Charleen T. Chu, MD, PhD
Associate Professor of Pathology and Ophthalmology
University of Pittsburgh School of Medicine
**Lecture Goals**

- Basic concepts and ongoing issues in **cell death pathways**, including necrosis, apoptosis and cell death associated with autophagy.

- **Integration of survival/death mechanisms** set into motion by pathologic cell injury.

- Mechanisms by which adaptive/reparative responses represent a **double-edged sword**.
Pathology

Stimulus > Process > Manifestation

Etiology (initiating cause)  Cellular/Molecular Mechanisms  Changes in function & morphology

DISEASE

Diagnosis & Prognosis

(Includes Response to Therapy, Individualized Rx)
Pathophysiology of Disease

Etiology → Pathogenic Mechanisms

Pathogenic Mechanisms → Physiologic Responses

Physiologic Responses → Loss of function

Loss of function → Decompensation

Decompensation → Adaptation

Adaptation → Repair

Repair → Etiology
Rudolf Virchow
1821-1902

- Organ injury begins with molecular or structural alterations in cells
- Cell-cell and cell-matrix interactions contribute to tissue injury responses
- Untested hypothesis is an anathema for the practice of medicine ...

Paraphrased from www.whonamedit.com
Cell Injury

Pathologic Stimulus → Adaptive and death mechanisms → Loss of function → Functional integrity → Survival → Commitment → Cell Death
Sublethal injury

- Transient, low level insult
  - Repair and recovery

- Sustained, gradual, or repetitive insults
  - Adaptation & new state of homeostasis
    - Minor muscle injury > hypertrophy in athletes
    - Cellular preconditioning
  - Functional trade-offs and new pathology
    - Atrophy
    - Metaplasia
Edema - most cell types
Normal corneal epithelium

Reversible Cell Injury

Edema - most cell types
Fatty change - liver, heart

Normal liver

Irreversible Cell Injury

Extensive loss of membrane integrity

Basis of Lab Tests for Heart Attack
LDH, creatine kinase-MB, troponin

Irreversible Cell Injury

Hypereosinophilia
Coagulation (aggregation) of proteins
Dissolution of ribosomes, loss of RNA

Normal liver
Ischemic cell Injury

- Impaired oxidative phosphorylation
- Adaptive change - glycolysis
  - Glycogen depletion
  - Drop in pH > chromatin clumping
- Failure of plasma membrane Na+ pumps > influx of Na+, Ca++, H₂O
  - ER and cell swelling
  - Calcium overload
- Ribosomes disassemble, unfolded proteins
  - Hypereosinophilia (e.g. red dead neuron)
- Oncosis - death by swelling (von Recklinghausen, 1910)
  - Commonly referred to as “necrotic”
Cell Injury & Cell Death

- Imbalance between intensity of injury and adaptive reserve of the cell leads to cell injury and potentially cell death

Outcome: Context dependent
Linguistics of cell death

- Necrosis - pathologic term referring to dead cells, independent of mechanism
- Common usage: passive cell death: Death by bombing or natural disaster

- Programmed cell death - physiologic, developmental “programs”
- Common usage: active mechanism(s) involving cell death “programs”: Suicide
Classifying cell death

Stimulus > Process > Manifestation

http://david.davies.name/weblog/2004/03/08.html
Classifying cell death

- **Stimulus**
  - Developmental/Physiologic vs. Accidental/Unscheduled/Pathologic

- **Process**
  - Active/regulated vs. Passive
  - Apoptosis vs. Oncosis (“necrosis”) (Caspase-dependent vs. -independent)

- **Morphology**
  - Geographic vs. single cell necrosis
  - Type I - apoptotic (condensation)
  - Type III - “necrotic” (swelling)
Disease-related Cell death

Beneficial
- Adapt cell number to need and nutritional status
- Eliminate cancer cells
- Eliminate autoreactive lymphocytes
- Eliminate viral infected cells

Detrimental
- Loss of non-regenerative cells
  - Ischemia, trauma
  - Viral infection
- Neurodegenerative diseases
- Bystander effects (inflammation, autoimmunity)

