Apoptosis (Programmed Cell Death)

Apoptosis, or programmed cell death, is a normal process that is unique to animal cells. Apoptosis occurs through an orchestrated sequence of events that leads to the death of a cell. Death by apoptosis is a neat, orderly process characterized by the overall shrinkage in volume of the cell and its nucleus, the loss of adhesion to neighboring cells, the formation of blebs at the cell surface, the dissection of the chromatin into small fragments, and the rapid engulfment of the "corpse" by phagocytosis.

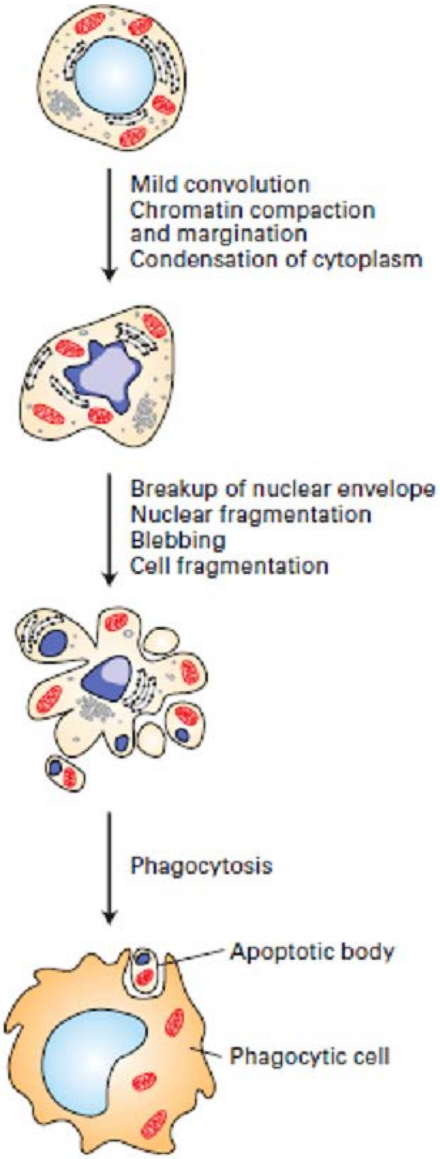

Fig- Schematic drawings illustrating the progression of morphological changes observed in apoptotic cells. Early in apoptosis, dense chromosome condensation occurs along the nuclear periphery. The cell body also shrinks, although most organelles remain intact. Later, both the nucleus and the cytoplasm fragment, forming apoptotic bodies, which are phagocytosed by surrounding cells.

Apoptosis vs Necrosis

Apoptosis is often contrasted with a different type of cell death called necrosis, which generally follows some type of physical trauma or biochemical insult. Like apoptosis, necrosis can also occur as a regulated and programmed process (called necroptosis), although much less orderly in nature. Necrosis is characterized by the swelling of both the cell

and its internal membranous organelles, membrane breakdown, leakage of cell contents into the medium, and the resulting induction of inflammation.

Comparison of morphological features of apoptosis and necrosis.

Apoptosis	Necrosis
Single cells or small clusters of cells	Often contiguous cells
Cell shrinkage and convolution	Cell swelling
Pyknosis and karyorrhexis	Karyolysis, pyknosis, and karyorrhexis
Intact cell membrane	Disrupted cell membrane
Cytoplasm retained in apoptotic bodies	Cytoplasm released
No inflammation	Inflammation usually present

Regulation of Apoptosis

Apoptosis can be triggered by both internal stimuli, such as abnormalities in the DNA, and external stimuli, such as certain cytokines. Studies indicate that external stimuli activate apoptosis by a signaling pathway, called the extrinsic pathway, that is distinct from that utilized by internal stimuli, which is called the intrinsic pathway . However, it should be noted that there is cross-talk between these pathways and that extracellular apoptotic signals can cause activation of the intrinsic pathway.

