Apoptosis

Definition

Apoptosis refers to a form of programmed cell death that occurs in multicellular organisms. Several biochemical events lead to characteristic cell changes (morphology) and death. These changes include blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and global mRNA decay.

Difference between Apoptosis and Necrosis

Edit	Apoptosis	Necrosis
Introduction	Apoptosis, or programmed cell death, is a form of cell death that is generally triggered by normal, healthy processes in the body.	Necrosis is the premature death of cells and living tissue. Though necrosis is being researched as a possible form of programmed cell death, it is considered an "unprogrammed" cell death process at this time.
Natural	Yes	Caused by factors external to the cell or tissue, such as infection, toxins, or trauma.
Effects	Usually beneficial. Only abnormal when cellular processes that keep the body in balance cause too many cell deaths or too few.	Always detrimental
Process	Membrane blebbing, shrinkage of cell, nuclear collapse (nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation), apoptopic body formation. Then, engulf by white blood cells.	Membrane disruption, respiratory poisons and hypoxia which cause ATP depletion, metabolic collapse, cell swelling and rupture leading to inflammation.
Symptoms	Usually no noticeable symptoms related to the process.	Inflammation, decreasing blood flow at affected site, tissue death (gangrene).
Causes	Self-generated signals in a cell. Generally natural part of life, the continuation of the cellular cycle initiated by mitosis.	Bacterial or fungal infections, denatured proteins that impede circulation, fungal and mycobacterial infections, pancreatitis, deposits of antigens and antibodies combined with fibrin.
Medical Treatment	Very rarely needs treatment.	Always requires medical treatment. Untreated necrosis is dangerous and can lead to death.

General Causes of Apoptosis

There are three mechanisms that cause cell death:

1. Self-generated signals in a cell, which may arise from age, infection, irregular mitosis (cell division), or other causes. This mechanism is known as the intrinsic or mitochondrial pathway, whereas the following two types of cell death are extrinsic pathways.
2. The triggering of death activators, receptors on a cell's surface that respond to external signals such as hormones or other chemical messengers.
3. External triggering by reactive oxygen species, such as free radicals, which are dangerous to the body.

Apoptosis Pathways

Apoptosis can be initiated by one of two major pathways; the intrinsic or extrinsic pathway. Both of these pathways end with a final common effector pathway, known as the execution phase.

Besides these two pathways, ER-stress induced apoptosis also takes place in some cells under specific stress.

I. Intrinsic Pathway

The intrinsic pathway mainly triggers apoptosis in response to internal stimuli such as:

a) Biochemical stress: DNA damage (this activates the p53 gene – which halts the cell cycle and initiates DNA repair. If this repair attempt is unsuccessful, apoptosis can be induced)

b) Lack of growth factors: The intrinsic pathway is modulated by two groups of molecules, Bcl-2 and Bax. Activation of Bax leads to the formation of Bax-Bax dimers, which in turn enhances the action of a variety of apoptotic stimuli – increasing a cell's susceptibility to apoptosis. The Bcl-2 family consists of both pro- and anti-apoptotic members, and it is the balance between these that determines how susceptible a cell may be to apoptosis.

The balance between these groups of molecules establishes a 'molecular switch' which determines whether a cell will survive or undergo apoptosis in response to internal stimuli,

II. Extrinsic Pathway

The extrinsic pathway triggers apoptosis in response to external stimuli, namely by ligand binding at 'death' receptors on the cell surface.

These receptors are typically members of the Tumour Necrosis Factor Receptor (TNFR) gene family, such as TNFR1 or FAS. Binding at these receptors leads to receptor molecules grouping up on the cell surface to initiate downstream caspase activation.

Execution Phase

The initiation of apoptosis by either pathway results in a cascade activation of caspases. These are specialised proteases which normally reside as inactive precursors within the cell.

Initiation of apoptosis first activates initiator caspases, such as caspase 8, the role of which is to cleave other pro-caspases into active "executioner" caspases. These executioner caspases then cause degradation of a variety of cellular structures, such as the cytoskeleton and nucleus. For example, caspase 3 activates DNAse – leading to fragmentation of DNA.