Diseases often reflect too little or too much cell death...
Necrosis

Typically yellow
Can be soft, firm or form a viscous liquid

Glioblastoma with necrosis

Spleen with infarct

Image courtesy of Larry Nichols
Coagulative necrosis

- Bioenergetic failure, physical damage
  - Confluent eosinophilic cells with loss of hematoxylin (nucleic acid) staining

- Leads to inflammatory clearance and fibrous scarring

Top: Embolic pituitary infarct; Bottom: Glioblastoma
Suppurative necrosis

- Infection
  - Nuclear and cytoplasmic debris
  - Leucocyte degranulation
  - A form of liquefactive necrosis

- Leads to fibrous scarring +/- chronic inflammation
Loss of calcium homeostasis

- Sources:
  - failure of membrane Ca\(^{2+}\), Mg\(^{2+}\) ATPases
  - release from mitochondria and ER
  - Increase from <0.1 \(\mu\)M to 1.3 mM (10,000 fold)

- Activation of:
  - ATPases
  - Phospholipases
  - Proteases (M-calpains)
  - Endonucleases
  - Kinases
Loss of membrane integrity

- Physical/chemical agents - not always lethal!
- Bacterial toxins, viral proteins, complement
- Calcium activated phospholipases > detergent effect > dystrophic mineralization
- Cytoskeletal detachment > stretch and rupture
- ATP depletion, decreased mitochondrial lipid synthesis
- Lysosomal leakage - RNase, DNase, cathepsins, phosphatase, glucosidase
Contagious cell death?

- Leakage of nuclear and cytosolic proteins
  - HMGB1, S100 family proteins
  - Purine metabolites (ATP, AMP, adenosine, uric acid)
  - Heat shock proteins

- Endogenous “danger” signal
  - RAGE (receptor for advanced glycation end products), Toll-like receptors
  - Recruit inflammatory cells > BYSTANDER cell death
  - Elicit cytokines
    - Epithelial, fibroblast and vascular proliferation
    - Secondary reparative pathologies
Reparative Pathology

- **Pseudotumors**
  - Florid reactions can simulate tumors
    - “Pyogenic granuloma” - exophytic mass

- **Fragile neo-vessels prone to rebleeding**
  - Subdural hematoma, subacute cerebral stroke

- **Extensive scarring or fibrous adhesions**
  - Interfere with tissue function
    - heart, lung, joints, anterior chamber of eye, cornea

- **Dystrophic mineralization**
  - Calcified plaques and loss of vessel wall compliance
Recurrent bleeding associated with reparative neovascularization

Fresh hemorrhage in an organizing subdural membrane
Pulmonary fibrosis

Normal lung

Asbestosis of the lung, Images courtesy of Dr. Tim Oury
Pathologic Calcifications

- **Dystrophic calcification** - normal blood calcium
  - Occurs with aging
  - Regions of necrotic tissue damage
  - Interferes with elasticity of tissues, transparency of ocular tissue

- **Metastatic calcification** - high blood calcium
  - Excess in: PTH (neoplasia), Vitamin D, bone resorption

Microcalcifications in radiographic assessment of retinoblastoma, high grade ductal breast carcinoma in situ, severe atherosclerosis, etc.

Retinoblastoma with punctate Ca^{2+}
“Single cell necrosis”

- Individual dying cells observed in many tissues
  - Eosinophilia
  - Pyknosis
  - Karyorrhexis
- No destructive inflammatory response, preservation of tissue structure
- Now recognized as apoptosis

Rat liver, Image courtesy of George Michalopoulos
Apoptosis

Liver ischemia - John Kerr 1960’s
- “Shrinkage necrosis” of individual cells
- Nuclear and cytoplasmic condensation
- Fragmentation into membrane bound apoptotic bodies
- Contents remain enclosed by membranes and no inflammatory response is elicited
- Final disposition - heterophagy

Kerr, Wyllie and Currie 1972
- Similar morphology in hormonal adrenocortical and breast CA death, and review of published developmental electron micrographs
- “Falling off” of petals or leaves
Hepatocyte apoptosis

