Apoptosis: Its Physiological Implication And Therapeutic Possibilities


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Abstract: Apoptosis is the process of programmed cell death (PCD) which usually occurs in multicellular organisms. In this case, biochemical events leads to morphological cell changes and death. Some of these changes are blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation and chromosomal Deoxyribonucleic Acid (DNA) fragmentation. Apoptosis is however distinct from necrosis which is a form of traumatic cell death that results from acute cellular injury. Apoptosis generally confers advantages during an organism's life cycle. One of the advantages can be seen in the differentiation of fingers and toes in a developing human embryo. This occurs because cells between the fingers apoptose and causes the digits to be separate. Unlike necrosis, apoptosis produces cell fragments called apoptotic bodies that phagocytic cells are able to engulf and quickly remove before the contents of the cell can spill out onto surrounding cells and cause damage. Also, between 50 and 70 billion cells die each day due to apoptosis in the average human adult. For an average child between the ages of 8 and 14, approximately 20 billion to 30 billion cells die a day. In addition to its importance as a biological phenomenon, defective apoptotic processes have been implicated in an extensive variety of diseases whereby excessive apoptosis causes atrophy and an insufficient apoptosis results in uncontrolled cell proliferation leading to cancer or tumour.

Key Words: Apoptosis, physiological Implications, Therapeutic possibilities.

1. Introduction And Literature Review

1.1 Introduction and Definition of Apoptosis

Apoptosis, which is Programmed Cell Death (PCD), 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 (Martin, 1993; Earnshaw, 1995). Apoptosis is an indispensable aspect of normal development and continues even into adulthood. The human body is composed of approximately 10^{14} cells and every day, billions of these cells die an altruistic death in order to secure the functionality of the whole organism. Therefore, we remain the same size only because cell division exactly balances cell death (Raff, 1992). Furthermore, during development, cell death helps sculpt organs, separate fingers and toes and eliminates structures that once served a function but are no longer needed, such as the tail of a tadpole during amphibian metamorphosis. Most of the neurons die during development before having any chance to function in the nervous system. Cell death also eliminates most newly formed lymphocytes, especially those that are useless or dangerous, by targeting self-antigens. Neutrophils, for instance, are produced continuously in the bone marrow, but the vast majority die within a few days. This apparently futile cycle of cell proliferation and cell death serves to maintain a supply of cells that can be readily mobilized when needed (Raff, 1992).

As cell death is intimately linked to tissue homeostasis, its disruption has been implicated in numerous pathological conditions. A reasonable estimate is that either too little or too much cell death contributes to approximately half of all medical illnesses, for many of which no adequate therapy exists. Abnormalities in cell death regulation can be a significant component of diseases such as cancer, autoimmune syndromes, AIDS, ischemia, liver diseases and neurodegenerative disorders including Parkinson’s and Alzheimer’s disease (Fischer and Schulzer-Osthoff, 2005).

1.2 History of Apoptosis

German scientist Carl Vogt was first to describe the principle of apoptosis in 1842, but it was not until 1965 that the topic was resurrected. While studying tissues using electron microscopy, John Foxton Ross Kerr at University of Queensland was able to distinguish apoptosis (Greek apo - from/off/without, piostis - falling) from traumatic cell death (Kerr, 1965). Following the publication of a paper describing the phenomenon, Kerr was invited to join Alastair R Currie, as well as Andrew Wyllie, who was Currie's graduate student at University of Aberdeen. In 1972, the trio published a seminal article in the British Journal of Cancer (Kerr et al., 1972). Kerr had initially used the term programmed cell necrosis, but in the article, the process of natural cell death was called apoptosis. Kerr, Wyllie and Currie credited James Cormack, a professor of Greek language at University of Aberdeen. This term was later changed to apoptosis due to a type of error. Kerr, Wyllie and Currie received the Nobel Prize in Medicine in 2002 for their discovery of the principle of apoptosis.
of Abardeen, with suggesting the term apoptosis. Kerr received the Paul Ehrlich and Ludwig Darmstaedter Prize on March 14, 2000, for his description of apoptosis. He shared the prize with Boston biologist Robert Horvitz (O’Rourke et al., 2000).

