FACULTY OF ENGINEERING & TECHNOLOGY

LT.18 Apoptosis: Morphological and biochemical features

Outline

1. Apoptosis
2. Major Pathways of apoptosis
3. Morphological changes in Apoptotic cells
4. Biochemical characteristic of apoptotic cells
5. Role of apoptosis in regulating lymphocyte development.
6.

Apoptosis & pathways

Apoptosis is a form of programmed cell death, or "cellular suicide." It is different from **necrosis**, in which cells die due to injury. Apoptosis occurs normally during development and aging and as a homeostatic mechanism to maintain cell populations in tissues. Apoptosis also occurs as a defense mechanism such as in immune reactions or when cells are damaged by disease or noxious agents.

Major pathways for Apoptosis

1. Extrinsic pathway (Death receptor mediated)
2. Intrinsic (Mitochondria mediated)

Death receptor mediated pathway

- The death receptor-mediated pathway is initiated by the interaction of the ligand with its death receptor, which leads to the activation of caspase-8 and caspase-3. The caspase-3 then cleaves various substrates leading to apoptosis. Mitochondria-mediated apoptosis is regulated by B-cell leukemia/lymphoma 2 (Bcl-2) family of proteins, which control mitochondrial membrane permeability. The chemopreventive agents are known to induce apoptosis by interfering with the extrinsic and the intrinsic apoptotic pathways.

Morphological changes in apoptotic cells

•Cell shrinkage, nuclear and cytoplasmic condensation, chromatin fragmentation, and membrane blebbing. With cell shrinkage, the cells are smaller in size, the cytoplasm is dense and the organelles are more tightly packed.

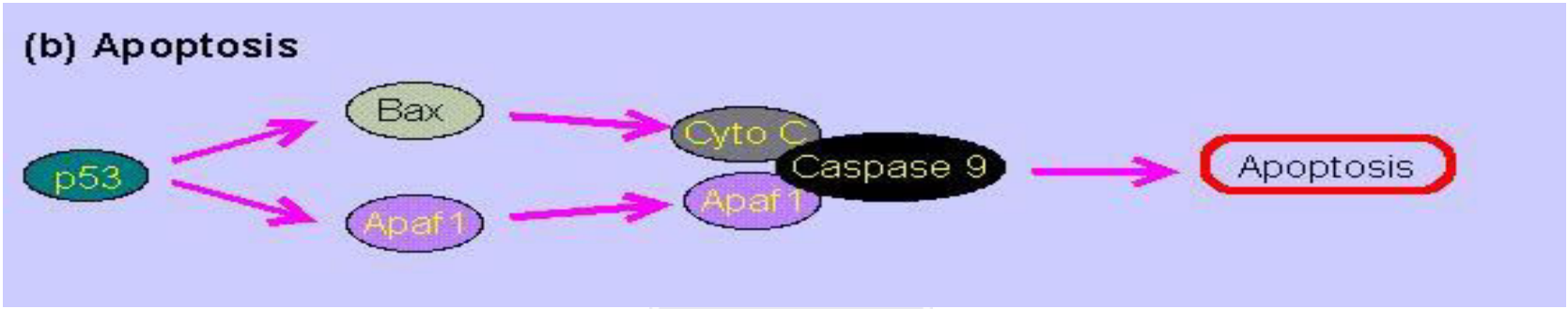
• Pyknosis is the result of chromatin condensation and this is the most characteristic feature of apoptosis. The apoptotic cell appears as a round or oval mass with dark eosinophilic cytoplasm and dense purple nuclear chromatin fragments.

•Extensive plasma membrane blebbing occurs followed by karyorrhexis and separation of cell fragments into apoptotic bodies during a process called “budding.” Apoptotic bodies consist of cytoplasm with tightly packed organelles with or without a nuclear fragment

Biochemical characteristics of apoptotic cells

•The biochemical hallmark of apoptotic cell death is the degradation of DNA into oligosome-sized fragments by specific endonucleases which cut the internucleosomal regions into double-stranded DNA fragments of 180–200 base pairs (bp) and the formation of small vesicles from the cell surface, also known as apoptotic bodies.