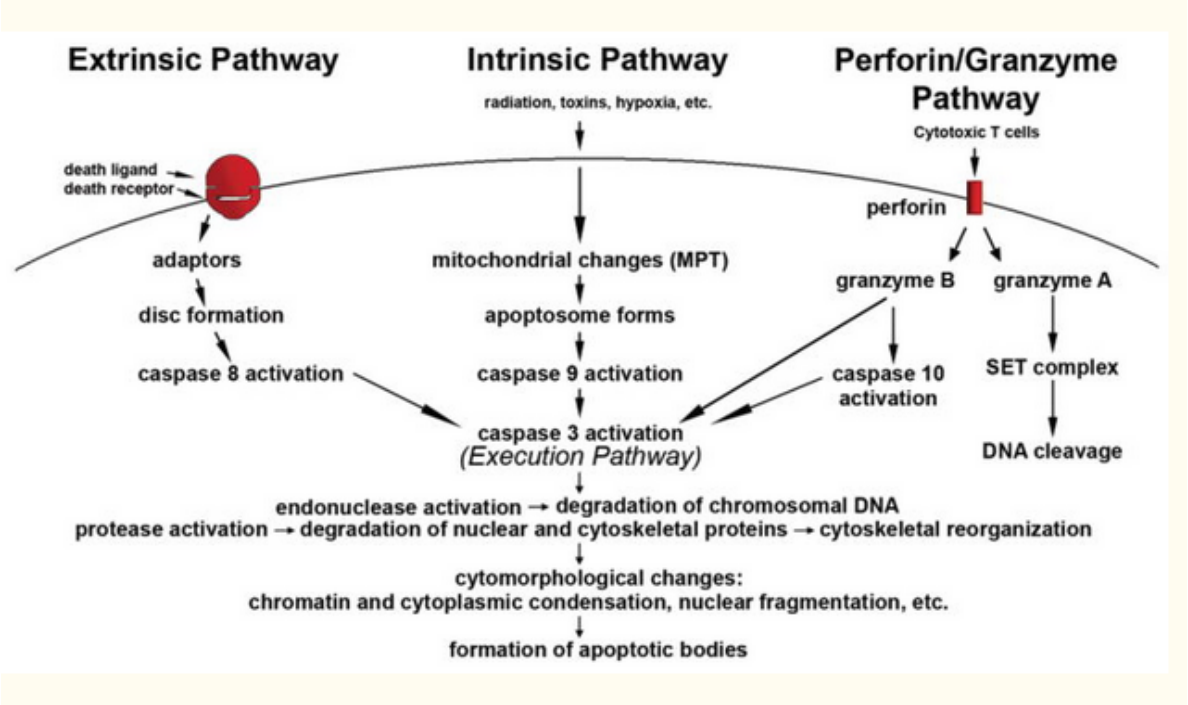

Fig- Schematic representation of apoptotic events. The two main pathways of apoptosis are extrinsic and intrinsic as well as a perforin/granzyme pathway. Each requires specific triggering signals to begin an energy-dependent cascade of molecular events. Each pathway activates its own initiator caspase which in turn will activate the executioner caspase-3. However, granzyme A works in a caspase-independent fashion. The execution pathway results in characteristic cytomorphological features including cell

shrinkage, chromatin condensation, formation of cytoplasmic blebs and apoptotic bodies and finally phagocytosis of the apoptotic bodies by adjacent parenchymal cells, neoplastic cells or macrophage

The Extrinsic Pathway of Apoptosis

The steps in the extrinsic pathway are illustrated in following figure. Here, the stimulus for apoptosis is carried by an extracellular messenger protein called tumor necrosis factor (TNF), which was named for its ability to kill tumor cells. Like other types of first messengers TNF evokes its response by binding to a transmembrane receptor, TNFR1.

The trimeric TNFR1 protein is a member of a family of related "death receptors" that turns on the apoptotic process. The cytoplasmic domain of each TNF receptor subunit contains a segment of about 70 amino acids called a "death domain" that mediates protein–protein interactions.

