

The memorandum of demise: programmed default

Abstract

A morphologically enunciated pattern of cell death comprising of genetically driven extermination of cells designated as 'APOPTOSIS' the process is a homeostatic process for controlling tissue and cell community. It manifests as a defence methodology in immune repercussions or cellular injury by disease/noxious agents. Appreciating the techniques of alternative methods of programmed cell death at the molecular level simultaneous to extensive apoptotic association at varied procedural checkpoints brings profound awareness of the evolution of miscellaneous diseases and can therefore impact therapeutic scenarios.

Keywords: cell community, apoptosis, DNA, TNF, CASPASES, pyknosis, karyorrhexis

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Abbreviations: TNF, tumour necrosis factor; ITP, immune thrombocytopenic purpura; APP, amyloid precursor protein; AIDS, autoimmune deficiency syndrome

Introduction

A morphologically enunciated pattern of cell death comprising of genetically driven extermination of cells designated as 'APOPTOSIS' by Kerr, Wyllie and Currie. Generally ensuing amidst developing and ageing, the process is a homeostatic process for controlling tissue and cell community. In addition, it manifests as a defence methodology in immune repercussions or cellular injury by disease/noxious agents. Diverse circumstances and physiological or pathological stimuli can elicit apoptosis; however the catalytic cellular reactions are not consistent. Irradiation and chemotherapy incite a partial, cellular DNA breakage i.e. an apoptotic annihilation via a p53 provision. Certain hormones (e.g. corticosteroids) induce specific cellular apoptosis (e.g. thymocytes), while alternative cells are not influenced or may be stimulated. Cellular significance of Fas or Tumour Necrosis Factor (TNF) receptors is recognized for apoptosis via ligand binding and protein cross linkages.¹ Alternatively, cells procure a default death pathway that necessitate obstruction by survival agents e.g. hormones or growth factors. Apoptosis needs to be demarcated from necrosis, two patterns that happen independently, simultaneously or sequentially.² Frequently, the category or the intensity of the stimulus predisposes to either apoptosis or necrosis. Varied and minimal amount of injurious stimuli such as heat, radiation, hypoxia and cytotoxic anticancer drugs encourage apoptosis but initiate necrosis when in excess.³ Apoptosis is a systematic, energy dependent operation engaging and commencing with an assembly of cysteine proteases called CASPASES and constituting a complex phenomenon connecting the initiating stimuli to eventual cellular demise.

Morphological changes

In the initial apoptotic phase, light microscopy demonstrates cell shrinkage and pyknosis. The cells are smaller in size, cytoplasm is dense, and organelles are more tightly packed. Consequent to chromatin condensation, pyknosis is the distinguishing feature of apoptosis. On histopathology, apoptosis affects single or cumulative cells. The cells resemble round to oval masses with dark, eosinophilic cytoplasm and dense purple nuclear chromatin. Electron microscopy defines the subcellular changes which are i) Chromatin condensation

with the peripheral aggregation of electron dense nuclear material just beneath the nuclear membrane along with uniformly dense nuclei. Extensive plasma membrane blebbing occurs, followed by karyorrhexis and cellular fractional detachment. The formation of apoptotic bodies impelled by channelized "budding" eventuates. Apoptotic bodies are constituted by cytoplasm with tightly packed organelles with or without a nuclear fragment. The plasma membrane is intact. Apoptotic bodies are prominently phagocytosed by macrophages, parenchymal cells or neoplastic cells and degraded with phagolysosomes. Macrophages that phagocytose and consume apoptotic bodies are designated as the tingible body macrophages and are chiefly located in the germinal centres of the reactive lymph nodes or the thymic cortex. Minimal inflammatory reaction coexists with apoptosis or induced by the eradication of apoptotic bodies considering⁴

- I. Apoptotic cells do not dispense their cellular constituents in the marginal interstitial tissues.
- II. The bodies are rapidly phagocytosed by the circumventing cells thereby arresting secondary necrosis
- III. Encircling cells do not manufacture anti inflammatory cytokines.

Necrosis: It is a toxic manifestation with the development of energy independent cellular death. Oncosis is a composition of necrosis accompanied with karyolysis and cell swelling.

