The Cellular Basis of Disease
Cell Injury 3

Apoptosis and Necrosis
Cellular Aging

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Objectives

** Discriminate** cell adaptation, reversible cell injury and irreversible cell injury (cell death) based on etiology, pathogenesis and histological and ultrastructural appearance.

** Compare and contrast** pathologic features and the clinical settings in which necrotic and apoptotic cell death occurs.

** List in temporal order** the genetic and biochemical steps in apoptosis.

** Contrast and compare** physiologic and pathologic apoptosis.

** Describe** the mechanisms and implications of cellular aging.
Necrosis

- Morphologic expression of cell death
- Progressive disintegration of cell structure
- Initiated by overwhelming stress
- Usually elicits an acute inflammatory cell response (neutrophils may be present).

Just because Dr. H mentioned it, inflammatory response doesn't occur in immunocompromised patients.
Apoptosis

• Pathway of cell death induced by a tightly regulated suicide program.
• Controlled by specific genes.
• Fragmentation of DNA in a regular pattern.
• Fragmentation of nucleus.
• Blebs form and apoptotic bodies are released.
• Apoptotic bodies are phagocytized.
• No neutrophils.

No inflammation in apoptosis.
Necrosis or Apoptosis?

Consequences of Cell Death

**Necrosis**
- Loss of functional tissue
- Impaired organ function, transient or permanent

**Apoptosis**
- Removal of damaged or unnecessary cells
Reversible injury

Recovery

NORMAL CELL

Swelling of endoplasmic reticulum and mitochondria

Myelin figure

Membrane blebs

Progressive injury

Breakdown of plasma membrane, organelles and nucleus; leakage of contents

Myelin figures

Inflammation

Amorphous densities in mitochondria

Necrosis

Apoptosis

NORMAL CELL

Condensation of chromatin

Membrane blebs

Cellular fragmentation

Apoptotic body

Phagocyte

Phagocytosis of apoptotic cells and fragments

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Causes of Apoptosis

Physiologic

Pathologic
Physiologic Apoptosis

Embryogenesis and fetal development.

Hormone dependent involution.
  Prostate glandular epithelium after castration
  Regression of lactating breast after weaning

Cell loss in proliferating cell populations.
  Immature lymphocytes
  Epithelial cells in the GI tract

Elimination of self-reactive lymphocytes.

Death of cells that have served their function.

Neutrophils, Lymphocytes
Apoptosis in Pathologic Conditions

DNA damage due to radiation, chemotherapy.

Accumulation of misfolded proteins leads to ER stress which ends with apoptosis.

Cell death in viral infections that induce apoptosis such as HIV and Adenovirus or by the host immune response such as hepatitis.

Organ atrophy after duct obstruction.
## General Characteristics

<table>
<thead>
<tr>
<th>NECROSIS</th>
<th>APOPTOSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usually affects <strong>large areas</strong> of contiguous cells</td>
<td>Usually affects <strong>scattered individual cells</strong></td>
</tr>
<tr>
<td><strong>Control</strong> of intracellular environment <strong>is lost early</strong></td>
<td><strong>Control</strong> of intracellular environment <strong>maintained in early stages</strong></td>
</tr>
<tr>
<td>Cells <strong>swell</strong> and organelles <strong>swell</strong></td>
<td>Cells <strong>contract</strong></td>
</tr>
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Compare and contrast Necrosis and Apoptosis. Look at the chart.
<table>
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<td><strong>NECROSIS</strong></td>
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<tr>
<td>Nuclear chromatin marginates early, while injury is still reversible</td>
<td>Nuclear chromatin marginates and chromatin condenses, becoming very compact</td>
</tr>
<tr>
<td>When DNA is cleaved, which is usually a late event, fragments are random in size (smear pattern in gels)</td>
<td>Chromatin condensation and DNA fragmentation occur together; DNA cleaved into multiples of 200 base pair units (ladder pattern in gels)</td>
</tr>
</tbody>
</table>
# General Characteristics

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<td>Cell membrane ruptures as terminal event and cell contents are released, which are chemotactic.</td>
<td>Blebs form and apoptotic bodies containing nuclear fragments are shed.</td>
</tr>
<tr>
<td>Chemotactic factors lead to neutrophil infiltration to degrade dead cells.</td>
<td>Phagocytosis of intact apoptotic bodies, no chemotactic factors are generated.</td>
</tr>
</tbody>
</table>
Apoptosis
Normal cell

