Pathology of Cell Death

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ABSTRACT:
Cell death is one of the prime events occurring in many of the pathological conditions. The causative or the etiologic agent acts at the cellular level to bring about molecular, biochemical and morphological changes. It can affect individual cell or group of cells producing changes at tissue and organ level. Not only under pathological conditions but cell death occurs under physiological conditions as well. It is highly required for the normal growth, development and maintenance of homeostasis of the body. There are various forms of cell death with each having its own mechanism and implications. Cell death is a part of periodontal disease spectrum too. The periodontal pathogens and their products can cause death of cells of the periodontium and even the immune cells there by impeding their defensive action against the disease. This article reviews the various modalities of cell death with a brief overview on cell death in periodontal diseases.

Key words: Cell death, Apoptosis, Necrosis, Proteases, Periodontitis, Microorganisms

INTRODUCTION

Cell being the structural unit of the body, normal functioning of all the tissues and organ systems in the body depends on the cell functioning in itself and in coordination with the neighboring cells in tissue or organ system to maintain a state of homeostasis. The normal cell is confined to its functions & structure by its genetic programs of metabolism, differentiation and specialization. Various pathological stimuli and even the physiologic stresses can bring about a number of physiologic and morphologic cellular adaptations. Due to these adaptations, new but altered steady states can be achieved, however with the preservation of viability of the cell & modulating its function.¹,²,³

Adaptive responses may occur in the form of hyperplasia, hypertrophy or atrophy. Inability to adapt to the stress or the stimuli produces cell injury, either reversible or irreversible, ultimately producing cell death. The type and extent of cell injury determines whether the cell and the tissue are restored to normal or the cell dies and disappears, or the residual effects persist as evidence of injury.¹,²,³,⁴,⁵

Cell injury is reversible up to a certain point. But if the stimulus persists or is severe from the beginning itself it produces irreversible cell injury, ultimately causing the cell death. Adaptation, reversible injury and cell death are considered as the stages of progressive impairment of cell’s normal function and structure. The consequences of cell injury depends on a number of factors, including the intrinsic nature of cells and tissues.
involved, in particular, their differentiating state, and their ability to divide and replicate. Cell death can result from diverse causes including ischemia, hypoxia, anoxia, infections, toxins and immune reactions.\textsuperscript{1,2,6} On the other hand it can be normal and essential part of embryogenesis, development of organs and maintenance of homeostasis and can be used intentionally as a part of cancer therapy.\textsuperscript{1,2,3}

Cell death can be classified according to its morphological appearance (which may be apoptotic, necrotic, autophagic or associated with mitosis), enzymological criteria (with and without the involvement of nucleases or of distinct classes of proteases, such as caspases, calpains, cathepsins and transglutaminases), functional aspects (programmed or accidental, physiological or pathological) or immunological characteristics (immunogenic or nonimmunogenic).\textsuperscript{4,7}

The Nomenclature Committee on Cell Death (NCCD) proposes that a cell should be considered dead when any one of the following molecular or morphological criteria is met: (1) loss of the integrity of its plasma membrane (2) complete fragmentation of cell and the nucleus discrete bodies (3) its fragments has been engulfed by an adjacent cell.\textsuperscript{4}

There are two principle patterns of cell death which are necrosis and apoptosis. While necrosis is the cell death occurring after abnormal stresses and is always pathological, apoptosis is the cell death through activation of an internally controlled suicide programme.\textsuperscript{3,4,8} Programmed cell death (PCD) is the death of a cell in any pathological format, when mediated by an intracellular program. It refers to apoptosis, autophagy and programmed necrosis. Apoptosis and programmed necrosis invariably contribute to cell death, whereas autophagy can play either pro-survival or pro-death roles. These three forms of PCD may jointly decide the fate of cells of malignant neoplasms.\textsuperscript{8}