Binding of TNF to the trimeric receptor produces a change in conformation of the receptor ' s death domain, which leads to the recruitment of a number of proteins. The last proteins to join the complex that assembles at the inner surface of the plasma membrane are two procaspase-8 molecules. These proteins are called "procaspases" because each is a precursor of a caspase; it contains an extra portion (called the pro region) that must be removed by proteolytic processing to activate the enzyme.

The synthesis of caspases as proenzymes protects the cell from accidental proteolytic damage. Unlike most proenzymes, procaspases exhibit a low level of proteolytic activity. When two or more procaspases are held in close association with one another, they are capable of cleaving one another's polypeptide chain and converting the other molecule to the fully active caspase.

The final mature enzyme (i.e., caspase-8) contains four polypeptide chains, derived from two procaspase precursors. Activation of caspase-8 is similar in principle to the activation of effectors by a hormone or growth factor. In all of these signaling pathways, the binding of an extracellular ligand causes a change in conformation of a receptor that leads to the binding and activation of proteins situated downstream in the pathway. Caspase-8 is described as an initiator caspase because it initiates apoptosis by cleaving and activating downstream, or executioner, caspases, that carry out the controlled self-destruction of the cell as described above.

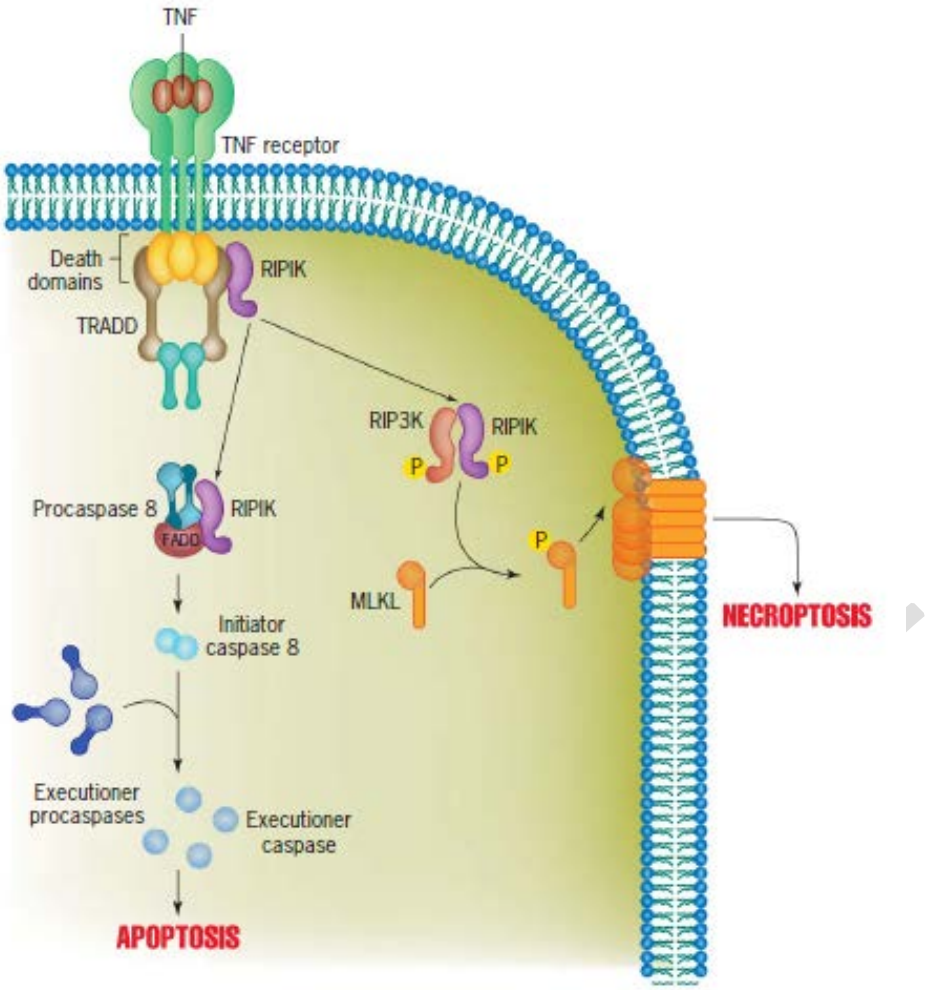

Fig- Simplified model of the extrinsic (receptor-mediated) pathway of apoptosis and the necroptotic pathway. When TNF binds to a TNF receptor (TNFR1), the activated receptor binds the cytoplasmic adaptor protein TRADD and additional signaling proteins including the kinase RIP1K. The cytoplasmic domains of the TNF receptor, FADD, TRADD, and RIP1K, interact with one another by homologous regions called death domains that are present in each protein. In the apoptotic pathway, RIP1K associates with the adaptor protein FADD and procaspase- 8. Once assembled in the complex, two procaspase molecules cleave one another to generate an active caspase-8 molecule containing four polypeptide segments. Caspase-8 is an initiator complex that activates downstream (executioner) caspases that carry out the death sentence. In the necroptotic pathway, the RIP1K associates with another kinase, RIP3K to form the active necroptosome. RIP3K phosphorylation of MLKL is thought to allow MLKL to translocate to the membrane and oligomerize to form channels that allow for cytoplasmic leakage. It can be noted that the interaction between TNF and TNFR1 also can lead to cell survival rather than self-destruction.