Apoptosis is a multi-pathway cell death programme that is inherent in every cell of the body. In cancer, the apoptosis cell-division ratio is altered. Cancer treatment by chemotherapy and irradiation kills target cells primarily by inducing apoptosis. In Greek, apoptosis translates to the dropping off of petals or leaves from plants or trees.

1.3 Hyperactive Apoptosis

Loss of control of cell death which can result to excess apoptosis, can lead to neurodegenerative diseases, haematologic diseases, and tissue damage. The progression of HIV is directly linked to excess, unregulated apoptosis. In a healthy individual, the number of Cluster of Differentiation 4+ (CD4+) lymphocytes is in balance with the cells generated by the bone marrow. However, in Human Immunodeficiency Virus (HIV) positive patients, this balance is lost due to an inability of the bone marrow to regenerate CD4+ cells. In the case of HIV, CD4+ lymphocytes die at an accelerated rate through uncontrolled apoptosis, when stimulated (Wikipedia, 2013).

1.4 Apoptosis and Necrosis

Cell death by apoptosis is a normal and energy dependent pathway caused by a number of endogenous as well as exogenous stimuli. During apoptosis, decrease in cell volume, nuclear changes with chromatin condensation, margination and fragmentation followed by blebbing and breakdown of intact cell and nuclear membranes takes place. It results into the formation of small fragmented apoptotic bodies having cytoplasmic contents surrounded by cell membrane which are removed by the process of phagocytosis in the extracellular environment avoiding the inflammatory reaction.

Necrosis is an unusual and unintended process caused by external cell injury by a number of stimuli. It is characterized by the increase in cell volume followed by enlargement of cell organelles including nucleus, loss of membrane integrity and release of cellular contents which consists of certain enzymes such as hydrolases that influence the adjoining cells leading to inflammatory reaction in the adjacent tissue.

Figure 1: Diagrammatic illustration showing the morphological distinctiveness occurring during apoptosis and necrosis.

Source: Rajesh et al. (2009).
1.5 Apoptosis, Autophagy and Necrosis
As the understanding of programmed cell death has evolved, it is clear that cells can die by various mechanisms. A recent classification explained eight different types of cell death, while some researchers describe as many as eleven pathways of cell death in mammals (Martin, 2005; Melino et al., 2005). Three types of cell death have been distinguished in mammalian cells by morphological criteria, they include:
1. Apoptosis, also called type I cell death, is characterised by changes in the nuclear morphology, including chromatin condensation and fragmentation, overall cell shrinkage, blebbing of the plasma membrane and formation of apoptotic bodies that contain nuclear or cytoplasmic material.
2. Autophagic cell death, also known as type II cell death, is characterized by a massive accumulation of double-membrane containing vacuoles known as autophagosomes, which subsequently fuse with lysosome vacuoles.
3. Necrosis, also called Type III cell death, is often defined in a negative manner as death lacking the characteristics of the type I and type II processes. Necrotic cells typically show cytoplasmic swelling and vacuolation, rupture of the plasma membrane, dilation of organelles (mitochondria, endoplasmic reticulum and Golgi apparatus), as well as moderate chromatin condensation. When cells swell and burst they spill their contents over their neighbours and elicit a damaging inflammatory response (Festjens, 2006).

![Figure 2: A) Apoptosis, Autophagy and Necrosis.](source: Hans-Jürgen (2008).)

1.6 Detection of Apoptosis
Since apoptosis occurs via a tightly regulated cascade, there are many possibilities to measure the activity of these regulators or the functional consequences of their action (Figure 3). A large number of apoptosis assays for detecting and counting apoptotic cells have been devised. All of these assays have
advantages and disadvantages. For instance, certain features of apoptosis might only appear transiently, while others might partially overlap with necrosis. It is therefore crucial to employ two or more distinct assays to confirm that cell death is occurring via apoptosis. In addition, certain assays might be suitable for cultured cells, but inappropriate for investigating apoptosis in tissue sections. Therefore, when choosing methods of apoptosis detection in cells, tissues or organs, we should understand the pros and cons of each assay.

