

1  **ANAT3231 Lecture 10**
Cell Death

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3  **Cell Death Lecture Overview**

1

- Cell Lifespan
- Cell Death
- Necrosis
- Apoptosis
- Apoptosis Detection
- Apoptosis in Development
- Apoptotic Cell Morphology
- DNA Fragmentation
- Apoptosis Signals

2

- The NGF/TNF Proteins
- Receptor Binding
- Death Receptor Pathway
- Mitochondrial Pathway
- Caspases
- Bcl2
- Apoptosis Inhibitors
- Neuronal Apoptosis
- Apoptosis Analysis
- References

4  **Cell Lifespan**

- Body Cell Types
 - About 210 types
- Lifespan
 - Born
 - Differentiate
 - Function
 - Die
 - daily in humans approx 5×10^{11} blood cells eliminated by programmed cell death
 - apoptosis

5  **Cell Lifespan**

- Neutrophil
 - 6-7 hours circulating
 - 4 days in tissue
- Red blood cell
 - 120 days
- Brain neuron

- 50 - 100 years

6 Lifespan Processes

- Birth
 - Mitosis and meiosis
- Death
 - Apoptosis and Necrosis
- Programmed Cell death
 - Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics.
 - Kerr, J. F., Wyllie, A. H. & Currie, A. R. Br. J. Cancer 26, 239-257 (1972).

7 Cell Death- Mechanisms

8 Necrosis

- Removes damaged cells from an organism
- Early stages
 - cells and organelles (mitochondria) swell
 - due to disruption of plasma membrane
 - cell contents leak out
 - leading to inflammation and necrosis
- Late stage
 - finally cell disintegration

9 Apoptosis

- tightly regulated
- selective process for deletion of
 - Superfluous
 - Infected
 - transformed cells
- involved in
 - Embryogenesis
 - Metamorphosis
 - Endocrine dependent tissue atrophy
 - Normal tissue turnover
 - Variety of pathologic conditions

10 Apoptosis (electron microscope)

- apoptotic cell in mammary gland
 - normal occurrence
 - balances proliferation
 - occurs each menstrual cycle
 - Breakdown of nuclear envelope
 - condensed chromatin
 - dark clumps

(Note: normal cell is visible right)

- EM Courtesy of David Ferguson

11 Apoptosis Detection Methods

- Haematoxylin and eosin
- Semi-thin plastic section showing apoptotic cells (dark)
- EM Apoptotic nuclei (arrows) shown in vacuoles of phagocytes (M)
- Nile blue sulfate
- Acridine orange
- TUNEL

12 Apoptosis in Development

- Limb development
 - Cell death between digits
- Ossification
 - Death of chondrocytes
- Nervous System
 - Death of neurons

13 Disruption of Brain Development

- Apoptosis blocked by caspase-9 or Apaf-1 KO
 - caspase 9-deficient mice
 - over proliferation of brain neurons
 - (A) 16 day embryo wild-type
 - (B) Caspase 9-KO
 - Enlarged brain protrudes
 - limbs still webbed
 - forebrain section day 13.5
 - (C) a normal mouse
 - (D) Caspase 9-KO mouse
 - Thickened ventricle walls
 - obliteration of ventricle space

From: Kuida et al. 1998

14 Apoptotic Cell Morphology

- loss of cell membrane phospholipid asymmetry
 - Annexin V
 - detection of exposed phosphatidylserine
- Condensation of chromatin
- Reduction in nuclear size
- Internucleosomal DNA cleavage
 - DNA ladder
- shrinkage of cell
- membrane blebbing
- breakdown of cell into membrane-bound apoptotic bodies
 - bodies then phagocytosed by other cells

15 DNA Fragmentation

16 Apoptosis Signals

- diverse group of signals induce apoptosis
 - UV or gamma-irradiation
 - Chemotherapeutic drugs
 - growth factor withdrawal
 - cytokines TNF-alpha and TGF-beta

17 The NGF/TNF Proteins

- trigger apoptosis by binding to receptors located on cell surface
 - death receptors
- Receptors
 - extracellular cysteine-rich domain
 - intracellular death domain (DD)

