

## Cell Death: Mechanisms and Pathways in Cancer Cells

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### Abstract

Programmed cell death is an essential physiological and biological process for the proper development and functioning of the organism. Apoptosis is the term that describes the most frequent form of programmed cell death and derives from the morphological characteristics of this type of death caused by cellular suicide. Apoptosis is highly regulated to maintain homeostasis in the body, since its imbalances by increasing and decreasing lead to different types of diseases. In this review, we aim to describe the mechanisms of cell death and the pathways through apoptosis is initiated, transmitted, regulated, and executed.

**Keywords:** *Apoptosis; Cell death; Extrinsic/ intrinsic pathway; Cancer*

**Received Date:** September 09, 2018; **Accepted Date:** October 05, 2018; **Published Date:** October 12, 2018

### Mechanisms of Cellular Death

In differentiated cells, it is necessary to control both death and cell division, in order to maintain a balance of the different cell populations, avoiding affecting the adjacent cells [1]. For this reason the apoptotic physiological process is produced, characterized by the decrease in cell size, vesicle formation and condensation of the nucleus. This series of transformations regulates the control of morphogenesis and organogenesis during embryonic development, in addition to tissue homeostasis in adult organisms [2].

Kerr et al. [2], almost half a century ago, defined apoptosis as a form of programmed cell death [3,4], which occurs as a consequence of tissue and cellular aging, or in response to different external agents such as ionizing radiation and agents chemotherapy [3,5,6]. It can be considered as a process that facilitates the elimination of defective cells, therefore the alteration in the regulation of genes involved in cell death by apoptosis can be cause and be associated with the development of different neoplasms, autoimmune diseases, viral infections and neurodegenerative diseases [5,7,8].

### Differentiation between apoptosis and necrosis

The alternative to apoptotic cell death is necrosis, which is considered a toxic process in which the cell is a passive victim and follows a mode of death independent of energy [9]. However, apoptosis is an active process, where the cells react and execute their death, programmed, by themselves [6,10].

**Citation:** Diego Fernández Lázaro, Cell Death: Mechanisms and Pathways in Cancer Cells. Cancer Med J 2018; 1(1) 12-23.  
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Although the mechanisms and morphologies of apoptosis and necrosis are different, there is an overlap between these two processes. The evidence indicates that necrosis and apoptosis represent morphological expressions of a shared biochemical network described as the "continuum of apoptosis-necrosis" [11]. Cell death due to necrosis or apoptosis depends in part on the nature of the biochemical signal of cell death, the type of tissue, the stage of tissue development and the physiological environment in which it is found [9,11].

Despite the use of conventional histology techniques, it is not always easy to distinguish apoptosis from necrosis, and they can occur simultaneously depending on factors such as the intensity and duration of the stimulus, the extent of energy depletion, and the availability of caspases enzymes [11]. Necrosis is an uncontrolled and passive process that generally affects large cell fields, which is mediated by two main mechanisms; interference with the cell's energy supply and direct damage to cell membranes. Conversely, apoptosis is controlled and energy dependent and can affect individuals or groups of cells [6].

### **Morphological changes in cell death**

Cell death occurs in two ways: apoptosis or necrosis. The apoptosis is physiological while the necrosis is pathological. In necrosis, the temporal sequence of the changes establishes that they occur first in the mitochondria, while the changes in the nucleus are much later and smaller. These alterations are followed by the dissolution of cellular organelles and the loss of selective permeability of the cell membrane. As a result, edema formation occurs and intracellular contents are released. As the lysosomes rupture, their hydrolase enzymes are released, which cause the degradation of the surrounding cells and a strong tissue inflammatory response [12]. It can be noted that the most important morphological changes occurring simultaneously with the inflammatory process are: the formation of cytoplasmic vacuoles; the distended endoplasmic reticulum; formation of cytoplasmic blisters; condensed, swollen or broken mitochondria; disaggregation and detachment of ribosomes; broken organic membranes; swollen and broken lysosomes; and finally, the alteration of the cell membrane [2,12,13].

