

Characteristics of normal cell division

- Anchorage dependence
 - Cells must be attached to a solid surface to divide
- Density dependence
 - Cells stop dividing when too dense
- Growth factors
 - Signals that regulate cell cycle
- Senescence
 - After a finite # divisions, cell self-destruct (**apoptosis**)
 - Irreparable DNA or membrane damage
 - Shortened telomeres

Primary culture of normal cells

- **Growth factor dependence**

EXPERIMENT

- 1 A sample of connective tissue was cut up into small pieces.
- 2 Enzymes were used to digest the extracellular matrix, resulting in a suspension of free fibroblast cells.
- 3 Cells were transferred to sterile culture vessels containing a basic growth medium consisting of glucose, amino acids, salts, and antibiotics (as a precaution against bacterial growth). PDGF (platelet-derived growth factor) was added to half of the vessels (T-flasks). The culture vessels were incubated at 37°C.
- 4 Cells cultured without PDGF did nothing. Cells cultured **with** PDGF attached to the vessel, flattened out, and began dividing. [**Growth-factor dependent growth**]

Figure 12.17

Primary culture of normal cells

- **Anchorage dependence; Density-dependent inhibition; & Senescence**

- 3 Cells cultured **with** PDGF attached to the vessel, flattened out, and began dividing. [**Growth-factor dependent growth**]

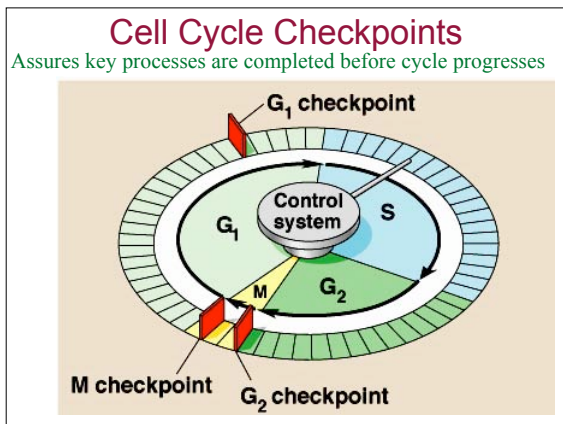
Cells anchor to dish surface and divide. [**anchorage dependence**]

When cells have formed a complete single layer, they stop dividing. [**Density-dependent inhibition**]

If some cells are scraped away, the remaining cells divide to fill the gap and then stop. [**Density-dependent inhibition**]

- 5 But even with low cell density, available surface, and added growth factors, after 20–50 cycles, the cells stop dividing, ball up, and detach. [**Senescence**]

Figure 12.18 A



Cell Cycle Checkpoints

Assures key processes are completed before cycle progresses

- **G₁ checkpoint:**
 - ✓ Sufficient growth & reserves to support replication
 - ✓ Pre-replication check for DNA damage
 - ✓ Internal clock
 - ✓ External growth factors and/or inhibitors
- **G₂ checkpoint:**
 - ✓ Sufficient growth & reserves to support mitosis & cytokinesis
 - ✓ Duplication of centrosomes
 - ✓ Replication of DNA
 - ✓ Pre-mitotic check for DNA damage
- **M checkpoint**
 - ✓ Spindle formed & functioning
 - ✓ Chromosome kinetochores correctly attached to spindle
 - ✓ Chromosomes properly aligned & untangled on metaphase plate

Transformation: damage to checkpoint mechanisms cause abnormal cell division

“Hallmarks of Cancer”:

- Growth independent of external growth regulators
 - Loss of anchorage & density dependence
 - Uncoordinated with surrounding tissues or the body
- Growth without stopping at checkpoints
- Avoidance of apoptosis despite cell/DNA damage
- Unlimited number of cell divisions
 - Activation of telomerase

Other indicators:

- De-differentiation
- □ cytoskeleton □ morphology & motility
- Angiogenesis — induced growth of blood vessels to support increased metabolic demands of hyper-growth

Primary culture of *transformed* cells

- Cancer cells exhibit neither density-dependent inhibition nor anchorage dependence
- And have only limited dependence on growth factors

Cancer cells usually continue to divide well beyond a single layer, forming a clump of overlapping cells.

Many transformed cell lines can also be cultured as liquid cell suspensions with no need for attachment substrate.

Transformed cells are also immortalized — showing no senescence

- E.g., the HeLa cell line was cultured from a tumor removed from Henrietta Lacks back in 1951. It is still growing in labs all over the world.

