Angiogenesis and Metastasis

(RPN 530)

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(Ph.D.)
Small localized tumor

Angiogenesis

Tumor that can grow and spread

Signaling molecule
Definition of Angiogenesis:

“Formation of new blood vessels/capillaries from the pre-existing ones”

• Involves
  - sprouting
    - splitting
    - Remodeling (of the existing vessels)

• Why is it important???
  - supply of oxygen and nutrients to tissues/organs
  - removal of waste products from tissues/organs
  - fuels tumor growth, progression and metastasis
Angiogenesis
• **Vasculogenesis:**
  - Blood vessel formation by endothelial cells (ECs) that differentiate from stem cells
  - Seen during embryonic development (*primary vasculature*)

• **Angiogenesis:**
  - New capillaries or vessels from the *pre-existing vessels*
  - Seen during the *embryonic development* and in *adult life*
  - Physiologic stimuli (wound/injury or reproductive cycle in women)

• **Arteriogenesis**
  - Growth of large arteries from pre-existing small vessels/capillaries

• **Lymphangiogenesis**
  - Formation of the lymphatic vasculature
Normal Angiogenesis in Children

Normal Angiogenesis in Adults

Angiogenesis in uterine lining

Angiogenesis in tissue during wound healing

What Is Tumor Angiogenesis?

Small localized tumor

Tumor that can grow and spread

Angiogenesis

Blood vessel

Signaling molecule
Vasculogenesis

Vessels formation by ECs differentiating from angioblasts in the yolk sac of embryo

- differentiation and proliferation of ECs in a non-vascularized tissue
- leads to formation of a primitive tubular network
- undergoes angiogenic remodeling to stabilize vascular system
Postnatal vasculogenesis

Hemangioblast → Angioblast → Endothelial cell (EC)
Structure of vessels and capillaries

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

**Capillary:** endothelial cell, basal lamina, pericytes
Angiogenesis is a multi-step process

4 major steps by endothelial cells during angiogenesis

1. Breaking through of the basal lamina that envelops existing blood vessels

2. Migration toward a source signal (chemotactic signal)

3. Proliferation (cell division)

4. Formation of tubes
Stage One: Endothelial cell activation in response to angiogenic factors.
- Basic Fibroblast Growth Factor (bFGF): a potent stimulatory factor for endothelial cell migration and proliferation.
- Vascular Endothelial Growth Factor (VEGF): initiates cell proliferation and migration.

Stage Two: Degradation of the capillary wall by extracellular proteinases.
- Matrix Metalloproteinases (MMPs): MMP1 (a collagenase) and MMP2 are expressed during angiogenesis and act to degrade extracellular matrix components.

Stage Three: Formation of a branch point in the vessel wall.
- Integrins: expressed on newly forming vessels.

Stage Four: Migration of endothelial cells into the extracellular matrix towards the angiogenic stimulus.
- Integrins: allow migrating endothelial cells to interact with specific components of the surrounding matrix. MMPs and urokinase: aid migration of endothelial cells into the surrounding matrix.

Stage Five: Re-organisation of endothelial cells to form tubules with a central lumen.
- Angiopoietin (Ang 1): produced by surrounding stromal cells; facilitates endothelial cell survival and stabilisation of new capillary tubes.

Stage Six: Interconnection of the new tubules to form a network (anastomosis).
- Platelet Derived Growth Factor (PDGF): produced by endothelial cells of the new capillaries; recruits pericytes which stabilize the new vessels.

Conti... Angiogenesis- multistep process
Life time of endothelial cells (major players):
- Months (lung, liver) to Years (brain, muscle)
- Slow repair and renewal of vascular wall

New vessel formation:
- Embryo, growth to adulthood,
- In uterus, during menstruation cycle
- Wound repair
- Tumor angiogenesis
Intussusceptive angiogenesis

Sprouting angiogenesis

Intussusceptive angiogenesis
A) Sprouting angiogenesis: formation of blood vessels is a multi-step process, which includes:
(i) reception of angiogenic signals (yellow spot) by endothelial cells (EC)
(ii) retraction of pericytes from the abluminal surface of capillary and secretion of protease from activated endothelial cells (aEC) and proteolytic degradation of extracellular membrane (green dash-line)
(iii) chemotactic migration of EC under the induction of angiogenic stimulators
(iv) proliferation of EC and formation of lumen/canalisation by fusion of formed vessels with formation of tight junctions
(v) recruitment of pericytes and deposition of new basement membrane and initiation of blood flow.

