Apoptosis: Death comes for the Cell

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From Ingmar Bergman’s The Seventh Seal

Mutations in proteins that regulate cell proliferation, survival and death can contribute to oncogenesis

From Okada and Mak, Nat. Rev. Cancer 4:592-603

Apoptosis: Programmed Cell Death

- morphological changes associated with programmed cell death.

- The term was originally used by Wyllie and his colleagues and is from the Greek meaning “dropping away” as the leaves from a tree.

Apoptosis

- **Active** cell death
- Requires energy and RNA and protein synthesis
- Characteristic morphological features
- DNA cleaved, chromatin condenses
- Cells shrink
- Formation of apoptotic body
- Cleared by phagocytosis
- No inflammation=**no tissue damage**

Necrosis

- **Passive** cell death
- Cells swell up
- Membrane breaks down and cellular contents leak out
- Nucleus disintegrates
- Cell ghosts
- Inflammatory=**tissue damage**
The function of Cell death

• **Multicellular development**
  - involved in deletion of entire structures,
  - sculpting of tissues,
  - and regulates the neuron number
• **The immune response**
• **The body’s defense against cancer**

Death and the mouse’s paw

Dark Green fluorescence indicates apoptotic cells.

Fig 18-18

Apoptosis regulates nerve cell targeting

Fig 18-20

Apoptosis in Lymphocyte development

How do we recognize Programmed Cell Death?

The Face of Cell Death: Apoptosis
Detection of apoptotic cells

- **Microscopy**
  - Cells have classic features (e.g., small darkly stained nuclei)
  - Detection of free 3’ ends of DNA by TUNEL assay (terminal deoxytransferase-mediated dUTP-biotin nick end labeling)

- **Gel electrophoresis**
  - Detect DNA ladder of 180 bp intervals caused by internucleosomal DNA cleavage

- **Flow cytometry**
  - Measure externalization of phosphatidylserine (PS) with fluorescently labeled Annexin-V
  - Measure DNA fragmentation with propidium iodide fluorescence

Analysis of DNA content with a flow cytometer

Recall the fluorescence intensity of the DNA dye (amount of DNA) is measured for each cell.

Triggers of apoptosis

- Programmed cell death in which many more cells are produced than survive (e.g., development of lymphocytes)
- Toxic stimuli (viruses, chemicals, ionizing radiation)
- Extracellular signals (Fas, p75 NGF-R, TNF)
- DNA damage (p53)

C. elegans has played a key role in our understanding of Apoptosis

<table>
<thead>
<tr>
<th>1000 total cells</th>
<th>131 die</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ced-3: no death</td>
<td>Ced-4: no death</td>
</tr>
<tr>
<td>Ced-9: all die</td>
<td>Ced-1/ced-3: no cells die</td>
</tr>
</tbody>
</table>

H.R. Horvitz and colleagues responsible for much of this work, 2002 Nobel Prize in Medicine with Sulston and Brenner.

C. elegans apoptosis

CED-9—Blocks apoptosis
CED-4—linker molecule forms activating complex with CED-3
CED-3—Protease that executes cell by chewing up proteins
EGL-1—Proapoptotic by blocking CED-9 function
Three classes of proteins function in the apoptotic pathway—conserved in vertebrates

<table>
<thead>
<tr>
<th>Regulator</th>
<th>Adapter</th>
<th>Effector</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. elegans</td>
<td>Ced-9</td>
<td>Ced-4</td>
</tr>
<tr>
<td>Vertebrates</td>
<td>Bcl-2</td>
<td>Apaf-1</td>
</tr>
</tbody>
</table>

Mammalian Bcl-2 can substitute for Ced-9 in C. elegans.

Death's Methods: A protease cascade

Caspases

- Caspases are Cysteine directed proteases that cleave after ASParate residues.
- Ced-3 is the C. elegans homologue.
- At least 14 family members.
- Synthesized as proenzymes with low levels of caspase activity (~1–2 % of active form).
- Activated upon aggregation or cleavage to mature form.
  - Caspases -8 and -9 are “initiator” caspases.
  - Caspase -3 is the “effector” caspase.
  - Caspase activation requires a stimulus.
  - They proteolyze cellular proteins to carry out cell death program.