- Budding, boiling
- Shrinking
- Round up
- Fragmentation
- Spread out

TNFα/Actinomycin D treated mouse hepatocytes, Image courtesy of Xiao-Ming Yin
Molecular basis of apoptosis

- Cancer (1988)
- **bcl-2**
  - Proto-oncogene in t(14:18) translocation
  - mediates cell survival rather than proliferation
- Developmental studies in C. elegans (1990s) - ced genes
Reactive follicle

Large germinal center cells

Secondary follicle in lacrimal gland, H&E

Small memory B and T cells

Tonsil, Ki-67 proliferation Ag

Bcl-2
Follicular B-cell lymphoma

Translocation (14:18)

- Bcl-2 gene on chromosome 18
- Immunoglobulin promoter on chromosome 14q
Apoptotic Mechanisms

(Lecture 2, Dr. Zinkel)

- **Intrinsic pathway** - “suicidal ideation”
  - Mitochondria

- **Extrinsic pathways**
  - Death receptors - “witch doctor”
  - Cytotoxic T lymphocytes - “euthanasia”

- Inhibitors of apoptosis
- Clearance of dead cells

Image courtesy of Donna Beer Stolz
Inhibitors of apoptosis

- Viral infected cells will try to kill themselves
- Viral inhibitors of apoptosis (IAP)
- Growing number of endogenous inhibitors
  - Combined deletion of SMN-1 and NAIP (neuronal apoptosis inhibitory protein) in Werdnig-Hoffman disease (SMA-1)
  - Death of motor neurons >> severe muscle atrophy

Image courtesy of David Lacomis
Clearance of dead cells

- Necrosis >> release of cell contents >> active inflammation and 2° tissue injury

- Orderly clearance of apoptotic cells
  - Cells undergoing apoptosis secrete factors that attract, but do not activate, phagocytes.
  - Phosphatidylserine externalization as an “eat me” signal
Alternative “Deathstyles”? 

**Perinatal ischemia**  
A apoptotic neurons abundant

**Adult ischemia**  
Red dead neurons

**Alzheimer Disease**  
Neurofibrillary tangle (*)  
Granulovacuolar degeneration (arrow)

Viable neuron
Neuronal cell death over a lifespan

(Lecture 3, Dr. Roth)

- Developmental wave of neuronal cell death by apoptosis
- Perinatal ischemia - pontosubicular necrosis - prominent apoptotic morphology
- Adult ischemia and neurodegenerative diseases - less clear
  - Biochemical markers of apoptotic pathways more frequently observed than morphologic apoptosis
Age effects in model systems

- The rat 6-OHDA neurotoxin model
  - Predominant morphology is apoptotic at 14, 21 and 29 days of age
  - 42-day old rats also exhibit a prominent non-apoptotic form of cell death
    *Brain Research (2002) 958: 185*

Cell type dependence

- K+ and serum withdrawal
  - Cerebellar granule cells - apoptosis
  - Purkinje neurons - autophagic
Adult post-mitotic cells

- Arrested apoptosis or diversion to alternative pathways?
  - Energetics
    - Switch to passive necrosis?
  - Expression of endogenous inhibitors
    - Sympathetic neurons must develop competence to undergo apoptosis - XIAP
- Fundamentally different pathways of regulated death?
# Programmed Cell Death

<table>
<thead>
<tr>
<th>Type 1</th>
<th>Type 2</th>
<th>Type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nuclear/Apoptotic</strong></td>
<td><strong>Autophagic</strong></td>
<td><strong>Cytoplasmic</strong></td>
</tr>
<tr>
<td>Condensation of chromatin and cytoplasm</td>
<td>Abundant autophagic vacuoles</td>
<td>“Regulated necrosis”</td>
</tr>
<tr>
<td></td>
<td></td>
<td>“Paraptosis*”</td>
</tr>
<tr>
<td></td>
<td></td>
<td>“Necroptosis†”</td>
</tr>
<tr>
<td>Caspase inhibitors</td>
<td>RNAi Atg genes</td>
<td>No universal concensus</td>
</tr>
<tr>
<td>Bcl-2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reviewed by Clarke 1990; †Junying Yuan lab; *Dale Bredesen lab
Autophagy

Autophagy: Physiologic
Starvation, Turnover of organelles, long-lived proteins, aggregates
Autophagic cell death?
Hormonally driven tissue regression
Oxidative injuries


EM of dying cells

Apoptotic cells – committed & beyond rescue

Autophagic morphology – failed adaptation vs. suicide?