When cell death is due to apoptosis, there are two stages to understanding it. In the first stage, the biochemical mediators try to repair a damaged cell. If they fail, the cell enters the second stage or execution phase where structural changes occur that lead the cell to death [7,12]. In 1972, these changes were described by Kerr et al. [2] in three morphological levels: nuclear; of cellular membrane and intra-cytosolic organelles. At the nuclear level, the chromatin becomes dense groups that move towards the nuclear membrane and although the nuclear membrane remains intact, the redistribution of nuclear pores occurs. Changes in the nuclear protein are also observed. In mitochondria there is degradation of DNA. The endoplasmic reticulum loses its structure. The cytoplasmic membrane of the apoptotic cell deforms and develops bubble formation. In the endoplasmic reticulum, the cisterns widen and fuse. The phospholipids of the cell membrane change their orientation and are exposed to the external environment. The cell membrane fragment forms apoptotic bodies that are actually cytoplasmic remains surrounded by a cell membrane. When the apoptotic bodies are released in an external environment, they are swallowed by phagocytes. As a result, there is no inflammatory reaction. At the molecular level there is the activation of proteolytic enzymes that provide for the cleavage of DNA into oligonucleosomal fragments and the excision of a multitude of specific protein substrates [12,14,15]. In parallel, a series of biochemical processes are produced in the cell that include a decrease in the mitochondrial membrane potential, loss of the asymmetry in the composition of the phospholipids of the plasma membrane, with exposure to the exterior of phosphatidylserine residues and DNA fragmentation, due to the activation of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  dependent endonucleases that cut genomic DNA through the internucleosomal spaces. Finally, membrane-covered nuclear fragments, called apoptotic bodies, are formed and phagocytosed without evidence of inflammatory reaction [7,16].

## **Molecular events**

At the molecular level, apoptosis constitutes a complex series of events with multiple regulators, both positive and negative, and integrated into other intracellular pathways as important as progression in the cell cycle, signals mediated by phosphorylation and repair of DNA damage [6]. Apoptosis is mainly carried out in two different ways. These two alternative ways of induction of apoptosis are divided into: 1) Apoptosis mediated by death receptors expressed on the cell surface or extrinsic pathway; and 2) Apoptosis mediated by the mitochondria or intrinsic pathway. Signaling by both pathways induces the activation of members of a family of proteins known as caspases (Caspases: Cysteine Aspartate-specific Proteases) [7,17], which specifically cut proteins in cysteine residues located close to aspartic acid. Caspases initiate a cascade of events that converge within a common pathway of effector caspases that lead to the execution of apoptosis, which results in the biochemical and morphological changes characteristic of this phenomenon described previously [18,19]. In addition, the intrinsic pathway also induces cell death through a caspase-independent pathway [20].

## **Chemotherapy and apoptosis**

Apoptosis plays an important role in the cellular response to chemotherapy, since most chemotherapeutic agents exert their anti-tumor effect through the induction of apoptosis [21-23]. In this sense, resistance to apoptosis is a cause of the decrease in the sensitivity of tumor cells to chemotherapeutic agents and the induction of apoptosis by chemotherapy is associated with the activation of pro-apoptotic genes and the suppression of anti-apoptotic genes, whereas the attenuation of pro-apoptotic genes and the increase of anti-apoptotic genes causes resistance to apoptosis [15,20]. In general, anti-apoptotic mechanisms do not prevent the entry of the drug into cells, inhibit the production of cell damage by the chemotherapeutic agent, or block its effects on proliferation, but these do not result in cell death. The survival mechanisms of the cell would allow, later, at the end of the treatment, the repair of the damage suffered. Although the identified genes are increasingly numerous that participate in the regulation, initiation and execution of apoptosis, and their corresponding proteins, in this introduction we will focus specifically on some proteins that intervene in the regulation of apoptosis through the extrinsic pathway and the intrinsic pathway that includes dependent and caspase-independent apoptosis [24-27].