Apoptotic cell with ladder pattern, reflecting cleavage at 200bp

Necrosis. DNA in all different lengths, smear pattern.
A - early apoptosis; chromatin margination & condensation

B - later in apoptosis; nucleus is fragmented

C - phagocytosis of apoptotic cellular remnants by adjacent cell

D - swollen, necrotic cell for comparison
Apoptosis

A. Caused by ischemia and inflammation.
B. Responsible for changing cell type in response to stress
C. Beneficial process to eliminate damaged cells
D. Reduces the size of an organ
E. Induced by retinoic acid
Apoptosis

A. Caused by ischemia and inflammation. [Necrosis]

B. Responsible for changing cell type in response to stress [Metaplasia]

C. Beneficial process to eliminate damaged cells

D. Reduces the size of an organ [Atrophy]

E. Induced by retinoic acid [Patterning during embryogenesis]
Mechanisms of Apoptosis

Death receptor *(Extrinsic)* pathway

Mitochondrial *(Intrinsic)* pathway

Execution Phase

Removal of dead cells

This slide indicates that there are 2 pathways (Intrinsic/Extrinsic) that lead to the execution phase and removal of cells.
I think this slide is pretty self-explanatory. Any important points are brought up again.
Mechanisms of Apoptosis

Cells contain **intrinsic death and survival signals** that are genetically regulated.

**Genes are highly conserved across species** and are homologous to **ced** (cell death abnormal) genes in nematodes that initiate or inhibit apoptosis.
Intrinsic - Mitochondrial pathway

Increased mitochondrial permeability with release of pro-apoptotic molecules into the cytoplasm (cytochrome c).

Synthesis of anti-apoptotic molecules (Bcl-2) promoted by Growth factors.

When cells are deprived of growth factors or subjected to stress anti-apoptotic molecules (Bcl-2) are lost.

*Bcl-2 is over expressed in most follicular B-cell lymphomas – allowing abnormal cells to proliferate.*

Mitochondrial membrane becomes permeable and proteins that activate caspase leak out.
Intrinsic (Mitochondrial) Pathway of Apoptosis

A. VIABLE CELL

- Survival signal (e.g., growth factor)
- Bcl-2 (or Bcl-x)
- No leakage of cytochrome c

Normal Cell

B. APOPTOSIS

- Lack of survival signals
- DNA damage
- Activation of sensors (BH3-only proteins)
- Antagonism of Bcl-2
- Leakage of cytochrome c, other proteins
- Activation of caspases

APOPTOSIS

Don’t memorize details unless you plan on getting a PhD.

Hi MSTPs!!
Essence of intrinsic 
(mitochondrial) pathway

Pro-apoptotic and protective molecules that regulate mitochondrial permeability and the release of death molecules sequestered in the mitochondria are maintained in balance normally.

Imbalance initiates the death pathway.
Extrinsic (Death receptor initiated) pathway

Death receptors are members of the tumor necrosis factor receptor family and a related protein called Fas (CD95).

These molecules contain a death domain.
Extrinsic (Death Receptor-initiated) Pathway of Apoptosis

Fas Expressed on cell surface linked to Death domain. If activated, apoptosis is initiated.
Execution Phase

The intrinsic and extrinsic pathways converge to a caspase activation cascade.

Caspases (cysteine-aspartic-acid-proteases) are conserved across species.

Synthesized as inactive precursors; activated by proteolytic cleavage.

Family of at least 12 proteases, a few of which are involved in inflammation, and many of which are involved in apoptosis
How Caspases Disassemble a Cell

Cleave structural proteins leading to nuclear breakdown.

Converts cytoplasmic DNase to active form.

DNase causes characteristic internucleosomal cleavage of DNA.
Removal of Dead Cells

Dying cells secrete factors that recruit phagocytes.
This facilitates prompt clearance before they undergo secondary necrosis. Dead cells disappear without a trace and do not produce inflammation.
Which of the following features is seen in apoptosis but not in necrosis?

A. Internucleosome cleavage of DNA
B. Inflammation
C. Pyknosis
D. Cytoplasmic hypereosinopililia
E. Karyolysis
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Reflection of cell injury and not death.

