

HSCI 440: Cell Pathophysiology Lab

Module 1: Cell Growth and Cell Death (Apoptosis)
Module 2: Cell Differentiation and Cell Invasion

Sept 6, 2012

SCIENCE, ENVIRONMENT & HEALTH SCIENCES CO-OPERATIVE EDUCATION

INFO SESSION

September 18, 2013
12:30-1:20PM
K 9500

EXPLORE CAREER OPTIONS WITH
CO-OP AS PART OF YOUR DEGREE!



HSCI 440: Cell Pathophysiology Lab

week 1	05-Sep-13	lecture/Bradford Assay
week 2	12-Sep-13	**MTS assay
week 3	19-Sep-13	**Preparing samples and running SDS-PAGE gels
week 4	26-Sep-13	Biochemical markers of apoptosis
week 5	03-Oct-13	**Comet assay-1
week 6	10-Oct-13	Comet assay-2
week 7	17-Oct-13	Test
	19-Oct-13	5:00 pm 1st Lab report due
week 8	24-Oct-13	lecture/cell differentiation
week 9	31-Oct-13	**Matrix invasion assay
week 10	07-Nov-13	**RT-PCR to monitor EMT – cDNA synthesis
week 11	14-Nov-13	RT-PCR to monitor EMT – realtime PCR
week 12	21-Nov-13	Glutamate and 6-OHDA toxicity experiments
week 13	28-Nov-13	Test
	30-Nov-13	5:00 pm 2nd Lab report due
		** quiz days
		□

HSCI 440: Cell Pathophysiology Lab

□ GRADING:

- 50% - Lab reports (module 1, module 2)
- 30% - Tests (2x)
- 10% - Quiz (5x)
- 10% - Participation

**** Lab reports submitted after 5:00 pm will automatically be deducted 10%. No exceptions!! Every additional day late will incur another 10% deduction.**

HSCI 440: Cell Pathophysiology Lab

Module 1: Apoptosis

Mechanisms and detection methods

Apoptosis and Diseases

Diseases Associated with the Inhibition of Apoptosis

1. Cancer
 - Follicular lymphomas
 - Carcinomas with *p53* mutations
 - Hormone-dependent tumors
 - Breast cancer
 - Prostate cancer
 - Ovarian cancer
2. Autoimmune disorders
 - Systemic lupus erythematosus
 - Immune-mediated glomerulonephritis
3. Viral infections
 - Herpesviruses
 - Poxviruses
 - Adenoviruses

Diseases Associated with Increased Apoptosis

1. AIDS
2. Neurodegenerative disorders
 - Alzheimer's disease
 - Parkinson's disease
 - Amyotrophic lateral sclerosis
 - Retinitis pigmentosa
 - Cerebellar degeneration
3. Myelodysplastic syndromes
 - Aplastic anemia
4. Ischemic injury
 - Myocardial infarction
 - Stroke
 - Reperfusion injury
5. Toxin-induced liver disease
 - Alcohol



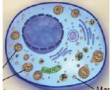
Fig. 4. Diseases associated with the induction or inhibition of apoptotic cell death.

Science 267, 1456-1462 (1995)

