Cancer Biology

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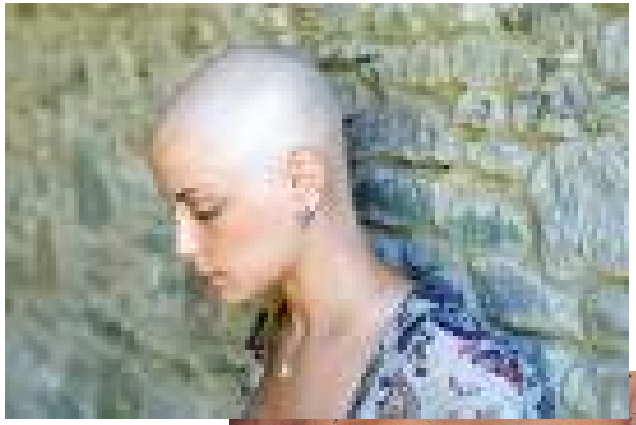
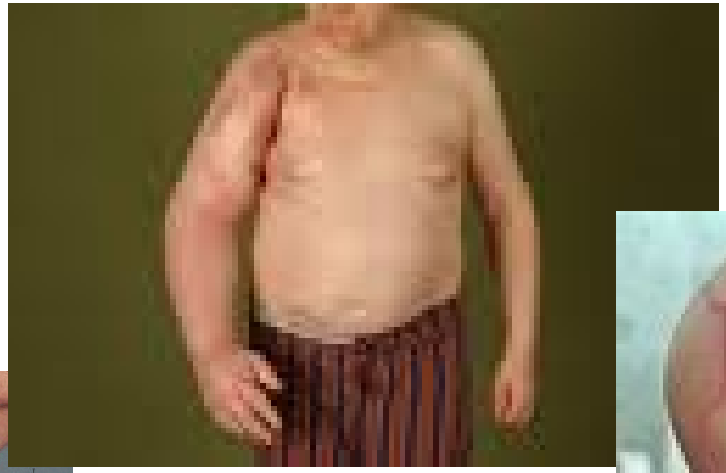
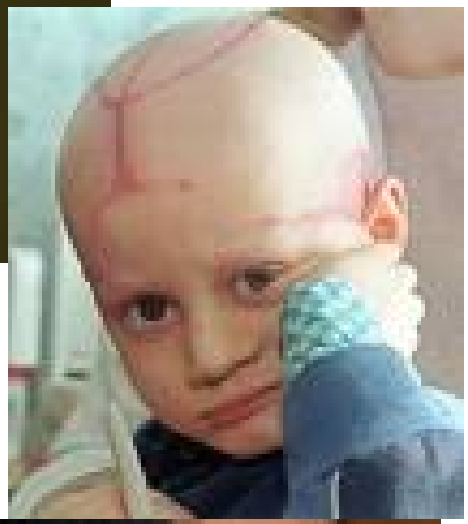
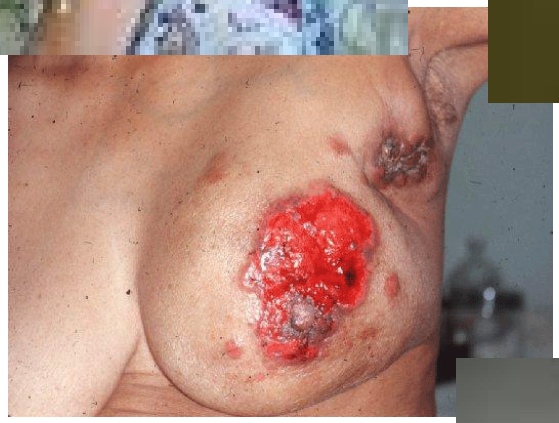
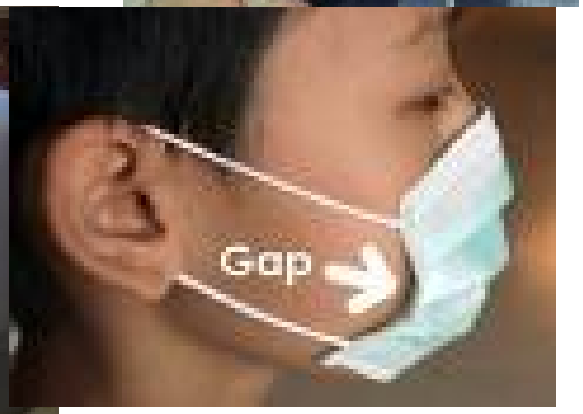
Why learn about cancer?







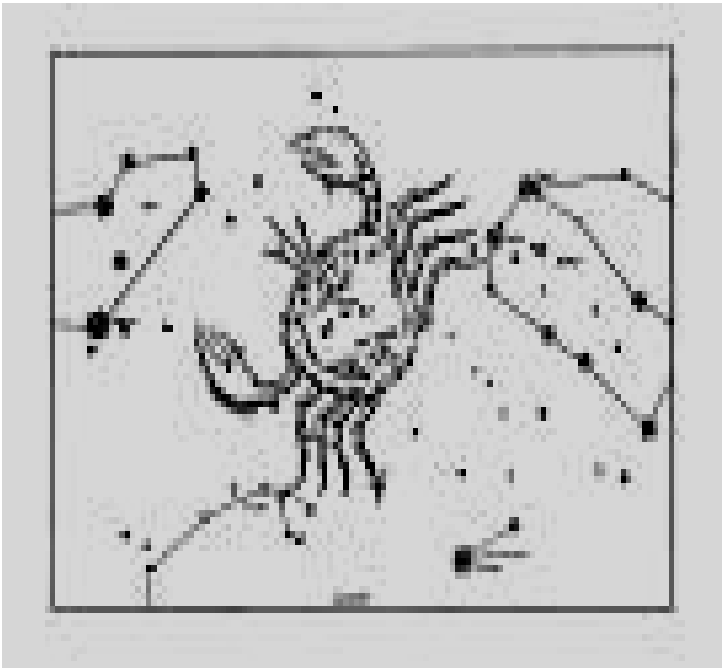




Most forms of cancers are sporadic!

Cancer

- Derived from Greek word for crab, *karkinoma*
- Malignant tumor are ambitious. They have two goals in life: to survive and to conquer new territory – metastasizing.



Definition of Cancer

A class of genetic diseases, in that aberration of key genetic and resultant molecular pathway are critical for carcinogenesis. Cancer is not a single disease, but many with related features

Such key events have been called hallmarks of cancer.

There is also increasing evidence for a major role of epigenetic aberrations in cancers

Hanahan D, Weinbery RA. The hallmarks of cancer. Cell 2000;100:57-70

Definition of Cancer

(Continued)

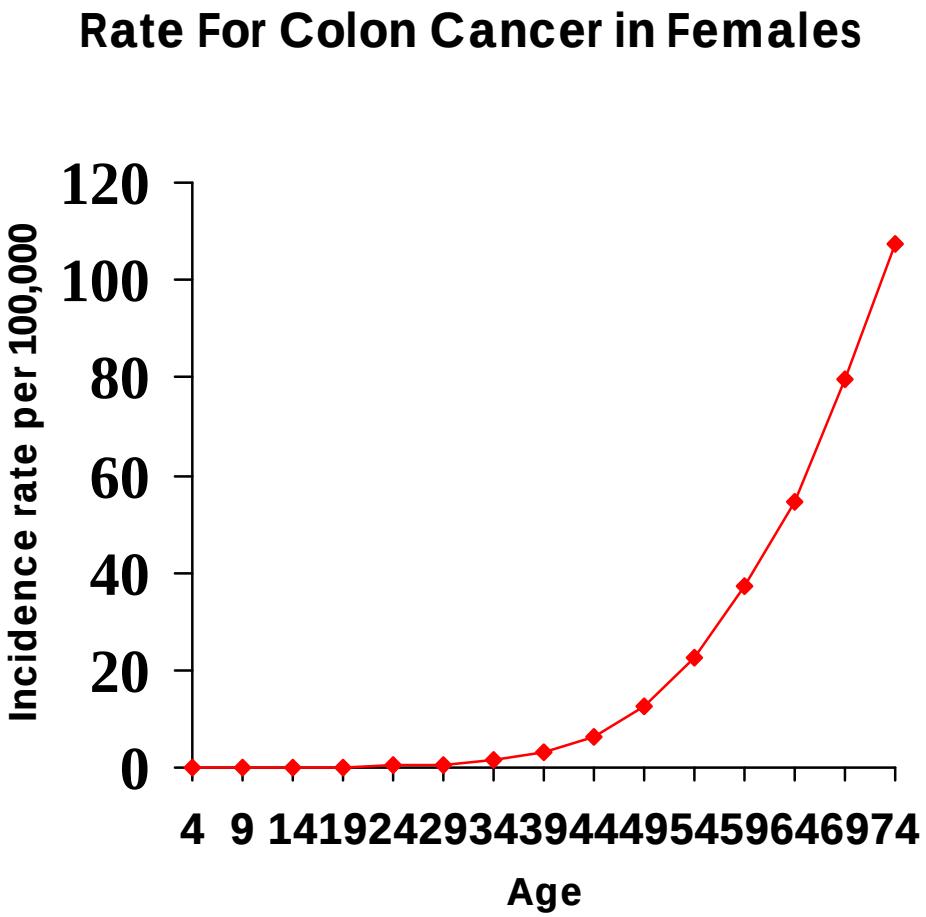
A class of genetic disease caused by disregulation of various cellular pathways that orchestrate cell growth and death.

It is clear that some of these pathways must modulate cellular metabolism.

Uogelstein B, Kinzler K.W., Nat Med.10,789 (2004)

Cancer is a disease of age

- Most cancers develop late in life.
- Cancer did not become a major societal challenge until the middle 20th century when life expectancy rose due to better nutrition, sanitation, and improving medical care.



Cancer

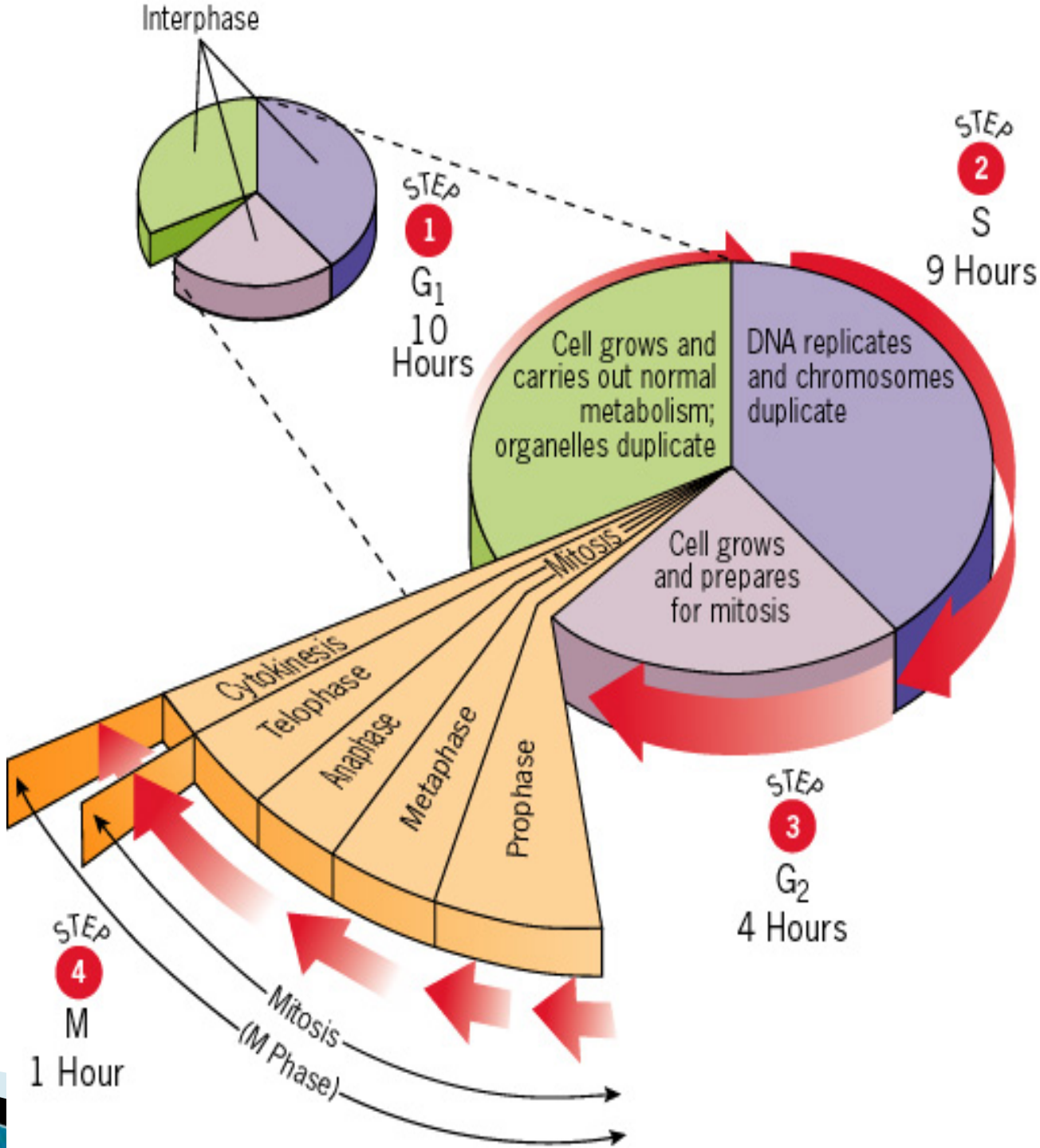
- Cancer is one of the most common diseases in the developed world:
- 1 in 4 deaths are due to cancer
- 1 in 17 deaths are due to lung cancer
- Lung cancer is the most common cancer in men
- Breast cancer is the most common cancer in women
- There are over 100 different forms of cancer

Cancer

- The division of normal cells is precisely controlled. New somatic cells are only formed for growth or to replace dead cells.
- Germ cells arise via meiosis , a process that uses many of the same intracellular components as mitosis.
- Cancerous cells divide repeatedly out –of– control maner even though they are not needed. They crowd out other, normal cells and function abnormally. They can also destroy the correct functioning of major organs.

Cause of Cancer

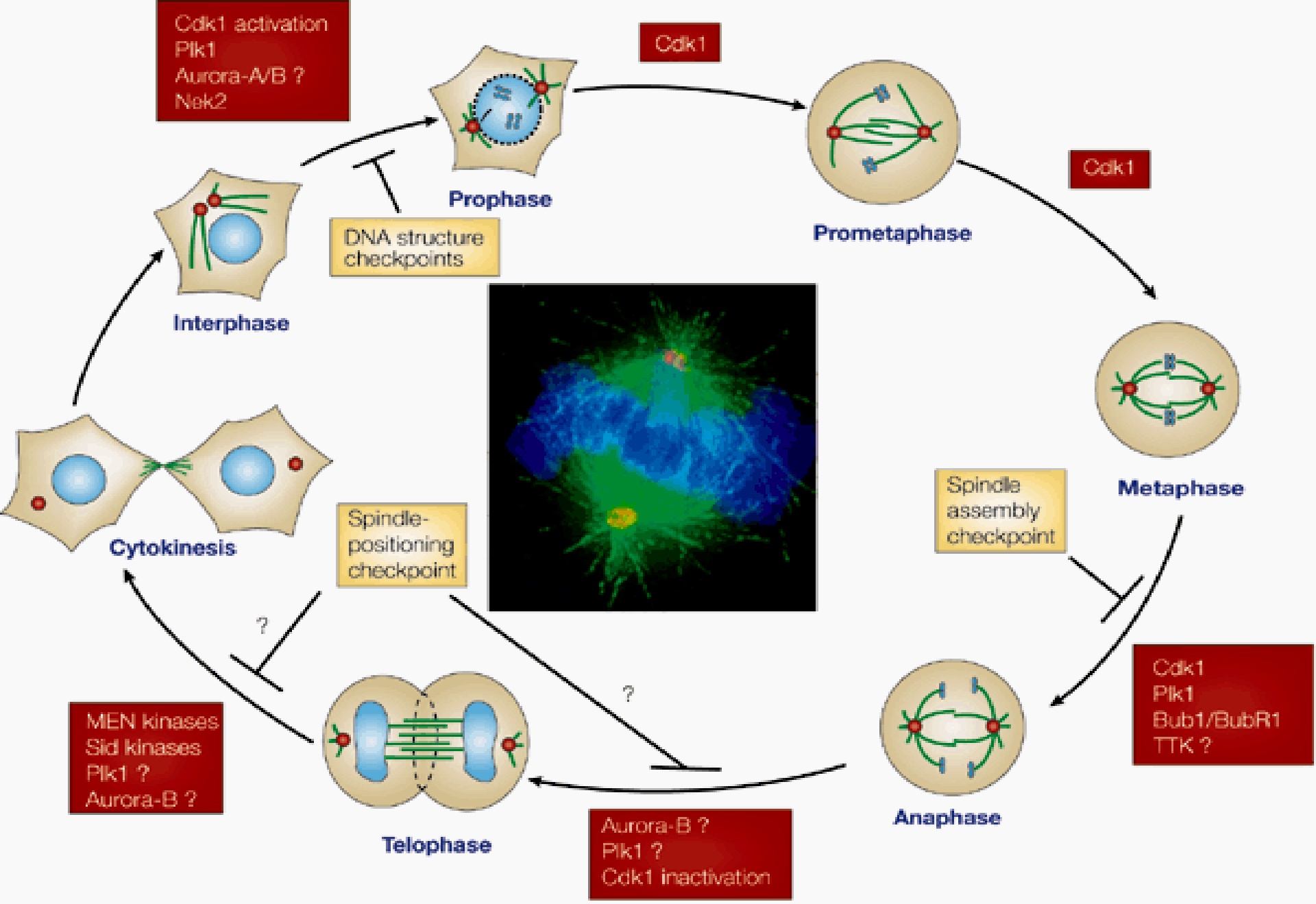
- **Mutations in genes that regulate:**
  - **Cell Division**
  - **Cell Growth**
  - **Cell Death**