These processes lead to a variety of morphological changes within the cell, such as nuclear shrinkage (pyknosis) and fragmentation (karyorrhexis). Alongside this the cell itself shrinks, but importantly, retains an intact plasma membrane. The dead cells are either immediately phagocytosed by neighbouring cells or break down into smaller, membrane bound vesicles, known as apoptotic bodies – which are eventually phagocytosed.

Intrinsic Pathway of Apoptosis

In the intrinsic pathway of apoptosis, the death-inducing stimuli originate inside the target cell itself. Mitochondria, the powerhouse of the cell, have a significant role in executing the intrinsic pathway of apoptosis. Thus, the intrinsic pathway of apoptosis is also known as the Mitochondria-mediated death pathway.

What are Bcl-2 (B-cell lymphoma-2) family proteins?

The intrinsic pathway of apoptosis is facilitated by the members of Bcl-2 family proteins. The members of the Bcl-2 family proteins are characterized by the presence of one or more BH domains (Bcl-2 Homology Domain). The first identified member of Bcl-2 family proteins is Bcl-2 itself. The Bcl-2 was first identified as a cancer-causing oncogene in some human lymphomas. The gene which codes for the Bcl-2 protein was over-expressed in these cancer cells due to translocation.

However, later studies have shown that Bcl-2 is not directly acting as an oncogene. They act as the oncogene by promoting the survival of the cancerous cells that would otherwise die by apoptosis.

The Bcl-2 family proteins were classified into THREE subcategories:

(1). Pro-apoptotic Bcl-2 proteins

(2). Anti-apoptotic Bcl-2 proteins

(3). BH3-only proteins

(1). Pro-apoptotic Bcl-2 members: Ø Pro-apoptotic Bcl-2 proteins promote apoptosis.

Ø In a normal cell, the pro-apoptotic Bcl-2 members are in inactive stage.

Ø Example: Bax and Bak

(2). Anti-apoptotic Bcl-2 members

Ø Anti-apoptotic Bcl-2 proteins inhibit apoptosis and ensure cell survival.

Ø Example: Bcl-2, Bcl-xL and Bcl-w

(3). BH3-only proteins

Ø BH3-only proteins only have a small BH3 domain.

Ø They can promote or inhibit apoptosis through an indict mechanism.

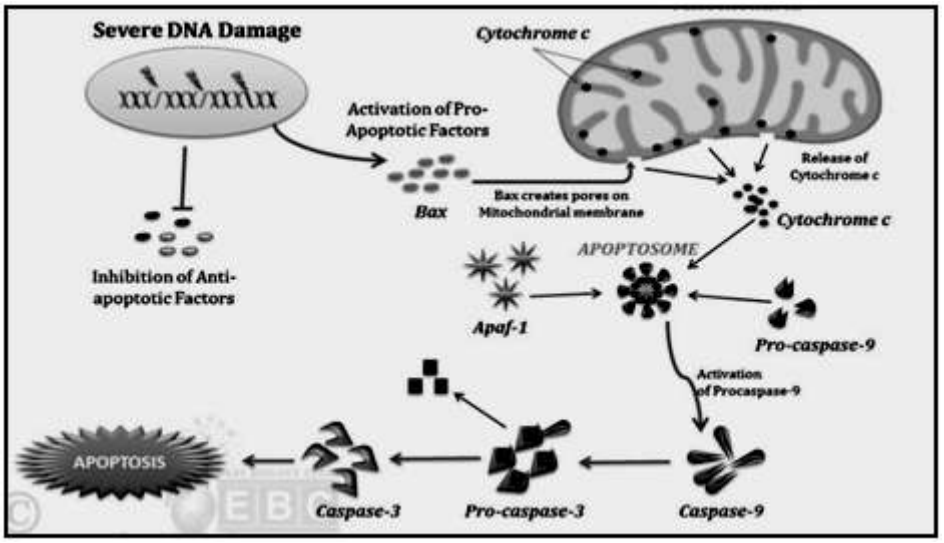
Ø Examples: Bid, Bad and Bim

The BH3-only proteins can be pro-apoptotic and they can promote apoptosis in two different ways. In some cases, they promote apoptosis by inhibiting the anti-apoptotic Bcl-2 members. In other cases, they stimulate apoptosis by activating pro-apoptotic Bcl-2 members. In both the cases, the BH3-only proteins are the key determinant in the cell survival or apoptotic cell death.

