OVERVIEW

APOPTOSIS IN ORAL DIAGNOSTICS: AN OVERVIEW

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ABSTRACT

Apoptosis describes the molecular and morphological processes leading to controlled cellular self destruction. In recent years, it has been investigated for its biological significance in numerous physiological processes including embryogenesis, differentiation, proliferation /homeostasis and in the regulation of immune system. Its dysfunction and deregulation seems responsible for variety of pathological conditions e.g immune deficiency, autoimmune diseases, neuro-degenerative diseases and cancer.

This communication updates molecular understanding of this natural phenomenon and its application in oral diagnostics. The present concept of signaling pathways for initiation of apoptotic characteristic changes is illustrated and the role of certain apoptotic genes identified so far is discussed. Abnormality of apoptosis and apoptotic regulatory genes during oral carcinogenesis, though conflictory, is presented. Further, clinical potential for monitoring reactions to chemo-radiotherapy is evaluated from human and animal studies and their usage as physiological markers for oral preneoplasia and squamous cell carcinoma is analyzed.

On the basis of oral cytology the application of new physical and molecular methodological techniques is outlined e.g cytophotometry, DNA cytometry in relation to molecular studies and their diagnostic and prognostic implications.

Understanding of molecular mechanisms provides development of newer therapeutic approaches in disease management and in future biomedical research. This overview updates molecular understanding of this natural phenomenon as its applications seem to have potential for managing common diseases in future.

Keywords: Apoptosis, cell death, oral mucosa

INTRODUCTION

Cell renewal or programmed cell death is an evolutionary, physiologically conserved pathway needed for embryogenesis, tissue homeostasis and regulation of immune system. Being a gene-directed, cellular self destruction method, apoptosis is a highly regulated process of cell death characterized by membrane blebbing, cell shrinkage, chromatin condensation, DNA fragmentation followed by immediate engulfment by surrounding cells in the absence of an associated inflammatory response.[1] It plays a fundamental role in the maintenance of tissue homeostasis as it is a normal component of the development and health of the multicellular organisms.[2] The number of cells in the adult organisms is maintained relatively constant by cell division and cell death. Diseased or malfunctioning cells are replaced and proliferation compensates cell death, thus balancing homeostasis.[3] Apoptosis in the average human adult (50-70 billion cells/day) amounts to an individual's body weight.

Cells die in response to a variety of stimuli (including irreparable DNA damage). Apoptosis is a form of cell death in which the cell itself initiates, regulates and

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executes using extensive components of cellular and molecular pathways,[4] making apoptosis distinct from the other type of cell death i.e. necrosis. The later involves uncontrolled cell death leading to inflammatory response, lysis of cells and potentially to serious health problems. Inhibition of apoptosis contributes to many disease states namely immunodeficiency, autoimmune disease, neurodegenerative diseases, viral infections and cancers,[5] whereas initiation of apoptosis is beneficial in prevention of cancers and transplant rejection. The molecular mechanisms responsible for death signals, genetic regulation and activation of effectors are involved in its present day usage as anti inflammatory and anti cancerous. In view of its increasing importance in future biomedical research, related to newer strategies for diagnostics, the phenomenon of apoptosis is reviewed.

Historical perspectives and Definition

Apoptosis is a Greek word meaning "falling off" as it occurs in leaves falling from trees, used by Hippocrates (460-370 BC) 2000 years ago as "falling of bones" and later on Gallen extended its meaning to "dropping of scabs".[6] Medical concept is that of a natural cell death involving an active and defined process essentially responsible for the regulation of cell population in tissues both under physiological and pathological conditions. It is defined as a type of cell death in which cell uses a specialized cellular apparatus to kill itself, a cell suicide mechanism that results in controlling cell number and eliminating the cells that threaten organism's survival. Though Embryologists were familiar with this term long before, but the mechanisms of apoptosis were recognized by pathologists after 1972.[7] It has emerged as one of the most exciting areas of research in Pathology, Medicine as well as in Physiology where its role is as significant as that of its counterpart mitosis. It plays a complementary but opposite role to mitosis in the regulation of cell population. During the last decade there has been an explosion of interest with apoptosis becoming most widely used word in biomedicine. Since 1990 enormous research is being undertaken because of its implications in many normal and disease processes.

Physiology of apoptosis

Programmed cell death is an integral part of both plants and tissue development. Its functions can be considered under different categories.