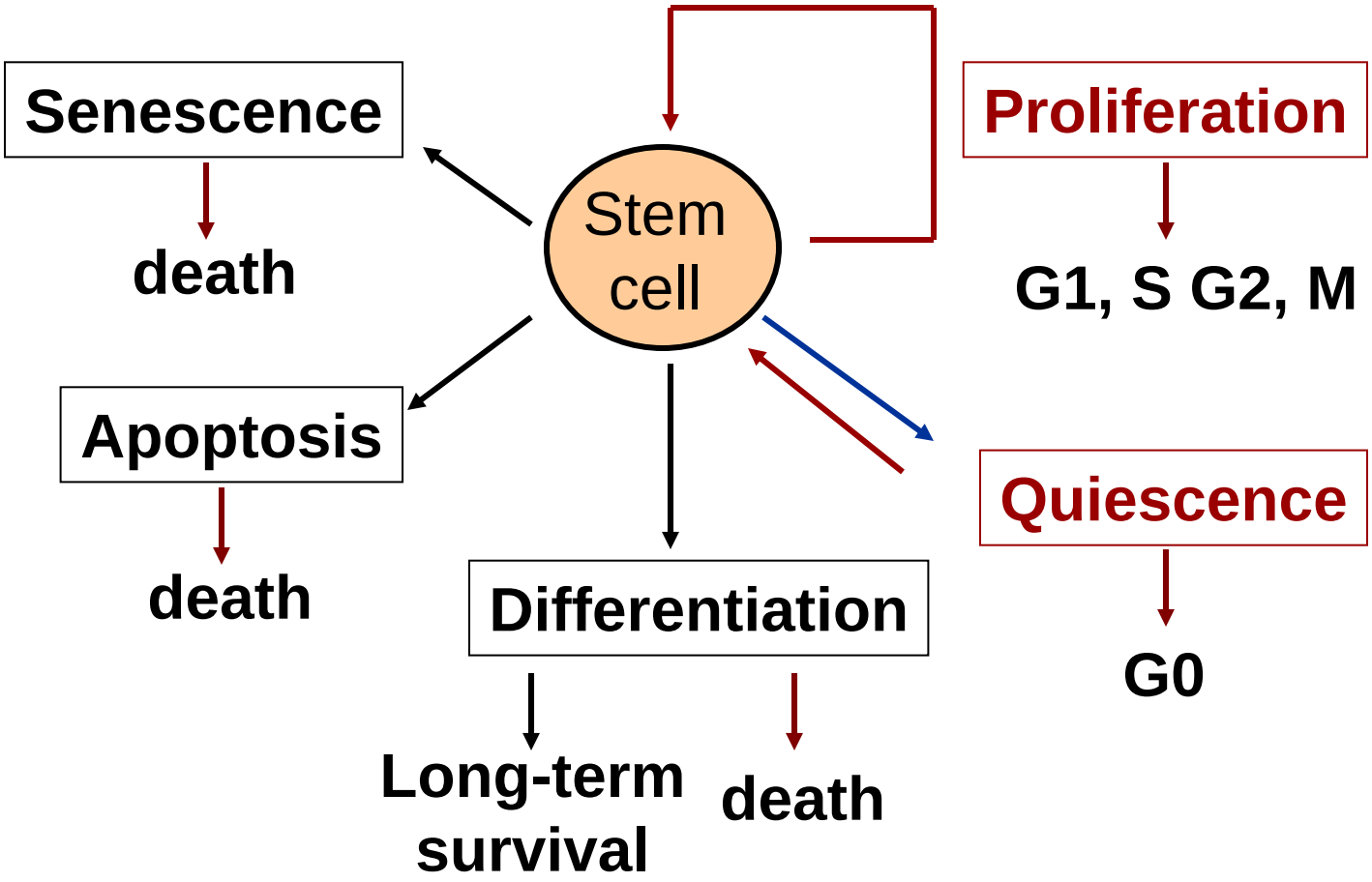
Mitosis

- One full cycle can vary but often last 16-30 hours.
  - For example skin and epithelial cells have a rapid turnover in the human body in order to replace the ones constantly being worn away.
  - Cells that make up organs such as the eye and the brain rarely multiply in adults.

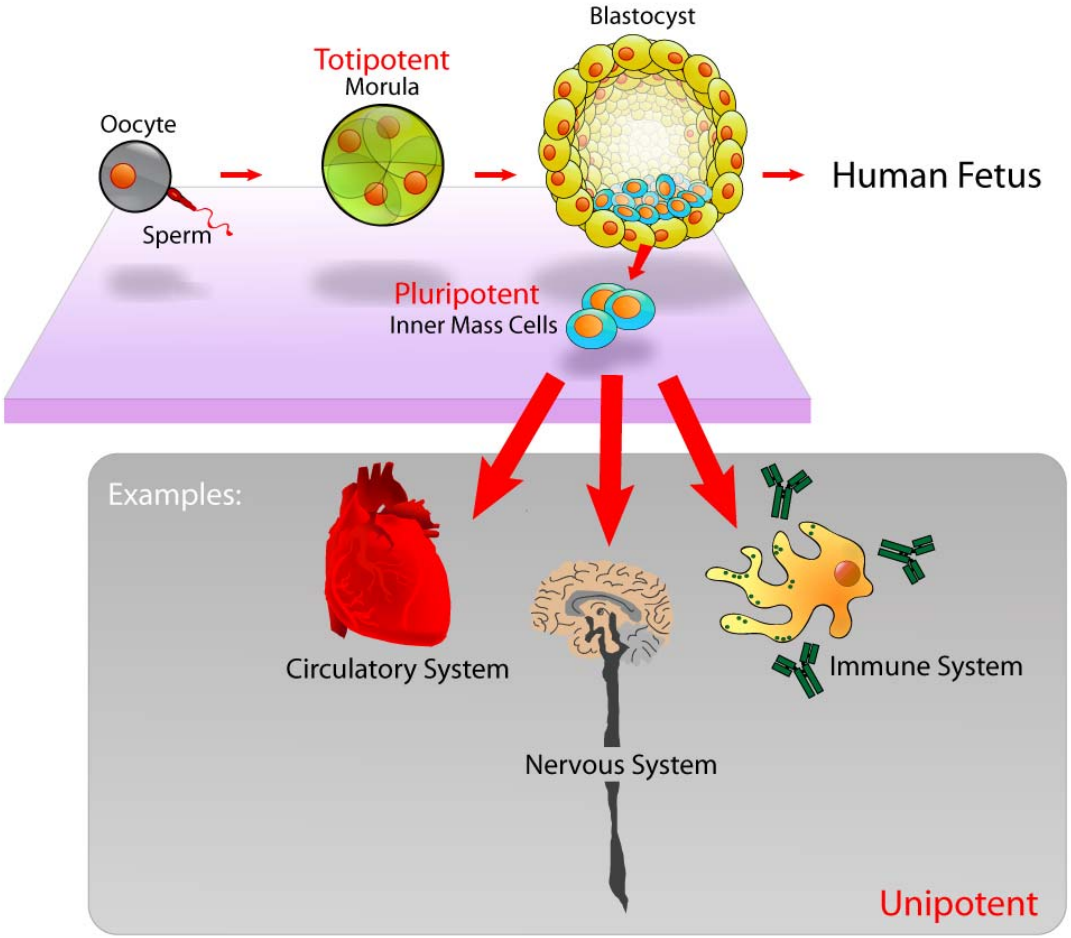
- Mitotic Stage: the nucleus and cytoplasm split to make two new cells known as DAUGHTER cells


Cdk1 activation
Plk1
Aurora-A/B ?
Nek2
Cdk1
Prophase
Prometaphase
Cdk1
DNA structure checkpoints
Interphase
Metaphase
Spindle assembly checkpoint
Cytokinesis
Spindle-positioning checkpoint
?
?
Cdk1
Plk1
Bub1/BubR1
TTK ?
MEN kinases
Sid kinases
Plk1 ?
Aurora-B ?
Telophase
Anaphase
Aurora-B ?
Plk1 ?
Cdk1 inactivation

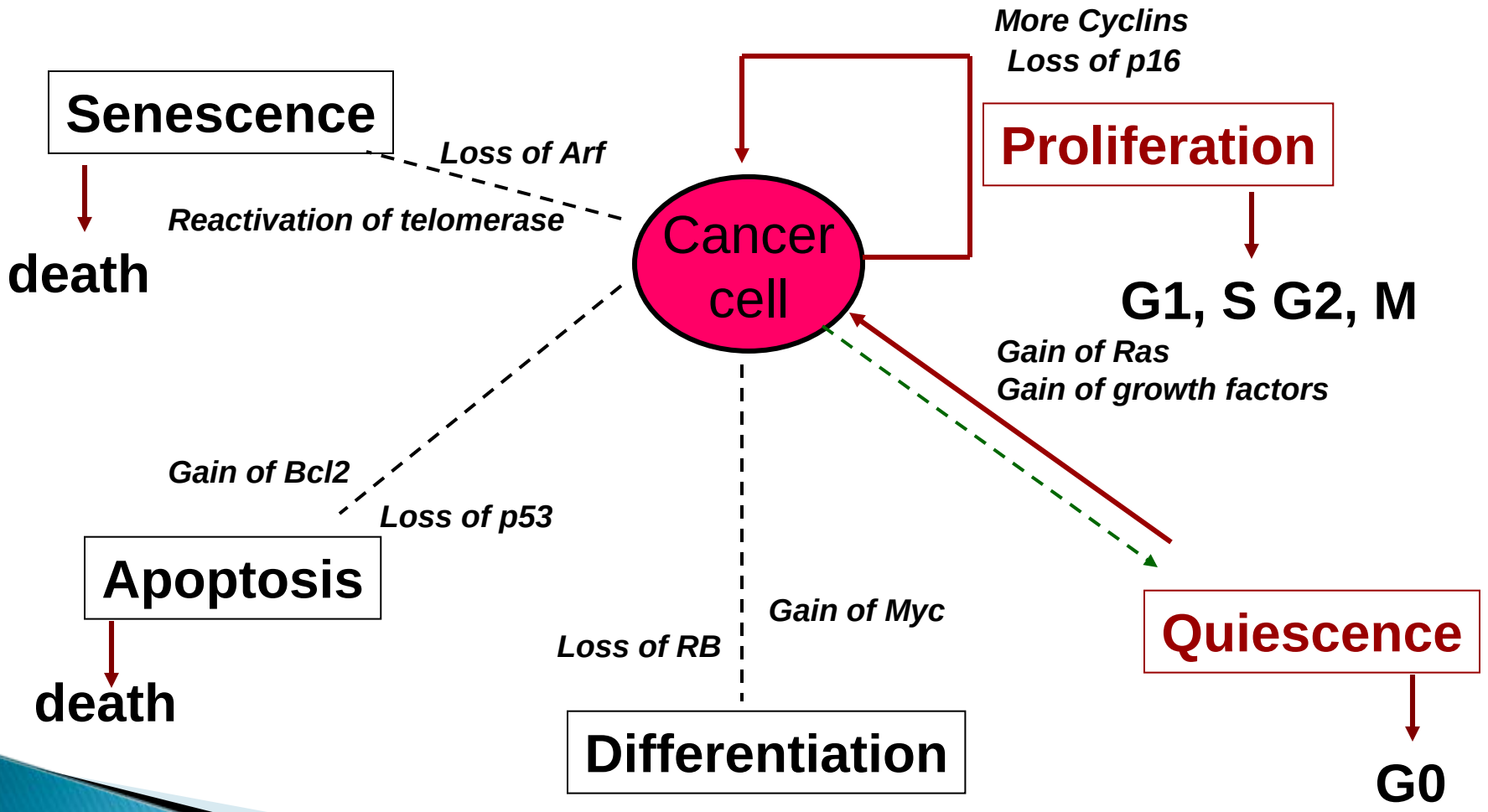
Normal Proliferation is Coupled to Multiple Choices



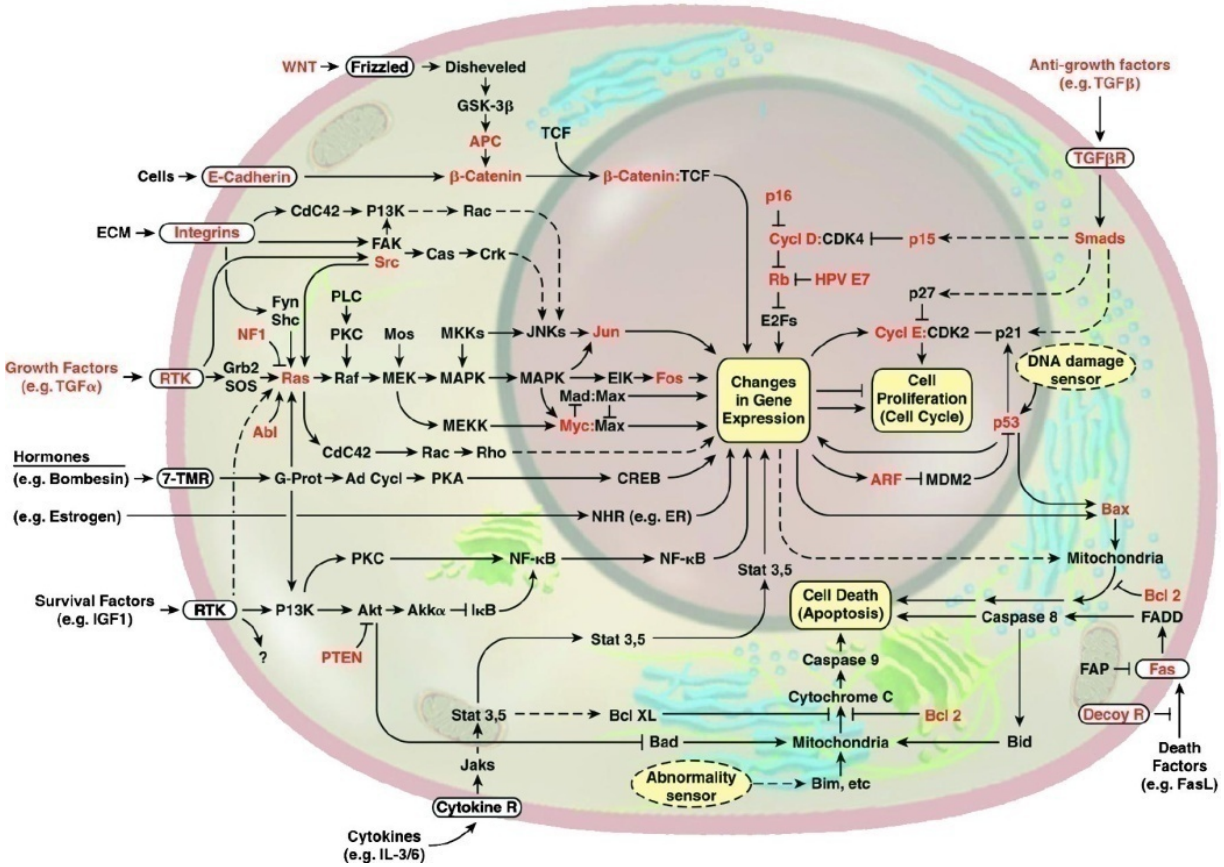
Potency Definition

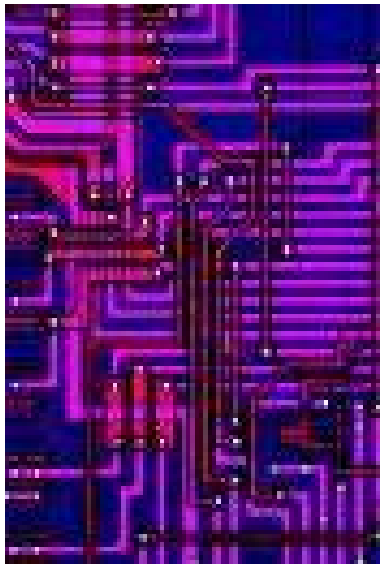


Normal Proliferation is Coupled to Multiple Choices



Normal cell


WNT
Frizzled
Disheveled
GSK-3β
APC
TCF
Cells
E-Cadherin
β-Catenin
β-Catenin:TCF
Anti-growth factors
(e.g. TGFβ)
TGFβR
ECM
Integrins
CdC42
P13K
Rac
FAK
Src
Cas
Crk
p16
Cycl D:CDK4
p15
Smads
Rb
HPV E7
p27
E2Fs
Fyn
Shc
PLC
NF1
PKC
Mos
MKKs
JNKs
Jun
Cycl E:CDK2
p21
DNA damage
sensor
Growth Factors
(e.g. TGFα)
RTK
Grb2
SOS
Ras
Raf
MEK
MAPK
MAPK
Elk
Fos
Changes
in Gene
Expression
Cell
Proliferation
(Cell Cycle)
Mad:Max
Myc:Max
MEKK
p53
Abl
CdC42
Rac
Rho
Hormones
(e.g. Bombesin)
7-TMR
G-Prot
Ad Cycl
PKA
CREB
ARF
MDM2
(e.g. Estrogen)
NHR (e.g. ER)
Bax
PKC
NF-κB
NF-κB
Stat 3,5
Mitochondria
Survival Factors
(e.g. IGF1)
RTK
P13K
Akt
Akkα
IκB
Cell Death
(Apoptosis)
Caspase 8
FADD
Bcl 2
?
PTEN
Stat 3,5
Caspase 9
FAP
Fas
Cytochrome C
Stat 3,5
Bcl XL
Bcl 2
Decoy R
Bad
Mitochondria
Bid
Jaks
Abnormality
sensor
Bim, etc
Death
Factors
(e.g. FasL)
Cytokine R
Cytokines
(e.g. IL-3/6)



Cancer cell

Genetic mutations can cause cancer

- What is a mutation?
  - A change in the sequence of DNA.
  - This changes the structure or regulation of proteins.

- Mutagen
  - Anything that produces mutations.
  - Radiation, chemicals, viruses.

