Definition of Angiogenesis:

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

- **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
Types of new vessel/ capillary formation

• **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
Tumor angiogenesis

Small localized tumor  

Tumor that can grow and spread

Angiogenesis

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

**Structure of vessels and capillaries**

- **Loose connective tissue**
- **Smooth muscle**
- **Elastic lamina (elastin fibers)**
- **Endothelial lining**

**Sections through the three types of blood vessels**

- **A capillary:** Very small lumen, wall made of a single layer of cells
- **A vein:** Thin layer of muscle and elastic fibers, fairly thin outer wall
- **A artery:** Thick outer wall, thick layer of muscles and elastic fibers
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
Conti... Angiogenesis- multistep process:

<table>
<thead>
<tr>
<th>Key Stage</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage One:</strong></td>
<td>Endothelial cell activation in response to angiogenic factors.</td>
</tr>
<tr>
<td><strong>Markers:</strong></td>
<td>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.</td>
</tr>
<tr>
<td><strong>Stage Two:</strong></td>
<td>Degradation of the capillary wall by extracellular proteinases (matrix metalloproteinases)</td>
</tr>
<tr>
<td><strong>Markers:</strong></td>
<td>Matrix Metalloproteinases (MMPs): MMP1 (a collagenase) and MMP2 are expressed during angiogenesis and act to degrade extracellular matrix components.</td>
</tr>
<tr>
<td><strong>Stage Three:</strong></td>
<td>Formation of a branch point in the vessel wall.</td>
</tr>
<tr>
<td><strong>Markers:</strong></td>
<td>Integrins: expressed on newly forming vessels.</td>
</tr>
<tr>
<td><strong>Stage Four:</strong></td>
<td>Migration of endothelial cells into the extracellular matrix towards the angiogenic stimulus.</td>
</tr>
<tr>
<td><strong>Markers:</strong></td>
<td>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.</td>
</tr>
<tr>
<td><strong>Stage Five:</strong></td>
<td>Re-organisation of endothelial cells to form tubules with a central lumen.</td>
</tr>
<tr>
<td><strong>Markers:</strong></td>
<td>Angiopoietin (Ang 1): produced by surrounding stromal cells; facilitates endothelial cell survival and stabilisation of new capillary tubes.</td>
</tr>
<tr>
<td><strong>Stage Six:</strong></td>
<td>Interconnection of the new tubules to form a network (anastomosis).</td>
</tr>
<tr>
<td><strong>Markers:</strong></td>
<td>Platelet Derived Growth Factor (PDGF): produced by endothelial cells of the new capillaries; recruits pericytes which stabilize the new vessels.</td>
</tr>
</tbody>
</table>
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

TYPE

Intussusceptive angiogenesis
A) Sprouting angiogenesis: formation of blood vessels is a multi-step process, which includes:

(i) angiogenic chemoattractant signals (yellow spot) activate endothelial cells (EC)

(ii) retraction of pericytes from the abluminal surface of capillary and secretion of proteases from activated EC (aEC) to degrade ECM (green dash-line)

(iii) chemotactic migration of EC under the 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)
Angiogenic factor production (VEGF, bFGF), secretion of proteases, resolution of basal lamina, migration towards chemotactic gradient, proliferation, tube formation

This endothelial cell will generate a new capillary branch.

Pseudopodial processes guide the development of the capillary sprout as it grows into the surrounding tissue.

Capillary sprout hollows out to form tube.

Red blood cell
Endothelial cell
Capillary lumen
Tip cell
Stem
Capillaries sprouting in the retina of an embryonic mouse
# Activators of Angiogenesis