The Caspase Family

Procaspsa activation

![Diagram of procaspsa activation](image)

Caspase cascade

![Diagram of caspase cascade](image)
Two Pathways that Initiate Apoptosis

- Intrinsic/Mitochondrial Apoptosis
  - Regulated by Mitochondria
  - Cytochrome c release
- Extrinsic/Death Receptor Apoptosis
  - Activated by ligation of Death Receptors
  - Fas, TNF alpha
- These pathways intersect at the effector caspases

Activation of the Intrinsic Pathway

Intrinsic Pathway: Apaf-1 Induced Apoptosis

Intrinsc/ Mitochondrial Pathway

Intrinsic Pathway: Apaf-1

Smac/Diablo and IAPs

Smac=Second mitochondrial activator of caspases
IAP=Inhibitor of Apoptosis Proteins
Bcl-2 family members

- A very large family with 19 members identified
- Bcl-2 (homologous to ced-9) is prototype
- All have the BH3 domain (Bcl-2 homology)
  - BH-3 is the pro-apoptotic domain exposed on activation
- Act as dimers—either hetero or homodimers
  - Pro-apoptotic dimers (Bax) increase mitochondrial permeability
  - Anti-apoptotic members (Bcl-2, Bcl-XL) form dimers with pro-apoptotic members to inactivate them

The Bcl-2 Family

Some trophic factors prevent apoptosis by inducing inactivation of a pro-apoptotic regulator

Figure 23-50

Cell, Vol 111, 331-342, 1 November 2002

Bid, Bax, and Lipids Cooperate to Form Supramolecular Openings in the Outer Mitochondrial Membrane

Tomomi Kuwana 1, Mason R. Mackey 2, Guy Perkins 2, Mark H. Ellisman 2, Martin Latterich 3, Roger Schneiter 4, Douglas R. Green 1, and Donald D. Newmeyer 1

Mitochondrial permeability

Bid and Bad have distinct functions to activate apoptosis

Kuwana et al., Molecular Cell, 17, 525-535, 2005
Extrinsic/Death Receptor Pathway

Death Receptors and Ligands

CD95=Fas

TNF receptor family

Fas-FasL Apoptosis

- In response to antigenic stimulation, peripheral T cells expand
- The antigen specific T cells generated must be eliminated (except for the memory cells)
- Upon repeated antigenic stimulation via the T Cell receptor: T cells upregulate Fas and FasL
- Eliminate neighboring T Cells expressing Fas

Activation of Apoptosis by Fas Ligand

Fas Induced Apoptosis

The Formation of the Death Initiating Signal Complex (DISC)
Adaptor Proteins contain conserved protein interaction domains

- CARD domains of Apaf-1
- DED domains of FADD
- DED domains of FLIP

Fas and the intrinsic pathway: Bid

Proteolytic targets of effector caspases

- Cytoskeletal regulatory proteins
  - Actin
- Nuclear Lamins
- Poly(ADP-ribose) polymerase (PARP)
  - PARP activity depletes ATP, thus cleavage of PARP may maintain store of ATP to drive apoptosis
- DNA-fragmentation factor (DFF)

Removal of apoptotic cell by phagocytosis

Removal of cell corpses

Phagocytosis tags and receptors
Two roads to activate apoptosis

Extrinsic

Intrinsic

TNFα receptors also signal to NFκB

Ubiquitylation is a common signal transduction mechanism (see regulation of cyclins for example)

IKKK=IKB Kinase kinase

NFκB activates transcription of several anti-apoptotic proteins including IAPs and Bcl-2.

PEA-15 Structure and Binding Partners

- 15-kDa protein containing 130 amino acids
- N-terminus consists of a Death Effector Domain and NES
- Regulated at Ser104 and Ser116 by phosphorylation

Characterization of phospho-epitope antibodies

Effect of PEA-15 phosphorylation on its binding to ERK

pS104

pS116

pS116 PEA-15 binds FADD

GST-PEA15 pulldown
**PEA-15 is Anti-apoptotic**

- PEA-15 blocks Fas and TNFα apoptosis in HeLa, MCF7, NIH3T3
- PEA-15 blocks TRAIL apoptosis in glioma lines
- PEA-15 null astrocytes more sensitive to TNFα

**Example Question**

- **Compare the formation of the Death Initiation Signaling Complex (DISC) of the extrinsic pathway to the formation of the apoptosome of the intrinsic pathway.** Drawings could help.  
  - What signal initiates the formation of each (an aggregation step)?
  - Where are the complexes formed in the cell?
  - What adaptor proteins mediate the formation of each complex?
  - What are the initiator and effector caspases for each?
  - How are the caspases activated? What do they do?