ApoPTOSIS-AN OVERVIEW

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ABSTRACT

Apoptosis is the term describing programmed cell death in vertebrates and plants. It represents a physiologic form of cell death distinct from necrosis and it is involved in homeostatic and pathologic process in the body. This paper reviews the basic concepts of programmed cell death along with its application in oncology.

Key Words: Apoptosis; Programmed Cell Death; TNF; Cell Cycle; CD 31

All living things are composed of number of cells. Cell is the structural and functional unit of the body. To maintain the cell has to undergo much physiological condition like mitosis, cell division. The damaged cells, in addition to cell-cycle arrest and repair machinery, where damage is beyond repair, may induce an physiologic form of cell death- Apoptosis. Apoptosis is the term describing programmed cell death in vertebrates and all eukaryotes. It represents a physiologic form of cell death distinct from necrosis, and it is involved in every homeostatic and pathologic process in the body. Apoptosis occurs normally during development and aging and as a homeostatic mechanism to maintain cell populations in tissues. Apoptosis also occurs as a defense mechanism such as in immune reactions or when cells are damaged by disease or noxious agents. Although there is a wide variety of stimuli and conditions, both physiological and pathologic, that can trigger apoptosis, not all cells will necessarily die in response to the same stimulus. On the other hand up-regulation of apoptosis could help fight autoimmune diseases and cancer. Because of the ubiquitous nature of this phenomenon combined with a timelinesscientific ability to efficiently characterize new gene products, apoptosis is now one of the most investigated areas in biologic sciences.

Finally, apoptosis is a coordinated and often energy-dependent process that involves the activation of a group of cysteine proteases called “caspases” and a complex cascade of events that link the initiating stimuli to the final demise of the cell. The control of apoptosis has therapeutic ramifications. Its inhibition could help physicians control events ranging from aging to ischemic heart and brain diseases. This paper reviews the basic concepts of programmed cell death along with its application in oncology.

Historical Background

The description of physiologic cell death, that is part of normal development, dates back possibly to Aristotle and definitely to the time of Galen based on descriptions of regression of the ductus arteriosus. Throughout the latter half of the last millennium there were similar descriptions of apoptosis in other areas of the body. By 1842, microscopes were widely available and a histologist named Carl Vogt performed his seminal work on “resorption” of notochord cells in the toad, which included distinct changes in the nuclei. Although it was not recognized at the time, this was the first published account of the histologic features of apoptosis. However, neither Vogt nor his contemporaries found great importance in the field of cell death. In the mid-20th century, Glucksman described naturally occurring cell death in both invertebrates and vertebrates. By the early 1960s, the term programmed cell death was coined to describe a predictable process both in terms of location and timing.

In 1972, three researchers described the term “apoptosis” to draw a distinction seen in homeostatic and pathologic states from that seen in ischemic or uncontrolled demise of the cell. Although the phenotypic features of apoptosis were described, the mechanisms involved did not start to be discovered until the early 1980’s when researchers in Robert Horvitz’s laboratory found three distinct genes controlling cell death in the nematodes. During development, this worm has only 1090 cells, and only 131 of these die, much in the same fashion seen in human development. Knocking out one of any of these three genes will lead to a change in the balance of cell death. The ced-3 and ced-4 gene products were found to be pro-apoptotic, whereas the ced-9 product was found to be anti-apoptotic.

The term programmed cell death was introduced in 1964, proposing that cell death during development is not of accidental nature but follows a sequence of controlled steps leading to locally and temporally defined self-destruction. Eventually, the term apoptosis had been coined in order to describe the morphological processes leading to controlled cellular self-destruction and was first introduced in a publication by Kerr, Wyllie and Currie.

Apoptosis

The term “Apoptosis” has a Greek origin, meaning “falling off or dropping off”, in analogy to leaves falling off trees or petals dropping off flowers. Apoptosis mechanism by which cells are physiologically removed and thus plays role in regulating tissues during embryogenesis and in normal homeostasis.
Apoptosis specifically refers to an energy-dependent, asynchronous, genetically controlled process by which unnecessary or damaged single cells self-destruct when the apoptosis genes are activated. It is part of normal development and maturation cycle, and is the component of many responding tissues to xenobiotic agents (i.e. microorganisms and chemicals) and to endogenous modulations, such as inflammation and disturbed blood supply. Cells die in response to a variety of stimuli and during apoptosis they do in a controlled, regulated fashion. In multicellular organisms, cells are continuously shed and replaced. Mitosis and apoptosis are the basic physiology processse cell number. In a healthy organism, 10 billion cells are lost daily through apoptosis and are replaced by mitosis. A dysfunctional apoptotic system can lead to either excessive removal or prolonged survival of cells. Therefore, dysregulation of apoptosis is involved in the pathogenesis of a variety of diseases such as carcinogenesis, viral infections and immunodisorders. An understanding of the role of cell death in the pathophysiologyissues is pertinent to the development of novel therapeutic approaches.