Necrosis

Necrosis refers to the cumulative morphologic range of changes indicative of cell death in a living tissue or an organism and is caused by the progressive degradative action of hydrolytic enzymes on a cell or cells and on tissues. It results in the loss of structural integrity, and the denaturation of proteins and other macromolecules. The enzymes can be derived either from the lysosomes of dead cells themselves, in which case it is referred to as autolysis, or from the lysosomes of the leukocytes in the inflammatory reactions, the heterolysis. Due to the inability of these cells to maintain the membrane integrity, the cell contents often leak out eliciting an inflammatory reaction.\textsuperscript{1,3} Fate of necrotic cells involves their enzymatic digestion, fragmentation and phagocytosis by the adjacent inflammatory cells and in certain cases they can undergo dystrophic calcification.\textsuperscript{3,6}

Core events in necrosis include bioenergetic failure and loss of plasma membrane integrity.\textsuperscript{2} There are various pathways to necrosis depending on the cause and the site of action. These are

- Cellular energy depletion that produces loss of membrane potentials, which further leads to the opening of death channel (ASIC-Acid sensing ion chandelles) in the cytoplasmic membrane. The opening which results in colloid osmotic forces and entry of cations that causes the cytoplasmic membrane swelling and cell rupture.\textsuperscript{2,3}
- A number of proteases such as calpains, cathepsins, lysosomes and caspases have been implicated in necrosis. Most of these enzymes cause lysosomal membrane permeability (LMP), which can lead to release of lysosomal enzymes and necrotic cell death. Calpains cause calpain-mediated cleavage of the Na+/Ca\textsuperscript{2+} exchanger in the plasma membrane resulting in the sustained secondary intracellular Ca\textsuperscript{2+} overload and subsequent necrotic cell death.\textsuperscript{3,7,9}
- Mitochondrial permeability transition (mPT) is the loss of transmembrane potential of the mitochondrial inner membrane. Permeability transition (PT) pores open in the mitochondrial inner membrane in response to stimuli such as increased intracellular Ca\textsuperscript{2+}, inorganic phosphate, alkaline pH, and ROS (Reactive Oxygen Species). The persistent opening of the PT pore has been proposed to amplify apoptosis by mediating release of mitochondrial apoptogenic factors. CypD is a key regulator of this mechanism.\textsuperscript{3,10}
- Generation of ROS, can causes necrosis by DNA damage, activation of poly(ADPribose) polymerase (PARP), lipid oxidation, which can lead to the loss of integrity of both the plasma membrane and intracellular membranes such as that of lysosomes and the ER, leading to an intracellular leak of proteases or an influx of Ca\textsuperscript{2+}, breakdown of aminoacids and proteins,
and adversely affecting Ca\textsuperscript{2+} channels on the endoplasmic reticulum and plasma membrane.\textsuperscript{2,3}

- Prolonged cytosolic Ca\textsuperscript{2+} can cause mitochondrial Ca\textsuperscript{2+} overload, there by inducing calcium mediated cell necrosis by depleting ATP, activating Ca\textsuperscript{2+}-dependent proteases and mPT.\textsuperscript{3}

The advantage with necrosis is that it allows cells to actively recruit a defensive or a reparative response to regions of multicellular organisms that have sustained damage or invasion and thus, it serve as an organismal warning system of the extent of a toxic exposure.\textsuperscript{3} Necrosis can be of different types and it in part is determined by richness of lysosomal enzymes in the cell. The type of necrosis occasionally provides a clue about the underlying cause of necrosis.\textsuperscript{2}

Though necrosis was considered to be accidental cell death, recent studies suggest it to be a regulated event that contributes to development and maintenance of organismal homeostasis.\textsuperscript{4,8} This form of regulated necrosis is termed as ‘necroptosis’.\textsuperscript{4} With the discovery of role of RIP (Receptor interacting protein) kinases and PARP in necrosis this concept of programmed necrosis is gaining profundence. RIP kinases, PARP1, NADPH oxidases and calpains have been identified as potential signaling components in programmed necrosis.\textsuperscript{4,8}