Apoptosis necrosis continuum: Ongoing apoptosis is transformed to necrosis, attributed to declining caspases and intracellular ATP. Cell death ascribed to apoptosis/necrosis relies on nature of cell death signal, tissue type, developmental stage of the tissue and physiologic milieu, along with the intensity and the duration of the stimulus. Necrosis is a disorderly, inert phenomenon influencing considerable zones. Apoptosis is controlled, depends on the amount of generated energy and the extent of ATP depletion and influences individual or cellular batches. Necrotic cell injury is moderated by restriction of the cellular energy reservoir and definitive corruption of the cell membrane.

Electron microscopy of necrosis: These are cell swelling, formation of cytoplasmic vacuoles and blebs, distended endoplasmic reticulum, disaggregation or detachment of ribosomes, disrupted organelle membranes, condensed, ruptured or swollen mitochondria, swollen and ruptured lysosomes and disrupted cell membrane.

Dynamics of apoptosis

Extrinsic pathway: introduces apoptosis, by compromising the transmembrane receptor moderated interaction. It constitutes of members of the TNF receptor gene super family (death receptors) with an 80 amino acid rich “death domain” e.g. FasL/FasR Apo3L/DR3, Apo2L/DR4, TNF alpha/ TNFR1. Receptor aggregation with repressed homologous trimeric ligand helps recruit cytoplasmic adaptor proteins and aids procaspase 8 via dimerization of death affector domain (Figure 1).⁵

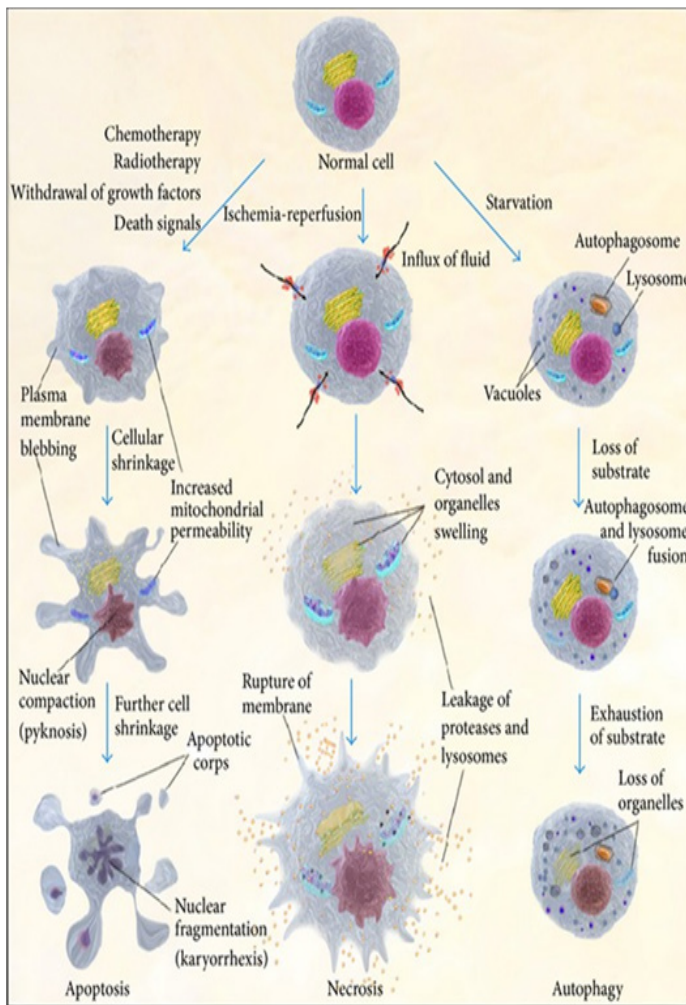


Figure 1 Causes and Types in Cellular Demise: The three major channels of cell death in which the cells are governed by various mediums of programmed cell death as established by definitive components. Apoptosis pathway elucidates the essential cellular shrinkage and configuration of apoptotic bodies devoid of the percolation of cellular contents. The necrotic route exhibits cytosol and organelle swelling with the breach of plasma membrane leading to escaped cellular contents. Autophagy is illustrated by the display of vacuoles with autophagosomes and its amalgamation with the lysosomes resulting in organelle digestion.

In order to adapt to a death actuating signalling complex with autocatalytic initiation of procaspase 8 which generates the execution phase of apoptosis and is constrained by the existence of c-FLIP and TOSO proteins.