Cellular Response to DNA Damage

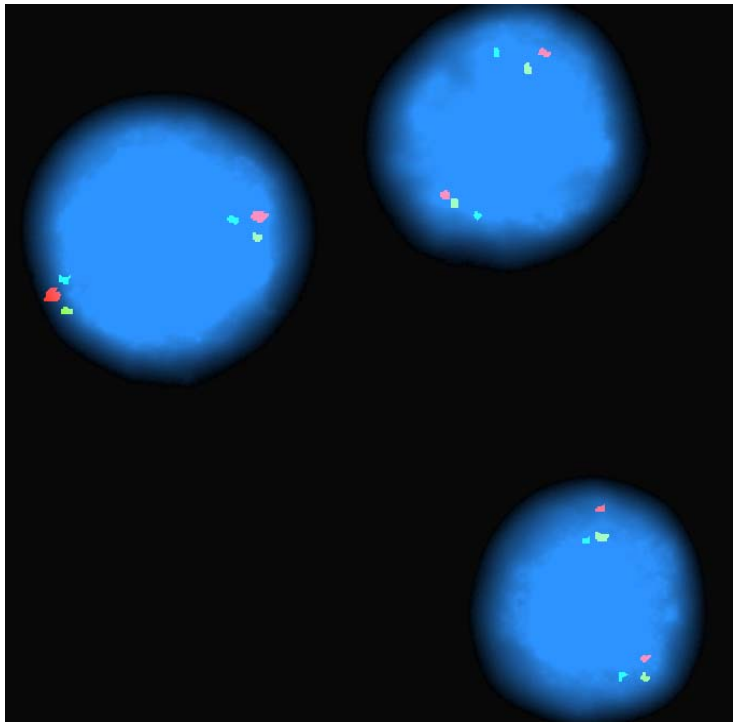
Response	Mechanism
Reversal of DNA damage	▸Repair of alkyl products ▸Ligation of DNA strand breaks
Excision of DNA damage	▸Base excision repair ▸Nucleotide excision repair ▸Mismatch repair
Tolerance of DNA damage	▸Translesion DNA synthesis ▸Replicative bypass and recombination

Mutations of Normal Genes

- Point mutations
  - Changes in one nucleotide base pair
- Chromosome translocation
  - A piece on one chromosome is transferred to another
- Gene amplification
  - Duplication of a small piece of chromosome over and over
  - May result in an increased expression of gene product either in simple amount or at inappropriate times; this can interfere with normal cell function (increase expression of oncogenes).

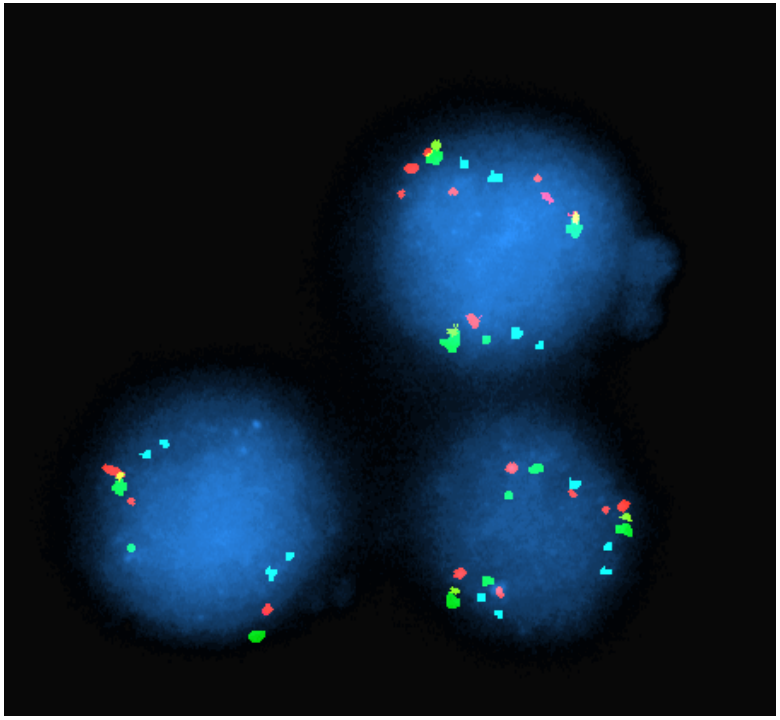
Gene amplification

A



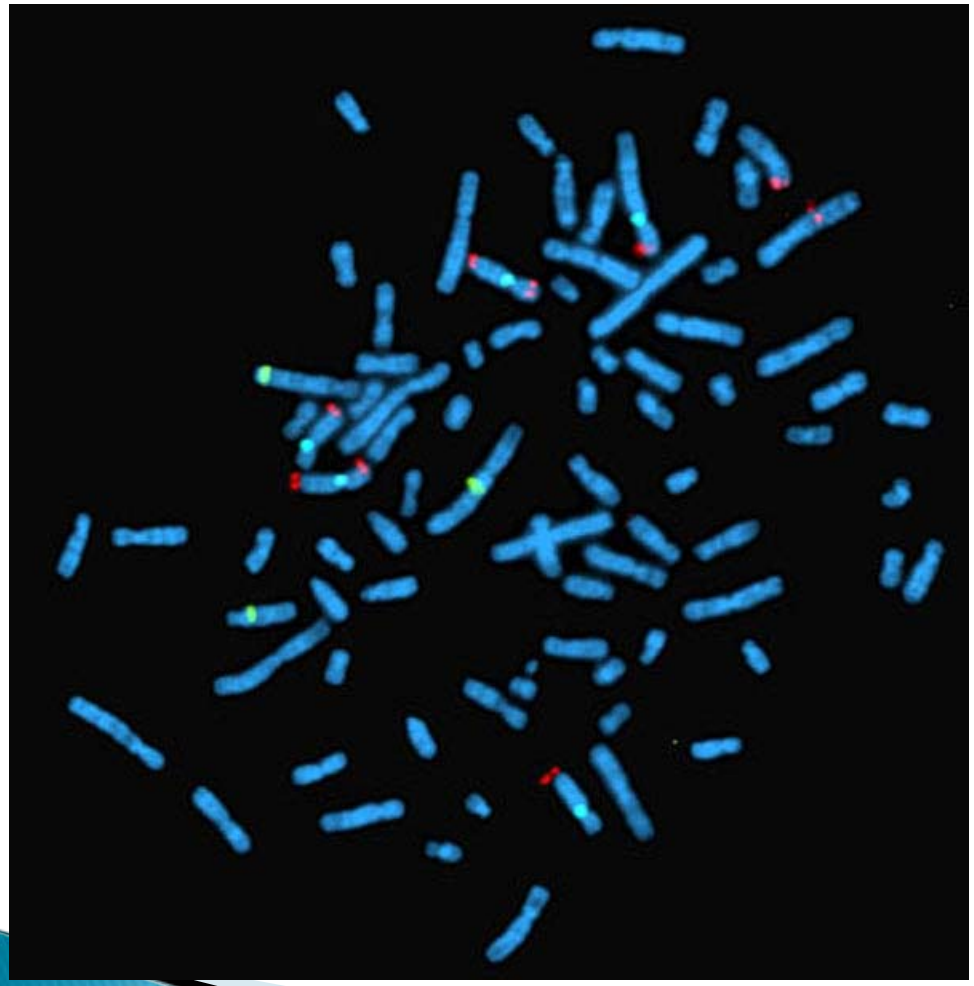
Lymphocytes

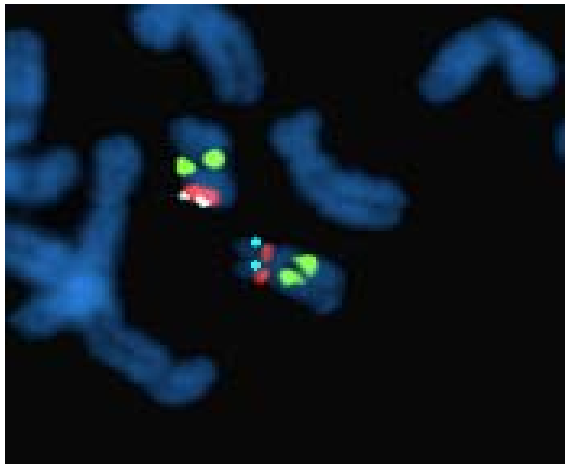
B

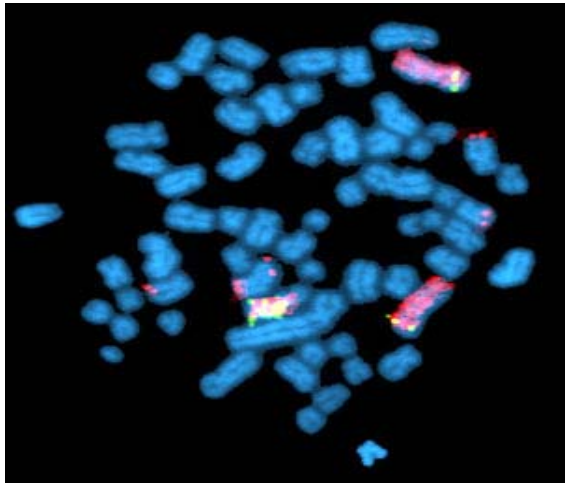


CTC

Gene amplification/translocation







Mutations of Normal Genes

- Mutation of tumor-suppressor genes
  - Allows unregulated cellular growth
- Loss of heterozygosity
  - Both chromosome copies of a gene are inactivated
- Gene silencing
  - Whole regions of chromosomes are shut off while the same regions in other cells remain active

Mutations of Normal Genes

- Caretaker genes
  - Encode for proteins that are involved in repairing damaged DNA
- Chromosome instability
  - Increased in malignant cells
  - Results in chromosome loss, loss of heterozygosity, and chromosome amplification

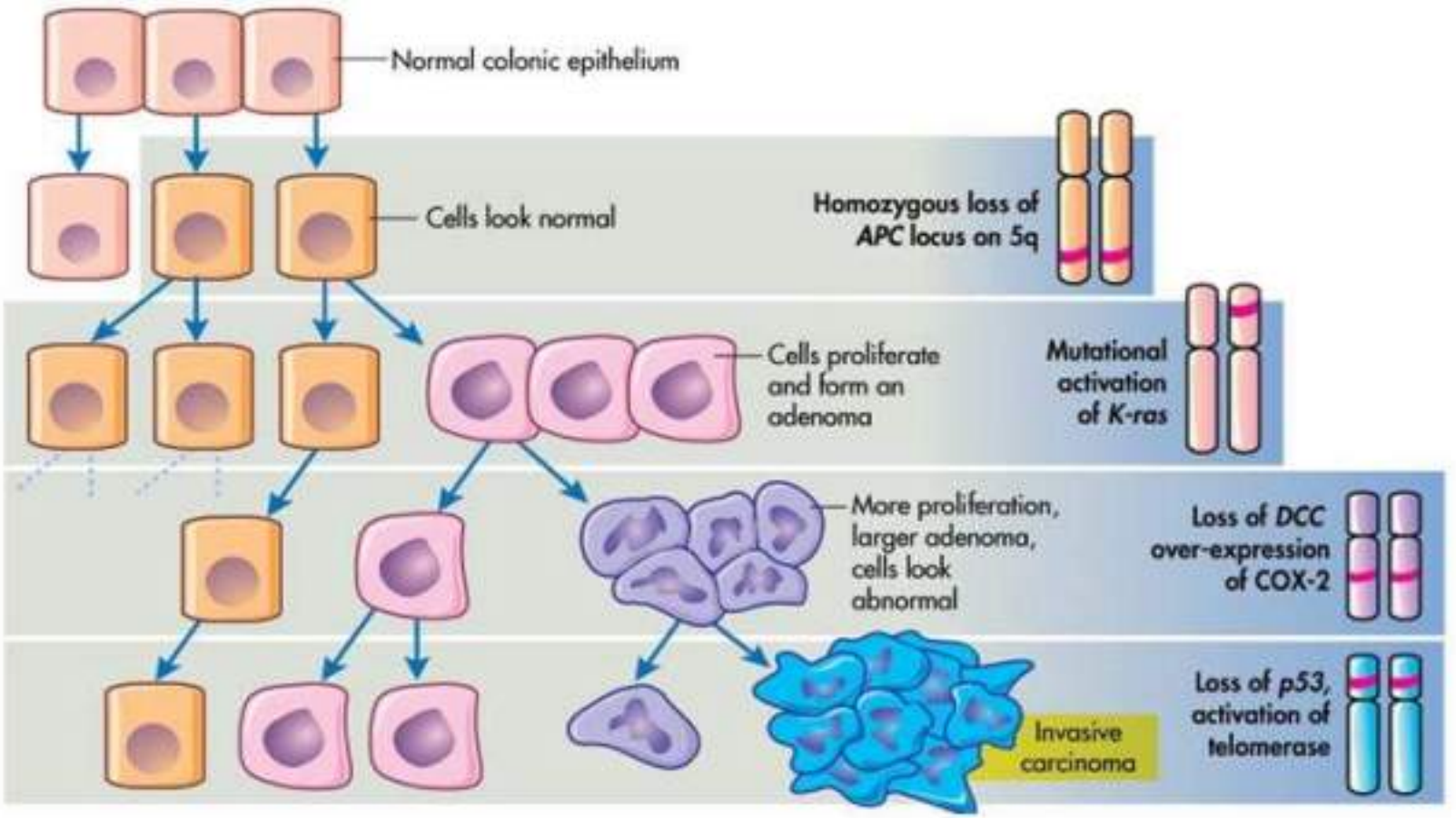
Genetic mutations can cause cancer

- Oncogene
  - Normal genes (proto-oncogenes) that have undergone mutation but remain actively producing gene products (oncogene products) that may help turn normal cells into cancer cells.

- Apoptosis
  - The destruction of cells which occurs normally.

- Supressor genes
  - Genes that regulate normal cell division by monitoring if extracellular conditions are suitable for cell division of by veryfying the correctness and completeness of DNA replication.

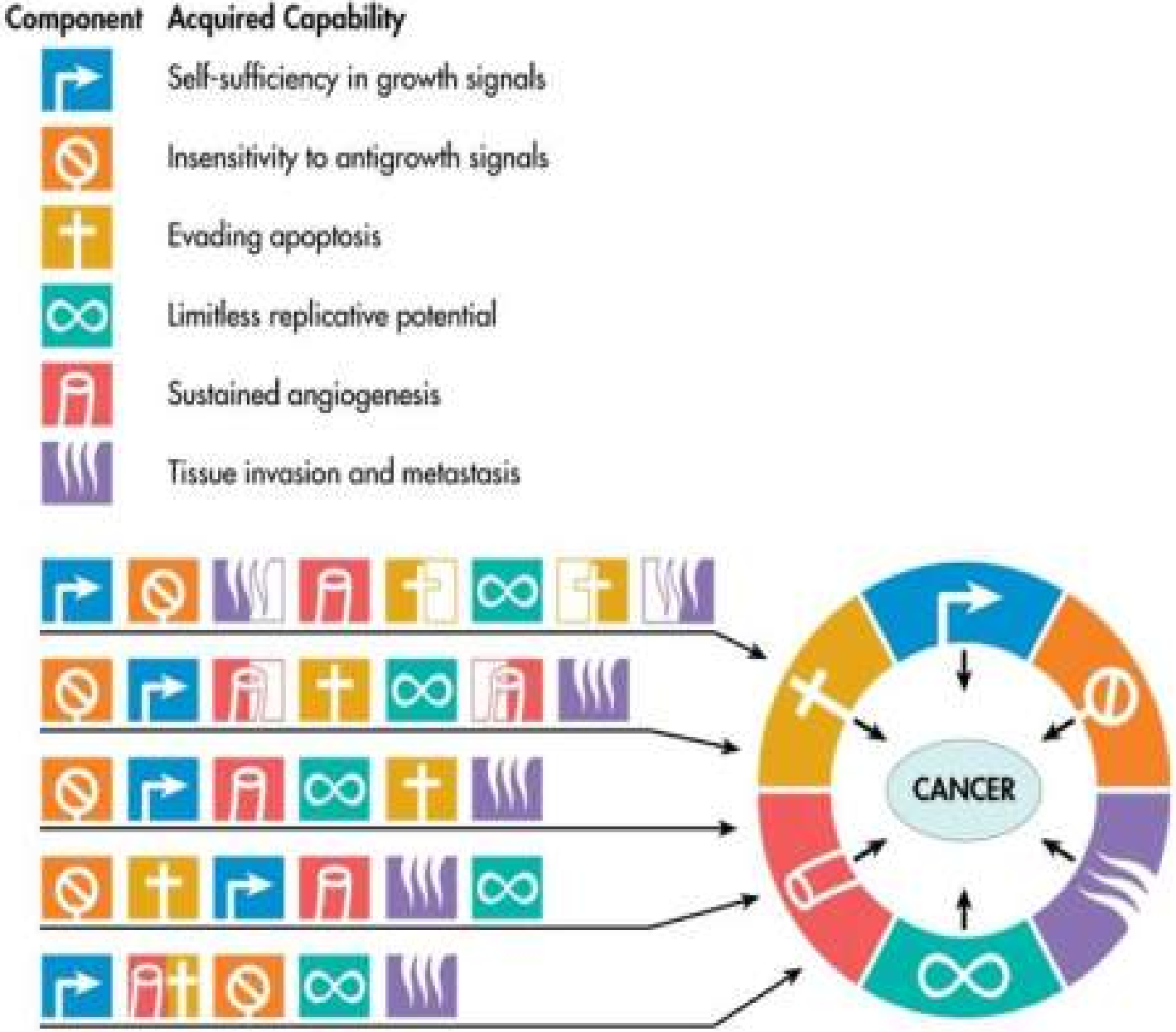
Types of Mutated Genes



(Modified from Kumar V, Cotran RS, Robbins SL: *Basic pathology*, ed 6, Philadelphia, 1997, Saunders.)

Nearly all known cancers arise gradually, as errors build up in the cancer cell and its progeny!!!

Hallmarks of Cancer



(Modified from Hanahan D, Weinberg, RA: *Cell* 100(1):57-70, 2000.)

