

Review Article

A Systematic Review of Apoptosis

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Abstract: Apoptosis is a programmed cellular death involved in morphogenesis and homeostasis of organs and tissues. The survival of organisms depends on balance between cell proliferation and cell death. Apoptosis occurs in tissues normally during development and aging to maintain cell populations and as a defense mechanism in immune reactions. T and B lymphocyte expansion, maturation and excessive accumulation after antigen-stimulation in peripheral lymphoid organs is regulated by apoptosis. Several mechanisms may trigger this process. The goal of this review is to provide an overview of apoptosis and its mechanisms.

Keywords: apoptosis, apoptoses- extrinsic pathway, apoptoses-intrinsic pathway, necrosis, tunel.

APOPTOSIS

Apoptosis is a kind of cellular death that plays an important part in the early development and growth of tissues and is necessary for the continuation of cellular hemostasis. Programmed cell death is mostly synonymous with the suicide of cell. In other words, apoptosis refers to controlled regulation of cycle and removal of unwanted cells at proper times without causing surrounding tissue damage [1, 2].

Apoptosis was first defined in 1972 [3], as physiological death pattern unlike necrosis. In Ancient Greek, apoptosis means falling of leaves from trees and petals from flowers. Apoptosis is an active procedure that is activated by various traumatic extra cellular lesions (hormonally active various agents, ionized radiation and traumatic agents including chemotherapy) or by genetic factors and that controls cellular death via a mechanism programmed by the cell itself [4]. As a physiological process, apoptosis is the most common morphology occurring in conditions in which cellular death is considered as physiological [5]. Apoptotic cells are continuously formed in some cells and tissues of organism and this process continues throughout all life. Thus death (apoptosis) and reformation (mitosis) proceeds in a dynamic balance by producing hemostasis in these tissues [6].

Apoptosis is responsible for morphogenic death of cells during embryonic and early

postembryonic development in addition to playing a fundamental role in the maturation of differentiated tissues, as it makes it possible to keep the number of cells in the body constant and to activate immune system. For example, if the outcome of an immune reaction is considered, it can be seen that at this point, activated lymphocytes eliminate their own antigens via direct apoptosis [7].

Morphological characteristics

After signal is received for apoptosis, many biochemical and morphological changes are observed in the cell. At first cell starts to get smaller and more condensed, the cellular skeleton is disintegrated and cellular membrane is dissolved. Nuclear DNA is divided into many pieces [8]. The disruption of cytoplasmic skeleton of the cell leads to apoptosis while the stabilization of cytoplasm inhibits apoptosis. Cytoplasm starts to shrink and get smaller. Subsequently, nucleus is divided into separate fragments. Nucleus is the focus of events in apoptosis [4]. Cell continues to shrink and become smaller and is divided into small vesicular parts surrounded by membrane and that can be recognized by macrophages. Apoptotic bodies containing cytoplasm, intact organelles and nucleus fragments (in some) may be formed [8, 9, 10]. Like nucleus, cell also shrinks and may be divided into a few parts surrounded by membrane [9]. Eventually, cell dies, plasma membranes are disintegrated and organelles are observed. The chain

of apoptotic events ranging from the separation of protoplasm from membrane to the formation of apoptotic body lasts a few minutes. However, phagocytosis of apoptotic cells may last 12-18 hours [11].

Biochemical changes

Alterations occur in cell membranes during apoptosis. The most marked change is that negative charged phospholipid units which are normally on the cytoplasmic surface of cellular membrane goes up to its outer surface. Consequently, various proteins called collectin (C1q) are attached to apoptotic cell membrane. Vesicles involving cell content and surrounded by membranes are separated from apoptotic cells. These small vesicles are also called apoptotic bodies. These changes occur near the end of apoptotic process [8]. The most important characteristics of apoptosis is that via the activation of intracellular Ca^{++} and Mg^{++} dependent endogenous endonuclease enzyme, chromosomal DNA is fragmented into nucleosomal units [12]. Thus, it is thought that this fragmentation is a key in apoptosis [13]. Fragmentation is carried out in apoptotic cells by CAD (caspase activated deoxyribonuclease) enzyme, separating DNA into nucleosomal units. CAD is found in normal cells in inactive form, but in cells receiving apoptosis signal, it is activated by caspase 3 and as this enzyme is DNase (endonuclease), it makes it possible for nuclear DNA to be divided rapidly into nucleosomal fragment with 180-200 base pairs [5, 8]. However, the disintegration of DNA is not a prerequisite for apoptosis. In an intact cell, a simple molecular or cellular event may initiate apoptosis at any moment. This is an indicator of the fact that cell should carry an inhibitor molecule determining whether they will carry out suicide program which they have already have [12, 14].

Genetics of apoptosis

As in all cellular procedures, there are certain stages in the genetics of apoptosis. Deciding upon death, fragmentation and phagocytosis follow. Bcl family is a oncoprotein group composed of antiapoptotic and proapoptotic members and has the most important role in the regulation of apoptosis. Bcl-2 and Bcl-XL carry out the function of preventing apoptosis by stopping the precursor forms of caspases or by impeding the flow of caspases (15). Bcl-2 defined as proto-oncogen has usually been demonstrated in human follicular B cell lymphomas. Bcl-2 long survival in early hemopoietic cell lines associated with IL-3, GM-CSF and IL-4. It has characteristics reminding "Anti-apoptosis" gene. For example, it can not protect target cells from cytotoxic T cells. However, it can not always prevent apoptosis [5].

Anti-oncogen p53 is another gene associated with apoptosis. P53 is classified as tumor suppressor

gene. The real role of p53 is to keep damaged cell in G1 phase until damage is repaired [11]. Many anti tumor drugs choose cellular DNA as target and increases p53 level. This activation leads to repair of damage or apoptosis [6] i.e. if Bcl-2 is an excessive amount in the cell and p 53 undergoes mutation, apoptosis can not take place.

The balance between cytoplasmic levels of oxidants and antioxidants also play a role in the regulation of apoptosis. Many agents inducing apoptosis are either oxidants or stimulators of oxidative metabolism. In addition, there are death receptors in the cell membrane that can control apoptotic signals. This receptor family is also known as tumour necrosis factor (TNF) and it has 24 members including 6 receptors (TNF-R, FAS, DR3, DR4, DR5, DR6) [16]. There is chain of 80 aminoacids on cell membrane, known as death area. This region has great significance in the activation of apoptosis. Fas has the same structure with cell surface molecule termed as APO-1. Anti APO-1, induces apoptotic cell death by completely blocking proliferation. Fas/APO-1 system activates normal tissue cycle. In addition, it makes apoptosis activation possible in many conditions including malignity [2, 5].

Caspases

Caspases are normally found in cytoplasm as inactive proenzymes. However, they become active after proteolytic disintegration and hence caspase activation chain is initiated. At the onset, caspases produce membrane injury at mitochondrium and as a consequence injuries leading to alteration in membrane, cell skeleton and nucleus arise [17].

Apoptosis and necrosis

Cellular death occurs in two different forms, i.e. necrosis and apoptosis.

Apoptosis mechanism with internal signals.

It is also termed as mitochondria mediated pathway. It is a pathway activated with extracellular signals or DNA injury. In the outer membrane of the mitochondria of healthy cells, Bcl-2 protein is expressed as a membrane surface protein. Bcl-2 protein is bound to Apaf-1 protein molecule. Internal damage in the cell influences Bcl-2 protein, leading to cleavage of Apaf-1 molecule. This event leads the cytochrome-c present in mitochondria to leak into cytoplasm. Together with released cytochrome-c and Apaf-1 they are attached to caspase-9 molecule. Cytochrome-c, Apaf-1 and caspase-9 molecules unite in the presence of ATP, forming a complex termed as apoptosome. These events take place in cytosol. Caspase-9 effector is bound to caspases, activating them. These reactions occur in a similar manner to complement and clotting mechanism and lead to digestion of structural proteins

in cytoplasm, fragmentation of chromosomal DNA and to phagocytosis of the cell [18].

Apoptosis mechanism with external signals

It is also called death receptor mediated apoptosis. This pathway assumes important roles particularly in the protection of internal balance of immune system.

Fas and TNF receptors are integral proteins on cell membrane termed as death cell receptor. Their receptors are on cell surface. With the bonding of death activators such as Fas l, TNF- α and β to these receptors, they transmit apoptotic signals to cytoplasm. Subsequently, caspase-8 is activated. Caspase-8, with a mechanism similar to that of caspase-9, activates effector caspases and finally leads to cell undergoing phagocytosis [19]. These processes in the cell require energy. Energy requirement is an important factor distinguishing apoptosis from necrosis. According to results of investigations, intracellular ATP level has a determining role in the selection of cellular death pattern.

Necrosis

It occurs due to external factors rather than events occurring within the cell itself. Although both necrosis and apoptosis are form of death, there are large differences between them. Typical characteristics of the necrosis is that death occurs in groups of cells and the most common cause of necrosis is hypoxia. However, toxic substances and heavy metals may also cause necrosis as well. Events causing necrosis play part in the increase in the permeability of membrane, leading to cell and organelle fragmentation, which in turn gives rise to release of cytoplasm and the content of nucleus to extracellular space [13, 20]. In consequence, inflammation develops. The typical characteristics of this event is that macrophages and neutrophils migrate to necrotic tissue, which undergoes phagocytosis by them. Therefore inflammation is an important indicator of necrosis. While mechanisms initiating apoptosis are endogenous, those initiating necrosis are exogenous. Apoptosis is event arising in a single cell, and results in with the formation of apoptotic body. Apoptotic cell loses the property of being a cell and is transformed into a completely lifeless mass. However, in necrosis, organelles are fragmented, swelling, membrane injury, and free radical damage are involved and necrosis influences a certain group of cells [14].

Table 1: General differences between apoptosis and necrosis [14]

Characteristics	Apoptosis	Necrosis
Stimulus	Physiological	Pathological (due to injury)
Appearance	Individual cells	Cellular groups
Reversibility	No (after morphological changes)	Yes (until irreversible changes)
Adhesions between cells and to basement membrane	Disappears at early period .	Disappears at late period
Cytoplasmic organelles	Swells at late period	Swells at early period
Lysosomal enzyme release	Absent	Present .
Nucleus	Disintegrates (caryorexis).	Disappears (caryolysis).
Nuclear chromatin	They are collected together in similar clusters	They are clustered with ill defined borders.
DNA fragmentation	Inter nucleosomal	Random
Cell	Apoptotic bodies form .	Swells and bursts at late stage
Phagocytosis by other cells	Present .	absent
Exudative inflammation	Absent	Present
Scar formation	Absent	Present

Relation of apoptosis with physiological events and diseases

Apoptosis plays an active role in many physiological and pathological processes. The most important physiological event in cellular turnover. In tissues with more rapid cellular turn over such as skin, intestinal epithelium and blood cells, aging cells are eliminated by apoptosis and they are replaced by new cells. It plays an important role in many development steps throughout life from implantation of fetus to organogenesis. In the embryological development of

humans, the disappearance of webs between fingers and death of more than 50% of neurons produced during the development of nervous system occurs by means of apoptosis [21]. Apoptosis is considered a vital component of various processes including normal cell turnover, proper development and functioning of the immune system, hormone-dependent atrophy, embryonic development and chemical-induced cell death. Inappropriate apoptosis (either too little or too much) is a factor in many human conditions including neurodegenerative diseases, ischemic damage,

autoimmune disorders and many types of cancer. As normal homeostasis mechanism, apoptosis plays an active role in many physiological events such as shedding of endometrium in menstruation, hormone associated changes such as the shrinking of mammary glands after lactation ceases, and development and proper functioning of immune system. In addition, apoptosis occurs in many other pathological processes, i.e. cellular injury caused by detrimental agents such as hyperthermia, radiation, cytotoxic chemotherapy and hypoxia, some viral diseases such as HIV-1 and HCV and cellular death produced by cellular death via cytotoxic T lymphocytes in response to immune reactions [22]. Due to slowing or accelerating apoptosis in tissue, viral infections such as HIV and HCV, Parkinson's disease, neurodegenerative diseases such as Alzheimer's disease, atherosclerosis and ischemic injury, autoimmune diseases and cancer may occur [6]. While apoptosis usually increases in tumor tissues owing to increase in proliferation, in some tumors, such as B cell lymphoma, decrease in apoptotic process may give rise to tumor development [23].

Methods used in the determination of apoptosis [24]

1. Hematoksilen-eozin staining
2. Giemsa staining
3. Fluorescent microscopy
4. Electron microscopy
5. Phase contrast microscopy
6. Annexin V method
7. TUNEL method
8. M30 method
9. Caspase-3 method
10. Agarose gel electrophoresis
11. Western blotting
12. Flow cytometry

TUNEL method

It makes it possible to recognize DNA breaks in situ. Free 3'OH part of DNA fragments may be determined by enzymatic labels modified via nucleotides such as biotin, digoxigenin or fluorescein after apoptotic fragmentation, DNA ends may be labeled using DNA polymerase or Klenow fragment. Nevertheless, labelling made using terminal deoxynucleotidyl transferase (TdT) has been found to be relatively more accurate method. In this technique, the percentages of apoptotic cells may be measured using flow cytometry. In order to avoid DNA loss, sections may be made with freezing microtome following in situ labelling carried out with conventional paraffin sections, TdT and non isotopic labelled nucleotides (frequently dUTP with biotin), fluorescent or enzymatic imaging is adequate in distinguishing apoptotic cells from others. This method is commonly called as TUNEL method, which is the abbreviation of the words "TdT-dUTP nick-end-labelling" [25, 26].

Apoptosis and Immune system

Apoptosis play part in maintaining cellular balance in the growth of tissues. According to a known theory, cells always die inherently unless they are stimulated on the contrary direction. Thus, immune system and control of hematopoiesis includes many examples in the survival of cell, corroborating this theory. In thymus, thymocytes enter a selection process and hence only cells recognizing self antigen with lower affinity are turned into mature CD4+ and CD8+ T lymphocytes. This is called positive selection. If thymocytes which recognize self antigen with high affinity continue to develop, autoreactive lymphocytes are formed, therefore they should be eliminated. This is negative selection and is mediated by induction of apoptosis [2]. This negative selection in thymus is the most reliable mechanism in the elimination of autoreactive T lymphocytes and hence self tolerance of organisms is protected.

B cells, like T cells, respond variously to antigen stimulation depending upon the stage of differentiation. When immune reaction is completed, lots of antigen specific lymphocytes are no longer required. Very few of these cells turn into memory cells. these cells show a faster and stronger reaction when they encounter a new antigen, while the other cells are destroyed via apoptosis. Apoptosis of T lymphocytes are used for the removal of old T lymphocytes in peripheral immune system [27].

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