I. Apoptosis is an important mean by which an organism not only shapes its tissues and organs during development but even in adult life: Examples include[8,9]

- Removal of webbed tissues between fetal fingers and toes during intra uterine development.
- Formation of synapses between neurons, shaping the brain by eliminating the surplus cells in young children.
- Formation of fetus's eyelids opening.
- Sloughing off the uterine endometrium at the start of menstruation.
- Continuous production and replacement of neutrophils.
- Migration of deeper skin cells to surface, forming protective layer of dead keratinocyte.
- Regression of lactating breast after weaning, atrophy of prostate after castration and ovaries after menopause.
- Resorption of the tadpole tail at the time of its metamorphosis into adult frog.

II. Apoptosis is needed to destroy the cells that represent a threat to the integrity of the organisms

That is cells commit suicide in times of distress for the benefit of organism as evidenced by following egs.[4, 10, 11]

- Damage of cell beyond repair.
- Stress conditions such as starvation.
- Viral infected cells killed by cytotoxic T lymphocytes.
- Removal of immature colonies of T and B lymphocytes to prevent them from attacking body constituents, leaving only a small population of memory cells for secondary response.
- Increase production of tumor suppressor gene p53 (a potent inducer of apoptosis) in cells with DNA damage.

- Radiotherapy and chemotherapy treated cells.

Factors inducing apoptosis

The decision for apoptosis can come from the cell itself, from the surrounding tissue, or from immune system cells. Normally there is a balance between diverse ranges of cell signals. Apoptosis occurs due to the withdrawal of the positive signals i.e. signals needed for cell survival and receipt of negative signals

Positive signals: The continued survival of cells, depend upon their continued adhesion to the surface on which they are growing and continuous stimulation from other cells, for examples hormones, growth factors, nitric oxide, and Cytokines (e.g. Interleukin-2 an essential factor for mitosis of lymphocytes).[12]

Negative signals: for survival include increased levels of oxidants within the cell; damage to DNA by any agent like ultra violet light, x- rays, chemotherapeutic agents and oxidants;[10] accumulation of abnormal proteins and presence of molecules that bind to specific receptors on the cell surface and signal the cell to begin the apoptosis program.

Regulation of apoptosis

Two types of signal molecules can cause the cell to undergo apoptosis.[13, 14] External signal molecules called death activator proteins e.g. tumor necrosis factor (TNF-á), lymphotoxins, Fas ligand (Fas-L) and reactive oxygen species (ROS) etc. Intracellular molecules that monitor the damaging events such as DNA damage by heat and radiations, nutrient deprivation, viral infection, hypoxia, binding of nuclear receptors by glucocorticoids.

Biochemical pathways leading to apoptosis

There are three mechanisms by which a cell undergoes apoptosis.

Intrinsic or mitochondrial pathway

- It is the major apoptotic mechanism (Figure-1)
- Outer mitochondrial membrane of a healthy cell contains protein Bcl-2.
- This protein is activated by initial damage to cell and in turn activates a related protein Bax, which causes holes formation in mitochondrial membrane, causing cytochrome-c to leak out.[15]
- Cytochrome-c in turn binds to protein apoptotic protease factor 1 (Apaf-1) forming complexes apoptosomes.
- Apoptosomes bind to and activate protease Caspase-9 (Cystein Aspartate Specific Prote ase) which activates other caspases in a cascade of reaction.[16]
- Activation of these caspases results in widespread proteolytic activity resulting in degradation of chromosomal DNA, digestion of structural proteins in cytoplasm and ultimately phagocytosis of cell.[8]

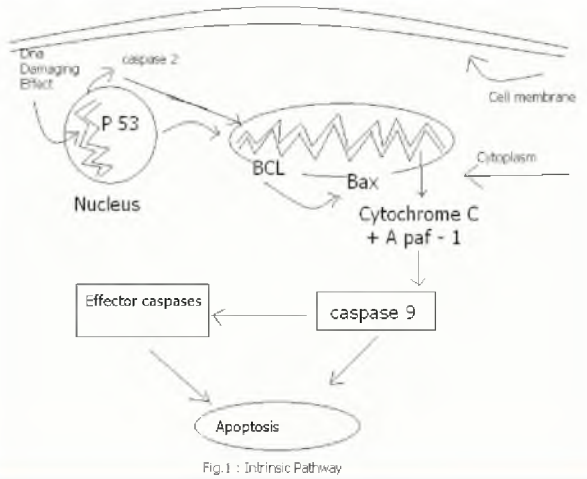

Figure 1: Intrinsic Pathway

2- Extrinsic / death receptor pathway:

- Cell surface has receptors for death activators TNF and Fas-L.[17]
- Binding of TNF and Fas to their receptors transmit a signal to cytoplasm to activate Caspase-8. (Figure-2).[18]
- This results in a series of reactions activating other caspases, leading to cell destruction and phagocytosis e.g. cytotoxic T lymphocytes after binding to their target produces Fas at their surface.

