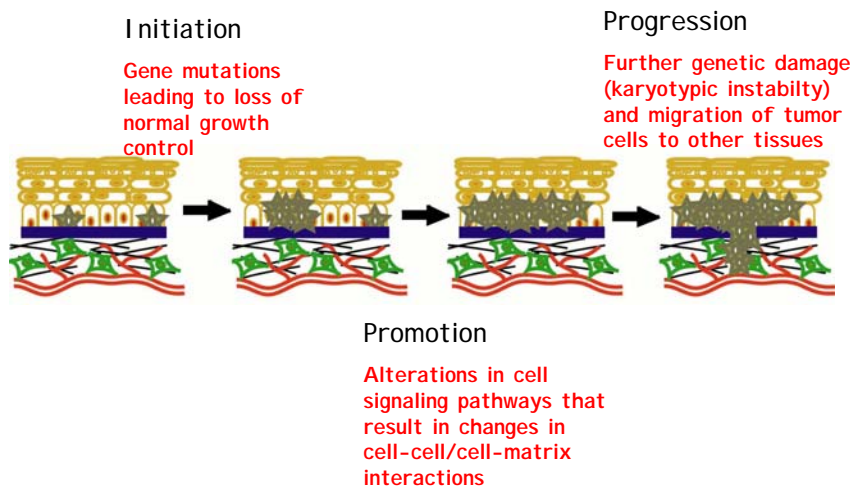


Angiogenesis and Metastasis

Lori A. White, Ph.D.
Biochemistry of Cancer (2006)
11:115:421

Stages of Carcinogenic Progression



Malignancy requires

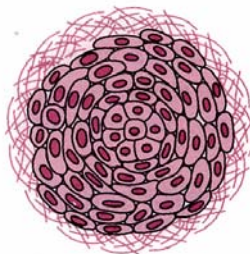
- Cells lose *adhesion* with the initial tumor
- Cells *migrate* out of the initial tumor
- Cells *enter the bloodstream/lymphatic systems*
- Cells *exit* the bloodstream/lymphatic system
- Cells are able to *form new tumor foci*

Invasion vs. Metastasis?

Benign versus Malignant Tumors

Characteristic	Benign	Malignant
Cell structure	Near normal	Abnormal shapes, larger cell and nucleus
Tissue structure	Orderly	Disordered, irregular
Growth rate	Above normal	Rapid
Invasive growth	Uncommon	Typical
Metastasis	Never	Typical
Capsule	Typical	Rare, incomplete if present
Anaplasia	Minimal	Typical
Prognosis	Good	poor

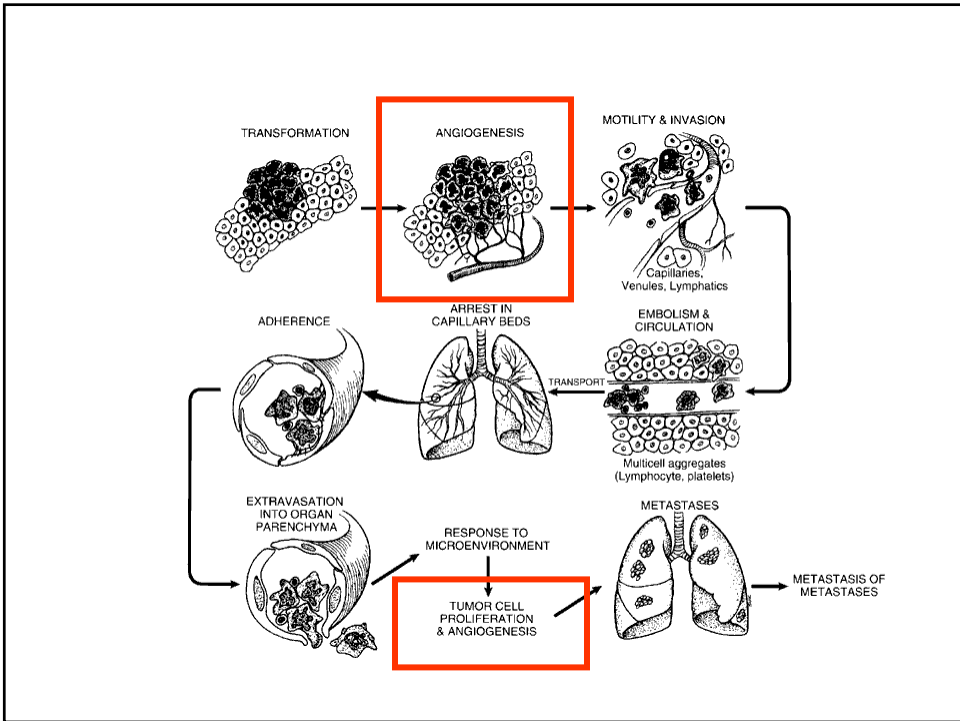
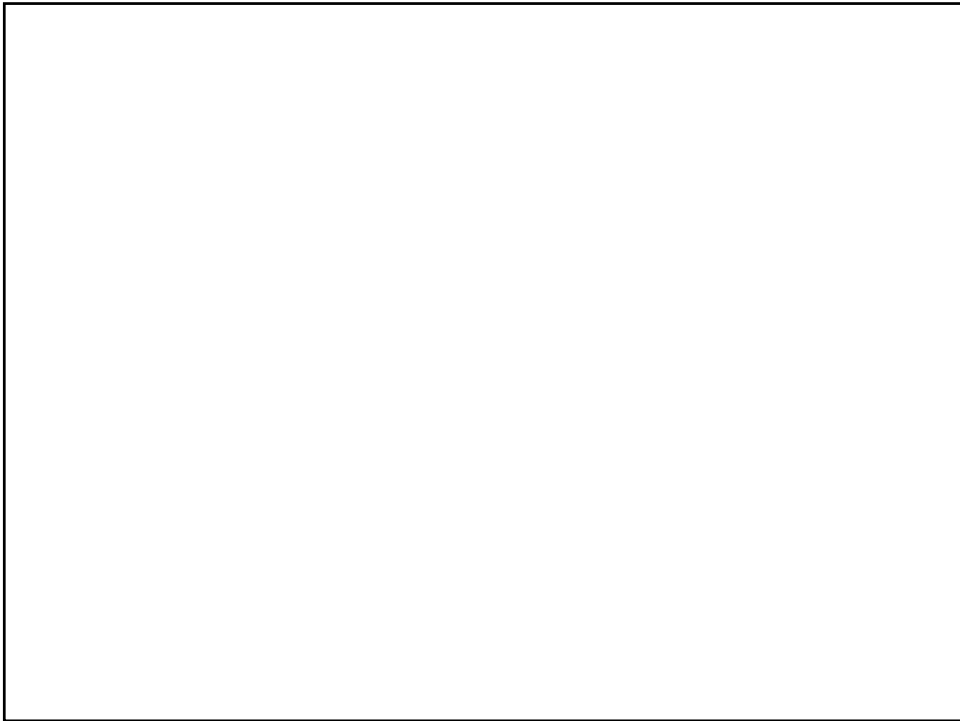
Characteristics of Benign and Malignant Tumors