B) Non-sprouting angiogenesis – intussusceptive microvascular growth: It is initiated by
(i) protrusion of opposing capillary walls towards the lumen
(ii) perforation of the EC bilayer and formation of many transcapillaries with interstitial core (red arrow)
(iii) formation of the vascular tree from intussusceptive pillar formation and pillar fusion and elongation of capillaries (green arrows)
Angiogenesis: Sprouting of cells from mature endothelial cells of the vessel wall

Angiogenesis - formation, maturation and differentiation of blood vessels from pre-existing vessels.

Observed in - physiological and pathological conditions (growth, injury, inflammation and cancer). Occasionally, angiogenesis is called neovascularization.

Angiogenic factor production (VEGF, bFGF), secretion of proteases, resolution of basal lamina, migration towards chemotactic gradient, proliferation, tube formation.
Capillaries sprouting in the retina of an embryonic mouse
Activators of Angiogenesis

Some Naturally Occurring Activators of Angiogenesis

Proteins
- Acidic fibroblast growth factor
- Angiogenin
- Basic fibroblast growth factor (bFGF)
- Epidermal growth factor
- Granulocyte colony-stimulating factor
- Hepatocyte growth factor
- Interleukin 8
- Placental growth factor
- Platelet-derived endothelial growth factor
- Scatter factor
- Transforming growth factor alpha
- Tumor necrosis factor alpha
- Vascular endothelial growth factor (VEGF)

Small Molecules
- Adenosine
- 1-Butyryl glycerol
- Nicotinamide
- Prostaglandins E1 and E2
ROLE OF VEGF

- VEGF production is under control of: hypoxia inducible factor (HIF)
- VEGF receptor expression is up-regulated under: hypoxic or ischemic conditions.
  - So, early involvement of VEGF in this process.

- VEGF is a major player in angiogenesis initiation because:
  i) it induces vasodilatation via endothelial NO production
  ii) it increases endothelial cell permeability
1. vasodilatation
2. increased vascular permeability
3. can induce the expression of proteases and receptors important in cellular invasion and tissue remodeling
4. prevent endothelial cell apoptosis

But angiogenesis is not completely dependent on VEGF production. Recently shown by: Hansen-Algenstaedt et al.
Chorioallantoic Membrane Assay (CAM)

(a) Serum free-media

(b) Serum free-media plus VEGF
**The VEGF Family and Its Receptors**

- **PIGF**
- **VEGF(-A)**
- **VEGF-B**
- **VEGF-C**
- **VEGF-D**

**Receptors:***
- **VEGFR-1 (Flt-1)**
- **VEGFR-2 (Flk-1/KDR)**
- **VEGFR-3 (Flt-4)**
- **NRP-1 (neuropilin)**

**Pathways:**
- Angiogenesis (RTK)
- Angiogenesis, lymphangiogenesis (RTK)
- Unclear but likely involved in tumor growth (Non-RTK)
- Lymphangiogenesis (RTK)

**Abbreviations:**
- RTK = receptor tyrosine kinase.
- Flt- FMS-like tyrosine kinase
- KDR- Kinase insert domain receptor (*KDR*, a type III receptor tyrosine kinase)
VEGF-VEGFR signaling

Angiogenesis

EC proliferation, survival, migration and invasion
VEGF Is a Key Mediator of Angiogenesis

Upstream activators of VEGF synthesis

Downstream signaling pathways

ANGIGENESIS
Hypoxia and Angiogenesis

• Hypoxia induces Angiogenesis:
  - during embryonic development
  - tumor growth
  - ischemia

