The Process of Carcinogenesis

Steps of tumor development

- **Initiation: essential step that is latent until next step. Often occurs as DNA damage in stem cells**
- **Promoting Agent: Typically not a carcinogen but a chemical that can induce cells to grow.**
- **Growth Inhibition: Normal control of cell growth can influence tumor growth .**

Initiating Agent ⟶ Promoting Agent ⟷ Growth Inhibitors

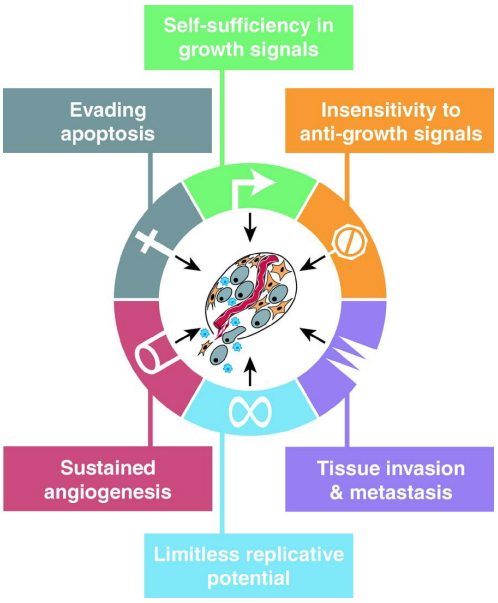
Initiating Agent	Promoting Agent	Growth Inhibitors
•Chemical-Carcinogens •Viruses •Radiation •UV Light •Replication Errors	•Specific Promoters •Inflammation •Hormones •Normal growth promoters	•Hormones - therapeutic or physiological •Normal Growth Inhibitors -age, surgery, radiation

1

6 Hallmarks of Cancer

Most, if not all cancers have acquired same set of functional capabilities during their development, albeit through various mechanisms

Invasion and Metastasis most heterogeneous and poorly understood



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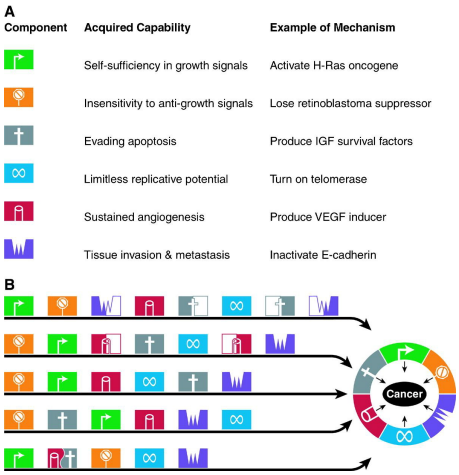

Means of achieving all six hallmarks will vary significantly, both mechanistically and chronologically

Thus, the order in which these capabilities are acquired seems likely be quite variable across the spectrum of cancer types and subtypes.

Moreover, in some tumors, a particular genetic lesion may confer several capabilities simultaneously, decreasing the number of distinct mutational steps required to complete tumorigenesis.

In other tumors, a capability may only be acquired through the collaboration of two or more distinct genetic changes, thereby increasing the total number necessary for completion of tumor progression.

Thus, in the eight-step pathway shown, invasion/metastasis and resistance to apoptosis are each acquired in two steps.

3

Definitions:

- Oncogene: a gene of cellular or viral origin that is responsible for rapid and uncontrolled growth in animal cells (c-onc indicates a cellular oncogene)
- Proto oncogene : A cellular gene that can undergo modification to a cancer causing gene (the conversion of a normal gene product to a mutated gene and it's resulting protein alteration)
- Transformation: conversion of a normal cell line to a cancerous growing cell

Cancer cells are characterized by 3 properties

1- lack or loss of control of cell growth
2- invasion of local tissue
3- spread or metastasis to distal tissues

4

Mutations Drive Cancer

Human Genome – 3 billion bp

- 1.5% codes for proteins ~ 45 million bp
- Rest is "junk" DNA
- ~550 "cancer genes"
- Ignore upstream regulators
- ~8250,000 bp "cancer coding"
- Only some bp will lead to cancer
- Multiple Genes must be altered 3-12
- Odds of getting a "random" mutation is difficult and cumulative

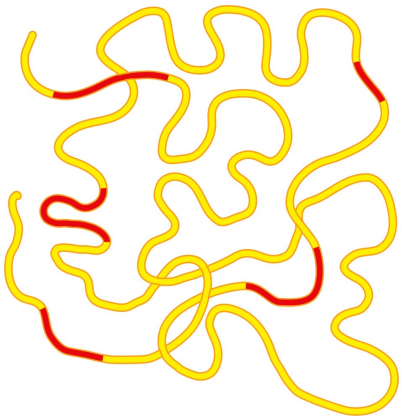

Figure 1.4 The Biology of Cancer (© Garland Science 2014)

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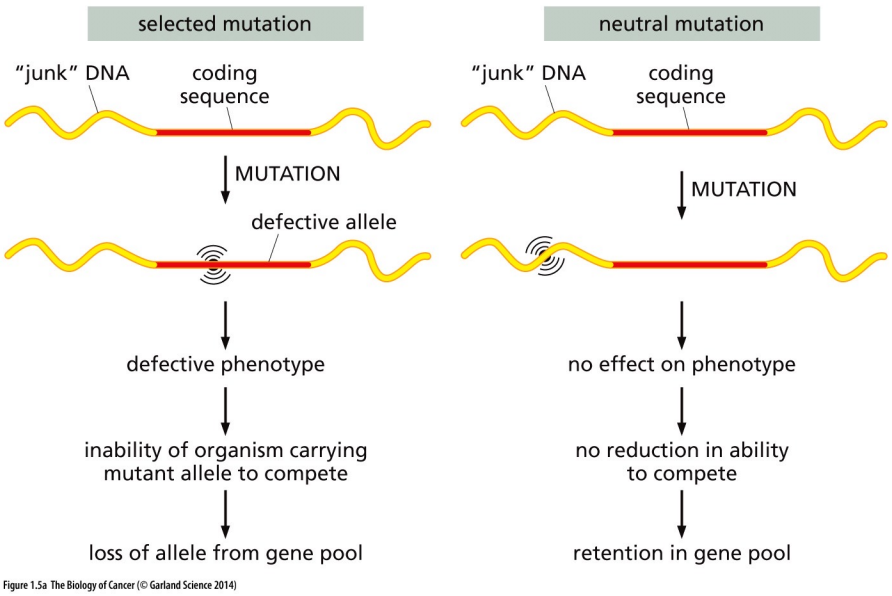

Figure 1.5a The Biology of Cancer (© Garland Science 2014)

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Transmission of Mutations

- Germ cell – sperm and egg: inheritable or new mutation must take place in gonadal tissue
- Somatic cell – All other cells are soma. Genetic alterations (mutations) are passed on to daughter cells but not to offspring

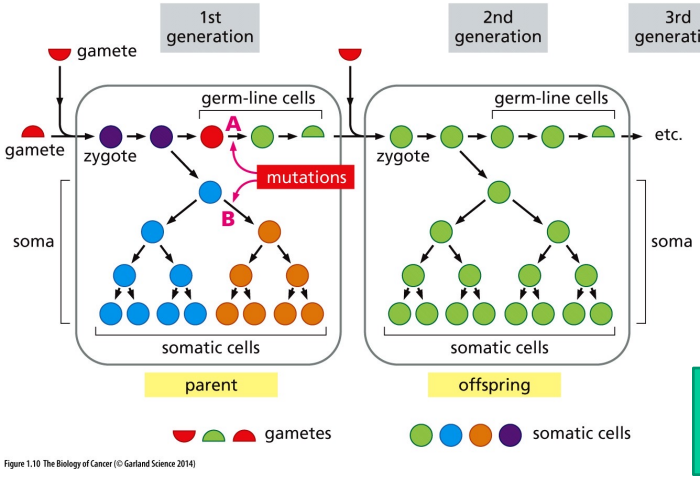

Figure 1.10 The Biology of Cancer (© Garland Science 2014)

Mutation A is inheritable, not mutation B

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Tumors arise from normal tissues

Tumor cells which have invaded and proliferate forming new colonies (tumors) are metastases

- Primary tumor is created from founding tissue
- Tumors which have not breached basal membrane or invaded other tissues are benign
- Tissues that spread are malignant
- Adult Stem Cells – can collect mutations and form tumors

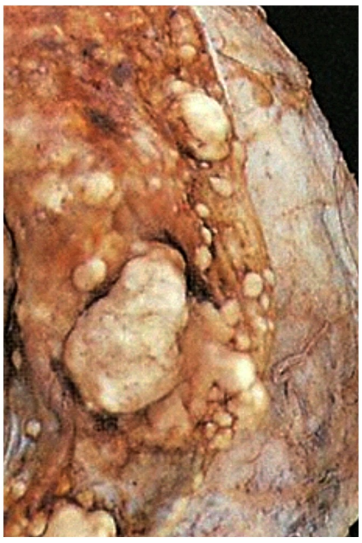

Figure 2.2c The Biology of Cancer (© Garland Science 2014)

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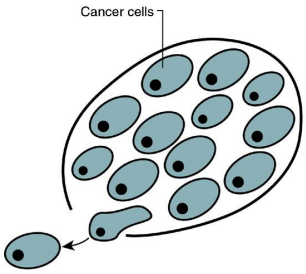
The Reductionist View

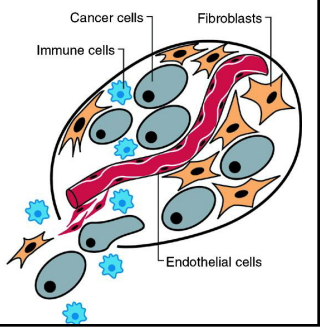


Which View?