Caspase are highly specific proteases that can cleave proteins after aspartate residues and regulate proteolysis during apoptotic cell death.

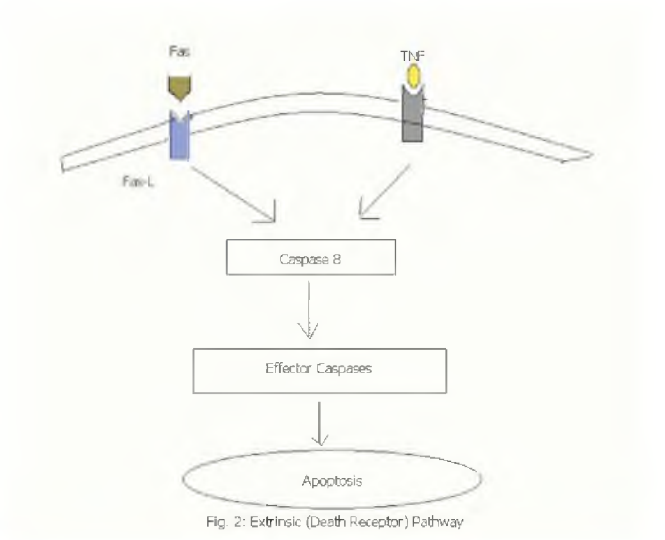

Figure 2: Extrinisic (Death Receptor) Pathway

3- Apoptosis inducing factors (AIF)

Neurons and some other cells do not use caspases for self destruction. AIF is a protein normally located in the mitochondrial inter-membrane space, is released upon receiving a signal (Figure 3). It enters into nucleus, binds to DNA and initiates destruction of DNA and cell death.[19] Whatever is the pathway the morphologic changes seen under microscope include:

- cell shrinkage
- appearance of bubble like blebs on cell surface called Zeiosis.
- nuclear chromatin is degraded creating a vacuolar nucleus.20
- mitochondrial breakdown with release of cytochrome-c.
- fragmentation of cell into apoptotic bodies.[21,22]
- membrane lipids are exposed on the surface, which attract phagocytes. Phagocytes recognize them and secrete cytokines to inhibit inflammation and engulf the cell fragments.[23]

The cascade of events in apoptosis leads to the ordered breakdown of components usually required for cell survival and undesirable effects such as inflammation are prevented.

Methods used in the detection of apoptosis

i Standard method for identification and quantification is morphological assessment by Electron microscopy.

ii Use of fluorescent dyes to stain for condensed nuclei or exposed cell surface phosphatidylserine is also employed. DNA Laddering detects fragments of

ii DNA from nuclear breakdown by terminal transferase mediated dUTP-biotin nick end labeling (TUNEL); method to detect the enzymes involved in nuclear breakdown but has a few limitations.

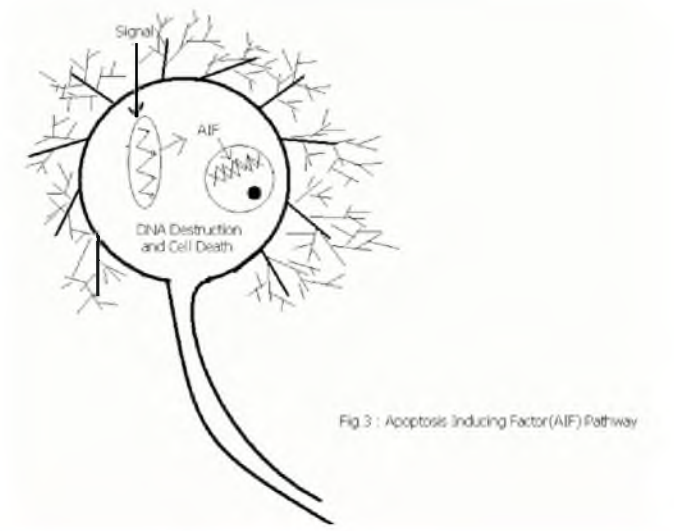

Figure 3: Appotosis Inducing Factor (AIF) Pathway

Apoptosis related to disease

Faliure of apoptotic mechanisms are considered important determinants of fetal abnormalities. During development of nervous and immune system, unwanted cells fail to undergo apoptosis resulting in multiple congenital abnormalities.[24] Likewise, if a cell is unable to undergo apoptosis, due to mutation or biochemical inhibition, it can continue dividing and develop into a tumor. Thus apoptosis has important role in preventing cancers.[25]