Example of when apoptosis does not occur

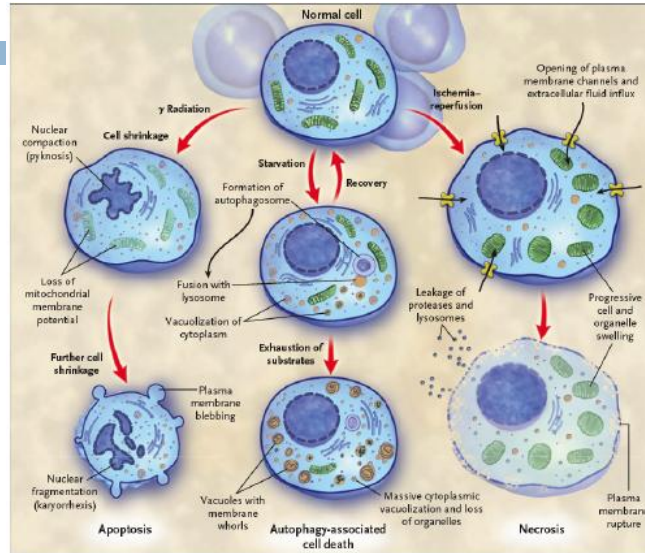


Classification of Cell Death

<u>Mode of death</u>	<u>Morphology</u>	<u>Biochemical attributes</u>
1. Apoptosis Programmed ancient Gk – falling off : • eg, leaves from trees • apo (away from); ptosis (falling)	 shrinkage	Caspase cascade
2. Necrosis Accidental corpse (nekros)	 swelling	Leakage of cytoplasmic proteases etc.
3. Autophagy Programmed to eat (phagy) oneself (auto)		Lysosomal degradation

Figures from
Hotchkiss et al. NEJM 361, 1570-83, 2009

Classification of Cell Death

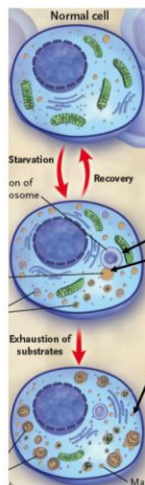


1. Apoptosis

2. Autophagy

3. Necrosis

Autophagy



- Greek: to eat (phagy) oneself (auto)
- Lysosomal degradation pathway
- Cells recycle their own non-essential, redundant, or damaged organelles and macromolecule components.

Formation of autophagosome
Fusion with lysosome
Massive cytoplasmic vacuolation
and loss of organelles

The role of autophagy in cell death is controversial.

Autophagy is a survival mechanism.

Figures from
Hotchkiss et al. NEJM 361, 1570-83, 2009

Mechanisms of cell death

□ Necrosis

▣ response cellular stress

■ intracellular pathways activated:

- turn off nonessential functions
- limit damage
- activate repair mechanisms

■ excessive injury leads to overactivation of these pathways

- depletion of energy stores
- loss of membrane integrity

Mechanisms of cell death

□ Necrosis

▣ cells and organelles swell until they burst

▣ leakage of cytoplasmic and organelle contents

▣ toxic to neighbouring cells

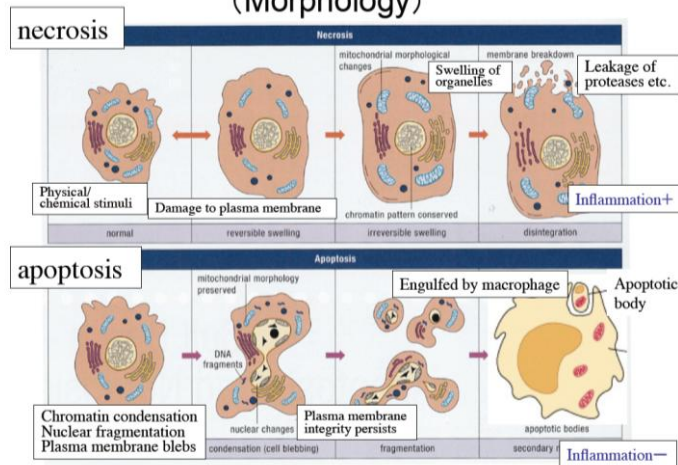
▣ leads to inflammatory response

Mechanisms of cell death

- Apoptosis
 - programmed cell death
 - PM maintained; DNA, nuclear and cytoplasmic proteins are degraded
 - cell does not lyse and becomes a target of phagocytosis to prevent inflammatory response
 - occurs normally to remove unwanted or harmful cells

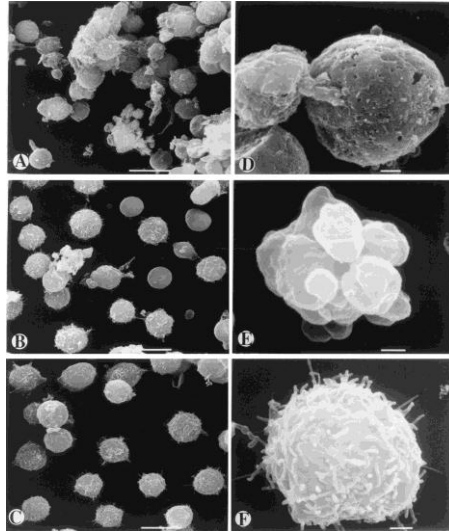
Mechanisms of cell death

Apoptosis and Necrosis (Morphology)