During karyorrhexis, endonuclease activation leaves short DNA fragments, regularly spaced in size. These give a characteristic laddered appearance on agar gel after electrophoresis. Tests for DNA laddering differentiate apoptosis from ischemic or toxic cell death (Iwata et al., 1994).

Figure 3: Methods to detect apoptosis. Hallmarks of apoptosis include caspase activation, DNA fragmentation as well as alterations of the plasma membrane and mitochondria, which can be assessed by a variety of methods in cell lysates, cell culture or tissue biopsies.


II. Apoptotic Process

2.0 Introduction

Apoptosis results from a collapse of the cellular infrastructure through internal proteolytic digestion, which leads to cytoskeletal disintegration, metabolic derangement and genomic fragmentation (Taylor et al., 2008). Following an appropriate stimulus, the first stage or decision phase of apoptosis is the genetic control point of cell death. This is followed by the second stage or execution phase, which is responsible for the morphological changes of apoptosis.

The process of apoptosis is controlled by a diverse range of cell signals, which may originate either extracellularly (extrinsic inducers) or intracellularly (intrinsic inducers). Extracellular signals may include toxins (Popov et al., 2002) hormones, growth factors, nitric oxide (Brune, 2003) or cytokines, that must either cross the plasma membrane or transduce in order to effect a response. These signals may positively (i.e., trigger) or negatively (i.e., repress, inhibit, or dampen) affect apoptosis.

2.1 Mitochondrial Regulation

The mitochondria are crucial to multicellular life because, without them, a cell ceases to respire aerobically and dies quickly. This fact forms the basis for some apoptotic pathways, where apoptotic proteins that target mitochondria affect them in different ways. They may cause mitochondrial swelling through the formation of membrane pores, or they may increase the permeability of the mitochondrial membrane and cause apoptotic effectors to leak out (Cotran et al., 1998). There is also a growing body of evidence indicating that nitric oxide is able to induce apoptosis by helping to dissipate the membrane potential of mitochondria and therefore make it more permeable (Brune, 2003). Nitric oxide has been implicated in initiating and inhibiting apoptosis through its possible action as a signal molecule of subsequent pathways that activate apoptosis (Brune et al., 1999). Mitochondrial proteins known as small mitochondria-derived activator of caspases (SMACs) are released into the cytosol following an increase in permeability. SMAC binds to inhibitor of apoptosis proteins (IAPs) and deactivates them, preventing the IAPs from arresting the apoptotic process and therefore allowing apoptosis to proceed. IAP also normally suppresses the activity of a group of cysteine proteases called caspases (Fesik and Shi, 2001), which carry out the degradation of the cell, therefore the actual degradation enzymes can be seen to be indirectly regulated by mitochondrial permeability.

Cytochrome c is also released from mitochondria due to formation of a channel called the mitochondrial apoptosis-induced channel (MAC), in the outer mitochondrial membrane (Laurent et al., 2006).
and serves a regulatory function as it precedes morphological change associated with apoptosis (Cotran, 1998). Once cytochrome c is released it binds with Apoptotic protease activating factor - 1 (Apaf-1) and Adenosine Triphosphate (ATP), which then bind to pro-caspase-9 to create a protein complex known as an apoptosome. The apoptosome cleaves the pro-caspase to its active form of caspase-9, which in turn activates the effector caspase-3.

2.2 Decision Phase

Apoptosis is controlled genetically where two genes, Bcl-2 and p53 are important. The first, Bcl-2, is a family of genes that regulates apoptosis (Tsujimoto et al., 1985; Cleary et al., 1986; Vaux et al., 1992; Brown, 1996); found on the mitochondrial membrane and endoplasmic reticulum where it may control calcium channels. It is now recognised that there is a family of mammalian proteins similar to Bcl-2 that promotes or inhibits apoptosis (Hockenbery et al., 1993; Yang et al., 1997). Proteins such as Bcl-2 and Bcl-xL prevent apoptosis, whereas Bcl-2 associated x proteins (Bax) such as Bax, Bad, Bak and Bcl-xS promote apoptosis (Olivetti et al., 1997; Haunstetter and Izumo, 1998; Savitz et al., 1998).