Mechanism of Apoptosis

Apoptosis is induced by a cascade of molecular events that may be initiated in distinct ways and culminates inactivation of caspases. The process of apoptosis may be divided into an initiation phase, during which caspases become catalytically active, and an execution phase, during which these enzymes act to cause cell death. Initiation of apoptosis occurs principally by signals from two distinct but convergent pathways — the extrinsic, or receptor-initiated, pathway and the intrinsic, or mitochondrial, pathway. Both pathways converge to activate caspases.

The Extrinsic (Death Receptor-Initiated) Pathway

This pathway is initiated by engagement of cell surface death receptors on a variety of cells. Death receptors are member-sumor necrosis factor receptor family that contain a cytoplasmic domain involved in protein-protein interaction called the death domain because it is essential for delivering apoptotic signals. Death receptors are members of the tumor necrosis factor receptor family that contain a cytoplasmic domain involved in protein-protein interactions that is called the death domain because it is essential for delivering apoptotic signals. The best-known death receptors are the type-1 TNF receptor (TNFR1) and a related protein called Fas (CD95).

When Fas is cross-linked by its ligand, membrane-bound Fas ligand (FasL), three or more molecules of Fas come together, and their cytoplasmic death domains form a binding site for an adapter protein that also contains a death domain and is called FADD (Fas-associated death domain). FADD that is attach death receptors in turn binds an inactive form of caspase-8 (and, in humans, caspase-10), again via a death domain. Multiple pro-caspase-8 molecules are thus brought into proximity, and they cleave one another to generate active caspase-8. The enzyme then triggers a cascade of caspase activation by cleaving and thereby activating other pro-caspase active enzymes mediate the execution phase of apoptosis.

The Intrinsic (Mitochondrial) Pathway

This pathway of apoptosis is the result of mitochondrial permeability and release of pro-apoptotic molecules into the cytoplasm, without a role for death receptors. Growth factors survival signals stimulate the panti-apoptotic members of the Bcl-2 family of proteins. This family is named after Bcl-2, which was identified as an oncogene in a B cell lymphoma and is homologous to the C. elegans protein, Ced-9. There are m20 proteins in this family, all of which function to regulate apoptosis; the two main anti-apoptotic ones are Bcl-2 and Bcl-x. These anti-apoptotic proteins normally reside in mitochondrial membranes and the cytoplasm. In the cytosol, cytochrome c binds to a protein called Apaf-1 (apoptosis activating factor-1, homologous to Ced-4 in C.elegans), and the complex activates caspase-9. (Bcl-2 and Bcl-x may also directly inhibit Apaf-1 activation, and their loss from cells may permit activation of Apaf-1). Other mitochondrial proteins, such as apoptosis inducing factor (AIF), enter the cytoplasm, where they bind to and neutralize various inhibitors of apoptosis, whose normal function is to block caspase activation. The net result is the initiation of a caspase cascade.

P53 Dependent Pathway

Activated P53 serves as a transcription factor that modulates transcription of several apoptosis related genes. E.g. P53 up-regulates the transcription of Bax but down regulates that of Bcl-2, thus favouring mitochondria dependent apoptosis. In addition it upregulates transcription of Fas to support Fas mediated apoptosis.

Execution Pathway

The extrinsic and intrinsic pathways both end at the point of the execution phase, considered the final pathway of apoptosis. It is the activation of the execution caspases that begins this phase of apoptosis. Execution caspases activate cytoplasmic endonuclease, which degrades nuclear material, and proteases that degrade the nuclear and cytoskeletal proteins. Caspase-3, caspase-6, and caspase-7 function as effectors or “executioner” caspases, cleaving various substrates including cytokeratins, PARP, the plasma membrane cytoskeletal protein alpha fodrin, the nuclear protein NuMA and others, that ultimately cause the morphological and biochemical changes seen in apoptotic cells.
then degrades chromosomal DNA within the nuclei and causes chromatin condensation. Caspase-3 also induces cytoskeletal reorganization and disintegration of the cell into apoptotic bodies. Gelsolin, an actin binding protein, has been identified as one of the key substrates of activated caspase-3. Gelsolin will typically act as a nucleus for actin polymerization and will also bind phosphatidylinositol bisphosphate, linking actin organization and signal transduction. Caspase-3 will cleave gelsolin and the cleaved fragments of gelsolin, in turn, cleave actin filaments in a calcium independent manner in disruption of the cytoskeleton, intracellular transport, cell division, and signal transduction.29

Removal of Dead Cells
At early stages of apoptosis, dying cells secrete soluble factors that recruit phagocytes.30 This facilitates prompt clearance of apoptotic cells before they undergo secondary necrosis and release their cellular contents (which can result in inflammation). As already alluded to, apoptotic cells and their fragments have marker molecules on their surfaces, which facilitates early recognition by adjacent cells or phagocytes for phagocytic uptake and disposal. Numerous macrophage receptors have been shown to be involved in the binding and engulfment of apoptotic cells.31 In addition, macrophages can also secrete substances that bind specifically to apoptotic but not live cells and opsonize these cells for phagocytosis. In contrast to markers on apoptotic cells, viable cells appear to prevent their own engulfment by macrophages through expression of certain surface molecules (such as CD31). This process of phagocytosis of apoptotic cells is so efficient that dead cells disappear without leaving a trace, and inflammation is virtually absent.32

Human Studies
The development and maintenance of multicellular biological systems depends on a sophisticated interplay of cells forming an organism. It sometimes even seems to involve an altruistic behavior of cells in favor of the organism as a whole. During development many cells are produced in excess which eventually undergo programmed cell death and thereby contribute to sculpturing many organs and tissues.27,34 Apoptotic processes are of widespread biological significance, being involved in e.g. development, differentiation, proliferation/homoeostasis, regulation and function of the immune system and in the removal of defect and therefore harmful cells. Thus, dysfunction or dysregulation of the apoptotic program is implicated in a variety of pathological conditions.7 Defects in apoptosis can result in cancer, autoimmune diseases and spreading of viral infections, while neurodegenerative disorders, AIDS and ischemic diseases are caused or enhanced by excessive apoptosis. The identification of apoptosis under pathological settings dates back to the 1960s, when John FR Kerr was studying ischemic liver damage. Apoptosis plays an important role in morphogenetic processes in early developmental stages of intrauterine life. It is associated with normal differentiation and formation of organs during organogenesis.29

Apoptosis in Physiologic Situations
Apoptosis in different adult cells: a) Germ cells,31 b) Hematopoietic system,30 c) Neurons,33 d) Muscle cells,30,31 e) Cardiomyocytes, f) Smooth muscle, g) Skeletal muscle, h) Fibroblasts,35 i) Endothelial cells,36 j) Tooth37

Apoptosis in Pathologic Conditions
Aberrations of the molecular mechanism that control and execute apoptosis leads to defects in the physiologic regulation of cell number balance and manifest as various pathologic states.38 The disease in which the apoptosis has been involved can be divided into a) Those in which there icell survival (diseases associated with the inhibition of apoptosis) and b) Those in which there is an increase in cell death (diseases associated with excess of apoptosis).39 Apoptosis is an active mode of cell death under molecular control that requires energy to proceed.39 Mitosis and apoptosis are the basic physiologic processes that regulate cell number.40,41 Apoptosis is regarded as a carefully regulated energy-dependent process, characterized by specific morphological and biochemical features in which caspase activation plays a central role. The importance of understanding the mechanistic machinery of apoptosis is vital because programmed cell death is a component of both health and disease, being initiated by various physiologic and pathologic stimuli.42 Moreover, spread involvement of apoptosis in the pathophysiology of disease lends itself to therapeutic intervention at many different checkpoints. Understanding the mechanisms of apoptosis, and other variants of programmed cell death, at the molecular level provides deeper insight into various disease processes and may thus influence therapeutic strategy.42,43

Future
With greater ability to understand and control the mechanisms involved in programmed cell death, both scientists and clinicians will be able to initiate novel therapies in multiple disease processes and even begin to intervene in physiologic conditions such as aging.44 From the oncologists’ point of view, targeting the myriad of tumor mechanisms of cell death evasion may become onpowerful tools in the future of anti-neoplastic therapy.45 The era of widespread clinical implementation of apoptotic mode treatment has not yet arrived, but it has the potential for tremendous impact on the prognosis of many important and challenging diseases.

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