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Small Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidic fibroblast growth factor</td>
<td>Adenosine</td>
</tr>
<tr>
<td>Angiogenin</td>
<td>1-Butyryl glycerol</td>
</tr>
<tr>
<td>Basic fibroblast growth factor (bFGF)</td>
<td>Nicotinamide</td>
</tr>
<tr>
<td>Epidermal growth factor</td>
<td>Prostaglandins E1 and E2</td>
</tr>
<tr>
<td>Granulocyte colony-stimulating factor</td>
<td></td>
</tr>
<tr>
<td>Hepatocyte growth factor</td>
<td></td>
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<tr>
<td>Interleukin 8</td>
<td></td>
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<tr>
<td>Placental growth factor</td>
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<tr>
<td>Platelet-derived endothelial growth factor</td>
<td></td>
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<tr>
<td>Scatter factor</td>
<td></td>
</tr>
<tr>
<td>Transforming growth factor alpha</td>
<td></td>
</tr>
<tr>
<td>Tumor necrosis factor alpha</td>
<td></td>
</tr>
<tr>
<td><strong>Vascular endothelial growth factor (VEGF)</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Artwork by Jeanne Kelly © 2002*
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

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:
- IL-6
- EGF
- IGF-1
- bFGF
- PDGF
- H$_2$O$_2$
- Hypoxia
- COX-2
- NO
- Oncogenes

Release VEGF

Binding and activation of VEGF receptor

Endothelial cell

Survival

Migration

Proliferation

Downstream signaling pathways

ANGIOGENESIS
Hypoxia and Angiogenesis

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

- How???

Hypoxia induces Vascular Endothelial Growth Factor (VEGF)
Role of hypoxia in angiogenesis:
(Hypoxia - HIF – VEGF module)

Angiogenic Switch

Or Mutant VHL

E3-ubiquitin Ligase complex

VBC: Von Hippel-Lindau (VHL)-containing VHL-elongin B/C and cullin-2
Role of hypoxia in angiogenesis:
(Hypoxia - HIF – VEGF module) conti...

HIF: hypoxia inducible factor
VEGF: vascular endothelial growth factor
Von Hippel-Lindau Tumor Suppressor, HIF and VEGF
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

- Naturally occurring protein found in several animal species, including humans.
- It is an endogenous angiogenesis inhibitor.
- Angiostatin is produced by autoproteolytic cleavage of plasminogen.
- Can be cleaved from plasminogen by different metalloproteinases (MMPs), elastase, prostatespecific antigen (PSA), 13 KD serine protease, or 24KD endopeptidase.
Tumor angiogenesis

Judah Folkman (1971)-

• Angiogenesis is a pre-requisite process for tumor growth and metastatic progression

• Angioprevention- Targeting angiogenesis advocated for cancer therapy
Small localized tumor

Angiogenesis

Tumor that can grow and spread

Signaling molecule
Tumor growth and angiogenesis

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
Carcinogenesis process

Normal Cells

Initiation

Dysplasia → Neoplasia → Focal Carcinoma → Invasive Carcinoma → Metastasis

Requirement of Angiogenesis

Angioprevention Strategies

VEGF Production → Blood Vessel Growth → Tumor Growth
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

Carcinoma cells can promote tumor angiogenesis through the release of chemotactic factors (MCP-1, CSF-1, PDGF). These factors attract circulating monocytes and tumor-associated monocytes to the tumor site. Macrophages, also known as tumor-associated macrophages (TAMs), play a crucial role in this process.

Macrophages can release EGF, which cleaves IGFBPs and liberates IGFs. They also secrete proteases (e.g., cathepsins, MMPs) to disrupt the extracellular matrix (ECM). Additionally, macrophages release angiogenic factors (VEGF, IL-8) that promote angiogenesis.

Angiogenesis is the process of creating space for new vessels and invading tumor cells, which is essential for the tumor's survival and invasiveness. EMT (epithelial-mesenchymal transition) is also involved in these processes, contributing to the tumor's invasiveness and metastasis.
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)

• Using anti-angiogenic agents (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 alternative angiogenic factors such as bFGF and PDGF
- Toxicity and dosage (off target effects)
- Normalize disorganized tumor blood vessels
- Side effects (high blood pressure, bleeding and coronary artery disease)
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

Hippocrates (460–375 B.C.)
Galen (131–201 A.D.)

Pre-1700: The Greek physician Hippocrates coined “carcinoma” from karkinos, the word for crab.

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

Takahashi: (1915)

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.
Paget (1889)

1700–1899: Paget proposed that metastases form specifically in organs that are “soil” to a metastatic cell’s “seed.”

The organ pattern of metastasis is characteristic of the tumor type and tissue of origin.