18 Receptor Binding

- Binding ligand to receptor

- Stimulates oligomerization of receptors through DDs
- receptors recruit and bind other DD-containing proteins (adaptor proteins)
 - FADD and RAIDD
 - adaptors bind a proximal caspase
 - activate caspase proteolytic cascade

19 Apoptosis - 2 Pathways

- 2 Pathways
 - death-receptor
 - mitochondrial
- Both pathways
 - Converge on caspase-3 activation
 - branch into many pathways
 - leading to eventual cell death

20 Death Receptor Pathway

- triggered by members of death-receptor superfamily
 - CD95 and tumour necrosis factor receptor I
 - CD95 ligand binding
 - induces receptor clustering
 - formation of death inducing signaling complex
 - Complex recruits by adaptor molecule FADD multiple procaspase-8 molecules
 - F associated death domain protein
 - then caspase-8 activation by induced proximity
 - blocked by recruitment of degenerate caspase homologue c-FLIP

21 Mitochondrial Pathway

- Extracellular cues and internal insults
 - DNA damage
- diverse pathways converge on mitochondria
 - activation of a pro-apoptotic member of Bcl-2 family
 - group II and group III
 - Not group I, Bcl-2, anti-apoptotic, mostly attached to intracellular membranes

22 Mitochondrial Pathway

- Bax, Bad, Bim and Bid
 - group II and group III
 - shuttle between cytosol and organelles
 - cytosolic forms represent pools of inactive proteins
- Apoptotic signal
 - redirect proteins to mitochondria
- Activation of pro-apoptotic members
 - Proteolysis
 - dephosphorylation
 - other mechanisms

23 Mitochondrial Pathway

- surface of mitochondria
 - Pro- and anti-apoptotic Bcl-2 family members meet
- compete to regulate cytochrome c exit
 - mechanism still debated
- If pro-apoptotic wins
 - array of molecules released from mitochondrial compartment
 - cytochrome c (CytC) main protein
 - associates with Apaf-1
 - then procaspase-9

- forms apoptosome

24 Caspases

- cysteine proteases
 - central regulators of apoptosis
- Initiator caspases
 - caspase 8, 9, 10 and 12,
 - closely linked to pro-apoptotic signals
 - once caspases activated
 - cleave and activate effector
- Effector caspases
 - caspases 3, 6, and 7
 - cleave cytoskeletal and nuclear proteins
 - induce apoptosis

25 Caspase Cleavage

- diverse results
 - depends on substrate and position of cleavage site
- A, B most frequent outcome is loss of biological activity
 - eg lamin network
- C, D Limited proteolysis by caspases result in a gain of biological activity
 - eg Bcl-2 or Bcl-x, PAK2, Bid and CAD/ICAD

26 Caspase Activation Mechanisms

- proteolytic cleavage by an upstream caspase
- Induced proximity
- holoenzyme formation

27 Caspase Activation Mechanisms

- proteolytic cleavage by an upstream caspase
 - activation of downstream effector caspases
 - induction of non-caspase proteases (granzyme B)

28 Caspase Activation Mechanisms

- Induced proximity
 - aggregation of procaspase-8 molecules somehow results in cross-activation

29 Caspase Activation Mechanisms

- holoenzyme formation
 - holoenzyme formation cytochrome c and ATP dependent oligomerization of Apaf-1
 - allows recruitment of procaspase-9 into apoptosome complex
 - Activation of caspase-9 is mediated by conformational change not proteolysis
 - Stoichiometry of apoptosome is not known
 - shown as a hexamer for aesthetic reasons

30 Apoptosome Formation

31 Caspases

32 Cytoskeleton- Nuclear Lamins

33 Apoptosis Regulation

- both positively and negatively regulated
- numerous factors induce apoptosis

- other factors prevent cell death
 - Anti-apoptotic ligands

34 **Anti-Apoptotic Ligands**

- Growth factors and cytokines
 - induce anti-apoptotic Bcl-2 family members
- Bcl-2 proteins
 - protect integrity of mitochondria
 - Prevent cytochrome C release
 - Block caspase 9 activation

35 **Bcl Proteins**

- First isolated as a gene involved in **B-cell** lymphoma
- Bcl-2 family
 - 12+ proteins
 - 3 functional groups
 - Hydrophobic C-terminal anchors to Mitochondria membrane