Cell with organelle swelling

Injured, but rescuable?

Beyond rescue

Type 1

Type 2

Type 3

Image courtesy of Donna Beer Stolz
Potential role(s) of autophagy in cell death

- Pro-survival - death from failed compensation.
  - Atrophic response to limiting resources.
  - Sequester damaged mitochondria?

- Pro-death
  - Gatekeeper role upstream of apoptosis
    - Mechanism of controlling death-related debris?
  - Alternative executionary system?

- Context dependent, “autophagic stress”
  - Excessive or imbalanced induction/clearance
  - Most stress responses lead to repair or cell suicide

Correlation ≠ Causation
Pathologic cell death can serve both beneficial and detrimental roles.

Necrosis describes cell corpses - typically with loss of membrane integrity.

Apoptosis
- Regulated set of events activated by developmental and pathologic signals.
- Controlled removal of superfluous, neoplastic, infected or otherwise damaged cells for the benefit of the tissue or organism as a whole.

AND...
Unscheduled cell death
Balance of adaptive/reparative & injurious mechanisms

APOPTOSIS

OTHER DEATH PATHWAYS?

PASSIVE “Necrosis”

ADAPTATION
Unscheduled cell death
Balance of adaptive/reparative & injurious mechanisms

APOPTOSIS

OTHER DEATH PATHWAYS?

PASSIVE “Necrosis”

ADAPTATION
Cellular Repair and Cell Death

Pathologic Stress → Reparative responses → Survival

DNA damage → Cell cycle arrest, p53, PARP
ER stress → Unfolded protein response

Suicide
DNA Damage
ROS, Radiation, Genotoxins

p53

Cell Cycle Arrest
DNA repair
Apoptosis

Failure
Failure
Failure

Proliferating Mutated Cell
Neoplastic Transformation
ER stress

- **ER functions**
  - Protein synthesis, post-translational modification, folding
  - Calcium homeostasis and lipid homeostasis

- **Accumulation of misfolded proteins**
  - Genetic defects in protein 1° structure
  - Protein overexpression
  - Many drugs/toxicants disrupt ER functions

- **Unfolded protein response**
Unfolded protein response

- Suppress initiation of protein synthesis
- Induce chaperone proteins
- Enhanced ER associated degradation
- Induce apoptosis if damage is overwhelming
  - ?Caspase 12 (but just a pseudogene in humans)
  - Casp 8 cleavage of ER protein > redistribution of Ca^{2+} > induction of intrinsic pathway?
Emerging directions

- It is clear that adaptive responses in multicellular organisms can lead to cell death.
- Emerging data indicate that agents with a proven role in cell death may also regulate cellular adaptation, differentiation and function.
Reactive Oxygen/Nitrogen Species

- Free radical - one unpaired electron
  - Superoxide •O$_2^-$, Hydroxyl radical •OH
  - Nitric oxide •NO
- Oxygen is a biradical
  - Energy barrier can be bridged by metal catalysis, heat
- Other oxidants
  - Hydrogen peroxide H$_2$O$_2$, Singlet oxygen
  - Peroxynitrite ONOO-
Reactive Oxygen Species

Evolution of thought
- Initially thought to not be biologically relevant

With the discovery of antioxidant enzymes...
- ROS as unfortunate by-product of respiration
- Mediator of cell death
- Implicated in aging and most diseases!