Failure of apoptosis may result from different types of defects in apoptotic pathways. Some cancer associated viruses use tricks to prevent apoptosis of cancerous cells resulting in increased number of tumor cells. e.g. human papilloma virus produces a protein (E6) that binds and inactivates protein p53 causing cervical cancer. Epstein Barr virus produces a protein similar to BCl-2 making the cell more resistant to apoptosis, resulting in mononucleosis. Other non-viral cancers like B-cell leukemia, melanoma etc also use methods to avoid apoptosis. Lungs and colon cancers secrete elevated levels of a molecule that binds to Fas-L preventing it to

bind with Fas, making cytotoxic T cells unable to kill the cancer cells.[26, 27] Genetic variation in caspase genes may play an important role in the etiology of non-hodgkins lymphoma.[28] Defects in apoptotic machinery may result in autoimmune disease such as rheumatoid arthritis and lupus erythematosis. Some viruses initiate apoptotic pathways e.g. in AIDS human immunodeficiency virus depletes CD4 T helper lymphocytes by various apoptotic mechanisms which lead to a compromised immune system.[29].

Apoptosis and oral epithelium

Progenitor basal cells continuously multiply,differentiate and mature into flattened squames which are finally shed off from the epithelial surfaces. Epithelial cells maintain their contacts with their neighbouring cells and their survival or shedding depends upon their interaction with mesenchyme. Loss of cell-cell contact deprives epithelial cells of necessary intergin –cadherin mediated survival signals (due to lack of organization of cytoskeleton).

Apoptotic findings in oral lesions

Apoptotic changes are seen in several oral conditions.The most commonly occuring periodontal condition involves destruction of periodontium leading to tooth loss due to involvement of cytokines and lysosomal enzymes. Oral ulcerations show resistance of lymphocytes to FAS-induced apoptosis and reduced p53, BAX, BCL-X.In oral lichen planus, basal layer apoptosis and lymphocytic infiltration is seen. Molecular findings include increased P53, TNF-x, FAS, FasL, MMP-9,granzyme-B and caspace-3; decrease BCL-2.Viral infections have viral BCL- 2,suppression of p53,encoding viral interleukin-6. Auto immune diseases like lymphomas,hemolytic anaemia,thrombocytopenia show massive depletion of CD4 T-cells. Squamous cell carcinoma where epithelial dysplasia and neoplasia are common show inactive p53, inactivation of BAX and BAD and aberrant BCL-2 include increase P53, TNF-x, FAS, FasL, MMP-9,granzyme-B and caspace-3; decrease BCL-2. Viral infections have viral BCL-2,suppression of p53,encoding viral interleukin-6 .[26,27]

Future prospects

Research on apoptosis has increased substantially since 1990 because of its increasingly identified roles in extensive variety of diseases including oncology and organ transplantation. It is now well recognized that many human diseases may be caused by death of cells that should not die or survival of others that should die.[1] Hence modulation of apoptotic process may offer useful strategies for treatment.

Cytotoxic drugs and radiotherapeutic approaches induce apoptosis in tumor cells and resistance to apoptosis is linked with treatment failure. However these therapies also induce apoptosis in normal cells, a side effect that limits the dose that can be used.[24]

Latest therapeutic strategies have shown to induce apoptosis in several tumor types e.g. simultaneous use of proteosome inhibition and death receptor ligand could represent a promising therapeutic strategy in the treatment of anaplastic thyroid carcinoma.[30] Likewise, induction of pancreatic acinar cell apoptosis protects mice against acute pancreatitis.[31] Caspase inhibitors are being investigated as a possible means to slow the progress of Huntington's disease, a degenerative brain disease.

Its importance in organ transplantation has recently been identified. The finding that some cells of the body express high levels of Fas-L at all times, thus antigen reactive T cells which express Fas are killed, raise the possibility of a new way of preventing graft rejection. If at least some of the cells on a transplanted kidney, liver, heart etc could be made to express high levels of Fas-L, T lymphocyte attack on graft can be prevented, and long life treatment with immunosuppressive agents can be avoided.[32]

In view of its importance in most of the challenging diseases of the present era the apoptosis has become the hottest field of biomedical research not only at present but also in future. Mankind is expected to be benefited from the latest research in this field, by better understanding of diseases where apoptosis plays an important role and by adopting methods to minimize the development of horror diseases like cancer and AIDS.

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