•A variety of caspase substrates are involved in the regulation of DNA structure, repair and replication . The DNase enzymes responsible for the fragmentation during apoptosis include DNA fragmentation factor (DFF40), caspase activated DNase (CAD) and in hematopoietic cells NUC70. DFF40 and CAD are present in normal cells as inactive heterodimers with the inhibitor proteins DFF45 [40] and ICAD (inhibitor of CAD). These enzymes are selectively activated upon cleavage by caspase 3 or by other members of the caspase family. Exposure of nuclei to activated CAD or DFF40 is sufficient to induce the nuclear morphologic changes typical of apoptosis. Formation of large size (50–300 kbp) DNA fragments precedes internucleosomal fragmentation, but the role of the above mentioned DNases in this process has not been studied.



Apoptosis can be induced by the binding of Caspase 9 to cytochrome c and Apaf1. p53 may activate the expression of Apaf1 and Bax. The latter can then stimulate the release of cytochrome c from mitochondria.

Role of apoptosis in regulating lymphocyte development

Lymphocyte apoptosis is crucial to proper immune function. It removes developing lymphocytes that fail to express an antigen receptor, thereby ensuring a functional repertoire of mature B and T cells, and it maintains tolerance toward self by eliminating lymphocytes with antigen receptors that recognize autoantigens. Apoptosis also regulates the size and duration of immune responses, activated lymphocytes being killed when an infection is cleared successfully.

Apoptotic pathways for T-lymphocyte

a. The intrinsic cell death pathway controlled by Bcl-2 family members and

b. The extrinsic cell death pathway controlled by death receptor signaling.

Role of apoptosis in regulating lymphocyte development

•The intrinsic cell death pathway is activated by a variety of apoptotic stimuli, such as genomic toxicity and cytokine withdrawal. Intrinsic cell death signals generally converge within the cell at the outer membrane of mitochondria. They result in a loss of mitochondrial membrane integrity and the subsequent activation of downstream apoptotic pathways.

•Bcl-2 family members control mitochondrial membrane integrity and are the major mediators in the intrinsic cell death pathway. Bcl-2 protein family members are divided into three subfamilies based on function and BH domain structure. These groupings include the anti-apoptotic BH1-4 subfamily, the pro-apoptotic BH1-3 subfamily and the pro-apoptotic BH3-only subfamily.

•Bcl-2 protects various cell types from apoptosis. During T cell development, the expression for Bcl-2 is tightly regulated, which suggests a critical role of Bcl-2 in T lymphocytes. Indeed, Bcl-$2^{-/-}$ mice exhibit an intrinsic defect in T lymphocyte development probably due to increased apoptosis. In Bcl-$2^{-/-}$ adult bone marrow hematopoietic stem cell reconstituted chimeric mice, donor-derived lymphocyte development is almost completely absent. IL-7 provides a crucial survival signal for lymphocyte precursors. It is believed that Bcl-2 plays an important anti-apoptotic role downstream of IL-7 signaling.