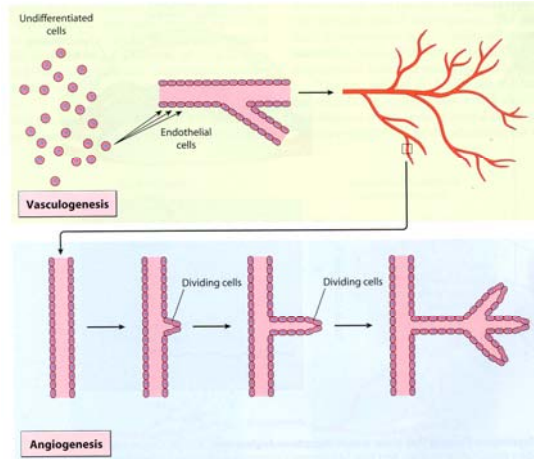
(a) Benign Tumor



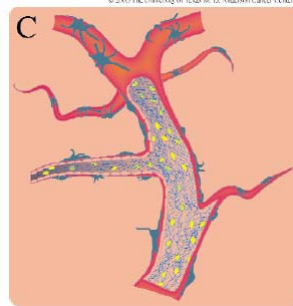
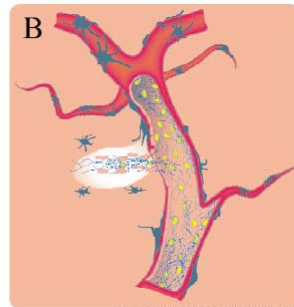
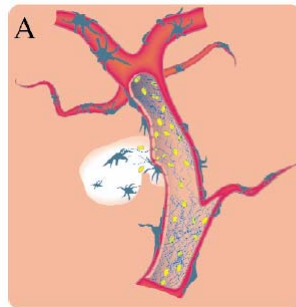
(b) Malignant Tumor



Angiogenesis

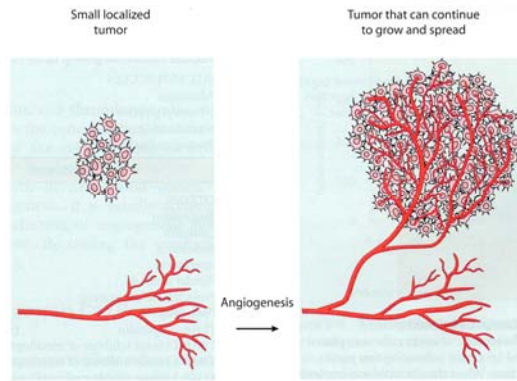


- is the formation of new blood vessels from existing vasculature
- Requires
 - endothelial cell proliferation
 - changes in adhesion
 - cell migration