• How???
  Inducing Vascular Endothelial Growth Factor (VEGF)
Role of hypoxia in angiogenesis:
(Hypoxia - HIF – VEGF module)

VBC: Von Hippel-Lindau (VHL)-containing VHL-elongin BC
Role of hypoxia in angiogenesis:
(Hypoxia - HIF – VEGF module) conti…

HIF: hypoxia inducible factor
VEGF: vascular endothelial growth factor
**VEGF-gene expression:** Regulated by HIF, HIF is continuously produced, ubiquitinylated, degraded in proteasome, therefore low concentration;

**Ubiquitinylation** is dependent on Hippel-Lindau tumor suppressor (part of an E3 ubiquitin-ligase complex)

HIF1α is modified by a prolyl hydroxylase, then better interaction with vHL protein, high turnover; Hydroxylase is regulated by O₂

**FIH:** Factor inhibiting HIF1α-Aaparaginyl hydroxylation leading to HIF inactivation
Angiogenesis-dependent diseases

**Excess:**
- Cancer
- Infantile hemangiomas
- Autoimmune diseases, chronic inflammatory diseases:
  - Rheumatoid arthritis
  - Psoriasis
- Age-related macular degeneration
- Atherosclerosis

**Deficiency:**
- Limb ischemia
- Myocardial ischemia
Angiogenic inhibitors:

• During the process of wound healing, the burst of angiogenesis must be shut down once the newly formed capillaries have reached a certain density.

TSP-1 produced by stromal fibroblasts, ECs and immune cells suppresses tumor progression by inhibiting angiogenesis through direct effects on EC migration and survival and through indirect effects on growth factor mobilization.
Inhibitors of Angiogenesis

Angiogenesis Inhibitors

Proteins
- Angiostatin
- Endostatin
- Interferons
- Platelet factor 4
- Prolactin 16Kd fragment
- Thrombospondin
- TIMP-1 (tissue inhibitor of metalloproteinase-1)
- TIMP-2 (tissue inhibitor of metalloproteinase-2)
- TIMP-3 (tissue inhibitor of metalloproteinase-3)
Angiogenesis Inhibitors

- Other angiogenesis inhibitors have been found in nature - in green tea, soy products, fungi, mushrooms, Chinese cabbage, tree bark, shark tissues, snake venom, red wine, and many other substances.
- Still other angiogenesis inhibitors have been manufactured synthetically in the laboratory.
- Some FDA-approved medicines have also been "re-discovered" to have anti-angiogenic properties.
ENDOSTATIN

- It was first discovered in 1995 in Dr. Folkman's lab
- Phase I clinical studies began at M.D. Anderson November 1999
- A naturally-occurring 20-kDa C-terminal fragment derived from type XVIII collagen.
- Interfere with the pro-angiogenic action of growth factors such as basic fibroblast growth factor (bFGF/FGF-2) and vascular endothelial growth factor (VEGF)
Inhibits EC migration, proliferation and induces EC apoptosis
Tumor angiogenesis

Judah Folkman (1971)-
• Angiogenesis is pre-requisite for tumor growth and metastatic progression

• Angioprevention- a critical target for cancer therapy
Small localized tumor

Tumor that can grow and spread

Angiogenesis

Signaling molecule
Solid tumors can grow in size up to ~1-2 mm diameter by simple diffusion of nutrients and gaseous exchange. However, beyond this size limit they require active supply of such components for tumor growth and progression.

**Angiogenic Switch**

- Hypoxia
- Stabilization of HIF-alpha
- HR-gene expression (VGF)
- Growth, proliferation, survival and migration of ECs
- Sprouting, tube formation and tumor vasculature formation
- Tumor growth and metastatic progression
Features of tumor angiogenesis

• Extreme and chaotic expression of angiogenic factors
  
• Disorganized vascular structure and Low adhesion and pericyte coverage
  
• Hypoxic stress, metabolic changes, cancer cell intravasation and lesser effects of chemotherapy
What Is Tumor Angiogenesis?

