Heterotypy and Angiogenesis
Tumors are perpetual wounds

1. Normally stroma and epithelia converse at a distance.

2. Juxtaposition of stroma and epithelia is indicative of tissue damage.

4. Activate strategies to fight infection and restore normal architecture.

5. Important strategy for wound-healing is to restore blood supply.
Program of wound healing

Figure 13-14 The Biology of Cancer (© Garland Science 2007)
The vasculature:

Capillaries supply every cell in the body with oxygen and nutrients.

One pound of fat contains one mile of capillary tubing.

Bone-marrow 6 billion cells divide per hour (whole marrow replaced every 5 days).

In contrast endothelium-replacement 3-5 years (10 years in the retina).

However, in proximity to tumors endothelial cells turn over at rates similar to bone marrow suggesting tumors regulate their own blood supply.
Wounding and tumors have leaky vasculature that provides a “provisional matrix”

A. Tumor leakiness (red dextran dye)
B. Leakage thrombin and fibrinogen into parenchyma-fibrin deposition
C. Fibrin bundles form ECM - support tumor growth, movement but also revascularization
Myofibroblasts are characteristic of wound tissue

Myofibroblasts – identified by smooth muscle actin (SMA) red infiltrate wound at 3 days.

Chronically inflamed tissue-
cirrhotic liver-
myofibroblasts (brown)

Hepatocellular carcinoma – stained for SMA (very similar)
Normal stroma becomes displaced by “desmoplastic” stroma in advanced carcinomas

Left: normal prostate (smooth muscle – pink).
Right: desmoplastic stroma rich in collagen I (purple) – myofibroblasts and fibroblasts rare.

Figure 13-17 The Biology of Cancer (© Garland Science 2007)
Stromal cells contribute to tumorigenesis

Changes in gene expression followed after addition of serum to serum-starved fibroblasts.

Core serum response (CSR) genes defined as genes that changed early and stably and were not associated with cell-cycle. CSR signatures in tumors indicative of CAF activity. The higher the activity the worse the prognosis.
Intensity of angiogenesis increases once cells breach basement membrane

prostate cancer (PIN; *in situ*)

human breast cancer (*in situ*)

invasive prostate cancer

invasive human breast cancer

Figure 13-41a The Biology of Cancer (© Garland Science 2007)

Figure 13-41b The Biology of Cancer (© Garland Science 2007)
Prostatic tumors develop in mice with genetically altered fibroblasts

Selective KO of TGF-β type II receptor in fibroblasts in tissues releases fibroblasts from TGF-β growth inhibition. Resulting hyperplasia of stroma and epithelium suggests that stroma is releasing growth signals to epithelium. Production of Hepatocyte Growth Factor (HGF), a potent epithelial growth factor, by stroma is increased by 3x. Mice eventually develop gastric carcinomas. Stroma controls epithelial growth.

Figure 13-19 The Biology of Cancer (© Garland Science 2007)
Admixed normal fibroblasts promote tumor growth

Human mammary epithelial cells transformed by SV40, hTERT and activated ras form tumors with long lag time. Addition of matrigel accelerates tumor development. Normal mammary tissue fibroblasts stimulate tumor development. Fibroblast recruitment important rate-limiting step.
Do carcinoma-associated fibroblasts promote tumor growth more efficiently?


![Graph showing tumor weight comparison](image)
Carcinoma-associated fibroblasts (CAF) initiate angiogenesis by recruiting endothelial cells.

MCF-7 tumors form highly vascularised tumors if admixed with CAF (myofibroblasts).

CAF but not normal fibroblasts recruit GFP-labeled endothelial precursor cells (EPC) from marrow.

Recruitment reduced by blocking SDF-1 (chemokine).

VEGF secreted by myofibroblasts promotes differentiation EPC into endothelial cells.

Angiogenesis is the rate limiting step in tumor formation.

Tumor-stimulating properties of CAF in large part due to stimulation angiogenesis.
Stromal cells recruit macrophages which stimulate angiogenesis

Tumor associated macrophages (red) stained for Hypoxia Induced Factor 2 alpha (HIF2α) in human breast cancer.

