What is Apoptosis?

Apoptosis: Programmed Cell Death

- Definitions: Apoptosis vs. Necrosis
  - Necrosis derived from Greek “nekrosis,” meaning “deadness.” Necrosis is lethal cell injury or accidental cell death in the living organism.
  - Apoptosis derived from Greek “apo,” meaning “away from,” and “ptosis,” meaning “to droop” or “to fall.” This is programmed cell death.
How apoptosis differs from necrosis

• Necrotic cell death is a pathological form of cell death resulting from acute cellular injury which is typified by rapid cell swelling and lysis
• Apoptosis is controlled autodigestion by activation of endogenous proteases resulting in cell shrinkage, membrane blebbing and nuclear condensation. This results in DNA fragmentation and DNA “ladder” formation

How apoptosis differs from necrosis (cont.)

• apoptosis results in loss of mitochondrial function unlike necrosis
• in apoptosis, the dying cell maintains its plasma membrane integrity
• in apoptosis, rapid clearing by phagocytes and formation of apoptotic bodies
• no inflammatory response with apoptosis

Oxidative Stress Apoptosis

• Apoptosis is involved in the morphogenesis of numerous structures
  – The addition of the antioxidants phenol and dimethyl sulfoxide to developing mouse limbs in culture prevents interdigital cell death and ergo digit individualization. This results in “webbing” between digits (Salas-Vidal et al. 1998)
  – In untreated limbs, the interdigital space stains for reactive oxygen species, suggesting that oxidative stress-induced apoptosis is necessary for normal embryonic development
  • Maybe true
Aging, oxidant production, antioxidant defenses and detection of oxidative damage

Examples of Diseases Associated with Decreased rates Apoptosis

- Cancer
  - Follicular lymphomas
  - Carcinomas with p53 mutations
  - Hormone-dependent tumors
- Breast cancer
- Prostate cancer
- Ovarian cancer
- Autoimmune disorders
  - (mixed increase and decrease)
- Viral infection

Examples of Diseases-Injuries Associated with Increased Apoptosis

- AIDS (non-infected cells often increase in apoptosis).
- Neurodegenerative disorders (Diseases of Aging)
  - Alzheimer’s
  - Parkinson’s
- Ischemic injury (I-R)
- Toxin-induced liver disease
  - Alcohol

**Other Conditions of Interest to us:**
- Sarcopenia, Atrophy, fiber loss, myocyte loss?

**Fig. 1.** The effect of different rates of cell death on homeostasis. In nature, organismic cell number is controlled as a result of the rates of cell proliferation and cell death. Organisms are adapted to the rate of cell proliferation. Changes in the rate of cell death can result in adverse cell accumulation or cell loss.

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Apoptosis and Viral Disease

• When viruses enter a cell, they shut down the production of all proteins except for those needed to make more viruses
  – Normally, inhibiting protein synthesis induces apoptosis
• Certain viruses produce substances that mimic Bcl-2 (anti-apoptotic) and/or induce host cells to produce more Bcl-2
• Other viruses inactivate or degrade p53, the apoptosis inducer

Inducers of Apoptosis

• Physiological activators
  – TNF family (Fas ligand), transforming growth factor Beta, neurotransmitters (glutamate, dopamine, N-methyl-D-aspartate), growth factor withdrawal, loss of matrix attachment, calcium, glucocorticoids
• Damage-related inducers
  – heat shock, viral infection, bacterial toxins, oncoproteins (myc, rel, E1A), tumor suppressors (p53), cytotoxic T cells, oxidants, free-radicals, nutrient deprivation
• Therapy-associated agents
  – Chemotherapeutic drugs (e.g., cispatin, nitrogen mustard), Anthracyclines (doxorubicin), gamma radiation, UV radiation
• Toxins
  – Ethanol, Beta-amyloid peptide
The Process of Cell Suicide

- Inducer causes a triggering signal which is conveyed via a series of transducers to the AGENTS OF DESTRUCTION
- The agents (proteins) of destruction are ICE-like proteases which, when activated by a transducer, attack the cell's structural “scaffolding” and disrupt nuclear chromatin