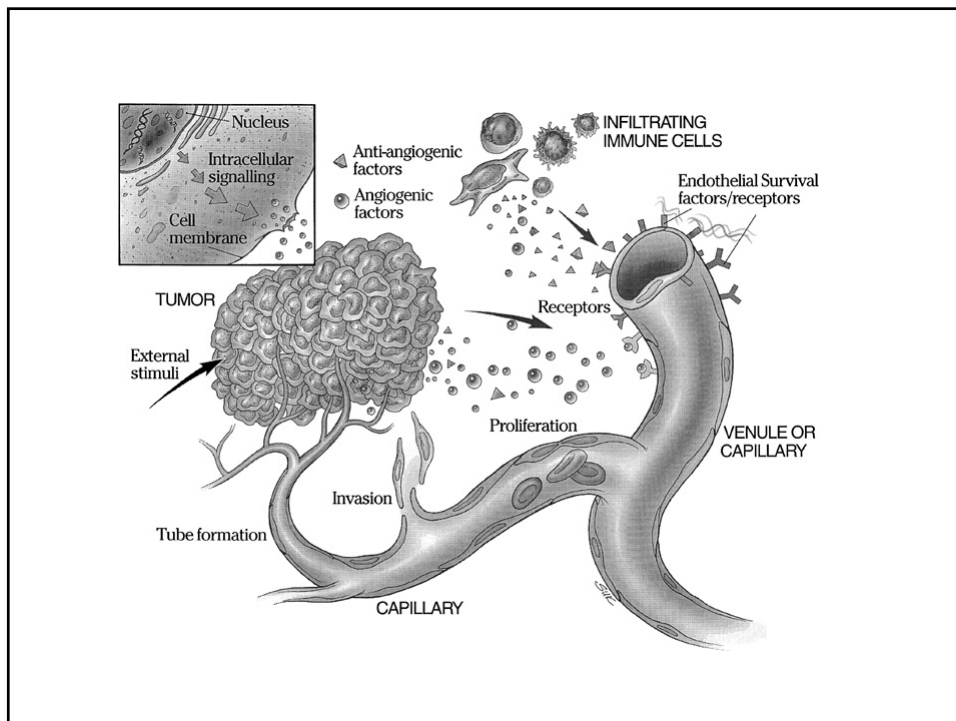


- Angiogenesis requires **detachment** of the pericytes from the blood vessel walls
- Degradation** of the vascular basement membrane and extracellular matrix
- Alteration** of cell-cell and cell-matrix **adhesions**
- Migration** of the endothelial cells

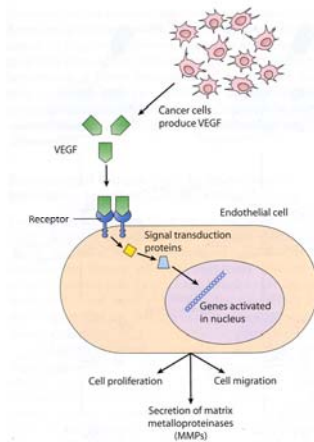
Without angiogenesis, tumor size is limited



(to a few millimeters)



Stimulation of Angiogenesis



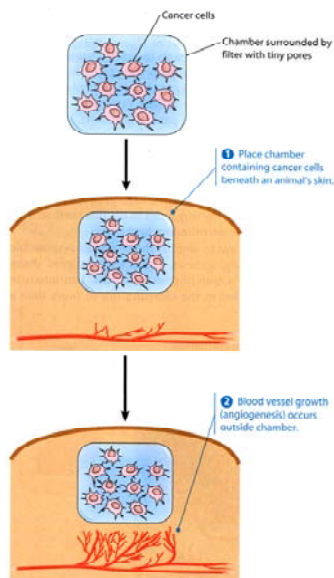
Stimulators of Angiogenesis

VEGF= vascular endothelial growth factor

•Growth factors: FGF, EGF, PGF, PDGF, GCSF

•Cytokines: IL 8, $TNF\alpha$, $TGF\alpha$

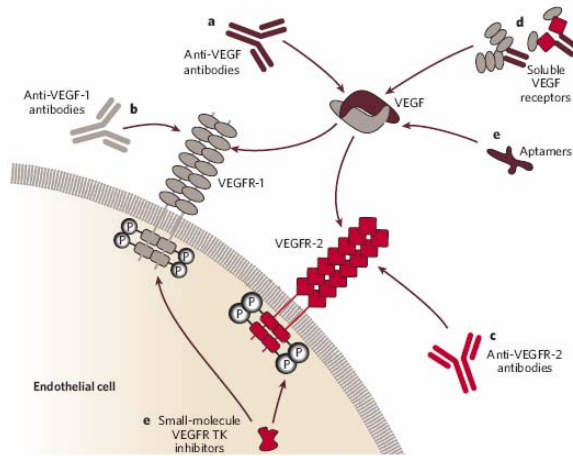
•Small Molecules: adenosine, nicotinamide, prostoglandins



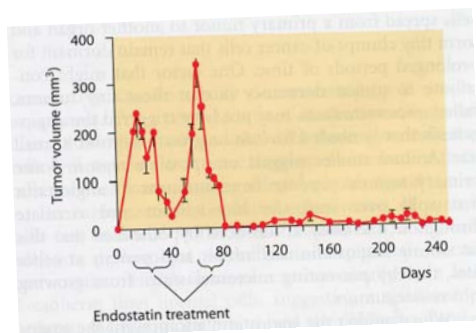
How do we know that cancer cells make factors that stimulate angiogenesis?