## **Pathways of Death Cellular**

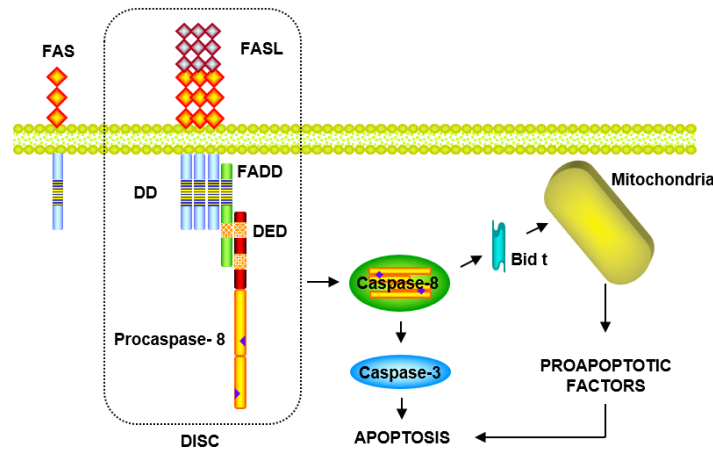
### **Extrinsic path: Recipients of death**

The extrinsic pathway is activated by ligands of the TNF-family that, when bound to their receptors, trigger caspase activation and apoptosis [28]. Death receptors are characterized by having extracellular domains rich in cysteine. All have in common a domain DD (Death Domain) in the cytoplasmic region. In general, the binding of ligands to death receptors induces their trimerization, and subsequently the DD domains recruit adapter molecules that will subsequently activate Caspase-8 and, when activated, activate Caspase-3 [28]. The best-studied death receptors are Fas (Apo-1 or CD95), TNF-R (Tumor Necrosis Factor Receptor) and TRAIL-R (TNF-Related Apoptosis-Inducing Ligand Receptor) [16,29,30].

### **Fas**

Fas/Apo-1/CD95 is ubiquitously expressed on the surface of the cell, is a membrane protein of 40 KDa, which is highly expressed in T lymphocytes and activated NK cells (Natural Killer) [29]. The Fas/FasL system participates in the elimination of T and B lymphocytes, of cells infected by viruses and of cancer cells. This path is initiated by the formation of the DISC complex (Death-Inducing Signaling Complex) in which an adapter molecule called FADD (Fas Associated Death Domain) and procaspase-8 intervenes. FADD binds to Fas through their respective DD domains and to procaspase-8 through a DED

domain (Death Effector Domain). The oligomerization of procaspase-8 in the DISC complex results in the activation of Caspase-8 and the subsequent activation of other caspases. Depending on the cell type, Caspase-8 can directly activate Caspase-3 or proteolyze the carboxy-terminal end of Bid (BH3 Interacting Domain Death Agonist), a proapoptotic protein of the Bcl-2 family. The translocation of the truncated form of Bid to the mitochondria will activate the mitochondrial pathway [31] (Figure 1). Some cytotoxic agents, such as doxorubicin and methotrexate, activate this pathway in order to achieve cell death on malignant cells in cancer disease [32,33].



**Figure 1:** Diagram of apoptotic signals of the extrinsic pathway: mediated by FAS receptors of cell death.

## TNFR

Like Fas, the type 1 receptor for TNF- $\alpha$  (TNFR1) is ubiquitously expressed, whereas its TNF- $\alpha$  ligand is only expressed in activated macrophages and in lymphocytes in response to infections [31,34]. Upon activation, TNF binds to its receptor by trimerization of TNFR1. Subsequently, a TRADD adapter molecule (TNFR-associated death domain protein) is added that induces the association with FADD and the activation of Caspase-8. In addition to the apoptotic pathway, TNF induces other signal transduction pathways from TRADD that trigger the activation of NF- $\kappa$ B and JNK (c-Jun Kinase)/Ap-1 [31,34] (Figure 2). Although TNF- $\alpha$  was capable of selectively eliminating tumor cells, because of its high inflammatory effect, systemic administration was not tolerated.