### Apoptosis

Apoptosis is the type I PCD and is the major type of cell death. It is characterized by specific morphological and biochemical changes in the dying cells. The apoptotic cell shows cell shrinkage, nuclear condensation and fragmentation, dynamic membrane blebbing and loss of adhesion with adjacent cell or with extracellular matrix. Biochemical changes include chromosomal DNA cleavage, phosphatidylserine externalization and a number of intracellular substrate cleavages by specific proteolysis.\textsuperscript{4,5,8,11,12}

It is induced by two mechanisms\textsuperscript{1,2,4,5}

- Extrinsic - death receptor pathway - It is triggered by binding of Fas to Fas-L that activates caspase cascade.
- Intrinsic pathway- it occurs under the control of mitochondrial pro-enzymes.
- In both cases if a cell becomes initiated by either extracellular stimuli or intracellular signals, outer mitochondrial membranes become permeable to internal cytochrome c, which is then released into the cytosol.
- Cytochrome c recruits Apaf-1 and pro-caspase-9 to compose the apoptosome, which triggers a caspase 9/3 signaling cascade, culminating in apoptosis.
- Apoptosis can also occur through a granzyme, perforin pathway.

Accumulating evidence has shown that abnormal expression of some key regulatory factors may lead to cancer, indicating the intricate relationships between apoptosis and cancer.\textsuperscript{6} In the absence of phagocytosis, the apoptotic bodies may lose their integration and undergo necrosis and is called as ‘apoptotic necrosis’ or ‘secondary necrosis’.\textsuperscript{5}

Many insults induce apoptosis at lower doses and necrosis at higher doses. Even in response to a certain dose of death-inducing agent, features of both apoptosis and necrosis may coexist in the same cell and such condition is referred to as ‘aponecrosis’.\textsuperscript{3,12}

- mPT may have opposite roles in inducing necrosis or apoptosis. While Bax and Bak function on the mitochondrial outer membrane to mediate the release of apoptogenic factors into the cytosol during apoptosis, CypD controls the PT pores on the inner membrane during necrosis.\textsuperscript{1,3,12}
- Increased cytosolic Ca\textsuperscript{2+} can initiate either apoptosis or necrosis. Low to moderate Ca\textsuperscript{2+} (200- 400 nM) triggers apoptosis, higher concentration of Ca\textsuperscript{2+} (>1 \mu M) is associated with necrosis. It is the reason why the Ca\textsuperscript{2+} released from the ER is mostly apoptotic, whereas Ca\textsuperscript{2+} influx through the plasma membrane is associated with necrosis.\textsuperscript{3,12}
- Sphingosine, a structural component of cell membranes has been shown to cause LMP and cell death in a dose-dependent manner. Sphingosine induces partial lysosomal rupture and apoptosis at low-to-moderate concentrations (<20 \mu M), and extensive lysosomal rupture and necrosis at high concentration (>20 \mu M).\textsuperscript{3}

Based on the nuclear morphology, Leist and Jaattela have classified cell death into four subclasses.\textsuperscript{9}

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• Apoptosis- Stage II chromatin condensation into compact figures, which are often globular or crescent shaped.

• Apoptosis-like PCD - Stage I chromatin condensation - less compact chromatin condensation.

• Necrosis-like PCD - No chromatin condensation, chromatin clustering to loose speckles

• Necrosis - Cytoplasmatic swelling and cell membrane rupture.

Oncosis

Oncosis refers to any cell death characterized by marked cell swelling. It is the prelethal pathway leading to cell death accompanied by cellular and organelle swelling and increased membrane permeability that result from active enzyme catalyzed biochemical processes. Oncotic cells, however, proceed to necrosis with lysis. The released contents of necrotic cells include molecules that act as signals to promote inflammation.