In a normal and healthy cell, the level of BH3-only proteins will be maintained in a very low concentration. Besides, in a healthy cell, the anti-apoptotic Bcl-2 proteins are able to restrain the pro-apoptotic members. If an apoptosis inducing stimulus is evoked in the cell, the levels of BH-3 only proteins are dramatically increased. This increase in the level BH3-only protein causes an imbalance in the level of pro-apoptotic and anti-apoptotic factors and beside that they shift the balance towards apoptotic cell death.

When this balance is lost, the inhibitory effects of the anti-apoptotic Bcl-2 proteins are also compromised. In this critical situation, a mitochondria-mediate signaling cascade is initiated inside the cell and that will eventually result in the execution of programmed cell.

Mechanism:



Ø When the cell lost the balance between pro-apoptotic and anti-apoptotic factors, a pro-apoptotic factor called Bax is translocated from the cytosol to the outer mitochondrial membrane.
Ø Bax protein undergoes a conformational change and gets inserted into the outer mitochondrial membrane.
Ø The assembly of Bax protein to the outer mitochondrial membrane is in such a way that it creates protein-lined channels or pores on the outer membrane.
Ø Due to the formation of these pores, the permeability of the outer mitochondrial membrane dramatically increased.
Ø Through these pores, cytochrome c is released out of the mitochondria to the cytosol.
Ø The increase in permeability of the membrane also causes a dramatic loss in the electrical potential of the mitochondria.
Ø The loss of mitochondrial membrane permeability is accelerated by the increased level of Ca2+ ions in the cytoplasm which are released by the endoplasmic reticulum.
Ø The membrane potential compromised mitochondria also release SMACs (second mitochondria derived activator of caspases) into the cytosol.
Ø SMACs bind to and inactivate all anti-apoptotic proteins in the cytosol.
Ø The complete release of cytochrome c to the cytoplasm is a ‘point of no return’. This means that the cell cannot be reverted back to its normal stage and moreover the cell should commit the apoptotic cell death.
Ø In the cytoplasm, the cytochrome c molecules combine together with Apaf-1 and Pro-caspase-9 in an ATP-dependent manner to form a multisubunit complex.
Ø This multi-subunit complex of cytochrome c, Apaf-1 and pro-caspase-9 is called Apoptosome
Ø A single apoptosome may contain several molecules of pro-caspase-9.
Ø The binding of Apaf-1 induce a conformational change in the procaspase-9 and it is activated to its fully proteolytic form (caspase-9).
Ø Caspase 9 is the initiator caspases in the intrinsic pathway of apoptosis.
Ø Caspase 9 activates the executioner caspases such as caspases -3 and Caspase-7.

Ø Activated caspase-3 and caspase-7 cleaves its target molecules in the cell and thus the apoptotic cell death is executed.