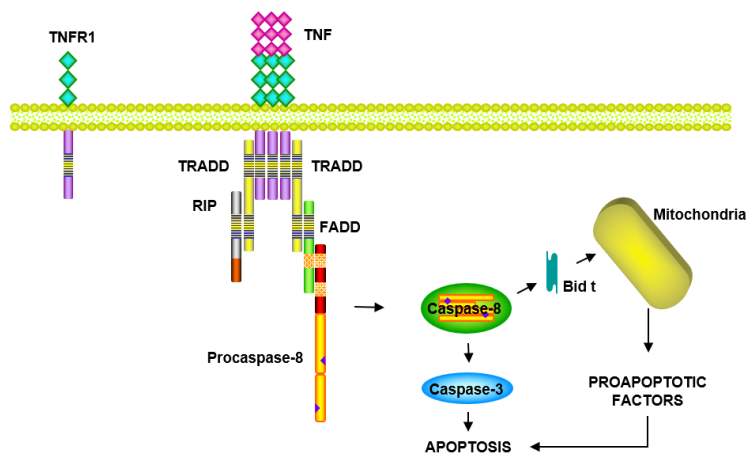
## TRAIL receptors

The expression of TRAIL (TNF-Related Apoptosis-Inducing Ligand) is found in normal cells and makes them resistant to cell death induced by TRAIL, whereas tumor cells are sensitive to this cytotoxic [17,31]. Four receptors have been described, of which two (TRAIL-R1 or DR4 and TRAIL-R2 or DR5) induce apoptosis and will determine whether a cell will be resistant or sensitive to TRAIL [17,31]. Studies in multiple myeloma (MM), on cell lines, have shown a synergism between the cytotoxic effect induced by TRAIL and etoposide [35]. In addition, in acute myeloid leukemia (AML), although the therapeutic effect of TRAIL is limited in monotherapy by the high expression of TRAIL-R3 and TRAIL-R4, it has shown some efficacy in combination with perifosine [36], bortezomib [37] and sorafenib [38] in different studies "*in vitro*" and "*ex vivo*".

## Caspases

Caspases are synthesized in the form of inactive (zymogen) 30-50 KDa precursors that have three domains: an amino-terminal domain (prodomain), one domain that will result in one large subunit and another domain that will result in a smaller subunit

[19,28]. In the presence of appropriate stimuli, there is a process of proteolysis between the domains, generating the active fragments.



**Figure 2:** Signaling pathway of the TNF receptor.

There are two types of caspases: the initiating caspases (Caspases 2,8,9 and 10) that are activated in response to signs of stress or cell damage and that protect and activate the effector caspases (Caspases 3,6 and 7), these will be responsible for the direct proteolysis of different substrates that will lead to the death of the cell. One of the first substrates identified was PARP (Poly (ADP-Ribose) Polymerase) [19,28].

The initiating caspases present in their N-terminal region one or two adapter domains that are essential for their function. In contrast, effector caspases do not have these domains. There are two fundamental ways in which caspases can be activated (intrinsic pathway and extrinsic pathway of caspase-dependent apoptosis), but although both pathways converge in effector caspases, they require different caspases to initiate the process. Thus, the activation of the extrinsic pathway mainly causes caspase 8 recruitment and the intrinsic pathway causes mainly caspase-9 recruitment [28].

The importance of caspases at the therapeutic level is evident in AML. In this malignant hemopathy, high levels of pro-caspase 3 are indicators of poor prognosis at diagnosis, which prevents the processing of caspase 3 and as a consequence to a resistance to apoptosis. However, patients who presented caspase 3 spontaneously processed at diagnosis had better survival [39].

### **IAPs, the caspase inhibitor proteins**

Proteins called IAPs (Inhibitors of Apoptosis Proteins), have the ability to inhibit apoptosis by selective binding and inhibition of caspase 3, and 7, but not caspase 8. IAPs block the caspase cascade and inhibit the cell death in response to proapoptotic stimuli [40]. There are currently 8 members of this family, but two of them, survivin and XIAP (X-linked Inhibitor of Protein Apoptosis) have received special attention.