Intrinsic pathway: Non receptor stimuli are interposed that furnish intracellular signals which operate precisely on the cellular targets

such as episodes initiated by the mitochondria. Corroborative incentives are radiation, toxins, hypoxia, hypothermia, viral infections and free radical injury. Antagonistic signals are deficiency of growth factors, hormones, cytokines and factor retraction with lack of suppression and mobilization of apoptosis. Distribution of normally sequestered pro apoptotic proteins from the mitochondrial inter membranous space into the cytosol is desirable to energize caspase dependent mitochondrial network. The manipulation and codification of apoptotic mitochondrial phenomenon occurs through Bcl₂ protein. Tumour suppressor p53 adapts the Bcl₂ which can be pro apoptosis or anti apoptosis. My concoprotein also potentiates apoptosis through p53 dependent and independent circuits.

Execution pathway: Propulsion of execution caspases is necessitated to induce cytoplasmic endonucleases and proteases to decompose nuclear material and cytoskeletal protein. Caspases 3, 6 and 7 decompose miscellaneous substrates containing cytokeratins, nuclear proteins, plasma membrane and cytoskeletal proteins. Phagocytic absorption is the ultimate component of apoptosis. Phospholipid asymmetry and externalization of phosphatidyl serine on the apoptotic cell exterior is the corroboration of this phase as it expedites non inflammatory phagocytic identification thereby permitting premature uptake and clearance.

Perforin/granzyme Pathway: T cell mediated cytotoxicity where sensitized CD8+ cells demolish antigen bearing/ target cells are modulated by the extrinsic transmission.⁶ FasL/FasR cooperation is the modus of cytotoxic T cell influenced apoptosis. Granzyme B splits proteins at the aspartate residues to stimulate procaspase 10.⁷ Granzyme B can employ the mitochondrial route for augmentation of death signal, along with specific stimulation of caspase 3 for categorical induction of the execution signals. Granzyme A is chiefly discernible in cytotoxic T cell activated apoptosis and prompts caspase independent pathways. It subscribes to apoptosis by obstructing the perpetuation of DNA and the structural coherence of the nuclear chromatin (Figure 2).

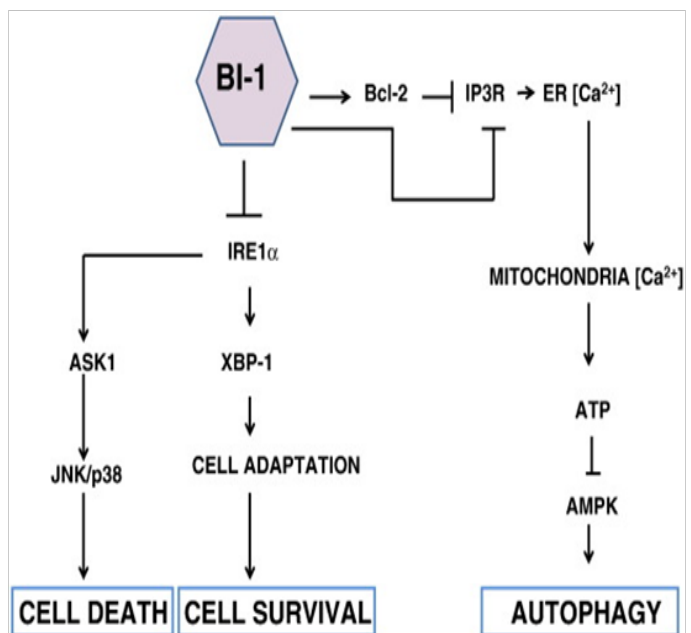


Figure 2 Molecular pathways and outcomes.