Autophagy

Autophagy is the type II PCD. It involves degradation of cellular components within the dying cell in autophagic vacuoles and is not inflammatory. It begins with the formation of autophagosomes, followed by autolysosomes. Over-activation of autophagy may result in autophagic cell death. It plays a crucial role in cell homeostasis, by controlling cell differentiation, cell survival and death especially under the conditions of starvation and stress. It also has role in both progression and prevention of cancer and it provides energy required for minimal cell functioning when nutrients are scarce. Its action is highly regulated by some autophagy-related genes (ATGs) and a number of pathways involving Beclin-1, Bcl-2, Class III and I PI3K, mTORC1/C2 and p53.

Pyroptosis

Pyroptosis is the proinflammatory pathway resulting from caspase-1 activity leading to membrane breakdown and proinflammatory cytokine processing by formation of a supramolecular cytoplasmic complex called the pyroptosome. It plays a significant role in local and systemic inflammatory reaction by release of IL-1β and IL-18. It is triggered by various pathological stimuli, such as stroke, heart attack or cancer, and is crucial for controlling microbial infections. However, the microbial organisms have evolved mechanisms to inhibit pyroptosis, due to which they persist and cause disease.

Paraoptosis

Parapoptosis is characterized by progressive swelling of mitochondria, endoplasmic reticulum (ER) and cytoplasmatic vacuolation. It is mediated by mitogen-activated protein kinases and can be trigged by the TNF receptor family member and the insulin-like growth factor I receptor. Unlike apoptosis, parapoptosis is inhibited by AIP1/Alix.

Mitotic catastrophe

Mitotic catastrophe occurs due to mitotic failure caused by defective cell cycle checkpoints and development of aneuploid cells and can be accompanied by morphological alterations including micronucleation and multinucleation. It is thought to be triggered by mitochondrial membrane permeabilization and caspase activation which still is controversial. It can lead either to an apoptotic morphology or to necrosis.

Pyronecrosis

Pyronecrosis is the necrotic cell death of macrophages in infections by S. flexneri at high bacteria/macrophage ratios and associated with the release of HMGB-1, caspase-1 and IL-1β. Pyronecrosis and pyroptosis are distinguished based on the fact that the latter requires caspase-1.

Anoikis

Anoikis is a form of apoptosis induced by the loss of the attachment to the substrate or to other cells. Besides its specific form of induction, its molecular mechanisms match those activated during classical apoptosis.

Entosis

Entosis is a form of cell death in which one cell engulfs one of its live neighbors, which then dies within the phagosome. However, in rare cases, internalized cells may divide within the engulfing cell or are released. Hence, it is difficult to know whether the cell-in-cell morphology (entosis) truly represents a novel cell death modality. Entosis is not inhibited by Bcl-2 or Z-VAD-fmk.

Though the different forms of cell death show varying morphological characteristics and physiological processes; still there exists an intricate interrelationships between them.
studied using various methods such as light and electron microscopy, IF microscopy, special stains, gel electrophoresis, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL), flow cytometry, western blotting, colorimetric and fluorometric assays, immunoblotting, polarography, co-immunoprecipitation studies, genetic studies and ultrasound.4,5,10,14

Cell death and Periodontal disease

Periodontal tissue is subjected to constant irritation by oral microorganisms and their toxic products that have an adverse effect on the tissue. Periodontitis is a chronic disease caused by chronic irritation, infection and inflammation that gradually destroys the supporting tissues.11 The causative organisms and their products act on the gingival epithelial cells, periodontal ligament, alveolar bone and the immune cells to bring about the disease and its further progression. Whether these effects cause the proliferation of cells or their death needs to be understood.15,16