Survivin is the only IAP that is associated with the mitotic spindle [41]. Its expression is cycle-dependent [42,43]. It has a double function, inhibits apoptosis through its direct and indirect interaction with caspases and regulates the cell cycle [44]. Survivin is expressed in embryonic tissue and is overexpressed in tumor cells, being associated with resistance to chemotherapy, but not in normal adult tissues [44,45].

XIAP is possibly the best-studied IAP both at a structural level and at the level of its mechanism of action [46]. In addition, XIAP is the only member of this family that can inhibit both effector caspases and initiators. XIAP is frequently elevated in tumor cells, causing resistance to chemotherapy [45]. XIAP, therefore, is a good therapeutic target, based on its functions, in addition, the inhibition of XIAP restores cell chemo-sensitivity [47,48].

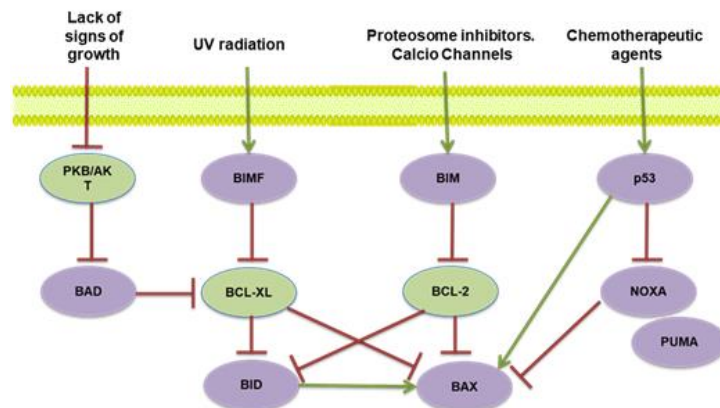
### Intrinsic route: The mitochondria

The mitochondrial death pathway is observed in response to oxidizing agents, drugs and growth factors. Initially, mitochondria were considered a passive element in cell death due to apoptosis, which only reflected damage to critical functions due to cell death. However, the fact that Bcl-2 was found in the outer mitochondrial membrane suggested that mitochondria could play a special role in apoptosis [49].

### Caspase-dependent apoptosis

#### Family of Bcl-2

The proteins of the Bcl-2 family contain at least one of the conserved domains, known as Bcl-2 homology domains (BH1-BH4) and are classified based on their function and structure in 1) Antiapoptotic proteins, which contain the BH1 and BH2 domains, 2) Proapoptotic proteins that contain the BH1, BH2 and BH3 domains, and 3) Proapoptotic proteins that contain only the BH3 domain [20,50-53]. The BH3 domain is essential for apoptotic activity. Within the first group of proteins that inhibit apoptosis and / or promote cell survival include Bcl-2, Bcl-XL and Mcl-1, among others, and are located in the outer mitochondrial membrane. Among the members of the family of Bcl-2 that induce apoptosis, with homology restricted to the BH3 domain, the following proteins are grouped: Bim (Bcl2-Interacting Protein BIM), Bad, Bid, Bnip3 (BCL2 adenovirus E1B 19 kDa-interacting protein 1 NIP3), Bax and Bak (Bcl2 Antagonist Killer). These proteins are located mainly in the cytosol and are translocated to the mitochondria in response to apoptotic stimuli (Figure 3). Location changes may be due to changes in conformation or oligomerization, such as occurs with the Bax protein, changes in its phosphorylation state described in the Bad protein or by proteolysis, as occurs in the Bid protein. Many of these proteins interact with each other forming a complex network that determines a greater or lesser susceptibility for the activation or inhibition of the activation of the intrinsic pathway of apoptosis [50,54]. The expression of the Bcl-2 oncogene plays an important role in line B lymphoid neoplasms [54]. The translocation t (14; 18) (q32; q21) leads to an abnormally increased expression of the Bcl-2 protein, which leads to the inhibition of cell death, contributing to increase the survival of B cells and thus perpetuate the Neoplastic process [54].