Cancer is a multi-step disease

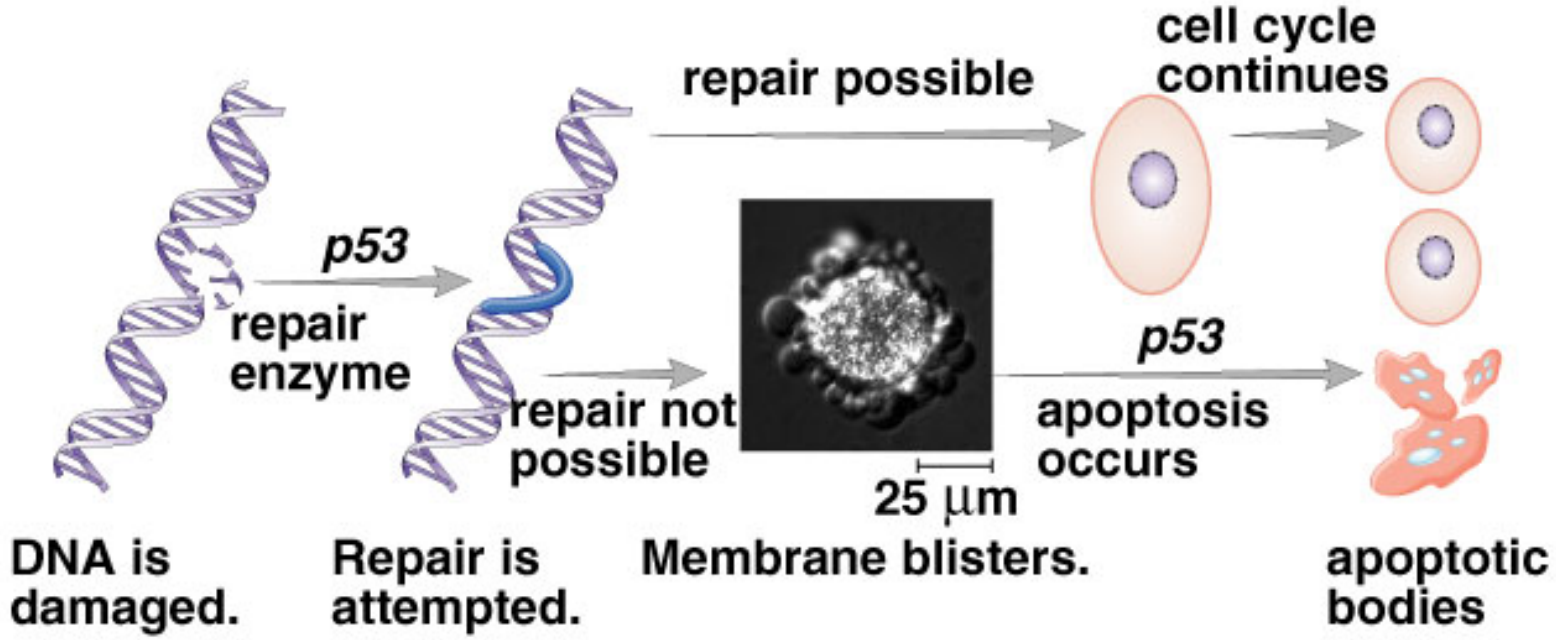
- **Multiple mutations in several distinct genes are usually required for normal cells to become cancerous.**

- **The progressive accumulation of these multiple hits explains the age-incidence of cancer and the tendency of some types of cancer to be dependent on genetic background .**

Cancers are clonal

- **All the cells in a tumor originate from a single ancestral cell.**
- **But, not all cells in a tumor have the same genotype because cancer cells are genetically unstable.**
- **Variation gives rise to selection.**
- **Clonal selection of variant progeny with the most robust growth properties play major contributing roles.**

Apoptosis



p53 is a tumor suppressor gene. It promotes apoptosis in mutated cells. Apoptosis is a normal and healthy process of cell death (leaves falling from trees).

Types of Mutated Genes

- Secreated growth factors (allow autocrine stimulation)
- Increased growth factor receptors
- Signal from cell-surface receptor is mutated in the "on" position (e.g. EGFR – erbB oncogene product)
- Inactivation of *Rb* tumor suppressor
- Activation of protein kinases that drive the cell cycle
- Mutation in the *p53* gene and the "proof-reading" function of the p53 gene product
- Mutation in the *ras* intracellular signaling protein

The Balance of Power Shifts in Cancer Cells

Proto-oncogenes (Oncogenes)

Overactive

Oncogenes : variant alleles with gain-of-function (activating) mutations.

Tumor suppressor genes

Disabled

Tumor suppressor genes : both alleles with loss-of-function (inactivating) mutations.

Examples of tumour suppressor genes

- **RB1 - retinoblastoma susceptibility gene**
- **WT1 - Wilm's tumour gene**
- **NF1 - neurofibromatosis type 1 gene**
- **NF2 - neurofibromatosis type 2 gene**
- **DCC - involved in colorectal cancer**
- **BRCA1, BRCA2 - involved in breast cancer**

Tumor suppressor

p53-a classic tumor suppressor

- “The guardian of the genome”
- Senses genomic damage
- Halts the cell cycle and initiates DNA repair
- If the DNA is irreparable, p53 will initiate apoptosis, the cell death process

Rb-a classic tumor suppressor

- Rb binds to a protein called E2F1
- E2F1 initiates the G1/S cell cycle transition
- When bound to Rb, E2F1 can't function
- Thus, Rb is a crucial cell cycle checkpoint

Oncogenes

HER2/neu an oncogene

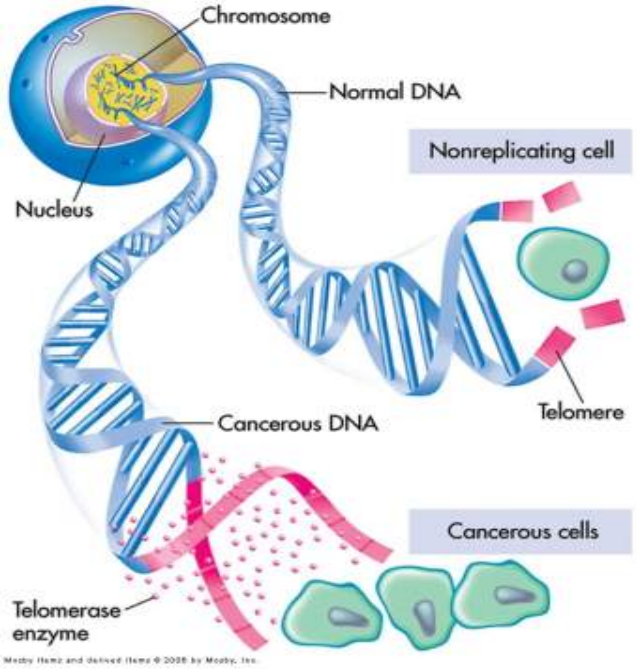
- A growth factor receptor
- 25-30% of breast cancers over-express HER2/neu
- Which hallmark of cancer does this lead to?
- Herceptin is used as a treatment

Ras oncogene

- Encoding small GTPases that are involved in cellular signal transduction.
- Activation of Ras signalling causes cell growth, differentiation, and survival.

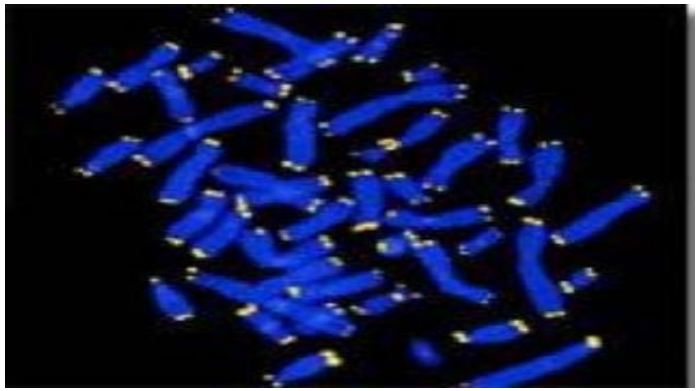
Telomeres and Immortality

- Somatic, non-stem, cells are not immortal and can only divide a limited number of times (the Hayflick limit)
- Telomeres are protective caps of repetitive DNA sequence that are elongated in stem cells by telomerase.
- Telomeres become smaller and smaller with each cell division in normal somatic Cells because telomerase is not active.

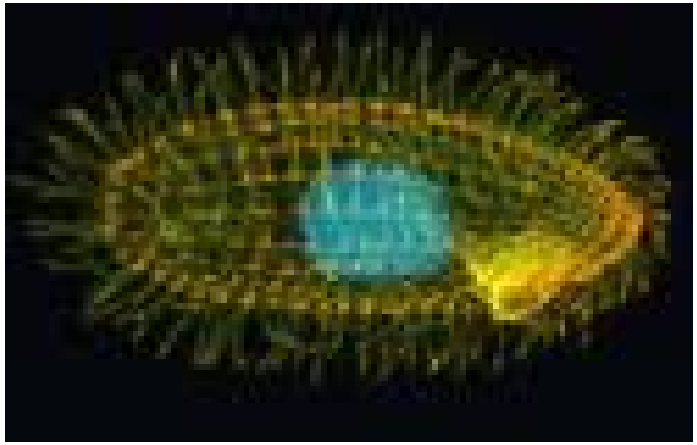


Telomeres

- Work on Telomeres Wins Nobel Prize in Physiology or Medicine for 3 U.S. Genetic Researchers
  - Elizabeth Blackburn
  - Carol Greider
  - Jack Szostak

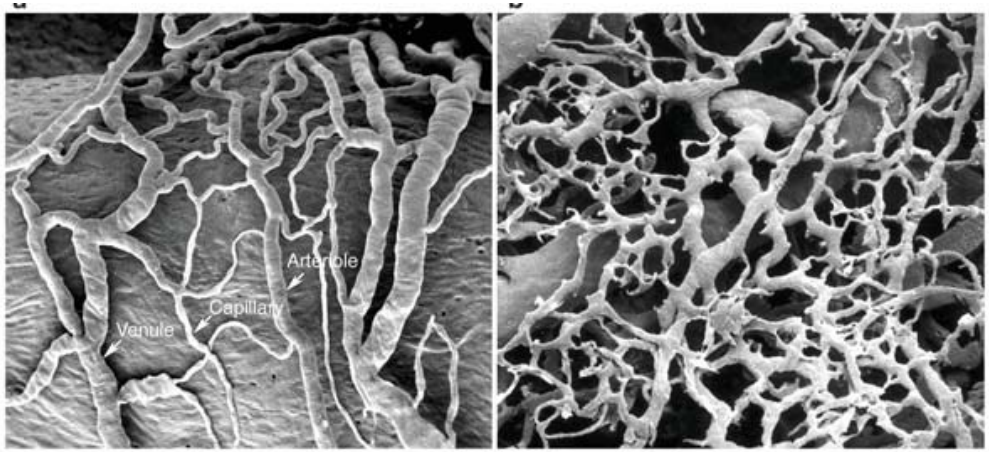


  - Elizabth and Carol discovered the existence of telomerase in 1985 in the ciliate Tetrahymena

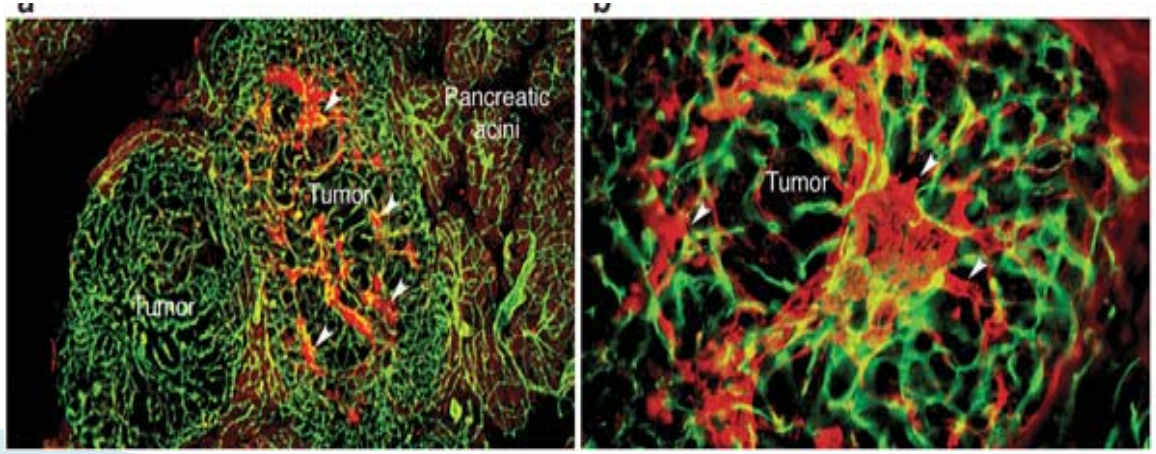


Angiogenesis

- Growth of new vessels
- Advanced cancers can secrete angiogenic factors



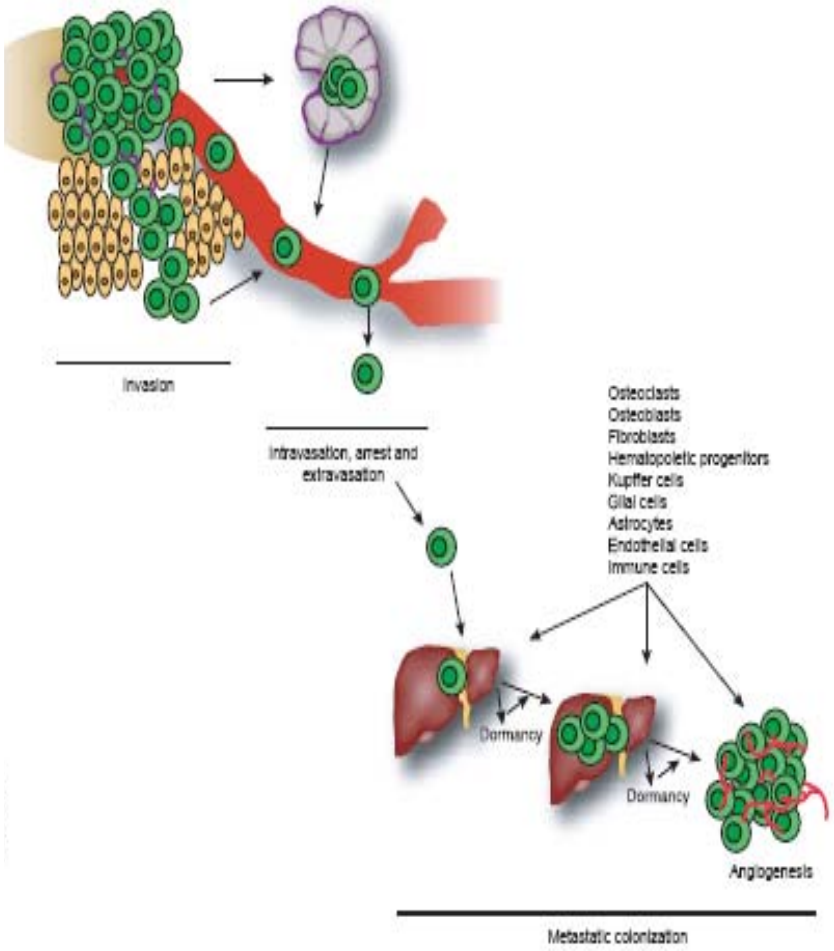
From McDonald and Choyke, 2003, Nature Medicine 9, 713-725



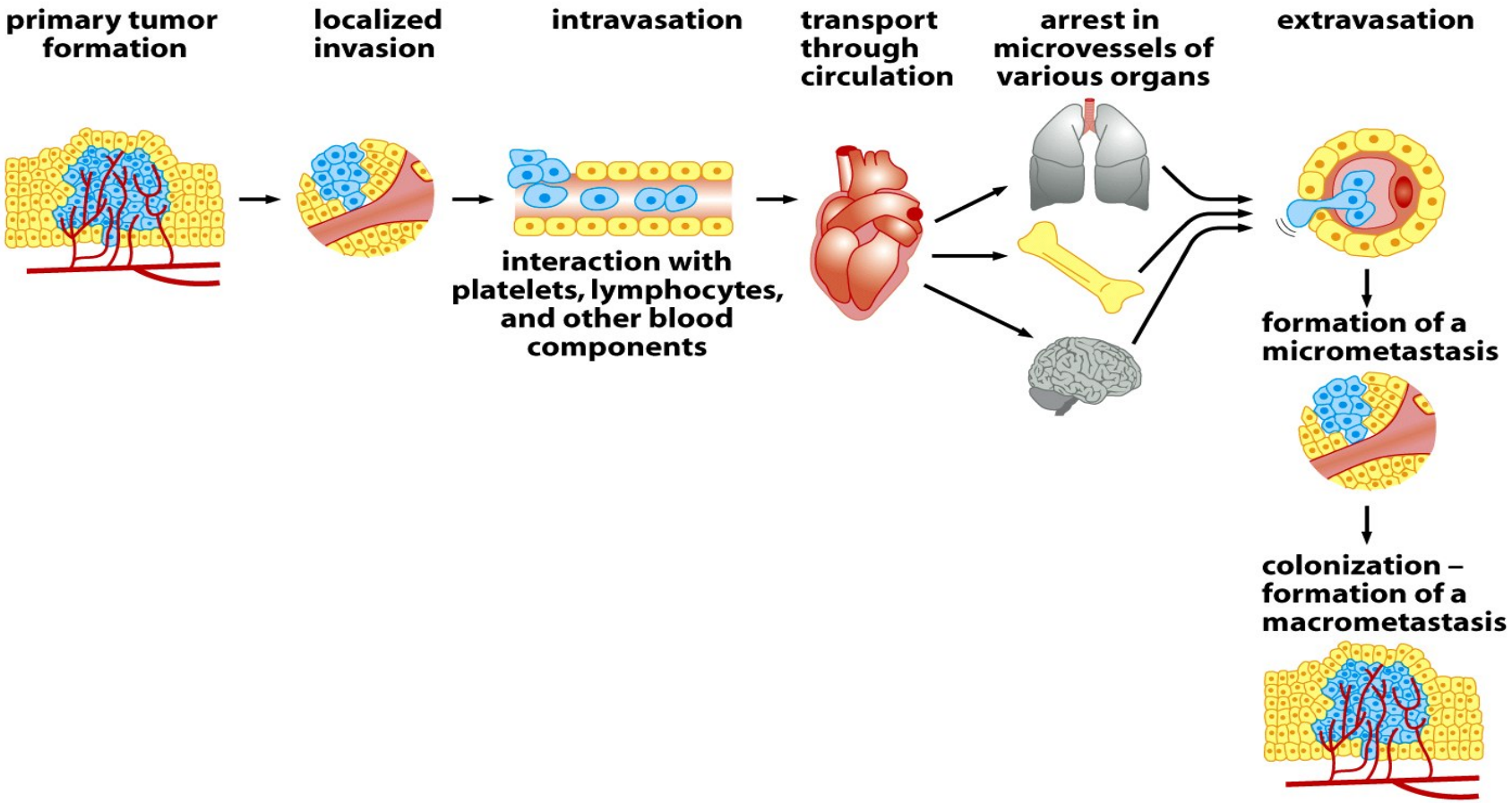
From McDonald and Choyke, 2003, Nature Medicine 9, 713-725

How do tumor vessels differ from normal vessels

- Increased vessel number
- Decreased endothelial cell–cell adhesion
- Leaky vessels
- Decreased vessel stability: decreased association of mural cells with endothelial cells
- Loss of close association of basement membrane with endothelial cells