Biochemical features

Contrasting adaptations are enumerated such as protein cleavage, protein cross linkage, DNA breakdown and phagocytic recognition. Procaspases can assemble and stimulate some caspases (inactivated proenzymes) which when incited, can mobilize other caspases thus influencing an irreversible commitment to cell death. Initiator caspases 2,8,9,10 effector or execution caspases 3,6,7 inflammatory caspases 1,4,5, caspase 11 coordinate apoptosis and cytokine progression, caspase 12 determines cytotoxic and endoplasmic specific apoptosis. Extensive protein cross linkage is an essential feature of apoptotic cell e.g. externalization of phosphatidyl serine Annexin I and Calreticulin.⁸

Types of apoptosis

Physiologic Apoptosis is an approved but contradictory aspect of mitosis. Apoptosis is an important phenomenon among maturation and evolution mechanisms (e.g. nervous/ immune system) where the cellular excess ensues with subsequent cell death, along with elimination of cells invaded by the pathogens. An essential component of wound healing, apoptosis is implicated in the elimination of inflammatory cells and transformation of granulation tissue into scar tissue. Poor modulation of apoptosis can be expressed as pathologically designed healing as is delineated with disproportionate scarring and fibrosis. Apoptosis is a vital aspect of termination of self perpetuating, aggressive immune cells in concurrence with the development of central lymphoid organs (bone marrow/ thymus) and is also active in cellular rejuvenation in adults as in follicular atresia in the post ovulatory follicles or post weaning mammary gland involution. Immense or imperceptible cellular demise stimulates pathology that encompasses developmental defects, autoimmune diseases, neurodegeneration etc.

Pathologic Apoptosis: Aberration and assimilation of cell death is a characteristic outcome of Cancer, Autoimmune Lymphoproliferative syndrome,⁹ AIDS, Ischaemia, Neurodegenerations like Parkinson's disease, Huntington's chorea, Amyotrophic lateral sclerosis etc which incorporates deficient to abundant apoptosis. In cancer the normal cell cycle is dysfunctional with extreme cellular propagation or displacement. Elimination of apoptosis is critical for induction and growth of certain malignancies. Tumour cells obstruct apoptosis by elucidating anti-apoptotic proteins such as Bcl₂ or induce deregulation / metamorphosis of pro apoptotic protein e.g Bax. Both Bcl₂ and Bax are managed by the p53 tumour suppressor gene. Specific human B cell lymphomas have over expression of Bcl₂. The p53 gene which is implicated in 50% of the cancers is a generic anomaly in human tumourigenesis induced by viruses (HPV), radiation or with chemical afflictions. Extensive rearrangement of phosphatidyl inositol 3 kinase. AKT pathway in tumourigenesis contributes to autonomous cell survival in addition to control of supplementary cellular mechanisms such as growth, multiplication, cytoskeletal organization etc. Inappreciable apoptosis e.g. in autoimmune lymphoproliferative syndrome amplifies the B cells with production of abundant immunoglobulin and induction of autoimmunity as in haemolytic anaemia, immune thrombocytopenic purpura (ITP), autoimmune neutropenia etc. Exuberant apoptosis as occurs in neurodegenerative diseases or ischemia induced injury is associated with formulation of Bax proto-oncogene. Neurodegenerative disorders such as Alzheimer's disease arise with altered proteins such as Amyloid precursor protein

(APP). Autoimmune deficiency syndrome (AIDS) commences with a viral infection of CD4+ helper cells and ensues with the binding of CD4 receptors, which are then internalized. Enhanced expression of Fas receptors is followed by profuse apoptosis of T cells.

Assays for apoptosis

- i. Cytomorphological alterations
- ii. DNA fragmentation
- iii. Detection of caspases
- iv. Cleaved substrate regulators with inhibitors
- v. Membrane alterations
- vi. Detection of apoptosis in whole mounts
- vii. Mitochondrial assays

Corollary apoptosis: It is observed as a stringently regulated energy dependent mechanism, distinguished by specific morphological and biochemical aspects in which caspase activation plays a cardinal role. Numerous pivotal apoptotic proteins which are activated or inactivated in the apoptotic agendum have been analyzed. However the molecular executions or initiation of these protein constituents has not been grasped in its entirety and necessitate further research. Comprehending the functioning and performance of apoptosis is essential, considering that programmed cell death is an element of health as well as disease.¹⁰ Divergent physiologic and pathologic stimuli are known to activate apoptotic phenomenon. Appreciating the techniques of alternative methods of programmed cell death at the molecular level simultaneous to extensive apoptotic association at varied procedural checkpoints brings profound awareness of the evolution of miscellaneous diseases and can therefore impact therapeutic scenarios.¹¹

Acknowledgement

None.

Conflict of interest

Author declares that there is no conflict of interest

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