Cell death

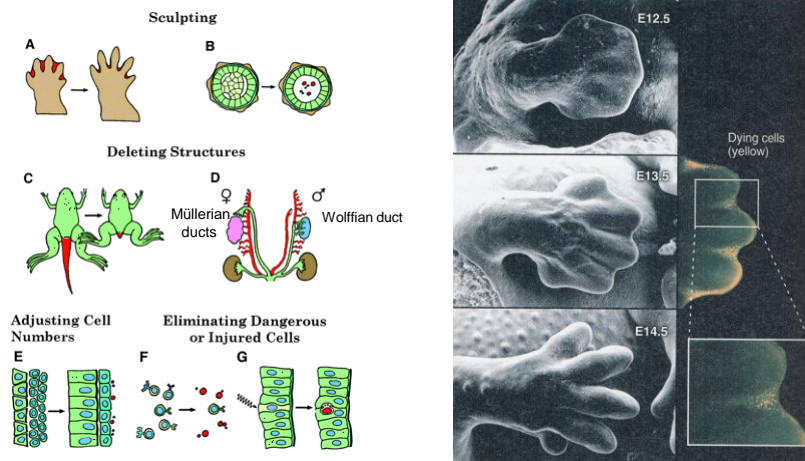
Cell death induced in Jurkat cells



Apoptosis

- endometrial cell breakdown during menstruation
- cell deletion in proliferating cell populations – intestinal crypt epithelia to maintain a constant number
- death of host cells that served its purpose – neutrophils (acute inflammation); lymphocytes (immune response)
- cell death induced by cytotoxic T cells – defense against viruses and tumors

Examples of Apoptosis

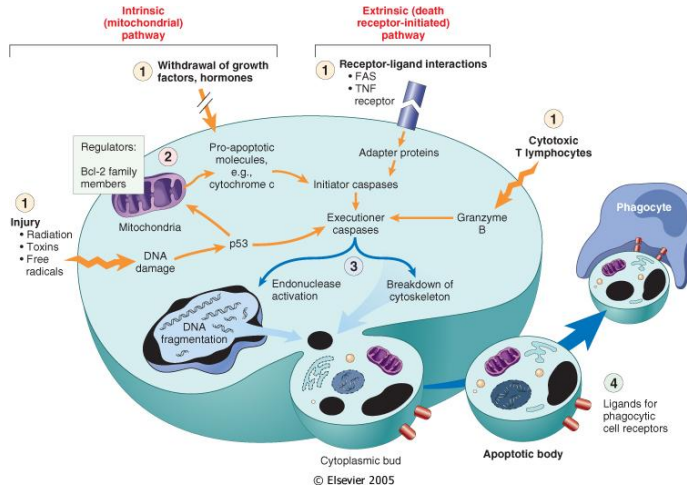


Apoptosis

- pathologic conditions
 - injurious stimuli – DNA damage induced by radiation, chemotherapy drugs; protein aggregation induced ER stress
 - viral infections
 - organs affected by duct obstruction (pancreas, kidney)