Although cells are unable to make contact with the surrounding tissue, blood vessel growth is still observed.

Angiogenesis is a target for chemotherapy



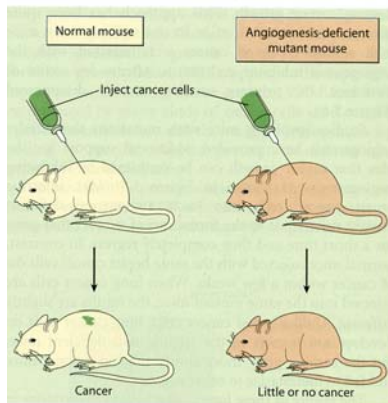
How effective are angiogenesis inhibitors?



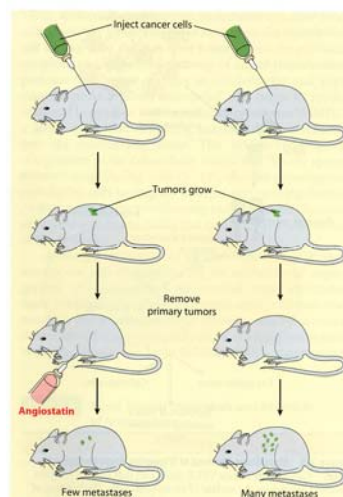
Natural Inhibitors of angiogenesis

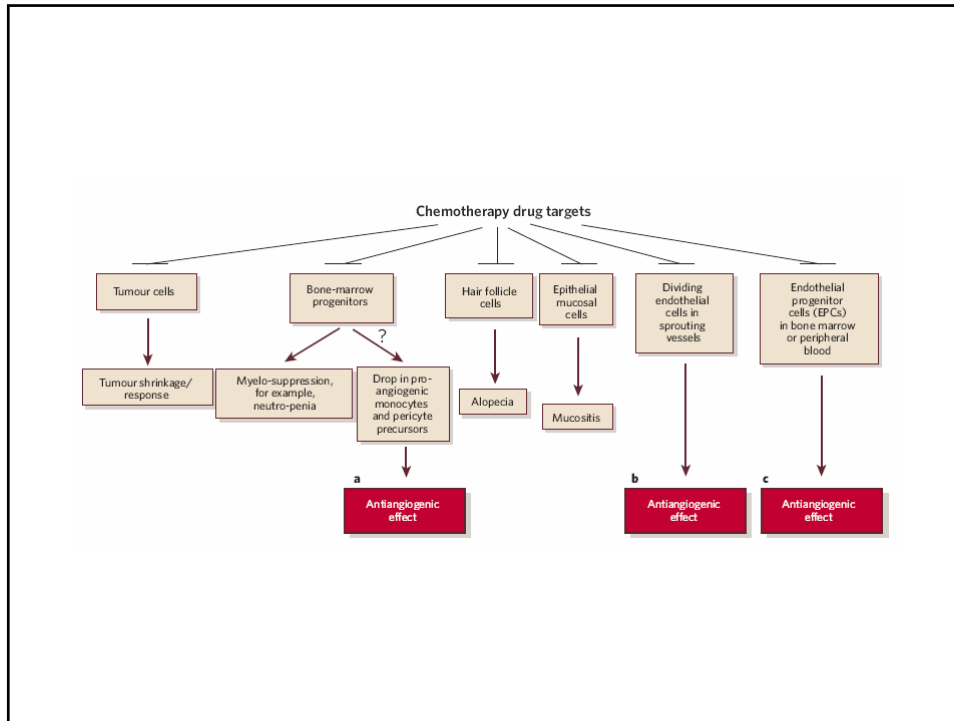
- Antiostatin
- Canstatin
- Endostatin
- Interferon
- TIMP-1,2,3