Features of Necrosis
Examples of Apoptosis
Lack of growth factor or hormone

Hormone sensitive cells deprived of hormone.

Lymphocytes that are not stimulated.

Neurons deprived of growth factor.

Remember Brain and Behavior?
Examples of Apoptosis
Specific activation of death receptors

**DNA damage** - Tumor suppressor gene p53 accumulates in damaged cells and arrests the cell cycle. p53 is mutated or absent in some cancers and can not initiate apoptosis in malignant cells.

**Protein misfolding** – unfolded protein response and ER stress – Alzheimer, Parkinson and Huntington diseases.

**TNF Receptor family.**

**Cytotoxic T lymphocyte**
Apoptosis in Pathologic Conditions

Ionizing radiation
Cytotoxic chemotherapeutic drugs
Mild thermal injury
Cell injury in some viral diseases
Pathologic atrophy after duct obstruction
Cell death in tumors
Glucocorticoids induce apoptosis in lymphocytes
Apoptosis Summary

“Programmed cell death” can be activated by moderate stress which has damaged the cell beyond its ability to recover fully or by viral infection. This has the desirable effect of removing damaged or infected cells. Selective manipulation of apoptotic pathways may be an important approach for treating cancer in the future.
We recognize that cell death has occurred by morphologic manifestations which are often influenced by the environment.

Is the distinction between necrosis and apoptosis absolute? **NO!!!**
We’re done with Apoptosis now. Here are the main points to focus on for cellular aging:

**Cellular Aging**

Structural and Biochemical Changes with Aging

- Decreased cellular replication
- Telomere shortening causes cell cycle arrest
- Accumulation of Metabolic and Genetic Damage
- Calorie restriction delays aging
Telomere shortening leads to replicative senescence. Environmental insults cause damage to proteins and organelles, leading to free radicals and DNA damage. DNA repair defects result in accumulation of mutations. DNA damage activates Sirtuins, suggesting mechanisms of cellular aging. Abnormal growth factor signaling (e.g., insulin/IGF) is not well understood. Caloric restriction is an uncertain factor in cellular aging. "?" is not a sufficient explanation in science.
Structural and Biochemical Changes with Aging

Oxidative phosphorylation is reduced

Synthesis of nucleic acids, structural proteins, enzymes, cell receptors and transcription factors are reduced

Decreased capacity for nutrient uptake and repair of DNA damage

Cytologic changes

Accumulation of abnormally folded proteins
Replicative Senescence

Cells have a limited capacity for replication.

Cultured human fibroblasts have limited division potential.

Werner’s syndrome is a rare disease characterized by premature senescence.

Werner's syndrome patients often die as early as age 20, but may look 100.
Oh hey, Robbins.

Fibroblasts from individuals, # of times they can reproduce.

(From Dice JR: Cellular and molecular mechanisms of aging. Physiol Rev 73:150, 1993.)
Replicative Senescence

With each cell division there is **incomplete replication of telomeres**.

**Broken telomeres signal cell cycle arrest.**

As cells age, the telomere becomes shorter.

**Telomerase normally adds nucleotides.**

Telomerase is **active in germ cells and stem cells** but **absent in somatic tissue**.

Telomerase may be **reactivated in cancers**
Parental strand

Newly synthesized (lagging) strand

Binding of telomerase

Extension of 3' end by telomerase

(Telomerase enzyme)

Genes that influence Aging

Insulin growth factor receptor. 

Decreased signaling of IGF-1 receptor is the result of decreased caloric intake or mutations and results in longer lifespan in *C. elegans*.

Evidence suggests that aging is genetically determined.
Accumulation of Metabolic and Genetic Damage

Reactive oxygen species (lipofuscin).
Over expression of SOD extends life span in *Drosophila*.

Werner's syndrome – defective helicase.

Ataxia telangiectasia – ineffective repair of dsDNA breaks.

Damaged organelles accumulate.
Which of the following cells has the highest telomerase activity?

A. Endothelial cells
B. Germ cells
C. Neurons
D. Neutrophils
E. Erythrocytes
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Summary

Necrosis and Apoptosis
Molecular mechanisms
Role in health and disease

Cellular aging