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AN OVERVIEW OF PROGRAMMED CELL DEATH: APOPTOSIS.

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INTRODUCTION:

A distinct and significant form of "planned" cell death, Apoptosis, derived from the Greek word "falling off[1]". Understanding apoptosis in illness settings is crucial since it can provide information about a disease's pathophysiology as well as possible treatment options. Apoptosis occurs as a defense mechanism, such as in immunological responses, as well as a homeostatic mechanism to maintain cell population in tissues[2].

WHY DO CELLS UNDERGO APOPTOSIS?

Apoptosis is essentially a common and practical method for getting rid of cells that shouldn't be a part of the body.

• Some cells must be "eliminated" during development, such as when a complex structure like a hand must be carved out of a larger block of tissue.

• Some cells, such as those with viral infections or DNA damage, are aberrant and, if they survive, could harm the rest of the body[3].

HISTORICAL ASPECTS

Weigert and Cohnheim used the term "coagulation necrosis" in 1884 to characterise the microscopic manifestation of cell death in necrotic tissue. In contrast, Flemming first documented the process of chromatolysis in 1885, in which the nuclei of mammalian ovarian follicles disintegrated before eventually dying spontaneously. Kerr coined the term "shrinkage necrosis" to characterise the appearance of single cell death in the liver in the beginning of the 1970s. In a seminal work published in 1972, Kerr et al. detailed the distinctive sequential changes in cell structure that take place during the dying process in healthy tissues, normal development, tumour regression, atrophy, and involution[4].

APOPTOSIS IS PART OF DEVELOPMENT

A common feature of development in many species is programmed cell death. Apoptosis can sometimes take place in highly predictable ways during development. For example, as the worm C. elegans grows from a single cell to an adult, cells will undergo apoptosis. Additionally, it's important for human growth. As we saw in the introduction, for instance, when you were an embryo, the beginning of your hand

was a tissue block that resembled a paddle. Figure 1 illustrates how the cells between the developing fingers "carved" the block into fingers by undergoing apoptosis[5].

Less apoptosis leads to increased webbing between the digits in all kinds of vertebrate species with digits that resemble fingers or toes. Apoptosis may occasionally be imperfect if a minor error occurs during the development of a finger or toe (leading, for instance, to fused toes). Other instances of apoptosis during normal development include the removal of surplus neurons during the "wiring" of neural circuits in the brain and the loss of a tadpole's tail as it transforms into a frog[5,6].

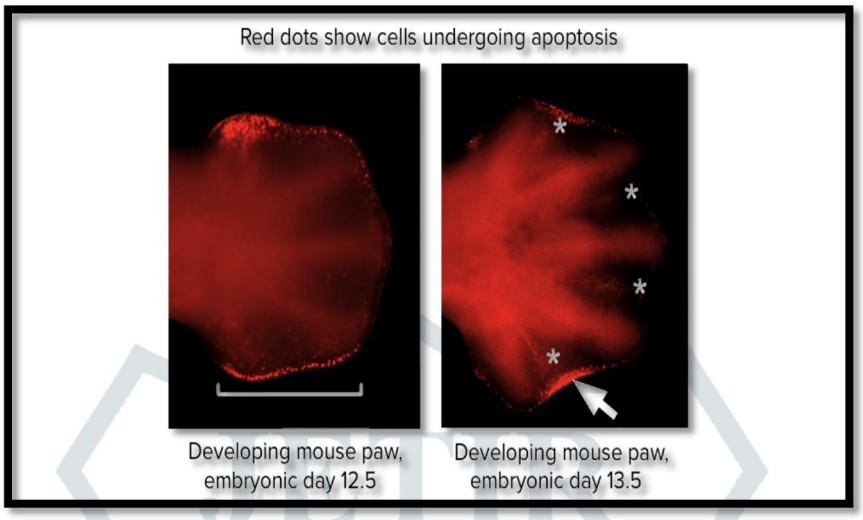


Figure:1. Image modified from Farin et al. 22squared, CC BY 4.0, "Duplication of digit 4 is preceded by decreased apoptosis and increased chondrogenesis in the posterior limb mesenchyme."

APOPTOSIS CAN ELIMINATE INFECTED CELLS

In rare circumstances, if a cell survives, it could endanger the rest of the body. When a cell's DNA is harmed, it usually recognises the harm and tries to repair it. The cell will typically initiate apoptosis if the damage is beyond repair, guaranteeing that it won't transmit its damaged DNA to other cells. As seen in figure 2, cells that have DNA damage but do not undergo apoptosis may be headed toward malignancy.

Immune cells occasionally find pre-cancerous cells that have evaded internal apoptosis cues and attempt to initiate apoptosis through an external signalling channel.

However, effective cancer cells are able to avoid the internal and exterior stimuli that would often cause apoptosis. This enables them to proliferate uncontrollably and develop mutations (changes in their DNA)[3].

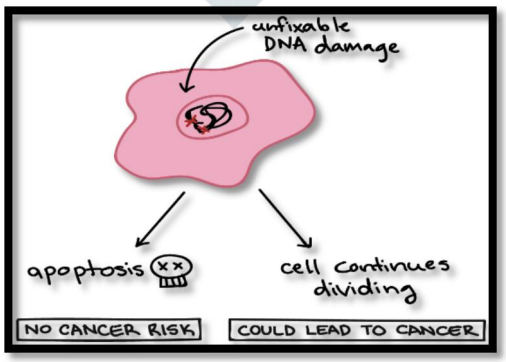


Figure :2

APOPTOSIS IS KEY TO IMMUNE FUNCTION

In addition, apoptosis is crucial for the growth and upkeep of a strong immune system. B and T cells, which are immune cells that bind particular molecules, are initially examined to check if they have any reactions to the body's own "self" substances. Apoptosis immediately kills any such cells. In the event that this procedure is unsuccessful, the body may produce self-reactive cells that might assault tissues and result in autoimmune diseases[3].

Additionally, apoptosis is crucial in enabling the immune system to stop responding to a pathogen. When a pathogen is found, the immune cells that recognise it divide rapidly, greatly increasing in number with the intention of eradicating the pathogen. Large numbers of pathogen-specific immune cells must be eliminated by apoptosis in order to keep the immune system's homeostasis (balance) after the pathogen has been eliminated from the body[3,7].

APOPTOSIS ASSAY'S

The scope of this page does not allow for a comprehensive overview of all approaches and assays for detecting apoptosis. However, a few of the tests that are used the most frequently are mentioned and briefly defined. According to methodology, apoptosis tests can be divided into six main classes. A subset of the assays that are now accessible in each area is listed and briefly reviewed.

1. Cytomorphological changes
2. Fragmentation of DNA
3. Identifying Caspases, Cleaved Substances, Regulators, and Inhibitors
4. Membrane Changes
5. The Identification of Apoptosis in Complete Mounts
6. Tests for mitochondria[8].

CYTOMORPHOLOGICAL ALTERATIONS

The nuclear and cytoplasmic condensation that takes place during apoptosis is what this methodology is based on. With this method, the cellular and tissue features are preserved, and surveys of vast tissue regions are clear benefits. Smaller apoptotic entities, on the other hand, won't be seen, and healthy cells with big, dense intracellular granules could be misinterpreted for apoptotic cells or other detritus, as seen in figure 3.

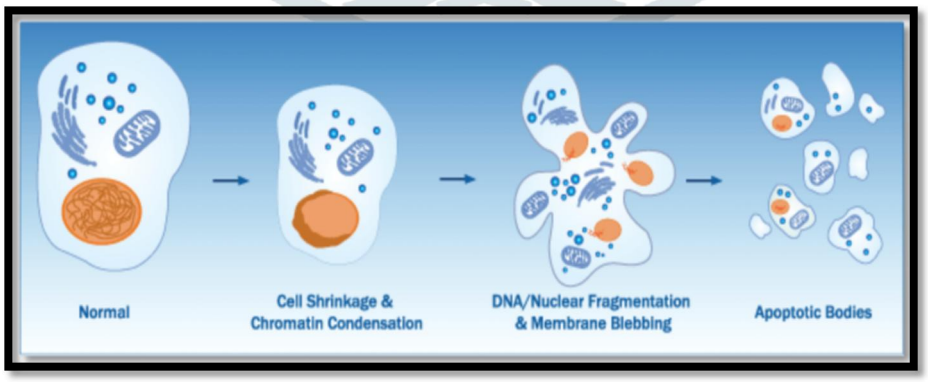


Figure: 3

With this technique, a single apoptotic cell can be found; further verification using other techniques may be required. A large amount of apoptosis may occur in some tissues before it is histologically evident because the morphological events of apoptosis occur swiftly and the fragments are phagocytized quickly. Additionally, because this approach only picks up on later apoptosis events, it cannot pick up on cells that have already undergone apoptosis [8,9].

DNA FRAGMENTATION

The endonuclease cleavage products of apoptosis are visible using the DNA laddering technique. In this test, DNA is extracted from a lysed cell homogenate and electrophoresed on an agarose gel. This produces a distinctive "DNA ladder" with each band in the ladder being separated by roughly 180 base pairs in size.

By enzymatically end-labelling the DNA strand breaks, the TUNEL (Terminal dUTP Nick End-Labelling) approach is utilised to assess the endonuclease cleavage products. The 3′end of the DNA fragments are added with tagged UTP using terminal transferase. In order to enable detection by light microscopy, fluorescence microscopy, or flow cytometry, the dUTP can subsequently be tagged with a number of probes. For tissues and cell cultures with high numbers of apoptotic cells per tissue mass or volume, respectively, DNA fragmentation technology is useful. It is simple to apply, has a sensitivity of 1X 10[6] cells, and is effective. On the other hand, it is not advised in situations where there are few apoptotic cells [8,9].

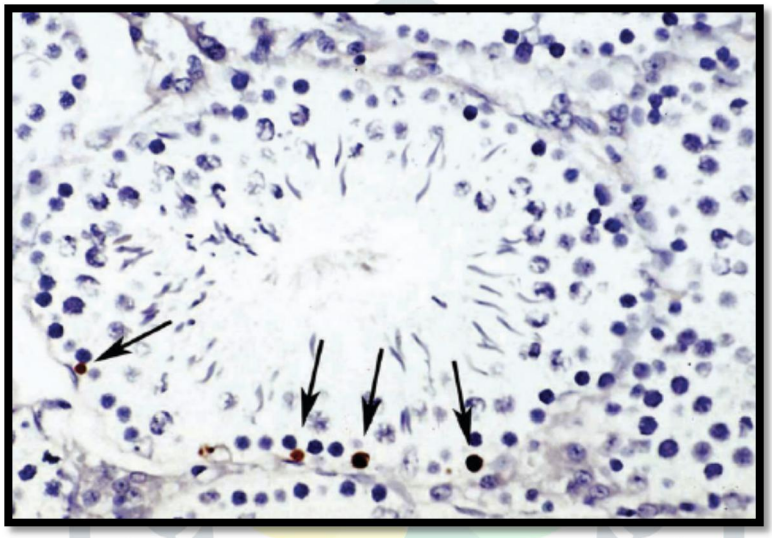
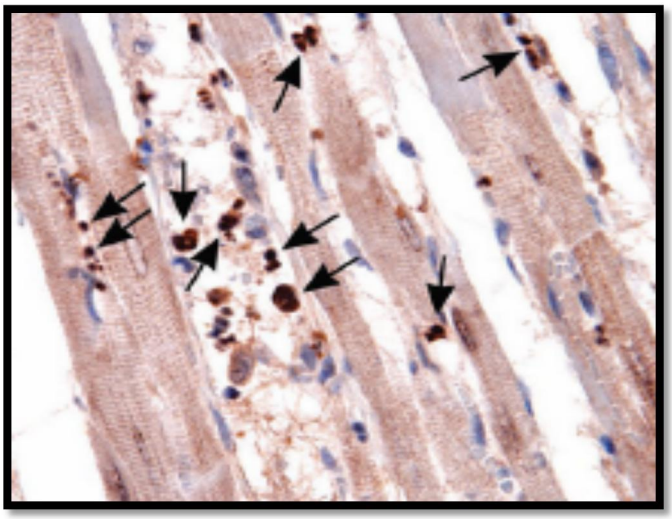


Figure: 4

DETECTION OF CASPASES, CLEAVED SUBSTRATES, REGULATORS, AND INHIBITORS

Using different kinds of assays for caspase activity, it is possible to identify more than 13 known caspases (procaspases or active cysteine caspases). Additionally, there are immunohistochemical techniques that can identify cell changes like phosphorylated histones and cleaved substrates like PARP (Figure 14A). Additionally, active caspases within cells can be marked with fluorescently attached caspase inhibitors.



Numerous techniques, such as western blot, immunological precipitation, and immunohistochemistry, can be used to identify caspase activation. Both procaspases and active caspases can be treated with polyclonal and monoclonal antibodies.

Real-time PCR is used in the relatively new technique known as the "apoptosis PCR microarray" to characterise the expression of at least 112 apoptosis-related genes. These PCR microarrays are made to analyse the gene expression patterns of important ligands, receptors, intracellular modulators, and transcription factors involved in the control of programmed cell death. Using this technology, genes involved in anti-apoptosis can also be evaluated[10].

MEMBRANE ALTERATIONS

Using Annexin V, apoptotic cells' externalised phosphatidylserine residues on the outer plasma membrane can be found in tissues, embryos, or cultured cells. Fluorescent microscopy can be used to see the apoptotic cells after they have been coupled with FITC-labeled Annexin V. The benefits are confirmation of initiator caspase activity and sensitivity (can identify a single apoptotic cell). The drawback is that necrotic cells' membranes are also marked.

Necrotic cells will stain with particular membrane-impermeant nucleic acid dyes like propidium iodide and trypan blue because loss of membrane integrity is a pathognomonic hallmark of necrotic cell death. The absence of these dyes can also show that apoptotic cells' membranes are intact[11].

DETECTION OF APOPTOSIS IN WHOLE MOUNTS

Embryos or tissue whole mounts can also be used to see apoptosis using dyes like acridine orange (AO), Nile blue sulphate (NBS), and neutral red. These dyes are concentrated in regions with significant lysosomal and phagocytic activity because they are acidophilic.

NBS and NR do not enter thick tissues and can be lost during sectioning preparation, but AO is poisonous and mutagenic, quenches quickly under ordinary conditions, and does not. Another dye that functions similarly is Lyso-Tracker Red, which can be used in conjunction with laser confocal microscopy to produce 3-dimensional images of apoptotic cells[12].

MITOCHONDRIAL ASSAYS

Cytochrome c release and mitochondrial assays make it possible to spot changes in the intrinsic pathway's early stages. Utilizing sub-micron thin optical slices made by laser scanning confocal microscopy (LSCM), it is possible to track different mitochondrial processes in living cells throughout time in intact single cells. This approach allows for the simultaneous monitoring of mitochondrial permeability transition (MPT), depolarization of the inner mitochondrial membrane, Ca2+ fluxes, mitochondrial redox state, and reactive oxygen species. The fundamental drawback of this technology is that the mitochondrial parameters it tracks can also happen during necrosis [13,14].

THE APOPTOSIS PROTEINS

Numerous elements, most of which are proteins, have been found to be crucial in apoptosis. The p53 gene, the caspases, the amyloid-B peptide, the Bcl-2 family of proteins, and the heat shock proteins are the most significant[15].

CASPASES

A family of aspartyl-specific cysteine proteases, the caspases. "Cysteine-dependent aspartate-specific proteases" is the name of the acronym. Two identical giant subunits and two identical small subunits make up each active caspase, which is a tetramer. "Death effector domains" (DED) or "caspase recruitment domains" may be present (CARD). These domains allow the active caspases to bind to substances both within and outside of the cell.

The caspase 8 or caspase 9 enzymes are activated to begin the death-receptor-induced apoptosis pathway. In turn, caspase-9 activates caspase-3 and caspase -7 virtually simultaneously, which in turn triggers the activation of more caspases, creating a cascade. This completes the recruitment of all necessary

caspases in the death-receptor-activation triggered apoptosis cascade. Caspase-3, in particular, activates both caspase-2 and caspase-6, whereas caspase-6 activates caspases 8 and 10.

Finally, it should be noted that mice that express a dominant negative mutant of caspase-1 and caspase-1 defective mice are protected against ischemia-induced brain injury. These mice may have a role in the processing of pro-inflammatory cytokines during the immunological response. Therefore, it is hypothesised that, at least occasionally, suppressing caspase-1 may aid in preventing apoptosis [16,17.]

AMYLOID-B PEPTIDE (ABETA)

Major neuropathological indicators of Alzheimer's disease are the abeta and tau proteins. Amyloid-B peptide (Abeta), a protein generated from the amyloid-B precursor protein, is one of the most significant ones (APP). Abeta is thought to be the primary cause of Alzheimer's disease pathogenesis because it triggers neuronal death and has a significant part in the illness's pathogenesis. Results on primary mouse cortical cells really show that Abeta causes neurons in neurosphere cultures to die through a caspase-independent route [16,18,19.]

Bcl-2 family

The Bcl-2 family, whose name is an acronym for the B-cell lymphoma/leukemia-2 gene, has about 15 members, some of which are pro- and some of which are anti-apoptotic. They are encoded by a gene on chromosomal segment 18q21 that was first discovered due to its role in B-cell malignancies [17]. The Bcl-2 family of proteins may typically be recognised by the presence of certain sequence patterns known as Bcl-2 homology domains (Table 1). (BH1 to BH4). All members of the Bcl-2 family have at least one Bcl-2 homology domain, and those that resemble Bcl-2 the most have all four BH domains. While pro-apoptotic Bcl-2 family members have four BH domains, the majority of anti-apoptotic members only have the BH1 and BH2 domains [16,12,20].

Table 1

Anti- apoptotic	Pro- apoptotic	
	BH3 domain	*multidomain*
Bcl-2	Bid	Bax
Bcl-xL	Bad	Bak
Bcl-w	Bik	Bok/Mtd [42]
A1/Bfl1 [41]	Hrk	Bcl-xS [23]
Boo/Diva [38]	Bim/Bod	
Nr13 [12]	Bnip3 [11]	
Mcl-1* [29]	Nix [11]	
	Noxa	
	Puma	
	Spike [30]	
Notes :		
* Mcl-1 = Myeloid cell leukemia 1 protein		

On the other hand, all members of the pro-apoptotic family have the BH3 domain, which is essential to their pro-apoptotic action, but not the BH4 domain. The pro-apoptotic members of the Bcl-2 family of proteins are divided into two subgroups: (a) the Bax subfamily members, which are made up of the proteins Bax, Bak, and Bok and contain combinations of the domains BH1, BH2, and BH3, and (b) the BH3-only proteins, which include the proteins Bid, Bim, Bik, Bad, Hrk, Noxa, Puma, Blk, BNIP [21].

P53 gene

The tumour suppressor gene p53 is a crucial component of the apoptotic process. It codes for a protein that is a member of the P53, P63, and P73 protein families. They can all cause apoptosis and share 60–70% of the amino acids in the DNA-binding region. P53 is activated by a wide range of factors,

including DNA damage, ionising radiation, UV irradiation, hypoxia, heat shock, oncogene activation, and cytotoxic medicines [22].

P53 sets off reactions such as differentiation, DNA repair, cell cycle arrest, and apoptosis. Some Bcl-2 family genes, such as those for Bax and numerous BH3-only proteins, including as Bid, Noxa, and PUMA, are stimulated. Additionally, P53 has the ability to bind to one or more anti-apoptotic mitochondrial proteins, such as Bcl-XL, preventing the development of the Bax/Bak mitochondrial pore and, consequently, the release of cytochrome c. Finally, P53 can activate apoptosis by suppressing the transcription of some genes that don't have consensus binding site motifs [23].

HEAT SHOCK PROTEIN

It is thought that heat-shock proteins including Hsp70, Hsp27, and Hsp90 are responsible for preventing apoptosis. In specifically, the Hsp70 protein interacts with Apaf-1 to directly block caspase activation. The release of cytochrome c from the mitochondria, which is necessary for the creation of the apoptosome, can be inhibited by the heat shock proteins.

There are two probable interactions for Hsp27: either it interferes directly with the release of cytochrome c or indirectly through an upstream signal, or it interacts with cytochrome c when it is released by the mitochondria. The anti-apoptotic function of the heat-shock proteins is the subject of numerous more recent research studies. By primarily inhibiting Bax activation and hence blocking the release of pro-apoptotic molecules from mitochondria, the heat shock protein Hsp70 inhibits apoptosis[24].

NEURONAL THREAD PROTEIN (AD7c-NTP)

According to research, AD7c-NTP protein was overexpressed early in the course of the disease in patients with Alzheimer's disease. Patients with early or moderately severe AD have elevated amounts of AD7c-NTP in both their CSF and urine, suggesting that the compound could serve as a biochemical indicator of the condition. A 41kD membrane protein called AD7c-NTP causes cell death through apoptosis and compromised mitochondrial activity. Moreover, AD7c-NTP expression raises the levels of apoptosis-related proteins such p53 and Fas/CD95[25].

CONCLUSION:

In conclusion, apoptosis is a long-standing evolutionary mechanism that has very comparable mechanisms in wildly dissimilar systemic groups. The molecular mechanism of action or activation of many of the important apoptotic proteins that are activated or inactivated by the apoptotic pathways have been found, but further study is needed to completely understand this process.

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