Mechanisms of cell death

□ Apoptosis



Mechanisms of Apoptosis

Mammalian ICE (Interleukin-1 β converting enzyme)
 \longrightarrow Caspase 1

Caspase
 Cys Asp protease

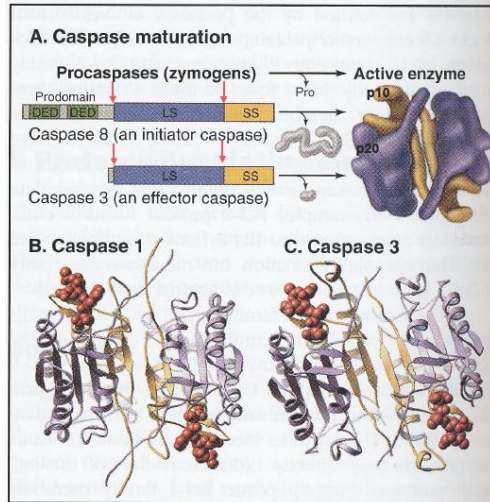
Active site
 QACRG

XXXD

Recognize specific sequence in substrate
 Cleave after Aspartic acid

Caspase 3
 xxxDEVDxxx

Activation of caspases

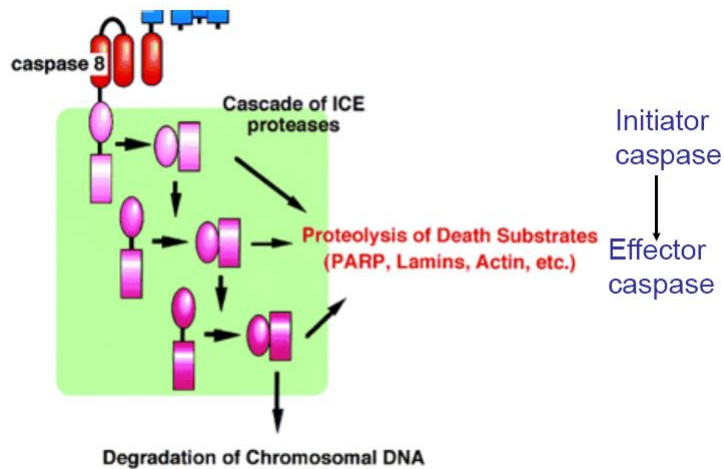


Consensus sequence
of cleavage site
xxxD

p10+p20
Hetero-dimer formation

(p10+p20)₂ tetramer
= active form

Caspase cascade



Substrates of caspases

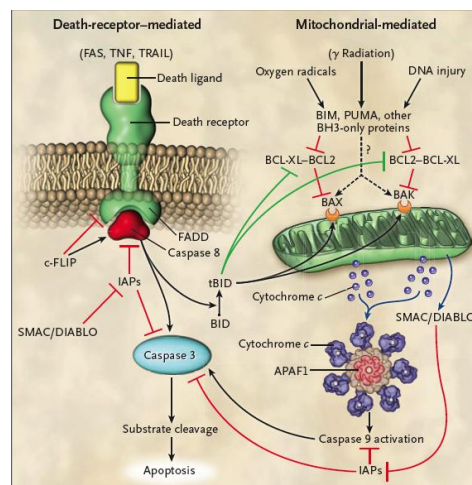
Increase activity by cleavage

Pro-caspase	active form
DFF45 (ICAD)	DNA fragmentation

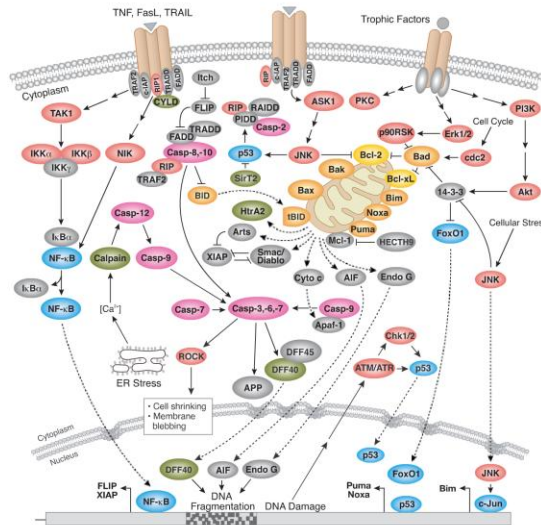
Decrease/lose activity by cleavage

DNA-PKcs	DNA repair
Rb	Cell cycle
MDM2	Modulation of p53
Lamins	Nuclear configuration
Action	Component of cytoskeleton
PARP	DNA repair