The Intrinsic Pathway of Apoptosis

Internal stimuli, such as irreparable genetic damage, lack of oxygen (hypoxia), extremely high concentrations of cytosolic Ca 2^{+}, viral infection, ER stress, or severe oxidative stress (i.e., the production of large numbers of destructive free radicals) trigger apoptosis by the intrinsic pathway.

Activation of the intrinsic pathway is regulated by members of the Bcl-2 family of proteins, which are characterized by the presence of one or more small BH domains. Bcl-2 family members can be subdivided into three groups:

(1) proapoptotic members (containing several BH domains) that promote apoptosis (Bax and Bak),

(2) antiapoptotic members (containing several BH domains) that protect cells from apoptosis (e.g., Bcl-x L , Bcl-w , and Bcl-2), 3 and

(3) BH3-only proteins (so-named because they contain only one BH domain), which promote apoptosis by an indirect mechanism.

According to the prevailing view, BH3 - only proteins (e.g., Bid, Bad, Puma, and Bim) can exert their proapoptotic effect in two different ways, depending on the particular proteins involved. In some cases they promote apoptosis by inhibiting antiapoptotic Bcl-2 members, whereas in other cases they promote apoptosis by activating proapoptotic Bax or Bak. In either case, the BH3 -only proteins are the likely determinants as to whether a cell follows a pathway of survival or death.

In a healthy cell, the BH3 -only proteins are either absent or strongly inhibited, and the antiapoptotic Bcl-2 proteins are able to restrain proapoptotic members. The mechanism by which this occurs is debated. It is only in the face of certain types of stress that the BH3 -only proteins are expressed or activated, thereby shifting the balance in the direction of apoptosis.

In these circumstances, the restraining effects of the antiapoptotic Bcl-2 proteins are overridden, and the proapoptotic protein Bax is free to translocate from the cytosol to the outer mitochondrial membrane (OMM). Although the mechanism is not entirely clear, it is thought that Bax molecules (and/or Bak molecules, which are permanent residents of the OMM) undergo a change in conformation that causes them to assemble into a multisubunit, protein-lined channel within the OMM. Once formed, this channel dramatically increases the permeability of the OMM and promotes the release of certain mitochondrial proteins, most notably cytochrome c, which resides in the intermembrane space.