**Tumor angiogenesis**
Proliferation of a network of blood vessels that penetrates into cancerous growths.

**Function**  
Supplying nutrients and oxygen and removing waste products.

**Mechanism**  
Cancer cells release molecules that send signals to surrounding normal host tissue. This signaling activates certain genes in the host tissue that, in turn, make proteins to encourage growth of new blood vessels.
Stroma contributes to tumor angiogenesis

**Tumor Microenvironment** (Tumor-associated stroma): induced by cytokines and chemokines secreted from tumor cells

- **Macrophage**: Tumor--Associated Macrophages (TAMs)
- **Fibroblast**: Carcinoma--Associated Fibroblasts (CAFs)
- **Myeloid cell**: Bone Marrow Derived Cells (BMDCs)
- **Extracellular matrix** (ECM)

**Tumor microenvironment complexity and degree of infiltration of various components correlates with the tumor angiogenesis and invasiveness**
Macrophage and tumor angiogenesis

- Macrophages and tumor angiogenesis
- CARCINOMA CELLS
- Chemotactic factors (MCP-1, CSF-1, PDGF)
- Circulating monocytes
- Tumor-associated monocytes
- Tumor-associated macrophages (TAMs)
- EGF
- Proteases (e.g., cathepsins, MMPs)
- Angiogenic factors (VEGF, IL-8)
- Cleave IGFBPs
- Disrupt ECM
- Liberating IGFs
- Liberating mitogenic factors
- Liberating angiogenic factors
- Create space for new vessels & invading tumor cells

Figure 13.25e The Biology of Cancer (© Garland Science 2014)
Cellular mechanisms of tumour angiogenesis

1. Host vascular network expands by budding of endothelial sprouts or formation of bridges (angiogenesis);
2. Tumour vessels remodel and expand by the insertion of interstitial tissue columns into the lumen of pre-existing vessels (intussusception); and
3. Endothelial cell precursors (angioblasts) home from the bone marrow or peripheral blood into tumours and contribute to the endothelial lining of tumour vessels (vasculogenesis).
4. Lymphatic vessels around tumours drain the interstitial fluid and provide a gateway for metastasizing tumour cells.
Dr. Judah Folkman proposed the concept of anti-angiogenic therapy (NEJM.1971).
Strategies for inhibition of tumor growth by anti-angiogenic drugs

- Inhibits production of angiogenic proteins
- Neutralizes angiogenic proteins
- Inhibits receptors for angiogenic proteins or induces endothelial cell apoptosis

Tumor

Angiogenic proteins: VEGF, bFGF and PDGF

Angiogenic vessel
Antiangiogenic Therapies

Potential Targets:

• Block pro-angiogenic molecules (e.g., VEGF)

• Add anti-angiogenic regulators (e.g. angiostatin, endostatin, TSP-1)

• Inhibit stroma-degrading enzymes (e.g., MMPIs)

• Target vascular antigens (e.g., avb3 integrin)

• Attack pericytes
Current Angiogenic Inhibitors in Clinical Use and Clinical Trials

- Bevacizumab (Avastin™)
- Sunitinib (Sutent™)
- Sorafenib (Nexavar™)
- Cederanib (Recentin™ - AZD- 2171)
- Cilengitide
- VEGF-Trap

Many others in development
“Avastin Bevacizumab- Reach Beyond Convention”

- Recombinant, humanized monoclonal antibody that binds to all isoforms of VEGF-A such that KDR signaling is inhibited

- Developed by Genentech BioOncology

- Not a chemotherapy drug: “Targeted Therapy”
Limitations of Anti-angiogenic therapy

- Resistance: expression of other angiogenic factors such as bFGF and PDGF
- Toxicity and dosage (off target effects)
- HIF-can induce EMT and promote invasiveness via cytokine expression
- Normalize disorganized tumor blood vessels
- Side effects (high blood pressure, bleeding and coronary artery disease, etc)
Boxed WARNINGS and ADDITIONAL IMPORTANT SAFETY INFORMATION

- Gastrointestinal (GI) perforation
- Wound healing complication
- Hemorrhage
- Neutropenia
Metastasis
When does metastasis begin?

Commitment to the metastatic phenotype:
- How early does it occur?
- Can it be reversed?

