(a) Survival cues and Proliferation cues.

Normal Cell
- Death mechanisms off
- Division checkpoints okay

Survival
Proliferation

(b) No survival cues and No proliferation cues.

Normal Cell
- Death programs activated
- Division checkpoints blocked

Cell death
No cell division

(c) Cancer Cell
- Death cues ignored
- Survival cues ignored
- Proliferation cues ignored

Uncontrolled survival and proliferation
Cell Proliferation

Regulated by **Cell Cycle**

Stages of cell cycle:

1. **M Phase** – Mitosis
2. **G₁ Phase** – End of mitosis and start of DNA replication
3. **S Phase** – DNA synthesis
4. **G₂ Phase** – Separates the end of the S Phase and the beginning of P Phase

- Phase S, G₂, and M have fixed durations
- Variation found in G₁ Phase
- Cell cycle checkpoints permit progression to next phase

**Cyclins and Cyclin-Dependent Protein Kinases**

**Cyclin**

- Proteins made by a transcription factor activated by a previous Cyclin-CDK Complex

**Cyclin-Dependent Protein Kinases (CDK)**

- Protein kinases that are activated and regulated by cyclins, CDK’s phosphorylate target proteins necessary in the cell cycle

**How do cyclins control cell cycle?**

- They mark the target proteins to be phosphorylated by the CDK
- Phosphorylation activates a transcription factor
- Transcription factors make genes required for the next phase of cell cycle

Each phase of cell cycle is regulated by the phosphorylation of certain target proteins. The cycle will halt without proper phosphorylations.

Cyclins are removed from the cell if:

- Inactivation of transcription factor
- High degree of instability of cyclin mRNA
- High degree of instability of cyclin

**Ex. Rb-E2F Pathway in Mammalian Cells**

- Rb – Target protein
- Cdk2-cyclinA – CDK-cyclin complex
- E2F – Transcription factor (Regulates Rb)
Regulation of Cell Proliferation

(1) **Intracellular Signal**

**Negative Control**
- “Checkpoints” in the cell cycle
- Ex.: [UV damaged DNA](#)
  - p53 detects damage
  - p21 binds to CDK-cyclin complex
  - Cell cycle stops, since target proteins of CDK are phosphorylated

**Positive Control**
- Cell cycle “block” removed
- Cyclin-CDK complexes activate target proteins
- Cell cycle continues

(2) **Extracellular Signals**

**Negative control**
- Secreted proteins inhibit cell division
- Ex.: TGF-β – ligand inhibits cell division
  - TGF-β binds to TGF-β receptor, activates serine/threonine kinase activity
  - Phosphorylation of SMAD’s
  - Rb protein is blocked

**Positive control**
- Mitogens (Growth factors) promote cell division
- Ex.: EGF – Epidermal growth factor

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**Cell Death**

- For every cell, there is a time to live and a time to die

**2 types of cell Death**

- **Necrosis**
  - uncontrolled cell death that leads to lysis of the cell, inflammatory responses, and potentially, to serious health problems

- **Apoptosis**
  - a process whereby cells play an active role in their own cell death - suicide
Apoptosis

- Programmed cell death
- Operated by a "SELF-DESTRUCT" mechanism
- Activation can occur for various reasons:
  - elimination of cells no longer needed for development
  - destruction of cells that represent a threat to the integrity of organism
    - cells infected with a virus
    - cells with DNA damage
    - cells of the immune system

Events of Apoptosis Pathway

1. Chromosomal DNA is fragmented
2. Organelle structures are degraded
3. Cell loses its shape
4. Cell breaks up into small, membrane-wrapped fragments called apoptotic bodies
5. Apoptotic bodies are phagocytosed by mobile scavenger cells

Overview of Events

Regulation of Apoptosis

- Caspases
- Intracellular Controls
  - positive - Cytochrome C
  - negative - Bcl Proteins
- Extracellular Controls
  - positive - Fas ligand
  - negative - Survival factors

(1) **Caspases**: the "engines" of self-destruction

- Enzymes that cleave other proteins (proteases)
- CASPases - "Cysteine-containing Aspartate-Specific Proteases"
- Cleaves target proteins at aspartate residues but need to be activated
- Present in normal cells
  - inactive state - zymogen form = precursor protein
  - contains longer peptide chain than activated enzymes
- proteolysis cuts zymogen into subunits which bind to form an active caspase