Stages of tumor progression

- 1. Hyperplasia:** over-production of normal-looking cells.
- 2. Dysplasia:** additional genetic/epigenetic changes lead to abnormal growth of malformed, disorganized cells.
- 3. Solid tumor *in situ*:** cells are even more malformed and de-differentiated. Growth extends from original mass into the tissue.
- 4. Malignancy (cancer):** cells detach and penetrate basal lamina into other tissues. May enter lymphatic or circulatory system and reach other organs to start new tumors.

Stages of tumor progression

- Metastasis: spread of malignant cells from original tissue

- 1 A tumor grows from a single cancer cell.
- 2 Cancer cells invade neighboring tissue.
- 3 Cancer cells spread through lymph and blood vessels to other parts of the body.
- 4 A small percentage of cancer cells may survive and establish a new tumor in another part of the body.

Types of tumors

Classification based upon tissue of origin

- “Solid tumors”
 - **Carcinoma:** epithelial cells
 - 80–90% of all cancers
 - **Sarcoma:** muscle or connective tissue
- Others
 - **Leukemia/Lymphoma/Myeloma:** bone marrow
 - **Glioma:** brain
 - **Choriocarcinoma:** placenta

Pulmonary carcinoma *in situ*

Cancer incidence & mortality in the U.S.

Estimated New Cases*					
	Men		Women		
Prostate	322,000	32%	Breast	211,240	32%
Lung and Bronchus	85,010	13%	Lung and Bronchus	79,580	12%
Colon and Rectum	71,820	10%	Colon and Rectum	73,470	11%
Urinary Bladder	47,610	7%	Uterine Cervix	40,860	6%
Melanoma of the Skin	35,580	5%	Non-Hodgkin Lymphoma	27,320	4%
Non-Hodgkin Lymphoma	29,070	4%	Melanoma of the Skin	26,000	4%
Kidney and Renal Pelvis	22,490	3%	Ovary	22,220	3%
Leukemia	19,640	3%	Thyroid	18,190	3%
Oral Cavity and Pharynx	18,100	3%	Urinary Bladder	16,200	2%
Pancreas	18,100	3%	Pancreas	16,200	2%
All Sites	718,040	100%	All Sites	662,870	100%

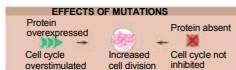
Estimated Deaths					
	Men		Women		
Lung and Bronchus	80,490	31%	Lung and Bronchus	73,000	27%
Prostate	30,260	10%	Breast	42,410	15%
Colon and Rectum	28,540	10%	Colon and Rectum	26,760	9%
Pancreas	18,820	8%	Ovary	16,210	6%
Leukemia	12,860	4%	Prostate	13,080	6%
Esophagus	10,530	4%	Leukemia	10,000	4%
Liver and Intrahepatic Bile Duct	10,230	3%	Non-Hodgkin Lymphoma	9,950	3%
Non-Hodgkin Lymphoma	10,160	3%	Uterine Cervix	7,210	3%
Urinary Bladder	8,970	3%	Multiple Myeloma	6,640	2%
Kidney and Renal Pelvis	8,020	3%	Brain and Other Nervous System	6,480	2%
All Sites	260,280	100%	All Sites	275,090	100%

*FIGURE 1. Ten Leading Cancer Types for the Estimated New Cancer Cases and Deaths, by Sex, U.S., 2005. Excludes basal and squamous cell skin cancers and all sarcomas except urinary bladder. Estimates are rounded to the nearest 10. Note: Percentages may not total 100% due to rounding. American Cancer Society, Surveillance Research, 2010.

From Jemal, A. et al. CA Cancer J Clin 2005;55:10-30. Copyright ©2005 American Cancer Society

Transformation requires a series of non-lethal mutations within a specific cell line

- Turn on “on-switches”
 - Dominant mutations: proto-oncogenes → **oncogenes**
 - Bypass checkpoints
- Turn off “off-switches”
 - Recessive mutations: inactivate **tumor suppressors**
 - Remove checkpoints
- All cancers involve mutations in one or more oncogene and one or more tumor suppressors.