Progenitor lesions:
- What are the key progenitor lesions?
- What is the efficiency of transition to invasion?
- Are all metastasis precursors clonal?
What is the role of the host?

- Under what conditions does the host drive or suppress the process?
- Does the transition from pre-invasive to invasive lesions require host participation?
- If so what are the molecular and cellular players that are functionally important?
- The circuitry of the tumor host communication may be the key to prevention of invasion.
Physiologic basis of metastasis

• Is metastasis a normal physiologic program which is disregulated or inappropriately activated?

• Does a physiologic motility and invasion program exist for development, angiogenesis morphogenesis and wound healing?

• Is metastasis colony formation a natural ongoing process conducted by stem cells?
What is the driving force?

• Is the metastatic phenotype pre determined within the primary tumor? Within the host microenvironment?

• Are malignant cells a product of adaptation and selection?

• What is the selection factor? If malignant cells are survival of the fittest, then what is the fitness test?

• Is cell survival in a foreign (non home) tissue the ultimate selection factor?
Pre-cellular theory of invasion and metastasis: recognition of malignant tumors and localized versus metastatic disease

LeDran 1757: Noted that malignant tumors begin as localized disease, then spread to regional lymph nodes and then enter the circulation to subsequently appear in the lung

Bichat 1801: Tumors contain both parenchyma and stroma

Recamier 1829: Used the term “Metastases”
Validation of the cellular theory of cancer metastasis

1900–1949: Takahashi found that the cells of various mouse carcinomas and sarcomas produce reproducible patterns of metastases when injected into other mice.

1950–1969: Rygaard and Povlsen showed in 1969 that human tumors can grow in nude mice, which lack a thymus and are T cell deficient. This experimental animal model of human cancer continues to be refined and used today.
The organ pattern of metastasis is characteristic of the tumor type and tissue of origin. 50-70% of the metastatic pattern can be predicted by the venous drainage blood flow. The remaining 30-50% may be caused by specific molecular homing mechanisms.

Potential molecular mechanisms:

a) Preferential adhesion in the vessels of the target organ
b) Selective extravasation
c) Organ attractants
d) Organ specific survival and growth
Pre-metastatic niche formation

Something secreted from primary tumor and changing the behavior of host tissue at distant sites
Cancer develops through gradual changes in cell morphology and properties.

- **Normal**
- **Hyperplasia**
- **Mild dysplasia**
- **Carcinoma in situ (severe dysplasia)**
- **Cancer (invasive)**

benign tumor
malignant tumor
Where do they go?

Metastatic tropism

Figure 14.42  *The Biology of Cancer* (© Garland Science 2007)
An organ is composed of several tissues

- Epithelial cells
- Connective tissue
- Muscle tissue
Cancer cells need to change their epithelial properties, to lose their adhesion and to penetrate through potent physical barriers.
Intravasation
Once lodged in the blood vessels of various tissues, cancer cells must escape from the lumina of these vessels and penetrate into the surrounding tissue—the step termed extravasation.
Platelet-mediated tumor cell extravasation

Formation of microthrombus (attachment of platelets) and Proliferation in the lumen of the capillary
The blood: a hostile environment

- Cells are normally anchorage-dependent (anoikis)
- Shear forces tear cells apart

http://www.cancerquest.org/
Chemokines regulate leukocyte recirculation and trafficking to sites of inflammation and infection.
Premise: Metastasis homing is dictated by relative abundance of chemokines and cognate receptors on the tumor cell.

Why do the tumor cells express the chemokine receptors in the primary tumor prior to dissemination?