**2 classes:**
- Initiators
The organization of the cleavage cascade is unclear

How They Work (Theories)
"Biochemical changes cause morphological changes"

- Initiators respond to cleavage by other types of enzymes
- Initiator caspases then cleave one of the executioner caspases, which then cleaves another - Domino Effect
- **Executioner caspases duties:**
  - Ex. 1: animation
    - Target » "sequestering" protein that holds endonuclease in cytoplasm
    - Caspase cleaves this sequestering protein
    - endonuclease is then free to enter nucleus and chops up DNA
  - Ex. 2:
    - Target » protein that will cleave actin
    - Caspase cleaves protein
    - protein cleaves actin
    - disruption of cytoskeleton (actin filaments)
    - loss of cell shape
- Other targets are assumed to be victims of caspases that correspond to the rest of the apoptotic pathway
- A model organism:
  - mutation that knockout genes that encode caspases have been seen in nematodes
  - apoptosis does not occur in this situation

(2) **Intracellular Controls**

- Idea was that leakage of material from mitochondria induced apoptosis
  - SWITCH - Cytochrome C
  - When organelle structure is degraded, cytochrome c leaks out
- **Normal conditions - Bcl Proteins**
  - Keep apoptotic pathway turned off by binding to a protein called Apaf-1 (apoptotic protease activating factor-1)
  - Block release of cytochrome c by making it more difficult for the mitochondria to burst
- Internal damage to cell causes Bcl-2 protein to release Apaf-1
- **Apoptotic stimulus - Bcl-x** (a related protein Bax)
  - penetrates mitochondrial membranes causing cytochrome c to be released
  - Cytochrome C interacts with Apaf-1; both bind to iniator caspase, caspase 9
  - Complex formed - Apoptosome
    - cytochrome c
    - Apaf-1
    - caspase 9
    - ATP
  - Caspase 9 cleaves other proteins which activate other caspases
Sequential activity causes cascade of proteolytic activity

(3) Extracellular Controls

**Ignition** (positive):

- **Fas system:**
  - transmembrane receptor (Fas)
  - membrane bound protein ligand (FasL)
    - trimerization of both complex and domain of the receptor
    - activation of Apaf, indirectly or directly
    - activation of the caspase cascade
  - Ex. Clogging of the immune system would occur if nonfunctional B and T cells were not eliminated through the Fas self-destruction signal

**Brake** (negative):

- Survival factors:
  - negative secreted factors necessary to block activation of apoptotic pathway
  - influence isn't clear

Pathway of Apoptotic Signals

**Conclusion**
Cancer

- Genetic disease due to rapid uncontrolled proliferation of cells within tissues of eukaryotes
- Most develop due to mutations in somatic cells
- Mutations create oncogenes and inactive tumor suppressing genes
- Determining specific oncogene and tumor suppressor gene mutations help in diagnosis of cancer type
- Multiple mutations lead to formation of neoplasia (tumors)

Types of tumors:
(1) Benign: Do not spread away from primary tumor
(2) Malignant:
  - Spreads away from primary tumor
  - Spreads via
    - the blood stream or lymphatic ducts,
    - cells lodge in the nearest narrow tube,
      - usually where there are capillaries, and tumors grow.
    - Spreads by crossing body cavities in fluids,
      - this type of spread is seen in abdomen, thorax,
        - and brain that is called metastasis spread.

Tumors develop due to mutations in:
- Oncogenes
- Tumor suppressor genes

Oncogenes

- 100 different types identified
- Normal counterparts - Proto-oncogenes
  - a class of proteins active only when proper regulatory signals activate them
- What happens in oncogene mutations?
  - Activity of mutant oncoprotein becomes uncoupled from normal regulatory pathway
  - Leads to continuous unregulated expression
- Categorized according to uncoupling of regulatory function
- Gain of Function
- positive control of cell cycle
- negative control of apoptosis

(1) Point Mutations

Recall - Ras protein is a G-protein subunit that takes part in signal transduction
- Normally: cycles between active GTP-bound state and inactive GDP-bound state
- Base-pair substitution (gly to val) at amino acid 12
• Creates oncoprotein in human bladder cancer
• **What Happens?**
  o Oncoprotein always binds GTP even without phosphorylation of Ras
  o Ras oncoprotein **constantly signaling** cell proliferation