Extrinsic Pathway of Apoptosis

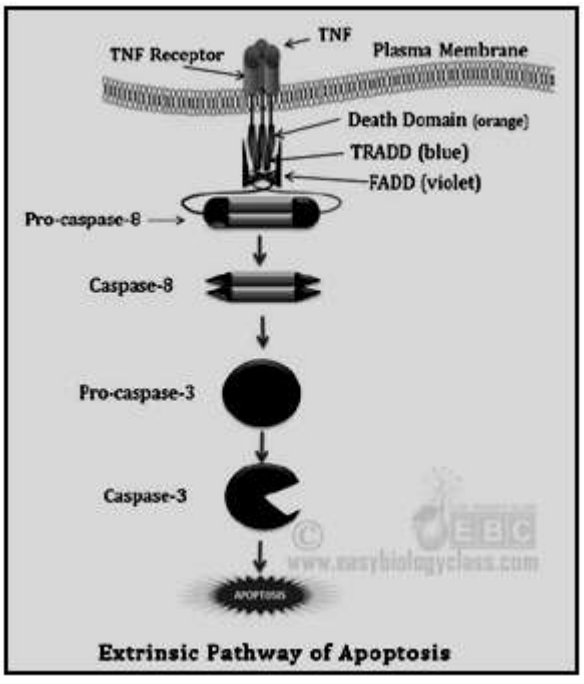
(The Receptor-Mediated Programmed Cell Death Pathway)

In the extrinsic pathway of apoptosis, the death-inducing signal for the programmed cell death is triggered by an external stimulus. For receiving such an external death-inducing signal, the cell possesses plasma membrane receptors specific to each stimulus and thus the extrinsic signalling of apoptosis is also known as the Receptor Mediated programmed cell death pathway.

The external stimuli for the apoptosis in most of the cases will be a cytokine. The most studied cytokine to induce extrinsic pathway of apoptosis is an extracellular messenger protein called Tumor Necrosis Factor (TNF). TNF is so named because it was first discovered as a protein factor which induces cell death in cancerous cells. The TNF cytokine is produced by the cells of the immune system in response towards the adverse conditions. The adverse conditions that can provoke the immune cells to produce TNF are:

Ø Exposure to radiation
Ø Introduction of viral toxins
Ø Exposure to elevated temperature
Ø Exposure to other toxic substances

Mechanism



Ø TNF first binds to its receptor called TNFR1 (Tumor Necrosis Factor Receptor-1) present on the plasma membrane.

Ø TNFR1 is a member of death receptor family proteins that turn on the apoptotic cell death process in eukaryotic cells.

Ø TNFR1 is a trans-membrane receptor with an external ligand binding domain and a cytosolic domain.

Ø The TNRF1 in the plasma membrane is presented as a preassembled trimer.

Ø The cytosolic domain of each TNFR1 subunit contains a segment of about 70 amino acids called ‘death domain’.

Ø Binding of TNF to the TNFR1 receptor cause a conformational change in the death domain.

Ø This conformational change in the ‘death domain’ cause the recruitment of many apoptosis-related adaptor protein factors.

Ø To the activated death domain, two cytosolic adaptor proteins (TRADD and FADD) and Pro-caspase-8 residues are binds to form a multi-protein complex.

Ø The cytosolic death domain of TNFR1, TRADD and FADD interact with one another by homologous regions present on each protein.

Ø Pro-caspase-8 and FADD possess a homologous region called ‘death effector domain’.

Ø The death effector domains of both pro-caspase-8 and FADD interacts each other.
Ø Due to these interactions, the two Pro-caspase-8 molecules cleave each other to generate an active caspase-8.
Ø A single active caspase-8 contains four polypeptide segments derived from two pro-caspases.
Ø Activated caspase-8 is an initiator caspase in the extrinsic pathway of apoptosis, they activate the downstream caspases
Ø Downstream caspases are called executioner caspases (caspase-3) that carry out the self-destruction process (apoptosis) of the cell.
Another commonly observed extrinsic pathway of apoptosis in human is by the killer lymphocytes through Fas ligand and Fas protein by the mechanism given below:
Ø Killer lymphocytes can induce apoptosis by producing a protein called Fas ligand.
Ø The Fas ligand binds to its receptor called Fas on the plasma membrane of the target cell.
Ø Similar to the death domain of TNFR1, the Fas protein can recruit intracellular adapter proteins that can aggregate Pro-caspase-8 molecules.
Ø The pro-caspase-8 molecules are then activated to caspase 8 and that in turn can activate the downstream executioner caspase (caspase-3) to induce apoptosis.