Nearly all of the cytochrome c molecules present in all of a cell's mitochondria can be released from an apoptotic cell in a period as short as five minutes. Cells lacking both Bax and Bak are protected from apoptosis, revealing the essential roles of these proapoptotic proteins in this process.

Release of proapoptotic mitochondrial proteins such as cytochrome c is apparently the "point of no return," that is, an event that irreversibly commits the cell to apoptosis.

Once in the cytosol, cytochrome c forms part of a wheel-shaped multiprotein complex called the apoptosome , that also includes several molecules of procaspase- 9. Procaspase-9 molecules are thought to become activated by simply joining the multiprotein complex and do not require proteolytic cleavage.

Like caspase-8, which is activated by the receptor-mediated pathway described above, caspase-9 is an initiator caspase that activates downstream executioner caspases, which bring about apoptosis.

The extrinsic (receptor-mediated) and intrinsic (mitochondria- mediated) pathways ultimately converge by activating the same executioner caspases, which cleave the same cellular targets. As cells execute the apoptotic program, they lose contact with their neighbors and start to shrink. Finally, the cell disintegrates into a condensed, membrane-enclosed apoptotic body. This entire apoptotic program can be executed in less than an hour.

The apoptotic bodies are recognized by the presence of phosphatidylserine on their surface. Phosphatidylserine is normally present only on the inner leaflet of the plasma membrane. During apoptosis, a phospholipid "scramblase" moves phosphatidylserine molecules to the outer leaflet of the plasma membrane where they are recognized as an "eat me" signal by specialized macrophages. Apoptotic cell death thus occurs without spilling cellular content into the extracellular environment. This is important because the release of cellular debris can trigger inflammation, which can cause a significant amount of tissue damage.

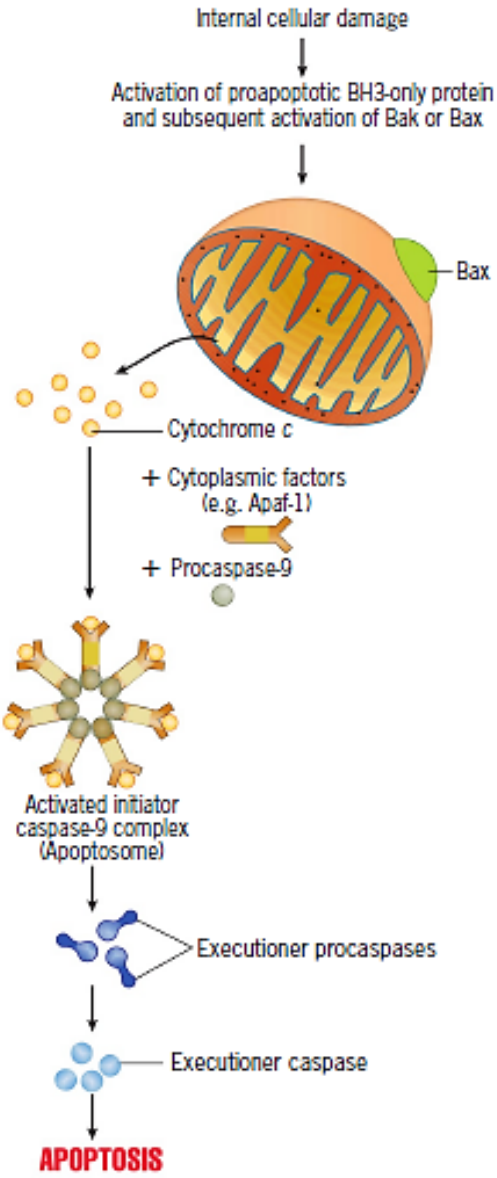

Fig- The intrinsic (mitochondria-mediated) pathway of apoptosis. Various types of cellular stress cause proapoptotic members of the Bcl-2 family of proteins–either Bax or Bak–to oligomerize within the outer mitochondrial membrane, forming channels that facilitate the release of cytochrome c molecules from the intermembrane space. Once in the cytosol, the cytochrome c molecules form a multisubunit complex with a cytosolic protein called Apaf-1 and procaspase-9 molecules. Procaspase-9 molecules are apparently activated to their full proteolytic capacity as the result of a conformational change induced by association with Apaf-1. Caspase-9 molecules cleave and activate executioner caspases, which carry out the apoptotic response. The intrinsic pathway can be triggered in some cells (e.g., hepatocytes) by extracellular signals. This occurs as the initiator caspase of the extrinsic pathway,