Therapeutic utility is limited because dissemination has already occurred at the time of diagnosis.
Colonization

First, micrometastases
Dormant micrometastases are viable
Eventually: macrometastases

Intravasation → Latency → Colonization

Steeg Nature Med 06
### Metastatic inefficiency

A sequence of inefficient steps

<table>
<thead>
<tr>
<th>escape from parent tissue</th>
<th>travel through circulation</th>
<th>colonization of remote site</th>
</tr>
</thead>
<tbody>
<tr>
<td>invasiveness causes entry into vessel</td>
<td>survival in the circulation</td>
<td>survival of cells in foreign tissue</td>
</tr>
<tr>
<td></td>
<td>arrest in capillary or other small vessel</td>
<td>initial growth of cells in foreign tissue</td>
</tr>
<tr>
<td></td>
<td>exit into remote tissue or organ</td>
<td>persistence of growth</td>
</tr>
</tbody>
</table>

Figure 20-44 Molecular Biology of the Cell (© Garland Science 2008)
## Metastasis Promoting Genes - I

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tissue Site</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARM-1</td>
<td>Lymphoma</td>
<td>Promotes adhesion of tumor cells to the endothelium</td>
</tr>
<tr>
<td>ATX</td>
<td>Breast, Liver, Lung, Melanoma, Teratocarcinoma</td>
<td>Cytoskeletal reorganization and motility; G-protein coupled receptor activation</td>
</tr>
<tr>
<td>CD44</td>
<td>Multiple sites</td>
<td>Cell-cell interactions; activates HGF/c-Met pathway</td>
</tr>
<tr>
<td>Cox2</td>
<td>Breast, Colorectal, Gastric</td>
<td>Prostaglandin synthase; induces VEGF</td>
</tr>
<tr>
<td>Cyr61</td>
<td>Breast</td>
<td>Mediates adhesion; Erb-B2/3/4 pathway</td>
</tr>
<tr>
<td>Ezrin</td>
<td>Liver, Ovary, Pancreas, Prostate, Uterus</td>
<td>Membrane-cytoskeletal linker; RHO and RAC interactions</td>
</tr>
<tr>
<td>HMG-I(Y)</td>
<td>Breast, Cervical, Colorectal, Prostate, Skin, Thyroid, Uterus</td>
<td>Regulated by EGF and MMP-9</td>
</tr>
<tr>
<td>Laminin-5</td>
<td>Multiple sites</td>
<td>EGF and TGF-α induce expression of laminin subunits; cell adhesion, motility</td>
</tr>
<tr>
<td>c-Met</td>
<td>Multiple sites</td>
<td>Activated by HGF; Modulates Ras and PI3 kinase</td>
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</table>
## Metastasis Promoting Genes - II

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tissue Site</th>
<th>Function</th>
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<tbody>
<tr>
<td>MTA1</td>
<td>Breast, Cervix, Melanoma, Ovary</td>
<td>Neucleosome remodeling; histone deacetylase complex</td>
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<tr>
<td>Oncostatin M</td>
<td>Lung</td>
<td>Activates PKA-dependent pathway</td>
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<tr>
<td>PP2A</td>
<td>Not determined</td>
<td>Activated by p38/MAPK; inhibits MEK1, MEK2, and MMP-1</td>
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<tr>
<td>RAGE</td>
<td>Gastric, Lung, Pancreatic, Renal</td>
<td>Transmembrane receptor; activates p21, MAPKs, NF-6B, cdc42/rac</td>
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<td>S100A4</td>
<td>Breast, Colorectal, Prostate</td>
<td>Calcium-binding protein; activates c-erbB-2</td>
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<tr>
<td>S100A9</td>
<td>Colon, Gastric, Skin</td>
<td>Calcium-binding protein; Modulates Mac-1 integrin receptor through G-protein</td>
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<tr>
<td>Semaphorins</td>
<td>Gastric, Leukemia, Lung, Skin</td>
<td>Cell-cell interactions; Receptor crosstalk with c-Met binding semaphorin receptor, plexin</td>
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<tr>
<td>Thymosin-β15</td>
<td>Prostate</td>
<td>Actin binding; motility</td>
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<tr>
<td>Wnt-5a</td>
<td>Breast, Colon, Lung, Melanoma, Pancreas, Prostate</td>
<td>PKC activation with associated changes in cytoskeleton, cell adhesion, and motility</td>
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</table>
## Metastasis Suppressor Genes - I

