Apoptosis: Insights into Pathways and Role of p53, Bcl-2 and Sphingosine Kinases

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Abstract Apoptosis or programmed cell death is characterized by certain morphological and biochemical characteristics. It is a vital process for normal cell turnover, proper development and functioning of the immune system, embryonic development and chemical-induced cell death. Disturbances of apoptosis play a key role in many diseases including neurodegenerative diseases, autoimmune disorders and many types of cancer. The field of apoptosis research has been moving forward at a rapid rate. Although many of the key proteins that play a role in apoptosis have been identified, the molecular mechanisms of action of these proteins had not been fully elucidated. The aim of this mini-review is to provide a general overview on the process of apoptosis including pathways, regulation, the role of apoptosis in health and disease as well as the role of p53, Bcl-2 and sphingosine kinase-1 in apoptosis.

Keywords: apoptosis, programmed cell death, cancer, p53, Bcl-2, sphingosine kinase-1

1. Introduction

Apoptosis is a process of programmed cell death that occurs in multicellular organisms in which biochemical events lead to characteristic cell changes and death. These changes include cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation and mRNA decay. Between 50 and 70 billion cells die each day due to apoptosis in the average human adult [1].

In contrast to necrosis, which is a form of traumatic cell death that results from acute cellular injury, apoptosis is a highly regulated and controlled process that has an important role during an organism's lifecycle [2]. For example, the separation of fingers and toes in a developing human embryo occurs because cells between the digits undergo apoptosis. 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 to surrounding cells and cause damage [3].

2. Regulation of Apoptosis

Because apoptosis cannot stop once it has begun, it is a highly regulated process. Apoptosis can be initiated through one of two pathways. In the intrinsic pathway the cell kills itself because it senses cell stress, while in the extrinsic pathway the cell kills itself due to signals from other cells [4]. Both pathways induce cell death by activating caspases, which are proteases, or enzymes that degrade proteins. The two pathways activate initiator caspases, which then activate executioner caspases, which then kill the cell by degrading its proteins [1].

In addition to its importance as a biological phenomenon, defective apoptotic processes have been implicated in a wide variety of diseases. Excessive apoptosis causes atrophy, while an insufficient apoptosis results in uncontrolled cell proliferation leading to cancer. Some factors like Fas receptors and caspases promote apoptosis, while some members of the Bcl-2 family of proteins inhibit apoptosis [5].

3. Activation Mechanisms of Apoptosis

The initiation of apoptosis is regulated by activation mechanisms. The two best-understood activation mechanisms are the intrinsic pathway (also called the mitochondrial pathway) and the extrinsic pathway [6]. The intrinsic pathway is activated by intracellular signals generated when cells are stressed and depends on the release of proteins from the intermembrane space of mitochondria. The extrinsic pathway is activated by extracellular ligands binding to cell-surface death receptors, which leads to the formation of the death-inducing signaling complex [1].

A cell initiates intracellular apoptotic signaling in response to cellular stress. The binding of nuclear receptors by glucocorticoids, heat, radiation, starvation, viral infection, hypoxia and increased intracellular calcium concentration can stimulate the release of intracellular apoptotic signals by the damaged cells. A number of cellular components, such as poly ADP ribose polymerase, may also help regulate apoptosis [7].

The apoptotic signals cause the regulatory proteins to initiate the apoptosis pathway. Several proteins are involved, but two main methods of regulation have been identified: the targeting of mitochondria functionality or directly transducing the signal via adaptor proteins to the apoptotic mechanisms [8]. Caspases play the central role in the transduction of the apoptotic signals. Caspases are proteins that are highly conserved, cysteine-dependent aspartate-specific proteases. There are two types of caspases: initiator caspases, caspase 2, 8, 9, 10, 11, 12, and effector caspases, caspase 3, 6, 7. The activation of the initiator caspases requires binding to specific activator protein. Effector caspases are then activated by these active initiator caspases through proteolytic cleavage. The active effector caspases then degrade the intracellular proteins to carry out apoptosis. Moreover, there is a caspase-independent apoptotic pathway that is mediated by apoptosis-inducing factor [5].
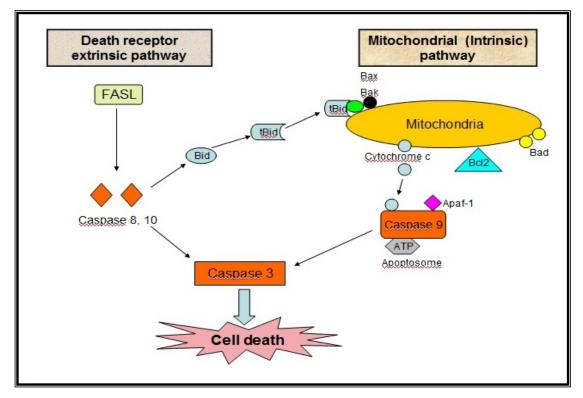