Knockout mice demonstrate that angiogenesis is critical in tumor formation

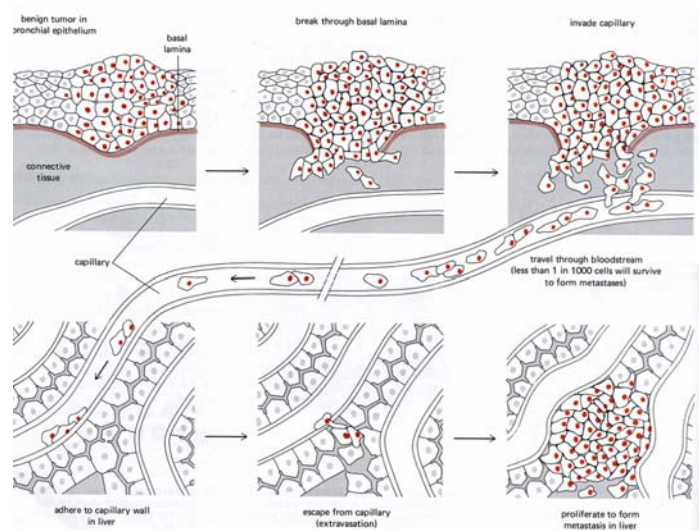


Angiogenesis also blocks metastasis





Cells must be able to cross the basal laminae to metastasize



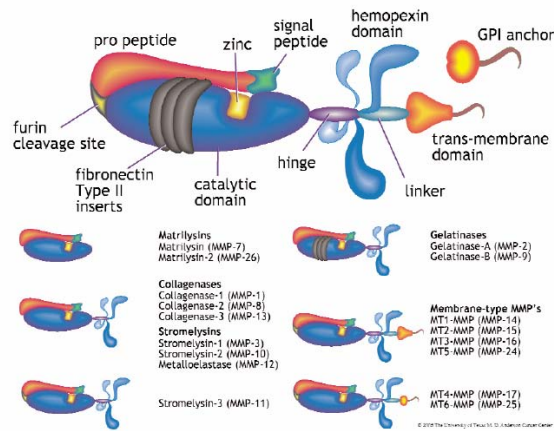
Protease activity is critical to invasion and metastasis

- Metastasis into new tumor foci accounts for more than 90% of all lethal outcomes in cancer.
- In order for cells to migrate, they must be able to degrade the protein barriers.
- Expression of proteases is necessary for this process.

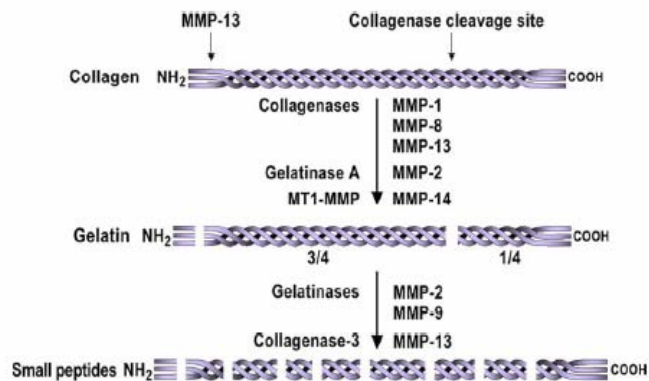
Matrix Metalloproteinases (MMPs)

- Zn^{2+} and Ca^{2+} dependent proteases.
- Degrade protein components of the extracellular matrix, basement membrane.
- Release and activate growth factors from ECM stores.
- Cleave proteins to reveal cryptic sites.
- Activity required for tissue remodeling and repair. Over/inappropriate expression associated with carcinogenic progression.

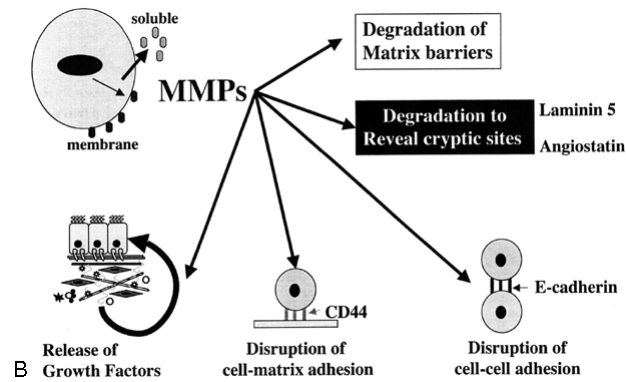
MMPs are a diverse family of proteases



MMPs were first identified as endopeptidases for structural proteins



MMPs have roles in many steps of tumor progression



- A variety of compounds can activate MMP expression including cytokines, pharmacologic agents, and growth factors

- MMP expression is regulated primarily at the level of transcription

- However, regulation of enzyme activity is also critical

- Propeptide cleavage
- TIMPs

- MMPs have been targeted for cancer therapy, as well as for other diseases involving excessive matrix degradation (rheumatoid arthritis) with little success

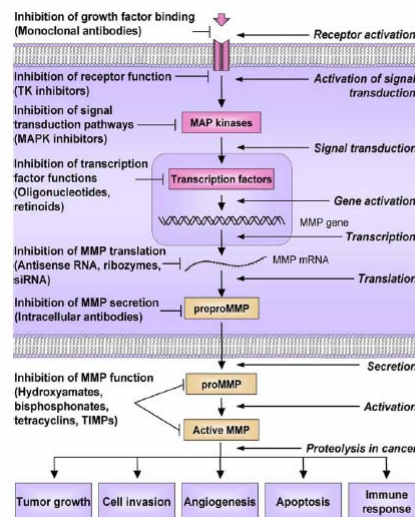


Fig. 6. Levels of regulation of MMP expression and activity and possible inhibition mechanisms.

