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Apoptosis in Health and Disease

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ABSTRACT

In recent year, the essential contribution of apoptosis to all stage of life of a multicellular organism including development and growth, homeostasis during adulthood and aging has been widely accepted and intensively studied. Beside the participation in physiologic processes, apoptotic mechanism is also involved in the pathogenesis of a broad spectrum of diseases including cancer, AIDS, and autoimmune conditions. The recognition of the significance of apoptosis in health and disease has fueled extensive research effort aimed at elucidation of the molecular mechanism of apoptosis and application of this knowledge for the discovery of new therapeutic regimen.

KEYWORDS : Apoptosis: Health and Disease: Cell Death

Introduction

In multicellular organism, the number of cells is determined by tightly regulated equilibrium between cell proliferation and cell death. Maintaince of this balance is a prerequisite for unhampered development and growth as well as for homeostasis of adult tissue. Although these principle date back to ancient Greek and Roman physicians and were broadly accepted since 19th century, the existence and importance of a physiologic cell death programme remained unrecognized until 1972[1]. That cells die had never been I doubt, and that some deaths are part of the enactment of a developmental programme had been recognized at least since the descriptive work of Glucksmann in 1951 and put on a firm experimental basis by Saunders. The unique character of this developmentally regulated death had been encapsulated by the term programmed cell death and was introduced by LOCKSHIN[2].

KERR and his associated introduced the term apoptosis derived from the Greek word for "dropping off" or" falling off" of petals from flower or leaves from trees[1].

Apoptosis is a process genetically programmed cell death by which senescent, DNA damaged and diseased cells are eliminated from the body.[3]

In contrast to necrosis, apoptosis represents a physiologic cellular mechanism or programmed cell death, which ensures the elimination of cells that have completed their life cycle or upon genetic damage have become useless or harmful for the organism.

Major inducer of Apoptosis[1]:

- Irreparable DNA damage
- Cell cycle perturbation
- Aberration in cell metabolism
- Loss of extra cellular molecule that inhibit cell death or promote cell survival
- Death ligand eg:Fas ligand
- Growth factor withdrawal
- Calcium influx
- Glucocorticoid
- Free radical
- Heat shock
- viral infection and bacterial toxin
- Radiation therapy
- Chemotherapeutic drug

Apoptosis occur in the following general setting:[4]

1. During development
2. As a homeostatic mechanism to maintain cell population in tissue
3. As a defense mechanism such as in immune reaction
4. When cells are damaged by disease or noxious agents
5. Aging

Morphological and biochemical features of apoptotic cell

Morphological feature of apoptotic cell seen with electron microscope are[5]:-

Cell shrinkage: cell is smaller in size, the cytoplasm is dense and the organelles relatively normal and more tightly packed

Chromatin condensation: the chromatin aggregates peripherally under the nuclear membrane into well delimitated dense masses of various shapes and sizes. The nucleus itself may breakup producing 2 or more fragment.

Formation of cytoplasmic blebs and apoptotic bodies: the apoptotic cell first shows extensive surface blebbing, then undergoes fragmentation into a number of membrane bound apoptotic bodies composed of cytoplasm and tightly packed organells, with or without nuclear fragmentation.

Phagocytosis of apoptotic cells or bodies: apoptotic cells are phagocytosed by adjacent healthy cells, either parenchymal cells or macrophages and rapidly degraded within lysosomes.

Apoptotic cells exhibit a biochemical modification are[5]:

Protein cleavage: protein hydrolysis involving the activation of several member of newly discovered family cysteine protease named caspase

Protein cross linking: protein cross linking by transglutaminase activation converts cytoplasmic protein into covalently linked shrunken cells that may break into apoptotic bodies.

DNA breakdown: Apoptotic cell exhibit a breakdown of DNA into large 50-300 Kilo base piece. There is inter nucleosomal cleavage of DNA into oligonucleosome in multiple of 180-200 base pairs by Ca+2 Mg+2 dependent endonuclease.

Molecular mechanism of apoptosis in human:

The molecular basis of apoptosis was first deciphered in the nematode worm *caenorhabditis elegans* through the discovery of cell death genes[1]. There are 3 sites in cell where apoptosis can be triggered i.e. the mitochondria, cell membrane receptor, chromosomal DNA[5].

There are two major pathway, intrinsic or mitochondrial pathway and extrinsic or receptor initiated pathway through which apoptotic signals are transmitted resulting in apoptosis[1]. (Fig 1)

Intrinsic pathway[1]:

In this process, mitochondria play a central role by undergoing structural and functional modifications, leads to apoptosis. There is disruption of the integrity of the outer mitochondrial membrane, which causes the release of various apoptotic molecules such as cytochrome C. In the cytoplasm, cytochrome C interact with the apoptotic factor Apaf-1(the human analogue of the product of ced-4 gene) in the presence of ATP and form a complex called apoptosome which initiate and activates the caspases and result in apoptosis.