Figure 1. Pathways of apoptosis

4. Bcl-2 and Apoptosis

Bcl-2 (B-cell lymphoma 2), encoded in humans by the Bcl-2 gene, is the founding member of the Bcl-2 family of regulator proteins that regulate programmed cell death (Apoptosis). Bcl-2 derives its name from B-cell lymphoma 2, as it is the second member of a range of proteins initially described in chromosomal translocations involving chromosomes 14 and 18 in follicular lymphomas [9]. Bcl-2 is found on the outer membrane of mitochondria, where it plays an important role in promoting cellular survival and inhibiting the actions of pro-apoptotic proteins. The pro-apoptotic proteins in the Bcl-2 family, including Bax and Bak, normally act on the mitochondrial membrane to promote permeability and release of cytochrome c and ROS, that are important signals in apoptosis. These pro-apoptotic proteins are inhibited by the function of Bcl-2 and its relative Bcl-Xl [10].

There are additional roles of Bcl-2 that are being explored. Bcl-2 is known to regulate mitochondrial dynamics, and is involved in the regulation of mitochondrial fusion and fission. Additionally, in pancreatic beta-cells, Bcl-2 may be involved in controlling metabolic activity and insulin secretion [11]. Damage to the Bcl-2 gene has been identified as a cause of a number of cancers, including melanoma, breast, prostate, chronic lymphocytic leukemia and lung cancer, and a possible cause of schizophrenia and autoimmunity. It is also a cause of resistance to cancer therapy [12].

Cancer can be considered as a disturbance in the homeostatic balance between cell growth and cell death. Over-expression of the anti-apoptotic genes, and under-expression of the pro-apoptotic genes, can result in lack of cell death that is characteristic of cancer. Simultaneous over-expression of the anti-apoptotic Bcl-2 protein and the proto-oncogene myc may produce aggressive B-cell malignancies including lymphoma [13]. In follicular lymphoma, a chromosomal translocation commonly occurs which places the Bcl-2 gene from chromosome 18 next to the immunoglobulin heavy chain locus on chromosome 14. This fusion gene is deregulated, leading to expression of excessively high levels of Bcl-2 which inhibits apoptosis of these cells and favors the development of malignancy [14,15].

5. Implications of Apoptosis in Various Diseases

The many different types of apoptotic pathways contain a multitude of different biochemical components, many of them not yet understood. The tumor-suppressor protein p53 accumulates when DNA is damaged due to a chain of biochemical factors [16]. Part of this pathway includes alpha-interferon and beta-interferon, which induce transcription of the p53 gene, resulting in the increase of p53 protein level and enhancement of cancer cell-apoptosis. P53 stops the cell cycle at G1 phase, to give the cell time to repair. However, it will induce apoptosis if damage is extensive and repair efforts fail. Any disruption to the regulation of the p53 or interferon genes will result in impaired apoptosis and the possible formation of tumors [17].

Inhibition of apoptosis can result in a number of cancers, autoimmune diseases, inflammatory diseases, and viral infections. Apoptosis in HeLa cells is inhibited by proteins produced by the cell which target retinoblastoma tumor-suppressing proteins. These tumor-suppressing proteins regulate the cell cycle, but they become inactive when bound to an inhibitory protein [18]. HPV E6 and E7 are inhibitory proteins expressed by the human papilloma virus (HPV) being responsible for the formation of the cervical tumor from which HeLa cells are derived. HPV E6 causes p53, which regulates the cell cycle, to become inactive. HPV E7 binds to retinoblastoma tumor suppressing proteins and limits its ability to control cell division [19].