MMPs are able to activate one another

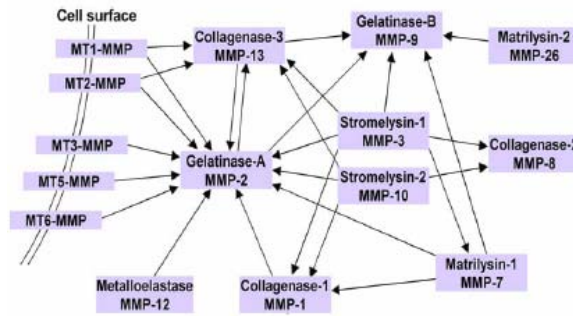


Fig. 4. Summary of MMP ability to activate each other. Arrows indicate the activation of one MMP by another.

MMPs can both promote and inhibit carcinogenic progression

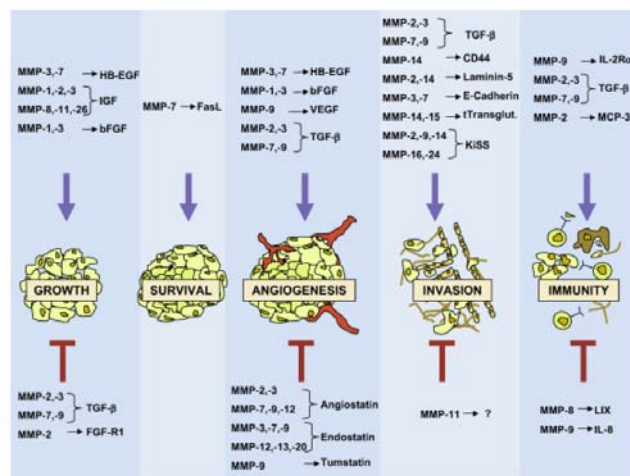
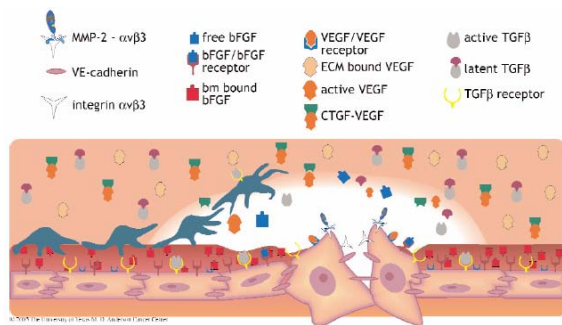


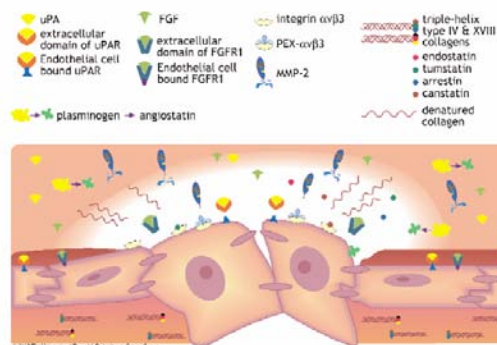
Fig. 2. Dual functions of MMPs in tumour progression. The opposite effects of bioactive molecule processing by MMPs on cancer development are shown.

MMPs are pro and anti-angiogenic



PRO-ANGIOGENIC

- Degradation of basement membrane and ECM to allow for cell detachment and migration
- Cleavage of VE-cadherin cell-cell adhesion
- Localization of MMP-2 to the migrating edge of the endothelial cell via binding to integrin $\alpha\beta 3$
- Exposure of cryptic integrin binding sites by cleavage of ECM
- Release of active VEGF from ECM stores
- Cleavage of basement membrane to release bFGF and to release and activate TGF β



ANTI-ANGIOGENIC

- Cleavage of ligand-binding domains of FGFR1 and uPAR (inhibits FGFR signaling and uPA localization)
- Inhibition of MMP-2 binding to $\alpha\beta 3$ integrin by release of MMP-2 PEX domain
- Generation of antiangiogenic factors
 - angiotatin from plasminogen
 - endostatin, tumostatin, arrestin, and canstatin from type XVII I and IV collagen