Some breast cancers produce VEGF (left)

In others VEGF produced by macrophages within stroma (right).
Macrophages correlate with angiogenesis

Non-small cell lung cancer – density of TAMs v. density microvessels.

Macrophages in human colorectal carcinoma produce MMP-9 (brown) a key enzyme in angiogenesis and invasion by releasing VEGF and other angiogenic factors from sequestration in the ECM.
Role of macrophages in tumorigenesis

- CARCINOMA CELLS
  - proliferation
  - chemotactic factors (MCP-1, CSF-1, PDGF)
  - circulating monocytes
  - tumor-associated monocytes
  - CARCINOMA CELLS

- EMT
  - invasiveness, metastasis

- tumor-associated macrophages (TAMs)
  - matrix metalloproteinases (e.g., MMP-9)
  - angiogenic factors (VEGF, IL-8)

- EGF
  - cleave IGFBPs
  - liberate IGFs
  - liberate mitogenic factors

- disrupt ECM
  - liberate angiogenic factors
  - create space for new vessels

- angiogenesis

Figure 13-26 The Biology of Cancer (© Garland Science 2007)
Hypoxia and angiogenesis

Left: Capillaries (green). Hypoxic (red).

Below: Tumor cells become necrotic if too far from capillary identified by endothelial marker CD31 (brown).

Limitations of diffusion in conveying oxygen and nutrients – perivascular cuffs.

The more active the tumor the more blood vessels it needs to grow and survive.
Hypoxia and necrosis in squamous cell tumor

Blood vessels (blue).
Areas of moderate hypoxia carbonic anhydrase (red).
Extreme hypoxia (green).
N: necrosis
VEGF

VEGF and notch cooperate to function as a “branching pattern generator”

VEGFR2 stimulates tip cell induction and filopodia
VEGFR3 embryonic – lymphatics . Tip cells re-express VEGFR3 .
VEGFR1 suppresses sprouting and vascularization

Feedback-loop between VEGF and Notch
Sequence of events that define angiogenesis

Myofibroblasts in tumor associated stroma release SDF-1 which recruits endothelial precursors.

VEGF assists development of ECP into mature endothelial cells.

Production of VEGF governed by availability of oxygen through VHL – dependent HIF-1 accumulation and transcription of VEGF.

VEGF produced by tumor cells, macrophages and myofibroblasts.
VEGF stimulates capillary formation

VEGF acts through tyrosine kinase receptors VEGF-RI and VEGF-RII to induce proliferation of endothelial cells.

Endothelial cells join thro’ tight junctions (arrows) to form capillaries.
The classical angiogenic switch
Blood vessel co-option precedes angiogenesis in astrocytoma progression

Low grade astrocytoma

Grade III astrocytomas progress to Grade IV they induce angiogenesis
Endothelial cells  Pericytes

Vascular basement membrane  Blood vessel/capillary

Extraction of basement-membrane collagen

MMPs, elastase or cathepsins (basement-membrane-degrading enzymes)

Anti-angiogenic activity

Baseline-membrane collagen-derived endogenous inhibitors

Integrin binding

- Arrestin (26 kDa): α1β1
- Canstatin (24 kDa): αvβ3, α3β1
- Tumstatin (28 kDa): αvβ3, α6β1
- Endostatin (20 kDa): α5β1, αvβ3

Type IV collagen

Type XVIII collagen

No anti-angiogenic activity in this form

Nature Reviews | Cancer
Endothelial cells also form lymph ducts

Same embryonic endothelial cell population forms lymph ducts. Drain fluid from interstices between cells and return to circulation. Serve to inform immune cells of local antigens by draining to lymph nodes.

Capillaries (green)
Lymphatics (orange)

VEGFA and B stimulate blood
VEGFC and D stimulate lymphatics
Recruitment of capillaries by implanted tumor

Left: Growth of subcutaneous human colorectal cancer cells over 20 days in mice

Right: Vascularization can be suppressed by ZD6474 (inhibitor of VEGF receptor) - bottom.
Tumor blood vessels are leaky

Above: normal vessel
Below: tumor vessel

Pericytes only loosely attached - walls of capillaries in tumors 10 leakier.