On the other hand, loss of control of cell death (resulting in excess apoptosis) can lead to neurodegenerative diseases, hematologic diseases and tissue damage [20]. The progression of human immunodeficiency virus (HIV) infection is directly linked to excess unregulated apoptosis. In a healthy individual, the number of CD4+ lymphocytes is in balance with the cells generated by the bone marrow. However, in HIV-positive patients, this balance is lost due to the inability of the bone marrow to regenerate CD4+ cells. In HIV patients, CD4+ lymphocytes die at an accelerated rate through uncontrolled apoptosis [21].

Viral induction of apoptosis occurs when one or several cells of a living organism are infected with a virus, leading to cell death [22]. Viruses can induce apoptosis of infected cells by several mechanisms including receptor binding, activation of protein kinase R (PKR), interaction with p53 or expression of viral proteins coupled to major histocompatibility proteins on the surface of the infected cells, allowing recognition by cells of the immune system (Such as natural killer and cytotoxic T cells) that then induce the infected cell to undergo apoptosis [23].

6. P53, Apoptosis and Cancer

P53 protein is a sequence-specific DNA-binding protein that is able to induce either cell cycle arrest or apoptosis at cell cycle checkpoints in damaged or transformed cells. It plays a crucial role in the cellular events that occur following DNA damage induced by exposure to ultraviolet (UV) radiation. Following exposure to UV radiation and DNA damage, cells increase production of P53 leading to cell-cycle arrest, DNA repair and, if the damage is too extensive to be corrected, apoptosis [17]. Mutations in P53 gene would result in inappropriate expression of S-phase genes. This, in turn, can lead to replication of damaged genes or chromosomes, a hallmark of cancer cells. It is reported that more than 50% of all cancers possess mutations in the P53 gene, making it the most frequently mutated gene in human cancers [24].

When the tumor suppressor genes such as p53 are inactivated, the cells become unable to respond normally to cell-cycle checkpoints and also become unable to undergo programmed cell death if DNA damage is extensive. This might lead to further increase in mutations and to the inability of the affected cell to leave the cell cycle when it should become quiescent and these cells may become tumorigenic. As examples of inactivation of tumor suppressor genes involved in cell cycle regulation are abnormalities affecting cyclin-dependent kinase inhibitors, or checkpoint regulators such as p53 proteins [25].

7. Sphingosine Kinases, Apoptosis and Cancer

Sphingolipids play a critical role in the cell biological functions. Among them, ceramide and sphingosine induce apoptosis and inhibit cell proliferation while sphingosine 1-phosphate (S1P) inhibits apoptosis and promotes cell survival and proliferation. The balance between ceramide/sphingosine and S1P forms the so-called "sphingolipid-rheostat", which decides the cell fate [26]. Sphingosine kinases are a group of enzymes that catalyze the phosphorylation of sphingosine to S1P [27]. There are two isoforms of sphingosine kinases known as SphK1 and SphK2 [28]. SphK1 was proven to increase the production of the proinflammatory cytokines such as TNF-α and IL-6 through activation of STAT3 and NF-κB which may participate in proliferation and growth of cancer cells [29]. Moreover, S1P which is produced by the action of SphK1 was proven to inhibit apoptosis, possibly through its effect on Phosphatidylinositol 3-Kinase/Akt pathway [30].

From the above mentioned data, inhibition of SphK1 activity might represent an effective targeted therapy for cancer. Nagahashi et al. [31] reported that specific inhibitor of SphK1 can suppress tumor-induced angiogenesis and lymphangiogenesis and can induce apoptosis. Paugh et al. [32] suggested that a selective sphingosine kinase 1 inhibitor may integrate multiple molecular therapeutic targets in human leukemia. The most prominent mechanisms of this effect were enhanced apoptosis and cleavage of Bcl-2 together with alteration of the Akt pathway. Also, Xu et al. [33] reported that concurrent targeting of Akt and SphK1 may inhibit the growth of tumor cells, possibly through affection of ceramide production, and subsequent cell death and apoptosis.

8. Conclusion

Apoptosis is a regulated energy-dependent process, characterized by specific morphological and biochemical

features in which caspase activation plays a key role. Although many proteins are involved in the apoptotic pathways, the molecular mechanisms of action of these proteins have not been fully understood. Sphingosine kinase-1, p53 and Bcl-2 play a crucial role in understanding the mechanistic machinery of apoptosis. Understanding the mechanisms of apoptosis at the molecular level provides deeper insights into various disease processes and may have a major influence on the therapeutic strategies.

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