophages In Response to LPS and Adenosine Receptor (AR) Agonists

Adenosine synergizes with TLR signaling to switch macrophages from an “inflammatory” to an “angiogenic” phenotype.
Regulation of HIF-1α Expression by LPS and NECA

Q-RT-PCR Analysis of HIF-1α mRNA

Western Blot Analysis of HIF-1α

C  L  N  L/N  H

HIF-1α

NPM
Regulation of Adenosine A\textsubscript{2A} Receptor Expression in Macrophages

- Induction is NF-\kappa B and STAT1 dependent
- Agonists of TLR2, 7 and 9 also induce A\textsubscript{2A}R expression
Ischemic Zone

Adenosine

TLR Agonists

Inflammatory Macrophage

Angiogenic Macrophage

VEGF
IL10
SK-1

TNFα
IL12
MIP1α
iNOS

TNFα
IL12
MIP1α
iNOS