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.
Where do they go?

Metastatic tropism

Figure 14.42  *The Biology of Cancer* (© Garland Science 2007)
Metastasis - a stepwise process

carcinoma in situ

PROGRESSION

invasive carcinoma

EMT

INVASION

INTRAVASATION

TRANSPORT through circulation

TRANSPORT through circulation

COLONIZATION

macrometastasis

micrometastasis

EXTRAVASATION
An organ is composed of several tissues: Epithelial cells, Connective tissue, and Muscle tissue.
Cancer cells change their epithelial properties, lose their adhesion and penetrate through 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

Figure 14.5d The Biology of Cancer © Garland Science 2014
Cells are normally anchorage-dependent (anoikis).
Shear forces tear cells apart.
After extravasation
what next???
Chemokines regulate leukocyte recirculation and trafficking to sites of inflammation and infection.
The CXCR4/CXCL12 axis is one of the most studied chemokine receptor axis and has been shown to play a vital role in metastasis

- Metastatic breast cancers selectively express chemokine receptor CXCR4 and migrate to organs that express high levels of its respective ligand CXCL12, also known as SDF-1.

- Chemokine receptor CXCR4 is consistently upregulated in metastatic breast cancer cell lines, lymph node metastases, and liver metastases while expression levels are undetectable in normal epithelial cells.

- Its ligand CXCL12, meanwhile, is preferentially expressed in the most common sites of breast cancer metastasis, lung, brain, lymph nodes, liver, and bone marrow.
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
\[\downarrow\]
Latency
\[\downarrow\]
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>survival in capillary or other small vessel</td>
<td>arrest in remote tissue or organ</td>
<td>initial growth of cells in foreign tissue</td>
</tr>
<tr>
<td>exit into remote tissue or organ</td>
<td>persistence of growth</td>
<td></td>
</tr>
</tbody>
</table>

DIFFICULT    EASY    DIFFICULT
## Metastasis Promoting Genes - I

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tissue Site</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTA1</td>
<td>Breast, Cervix, Melanoma, Ovary</td>
<td>Neucleosome remodeling; histone deacetylase complex</td>
</tr>
<tr>
<td>Oncostatin M</td>
<td>Lung</td>
<td>Activates PKA-dependent pathway</td>
</tr>
<tr>
<td>PP2A</td>
<td>Not determined</td>
<td>Activated by p38/MAPK; inhibits MEK1, MEK2, and MMP-1</td>
</tr>
<tr>
<td>RAGE</td>
<td>Gastric, Lung, Pancreatic, Renal</td>
<td>transmembrane receptor; activates p21, MAPKs, NF-6B, cdc42/rac</td>
</tr>
<tr>
<td>S100A4</td>
<td>Breast, Colorectal, Prostate</td>
<td>Calcium-binding protein; activates c-erbB-2</td>
</tr>
<tr>
<td>S100A9</td>
<td>Colon, Gastric, Skin</td>
<td>Calcium-binding protein; Modulates Mac-1 integrin receptor through G-protein</td>
</tr>
<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>
</tr>
<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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<tr>
<td>Gene</td>
<td>Tissue Site</td>
<td>Function</td>
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<tr>
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<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>
</tr>
<tr>
<td>CC3</td>
<td>Colon, Lung</td>
<td>serine/threonine kinase</td>
</tr>
<tr>
<td>CEACAM1-4S</td>
<td>Breast, Colon</td>
<td>Bax pathway</td>
</tr>
<tr>
<td>CRSP3</td>
<td>Melanoma</td>
<td>transcriptional co-activator</td>
</tr>
<tr>
<td>DAP-kinase</td>
<td>Multiple sites</td>
<td>calcium/calmodulin-dependent serine/threonine kinase; pro-apoptotic pathway</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>Multiple sites</td>
<td>Wnt signaling; cytoskeleton; cell-cell adhesion</td>
</tr>
<tr>
<td>HEPSIN</td>
<td>Ovarian, Prostate, Renal</td>
<td>transmembrane serine protease</td>
</tr>
<tr>
<td>HPI$^\text{HS}_\alpha$</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>
</tr>
<tr>
<td>KiSS1</td>
<td>Breast, Melanoma</td>
<td>Modulates Rho, Rac, and MAPK signaling</td>
</tr>
<tr>
<td>Maspin</td>
<td>Breast, Colon, Oral Squamous Cell, Prostate</td>
<td>Serine protease inhibitor; binds collagen and can modulate integrins</td>
</tr>
<tr>
<td>Melastatin</td>
<td>Melanoma</td>
<td>Calcium channel protein</td>
</tr>
</tbody>
</table>
# Metastasis Suppressor Genes - II