The process of apoptosis is controlled by bcl-2. Although the exact mechanism of bcl-2 molecule is not clearly understood, it may be act-

ing by altering the permeability of the mitochondrial membrane, thus reducing the release of cytochrome c and other apoptotic mediator in the cytoplasm for the initiation of apoptotic casacade. It also has the capacity to bind to the Apaf-1 result in the antiapoptotic action

Extrinsic pathway[1]:
Triggers the apoptotic cascade involves the activation of specific cell membrane receptors which belongs to the tumor necrosis factor family (TNF) i.e TNF-receptor1, Fas, DR4,DR5,DR6. Upon binding of specific ligands such as TNF-α, lymphotoxin,Fas ligand, APO3 ligand and TNF –related apoptosis inducing ligand (TRAIL). These receptor undergoes conformational changes that allow them to interact with specialized intracellular adopter proteins and formation of these complexes. Eg: death inducing signaling complex initiates the casacade of caspase activation, which terminates with apoptosis.

Eg: T-cell receptor of the cytotoxic T lymphocyte recognizes an antigen presented by the class –I major histocompatibility complex (MHC) of the target cell. This interaction stimulate the cytotoxic T cell to express Fas Ligand which binds to the Fas receptor on the surface of the target cell through the mediation of adoptor molecule (FADD), the procaspase 8 (procaspase-8) is cleaved to intiator caspase 8 (Casp-8), which in turn cleaves and activates caspase to excute the apoptosis. An addition mechanism, that degranulation of the cytotoxic Tcell to release of perforin and granzyme which form the pore in the membrane of the target cell,and allow the granzyme to enter the the target cell and directly activates caspases[1].(Fig.2)

Major player in apoptosis:
The major players participating in execution and control of apoptosis are concisely highlighted.[1]

Caspases:
Caspases are cysteine proteases that function as the executioner of apoptosis.[1] At least fourteen different caspases have been described in human cells, which are structurally and functionally similar to the product of ced-3 gene. The term caspase is based on two catalytic properties of this family of enzyme, the C reflect a cysteine protease mechanism, and aspase refers to their unique ability to cleave protein at aspartic acid residues.[4] Caspase which cleaves a procaspase molecule into its active form[1].

bcl-2:
Studies of human B cell lymphoma (Bcl) led to the discovery of proto-oncogene bcl-2, which is situated on chromosome 18. The bcl-2, the mammalian homolog of the antiapoptotic ced-9 gene in C.elegans, is situated in the outer mitochondrial membrane, endoplasmic reticulum and nuclear envelope.[4] The member of this family are able to either promote (eg: bax, bcl-xs, bad,bid) or inhibit (eg: bcl-2,bcl-xL, bcl-w) the apoptosis.[1] Several proteins of Bcl-2 family regulates the mitochondrial permeability events in apoptosis.

Tumor necrosis factor receptor:
Member of tumor necrosis factor receptor family have pleiotrophic action. Depending on the cell type and the other signals that the cell receives, these receptor can trigger proliferation survival, differentiation, or death. These receptors are activated by a group of structurally related ligands that belong to the TNF ligand family.[6]

Granzyme B:
Granzymes are family of protease that cleaves a number of protein substrate which in turn induces apoptotic cell death.[7] This granule released by cytotoxic T lymphocyte can kill target cells.[7]

Methods used for detecting apoptotic cells:
Various methods used to detect apoptotic cells are:
1. Light microscopy[3]
2. Electron microscopy
3. Immunohistochemical technique[9]
4. In Situ End Labeling (ISEL)[10]

Apoptosis in physiology[4]:
- The programmed destruction of cells during embryogenesis including implantation, organogenesis, development, involution and metamorphosis.
- Hormone dependent involution in the adult such as endometrial cell breakdown during menstrual cycle, ovarian follicular atresia in the menopause, regression of the breast after weaning
- The formation of the proper connection between neuron in the brain requires that surplus cells be eliminated by apoptosis.
- Apoptosis regulates mechanism of the wound healing.
- Apoptosis has an important role in the tooth development which shown to occur in the ameloblast, odontoblast, and pulp cells
- Oral epithelial cell are regulated by the proliferation of basal cells and shedding of the apoptotic cells.

Apoptosis in oral disease
Aberration of the molecule mechanism that control and execute apoptosis lead to defect in the physiologic regulation of cell number balance and manifest as various pathologic states. A number of human diseases have been related to pathologic acceleration/ retardation of the physiologic apoptotic rate.

Oral Lichen Planus has been reported that there was increase in the apoptotic rate as compare to normal epithelium. It was thought that activated T cells are responsible for induction of keratinocyte apoptosis in oral lichen planus. The mechanisms used by CD8+ cytotoxic T cells to trigger keratinocyte apoptosis in oral lichen plannus are unknown. But possible mechanism includes

1. T-cell secretes TNF-α binding TNF- α receptor 1 on the keratinocyte surface.
2. T-cell surface CD-95L binding CD-95 on the keratinocyte surface.
3. T-cell secreted granzyme B entering the keratinocyte via perforin induced membrane pores.

