Mechanisms of Cell Injury & Cell Death

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Lecture Goals

- Basic concepts in cellular and tissue responses to injury
- Morphologic and biochemical definitions of major cell death pathways
- Mechanisms by which adaptive/reparative responses represent double-edged swords.
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

Compensatory Responses

Disease

Adaptation
Cell survival

Decompensation
Cell Death

Functional integrity
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
Stress

Adaptation

OR

Injury

Transient, mild insult
Sustained, gradual, repetitive insults

Pre-conditioning

What does not kill me...

Functional trade-offs
Dysregulated repair

Cell death, impaired tissue fxn
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

Normal

Ki-67 proliferation Ag
Image from CT Chu

Robbins and Cotran Pathologic Basis of Disease, 8/E
(Fig. 12-1) with permission from Saunders an imprint of Elsevier Inc.
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

Bronchial irritation, smoking

Protection
Squamous epithelium is tough

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

Continued irritation
>> squamous cell carcinoma

H&E, Lung images courtesy of Dr. Tim Oury
Atrophy

- Loss of stimulation
  - Disuse atrophy
  - Neurogenic atrophy (left)

- Loss of support
  - Diminished blood supply
  - Malnutrition
  - Loss of growth factors
  - Pressure atrophy

Protection
Decreased metabolic demand
> Cell survival

Trade-offs?
Loss of fxn > disease
Cell Injury & Cell Death

- Tissue adaptations are reversible, and reflect changes in individual cells.
- The balance between intensity of injury and adaptive reserve of the cell determines outcome.
Reversible Cell Injury

Normal corneal epithelium

Edema - most cell types

Irreversible Cell Injury

Extensive loss of membrane integrity

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

Fatty change - liver, heart

Normal liver
Image courtesy of George Michalopoulos

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

Image courtesy of Larry Nichols
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”
Linguistics of cell death

- Necrosis - pathologic term referring to dead cells, independent of mechanism

- Programmed cell death - physiologic, developmental “programs”

- Common usage: passive cell death: Death by bombing or natural disaster

- 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 auto-reactive 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 – gross appearance

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

Embolic pituitary infarct

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 μM 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 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 from elevated PTH (neoplasia), Vitamin D, bone resorption

Microcalcifications in radiographic assessment of retinoblastoma, high grade ductal breast carcinoma in situ, severe atherosclerosis, etc.
“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: a historical perspective

- Liver ischemia - John Kerr 1960’s
  - “Shrinkage necrosis” of individual cells
  - Contents remain enclosed by membranes and no inflammatory response is elicited

- Kerr, Wyllie and Currie 1972
  - Hormonal (adrenocortical and breast CA) and developmental cell death
  - “Falling off” of petals or leaves

- Vaux, Cory & Adams 1988
  - Bcl2 – first survival oncogene

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
Primary articles on Apoptosis
Neuronal cell death over a lifespan

- 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
Alternative “Deathstyles”?  

**Perinatal ischemia**
- Apoptotic neurons abundant

**Adult ischemia**
- Red dead neurons
  - Viable neuron

**Alzheimer Disease**
- Neurofibrillary tangle (*)
- Granulovacuolar degeneration (arrow)
## Programmed Cell Death

<table>
<thead>
<tr>
<th>1970s</th>
<th><strong>Type 1</strong></th>
<th><strong>Type 2</strong></th>
<th><strong>Type 3</strong></th>
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<tbody>
<tr>
<td></td>
<td>Nuclear (Apoptotic)</td>
<td>Autophagic</td>
<td>Cytoplasmic</td>
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<tr>
<td></td>
<td>Condensation of chromatin and cytoplasm</td>
<td>Abundant autophagic vacuoles</td>
<td>3a - general disintegration</td>
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<tr>
<td></td>
<td>Caspase inhibitors Bcl-2</td>
<td>RNAi Atg genes</td>
<td>3b - dilated ER &amp; mitochondria</td>
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<td>*<em>Reviewed by Clarke 1990; †Junying Yuan lab; <em>Dale Bredesen lab</em></em></td>
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<td>No universal consensus “Regulated necrosis” “Paraptosis*” “Necroptosis†”</td>
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Guilt by association?

Or is cell death a fail-safe for repair?
### Cellular Repair and Cell Death

<table>
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<th>Pathologic Stress</th>
<th>Reparative responses</th>
<th>Survival</th>
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<tr>
<td>DNA damage</td>
<td>Cell cycle arrest, p53, PARP</td>
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<tr>
<td>ER stress</td>
<td>Unfolded protein response</td>
<td>Suicide</td>
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</tbody>
</table>
DNA Damage
ROS, Radiation, Genotoxins

p53

- Cell Cycle Arrest
- DNA repair
- Apoptosis

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
  - Proteasome
- Enhanced autophagy (lysosomal degradation)
- Induce apoptosis if damage is overwhelming
Emerging directions

- Adaptive responses in multicellular organisms can lead to cell death through active mechanisms.

- Agents with a proven role in cell death may also regulate cellular adaptation, differentiation and function.
  - Reactive oxygen species
  - Caspases
Summary

- Pathologic cell death serves both beneficial and detrimental roles
- Necrosis describes cell corpses - typically with loss of membrane integrity
- Apoptosis - controlled removal of superfluous, neoplastic, infected or damaged cells
- In multicellular organisms, tissue & cellular adaptations can be double-edged swords
  - Poised to activate Cell Death programs
  - Functional trade-offs/costs
    - >> Chronic Inflammation & Dysregulated Repair as major mechanisms of human disease
Unscheduled cell death
Balance of adaptive/reparative & injurious mechanisms

APOPTOSIS

 outros death pathways?

PASSIVE "Necrosis"
Unscheduled cell death

Balance of adaptive/reparative & injurious mechanisms

APOPTOSIS

OTHER DEATH PATHWAYS?

PASSIVE “Necrosis”
What tips the balance?
Upstream modulation of survival/death decisions

Additional Reading: