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Morphologic and Biochemical Criteria of Cell Death: Apoptosis and Necrosis

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ABSTRACT

Two hundred billion cells are dying every day. They need to be renewed. Dying or dead cell are involved a series of apoptotic events or eventually displays necrosis. Four basic modes of cell death have been identified. Apoptosis purges no-longer useful cells via phagocytosis, eliminating old cells, and/or stimulate tissue regeneration. Apoptotic cells are reflecting a suicidal event, whereas necrosis is playing role as a homicide. Active programed processes are characterized as being an autonomous happening. The process concerns caspases, which are inhibitors of apoptosis. According to morphologic and biochemical criteria, caspases contribute to membrane blebbing, intra-cytoplasmic alterations and formation of cysteine-rich domains. They are implicated in the translocation of nuclear proteins, play role in the control of the calcium efflux, regulate membrane permeability and inhibit the translocation of mitochondrial membranes associated to apoptosis. DNA damages, nuclear fragmentation leading to the formation of apoptotic bodies, autophagic cell death, necrosis and other forms of cellular death are instrumental in the reliability of apoptotic events leading to cells death. Apoptosis appears to be crucial in morphogenesis, and actually guide a series of measurable biochemical features involved in tissue renewal and regeneration.

INTRODUCTION

Apoptosis

In 2009, the Nomenclature Committee on cell death proposed a set of recommendations for the definition of distinct cell death morphologies including 'apoptosis', 'necrosis' and mitotic catastrophe [1]. A functional classification of cell death sub routes includes extrinsic apoptosis, caspase-dependent or -independent intrinsic apoptosis, regulated necrosis, autophagic cell death and mitotic catastrophe. Functional classification of cell death modalities have established links with anoikis, autophagic cell death, caspase-dependent intrinsic apoptosis, caspase-independent intrinsic apoptosis, cornification, entosis, extrinsic apoptosis by death receptors, mitotic catastrophe, necroptosis, netosis and pyroptosis [1] **(Figures 1-3)**.

Apoptosis and necrosis comprise different modes of cell death, respectively implicated in cell withdrawal and recovery. Different types of cell death have been described according to morphologic and biochemical criteria [2]. Four basic modes of cell death have been identified following the biomedical literature, together with ad-hoc variants adapted to different situations [3].

These various situations include 1) apoptosis and 2) senescent death (SD). 3) Necrosis and 4) stress-induced cell death (SICD), that are actual procedures identifying cell death. Scavengers engulf SICD and necrosis. Four basic cells death types and their variant-derived are involved in purging no-longer useful cells from the host tissue or organ. These processes are followed by regeneration, wound healing, and also by scar formation. Biochemical methods have many advantages over morphological techniques **(Figure 3)**.

Apoptosis purge no-longer useful cells from a tissue via phagocytosis, using scavengers and including macrophages. A crude estimation suggests that in the human body 60 billion cells die every day. The human body needs to yield 60-86.4 billion new cells per day, compensating the cell loss. Cell renewal implicates that ~200 billion of cells/per day die and therefore have to be renewed. Signals from apoptotic cells to scavengers indicates that apoptosis is a suicidal event, whereas necrosis is an homicide.

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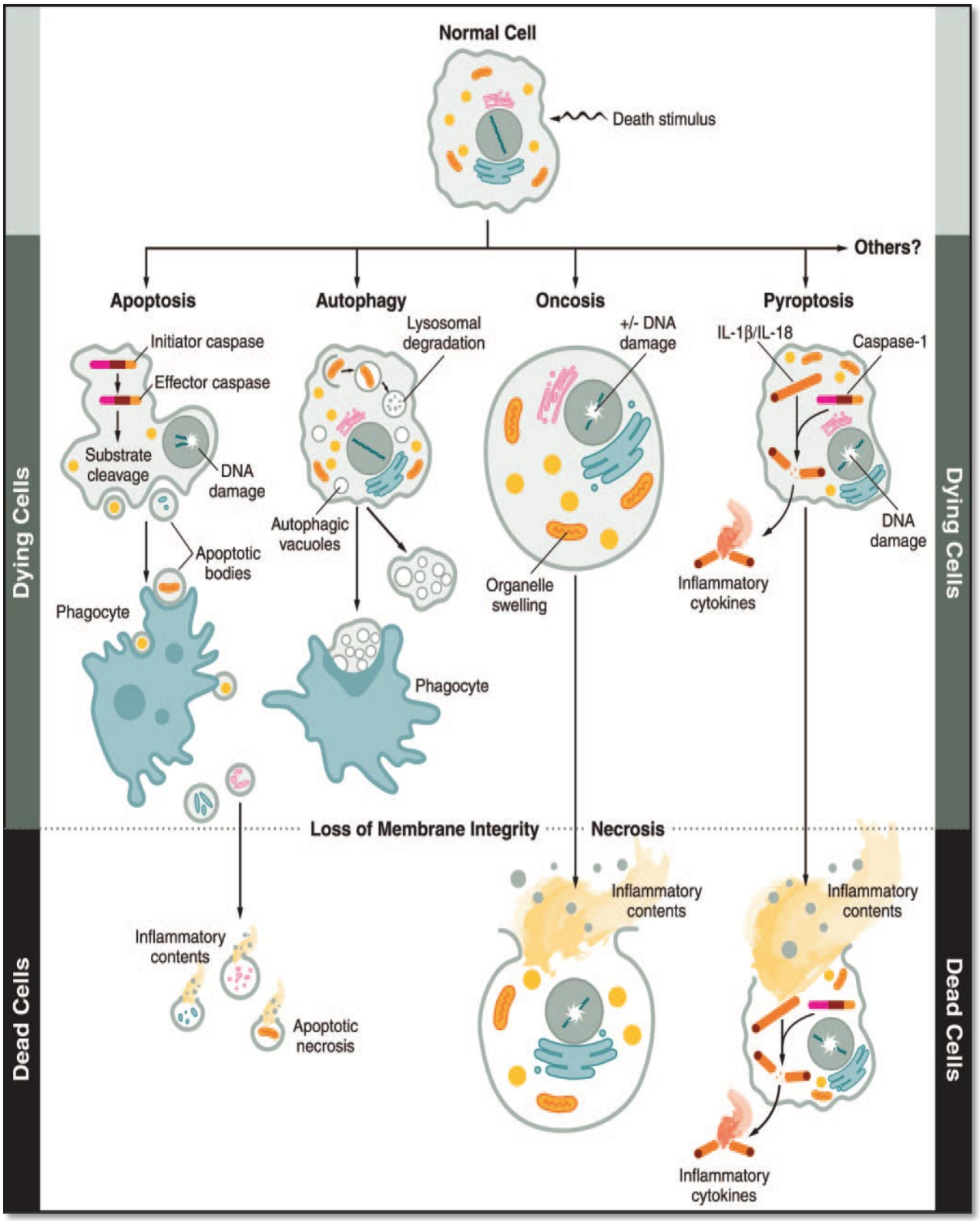

Figure 1. Pathways leading to cell death.

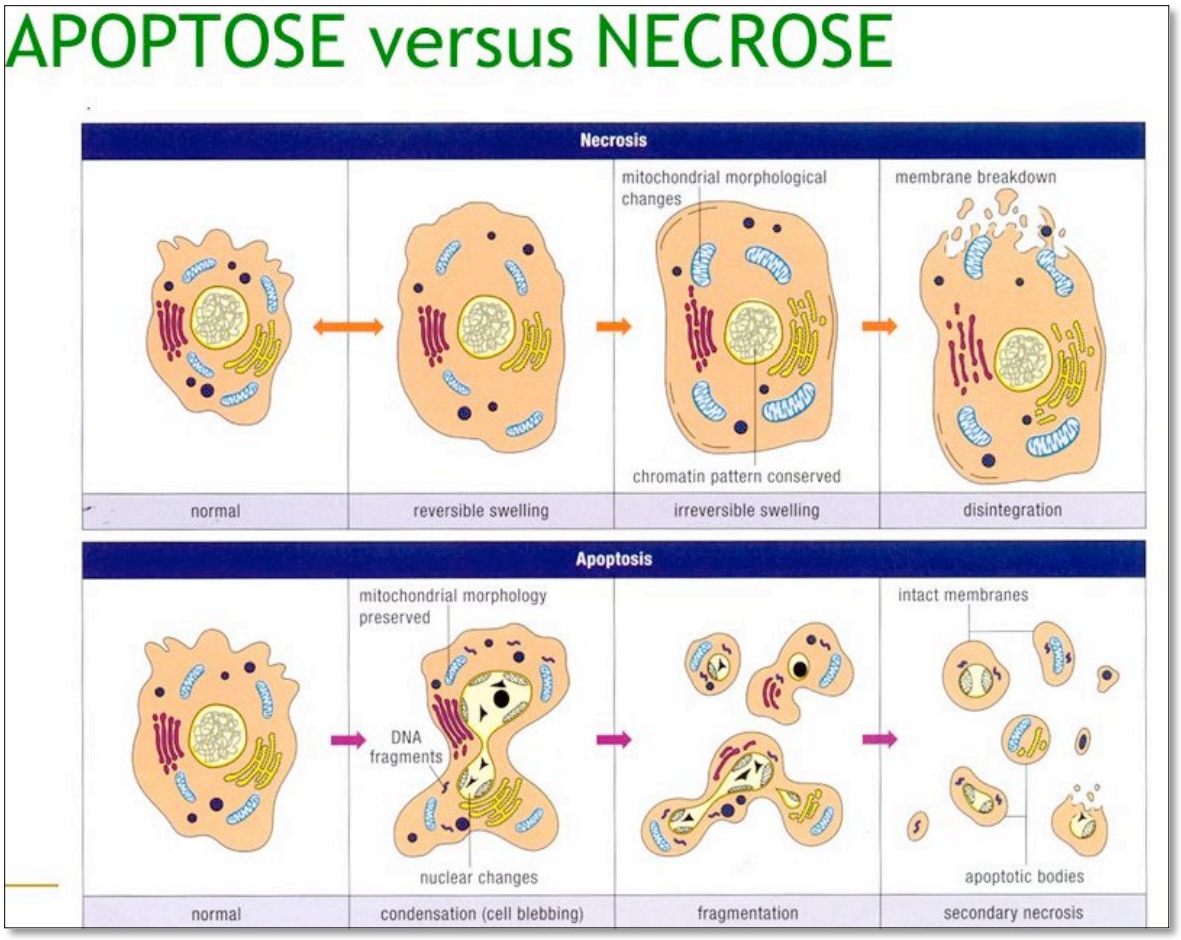

Figure 2. Apoptosis versus Necrosis.

Mild inflammation	>pulp healing	>pulp regeneration
Severe inflammation	> Dendritic cells Histiocytes/macrophages T-lymphocytes Latent or dormant pulp stem cell progenitors	> pulp repair
	Necrosis	Loss of protein functions & plasma membrane integrity. Coagulation necrosis Pyroptotic cell death
	Apoptotic caspases Inflammatory caspases	Programed necrosis-like cell death
	Nemosis	Pro-inflammatory cytokines and cyclooxygenase-2. Does Human Dental Pulp Fibroblasts (HDPFs) leads to spheroid formation: release of cyclooxygenase-2 and Prostaglandin E2

Figure 3. Mild to severe inflammation leads to pulp healing or to pulp regeneration.
Schematic representation of events contributing to necrosis, apoptosis and nemosis

They are both irreconcilable to each other. In contrast with apoptosis, necrosis eliminate plethoral cells and consequently does not trigger regeneration [4].

Cell death with some of the features of apoptosis may result from a variety of molecular pathways. Cell death follows either apoptosis or necrosis. Apoptosis is described as an active, programmed process of autonomous cellular that avoids inflammation. Necrosis has been characterized as a passive, accidental cell death resulting from environmental perturbations with uncontrolled release of inflammatory cellular contents. Other types of cellular death are necroptose, self-digestion, mitotic catastrophe, anoikis, cannibalistic cell death, cornification or keratinization, pyroptosis, parthanatose and ferroptose **(Figure 4)**.

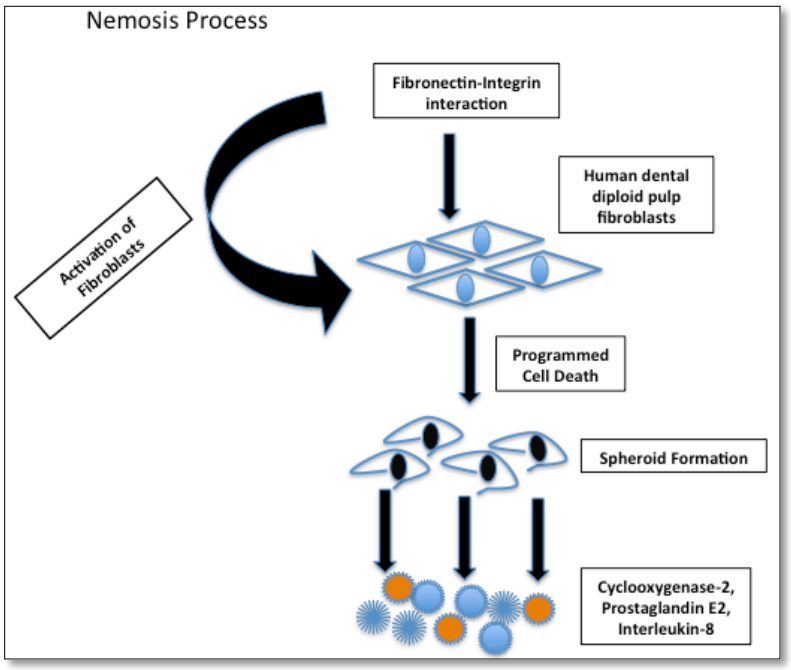

Figure 4. Nemosis process leading to the formation of spheroid and the expression of cyclooxygenase-2, prostaglandin E2, and interleukin-8.

Control of proliferation or cell cycle implicates caspases, Bcl-2, inhibitor of apoptosis (IAPs), FAK, membrane blebbing (ROCK1), gelsoline, lamines A, B, C, β-caténine, γ-caténine, topoisomerase I and II. Extrinsic pathway of apoptosis involves death ligands (Fas L, TNF, TWEAK, TRAIL), activation of death receptors (intracytoplasmic death domain and cysteine-rich domain) and intrinsic apoptosis (DNA damage, ROS, stress response), Anti-apoptotic (BCL-2) anti- and pro-apoptotic events, pro-apoptotic (Bax) seems to play role in apoptotic events. Anti-apoptotic action of the Bcl-2 family inhibits the translocation of some nuclear proteins in the nucleus, control the calcium efflux from the rough endothelium, whereas antagonists of ROS control the permeability or inhibit the translocation of mitochondrial membranes, which are also implicated in this phenomenon.

Apoptotic fragmentation

Proposed by Kerr et al. [5], apoptosis was characterized by nuclear and cytoplasmic condensation and cellular fragmentation into membrane-bound fragments (also named apoptotic bodies). Apoptosis is accompanied by the rounding-up of the cell, retraction of pseudopodes, reduction of cellular volume (pyknosis), chromatin condensation, nuclear fragmentation (karyorrhexis), little or no ultrastructural modification of cytoplasmic organelles, plasma membrane blebbing and engulfment by resident phagocytes (*in vivo*). It is worth noting that it is not correct to assume that "programmed cell death" and "apoptosis" are synonyms because cell death can manifest non-apoptotic features.

Specific biochemical analyses (such as DNA ladders) should not be employed as an exclusive means to define apoptosis, because this type of cell death may occur without oligonucleosomal DNA fragmentation. The presence of caspases or the cleavage product of their substrates is not sufficient to define apoptosis. The measurement of DNA fragmentation and/or caspase activation helps to better define the type of cell death, through the 'intrinsic' or the 'extrinsic' pathway, with or without the contribution of mitochondria.

Cell death is frequently considered to be 'caspase-dependent', suppressed by caspase inhibitors. Different forms of apoptosis have been identified including apoptosis, necroptosis, anoikis, pyronecrosis, death with autophagy or

mitotic catastrophe, pyroptosis, cornification or keratinization, entose (self-cannibalism), wallerian degeneracy, excitotoxicity, netose, parthanose and ferroptose [6].

Autophagy and autophagic cell death

Degraded in phagosomes by caspases or cysteine-dependent aspartate specific proteases. Interleukin-1β xov-1. Initiator caspases (caspase-2, -8, -9 and -10) contain a small prodomain. Activated effector caspases selectively cleave a restricted number of target proteins. One of the morphological and biochemical associated with apoptosis, the DNA ladder produced by cleavage of genomic DNA between nucleosomes generates fragments with length corresponding approximately to 180 base pairs [7].