Leakiness also due to overproduction VEGF and imbalance between angiopoietin I and II
Tumor vasculature is disorganized as well as leaky.

Resin impregnated blood vessels

endothelial cells overlap and separate

normal tissue
tumor
Tumor vessels are chaotic

- EC’s lack cobblestone and may be multilayered

- Arterio-venous identity ill-defined- shunting

- Basement membrane irregular –fewer mural cells

- Uneven delivery of chemotherapeutics and reduced oxygenation decrease therapeutic efficacy

- Tumors may co-opt alternative vascular growth requirements by reducing dependence on VEGF.
Anti-angiogenesis versus vessel normalization as a therapeutic stratagem

Cell 146: 873-887 2011
Role of non tumor cells in tumor vascularization:

• Precursors of TIE2-expressing monocytes (TEM’s) release growth factors. Tumor EC’s express ANG2 activating TEMs to stimulate angiogenesis. Tumor associated macrophages release PIGF- vessel de-organization

• Mast cells secrete proteases that release pro-angiogenic factors from ECM.

• CXCR4 (SDF-1 R) +ve bone marrow derived cells are retained by tumor derived SDF-1 and release angiogenic factors into the tumor

• Myeloid cells believed to account for resistance to VEGF R inhibitors.

• Cancer associated fibroblasts originate from local mesenchyme or recruited from bone marrow

• Recruit endothelial progenitor cells and release pro-angiogenic factors
Tripping the angiogenic switch: insight from the Rip-Tag mouse

Most tumors initially lack the ability to attract blood vessels. The blood supply therefore limits growth of the primary tumor. Hypoxia is not sufficient to promote vascularization.

Rip-Tag transgenic mouse: insulin gene-dependent SV40 large T and small T antigen. Restricts expression of tumor to islets of Langherhans where tumor progression easily followed. Until tumors develop a blood supply hypoxia triggers p53-dependent apoptosis. Islets “suddenly” acquire the capacity to develop a blood supply and tumors start to grow.

Figure 13-37 The Biology of Cancer (© Garland Science 2007)
Angiogenic switch and recruitment of inflammatory cells

N.B. MMP9 not VEGF is limiting
Angiogenic switch is effected by recruitment and activation of macrophages
Different tumors depend on different angiogenic factors

<table>
<thead>
<tr>
<th>Name</th>
<th>Mol. wt. (kD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular endothelial GF (VEGF)</td>
<td>40–45</td>
</tr>
<tr>
<td>Basic fibroblast growth factor (bFGF)</td>
<td>18</td>
</tr>
<tr>
<td>Acidic fibroblast growth factor (aFGF)</td>
<td>16.4</td>
</tr>
<tr>
<td>Angiogenin</td>
<td>14.1</td>
</tr>
<tr>
<td>Transforming growth factor-α (TGF-α)</td>
<td>5.5</td>
</tr>
<tr>
<td>Transforming growth factor-β (TGF-β)</td>
<td>25</td>
</tr>
<tr>
<td>Tumor necrosis factor-α (TNF-α)</td>
<td>17</td>
</tr>
<tr>
<td>Platelet-derived growth factor (PDGF)</td>
<td>45</td>
</tr>
<tr>
<td>Granulocyte-colony–stimulating factor</td>
<td>17</td>
</tr>
<tr>
<td>Placental growth factor</td>
<td>25</td>
</tr>
<tr>
<td>Interleukin-8 (IL-8)</td>
<td>40</td>
</tr>
<tr>
<td>Hepatocyte growth factor (HGF)</td>
<td>92</td>
</tr>
<tr>
<td>Proliferin</td>
<td>35</td>
</tr>
<tr>
<td>Angiopoietin</td>
<td>70</td>
</tr>
<tr>
<td>Leptin</td>
<td>16</td>
</tr>
</tbody>
</table>
Clinical outcome and the intensity of angiogenesis

Breast cancer patients with low microvessel density exhibit better survival. VEGF production correlates negatively with survival.
Tumor cells vary greatly in angiogenic potential

Liposarcoma cell line—subcloned and implanted into nude mice.
Because tumors are very heterogeneous in angiogenic potential the weakly angiogenic cells are supported by their highly angiogenic neighbors.
Angiogenesis is suppressed by physiological inhibitors

In wound healing important to shut down capillaries once reach density to support normal tissue (for example by suppressing HIF).