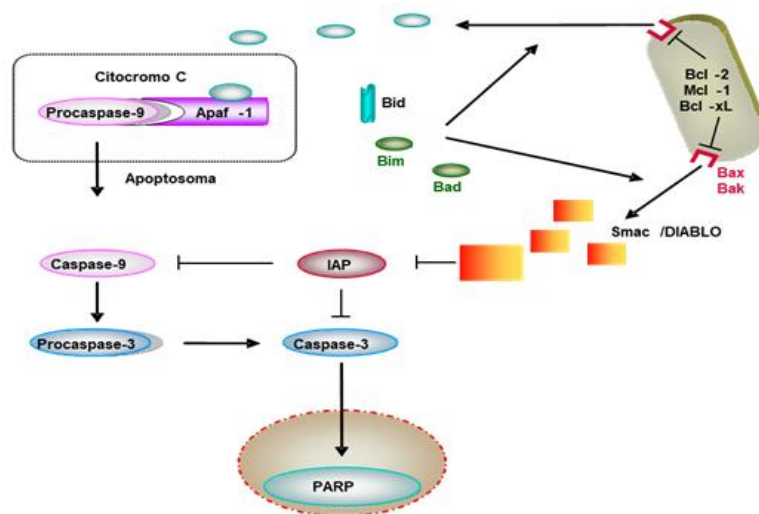
**Figure 3:** Activating signals of the mitochondrial pathway of apoptosis.

The three antiapoptotic proteins of the Bcl-2 family, Bcl-2, Bcl-XL and Mcl-1, prevent the activation of the mitochondrial apoptosis pathway, as demonstrated in MM and AML. In these neoplastic diseases such as MM, the defect of cell death pathways is frequently caused by imbalance in the expression of Bcl-2 family proteins [55]. The Bcl-2 gene has been implicated in the resistance to apoptosis induced by dexamethasone but not by melphalan in patients with MM [56,57].

Bcl-XL is expressed in most MM cell lines and patient cells; increased expression is frequently detected in the patient's relapse and correlates with resistance to chemotherapy [58]. Mcl-1 is expressed in virtually all MM lines and fresh patient cells. The induction of apoptosis in myeloma cells has been related to the decrease of Mcl-1 expression [58]. Also, for LMA, a higher expression of Bcl-2, Bcl-XL and Mcl-1 and a lower expression of Bax [59,60] increases the resistance to apoptosis in CD34+ populations than CD34- populations and is mainly due. In addition, the increase in Bcl-2 and Bcl-XL expression blocks the apoptosis induced by doxorubicin [61]. Mcl-1 levels increase in patients relapsing from AML [62,63].

### Cytochrome-C

It is a protein that participates in the electron transport chain and is located in the intermembrane space of the mitochondria. When released to cytosol it binds to the Apaf-1 protein (Apoptotic Protease Activating Factor-1) and to procaspase-9, forming the complex called apoptosome, inducing the activation of caspase-9 and caspase activation cascade, [28, 64] (Figure 4).