Sources of mutations

- Spontaneous
- Induced — mutagens/carcinogens
 - Radiation
 - UV — mostly point mutations
 - X-rays — translocations
 - Endogenous chemicals
 - Reactive oxygen species (ROS) → alter DNA bases
 - Chronic inflammation
 - Fat metabolism
 - Exogenous chemicals
 - Bind to DNA → replication & transcription errors
 - Benzo-pyrene from tobacco smoke
 - Aflatoxins from food-borne fungi
 - Viruses
 - Inserted pro-viruses
 - Viral-induced growth factors
 - Genetic carry-over from prior host cells

Oncogenes

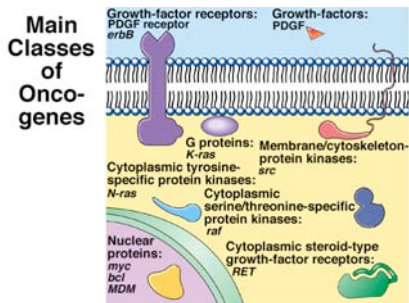
- **Proto-oncogene**: normal gene functions in stimulating cell growth or viability, esp. for embryogenesis & organogenesis.
- Mutated form = **oncogene**: stimulates unregulated cell division or immortalization.
- Presence of the oncogene or oncogene product → ↑probability of transformation

Oncogenes

- Presence of the oncogene or oncogene product → ↑probability of transformation
- **Types of oncogenes**:
 - Growth factors
 - Growth factor receptor (**HER2**)
 - G-proteins (**Ras**)
 - Receptor-associated kinases (**Src**)
 - Transcription factors (**Myc**)
 - Telomerase activators
 - Apoptosis-regulating proteins (**Bcl-2**)

Oncogenes

- Mutated proto-oncogenes may become oncogenes — tell cells to proliferate inappropriately.



Genetic changes that can turn proto-oncogenes into oncogenes

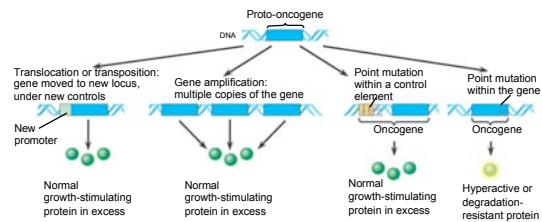
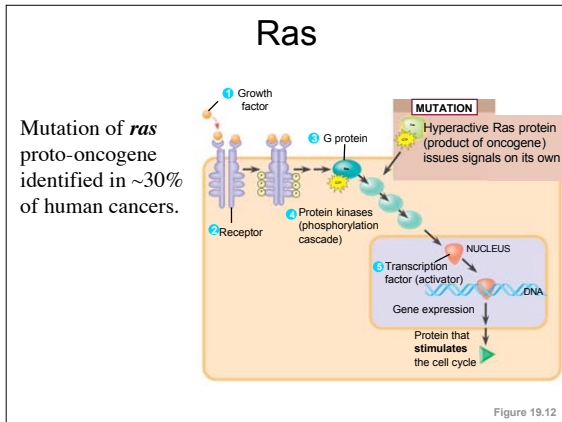
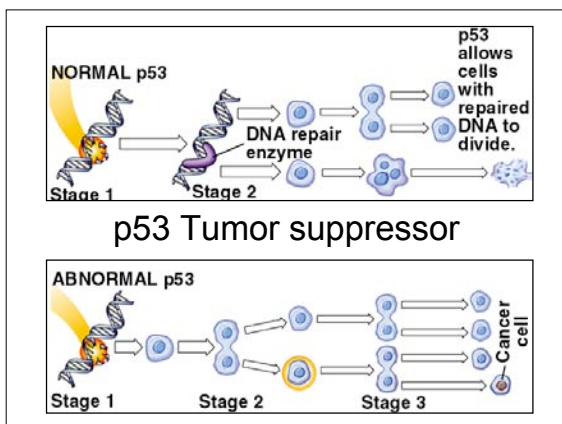
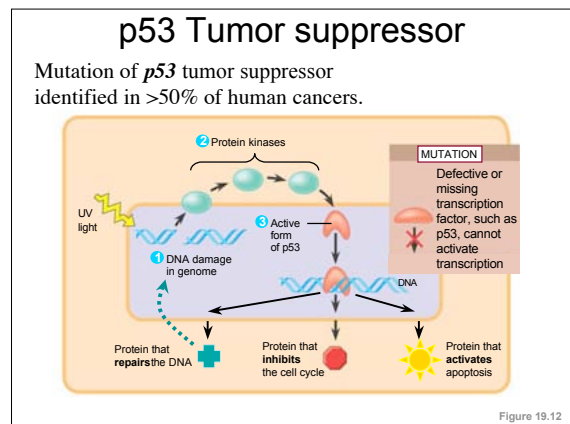