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tissue Site</th>
<th>Function</th>
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<tbody>
<tr>
<td>Annexin7</td>
<td>Prostate</td>
<td>calcium-dependent GTPase; substrate for PKC and other kinases associated with proliferation</td>
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<tr>
<td>BRMS1</td>
<td>Breast, Melanoma</td>
<td>gap-junctional communication</td>
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<tr>
<td>CC3</td>
<td>Colon, Lung</td>
<td>serine/threonine kinase</td>
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<td>CEACAM1-4S</td>
<td>Breast, Colon</td>
<td>Bax pathway</td>
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<tr>
<td>CRSP3</td>
<td>Melanoma</td>
<td>transcriptional co-activator</td>
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<tr>
<td>DAP-kinase</td>
<td>Multiple sites</td>
<td>calcium/calmodulin-dependent serine/threonine kinase; pro-apoptotic pathway</td>
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<tr>
<td>E-cadherin</td>
<td>Multiple sites</td>
<td>Wnt signaling; cytoskeleton; cell-cell adhesion</td>
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<tr>
<td>HEPSIN</td>
<td>Ovarian, Prostate, Renal</td>
<td>transmembrane serine protease</td>
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<tr>
<td>HPI[^{HSα}]</td>
<td>Breast</td>
<td>non-histone heterochromatin-associated protein</td>
</tr>
<tr>
<td>KAI-1</td>
<td>Breast, Prostate</td>
<td>Transmembrane tetraspondin; role in adhesion, motility, growth regulation, and differentiation; integrin interaction</td>
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<tr>
<td>KiSS1</td>
<td>Breast, Melanoma</td>
<td>Modulates Rho, Rac, and MAPK signaling</td>
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<tr>
<td>Maspin</td>
<td>Breast, Colon, Oral Squamous Cell, Prostate</td>
<td>Serine protease inhibitor; binds collagen and can modulate integrins</td>
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<td>Melastatin</td>
<td>Melanoma</td>
<td>Calcium channel protein</td>
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<tr>
<td>Gene</td>
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<td>Function</td>
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<tr>
<td>MKK4</td>
<td>Ovary, Prostate</td>
<td>MAPK; phosphorylates and activates p38 and JNK kinases</td>
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<td>NESH</td>
<td>Lung, Prostate</td>
<td>src homology 3 adapter protein; down regulates p21 pathway</td>
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<td>NM23-H1</td>
<td>Breast, Colon, Melanoma, Oral Squamous Cell</td>
<td>histidine kinase; phosphorylates KSR, which might reduce ERK 1/2 activation</td>
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<td>PTEN</td>
<td>Multiple sites</td>
<td>phosphatase; growth regulation, cell motility</td>
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<tr>
<td>RhoGD12</td>
<td>Bladder</td>
<td>Inhibits GTP binding; regulates RHO and RAC</td>
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<tr>
<td>SFRP1</td>
<td>Breast, Colorectal</td>
<td>Modulates Wnt signaling pathway</td>
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<td>SHPS-1</td>
<td>Breast, Leukemia</td>
<td>glycoprotein; may regulate RAS-MAPK signaling; suppresses anchorage independent growth</td>
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<td>Syk</td>
<td>Breast, Colon, Pancreas, Skin</td>
<td>Tyrosine kinase; inhibits PI3 kinase; necessary for MAPK activation</td>
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<tr>
<td>TSP-1</td>
<td>Multiple sites</td>
<td>inhibits endothelial cell proliferation and migration; c-Myc expression inhibits TSP-1</td>
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<tr>
<td>tropomyosins</td>
<td>Breast</td>
<td>interacts with e-cadherin/catenin complex</td>
</tr>
<tr>
<td>VDUP1</td>
<td>Melanoma</td>
<td>Thioredoxin inhibitor; upregulates KiSS1; interacts with CRSPs</td>
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</table>
How do cells become invasive???
EMT
Epithelial to Mesenchymal Transition

sea urchin embryo

Figure 14.13a  The Biology of Cancer (© Garland Science 2007)
Major changes during EMT

- Loss of E-cadherin
- Cell shape changes driven by Rho GTPases
- MMPs
Cell polarity