The above events may activate the caspase cascade resulting in apoptosis[11].
Keratinocyte cell death in erythema multiforme was found to be associated with altered expression of p53 and protein of the bcl-2 family but not the Fas/FasL system[12] and mutation in the *Fas* gene have been associated with defective apoptosis in systemic lupus erythematosus[13]. Some author suggested that Fas/FasL signaling might play an important role in the pathogenesis of disease-like pemphigus vulgaris,bullous pemphigoid and erythema multiforme[14]. A recent study on an in vitro epidermolysis bullosa model showed that the mutant keratin-5 and 14 protein form aggregates, which induce cell death by stimulation of TNF-R signaling. Other molecular defects in epidermolysis bullosa include integrin-α6β4, a major component of hemidesmosomess involved in proliferation, apoptosis and differentiation[15].

Pyogenic granuloma in pregnancy, a reactive oral lesion associated with hormonal changes during pregnancy. A recent study showed that regression of pyogenic granuloma after parturation with increased apoptosis and lack of vascular endothelial growth factor[16].

Poly methyl methacrylate resin, used as base material for removable dentures, have been shown to induce both apoptotic and necrotic cell death in human monoblastoid cells and in murine fibroblast.[17]

Recurrent aphthous ulceration is a common inflammatory condition of the oral mucosa and consistently seen in Behcet's syndrome. Apoptotic degeneration of the prickle cell layer was demonstrated microscopically in RAU. More recently, lymphocyte obtained from with Bechet's syndrome were relatively resistant to Fas-induced apoptosis, suggesting that a defect in the Fas pathway may be involved in this condition.[18]

HIV infection causes apoptosis of infected lymphocytes and increased spontaneous apoptosis of uninfected lymphocytes leading to massive CD+T cell depletion in patients. Lymphocyte apoptosis in AIDS is induced by viral components such as gd120, tat,nef,and cell surface death receptors.[19]

A recent study indicated that p53, bax, bcl-2 and caspase-3 were involved in the pathophysiology of radicular cyst. Heat shock protein (HSP27), an inhibitor of cell death, was found over expressed in epithelial cell rests, islands of epithelium and epithelial lining of microcysts.[20]

Several studies have shown that keratocyst have a higher proliferative rate compared with radicular or dentigerous cyst, a fact that may explain why the lumen of keratocysts are filled with desquamated apoptosed keratinocytes. The desquamated keratinocyte in turn increase

the protein content of the cyst lumen raising the osmotic pressure and thus contributing to an increase in cyst size.[21]

Fas signaling has been proposed as an important mechanism of salivary gland apoptosis in sjogren's syndrome.[22] A number of hematological disease which characterized by the number of blood cells owes to decrease or increase in the production or destruction.[23]

Diagnostic prognostic and therapeutic application:

Understanding the physiological process apoptosis at the molecular level helps not only to know the disease pathogenesis but also developing diagnostic, prognostic and therapeutic tools. There are intense investigations going on, for the development of new therapeutic strategies that will allow manipulation of the apoptotic machinery.

Insight into the mechanism of the apoptosis is already led to a surge in research, potential therapies which fall into 3 broad catergories[23].

1. Gene therapy
2. Injectable molecule target at the upstream modulator of apoptosis
3. Small molecule pharmaceutical designed to regulate expression of apoptosis related genes.

CONCLUSION:

Apoptosis is a distinctive form of cell death manifested by characteristic chromatin condensation DNA fragmentation whose function is the deletion of cell in normal development organogenesis immune function and tissue growth but which can also be induced by pathologic stimuli. The mechanism of apoptosis has several key stages. Deregulation of this process may contribute to variety of disease state including cancer. Knowledge of apoptosis is very important for the development of new understanding and new treatment of disease.

Fig 1: Diagram shows basic molecular mechanism of apoptosis:[1]

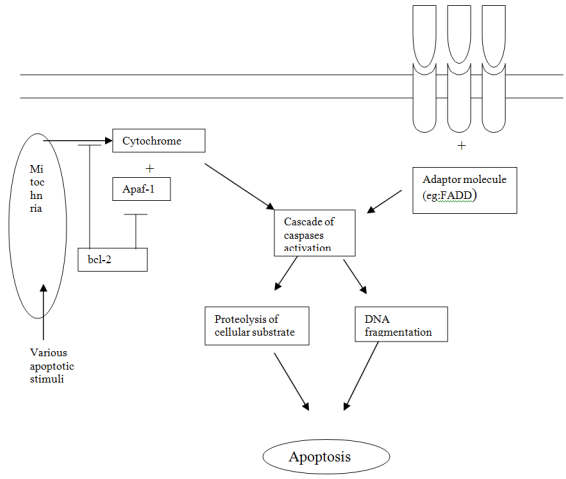
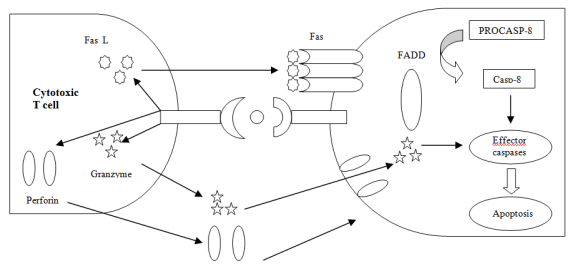


Fig 2: Diagram shows extracellular apoptotic pathway :[1]



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