36 **Bcl-2 Protein Family**

37 **Possible Bcl Mechanisms**

- Formation of a pore
 - cytochrome c and other intermembrane proteins escape
- Heterodimerization
 - between pro- and anti-apoptotic family members
- Direct regulation of caspases
 - by adaptor molecules
- Interaction with mito proteins
 - generate a pore or to modulate mitochondrial homeostasis
- Oligomerization
 - form a weakly selective ion channel

38 **Apoptosis Inhibitors**

- directly inhibit caspases or prevent activation
- Akt
 - serine/threonine protein kinase
 - important anti-apoptotic factor
 - inhibits pro-apoptotic Bcl-2 family member, Bad
 - directly inhibits caspase 9


39 **Akt Signaling**

40 **TNF-alpha**

- Tumour Necrosis Factor -alpha
 - both anti-apoptotic and pro-apoptotic effect
- Activates transcription factor NF-kB
 - then induces expression of IAP
 - an inhibitor of caspases 3, 7, and 9

41 **Neuronal Apoptosis**

- sculpts the developing brain
- role in neurodegenerative diseases
- apoptosis involves
 - Apaf-1
 - apoptotic protease-activating factor 1
 - Bcl-2 and caspase families
- Neurotrophins
 - regulate apoptosis through protein kinase cascades
 - phosphoinositide 3-kinase/Akt
 - mitogen-activated protein kinase pathways

42  **Neuronal Survival Pathway**43  **Neuronal Diseases**44  **Alzheimer's Disease**45  **Amyotrophic Lateral Sclerosis**46  **Apoptosis Analysis**

- Annexin
- Br-dUTP
- TUNEL
- Immunohistochemistry
- Mitochondrial Function
- Microarrays

47  **Annexins**

- ubiquitous homologous proteins
- bind phospholipids in presence of calcium
 - cellular changes involved in the apoptotic process
 - include loss of phospholipid asymmetry during early stages
- Universal
 - not limited to mammalian cells, also in insect and plant cells
 - PAP-1; Calphosbindin I; Lipocortin V
- Annexin V conjugates
 - used to detect apoptotic cells
 - significantly earlier than DNA-based assays
 - Fluorescent dye labeled annexin V
 - applications in flow cytometry, fluorescence microscopy, and laser scanning cytometry

48  **Annexin V**49  **DNA Laddering**

- DNA laddering
 - degradation of genomic DNA into fragments of 180-200 bp
 - fragmentation creates 3'-OH (hydroxyl) sites at DNA breaks
 - label sites with bromodeoxyuridine triphosphate
 - Br-dUTP
 - enzymatically attaches to 3'-OH sites of double- or single-stranded DNA by terminal transferase (TdT)

50  **Microarray Analysis**

- Microarrays

- analysis of differential gene expression
 - monitor expression of hundreds of genes simultaneously
- genes grouped into gene families
 - concurrent analyses of hundreds of relevant genes
 - complex signal transduction events that occur during apoptosis

51 Apoptosis Array Specifications

52 Apoptosis in the Clinic

- Humans approximately 10^{14} cells
 - each capable of committing suicide by apoptosis
- Compromised apoptosis process
 - inappropriate cell death
 - disease pathogenesis
 - either too little or too much
- Potential for therapeutic use
 - modulating apoptosis for treatment of human diseases

53 Apoptosis Pathways

(A) *C. elegans*

- CED-4 protein is a protease activating factor
 - activates CED-3 protease

(B) Mammals

- similar pathway exists
- Bcl-xL binds Apaf-1
 - prevents it from activating precursor of caspase-9

(C) Other pathways

- initiated by CD95 protein in cell membranes of lymphocytes
After Adams and Cory 1998

54 Apoptosis Summary

- complex process
 - involving many factors
 - operating in multiple pathways
 - must be carefully regulated for the proper growth and development of an organism
- Failure of apoptosis
 - can lead to a number of disease states
 - Cancer
 - Parkinson's
 - AIDS