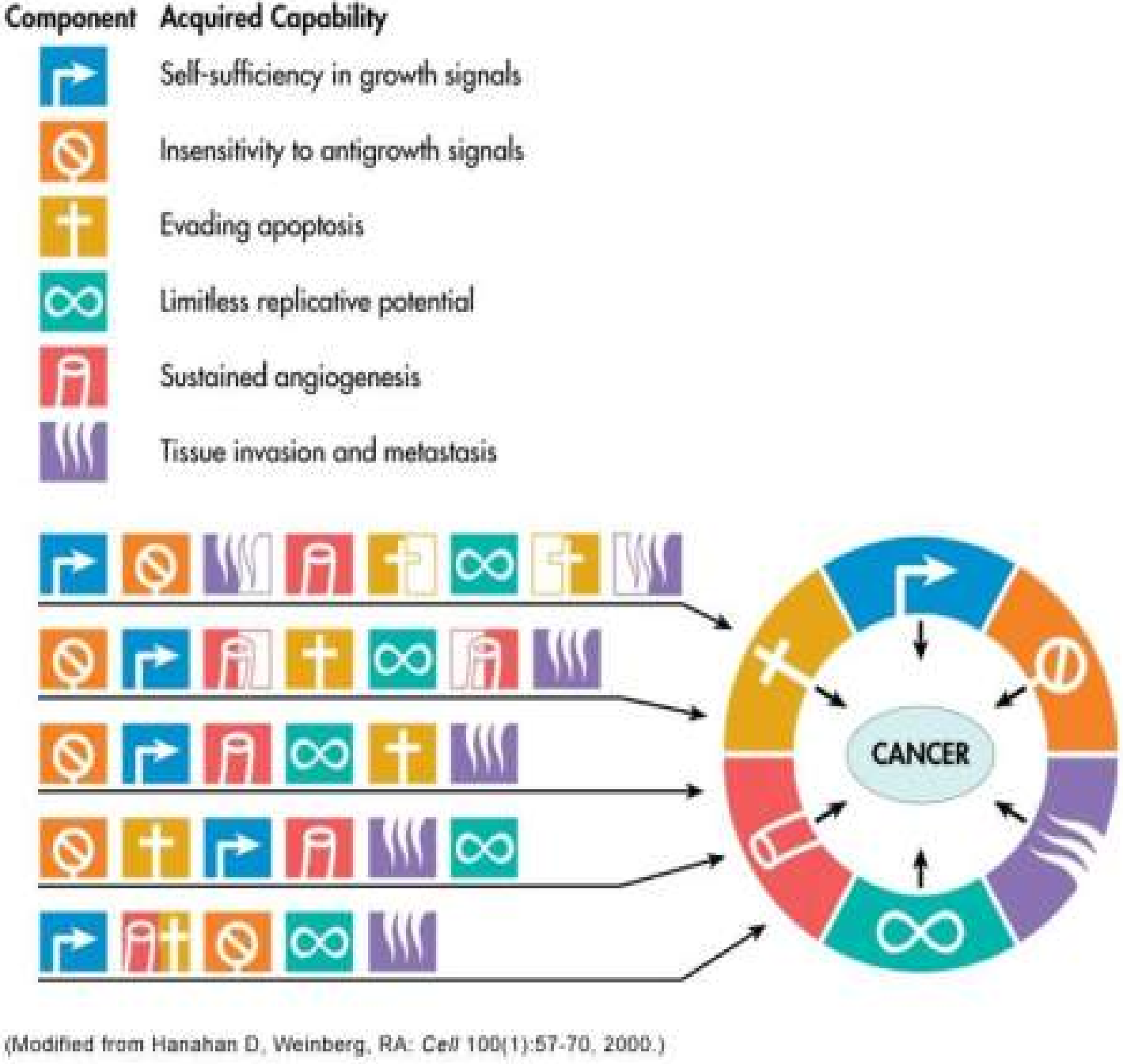


Secondary Tumor Formation: Metastases



PART II

Hallmarks of Cancer



(Modified from Hanahan D, Weinberg, RA: *Cell* 100(1):57-70, 2000.)

Tumors – Neoplasm
New growth

Benign	Malignant
Grow slowly	Grow rapidly
Well-defined capsule	Not encapsulated
Not invasive	Invasive
Well differentiated	Poorly differentiated
Low mitotic index	High mitotic index
Do not metastasize	Can spread distantly (metastasis)

Cancer

- Cancer is a complex and diverse disease
- Enormous progress has been made in understanding tumor cells
- Emerging focus: understanding and modeling the tumor as an organ with many interacting systems, such as:
  - Gene networks in cancer cells
  - Signaling pathways
  - Tumor and its microenvironment

Initiation

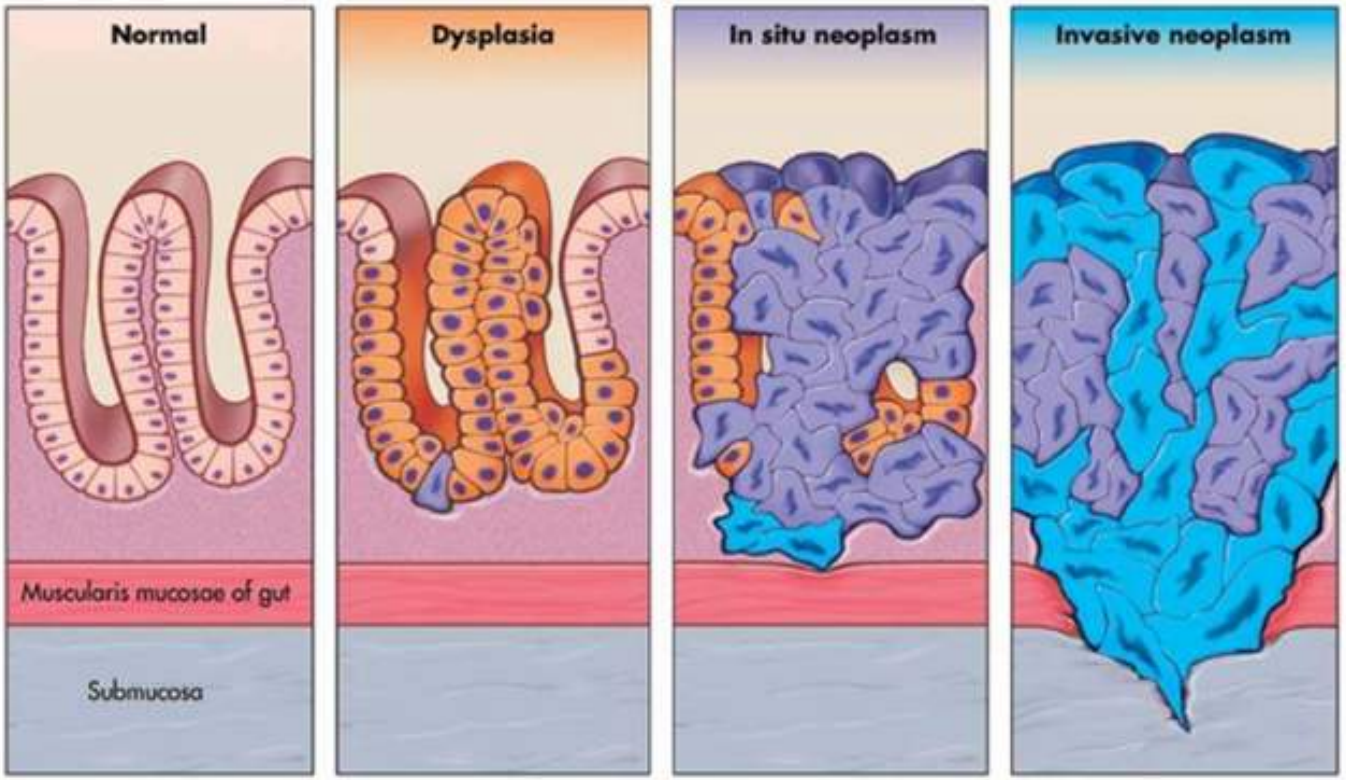
- Irreversible DNA damage in genes is critical to control of cell replication.

Promotion

- **Expansion of initiated cells**
- **Reversible, at least initially**
- **Repetitive process**

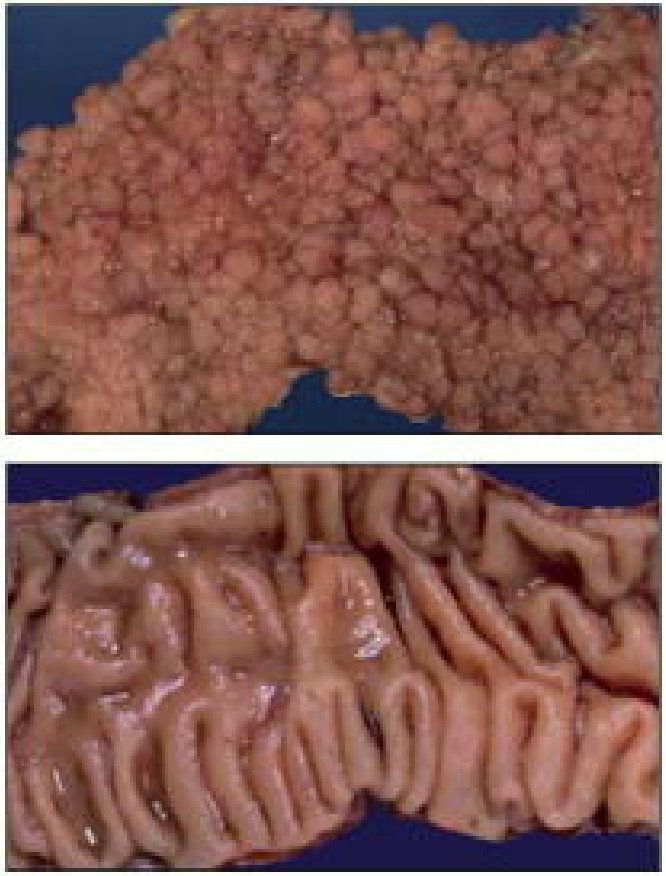
Progression

Classification and Nomenclature

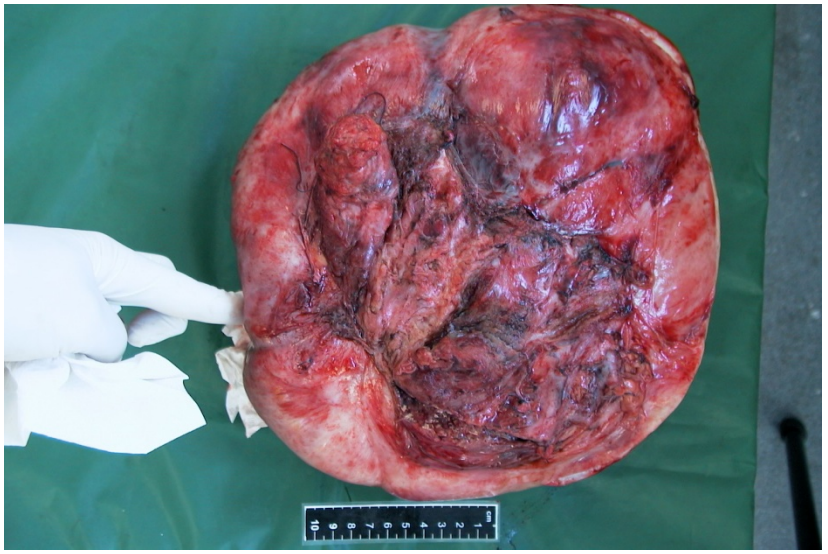


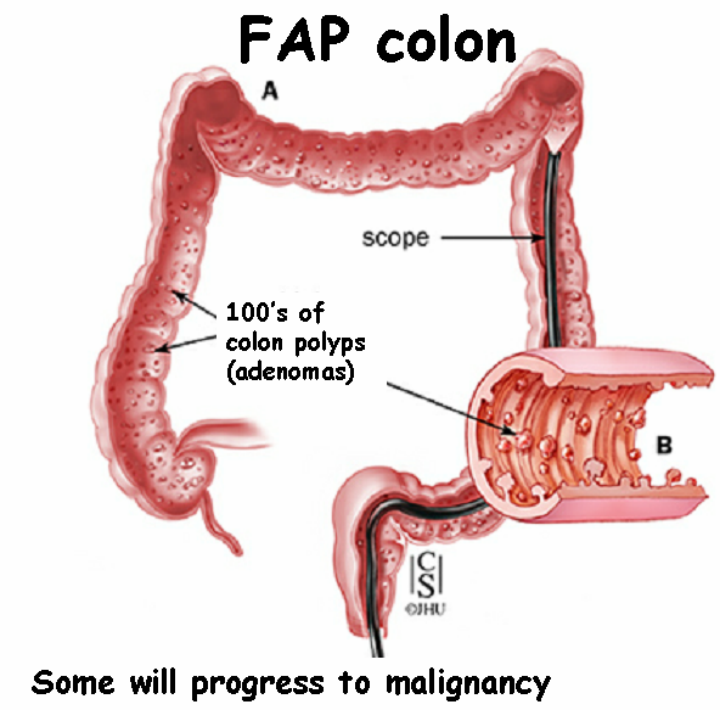
(Modified from Stevens A, Lowe J: *Pathology*, ed 2, London, 2000, Mosby.)

FAP phenotype



Polyps and normal colon





RISKS?

Risks Factors

- Viruses:
  - Hepatitis B and C viruses
  - Epstein–Barr virus (EBV)
  - Kaposi sarcoma herpesvirus (KSHV)
  - Human papillomavirus (HPV)
  - Human T cell leukemia–lymphoma virus (HTLV)

- Bacterias:
  - Helicobacter pylori – Chronic infections and their associated inflammations associated with:
    - Peptic ulcer disease
    - Stomach carcinoma
    - Mucosa–associated lymphoid tissue lymphomas

Risk Factors

- Tobacco
  - Multipotent carcinogenic mixture
  - Linked to cancers of the lung, lower urinary tract, aero digestive tract, liver, kidney, pancreas, cervix uteri, and myeloid leukemia

- Alcohol consumption
  - Risk factor for oral cavity, pharynx, hypopharynx, larynx, esophagus, and liver cancers
  - Cigarette/alcohol combination increases a person's risk

Risk Factors

- Ionizing radiation
  - Emission from x-rays, radioisotopes, and other radioactive sources
  - Exposure causes cell death, gene mutations, and chromosome aberrations
  - Poor gene repair
  - Changes in gap junction intercellular communication

- Ultraviolet radiation
  - Causes basal cell carcinoma, squamous cell carcinoma, and melanoma
  - Principal source is sunlight
  - Ultraviolet A (UVA) and ultraviolet B (UVB)
  - Promotes skin inflammation and release of free radicals

Environmental Risk Factors

- Sexual reproductive behavior
  - Carcinogenic types of human papillomavirus
  - High-risk HPV

- Occupational hazards
  - Substantial number of occupational carcinogenic agents
    - Asbestos
    - Dyes, rubber, paint, explosives, rubber cement, heavy metals, air pollution, etc.
    - Radon

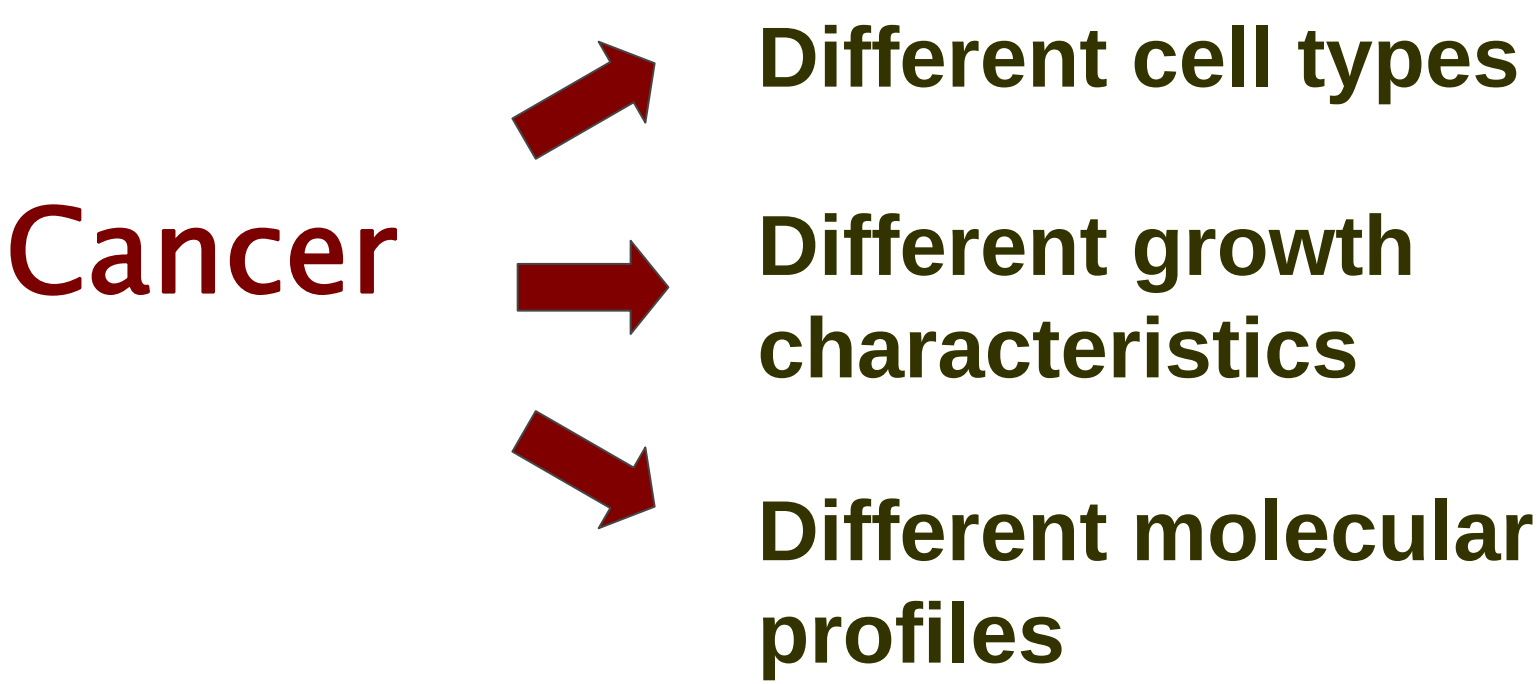
Environmental Risk Factors

- Diet
  - Xenobiotics
    - Toxic, mutagenic, and carcinogenic chemicals in food
    - Activated by Phase I activation enzymes
    - Defense mechanisms
      - Phase II detoxification enzymes
  - Examples
    - Compounds produced in the cooking of fat, meat, or proteins
    - Alkaloids or mold by-products