Components of ECM are ALSO important antagonists of angiogenesis.

### Table 13.3 Endogenous inhibitors of angiogenesis

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Derived from extracellular matrix</strong></td>
<td></td>
</tr>
<tr>
<td>Arresten</td>
<td>fragment of type IV collagen α₁ chain of vascular basement membrane</td>
</tr>
<tr>
<td>Canstatin</td>
<td>fragment of type IV collagen α₂ chain of vascular basement membrane</td>
</tr>
<tr>
<td>EFC-XV</td>
<td>fragment of type XV collagen</td>
</tr>
<tr>
<td>Endorepellin</td>
<td>fragment of perlecan</td>
</tr>
<tr>
<td>Endostatin</td>
<td>fragment of collagen type XVIII</td>
</tr>
<tr>
<td>Anastellin</td>
<td>fragment of fibronectin</td>
</tr>
<tr>
<td>Fibulin</td>
<td>fragment of basement membrane protein</td>
</tr>
<tr>
<td>Thrombospondin-1 and -2</td>
<td>ECM glycoproteins</td>
</tr>
<tr>
<td>Tumstatin</td>
<td>fragment of type IV collagen α₃ chain</td>
</tr>
<tr>
<td>Chondromodulin-I</td>
<td>component of cartilage ECM</td>
</tr>
<tr>
<td>Troponin I</td>
<td>component of cartilage ECM</td>
</tr>
<tr>
<td><strong>B. Non-matrix–derived</strong></td>
<td></td>
</tr>
<tr>
<td>Interferon-α (IFN-α)</td>
<td>cytokine</td>
</tr>
<tr>
<td>Interleukins (IL-1β, -12, -18)</td>
<td>cytokines</td>
</tr>
<tr>
<td>Pigment epithelium-derived factor (PEDF)</td>
<td>growth factor</td>
</tr>
<tr>
<td>Platelet factor-4</td>
<td>released by platelets during degranulation</td>
</tr>
<tr>
<td><strong>Other types</strong></td>
<td></td>
</tr>
<tr>
<td>Angiostatin</td>
<td>fragment of plasminogen</td>
</tr>
<tr>
<td>Antithrombin III</td>
<td>fragment of antithrombin III</td>
</tr>
<tr>
<td>2-Methoxyestradiol</td>
<td>endogenous metabolite of estrogen</td>
</tr>
<tr>
<td>PEX</td>
<td>fragment of MMP-2</td>
</tr>
<tr>
<td>Plasminogen kringle 5</td>
<td>fragment of angiostatin</td>
</tr>
<tr>
<td>Prolactin fragments</td>
<td>specific cleavage fragment</td>
</tr>
<tr>
<td>Prothrombin kringle 2</td>
<td>fragment of prothrombin</td>
</tr>
<tr>
<td>sFlt-1</td>
<td>soluble form of VEGF-R1 (= Flt-1)</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>inhibitor of metalloproteinase -2</td>
</tr>
<tr>
<td>TrpRS</td>
<td>fragment of tryptophan yl-tRNA synthetase</td>
</tr>
<tr>
<td>Vasostatin</td>
<td>fragment of calreticulin</td>
</tr>
</tbody>
</table>

Adapted from P. Nyberg, L. Xie and R. Kalluri, Cancer Res. 65:3967–3979, 2005.

Table 13-3  The Biology of Cancer  (© Garland Science 2007)
Treatment of tumors with endostatin decreases density of vascularity.

However, some tumors regress without reduction in vascularity indicating that endostatin can also inhibit tumor growth through non-angiogenic mechanisms.
Balancing the angiogenic switch

- **Activators**
  - VEGF-A
  - VEGF-B, -C
  - FGF1 (aFGF)
  - FGF2 (bFGF)
  - Other FGFs etc.

- **Inhibitors**
  - Thrombospondin-1, -2
  - Interferon α/β
  - Angiostatin
  - Endostatin
  - Collagen IV fragments etc.