2.3 Execution

Many pathways and signals lead to apoptosis, but there is only one mechanism that actually causes the death of a cell. After a cell receives stimulus, it undergoes organized degradation of cellular organelles by activated proteolytic caspases. A cell undergoing apoptosis shows a characteristic morphology:

1. Cell shrinkage and rounding are shown because of the breakdown of the proteinaceous cytoskeleton by caspases (Bohm, 2003).
2. The cytoplasm appears dense, and the organelles appear tightly packed.
3. Chromatin undergoes condensation into compact patches against the nuclear envelope (also known as the perinuclear envelope) in a process known as pyknosis, a hallmark of apoptosis (Santos et al., 2000; Madeleine et al., 2001).
4. The nuclear envelope becomes discontinuous and the Deoxyribonucleic acid (DNA) inside it is fragmented in a process referred to as karyorrhexis. The nucleus breaks into several discrete chromatin bodies or nucleosomal units due to the degradation of DNA (Nagata, 2000)
5. The cell membrane shows irregular buds known as blebs.
6. The cell breaks apart into several vesicles called apoptotic bodies, which are then phagocytosed. Apoptosis progresses quickly and its products are quickly removed, making it difficult to detect or visualize.

Figure 4: The two major routes to apoptosis. The intrinsic mitochondrial pathway is triggered in response to various forms of cellular stress, such as DNA damage, which provokes the activation of one or more proapoptotic BH3-only proteins.

2.4 Removal of Dead Cells

The removal of dead cells by neighboring phagocytic cells has been termed efferocytosis (Vandivier, 2006). Dying cells that undergo the final stages of apoptosis display phagocytotic molecules, such as phosphatidylserine, on their cell surface (Li et al., 2003). Phosphatidylserine is normally found on the cytosolic
surface of the plasma membrane, but is redistributed during apoptosis to the extracellular surface by a protein known as scramblase (Wang et al., 2003). These molecules mark the cell for phagocytosis by cells possessing the appropriate receptors, such as macrophages (Savill et al., 2003). Upon recognition, the phagocyte reorganizes its cytoskeleton for engulfment of the cell. The removal of dying cells by phagocytes occurs in an orderly manner without eliciting an inflammatory response (Savill et al., 2003).

III. Physiological Implication And Relevance Of Apoptosis

3.1 Central Nervous System

During embryonic development of the nervous system, surplus of cells are produced. PCD eliminates those neurons whose axons fail to reach the target. It occurs with the withdrawal of trophic substances, such as nerve growth factor, or with a loss of synaptic contact or afferent input (Louis et al., 1993). Oxidative stress, glutamate excitotoxicity and calcium influx can induce apoptosis in the mature central nervous system.

3.2 Viral Infection

Many viruses inhibit apoptosis in their target cells thereby prolonging host cell life and in order to permit viral replication. Clem et al. (1996) explained that Viruses such as the baculovirus IAPs and the baculovirus p35 may encode anti-apoptotic proteins, which promotes the development of certain Cancers. DNA viruses like papilloma virus and adenovirus also contain anti-apoptotic genes that encode a p53 inhibitor.

Human immunodeficiency virus (HIV) infection is characterised by a decreased proliferation of T cells with loss of CD4+ cells initially, and loss of CD8+ cells, natural killer cells and neurons later. Inappropriate induction of apoptosis in HIV-infected CD4+ cells is triggered by the virus (Terai et al., 1991).

3.4 Immune System

Usually, any dysfunction of the apoptotic pathway causes autoimmunedisease, immunodeficiencies and lymphoid malignancies. During development, large numbers of precursor cells from the bone marrow migrate to the thymus. The majority (90-95%) fail to produce T-cell receptor (TCR) and die via the apoptotic pathway (Surh and Sprent, 1994).

3.5 Renal System

Embryological development of the kidney involves periods of growth and apoptosis which are reflected by the levels of Bcl-2 present (Savill, 1994). Mice deficient in Bcl-2 develop polycystic kidney disease (Veis et al., 1993), whereas, Bcl-2 levels are high in all renal tumours (Chandler et al., 1994).

3.6 Gastrointestinal Tract

Gastrointestinal diseases may be associated with excessive or defective apoptosis. Shigella dysenteriae causes excessive apoptosis of macrophages in the lamina propria of the intestine. Progressive inhibition of apoptosis appears to be involved in the pathogenesis of gastrointestinal neoplasia, in particular colorectal cancer (Bedi et al., 1995).