Growing recognition of ROS and \( \cdot \text{NO} \) as signaling mediators
- Learning and memory, vasodilatation
- Pro-survival adaptations
Preconditioning

n Transient sublethal insult >> resistance to subsequent “lethal” insults

n Ischemic and hyperoxic preconditioning - heart, lung, kidney, liver, brain
Shared mechanisms with adaptive responses to
  n Chronic hypoxia
  n Changes in temperature

n Transcriptional induction of antioxidants, detoxifying enzymes, heat shock proteins (chaperones), and kinases
Emerging roles for caspases

- Caspases may have non-death related functions
  - Caspase 3 activation is essential for anoxic pre-conditioning \textit{in vitro}
  - Localized “apoptotic” signaling and neurite remodeling?
Too much of a “good” thing

- Growth factors promote proliferation, differentiation, and survival
- The same growth factor, receptor, or kinase can sometimes promote cell survival and sometimes cell death
- Too much, too little? Cell type, context?
Dual role of a growth factor receptor

Dual role of a “death receptor”

TNF-α

TNFR

TRADD

FADD

cIAP

TRAF

NF-KB

FADD
Defects in NF-kB activation or protein synthesis

TRAF cIAP
ERK can mediate death or survival

Extracellular signal Regulated protein Kinase

ROS

GTP

Ras

Grb2

Sos

Raf

MEK

ERK

Nuclear

Cytoplasmic/ Mitochondrial

Transcription

Eur J Biochem, 271: 2060-2066
Mitochondria: Integration of survival-death signals

- Apoptosis inducing factor
- Endonuclease G
- Cytochrome C
- SMAC/DIABLO
- HtrA2/Omi
- ROS
- Ca^{2+}, Fe^{2+}
- PKA
- PKC
- MAPK
- PI-3K

Metabolic stimuli

Bcl-2

Bax

+/− Permeability transition
Integrating the BALANCE of multidimensional continuums

CELLULAR CONTEXT SUBCELLULAR LOCATION
Summary - Part 2

- Multiplicity of adaptive responses and death pathways activated
- What factors determine if cells commit to death and the death pathway used?
  - Severity or type of insult
  - Pre-existing cellular context
    - Aging, genetic or nutritional background
    - Pre-conditioning and adaptive reserve of the cell type
- Shifting emphasis from execution to integration of upstream factors that regulate survival/death decisions
Cell/Tissue Injury

Stress

Adaptation

OR

Injury

What does not kill me...
Pre-conditioning

Cell death, Impaired tissue function

Functional trade-offs
Dysregulated repair
**Adaptation: new state of homeostasis**

- Increased functional demand, growth signals
  - Hypertrophy and Hyperplasia
  - Cellular preconditioning (minor injury needed)
- Altered functional demand, irritation
  - Metaplasia
- Decreased demand or nutrition/energy
  - Atrophy

**Loss of function**
- Metaplasia
- Atrophy

**Gain of undesirable effects**
- Cardiac hypertrophy
- Hyperplasia/metaplasia and cancer
Hypertrophy vs. Hyperplasia

Myocardial hypertrophy

Tonsillar hyperplasia

Ki-67 proliferation Ag

Normal

http://biomedicum.ut.ee/aran/Poster/hypertrophic_myocardium.htm

H&E, Image courtesy of Dr. Jennifer Hunt
## Physiologic vs. Pathologic Hyperplasia

<table>
<thead>
<tr>
<th>Physiologic -</th>
<th>Pathologic -</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regulated &amp; Reversible</td>
<td>Still Reversible!!</td>
</tr>
<tr>
<td>Hormonal or growth factor stimulus</td>
<td>Disturbed hormonal or growth factor stimulus</td>
</tr>
<tr>
<td>Breast at puberty</td>
<td>Endometrial hyperplasia</td>
</tr>
<tr>
<td>Compensatory to loss of functional tissue mass</td>
<td>Benign prostatic hyperplasia</td>
</tr>
<tr>
<td>Liver, Kidney</td>
<td>Viral infection</td>
</tr>
<tr>
<td>Wound healing</td>
<td>Papillomavirus (warts)</td>
</tr>
<tr>
<td></td>
<td>Increased risk of Neoplasia (“cancer”)</td>
</tr>
</tbody>
</table>
Metaplasia

- Chronic irritation converts one cell/tissue type to another
- An adaptive compromise
  - Protection from irritation
  - Loss of normal function
  - Gain of susceptibility to other pathologies
    - Infection
    - Cancer (although this may be due to chronic irritation rather than metaplasia itself, the cancer often arises from metaplastic cell type)
Squamous metaplasia