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tissue Site</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MKK4</strong></td>
<td>Ovary, Prostate</td>
<td>MAPK; phosphorylates and activates p38 and JNK kinases</td>
</tr>
<tr>
<td><strong>NESH</strong></td>
<td>Lung, Prostate</td>
<td>src homology 3 adapter protein; down regulates p21 pathway</td>
</tr>
<tr>
<td><strong>NM23-H1</strong></td>
<td>Breast, Colon, Melanoma, Oral Squamous Cell</td>
<td>histidine kinase; phosphorylates KSR, which might reduce ERK 1/2 activation</td>
</tr>
<tr>
<td><strong>PTEN</strong></td>
<td>Multiple sites</td>
<td>phosphatase; growth regulation, cell motility</td>
</tr>
<tr>
<td><strong>RhoGD12</strong></td>
<td>Bladder</td>
<td>Inhibits GTP binding; regulates RHO and RAC</td>
</tr>
<tr>
<td><strong>SFRP1</strong></td>
<td>Breast, Colorectal</td>
<td>Modulates Wnt signaling pathway</td>
</tr>
<tr>
<td><strong>SHPS-1</strong></td>
<td>Breast, Leukemia</td>
<td>glycoprotein; may regulate RAS-MAPK signaling; suppresses anchorage independent growth</td>
</tr>
<tr>
<td><strong>Syk</strong></td>
<td>Breast, Colon, Pancreas, Skin</td>
<td>Tyrosine kinase; inhibits PI3 kinase; necessary for MAPK activation</td>
</tr>
<tr>
<td><strong>TSP-1</strong></td>
<td>Multiple sites</td>
<td>inhibits endothelial cell proliferation and migration; c-Myc expression inhibits TSP-1</td>
</tr>
<tr>
<td><strong>tropomyosins</strong></td>
<td>Breast</td>
<td>interacts with e-cadherin/catenin complex</td>
</tr>
<tr>
<td><strong>VDUP1</strong></td>
<td>Melanoma</td>
<td>Thioredoxin inhibitor; upregulates KiSS1; interacts with CRSPs</td>
</tr>
</tbody>
</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
EMT

EPITHELIAL

- Cell polarity
- Cell adhesion (to each other and to Extra Cellular Matrix)
- Stationary
- High level of E-cadherin
- Low level of N-cadherin

MESENCHYMAL

- No cell polarity
- Loss of cell adhesion
- Ability to migrate and invade
- Low level of E-cadherin
- High level of N-cadherin

Buddhini Samarasinghe
<table>
<thead>
<tr>
<th>Name</th>
<th>Where first identified</th>
<th>Type of transcription factor</th>
<th>Cancer association</th>
</tr>
</thead>
<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 (SIP1)</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.*
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

[Diagram showing various signaling pathways and molecular interactions involved in EMT (Epithelial-Mesenchymal Transition), including roles of fibroblasts, macrophages, myofibroblasts, and mesenchymal stem cells. Key molecules and pathways such as FGF, HGF, TGF-α, EGF, Wnt, TGF-β, PGE2, cathepsin, PI3K, GSK-3β, NF-κB, and the EMT process are highlighted.]
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

Snail or Slug functions

Survival
- PI3K activity
- ERK activity
- Caspases
- P53
- BID
Small GTPase family plays a key role in cancer cell motility.

- Cytokines
- Chemokines
- Extracellular matrix

Rho family
  - Rho
  - Rac
  - Cdc42

Stress fiber
- Focal adhesion
- Lamellipodia
- Filopodia

---

Figure 14.39: The Biology of Cancer (© Garland Science 2014)
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?????