Phosphatidylserine (PS) exposure is another caspase-dependent process is actively localized on the inner leaflet of the plasma membrane. Cytoplasmic and nuclear condensation, chromatin cleavage, formation of apoptotic bodies, maintenance of an intact plasma membrane and exposure of surface molecules target cell corpses for phagocytosis. Caspases -2, -6, -7, -8, -9 and -10 mediate the process of apoptotic cell death.

Sequestration of cytoplasmic material within autophagosomes leads to bulk degradation by lysosomes. Autophagosomes are formed by two membranes and contain degenerating cytoplasmic organelles or cytosol allowing distinguishing by transmission electron microscopy between autophagosomes and other types of vesicles such as endosomes, lysosomes or apoptotic blebs.

Caspase-9, caspase-3 and caspase-7 have distinct roles during intrinsic apoptosis [8]. Apoptosis is a form of programmed cell death that is regulated by the Bcl-2 family and caspase family of proteins. Specific inhibition of caspase-9 allows the efficient release of cytochrome c, but blocks change in mitochondrial morphology and ROS production. Caspase-3-deficient MEFs are not resistant to intrinsic cell death. Altogether, the data suggest that caspase-9 is required for morphological change and ROS production by cleaving and activating Bid into tBid. After activation by caspase-9, caspase-3 inhibits ROS production and is required for efficient execution of apoptosis while effector caspase-7 is required for apoptotic cell detachment.

Signalization implicates the release of a number of signal molecules, implicated in cellular damages and loss of adhesion. They are also involved in the release of a number of signal molecules, including receptors such as Fas/TNF, and glucocorticoids.

Regulation of intracellular mediators leads to the release of effectors (proteins rich in cysteine, and nucleases leading to survival). Molecules such as P53, Bcl2, kinases, phosphatases, ceramides, proteins regulating the cell cycle and transcription factors guide to deficits in growth factors (NGF, IL-2). In summary, intracellular mediators regulate debits (death, proteolysis, DNA degradation and destruction). The morphological hallmarks of apoptosis include DNA fragmentation and membrane blebbing (**Figures 1, 2, 5 and 6**).

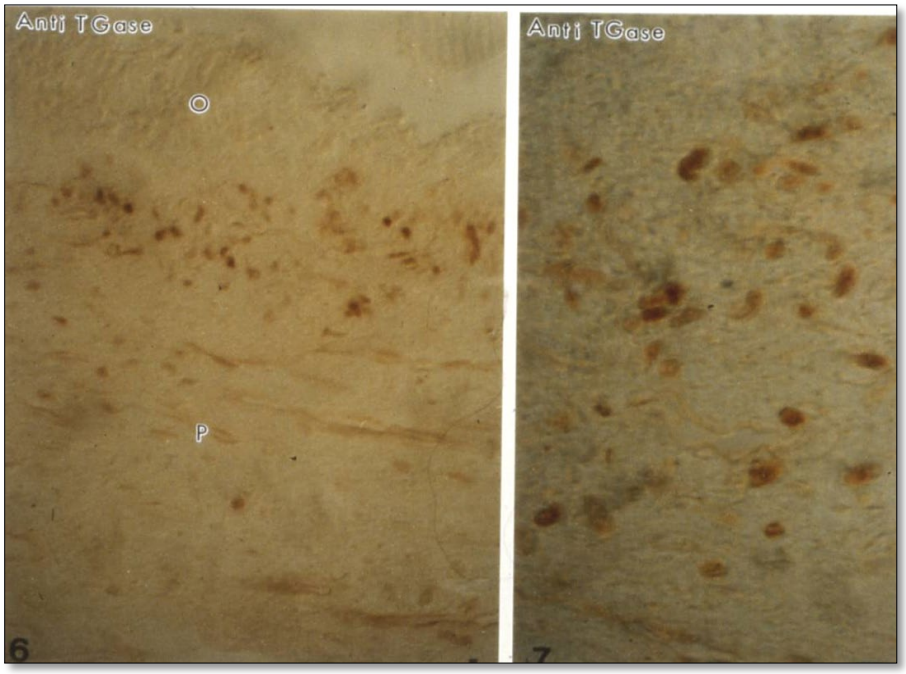

Figure 5. Immunostaining of apoptotic cells stained with anti-transglutaminase (Anti-TGase).
Odontoblasts (O) are unstained, as well as pulp cells (P)