Figure 13-46 The Biology of Cancer (© Garland Science 2007)
Angiogenesis is an attractive therapeutic target because it involves the growth of normal cells which are genomically stable and therefore unlikely to rapidly develop resistance.
Angiogenesis inhibitors and clinical trial

Table 13.4 Angiogenesis inhibitors and their development and use in clinical trials

<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 severe vascular toxicities</td>
</tr>
<tr>
<td>SU5416 inhibitor of VEGF-R2 (Flk-1)</td>
<td>trial abandoned</td>
<td></td>
</tr>
<tr>
<td>ZD6474 inhibitor of VEGF-R2</td>
<td>under clinical test</td>
<td></td>
</tr>
<tr>
<td>CP547, 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 cytoskeleton</td>
</tr>
<tr>
<td>Fumagillin and TNP-470</td>
<td>in trial; slowed tumor growth</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)
Clinically approved antiangiogenic therapy

- Avastin (anti-VEGF antibody) ~ combination with chemo/cytokine therapy treatment of metastatic cancers (non small cell lung cancer, colorectal cancer, renal cell, breast cancer).

- Pan VEGF- receptor tyrosine kinase inhibitors (Sunitinib) – metastatic RCC

- anti-VEGF R inhibitors block vascular branching and homing of BMDC’s Deprive tumor vasculature of VEGF-survival. Prune immature pericyte devoid vessels.

- Clinical benefit limited. Months. Subsets of patients refractory and other acquire resistance.

- Some tumors produce other angiogenic factors Hypoxia upregulates proangiogenic molecules (PIGF, IL-8).

- VEGF receptor inhibitors induce hypoxia and create pro-inflammatory environment
Alternative anti-vascularization strategies

- VEGF inhibitors more effective on peri-cyte free vessels (capillaries). Targeting both EC and pericytes may increase efficacy but not promising.

- Sustained vascular normalization – restore structure thereby increasing oxygenation and preventing hypoxia induction of vascularization genes.

- Role of VEGF (R) inhibitors in micrometastatic disease?

- Development of novel anti-angiogenic drugs to use in combination with VEGF R inhibitors.
Angiogenesis inhibitors as treatment of islet cell carcinogenesis

SU6668 (PDGF-R inhibitor) SU5416 (VEGF-R inhibitor). Treatment most effective when 2 inhibitors used in combination.
**ENDOTHELIAL CELL**
Inhibitors of VEGF, FGF, etc., signaling, e.g., anti-VEGF and anti-VEGF-R antibodies, small-molecule VEGF-R inhibitors, VEGF-Trap, Ang2/Tie2 blocking antibodies. Endogenous angiogenesis inhibitors, e.g., endostatin, tumsatan. Inhibitors of EPC recruitment.

**BASEMENT MEMBRANE EXTRACELLULAR MATRIX**
Inhibitors of matrix turnover, e.g., suramin, dalteparin and matrix-degrading enzymes, e.g., proteases (cathepsins, MMPs, uPA etc.), endoglycosidases (e.g., heparanase). Inhibitors of ECM contact, e.g., integrin αvβ3, αvβ5, α5β1, or α6β4 antibodies.

**PERICYTE**
Inhibitors of PDGF signaling, e.g., anti-PDGF antibodies, PDGF-R inhibitors. Inhibitors of Ang-1/Tie2 signaling.

**NEUTROPHIL MACROPHAGE MAST CELL**
Anti-inflammatory inhibitors, e.g., cytokine and chemokine inhibitors, NF-κB, IKK, TNF-α inhibitors.

**FIBROBLAST**
Inhibitors of HGF or its receptor c-Met, inhibitors of CXCL12/SDF-1, PDGF/PDGF-R, of fibroblast activation protein, e.g., sibrotuzumab.

**LYMPHATIC CELL**
Anti-lymphatic targeting: inhibitors of VEGF-C, VEGF-D, VEGF-R3, or PDGF/PDGF-R.

*Figure 13-49 The Biology of Cancer (© Garland Science 2007)*
Supplementary reading

1. Basic and Therapeutic Aspects of Angiogenesis: M. Potente, H. Gerhardt and P. Carmeliet Cell 146: 873-887 2011


2. Weinberg Chapter 13.