Environmental Risk Factors

- Obesity
  - Correlates with the body mass index (BMI)
  - Adipose tissue is active endocrine and metabolic tissue
  - In response to endocrine and metabolic signaling, adipose tissue releases free fatty acids
    - Increased free fatty acids gives rise to insulin resistance and causes chronic hyperinsulinemia
    - Correlates with colon, breast, pancreatic, and endometrial cancers

Cancer as a 3-D Disease



Cancer is a heterogeneous group of diseases and/or syndromes

Prognostic Factors

- Grade
- Stage
- Tumor type
- Biomarkers (slide based and molecular techniques)

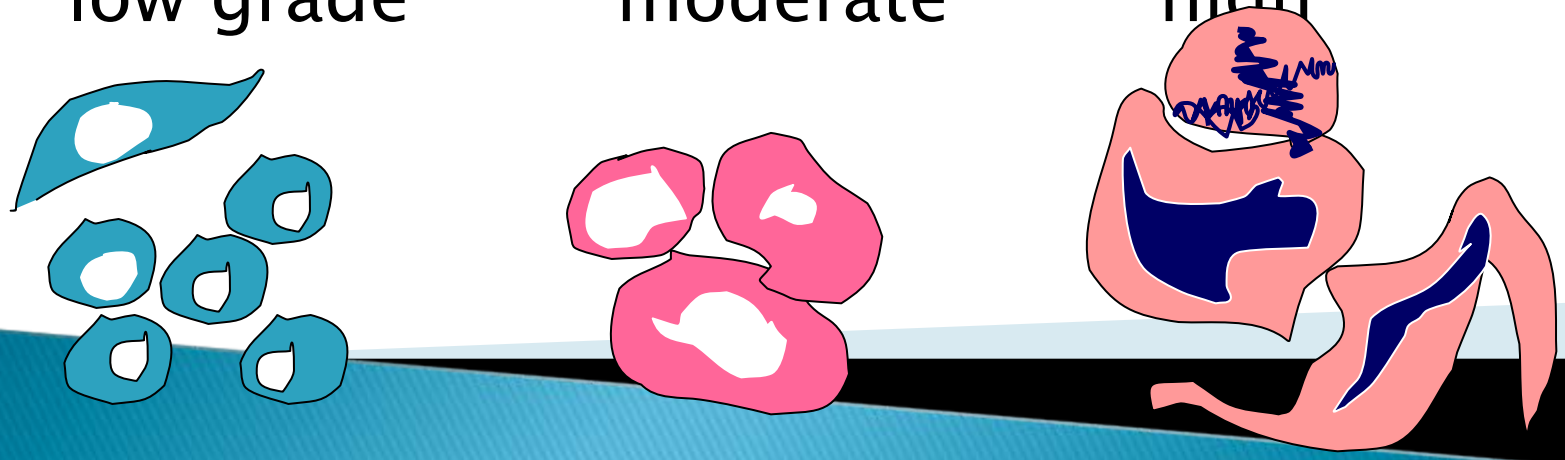
Prediction of Outcome

- Criteria are different for each cancer type
- Grade, stage, histology routine criteria
- Patient characteristics are important
- Treatment considerations critical

Cancer Grade

- Alternate term “tumor grade”
- Based on microscopic features (cytology or histology)

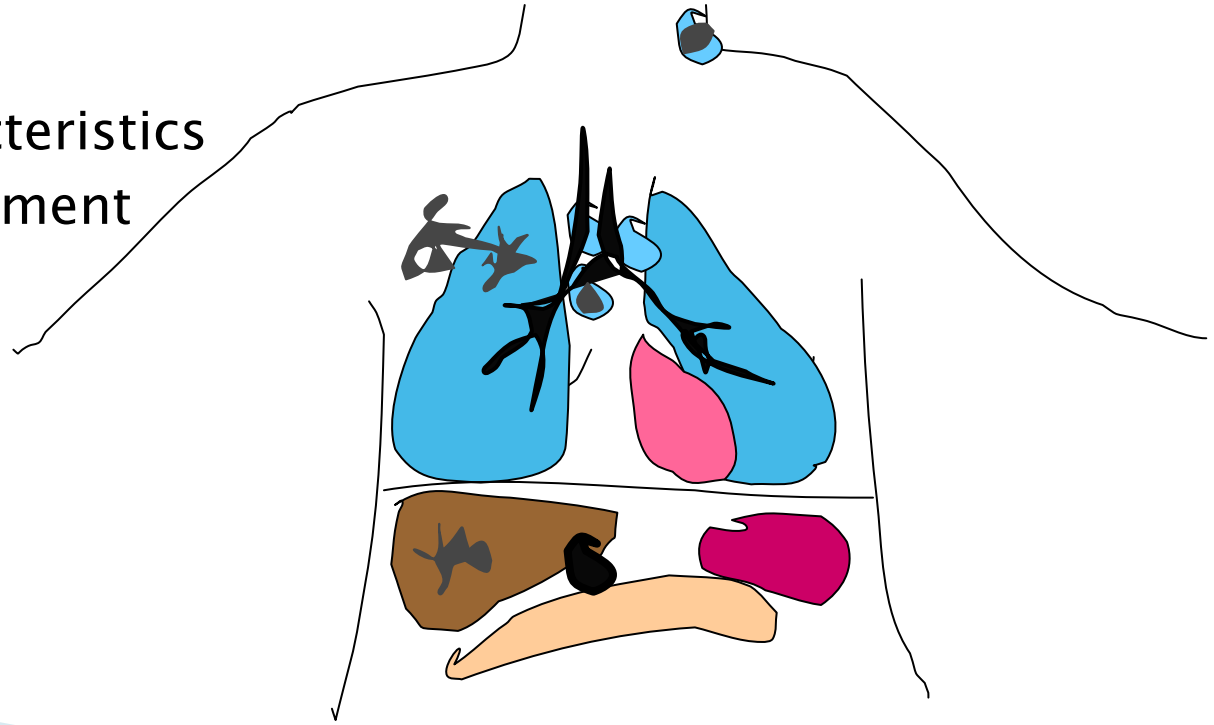
low grade moderate high



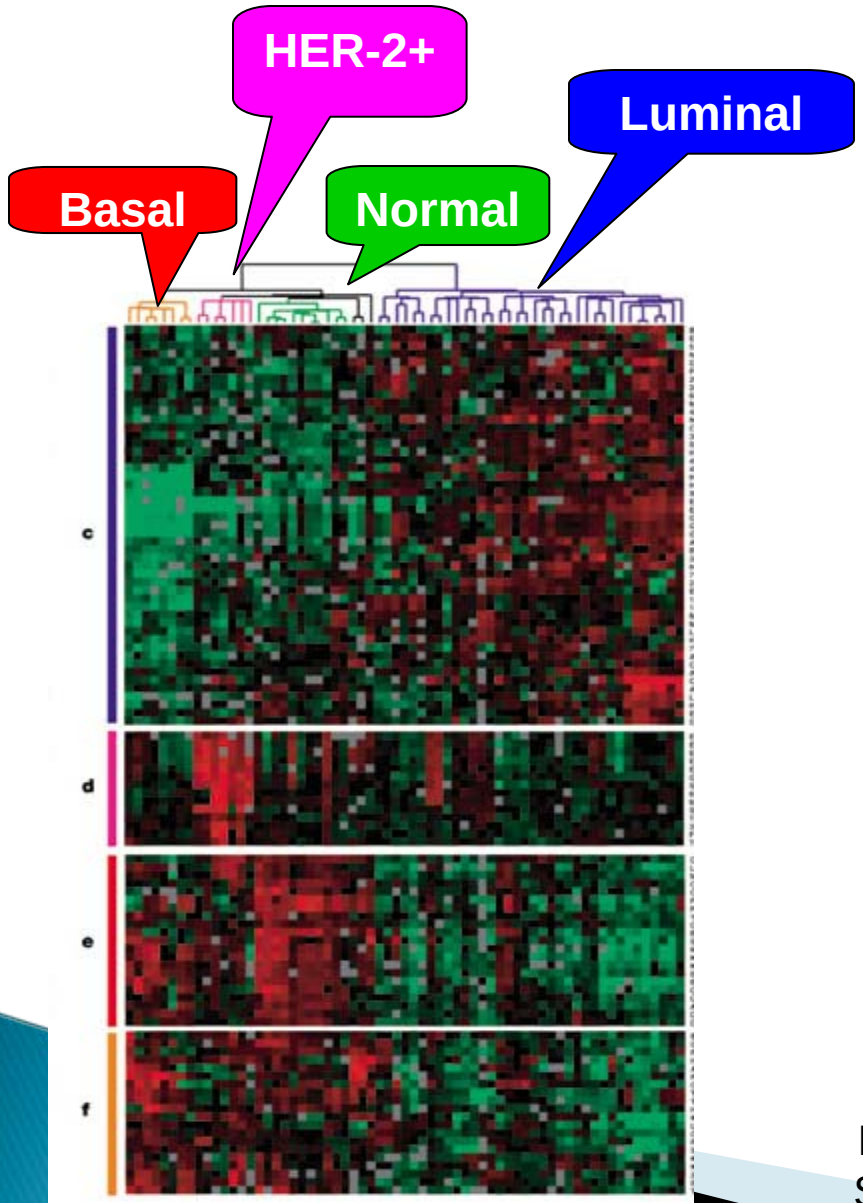
Cancer Stage

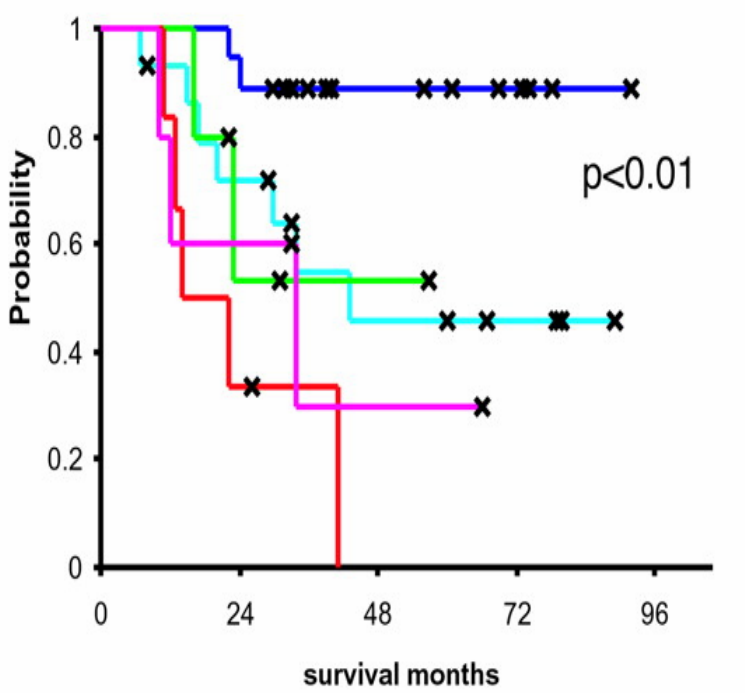
- Reflects degree of spread, for an individual cancer patient
- Assigned at the time of diagnosis, may be updated as patient progresses

T Tumor characteristics
N Nodal involvement
M Metastasis



Cancer Type





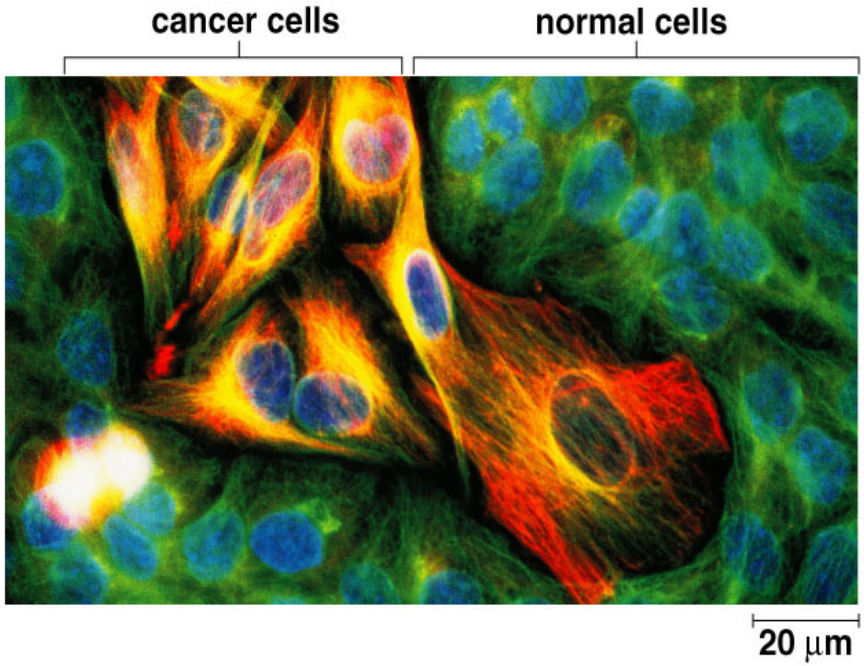
Perou, Sorlie, et al *Nature* **406:**747 2000
Sorlie, Perou et al, *PNAS* 98:10869 2001

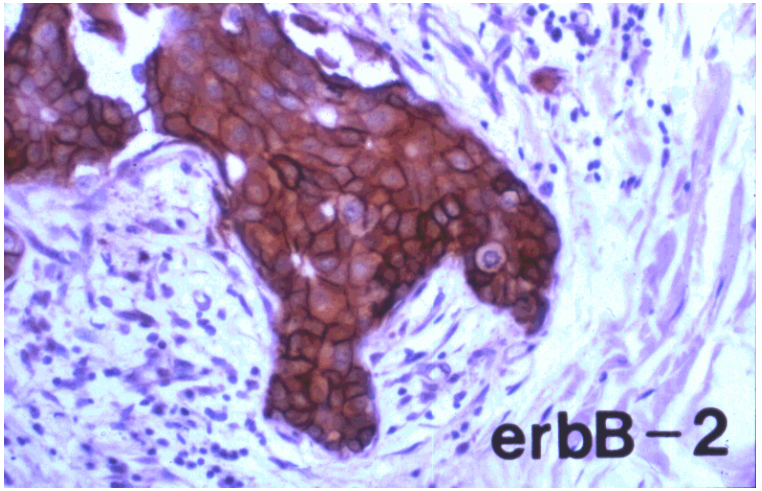
Tumor Markers

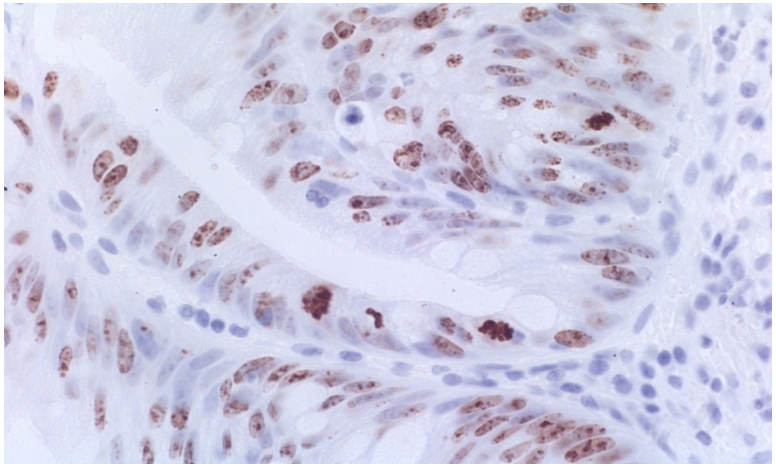
- Tumor cell markers (biological markers) are substances produced by cancer cells or that are found on plasma cell membranes, in the blood, CSF, or urine:
  - Hormones
  - Enzymes
  - Genes
  - Antigens
  - Antibodies

- Tumor cell markers are used to:
  - Screen and identify individuals at high risk for cancer
  - Diagnose specific types of tumors
  - Observe clinical course of cancer

Biomarkers







Growth Factors

Ligands which bind enzyme linked receptors
Signal diverse cellular responses including:
Proliferation
Differentiation
Growth
Survival
Angiogenesis

Can signal to multiple cell types or be specific

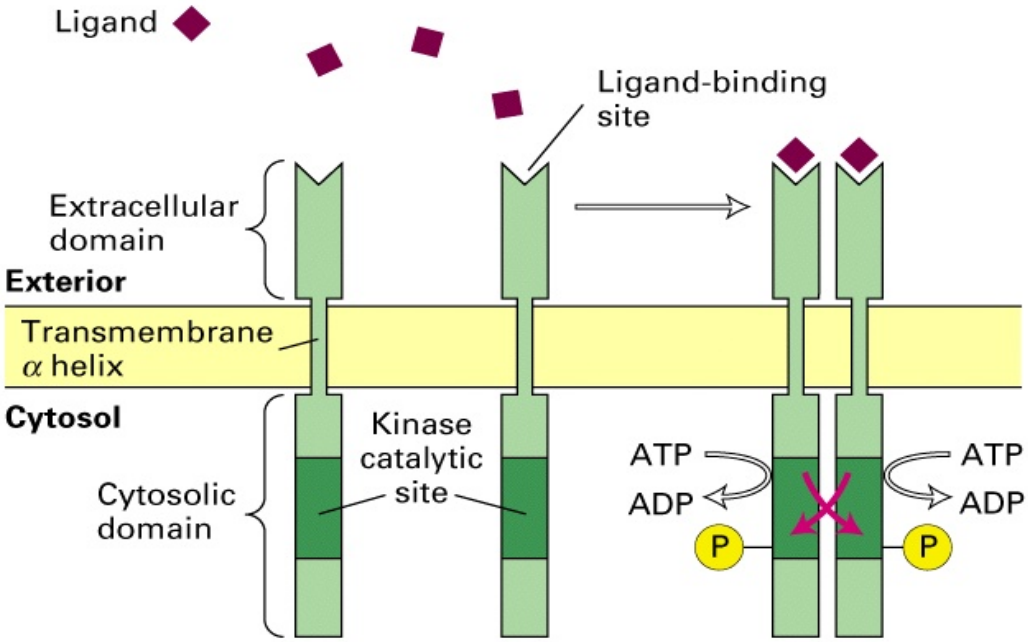
Growth Factors

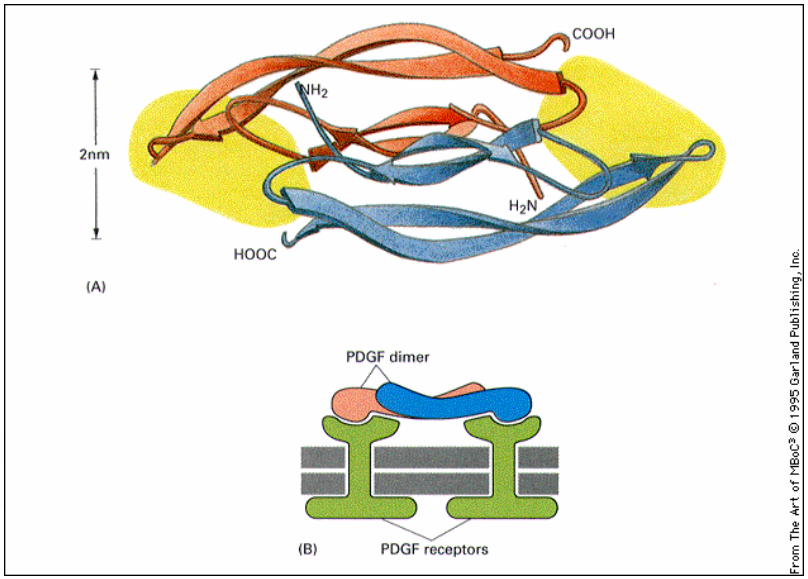
Factor	Principal Source	Primary Activity	Comments
PDGF	platelets, endothelial cells, placenta	promotes proliferation of connective tissue, glial and smooth muscle cells	two different protein chains form 3 distinct dimer forms; AA, AB and BB
EGF	submaxillary gland, Brunners gland	promotes proliferation of mesenchymal, glial and epithelial cells	
TGF-α	common in transformed cells	may be important for normal wound healing	related to EGF
FGF	wide range of cells; protein is associated with the ECM	promotes proliferation of many cells; inhibits some stem cells; induces mesoderm to form in early embryos	at least 19 family members, 4 distinct receptors
NGF		promotes neurite outgrowth and neural cell survival	several related proteins first identified as proto-oncogenes; trkA (***trackA***), trkB, trkC
Erythropoietin	kidney	promotes proliferation and differentiation of erythrocytes	
TGF-β	activated TH_1 cells (T-helper) and natural killer (NK) cells	anti-inflammatory (suppresses cytokine production and class II MHC expression), promotes wound healing, inhibits macrophage and lymphocyte proliferation	at least 100 different family members
IGF-I	primarily liver	promotes proliferation of many cell types	related to IGF-II and proinsulin, also called Somatomedin C
IGF-II	variety of cells	promotes proliferation of many cell types primarily of fetal origin	related to IGF-I and proinsulin