Protection
Squamous epithelium is tough

Trade-offs?
Loss of ciliated columnar epithelium
>> infection, hacking cough

Continued irritation
>> squamous cell carcinoma

Bronchial irritation, smoking

H&E, Lung images courtesy of Dr. Tim Oury
**Intestinal metaplasia**

Barrett’s esophagus, acid reflux

**Protection**

Intestinal type columnar epithelium is more resistant to acid than squamous

**Trade-offs?**

Continued irritation >> glandular adenocarcinoma

There are also less defined examples of mesenchymal metaplasia - including osseous metaplasia

Image courtesy of Dr. Antonia Sepulveda
Atrophy

- Loss of stimulation
  - Disuse atrophy
  - Neurogenic muscle atrophy (left)
- Loss of support
  - Diminished blood supply
  - Malnutrition
  - Loss of growth factor support
- Pressure atrophy
- Idiopathic - aging, neurodegeneration
Atrophy: Tissue vs. Cell level

- Tissue level atrophy can be accomplished by
  - Cellular atrophy (shrinkage in cell size)
  - Cell death (apoptosis)

- Cellular atrophy likely due to
  - decreased protein synthesis and
  - increased autophagic degradation
sER proliferation in liver

The smooth ER contains detoxifying enzymes including the Cytochrome P450 system.

Benefit.
sER “hyperplasia” allows more effective detoxification of ethanol, barbiturates, corticosteroids and their conjugation for secretion

Trade-offs
Resistance to therapeutic drugs
Some chemicals are made more toxic by the P450 system modification (CCl₄, Tylenol)
Sequestration from danger

Tissue level
- Elimination preferred
  (liver, kidney, innate and adaptive immunity)
- Sequestration
  - Inflammatory
    - Epithelioid macrophages and granulomas
  - Other?
    - Asbestos bodies

Cellular level
- Elimination preferred
  (detoxification enzymes, autophagy, exocytosis)
- Sequestration
  - Membranous
    - Vesicular uptake
  - Lysosomal
  - Aggregation?
Lysosomes - Importance of garbage disposal

- Oxidative stress, protein and organelle damage
- Pathogenic organisms
  - Destroy organism
  - Used by organism for life cycle/replication
- Protein aggregates (+/- ubiquitin)
- Expansion due to undigestible remnants
  - Lysosomal storage diseases
  - Drug induced deficits - chloroquine
  - Lipofuscin - lipid peroxidation
Major Degradative Pathways

Proteasomal
- Short-lived proteins
  - Cell cycle, transcription factors, apoptotic mediators
- Polyubiquitination regulates proteasomal degradation
  - E1, E2 and E3 conjugating enzymes

Lysosomal
- Long-lived proteins, organelles, membranes
- Nutrient or trophic factor deprivation
- Ubiquitin-like conjugations bring Atg proteins to membranes to initiate sequestration
- Selective targeting of cargo?
  - Chaperone-mediated autophagy - KFERQ
  - ?Damaged mitochondria
Altered protein degradation

- Proteasome inhibitors elicit apoptosis in primary neuronal cultures (yet proteasomal degradation of IAPs may be necessary)
- Lysosomotropic agents or agents that block cargo delivery to the lysosome elicit cell death
  - Chloroquine myopathy
  - X-linked vacuolar cardiomyopathy and myopathy (Danon disease) - lysosome-associated membrane protein-2 (LAMP2)
- Autophagy: clears aggregates, but can mediate neurite degeneration
Summary - Part 3

- In multicellular organisms, cellular adaptation can be a double-edged sword
  - Poised to activate **Cell Death** programs
  - Functional trade-offs
- Hypertrophy, Hyperplasia, Atrophy can be physiologic; as with Metaplasia, sustained stimuli can make them pathologic
- Similarly, tissue adaptations may come at a cost >> **Chronic Inflammation** & **Dysregulated Repair** as mechanisms of human disease
A better understanding of adaptive responses to injury and the factors that tip the balance to favor dysfunction & cell death are needed to guide development of effective therapies.