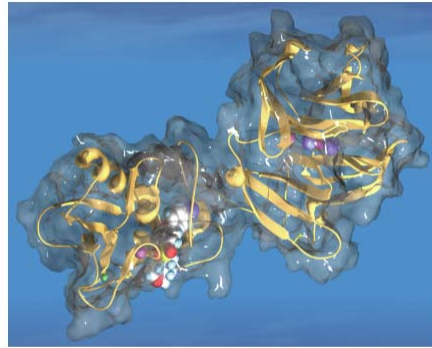
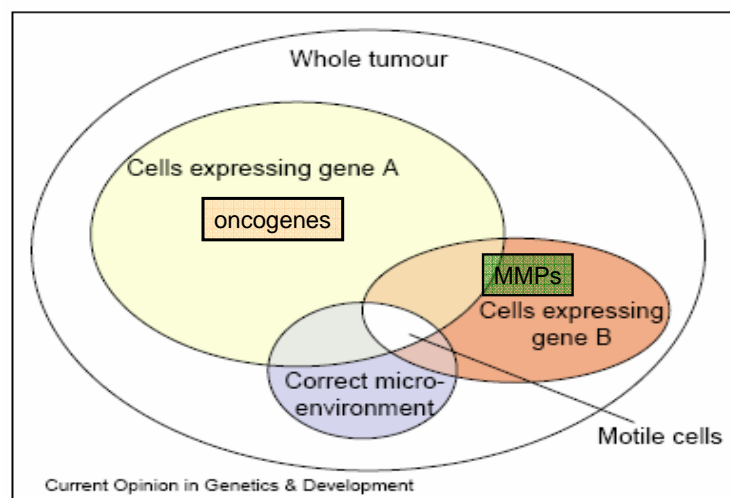
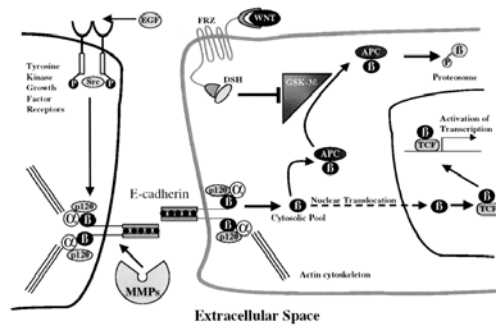


Fig. 3. Structural model of human collagenase-3 bound to a selective inhibitor. The model was created combining the structural data from the catalytic domain (pdb code 830C) and the hemopexin domain (pdb code 1PEX). Zn²⁺ ions are shown in green, Ca²⁺ ions in pink, and Cl⁻ ions in blue.

Multiple factors are required for cell motility



Disruption of cell-cell adhesion is needed for tumor progression



- E-cadherin is a homotypic cell adhesion molecule that is found in desmosomes, tight junctions, and adherens-type junctions.

- E-cad cytoplasmic tail interacts with α & β catenin and p120.

- Loss of E-cad function results in free cytosolic β-catenin levels

- Translocation of β-catenin to the nucleus results in association with TCF/LEF transcription factors

- The β-catenin & TCF/LEF complexes activate transcription of genes involved in transformation and tumor growth (ie c-myc and cyclin D1)

Integrins in tumor cell invasion and metastasis

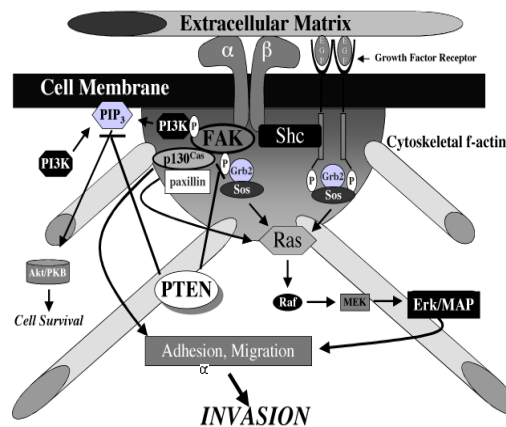
- Interaction of ECM and integrin receptors leads to integrin clustering and association with signal transduction molecules

- FAK is phosphorylated (tyr 397)

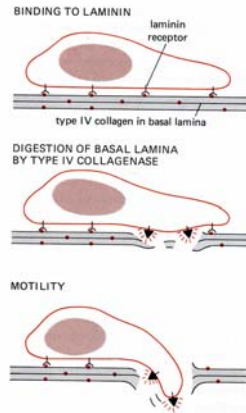
- Src is recruited to phosphorylated FAK

- Ultimately this leads to activation of the MAP kinase signaling pathway which activates adhesion and migration.

- PTEN, a tumor suppressor, can inhibit this pathway by dephosphorylation of FAK and PI3K.