**Figure 4:** Intrinsic pathway of caspase-dependent apoptosis.

### Smac/DIABLO

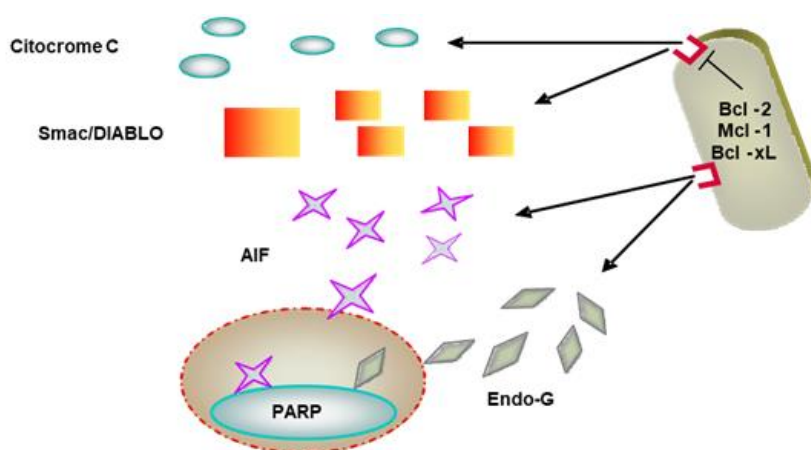
Smac/Diablo (Second Mitochondria-derived Activator of Caspase) binds by its N terminal end to the mitochondria and in the intermembranal space proteolizes leaving free the domain that allows its union with the IAPs [65]. Due to the loss of the mitochondrial potential, and at the same time that the release of Cytochrome-C occurs, Smac/Diablo is released that binds to the IAPs (XIAP, c-IAP1, c-IAP2, and survivin) in the cytoplasm and blocks them, inhibiting its function and potentiating the activation of caspases and the mitochondrial apoptosis pathway [66]. The release of Smac/Diablo is inhibited by Bcl-2 and Bcl-xL [67]. In MM cells, Smac plays a functional role in mediating the activation and apoptosis of caspase-9 induced by dexamethasone treatment [68]. It has been shown that APAF levels are low in a high proportion of patients diagnosed with AML, and that their expression pattern correlates with the induction of apoptosis [69].

### Apoptosis independent of caspases

The loss of mitochondrial potential increases the permeability of the mitochondrial membrane and the result is the release of small molecules such as Cytochrome-C and Smac/Diablo that activate the caspase-dependent pathway. But in addition to these proteins, other molecules are also released, such as AIF (Apoptosis-inducing factor) and Endonuclease G (Endo-G) that activate an independent caspase apoptosis pathway [28,64,70].

### AIF (Apoptosis inducing factor)

AIF is a highly conserved protein phylogenetically, essential for embryonic development. Like Smac, it is synthesized in the form of an immature precursor, [71] it is translocated to the mitochondria and in the intermembrane space it is proteolyzed, the mature form has oxidase activity [72,73]. In response to signals of death, AIF leaves the mitochondria and moves through the cytosol to the nucleus where it binds to the DNA causing condensation of the chromatin and fragmentation of DNA into fragments of approximately 50 Kb [71] (Figure 5).



**Figure 5:** Proapoptotic factors released from the mitochondria.

Recent studies indicate that AIF is necessary to induce PARP-dependent death. The processing and activation of PARP occurs in response to DNA damage. PARP initiates a signal in the nucleus that induces the release of AIF from the mitochondria. AIF then moves from the mitochondria to the nucleus and induces chromatin condensation and DNA fragmentation [6,74,75].

### Endonuclease G

Endo-G is an essential protein for mitochondrial DNA replication. It was isolated from the mitochondrial fraction treated with the proapoptotic active form of Bid: tBid. [76,77] Once released to the cytosol it is transferred to the nucleus where it fragments DNA, even in the presence of caspase inhibitors [76] (Figure 5). Endo-G cooperates with exonuclease and DNase facilitating DNA processing.

### Conclusion

In the human body a homeostasis is maintained between cells produced by mitosis and cell death by apoptosis. For this reason we believe that it is necessary to understand the mechanisms of apoptotic signaling, because its alteration contributes to a wide variety of diseases, one of which is cancer, which constitutes one of the most important mortality threats in world public health.



One of the fundamental characteristics of human cancers is the evasion of apoptosis, which contributes to both tumor progression and resistance to drug treatment. In this review, we explain the mechanisms of cell death, the pathways that initiate, transmit, regulate and execute apoptosis. With the future purpose of being able to develop effective and specific therapeutic approaches such as targeted activation of proapoptotic tumor suppressors or blockade of antiapoptotic oncogenes in cancer disease.

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