55 Online References

- UNSW Cell Biology
 - ANAT3231 Cell Death Lecture
 - <http://cellbiology.med.unsw.edu.au/units/science/lecture0510.htm>
- Other References
 - Molecular Biology of the Cell (Ch22)
 - <http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?db=Books&cmd=search&doptemdl=DocSum&term=apoptosis+AND+cell%5Bbook%5D>
 - Developmental Biology (Ch6)
 - <http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?db=Books&cmd=search&doptemdl=DocSum&term=apoptosis+AND+dbio%5Bbook%5D>
 - Molecular Cell Biology (Ch23)
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 - The Cell- A molecular approach (Ch13)
 - <http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?db=Books&cmd=search&doptemdl=DocSum&term=apoptosis+AND+cooper%5Bbook%5D>

- Sigma Apoptosis Brochure
 - www.sigma-aldrich.com/cellsignaling
- Reproductive & Cardiovascular Disease Research Lab
 - <http://www.sghms.ac.uk/depts/immunology/%7Edash/apoptosis/>

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- Apoptosis in the nervous system.
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 - http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=11048732&dopt=Abstract
- Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells.
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- The role of apoptosis in cancer development and treatment response.
 - Brown JM, Attardi LD. Nat Rev Cancer. 2005 Mar;5(3):231-7. Review.

57 Caspase Family

1

- Caspase-1 (ICE)
- Caspase-2 (Ich-1, Nedd2)
- Caspase-3 (CPP32, Yama, Apopain, SCA-1, LICE)
- Caspase-4 (ICErel-II, TX, ICH-2)
- Caspase-5 (ICErel-III, TY)
- Caspase-6 (Mch-2)
- Caspase-7 (Mch-3, ICE-LAP-3, CMH-1)

2

- Caspase-8 (FLICE, Mach-1, Mch5)
- Caspase-9 (ICE-LAP6, Mch6, Apaf-3)
- Caspase-10 (FLICE-2, Mch4)
- Caspase-11
- Caspase-12
- Caspase-13 (ERICE)
- Caspase-14 (Mini-ICE)

58 Caspases

- Apoptosis caspases either initiators or effectors
- Cytokine processing caspases
 - Caspase-1
 - first caspase to be purified, identified by ability to cleave precursor of IL-1b to mature form
 - Caspase-11
 - is required for activation of caspase-1
 - Caspases-4, -5, -12, -13, and -14
 - generally involved in cytokine processing
 - amino acid (aa) sequence homology closer to caspase-1 than to apoptosis caspases

59 Caspase Signaling

- family of proteases that signal in a step-wise manner
 - Similar to signaling cascade by kinases
- present in all cells as latent enzymes
- recruited to receptor-associated cytosolic complexes
 - whose formation is initiated by receptor oligomerization (e.g., TNF receptors, FAS, and TRAIL receptors) and to other cytoplasmic adaptor proteins, such as APAF-1
- Recruitment of caspases to oligomerized receptors leads to activation via dimerization or dimerization accompanied by autoproteolytic cleavage
- Active caspases can proteolyze additional caspases generating a caspase cascade that cleaves proteins critical for cell survival

- final outcome of controlled cell death, apoptosis

60 Caspases

- apoptosis caspases either initiators or effectors
 - Initiators
 - Caspases -8, -9, and -10 (possibly, -2 and -5) can initiate the caspase activation cascade and are therefore called initiators. Based on the prototypes, caspases -8 and -9, initiators can be activated either by dimerization alone (caspase-9) or by dimerization with concomitant autoproteolysis (caspase-8).
 - Effectors
 - The effector caspases -3, -6, and -7 propagate the cascade and are activated by proteolytic cleavage by other caspases. Although an initiator caspase may not be responsible for starting the caspase cascade, it can become activated and involved in later steps of the cascade. Thus, in the latter scenario, the caspase would be more appropriately termed an effector.
- Caspases involved in processing a select group of cytokines
 - Caspase-1, the first caspase to be purified, was identified by its ability to cleave the precursor of IL-1b to its mature form.¹¹ In caspase-1 deficient mice, IL-1b and IL-1a are not proteolytically processed and the mice are resistant to endotoxic shock.¹² a process involving IL-1b. Caspase-11 deficient mice are also resistant to endotoxic shock and fail to process IL-1b and IL-1a.¹³ Caspase-11 is required for activation of caspase-1 and subsequently, caspase-11 deficient mice fail to activate caspase-1. Caspases -4, -5, -12, -13, and -14 are generally expected to be involved in cytokine processing. These caspases have an amino acid (aa) sequence homology closer to caspase-1 than to the caspases involved in apoptosis.