Growth Factor Receptors

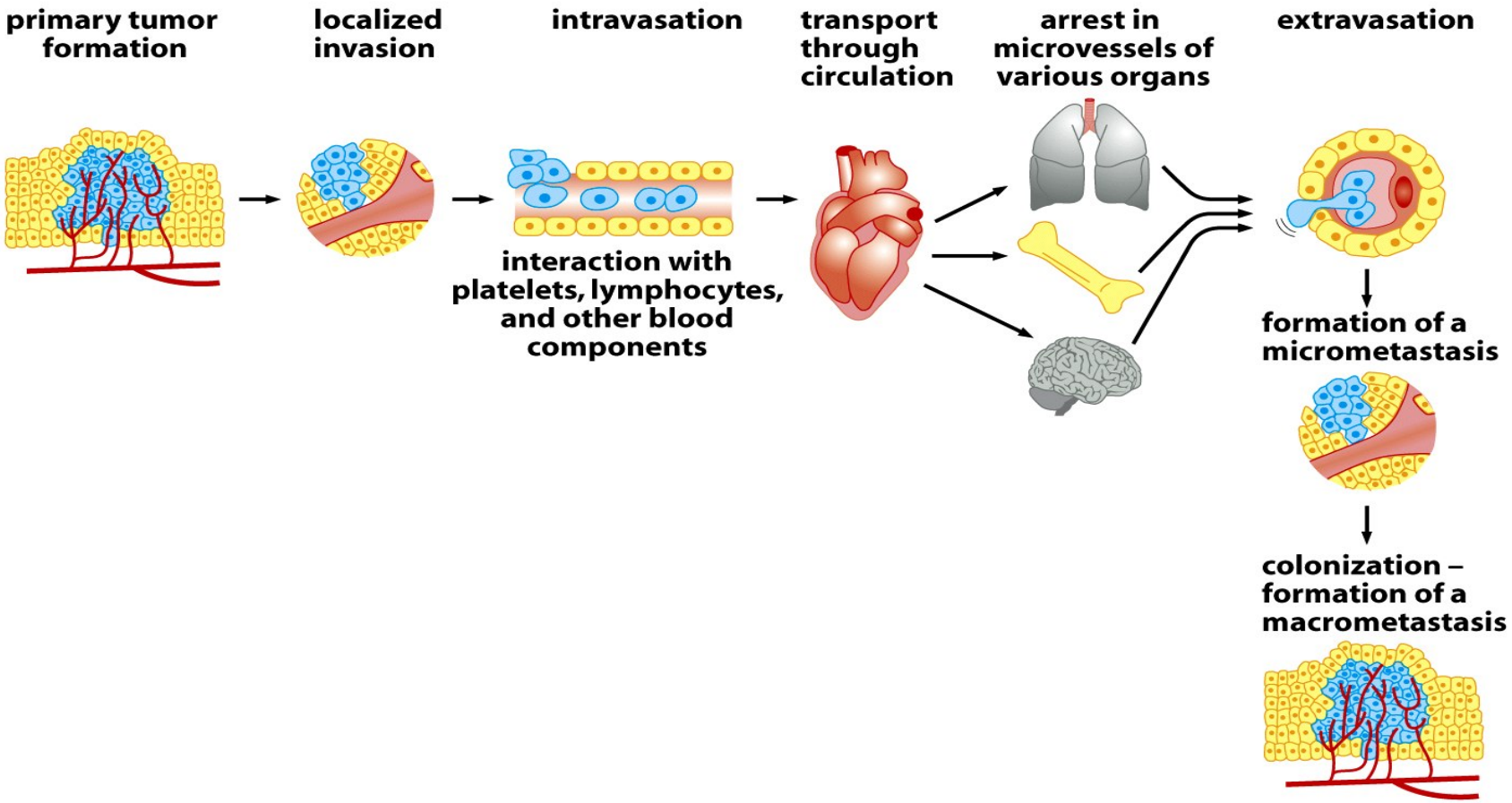
Table 10-2 Examples of Growth Factor Families

Growth Factor	Target Cells	Type of Receptor Complex
Epidermal growth factor (EGF)	Wide variety of epithelial and mesenchymal cells	Tyrosine kinase
Transforming growth factor-α (TGF-α)	Same as EGF	Tyrosine kinase
Platelet-derived growth factor (PDGF)	Mesenchyme, smooth muscle, trophoblast	Tyrosine kinase
Transforming growth factor-β (TGF-β)	Fibroblastic cells	Serine-threonine kinase
Fibroblast growth factor (FGF)	Mesenchyme, fibroblasts, many other cell types	Tyrosine kinase
Interleukin-2 (IL-2)	Cytotoxic T lymphocytes	Complex of three subunits
Colony stimulating factor-1 (CSF-1)	Macrophage precursors	Tyrosine kinase
Wnts	Many types of embryonic cells	Seven-pass protein





Secondary Tumor Formation: Metastases



Invasion and Metastasis

- Characteristics that are unique to malignant neoplasms (cancer)
- The major cause of morbidity and mortality

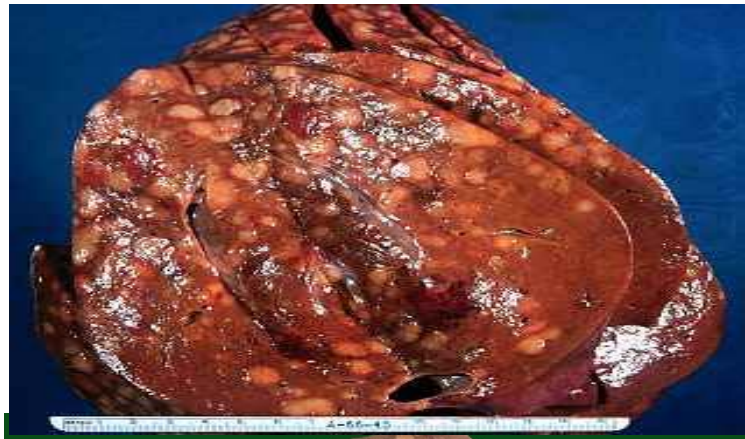
Invasion:

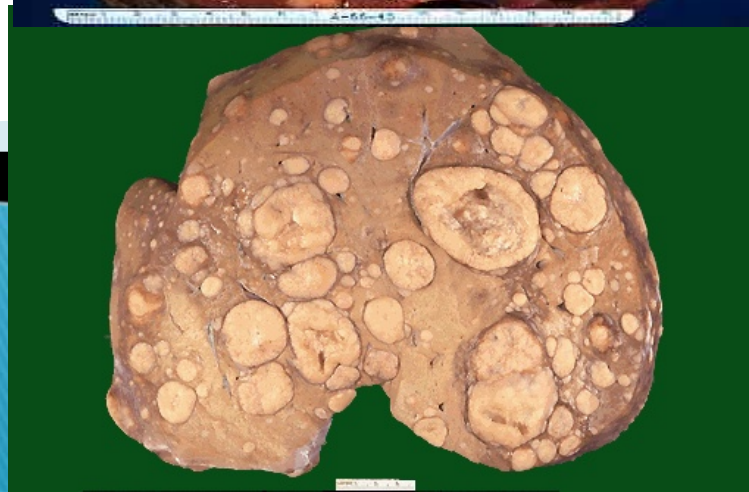
- Associated with activated motility and local tissue independence *in vitro*
- Balance between tissue destruction and synthesis
- Cell surface and extracellular matrix play important roles

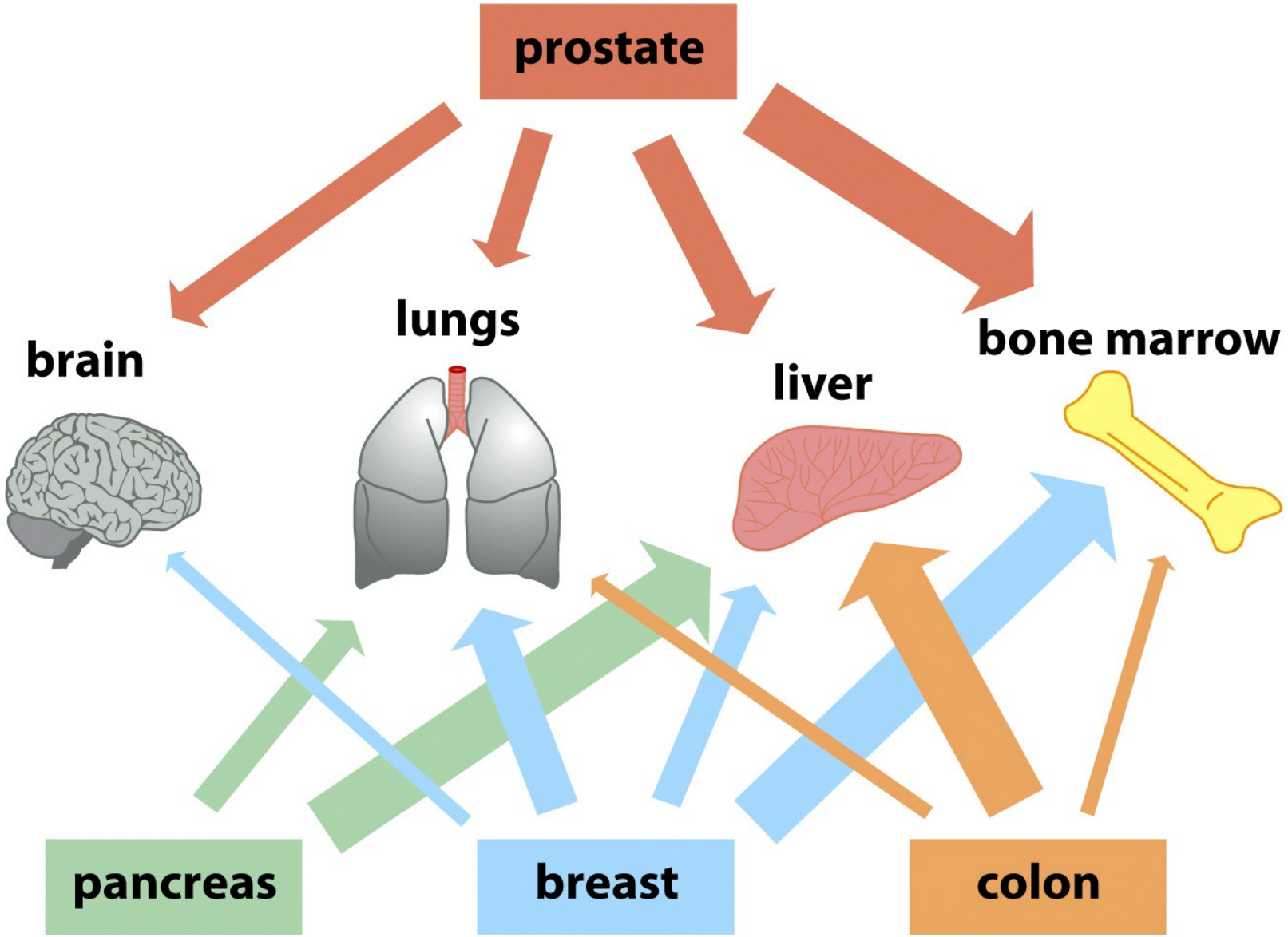
Metastasis

Mechanisms of Spread:

- **Hematogenous**
- **Lymphatics**
- **Require acquisition of additional tumor characteristics beyond those necessary for invasion**
- **Multiple lesions**
- **Organ specificity**






prostate
brain
lungs
liver
bone marrow
pancreas
breast
colon

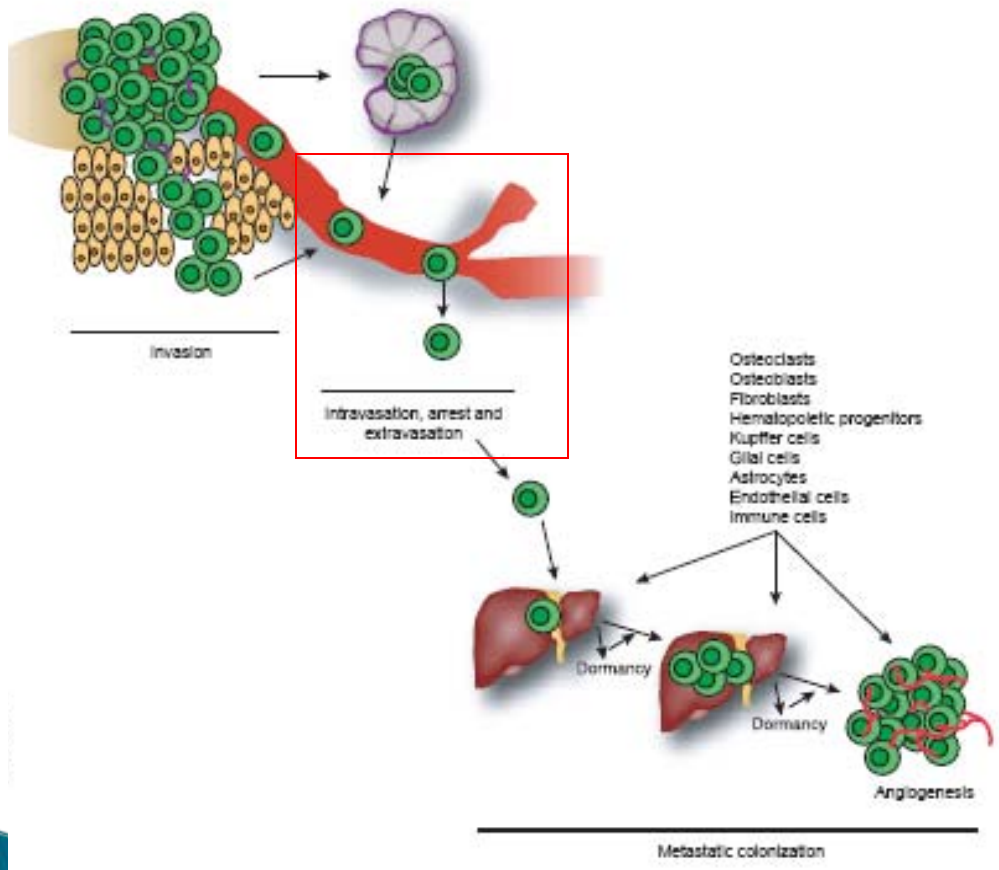
Tumor metastasis and CTCs

- **Metastatic disease is the primary cause of death in most cancer patients.**
- **It is difficult to obtain metastatic cells with conventional biopsies.**
- **Our understanding of early metastatic events is limited due to lack of detection tools.**
- **CTCs allow us to study metastatic disease and monitor the disease in "real time".**

Methods to isolate metastatic cells

- **Core biopsy**
- **FNA**
- **Resection**
- **Circulating Tumor Cells (CTC)**
  - **Flow Cytometry**
  - **Centrifugation**
  - Density gradient methods
  - **Magnetic beads**
  - **Veridex LLC**
  - **CHIP platform from MGH**

Circulating Tumor Cells



Once in circulation, cells must

1. **Survive—harsh environment**
   -shear forces
   -lack of substratum
   -immune cells

2. **Attach**

3. **Extravasate**

Nature Medicine 12, 2006

Possible applications for CTC

- **Current trials in Europe examine the direct role of CTC in patient treatment (CEK).**
- **CTC have a large number of applications for investigating the biology of metastatic cancer (CPK).**
- **CTC have a large number of applications in drug development where they can be used to:**
  - **Identify predictive biomarkers**
  - **Identify mechanisms of resistance**
  - **Identify mechanisms of acquired resistance**
  - **Facilitate pharmacodynamic studies.**