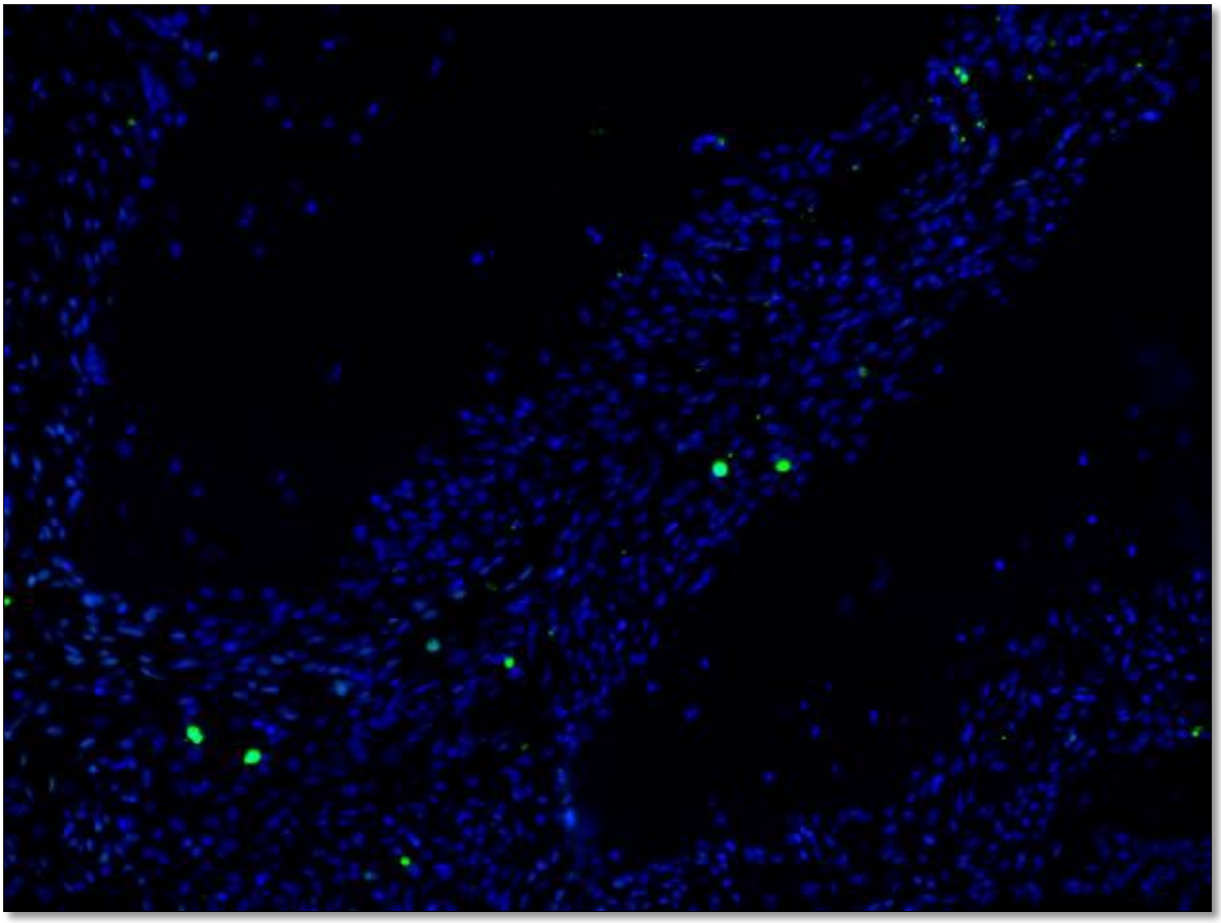

Figure 6. 28 days after BIO implantation, apoptotic cells are stained in the root and in the apical cell-rich zone.

Extrinsic pathway of apoptosis: Death receptors are members of the tumor necrosis factor (TNF) family that include TNF-receptor-1 (TNFR1), CD95, death receptor 3, TNF-related apoptosis-inducing ligand receptor-1, and TRAIL-R2.

Intrinsic pathway of apoptosis: Depends on factors released from mitochondria. Caspase deficient mouse phenotypes.