Extrinsic cell death pathways

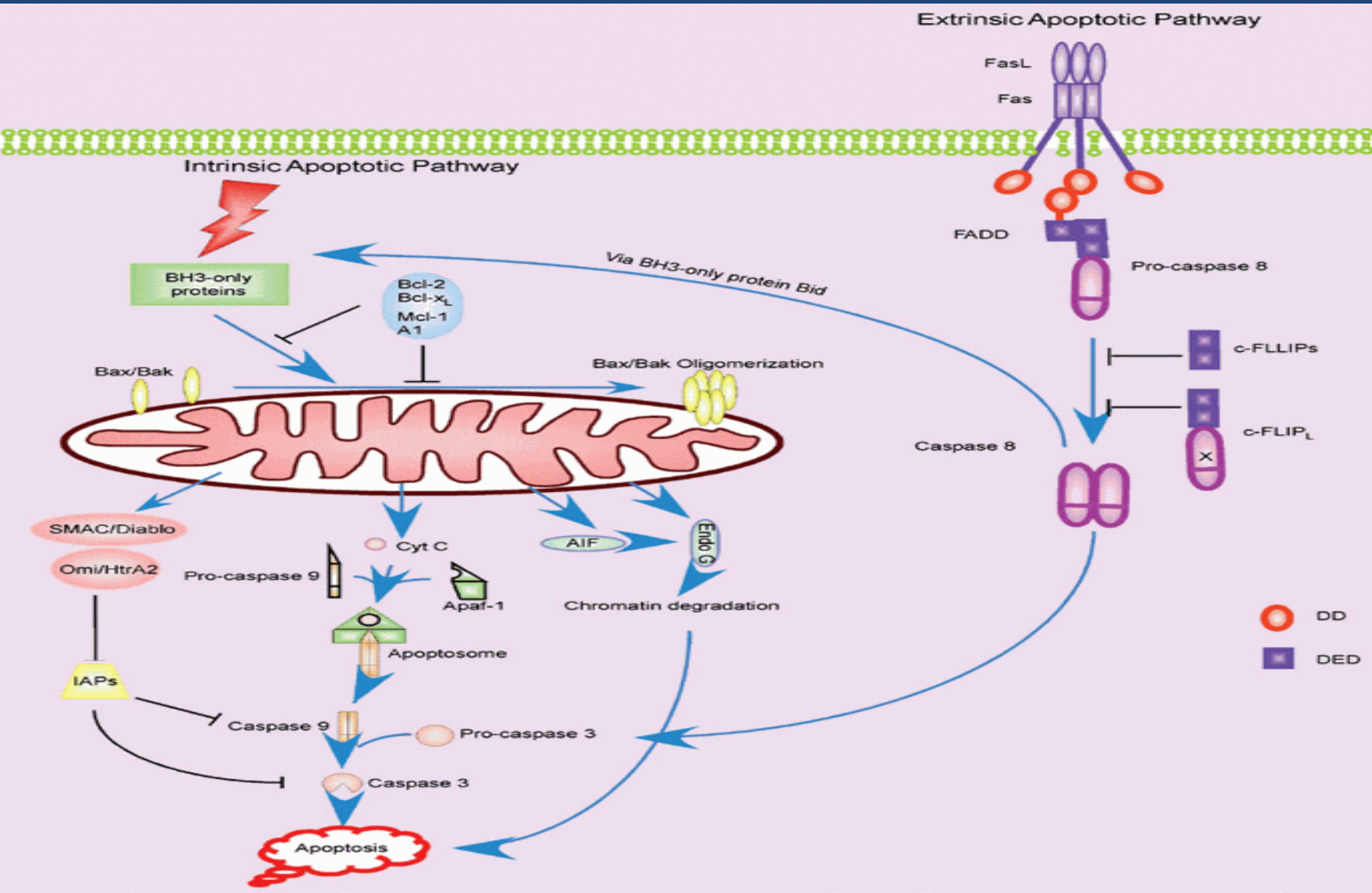
•The primary receptors that initiate these death signals in the immune system are the members of the TNF receptor family, such as Fas and TNFR-1. Death receptors (Group I) include Fas, TNFR1, death receptor 3 (DR3), tumor necrosis factor apoptosis related ligand (TRAIL) receptor1, TRAIL-R2 and DR6. Death receptors all contain a death domain (DD) in their cytoplasmic tail. The death domain is an evolutionarily conserved domain composed of approximately 60 amino acids that forms a globular bundle of six α helices. After ligands bind to death receptors, the DD mediates homotypic interaction with other DD-containing adaptor proteins with high specificity.

FAS MEDIATED EXTRINSIC CELL DEATH PATHWAY

•Fas and FasL have been extensively studied in the immune system. It is well-known that Fas-FasL interactions play an essential role in maintaining homeostasis of the immune system.

•Activation of Fas delivers an apoptotic signal through FADD and caspase 8/10. After FasL binds to Fas, the DD domain in the cytoplasmic tail of Fas recruits the adaptor protein FADD. FADD is a 26 kDa cytosolic adaptor protein composed of two functional domains: an N-terminal death effector domain (DED) and a C-terminal death domain (DD). After the DD domain of FADD binds to Fas, the DED domain of FADD recruits pro-caspase 8 and also pro-caspase 10 in human cells, which leads to the activation of caspase 8/10. Caspase 10 is highly homologous to caspase 8 at both a sequence level and a function level. However, there is no murine homolog for caspase 10. Active caspase 8 activates a downstream caspase cascade and leads to cell death.

Extrinsic Apoptotic pathways



References & Further reading

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