Apoptosis pathways

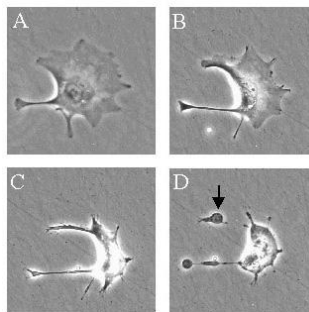


Apoptosis

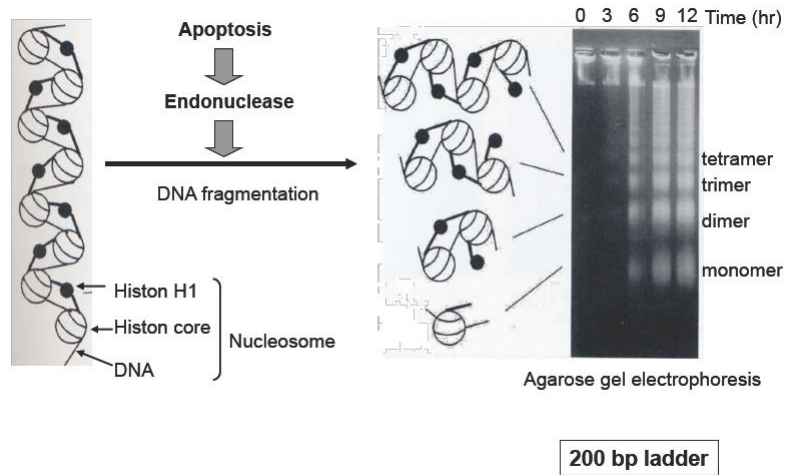


Mechanisms of cell death

- Apoptosis
 - morphologic changes in apoptosis
 - cell shrinkage
 - DNA condensation/fragmentation
 - cytoplasmic blebs, apoptotic body formation



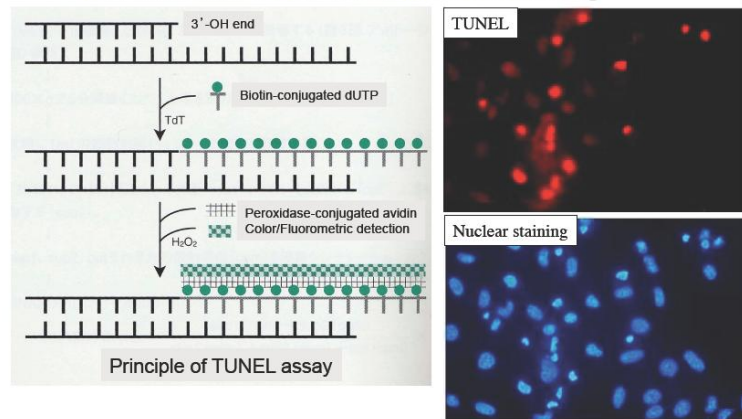
Detection of DNA fragmentation



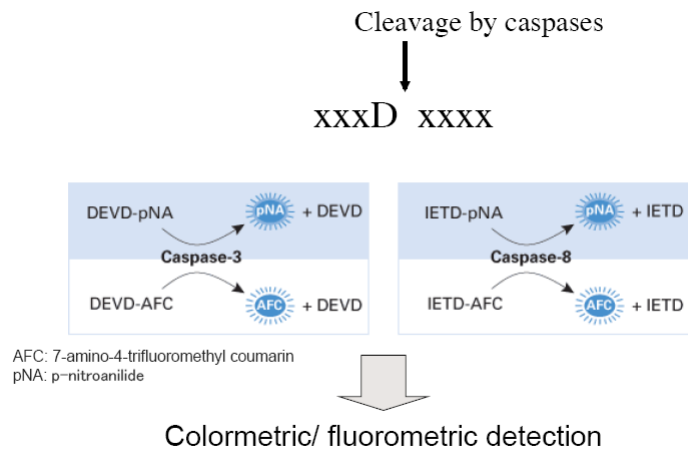
Detection of fragmented DNA (TUNEL)

Terminal deoxynucleotidyl transferase (TdT)

TdT-mediated d-UTP-biotin nick end labeling



Detection of caspase activation



Detection of caspase substrate cleavage