Figure 19.11



- ### Tumor suppressors
- **Tumor suppressors:** normal gene functions in delaying cell growth, locating or repairing damaged DNA, or initiating apoptosis of irreparably damaged or senescent cells.
 - Mutated form is inactive: unable to regulate cycle, detect or repair genetic damage, or divert to self-destruct.
 - Absence of the tumor suppressor
 - ↑probability of transformation

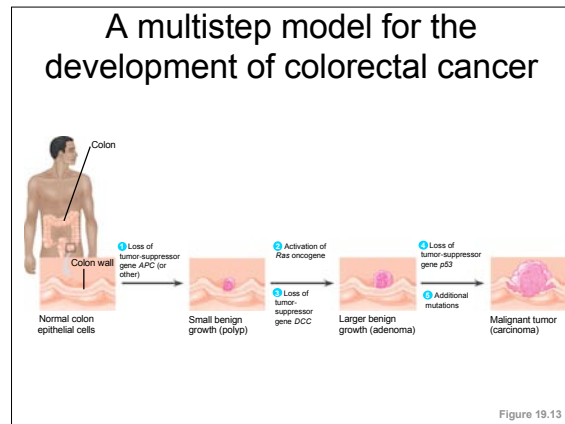
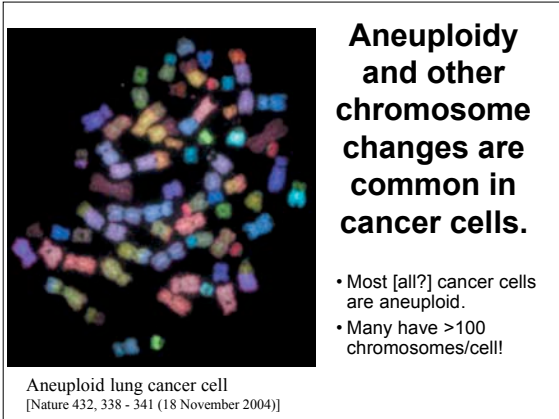
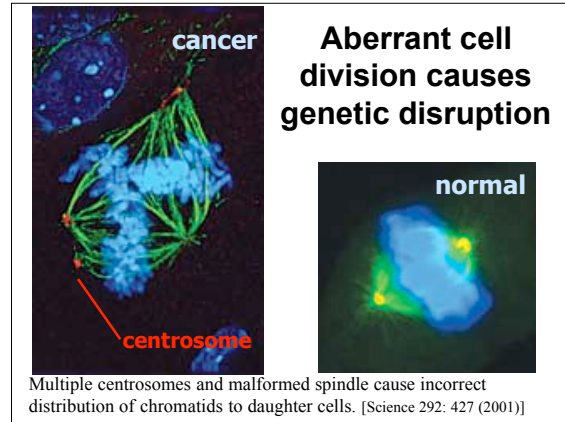
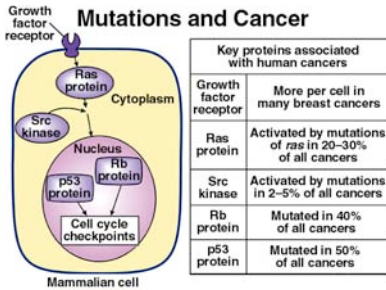
- ### Tumor suppressors
- Absence of the tumor suppressor
 - ↑probability of transformation
 - **Types of tumor suppressors:**
 - Transcription factors (**p53**)
 - Factors that restrict access of transcription factors (**APC**)
 - Factors that block effects of transcription factors (**Rb**)
 - DNA repair (**BRCA**)



- ### Clonal evolution (multistep) model of cancer development
1. Point mutations in tumor suppressor gene allows cell divisions without adequate checks & repair of DNA damage
 2. Aberrant cell divisions result in further genetic damage, including translocations, deletions, and aneuploidy.
 3. Major genetic rearrangements create further disruptions of proto-oncogenes and tumor suppressors to produce transformation and further stages of tumor progression.
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- 5
- Mutation inactivates tumor suppressor gene
- CELLS PROLIFERATE
- Mutation inactivates DNA repair gene
- Mutation of proto-oncogene creates an oncogene
- Mutation inactivates several more tumor suppressor genes
- CANCER

Multistep model of cancer development

- Carcinogenesis requires both oncogene and tumor suppressor mutations.



What is cancer?

1. Unrestricted proliferation of a cell line
 - Displacement of healthy tissues
 - Pressure on confined tissues
 - Over-consumption of resources
2. Metastasis
 - Spreading
3. Disrupted gene expression
 - De-differentiation
 - Loss of normal function
 - Inappropriate production of bioactive substances