(2) **Loss of Protein Domains**

Deletion of parts of normal protein can also produce an oncoprotein

Ex.: *v-erbB* oncogene (found in erythroblastosis tumor virus infecting birds)

- Encodes a mutated RTK P EGFR (Epidermal Growth Factor Receptor)
  - **Recall- RTK** (receptor tyrosine kinases):
    - Receptor/ligand complex that requires dimerization to activate signaling
    - The cytoplasmic domain is activated- phosphorylates tyrosine residues on target protein
    - Autophosphorylation initiates signal transduction cascade
    - Modification of transcriptional activators and repressors
  - This lacks the extracellular binding domain & some regulatory parts of cytoplasmic domain
  - Oncoprotein is able to dimerize in the absence of ligand (EGF)
  - Constitutive EGFR dimer is always autophosphorylated
  - Continuously initiates a signal transduction cascade

(3) **Gene Fusions**

Causes the most remarkable type of structural alteration to a protein

Classic Ex.: Philadelphia chromosome

- Diagnostic feature of Chronic Myelogenous Leukemia (CML)
- Translocation between chromosomes 9 and 22
- Break points of translocation among CML patients are very similar
- Cause the **fusion of bcr1 and abl**
  - The *abl* proto-oncogene encodes a cytoplasmic tyrosine-specific protein kinase
  - The Bcr1-Abl oncoprotein has permanent protein kinase activity

Some oncogenes cause Misexpression:

- When the oncoprotein and normal protein have identical structure
- Protein is expressed in cell types where it is normally absent
- Ex.: B-cell oncogene translocations (diagnostic of B-lymphocyte tumors)
  - No protein fusion
  - Causes a gene (near a break point) to be turned on in the wrong tissue

- Ex.: **Follicular lymphoma**
  - Translocation between chromosomes 14 and 18
  - Enhancer from an immunoglobulin gene is fused with *bcl2* (negative regulator of apoptosis)
Tumor-Suppressor Genes

- Genes that slow down cell division, repair DNA, tell cells when to die
- Nonfunctioning genes cause cells to grow out of control that leads to cancer
- Genes can be inherited or acquired
- Cause cancer when they are inactive

Functions

- Genes that control cell division
  - There are two copies of every gene; if one copy is mutated and one is normal – no cancer develops
  - Both mutated; loss of heterozygosity – cancer develops

- Genes that control cell death
  - p53 genes destroy cells when the DNA is badly damaged

- Genes that repair DNA
  - DNA proofreading genes; malfunction – DNA is mutated and can lead to cancer

Indirect Effects

- Inherited tumor phenotype
  - p53 tumor suppressor gene

Indirect effects:

Ex. 1: Inheritance of tumor phenotype

- Retinoblastoma – codes for mutated Rb gene protein
  - Sporadic retinoblastoma is most common
    - Mutation in the retina is not inherited
    - Multiple tumors in one eye
    - rb mutation(s) inactivate the Rb gene
    - Cancer develops by chance during developmental stages
  - Inherited retinoblastoma (hereditary binocular retinoblastoma, HBR)
    - Tumors in both eyes

Why does the absence of RB gene promote tumor growth?

- Back to cell cycle!
  - Rb binds to E2F, preventing transcription of genes for S phase
  - Inactive Rb, can’t bind E2F so genes are transcribed
  - Continuous growth w/out Rb gene!!

Ex. 2: p53 tumor suppressor gene
Recessive tumor-promoting mutation
50% of tumors lack functional p53 gene
p53
  - Transcriptional regulator
  - Activated by DNA damage
  - Can “stall” the cell cycle or induce apoptosis
  - W/out p53, apoptosis is not activated and cell cycle continues indefinitely

The Complexities of Cancer

- Mutations arise from alterations in cell cycle or apoptosis
- Benign tumors can become malignant
- Malignant tumors evolve by successive mutations
- More work on tumors is needed

A Cure?

- **Chronic myelogenous leukemia** (CML)
- Overexpression of tyrosine kinase
- *Gleevec* successfully treats 90% of CML patients
- ST1571 inhibits tyrosine kinase
- Prevents phosphorylation of target proteins
- Inhibits cell proliferation in *bcr-abl* cell lines
- Long term effects unclear

Conclusion

A Different Perspective on Cancer