Historical bias of cancer – homogeneous tumor cells break free from basal membrane to form metastasis

A Heterotypic Cell Biology

- Tumors are complex tissues: conscription of normal stromal and inflammation cells to act as collaborators of neoplasmic programing.



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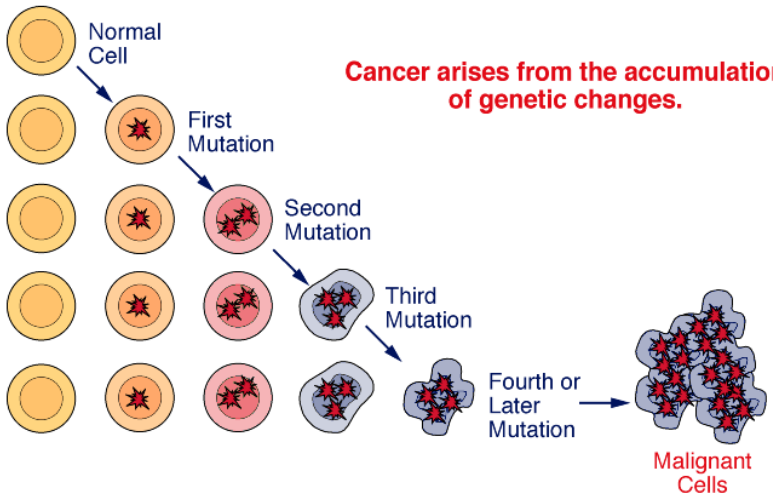
Understanding the Molecular Basis of Cancer



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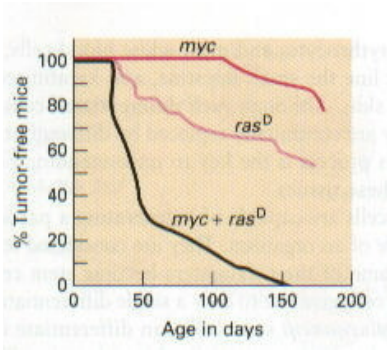
The Process of Carcinogenesis

Carcinogenesis is a multistage event

- **Application of a single cancer-producing agent (carcinogen) does not lead to tumor development**
- **Most cancers are collaborative events**



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Forces That Influence Cancer

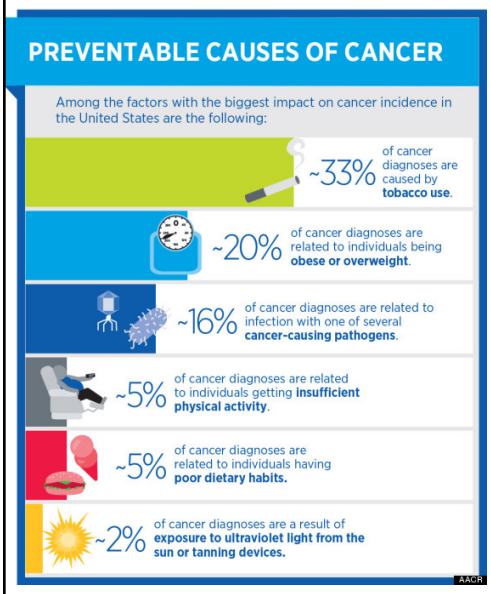
Intrinsic risk factors	Non-intrinsic risk factors	
	Endogenous risk factors	Exogenous risk factors
❖ Random errors in DNA replication	❖ Biologic aging ❖ Genetic susceptibility ❖ DNA repair machinery ❖ Hormones ❖ Growth factors ❖ Inflammation ❖ etc.	❖ Radiation ❖ Chemical carcinogens ❖ Tumour causing viruses ❖ Bad lifestyles such as smoking, lack of exercise, nutrient imbalance ❖ etc.
[Unmodifiable]	[Partially modifiable]	[Modifiable]

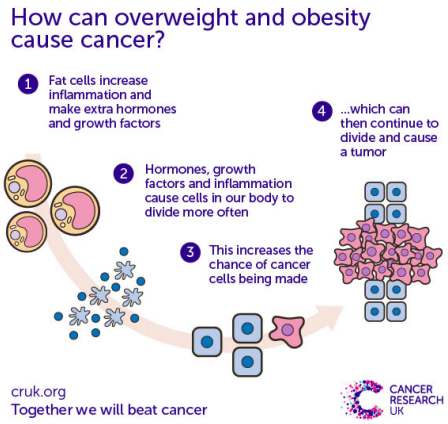
Absolute risk is the chance that a person will develop a disease during a given time. This identifies how many people are at risk for a disease in the general population.

Relative risk compares the risk of disease between two groups of people. It compares one group with a certain risk factor for a disease to another group's risk.

Factor	%
Tobacco	33
Diet	30
Infection	9
Hormones	7
Radiation	6
Occupation	3
Alcohol	3
UV light	1

12





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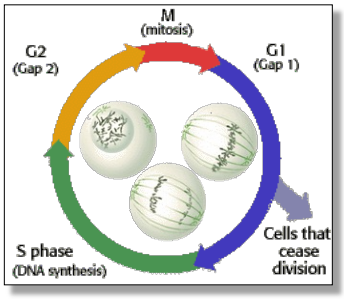
Progression of Cancer Control of the Cell Cycle

Oncogene: "gene capable of causing cancer"

Tumor Suppressor: "anti-oncogene"

On Switch



Off Switch

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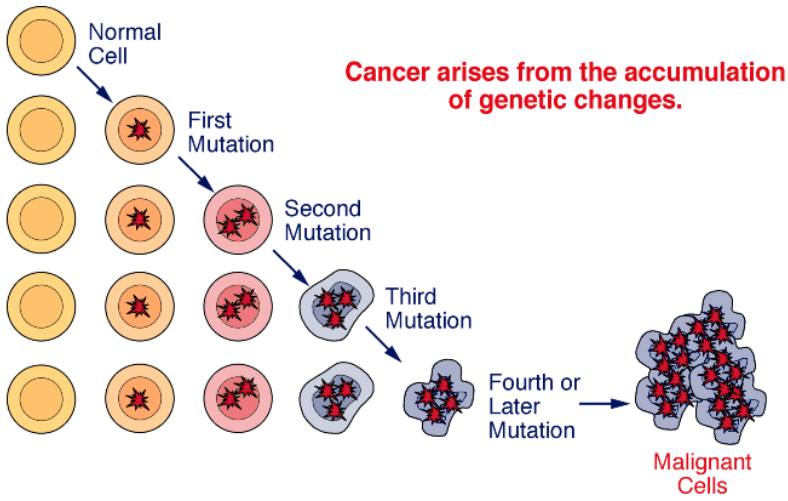
Understanding the Molecular Basis of Cancer



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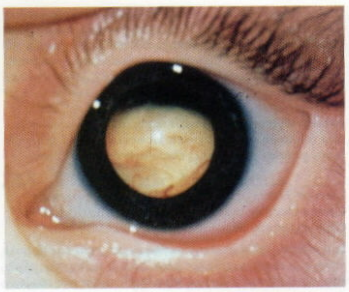
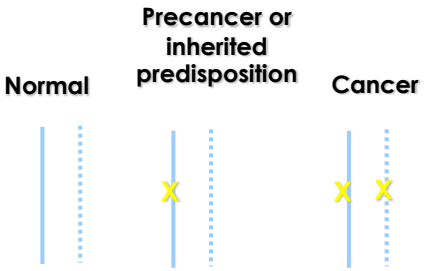
Multiple Hit Theory of Tumor Suppressor Cancer



- 5/100,000 children get retinoblastoma
- 40% of cases are familial remainder result from both genes being mutated



Model for the two hit recessive control of cancer

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Simple Definition

Tumor suppressor gene (aka anti-oncogene) stops cell growth protecting cell from cancer like behavior

- Act in recessive behavior giving rise to the "two hit" hypothesis. Loss of both genes must take place before phenomenon is observed.
- Retinoblastoma is a classic example of tumor suppressor genes
- Some tumor suppressor genes are not recessive but "dominant negative" Mutation of one gene copy will prevent normal function. Typically a dimer were mutation allows dimer to form with wild-type protein but block its effect– p53 is an example

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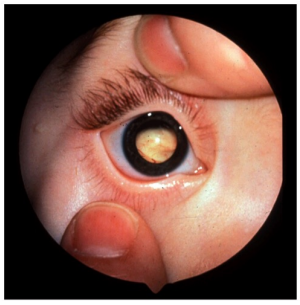
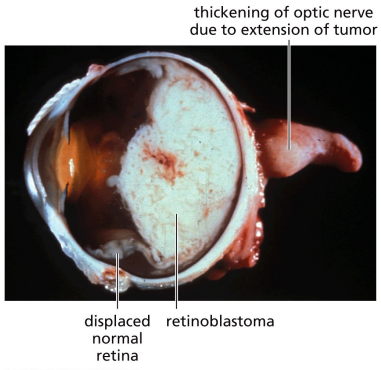
Retinoblasoma

- Tumor arising in youth very rare – 1 in 20,000. Occurs early. Tumor within the eye causing blindness.
- Most treatment by radiation or removal of the eye





Two forms.