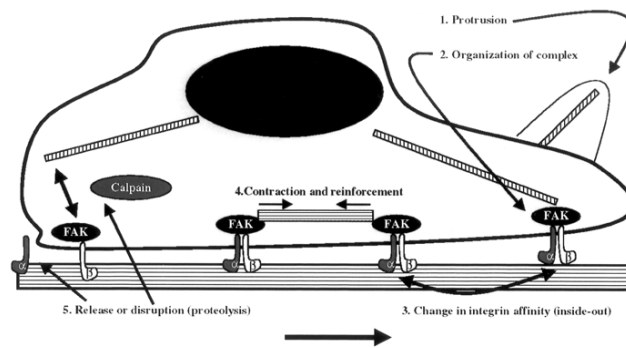


Cancer cell motility=metastasis



- Changes in integrin receptor subtypes are associated with cancer progression
- Expression of proteases that degrade the protein components of the basal laminae and the extracellular matrix (MMPs) are required for cell migration.

Cell migration requires transmission of force from the ECM to the cytoskeleton

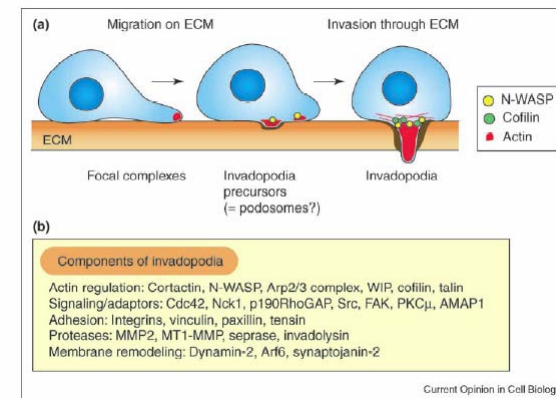
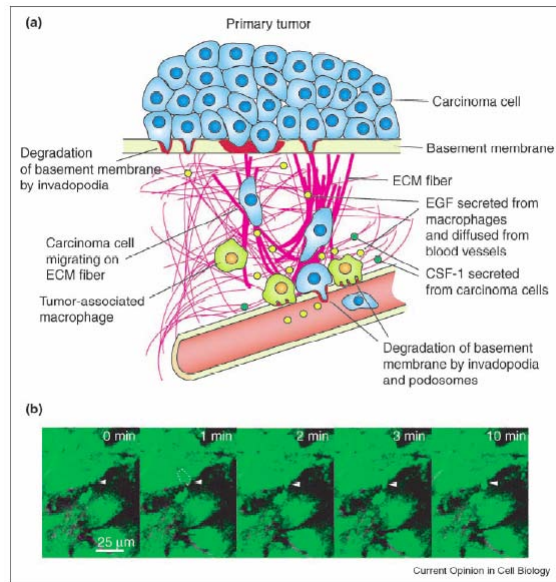


Net force and direction of movement

Steps in migration include:

protrusion of a cellular projection (filopodia or lamellipodia) that is mediated by actin polymerization
organization of the focal adhesion complex and connection with actin cytoskeleton

1. unactivated integrin subunits continually recycle along the cell surface
2. change in integrin affinity engages the ECM and traction force is generated
3. the focal adhesion complex is disrupted at the trailing edge of the cell
4. integrin complexes release (proteolytic disruption of FAK)



All tumors do not metastasize the same....

Primary Tumor	Typical metastatic sites
Carcinoma and breast adenocarcinoma	Bone, brain, liver, adrenals, lymph nodes
Bronchogenic carcinoma	Brain, spinal cord, bone, lymph nodes
Osteosarcoma	Lung, brain
Renal carcinoma	Lung, liver, bone, brain
Prostatic carcinoma	Bone
Colon carcinoma	Liver, brain, ovary, lung, lymph nodes
Neuroblastoma	Bone, lymph nodes
Melanoma	Lung, brain, liver, spleen, lymph nodes

Why???

- Patterns of blood flow
- Organ specific factors
 - I.e melanoma metastasizes primarily to the brain because melanocytes are (originally) from a neuronal cell lineage.
- Immune system (next week!)
- Specific genes
 - MMPs, adhesion molecules
 - Metastasis promoting genes
 - *Twist* -involved in promoting cell migration during development
 - Metastasis suppressor genes (E-cadherin, NM23, KISS1, KA11)
 - Encode for proteins involved in cell adhesion

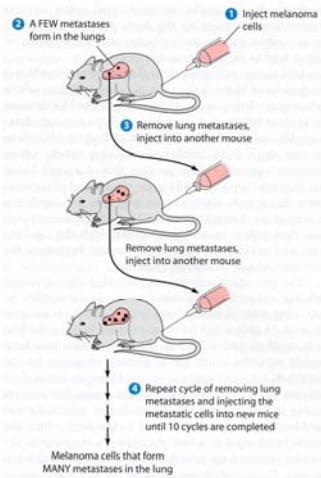


Figure 3-10 Selection of Melanoma Cells Exhibiting an Enhanced Ability to Metastasize. In this experiment, mouse melanoma cells were injected into the tail vein of a mouse. After a small number of metastatic tumors developed in the lungs, cells from some of the lung metastases were removed and injected into another mouse. When this cycle was repeated ten times, the final population of melanoma cells was found to produce many more lung metastases than the original cell population produced.

Interaction with normal cells is critical for tumor progression