caspase 8, cleaves a BH3-only protein called Bid, generating a protein fragment (tBid) that binds to Bax, inducing insertion of Bax into the OMM and release of cytochrome c from mitochondria.

Caspases - the executioner

Caspases are a distinctive group of cysteine proteases that are activated at an early stage of apoptosis and are responsible for triggering most, if not all, of the changes observed during cell death. Caspases accomplish this feat by cleaving a select group of essential proteins. Among the targets of caspases are the following:

● More than a dozen protein kinases, including focal adhesion kinase (FAK), PKB, PKC, and Raf1. Inactivation of FAK, for example, is presumed to disrupt cell adhesion, leading to detachment of the apoptotic cell from its neighbours. Inactivation of certain other kinases, such as PKB, serves to disrupt prosurvival signaling pathways. Caspases also disrupt the generation of survival signals by inactivating the NF-kB pathway.

● Lamins , which make up the inner lining of the nuclear envelope. Cleavage of lamins leads to the disassembly of the nuclear lamina and shrinkage of the nucleus.

● Proteins of the cytoskeleton , such as those of intermediate filaments, actin, tubulin, and gelsolin. Cleavage and consequent inactivation of these proteins lead to changes in cell shape.

● An endonuclease called caspase activated DNase (CAD), which is activated following caspase cleavage of an inhibitory protein. Once activated, CAD translocates from the cytoplasm to the nucleus where it attacks DNA, severing it into fragments.

Necroptosis

In contrast to apoptosis, necroptotic cell death results in the rupturing of the plasma membrane, causing cellular contents to be spilled into the environment, and often triggering an inflammatory response. This inflammatory response may often be beneficial, as a means to guide immune cells to a site of infection, for example. However, misregulation of the necroptotic pathway is suspected of triggering inflammatory diseases such as Crohn ’ s disease and inflammatory bowel disease.

Understanding the molecular mechanisms underlying necroptosis is currently an active area of research. Recent studies have demonstrated that necroptosis can be triggered by the same signal—TNF binding to TNF receptor—that also leads to the extrinsic pathway of apoptosis. In the necroptotic pathway, however, caspase 8 is not ultimately activated. Rather, a series of interactions leads to the recruitment of the kinases RIPK1 and RIPK3, which are thought to oligomerize into a structure dubbed the necrosome, and the activation of RIPK3 kinase activity. Recent evidence suggests that a substrate of RIPK3, called MLKL, is targeted to the plasma membrane after its phosphorylation. It is thought that MLKL can oligomerize to form transmembrane pores that result in loss of plasma membrane integrity and cell death.

Signaling Cell Survival

Just as there are signals that commit a cell to self-destruction, there are opposing signals that maintain cell survival. In fact, interaction of TNF with a TNF receptor often transmits two distinct and opposing signals into the cell interior: one stimulating apoptosis and another stimulating cell survival. As a result, most cells that possess TNF receptors do not undergo apoptosis when treated with TNF. This was a disappointing finding because it was originally hoped that TNF could be used as an agent to kill tumor cells. Cell survival is typically mediated through the activation of a key transcription factor called NF-κB, which activates the expression of genes encoding cell-survival proteins. It would appear that the fate of a cell depends on a delicate balance between signals promoting survival, apoptosis and necroptosis.