- Those with no family history (**unilateral**) have tumors in one eye and is considered sporadic. No further risk of other tumors later
- Familial form (**bilateral**) often have tumors in both eyes and have greater risk of other tumors forming at distal sites

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non-retinal tumors of retinoblastoma patients

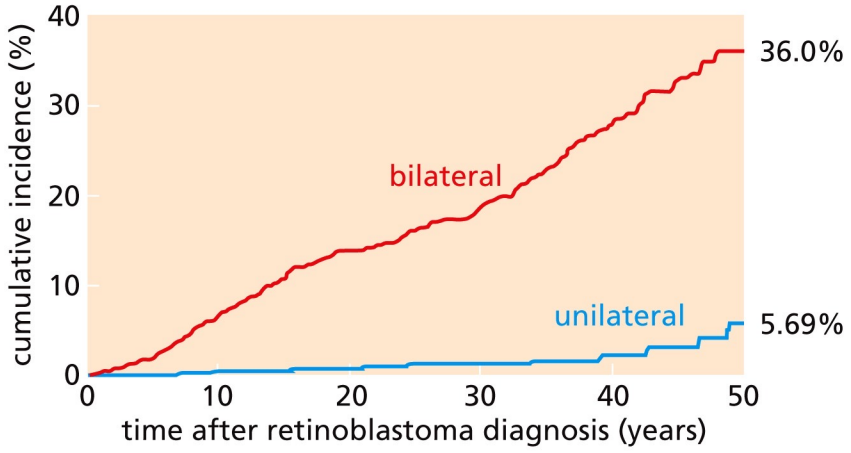

Figure 7.4a The Biology of Cancer (© Garland Science 2014)

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Kinetics of age and frequency

Suggest unilateral result from a single event while sporatic tumors require two random mutations/events.

Lead to speculation of an unknown gene called Rb.

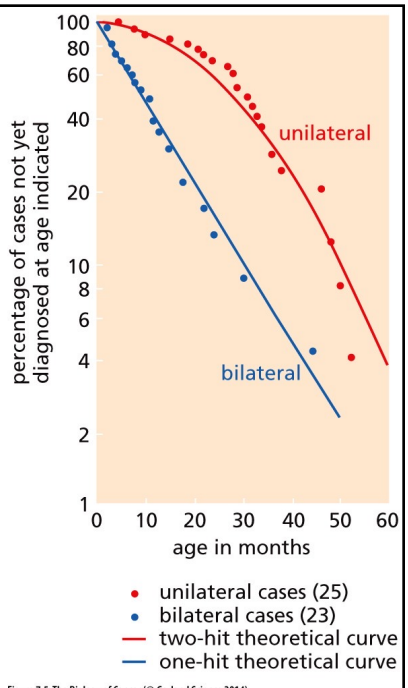

Figure 7.5 The Biology of Cancer (© Garland Science 2014)

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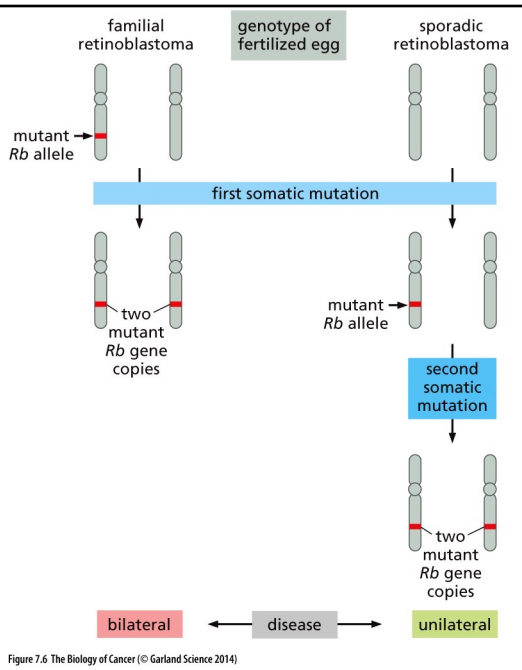

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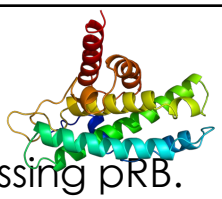
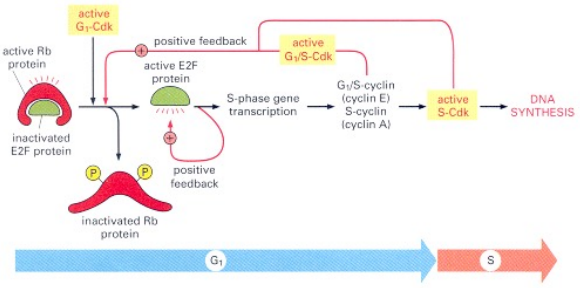
Rb function



- Retinoblastoma – RB1 gene expressing pRB.
- Phosphorlyated transcription REPRESSOR that blocks Go-G1 transition.
- Binds and blocks E2F TF which drives DNA replication. E2F is target of several oncogenes



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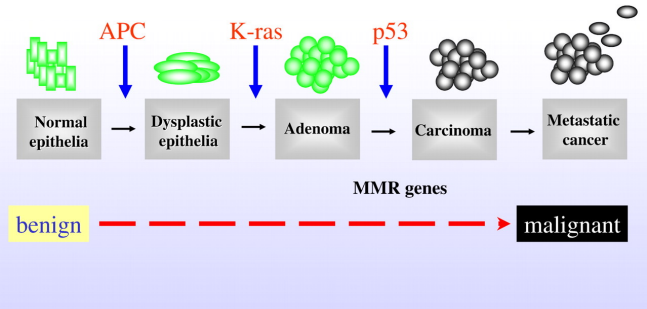
Another Example of Tumor Suppressor Genes
APC – Colon Cancer

Colon cancers – longest part of the intestine. Most are secreting mucus cell cancers (adenocarcinomas)

Often combined with rectal cancer (last several inches of intestine) for colorectal cancer

Most CR cancers show no or little familial association ~5% are familial adenomatous polyposis (FAP)



APC- Adenomatous polyposis coli. Tumor suppressor that when defective, makes the cell more susceptible to cancer

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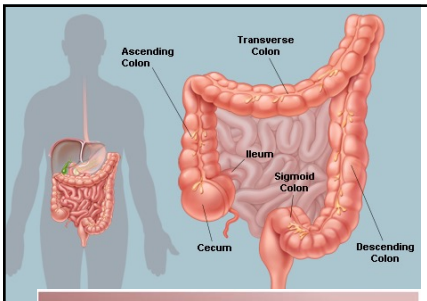

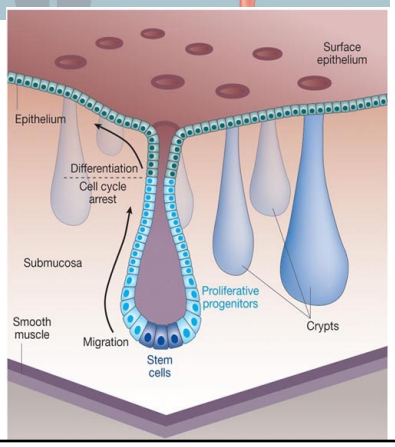

Your Colon

Colonic crypt cells provide new cells as stem cells at the bottom of the deep "cave" divide – retaining one daughter stem cell and a second daughter cell that is differentiated

Differentiated cells migrates to luminal surface (epithelial cells) of the colon to secrete mucus and serve as the lining of the gut

Most cells die within 3-4 days.

Cells at surface face harsh environment of mutagenic compounds from diet, radicals from oxidation and other harsh typical conditions of intestine (pH ect...)

Mutagens occur often in these cells but quickly die before progressing to cancer cells – thus only cancers that can happen will stop the out-migration of epithelial cells where additional lesions can induce proliferation and tumor formation can take place… APC

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Wild-type stromal cells (fibroblasts, inflammatory cells and endothelial cells) secrete growth factors, cytokines and other agonists including Wnt, to induce cell growth.