The pathogenic microorganisms inducing periodontal pathology act by inducing cytotoxicity of various cellular components and subsequently cell death.15 Butyrate produced by anaerobic bacteria and present in mature dental plaque of periodontitis patients can induce death of gingival epithelial cells by apoptosis and autophagy.11,17 It activates apoptosis by increasing caspase-3 activity, phosphatidylserine redistribution and bcl-2 downregulation.17 There is also release of high-mobility group box-1 (HMGB1) from the gingival cells by butyrate induced autophagic cell death. This molecule when released extracellularly acts as a group of proinflammatory cytokine.11,18 These dead cells also displays necrotic features including loss of cellular membrane integrity and cell swelling.15 Actinobacillus actinomycetemcomitans play an important role in periodontal diseases by inducing apoptosis in alveolar bone cells.19 A Fas ligand associated mechanism may be one of the method involved in apoptotic cell death.15

Periodontal pathogens by their immunosuppressive action can induce the progression of the disease. They cause depletion of immune cells at the infection site by inducing cell death, thereby providing a conducive environment for the further recruitment of other microorganisms.16,20 The functioning of PMNs is very crucial in periodontitis. These cells undergo apoptosis which is modulated by various factors. While inflammatory mediators, the GM-CSF, IFN-γ or LPS, delay the apoptosis by increasing mitochondrial stability, reducing caspase-3 activity and down regulating apoptotic gene expression, the anti-inflammatory cytokines accelerate the apoptosis of LPS activated neutrophils.15

Apoptosis of T cells and gingival epithelial cells can be caused by short chain carboxylic acids (SCCA) present in Porphyromonas gingivalis, Prevotella loescheii and Fusobacterium nucleatum.21 Toxin from the Actinobacillus actinomycetemcomitans induces apoptosis of B lymphocytes present in the periodontal tissue.22 A component of the cell wall of gram negative bacteria, lipopolysaccharide (LPS), stimulate butyric acid induced apoptosis in human peripheral blood mononuclear cells.23 Porphyromonas gingivalis can induce apoptosis and necrosis of peripheral blood mononuclear cells in periodontal diseases.16 P. gingivalis can also utilize a part of the host autophagy mechanism for their own protection from being damaged by host antibacterial system.18 F. nucleatum as well can induce the apoptotic cell death in peripheral blood mononuclear cells (PBMCs) and in polymorphonuclear cells (PMNs) through a heat-labile surface protein. Inhibitory signals mediated via programmed death-1 (PD-1) play a critical role in downmodulating immune responses and maintaining peripheral tolerance. In chronic periodontitis, the programmed death-1 can modulate the production of IFN-gamma by T cells.24

Study of cell division, synthetic capacity and apoptosis in periodontal lesions showed apoptotic cells to be consisting mainly of connective tissue cells, mainly fibroblasts with few polymorphonuclear leukocytes and other leukocytes. Fibroblast apoptosis and cell division occur within the periodontium and they are essential for the normal turnover and remodelling of these tissues. The low turnover of infiltrating leukocytes indicate that they probably arrive at this site by recruitment from distant lymph nodes, and that neither cell division nor programmed cell death significantly alter the numbers of inflammatory cells.25 Other study on dental periapical lesions implies the same as well. In periapical lesions, the apoptosis occurs predominantly in PMN. Most cells apart from PMN though exhibit synthetic activity, only epithelial cells undergo proliferation. Thus the immune cells proliferate at distant lymph nodes and travel to the periapical lesion rather than proliferating within the lesion.26
Volatile sulfur compounds, the main compounds causing halitosis has been shown to activate the apoptotic process in different tissues thus inducing the development of periodontitis. It induces apoptosis in human gingival epithelial cells by activating the mitochondrial pathway.

CONCLUSION

Though there are different forms of cell death, they can be induced by the same pathological agent and in some conditions more than one form of cell death co-exists. Cell death can be advantageous, as it has therapeutic implications too, including the treatment of cancer. With only necrosis and apoptosis in the beginning, other forms of cell death have been added to the literature. With researches on way, still much needs to be explored.

REFERENCES