Sequela Summary

- **Necrosis**
  - release of intracellular enzymes into extracellular milieu
  - release of pro-inflammatory cell breakdown products
  - ingress of neutrophils followed by macrophages
  - active inflammation with scarring

- **Apoptosis**
  - retention of intracellular enzymes within the apoptotic bodies
  - no release of pro-inflammatory products
  - ingestion by adjacent cells or by tissue macrophages
  - atrophy with stromal collapse but no scarring

Apoptosis Inhibitors

- Physiological
  - growth factors, extracellular matrix, CD40 ligand, neutral amino acids, zinc, estrogen, androgens
- Viral Genes (to be discussed)
  - adenovirus E1B, cowpox crmA, Epstein-Barr BHFR-1, herpesvirus, baculovirus, African swine fever virus, etc.
- Pharmacological agent
  - calpain inhibitors, cysteine protease inhibitors, tumor promoters (PMA, phenobarbital, alpha-hexachlorocyclohexane), cyclosporin

Figure 3: Cartoon representation of the key molecular mechanisms of an event to underscore the definition of death as apoptosis. The process of histone aggregation is in at least one frame. These mechanisms are outlined by an abstract number, which allows identification of the mechanism of apoptosis described in detail. They are presented in the right-hand pathway of the mechanism (the flowchart). The flowchart is represented by a series of arrows, with the cell undergoing apoptosis shown at the center. The process of histone aggregation is maintained by form, whereas histone is expressed by the transit of the cell from the process of apoptosis. The figure of this chapter provides a representation of the activation of a transducer to allow formation of an active form of the classical caspase system. The classical caspase system includes the cell's structural “scaffolding” and disrupt nuclear chromatin.
Early in apoptosis, mitochondria are triggered by multiple stimuli to release proteins that induce apoptosis. These include: oxidants, BAX (a pro-apoptotic protein that targets mitochondrial membranes), Ca^{2+} overload, active caspases, and perhaps ceramide.

The following caspase-activating proteins are then released from the intermembrane space:
1. Cytochrome c (SMAC)
2. AIF (apoptosis-inducing factor) OMMI
3. And procaspases like procaspase-3 and caspase-2

Cytochrome c binds with Apaf-1, which then associates with procaspase-9. This triggers caspase-9 activation. The complex of cytochrome c–Apaf-1–caspase-9 then activates caspase-3 proteolytically.

AIF also processes procaspase-3 to initiate caspase-3 activation. This cascade by caspases (cysteine proteases that cleave substrates at aspartic acid residues) culminates in apoptosis.

How are cytochrome c and other caspase-activating proteins released from mitochondria?

There are two general mechanisms:
1. The outer mitochondrial membrane ruptures due to expansion of the matrix space and organelar swelling.
2. This releases cytochrome c, AIF, etc.

In this scenario, the mitochondrial inner membrane potential drops, indicating the openings of channels known as permeability transition pores. These pores are composed of both inner and outer membrane proteins. When the pores open, water and solutes enter the matrix, causing matrix swelling and outer membrane disruption.
The other mechanism also involves the opening of channels. But, in contrast, these permeability transition pores open only in the outer membrane and do not result in organellar swelling. These transition pores allow cytochrome c and other proteins to move from the intermembrane space into the cytosol.

- Permeability of the membranes appear to be enhanced by calcium, pro-oxidants, and several apoptosis-related proteases (caspases) [Marzo et al. 1998]
- Bcl-2 and Bcl-2-like proteins increase resistance to pore opening

Lecture Summary
- Apoptosis is programmed cell death involving a signaling event (inducer) and a cascade of "messengers" (transducers) which ultimately activate the agents of destruction (proteases)
- The mitochondria appear to play a critical role in the apoptotic process
  - protease precursors may be stored and activated in mitochondria
  - mitochondrial damage induces apoptosis
  - mitochondrial membrane permeability increases early in apoptotic process

Summary
- Excessive or Deficient apoptosis is involved in numerous disease states
- Reactive oxygen species are involved in the apoptotic process
- Mitochondria may be a critical organelle controlling apoptosis with exercise