The paracrine stromal cell signaling in crypt activates Wnt signaling leading to growth of stem and differentiated cells

Wnt increases ß-catenin and decreases APC protein

ß-catenin ***increases*** proliferation and decreased differentiation (more stem cell like)

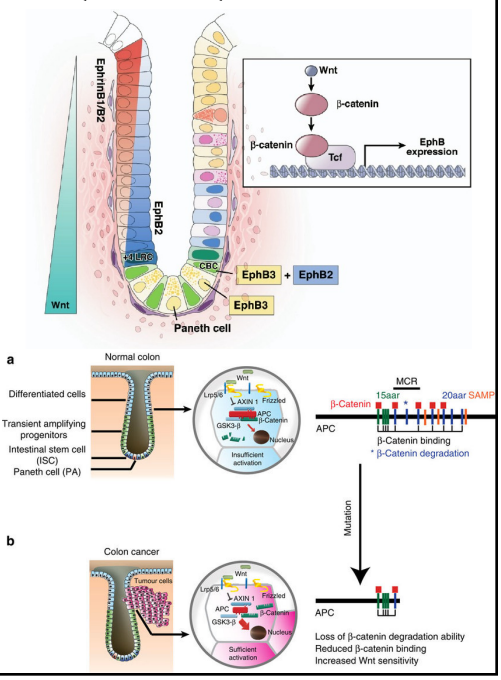
APC – causes the degradation of ß-catenin

Luminal cells less Wnt, -> less ß-catenin and more APC

Leading to loss of proliferation (cell cycle) and more differentiated (less stem cell/cancer like) behavior

Cancer Stromal Cells Tumor suppressor APC mutations do not reduce ß-catenin causing greater proliferation and less differentiation. Allow build up of additional mutations for full development of tumor mass – multiple polyps

Wnt, catenin, APC & cancer



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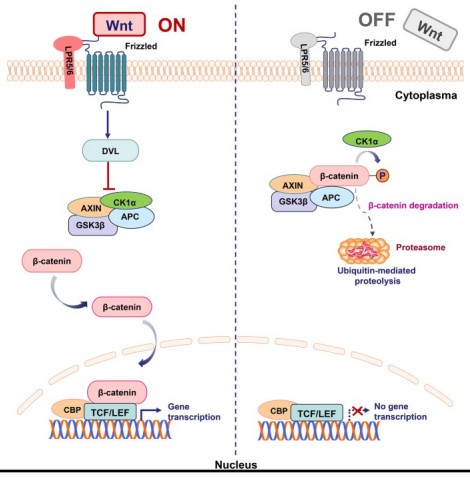
Wnt and ß-catenin Signaling

In the **ABSENCE** of Wnt, ß-catenin is phosphorylated and targeted for proteolysis

In the **PRESENCE** of Wnt, ß-catenin is not phosphorylated and remains intact and binds to TF factors



Half life of ß-catenin is about 20 min – w/phosphorylation; 1-2 hours after phosphospo.

Together Wnt signaling and ß-Catenin are involved in a large number of cell regulation events and loss of control leads to many diseases/disorders

APC in polyps, high Wnt signaling in several breast cancers

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Tumor Suppressor APC Gene Inactivation

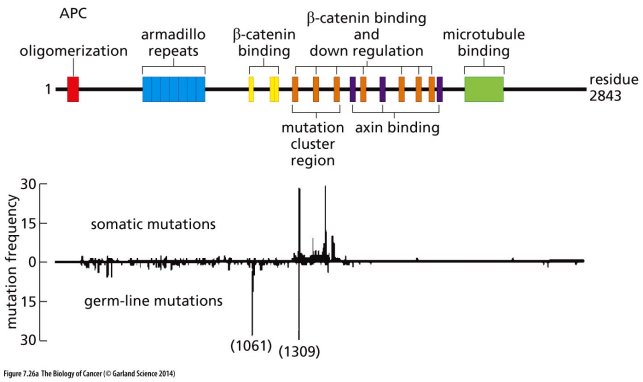

Figure 7.26a The Biology of Cancer (© Garland Science 2014)

Most common mutations are mapped to regulatory sites of APC protein -> truncated APC or non-protein binding APC

Some colon polyps show hypermethylation in APC gene decreasing APC expression

Results in accumulation of ß-catenin in 90% of sporadic colon carcinomas

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Cancer Staging

Staging is used to determine the severity of the cancer – use TMN system

Tumor – size extent and location (0-4)

Nodes – lymph node involvement (0-3)

Metastasis – Presence or absence of distant metastasis (0 or 1)

Primary Tumor (T)	TX	Tumor cannot be evaluated
	T0	No evidence of primary tumor
	Tis	Carcinoma in situ (early cancer that has not spread locally)
	T1, T2, T3, T4	Size and/or extent of tumor
Regional Lymph Nodes (N)	NX	Nodes cannot be evaluated
	N0	No nodal involvement
	N1, N2, N3	Nodal involvement (number/extent of spread)
Distant Metastasis (M)	MX	Cannot be evaluated
	M0	No evidence of metastasis
	M1	Metastasis

A number may be added to each letter to indicate size or spread of tumor

A T3N2M0 tumor is large, with local nodes but no evidence of metastasis

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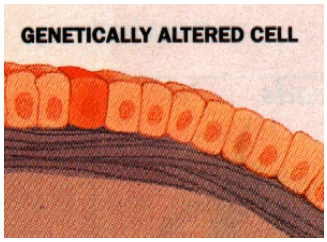
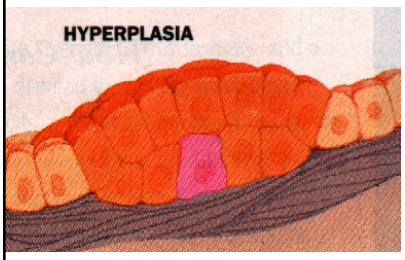
Tumor development occurs in stages



1) **Genetically altered cell -** tumor development begins when a single cell within a normal population sustains a genetic mutation that increases when it would normally rest



2) **Hyperplasia**
The altered cell continues to grow and the daughter cells continue to look normal but they produce too much - after years some of these cells suffer another mutation that further loosens controls on cell growth

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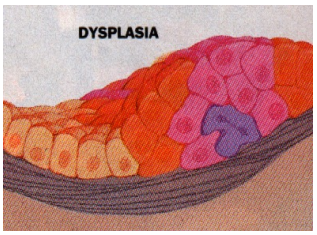
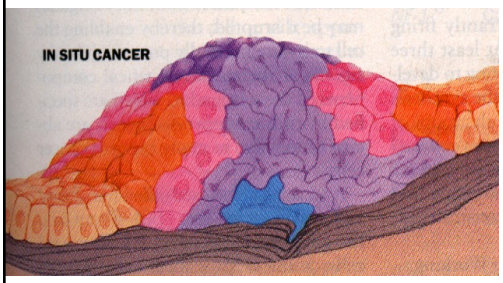


3) **Dysplasia**
In addition to proliferation excessively, the mutated cells begin to appear abnormal in shape and orientation - morphology changes; After time an additional mutation occurs



4) **In situ cancer**
The effected cells become still more abnormal in growth and may or may nor have begun to lose containment in the original tissue. Additional cells gain another mutation

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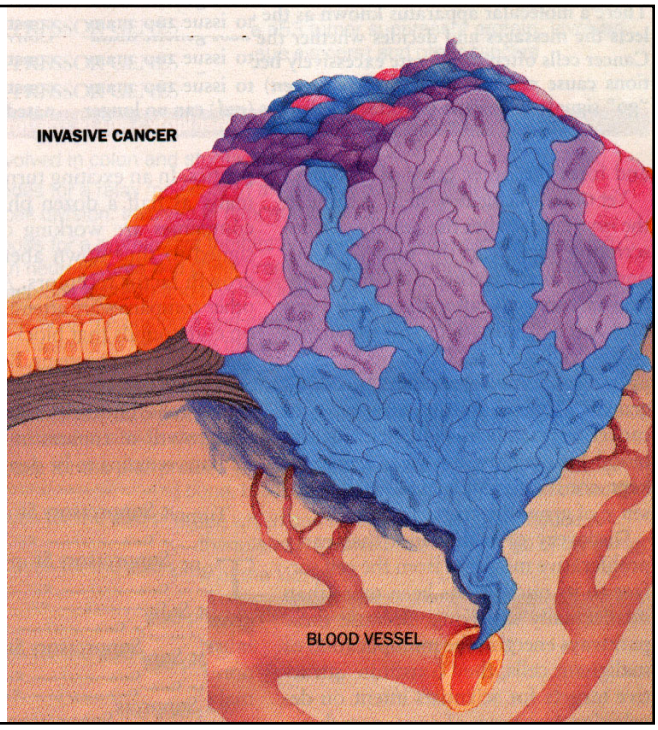
5) **Invasive cancer** if the genetic changes allow the tumor to begin invading underlying tissue and to shed cells into the blood stream or lymph, the mass is considered to have become malignant. The renegade cells are likely to establish themselves throughout the body; these may become lethal by disrupting a vital organ