3.7 Cancer

Evidence shows that failure to initiate apoptosis following DNA damage may cause cancer (Spencer and Groudine, 1991). Other mutations may also be involved in carcinogenesis. A notable example involves the tumour suppressor p53 that represses Bcl-2 expression. The p53 gene is deficient in over half of human cancers (Lowe et al., 1994).

3.8 Reproductive System

Apoptosis is continually inhibited in many tissues of the reproductive system owing to the presence of trophic hormones from the pituitary, gonads(testes and ovary) and uterus. When the hormones are removed, the tissues undergo atrophy. Ovarian follicles undergo growth or atresia in response to cyclic changes in luteinizing hormone and follicle stimulating hormone; the endometrium, breast and prostate are dependent on the steroid hormones and regress when these are removed (Gosden and Spears, 1997).

IV. Therapeutic Possibilities And Future Directions

4.1 Inflammatory Disease

Corticosteroids induce eosinophil apoptosis but inhibit neutrophil apoptosis. The treatment of asthmatic patients using corticosteroids causes eosinophil death and macrophage engulfment (Meagher et al., 1996). The detection of this process in airway secretions of asthmatic patients is associated with clinical improvement (Wooley et al., 1996).
4.2 Gastrointestinal Tract
Cytotoxic drugs induce apoptosis in studies of human gastrointestinal cancer cells as well as normal mouse intestine, which may account for their therapeutic action. Chronic ingestion of nonsteroidal anti-inflammatory drugs may be useful in preventing colon cancer, possibly by induction of apoptosis (Pritchard and Watson, 1996).

4.3 Cancer
Kerr et al. (1994) found that anticancer agents induce apoptosis in tumours. Chemotherapeutic agents reported to induce apoptosis include the alkylating agents (cyclophosphamide, mitomycin C, nitrogen mustard), topoisomerase II inhibitors (daunorubicin, Adriamycin), dexamethasone, antimetabolites (methotrexate, 5-fluorouracil, 5-azacytidine), cisplatin, microtubule disrupters (vincreistine, vinblastine, taxol), cycloheximide, bleomycin, cisplatin, tamoxifen and cytosine arabinose (McConkey et al., 1996; Savitz et al., 1998).

Irradiation and cytotoxic agents produce DNA damage which predisposes to p53 enhancement of apoptosis. If p53 is defective, then resistance to chemotherapy may result (Bellamy, 1997). The activation of the p53 pathway in neoplasms to regain chemosensitivity is a potentially powerful therapeutic tool that can render the tumour apoptotic. This may be possible through p53 gene-specific therapy. Such therapy has been attempted using retrovirally introduced wild-type p53 on non-small cell-lung cancer with encouraging results (Bellamy, 1997). Nicotine has been shown to suppress apoptosis in lung cancer in humans (Maneckgee et al., 1994).

V. Conclusion
Apoptosis which is a complicated phenomenon, involves an energy-dependent flow of molecular events accomplished by two types of pathways such as intrinsic and extrinsic which involves the activation of a set of cysteine proteases known as caspases. The process of cell death by means of apoptosis (PCD) and necrosis (Accidental Cell Death) is accompanied by a number of distinctive morphologic and metabolic changes. However, apoptosis plays a significant role in survival by maintaining homeostasis in multicellular organisms and the management of many diseases. Evidence has shown that the malfunctioning of apoptotic pathway may cause several human diseases like cancer, neurodegenerative as well as several types of autoimmune disorder. Presently, large numbers of synthetic and natural compounds have been discovered to be pharmacologically effective against certain diseases through the induction of apoptosis in their target cells (e.g. cancerous cells). These compounds may promote the development of novel remedy based on the inflection of apoptosis. As of now, the basic mechanisms of apoptosis have been established, but its implications for therapeutic purposes have still to be worked out.

Acknowledgement
I am indebted to Mr Asogwa Chinweike who was the brain behind the suggestion of this topic and the proof-reading of the work. I also want to thank my best friend, Mr. Teleola Oyeleke for his support and unflinching love throughout the writing up of the work.

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