Significance

Defective pathways

As a pathway is more or less sequential in nature, removing or modifying one component leads to an effect in another. In a living organism, this can have disastrous effects, often in the form of disease or disorder. The normal functioning of the pathway has been disrupted in such a way that it impairs the ability of the cell to undergo normal apoptosis. This results in a cell that lives past its "use-by-date" and is able to replicate and pass on any faulty machinery to its progeny, increasing the likelihood of the cell's becoming cancerous. Defects in regulation of apoptosis in cancer cells occur often at the level of control of transcription factors. As a particular example, defects in molecules that control transcription factor NF-κB in cancer change the mode of transcriptional regulation and the response to apoptotic signals, to curtail dependence on the tissue that the cell belongs. This degree of independence from external survival signals, can enable cancer metastasis.

Dysregulation of p53

The tumor-suppressor protein p53 accumulates when DNA is damaged due to a chain of biochemical factors. 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 prevents the cell from replicating by stopping the cell cycle at G1, or interphase, 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.

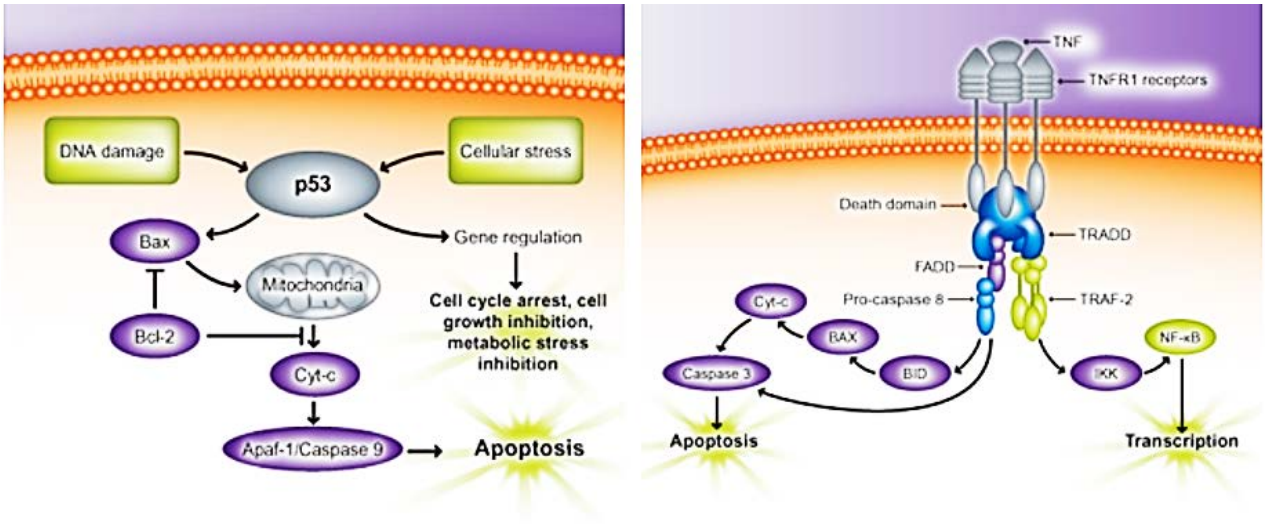

Fig-p53 and death receptor signaling in apoptosis

Hyperactive apoptosis

On the other hand, loss of control of cell death (resulting in excess apoptosis) can lead to neurodegenerative diseases, hematologic diseases, and tissue damage. It is to note that neurons that rely on mitochondrial respiration undergo apoptosis in neurodegenerative diseases such as Alzheimer's and Parkinson's. Moreover, there is an inverse epidemiological comorbidity between neurodegenerative diseases and cancer. The progression of HIV 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 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. At the molecular level, hyperactive apoptosis can be caused by defects in signaling pathways that regulate the Bcl-2 family proteins. Increased expression of apoptotic proteins such as BIM, or their decreased proteolysis, leads to cell death, and can cause a number of pathologies, depending on the cells where excessive activity of BIM occurs. Cancer cells can escape apoptosis through mechanisms that suppress BIM expression or by increased proteolysis of BIM.