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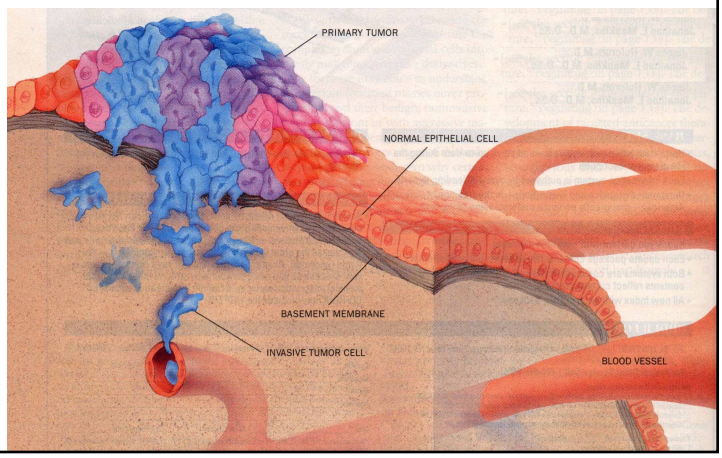
Invasion and Metastasis - the method which spreads cancer through out the body. First cancer cells detach from the primary tumor and breach the basal membrane surrounding a blood vessel and are free to circulate via the blood stream. Eventually a cancer cell may lodge in a capillary or lymph and create a secondary tumor.

- Less than one in 10,000 cancer cells that escape the primary tumor survives to colonize another tissue



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Organization of Tumor cells

Epithelial tissues give rise to most cancer types

- Sheets of cells that line inner or outer walls of organs and surfaces of the body
- These cells come from endodermal / ectodermal germ layers
- ***Cancers from epithelial tissues are called "carcinomas"***

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Epithelial Carcinomas

Protective layer cells which form tumors are squamous cell carcinoma – skin (keratinocytes) are an example

Epithelial cells which secrete substances form adenocarcinoma cancers

Tissue sites of more common types of adenocarcinoma	Tissue sites of more common types of squamous cell carcinoma	Other types of carcinoma
lung colon breast pancreas stomach esophagus prostate endometrium ovary	skin nasal cavity oropharynx larynx lung esophagus cervix	small-cell lung carcinoma large-cell lung carcinoma hepatocellular carcinoma renal cell carcinoma transitional-cell carcinoma (of urinary bladder)

Table 2.1 The Biology of Cancer (© Garland Science 2014)

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More definitions

Stromal cells – connective tissues of any organ and supports tissue surrounding other tissues and organs – produce connective proteins, extracellular matrix and secrete factors

- In cancer, changes in the stroma drive invasion and metastasis – malignancy. Together with the tumor cells, stromal cells are a critical part of the tumor cell microenvironment.
- These cells are not cancerous but support cancer growth and determine location of metastatic disease. Bone, breast, other tissue stromal cells secrete different proteins and factors supporting specific tumor metastasis
- Potential target for chemotherapy

Basement membrane – non cellular region of tissues that separates epithelium from underlying connective tissue – matrix (mixture of proteins – not cells)

Lumen cells – Those epithelial cells which face the hollow core (inside space) of a cavity

Fibroblast cell – Synthesize extracellular matrix and collagen providing structural framework for stromal cells to grow – have potential to mature into different cells "blast" chondroblast, collagenoblast or osteoblast

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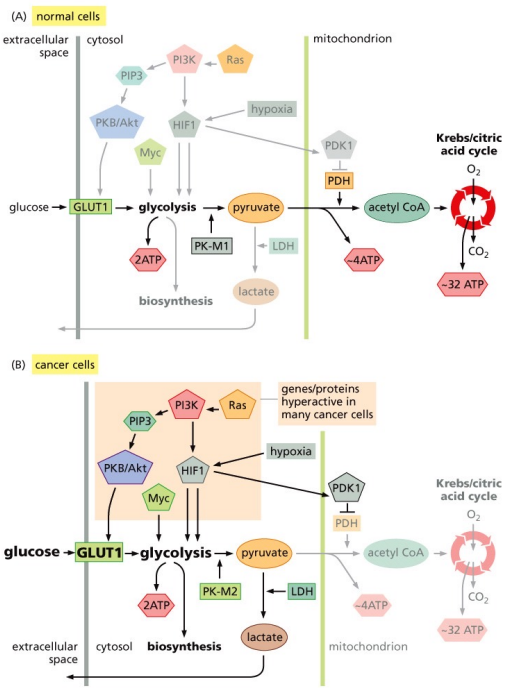
Warburg Effect

Hypoxia – anaerobic metabolism in tumor cells before angiogenesis

- Low ATP production but faster than Krebs cycle
- Formation of glycolytic intermediates for biosynthesis
- Takes place in tumor cells even when oxygenated

Pyruvate Kinase – M1 isoform usually expressed in most tissues (not liver) M2 form mostly in embryonic tissue

- M2 is expressed in carcinomas – M2 has slower Kcat – turnover rate: "backs up" glycolysis forcing lactate production
- Resulting in acid production



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Otto Warburg

Observed in 1924 that cancer cells use aerobic glycolysis to fuel growth instead of oxidative phosphorylation

Won the Nobel Prize in 1931

Advocated that: "replacement of oxygen-respiration by fermentation is the prime cause of cancer"

The metabolic view of cancer predominated thinking from 1920's up to the 1960's and most cancer therapies were called "anti-metabolites"

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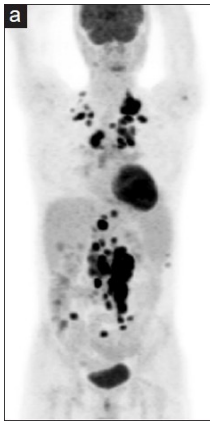
Cancer is a Metabolic Disease

Cancer cells consume 100-200X more glucose that other cells in the body

This unique metabolism is the basis to PET (positron emission tomography) scans for cancer using fluorinated deoxyglucose

This metabolic shift is called the Warburg effect or cytosolic aerobic glycolysis



Tumors are marked in black in this PET image (lots of glucose)

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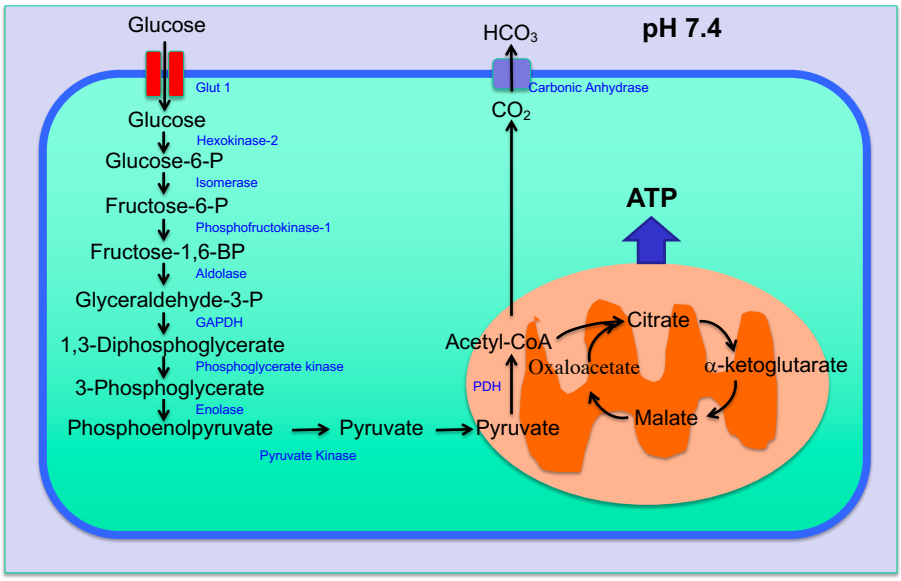
Normal Cell Metabolism



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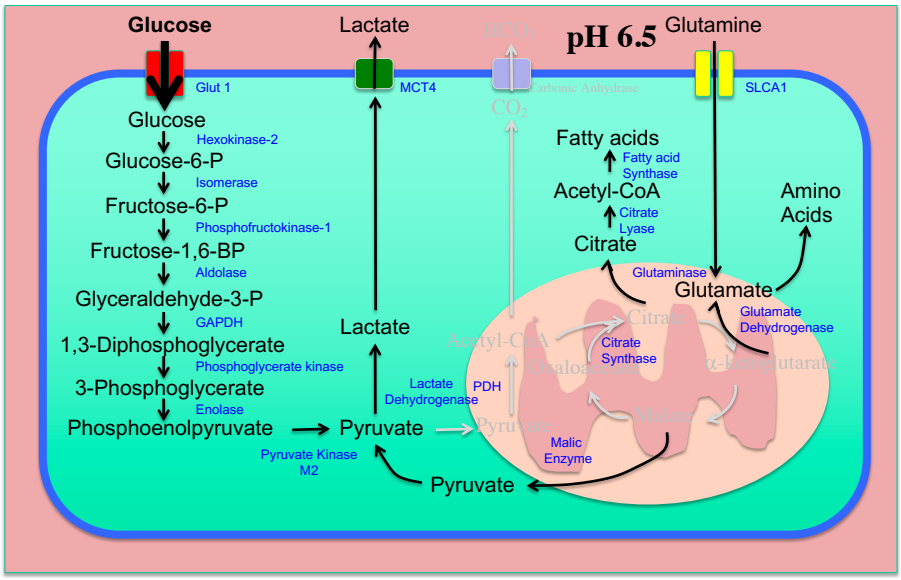
Cancer Cell Metabolism



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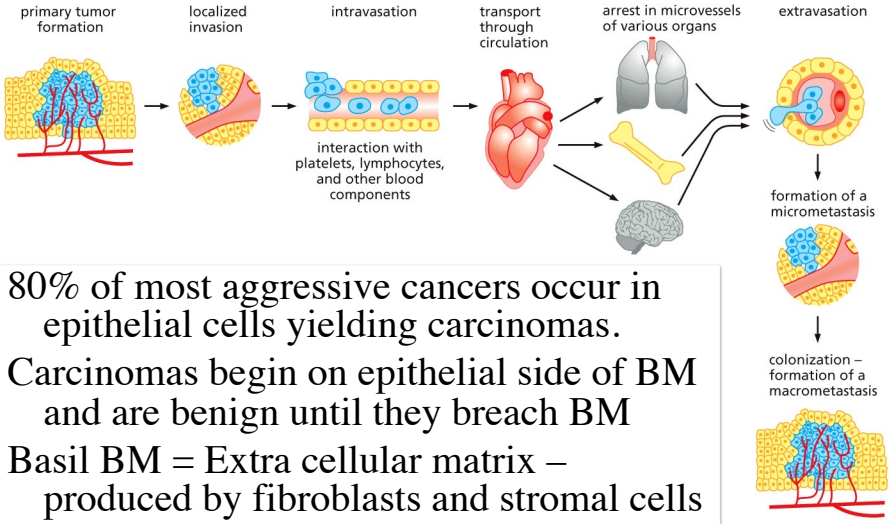
Invasion – Metastasis Cascade



80% of most aggressive cancers occur in epithelial cells yielding carcinomas.

Carcinomas begin on epithelial side of BM and are benign until they breach BM

Basil BM = Extra cellular matrix – produced by fibroblasts and stromal cells

Once crossing BM cells are malignant

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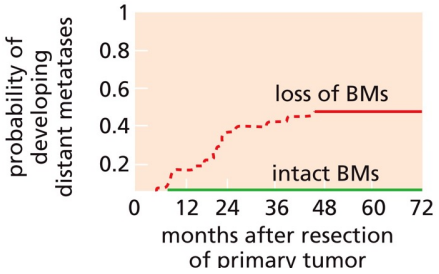
Invasion and Metastasis: Breaching the BM

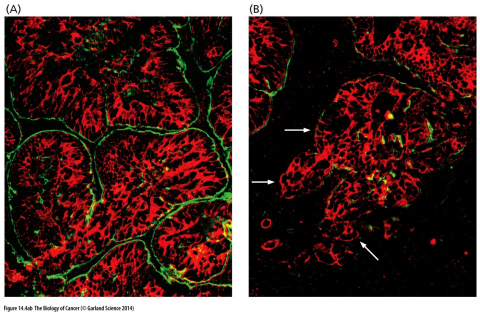
Angiogenesis begins before BM is degraded

But once invading through BM carcinomas gain access to stromal cells, agonists and better involvement with blood vessels and lymphatic system

Loss of BM can predict future course of metastatic disease



High grade carcinomas T3 and correlation with BM loss. Survival after surgical removal of primary tumor



A) Less aggressive/differentiated colorectal carcinoma islands (red anti cytokeratin Ab) and BM (green laminin Ab). Stromal cells are not labeled (black)

B) Less differentiated aggressive carcinoma staining – arrows highlight loss of BM and stromal/carcinoma interaction

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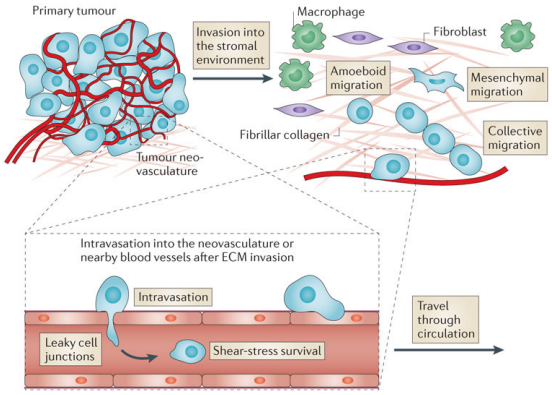
Intravasation



Primary cancer cells entering the blood (or lymph) system is Intravasation.

Crossing the endothelial barrier during metastasis
Nicolas Reymond, Bárbara Borda d'Água & Anne J. Ridley
Nature Reviews Cancer **13**, 858–870 (2013) doi:10.1038/nrc3628

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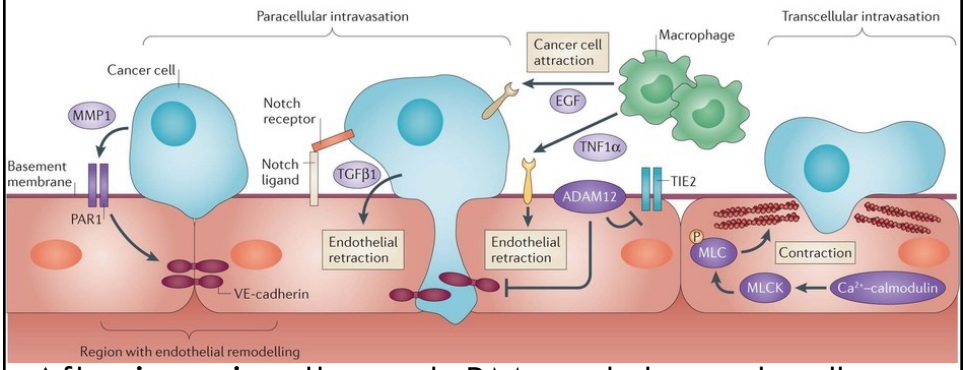

After invasion through BM and stromal cells, cancer cells enter by remodeling endothelial lining of blood vessel – between or through cells

Proteases degrade cadherin and gap junctions inducing cell retraction

Actin contraction is stimulated by MLC kinase and calcium calmodulin signaling

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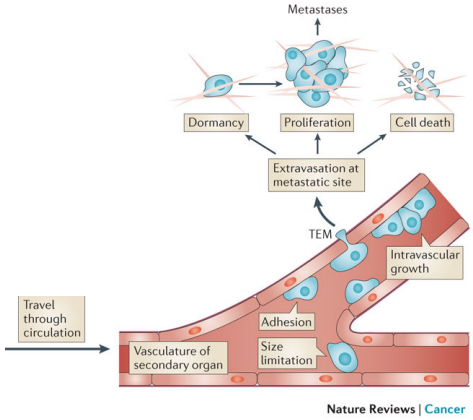
Rough Travels



Lack of contact (many tumor cells maintain some requirement), loss of stromal cell factors, sheer factors and size of vessels (cancer cells > 20μM while capillaries ~3-8μM) create hostile environment for most escaping tumor cells

Most cells die by anoikis (form of apoptosis)

Circulating tumor cells often become trapped in lungs – but many do not stay as they find through to tissue capillaries or move within tissues

Complicating passage is many tumor cells recruit and macrophages/platelets – found as multicellular aggregate

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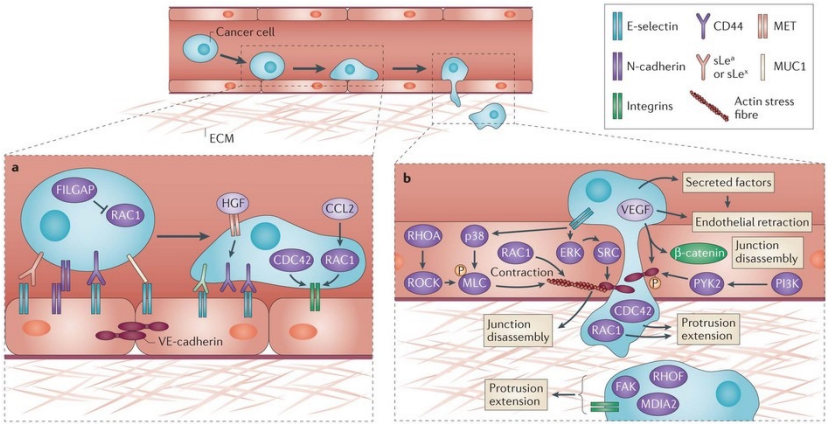
Time to leave - Extravasation



Receptor mediated attachment of cancer cell to endothelial cells induce small G proteins (Ras, Rac, Rho) to re-arrange cancer cell shape and allow protrusion and tail retraction

Receptors also induce expression and secretion of factors (integrin) and proteases to allow for junction disassembly of endothelial cells.