Deregulation of caspases underlines human diseases including cancer and inflammatory disorders.

Necrosis

Designate non-apoptotic accidental cell death. In the absence of phagocytosis, apoptotic bodies may lose their integrity and process to secondary or apoptotic necrosis.

This allows discriminating between programmed and fortuitous forms of necrosis, characterized by a gain in cell volume (oncosis), swelling of organelles, plasma membrane rupture and loss of intracellular contents **(Figure 1)**. In the presence of caspase inhibitors, necroptosis or necrosis constitute a type of cell death discriminating between programmed and fortuitous forms of necrosis.

Mitochondrial alterations, lysosomal changes, nuclear changes, lipid degradation, increase in the cytosolic concentration of calcium (Ca^{2+}) result in mitochondrial overload and activation of caspase proteases. A crucial role for the serine/threonine kinase RIP1 has been demonstrated. Necrotic cell death is identified by the absence of apoptotic or autophagic markers, especially when the cells undergo early plasma membrane permeabilization **(Figures 1 and 2)**.

Caspases are a family of genes (endoproteases) maintaining homeostasis through regulating cell death and inflammation (6). These endoproteases hydrolyze peptide bounds in a reaction that depends on catalytic cysteine residues. Caspase-3, -6, -7, -8 and -9 contains a caspase-recruitment domain (CARD), that displays a death effector domain.

Cornification

Occurs in the epidermis, morphologically and biochemically distinct from apoptosis. Often referred to as “keratinization” or cornified envelope formation, it is considered as a terminal differentiation program similar to other nucleated

tissues. Activation of caspases cornification should be regarded as a bona fide cell death program. Obtained by the cross linking enzymes (transglutaminase types 1, 3 and 5) acting on several substrates (loricrine, involucrine and SP100), the cornified envelope proteins and proteases are required for impermeability and desquamation, respectively.

Pyroptosis

Proinflammatory pathway resulting from caspase-1 activity leading to membrane breakdown is leading to proinflammatory cytokine processing. It is characterized by pore formation in the plasma membrane, cell swelling and membrane disruption [7]. Pyroptosis has been defined by the following 4 criteria: 1) Programmed by an inflammatory caspase activation, 2) Pore formation in the plasma membrane, 3) DNA damage with terminal deoxynucleotidyl transferase dUTP nick-end labeling positivity at a lower intensity than apoptosis, and 4) ADP-ribose polymerase activation after the pyroptosis-mediated DNA damage. A critical role for the caspase-1-inflammasome suggests that the inflammasome plays a crucial role in regulating hyperhomocysteinemia-induced endothelial dysfunction.

Caspases 3, 6, and 7 are executioner caspases with prodomains in the NH2-terminus that cleaves substrates essential for cellular homeostasis. Both executioner and initiator caspases contribute to apoptotic cell death. Necrosis was once recognized as an accidental or physical cell death induced by physiochemical stress [8].

OTHER TYPES OF CELL DEATHS

A variety of cell death has been identified including:

Atypical cell death modalities, mitotic catastrophe

Which can be accompanied by morphological alterations including micronucleation. Mitotic catastrophe can lead either to apoptotic morphology or to necrosis.

Anoikis

This form is induced by the loss of attachment to the substrate or to other cells. Excitotoxicity Wallerian degeneration

Neurons or axon degenerates without affecting the main cell body of the nervous system. Neurons affected by Wallerian degeneration remain alive.

Paraptosis, Necroptose, Entose, Oncosis, Autophagy and Programmed cell death have been also identified as forms of cell death.

Table 1. Terms for describing dead and dying cells.

Term	Characteristic(s)
Programmed cell death	Dependent on genetically encoded signals or activities within the dying cell; a sequence of potentially modifiable events leading to the death of the cell.
Apoptosis	Mediated by a subset of caspases **(Figure 1)**; morphology includes nuclear and cytoplasmic condensation and formation of membrane-bound cellular fragments or apoptotic bodies; not inflammatory.
Autophagy	Degradation of cellular components within the dying cell in autophagic vacuoles; not inflammatory.
Oncosis	Prelethal pathway leading to cell death accompanied by cellular and organelle swelling and increased membrane permeability; proinflammatory.
Necrosis	Postmortem observation of dead cells that have come into equilibrium with their environment.

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