I'd rather die than allow stem cell research!
funny you should say that...
ED Fisher
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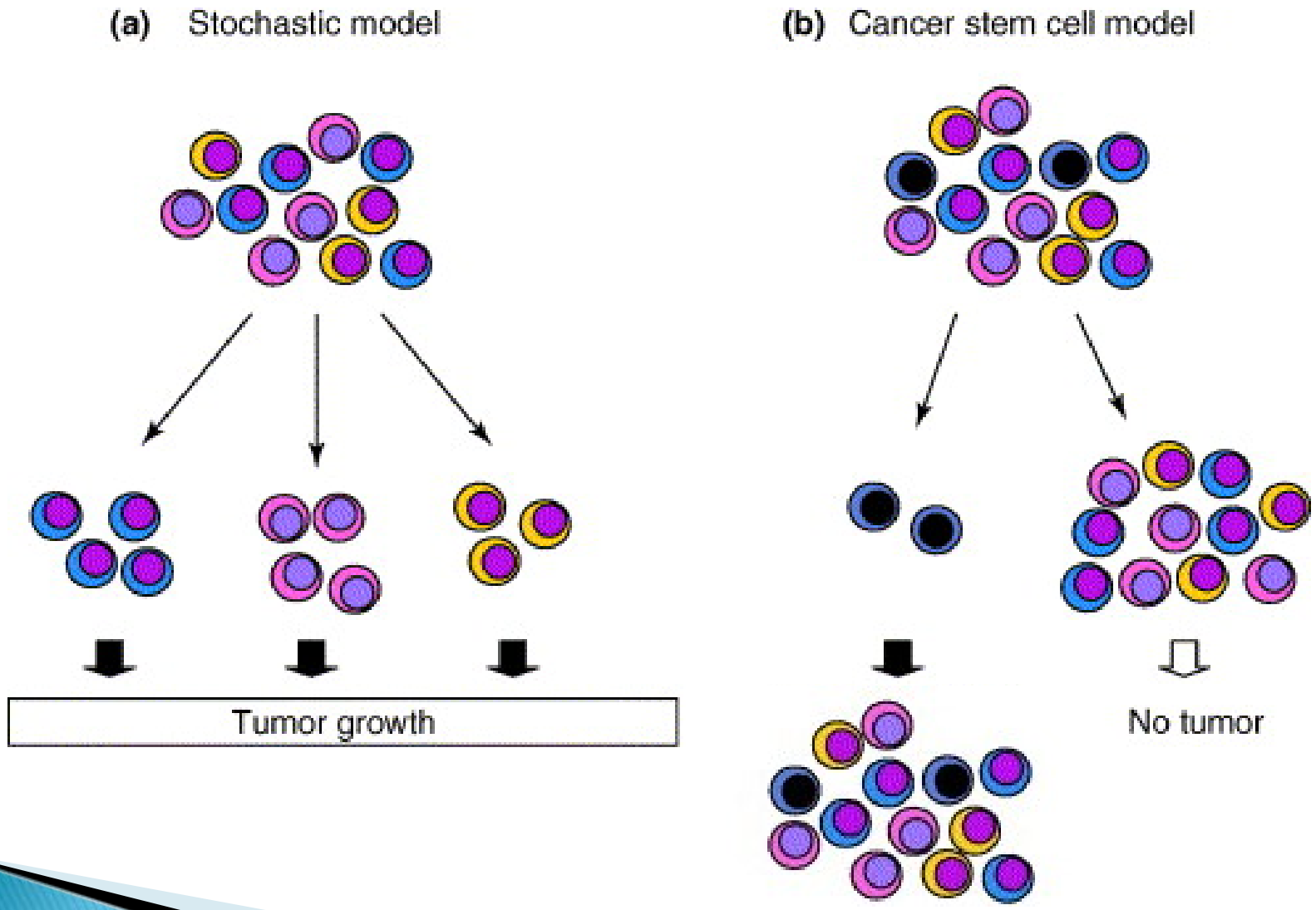
Cancer stem cell hypothesis



Adapted from Trends Cell Bio 15:4

Key features of the cancer stem cell hypothesis

- CSC represent small population of total cells
- CSC have capacity for infinite self renewal while non-CSC have limited replicative capacity
- CSC can undergo asymmetric division -providing one differentiated cell and one CSC
- CSC must balance self-renewal and differentiation to maintain homeostasis
- CSC have generally low rates of cell division

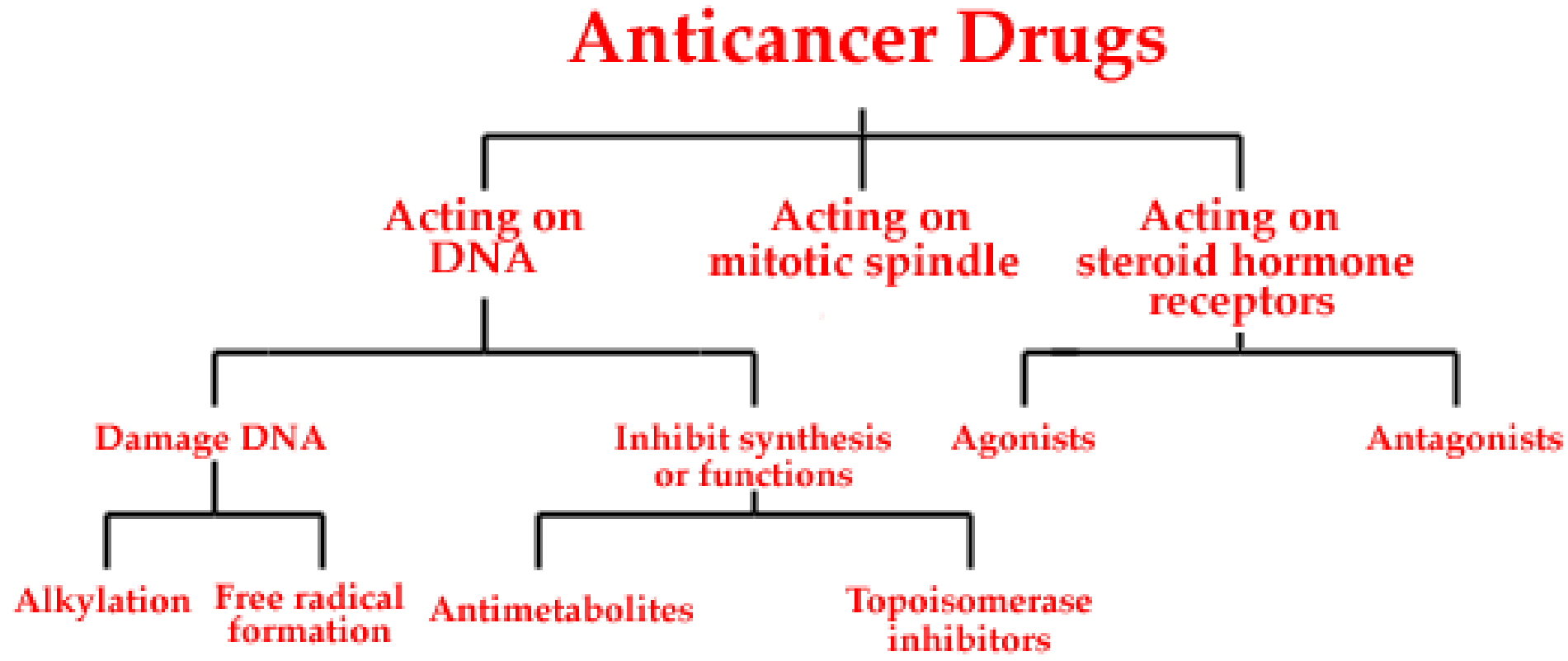
The Goals of Cancer Control

- To prevent future cancers
- To diagnose cancers early
- To provide curative therapy
- To ensure freedom from suffering
- To reach all members of the population

Chemotherapy

- Provides maximum cell kill within the range of toxicity tolerated by the host for each drug;
- Offers a broader range of coverage of resistant cell lines in a heterogeneous tumor population
- Prevents or slows the development of new drug resistant cell lines.

Treatment


Anticancer Drugs
Acting on DNA
Acting on mitotic spindle
Acting on steroid hormone receptors
Damage DNA
Inhibit synthesis or functions
Agonists
Antagonists
Alkylation
Free radical formation
Antimetabolites
Topoisomerase inhibitors

Targeted Treatment

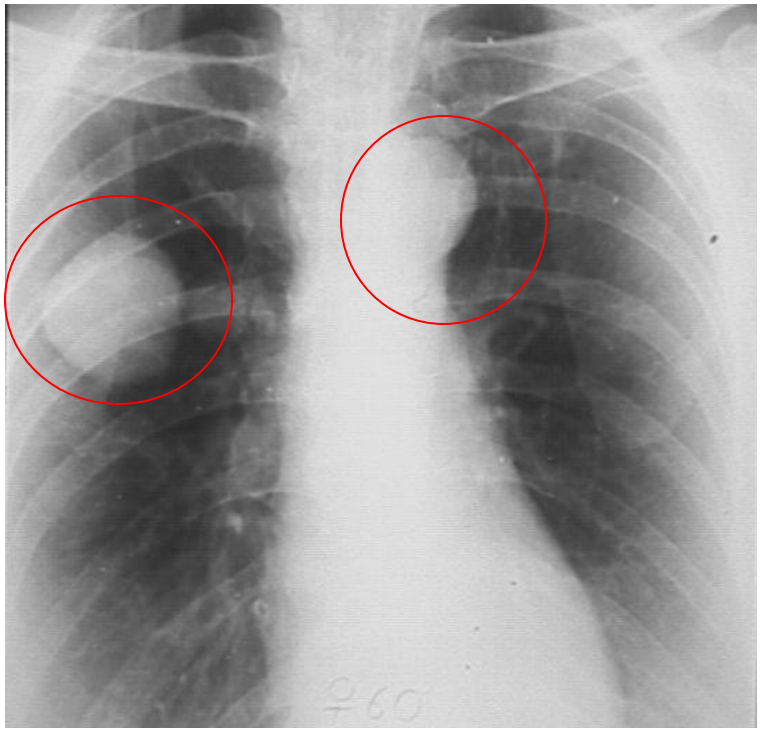
- **Herceptin** (Trastuzumab)– targets HER2/neu, a growth factor receptor
- **Gleevec** (Imatinib) – targets tyrosine kinase enzymes
- **Iressa** (Gefitinib) – targets the epidermal growth factor (EGFR)
- **Tarceva** (Erlotinib) targets epidermal growth factor receptor (EGFR)
- **Lapatinib** – HER2/neu and epidermal growth factor receptor (EGFR)
- **Avastin** (Bevacizumab) – targets circulating VEGF ligand

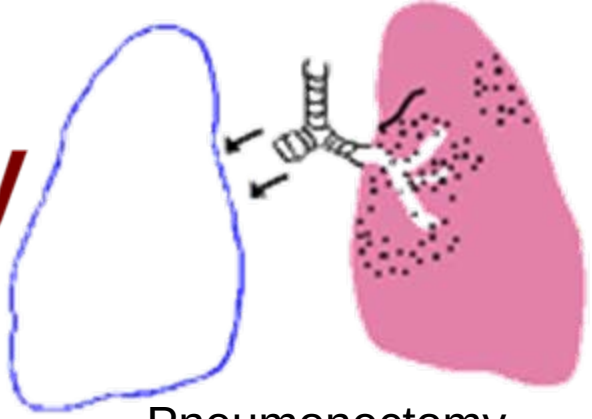
Surgery

Definitive surgical treatment of primary cancer, selection of appropriate local therapy, integration of surgery with other adjuvant modalities;

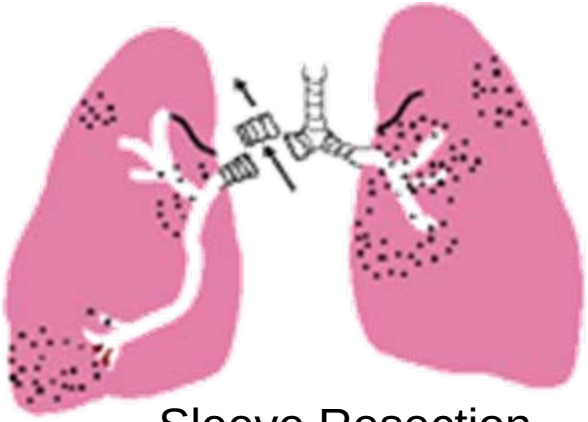
- Surgery to reduce the bulk of residual disease;
- Surgical resection of metastatic disease;
- Surgery for the treatment of oncologic emergencies
- Surgery for palliation;
- Surgery for reconstruction and rehabilitation.

Surgery

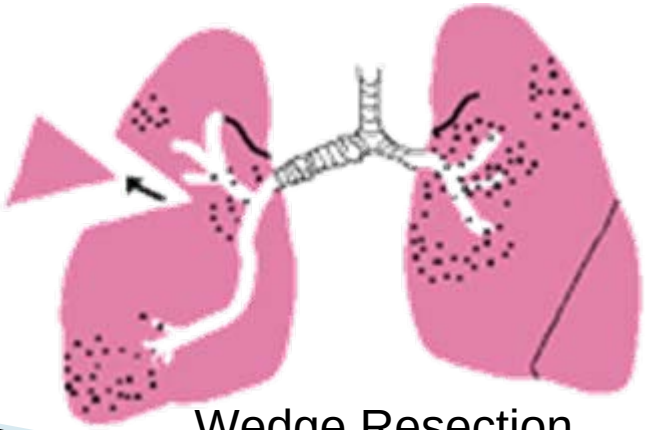




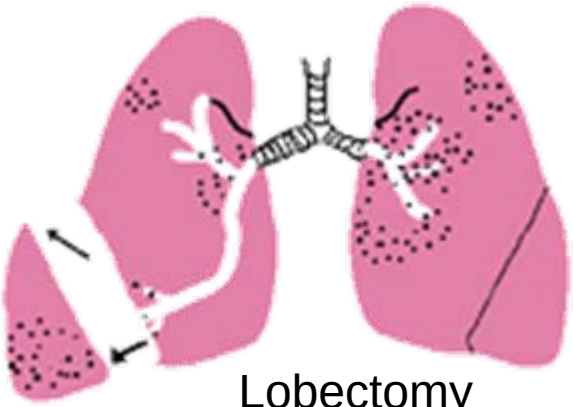
Pneumonectomy



Sleeve Resection



Wedge Resection



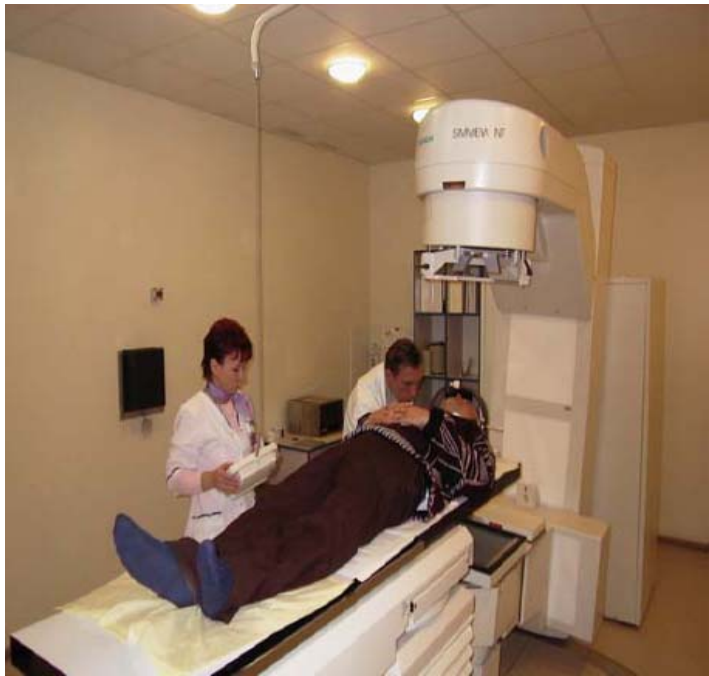
Lobectomy

Radiation

- The principle goals of radiotherapy is to deliver a tumoricidal dose of radiation to the cancer with sparing of the surrounding normal tissue

- The field of radiotherapy involves the use of ionizing radiation to treat cancer through several mechanisms of inducing cell death

- Radiotherapy can be used radically with a curative intent or to achieve palliation.



Why current therapies fail

- Tumor cells are genetically unstable
- Tumors can become drug resistant
- Tumors can remain dormant for years
- Tumor metastases are hard to find and difficult to access
- Current therapies are toxic to the patient

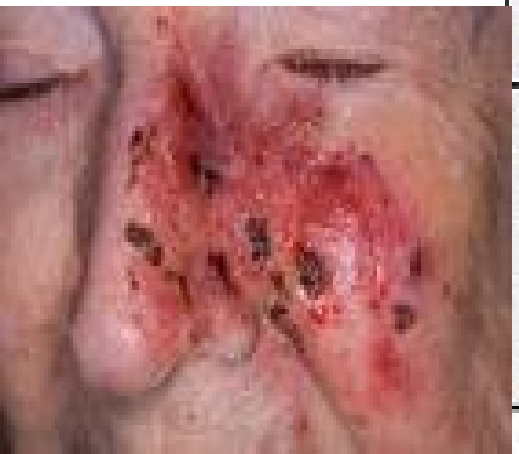
Morbidity and Mortality

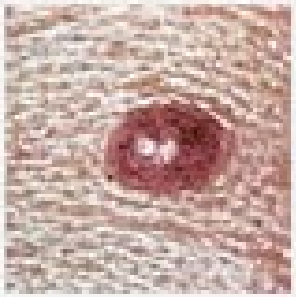
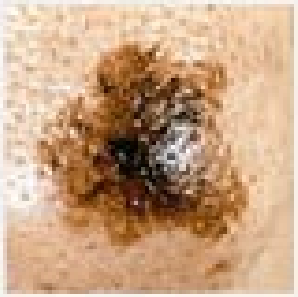
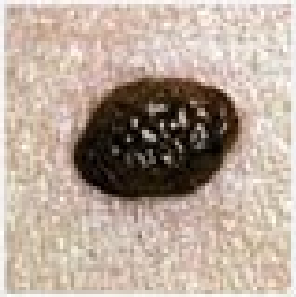
- Metastases
- Rupture into major vessels, structure
- Starvation
- Infection
- Compression of vital organs
- Organ failure

Prevention

- Physical activity reduces cancer risk
  - Decreases insulin and insulin-like growth factors
  - Decreases obesity
  - Decreases inflammatory mediators and free radicals
  - Increased gut motility
- Healthy diet reduces cancer risk
- Good night sleep boost immune system
- Self-examination – Early detection
- Annual doctor visits – Early detection
- Education: Its achievement requires knowledge of the disease process and understanding of the social and economic facto

ABCD of a Skin Cancer



Normal Mole	Melanoma	Sign	Characteristic
		Asymmetry	when half of the mole does not match the other half
		Border	when the border (edges) of the mole are ragged or irregular
		Color	when the color of the mole varies throughout
		Diameter	if the mole's diameter is larger than a pencil's eraser

Photographs Used By Permission: National Cancer Institute

Breast Cancer