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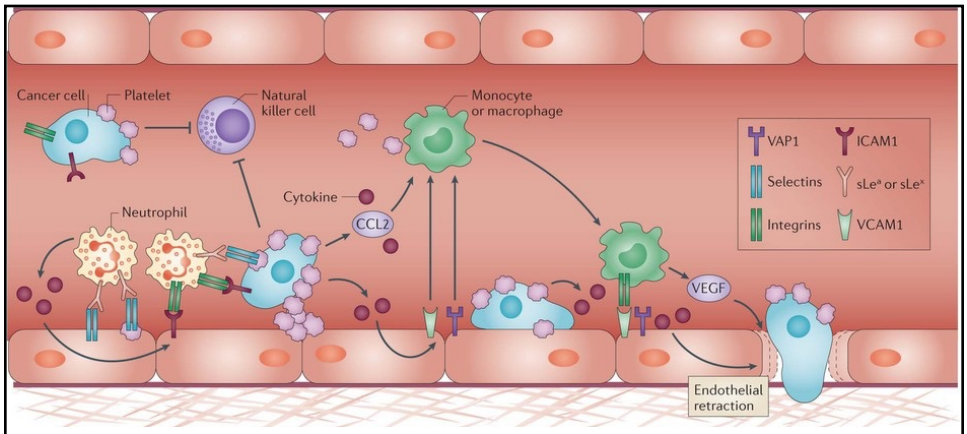

Cancer cell interaction with platelets protect tumor cells against immune lysis by natural/hunter killer cells.

Platelets secrete factors and help recruit neutrophils which secrete pro-inflammatory cytokines and help adhesion.

Cancer cells help the process by release of cytokines which drive endothelial retraction and extravasation by VEGEF and other factors

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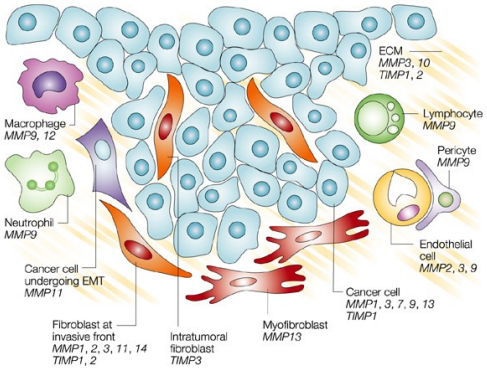
Proteases and Invasion/Metastasis

The ability to invade and metastasize depends on removal of the ECM barrier for cells to "move into" during EMT transition

MMPs matrix metalloproteinases – degrade extracellular protein of ECM

- ECM includes growth factors and other proteases inactive as pro-hormones/enzymes that are released after degradation
- Stromal cells, macrophages, mast cells, fibroblasts and tumor cells secrete MMPs
- Targets include collagen, fibronextin, tenascin, and proteoglycans



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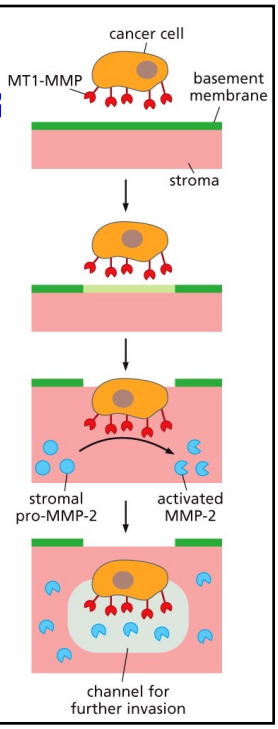
MMPs Produced by tumor/tumor associated cells

- BM creates network of proteins with small pore size – too small for most cancer cells to fit through
- Invadapodia – regions at leading edge of cell where MMP is secreted or tethered to membrane producing localized BM degradation
- Stromal cells release or activate MMP2/9 to degrade collagen coordinating with tumor cells MT-MMP
- Acidification of ECM by tumor cells activates MMP and supports secretion



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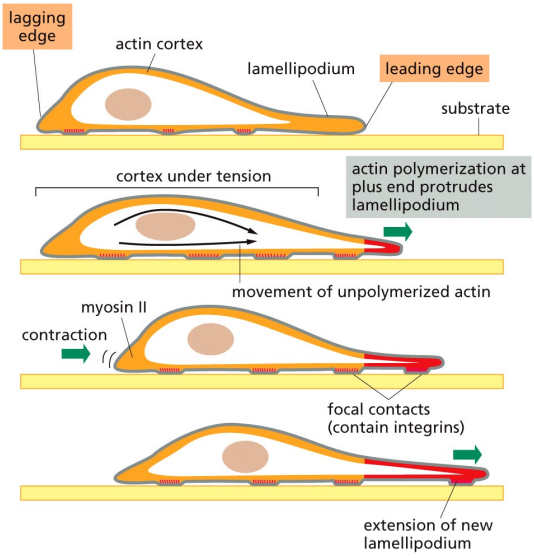
Cell motility requires coordination of many proteins – driven by small G proteins

Cell motility involves cytoskeletal protein at both leading and trailing edges.



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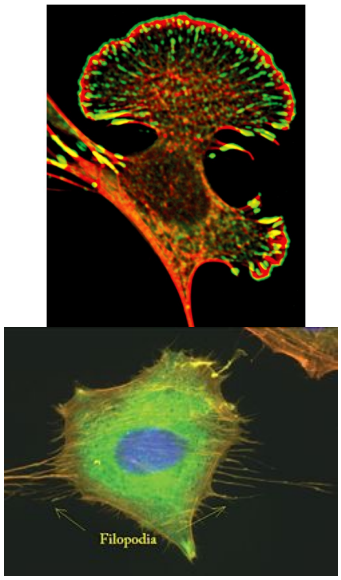
Grab on, Let go, Get away

- At the leading edge of a motile cell – actin polymerizes (stress fibers) to form subcell structures and form focal adhesions.
- MMPs lead the edge
- Membrane proteins anchor stress fibers by linker proteins
  - Often exchange proteins
  - Linker proteins ERM family
- Lamellipodia – broad sheetlike protrusion (ruffles)
- Filopodia are small spikes from lamellipodia



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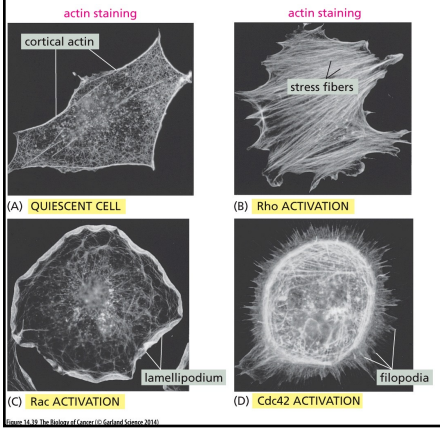
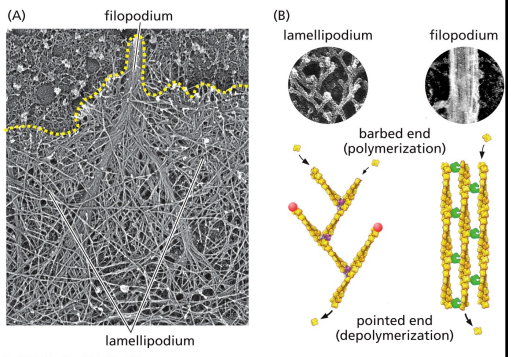
Actin Stress Fibers – regulated by SMG