Cell adhesion (to each other and to Extra Cellular Matrix)

Stationary

High level of E-cadherin

Low level of N-cadherin

No cell polarity

Loss of cell adhesion

Ability to migrate and invade

Low level of E-cadherin

High level of N-cadherin

Buddhini Samarako
<table>
<thead>
<tr>
<th>Name</th>
<th>Where first identified</th>
<th>Type of transcription factor</th>
<th>Cancer association</th>
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<tbody>
<tr>
<td>Snail (SNAI1)</td>
<td>mesoderm induction in <em>Drosophila</em>; neural crest migration in vertebrates</td>
<td>C2H2-type zinc finger</td>
<td>invasive ductal carcinoma</td>
</tr>
<tr>
<td>Slug (SNAI2)</td>
<td>delamination of the neural crest and early mesoderm in chicken</td>
<td>C2H2-type zinc finger</td>
<td>breast cancer cell lines, melanoma</td>
</tr>
<tr>
<td>Twist</td>
<td>mesoderm induction in <em>Drosophila</em>; emigration from neural crest</td>
<td>bHLH</td>
<td>various carcinomas, high-grade melanoma, neuroblastoma</td>
</tr>
<tr>
<td>Goosecoid</td>
<td>gastrulation in frog</td>
<td>paired homeodomain</td>
<td>various carcinomas</td>
</tr>
<tr>
<td>FOXC2</td>
<td>mesenchyme formation</td>
<td>winged helix/forkhead</td>
<td>basal-like breast cancer</td>
</tr>
<tr>
<td>ZEB1 (δEF1)</td>
<td>postgastrulation mesodermal tissue formation</td>
<td>2-handed zinc finger/homeodomain</td>
<td>wide variety of cancers</td>
</tr>
<tr>
<td>ZEB2 (Sie1)</td>
<td>neurogenesis</td>
<td>2-handed zinc finger/homeodomain</td>
<td>ovarian, breast, liver carcinomas</td>
</tr>
<tr>
<td>E12/E47 (Tcf3)**</td>
<td>associated with E-cadherin promoter</td>
<td>bHLH</td>
<td>gastric cancer</td>
</tr>
</tbody>
</table>

*It remains unclear whether E12/E47 can function on its own to induce an EMT, or whether this bHLH functions as a subunit of a heterodimeric TF complex formed with other well-validated EMT-TF proteins such as Twist.

Table 14.3 The Biology of Cancer (© Garfield Science 2014)
EMT in Tumor Progression
**Adopting changes typical to EMT**

**Twist** plays an essential role in cancer metastasis. Over-expression of Twist is common in *metastatic carcinomas*.
MMPs (matrix metalloproteinases) help the cancer cells to **invade the ECM**
Signals from stroma controlling EMT
Snail or Slug functions

Epithelial Markers
- E-cadherin
- Claudins
- Occludins
- Desmoplakin
- Cytokeratins

Proliferation
- Cyclin D
- CDK4
- Rb phosph
- p21

Mesenchymal markers
- Fibronectin
- Vitronectin
- Vimentin

Cell shape changes
- Cell movements, invasion
- RhoB
- MMPs

Survival
- PI3K activity
- ERK activity
- Caspases
- P53
- BID

Snail or Slug functions

Proliferation

Mesenchymal markers

Cell shape changes

Survival

Epithelial Markers

Proliferation

Mesenchymal markers

Cell shape changes

Survival
Small GTPase family plays a key role of cancer cell motility

Cytokines
Chemokines
Extracellular matrix

Rho family
• Rho
• Rac
• Cdc42

Stress fiber
Focal adhesion
Lamellipodia
Filopodia
EMT and cancer progression

Correlation between EMT inducing TFs with the malignant behavior in cancer patients
What we learn today!!

Angiogenesis
(role of VEGF etc.)

Tumor Angiogenesis

Metastasis and role of Tumor Angiogenesis

Invasion and migration

EMT process

Angioprevention OR anti-angiogenic therapy in preventing tumor metastasis

Targeting Tumor Metastasis
Thank you

Questions????