- RhoA kinase activates machinery to grow the barbed end





- Rac and CDC42 both differently impact cell structure

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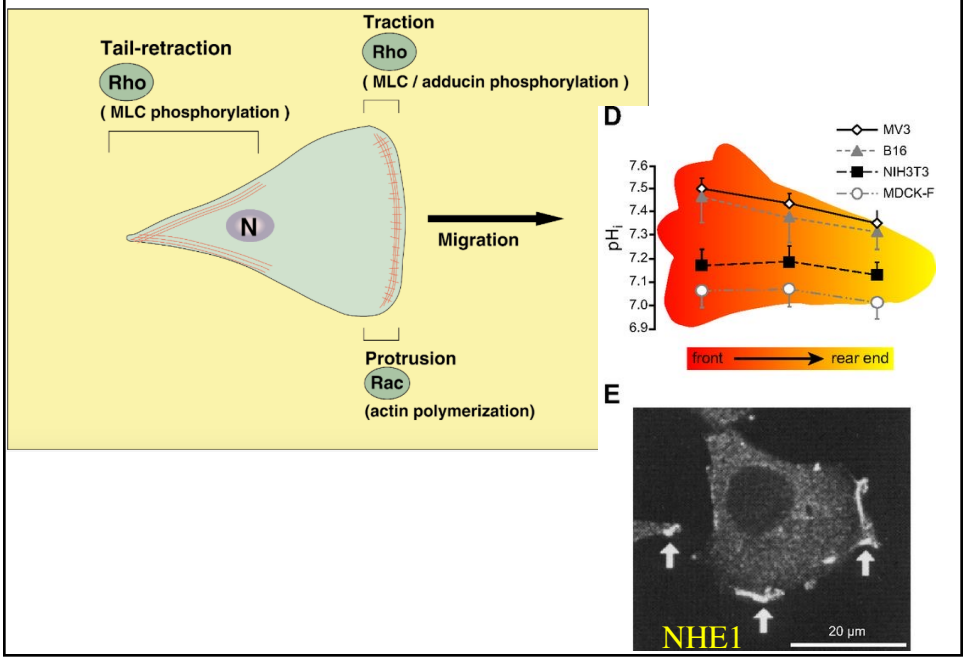
Cell Polarity and Motility



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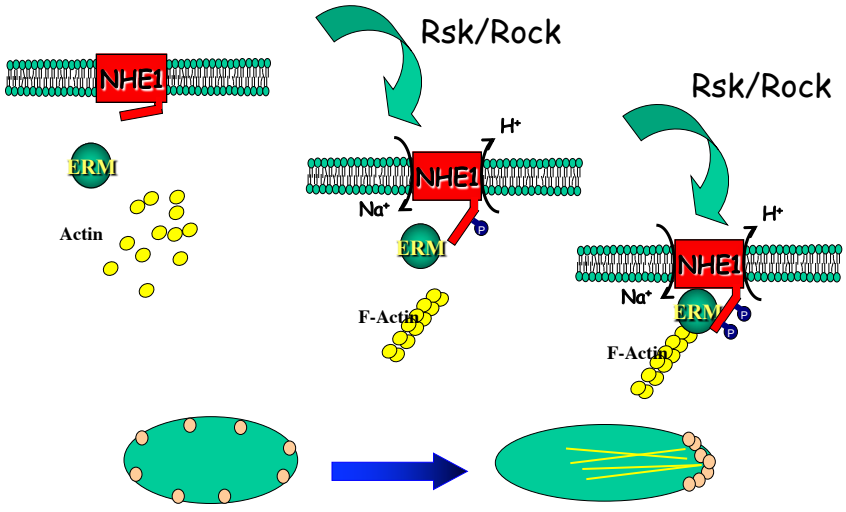
NHE and Migration



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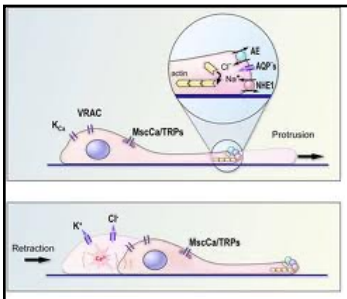
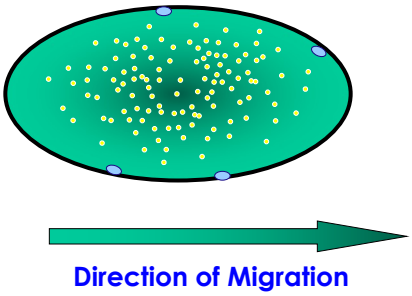


Focal Adhesions and Stress Fibers - Invadapodia

- NHE1 collects at the leading edge of migrating cells
  - ✓ Activates actin polymerization
  - ✓ binds actin filaments driving cell migration
- NHE1 is a major participant supporting the hallmarks of cancer



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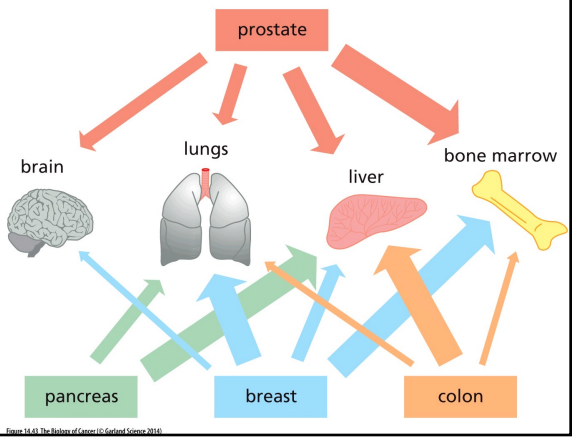
Now that tumors are moving… where do they move to?

Colonization of micromatastasis is very inefficient where most never survive or remain in stasis.

Tumors seem to target organs with highly specific stromal cell/supportive environment.

- Endothelilial cells exert an influence on ability of carinoma cells to form petastasis in specific organs
- Some tissues require fewer changes for metastasis to adapt
- Seed and Soil Hypothesis



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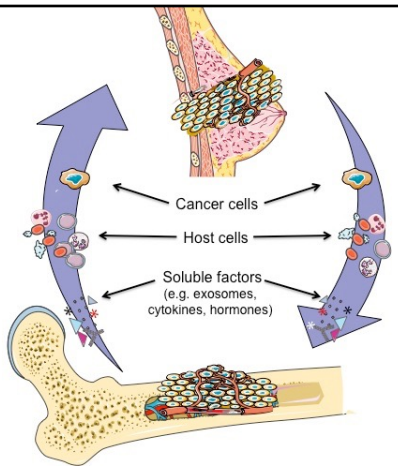

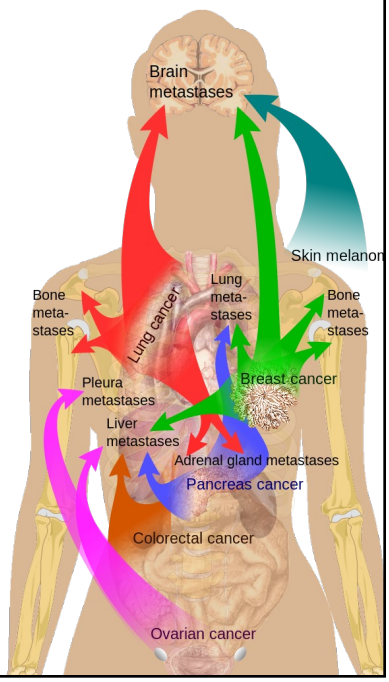

Hypothesis is that patterns of metastasis formation in patients could not be explained by random scattering or by patterns of cells through circulation

A plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil

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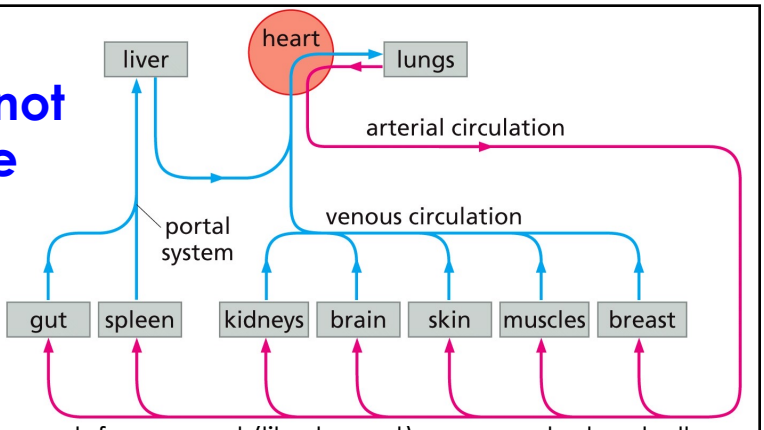
…but not quite



This seems to work for several (like breast) cancers but not all.

Colon cancers may settle in liver simply due to proximity via portal vein – trapped in small capillary beds – sheer numbers of cells influence ability to form distal tumor sites

Secondary breast tumor development in lungs is poor but happens due to frequency of implantation/extravasion

Secondary issue is more likely metastatic tropism – which involves both filter and microenvironment creating a permissive site for colonization

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Metastasis to bone – an example of seed and soil

- Osteotropic cancers: ~50-70% of patients with breast and prostate cancer metastasize to bone (lung and kidney cancer at high levels too)
- Once bone is colonized, metastasis are incurable and result in morbidity (rate of sickness or symptoms of disease) prior to patient death.
- Bone metastases leads to significant pathological fractures, pain, nerve compression and hypercalcemia.
- Treatments are mostly palliative (treat pain not disease)

Bone remodeling is a constant event – bone (collagen matrix with calcium phosphate and metal ppt – hydroxyapatite) is dissolved and replaced by two sets of cells – up 10% of skeletal mass is dissolved and replaced each year

Osteoclasts – derived from precoursor phagocytes and are responsible for bone resorption

Osteoblasts – specialized cell from stromal lineage responsible for laying down new matrix (collagen and other matrix)

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