

Apoptosis And The Role Of Caspases In The Regulation Of Cellular Stress

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Abstract: Apoptosis is programmed cell death triggered by the destruction of the cytoskeleton by proteases and DNA cleavage by endonucleases. This process occurs through various homeostatic and pathological pathways and plays a vital role in maintaining the body's homeostasis, eliminating cells that pose a threat to its survival. Caspases play a key role both in triggering apoptosis (programmed cell death) in the event of irreversible damage and in adaptive cellular responses to moderate stress. More precisely, apoptosis is the controlled elimination of cells deemed unnecessary or potentially dangerous to the body.

Keywords: Necroptosis, pyroptosis, apoptosis, ferroptosis, panoptosis, ROCK-1 kinase, TUNEL method.

INTRODUCTION:

Disruption of the proper rate and sequence of apoptosis can lead to dysregulation of this process. Caspases are key enzymes that mediate apoptosis through cascaded activation reactions. They are activated via intrinsic and extrinsic pathways, which can act independently or in concert.

Modern research shows that apoptosis is one of the major mechanisms of cell death, summarized below:

Necroptosis: occurs following activation of tumor necrosis factor alpha (TNF- α), activating several cell death receptors.[5]

Pyroptosis: Primarily disrupts the integrity of the cell membrane by engaging inflammasomes to activate caspases.[5][6]

Apoptosis: Characterized by the release of cytochrome c from mitochondria, is immunologically silent and nonlytic.[5][6]

Ferroptosis: characterized by the accumulation of iron-dependent phospholipid peroxides in cell membranes, leading to non-apoptotic cell death.[7][8]

Given the shared mediators of the middle pathway, some researchers do not distinguish necroptosis and apoptosis as separate mechanisms. Instead, the simultaneous process is called panoptosis, in which

pyroptosis, apoptosis, and necroptosis occur as programmed cell death.[9] Additionally, autophagy refers to the process of digestion of organelles or other cellular parts by the lysosomal apparatus, which can also lead to cell death.[10] Some steps of apoptosis are thought to be reversible, a phenomenon known as anastasis, particularly observed in cancer cell lines.[11]

The mechanisms of cell death involve apoptosis, the key pathway for cellular demise. Beyond apoptosis, there are other types of programmed cell death, each with unique biochemical pathways:

1. Necroptosis: is triggered by the increase in tumor lysis factor alpha (TNF- α) and entails the activation of receptor complexes responsible for the death of cellular units.
2. Pyroptosis: is associated with the loss of membrane integrity and the participation of inflammasomes in the initiation of caspase cascades.
3. Ferroptosis: characterized by the accumulation of iron-dependent phospholipid peroxides in cellular structures, which leads to subcellular damage without triggering apoptosis.
4. Some scientists propose necroptosis and apoptosis as associated processes rather than independent entities. This combined cascade is called

"panoptosis," in which pyroptosis, apoptosis, and necroptosis are recognized as part of a general, planned cell death. Autophagy—the process of engulfing cellular structures or cell parts by the lysosomal complex, which has the potential to lead to cell death—should also be mentioned.

5. It is critical to emphasize that certain stages of apoptosis can be considered reversible. This phenomenon, known as anastasis, is often observed in some cancer cells.

Apoptosis is a tightly regulated process of programmed cell death characterized by a specific cascade of events. During apoptosis, cytoskeletal fragmentation occurs under the influence of caspases, leading to a decrease in cell size. Cells become highly eosinophilic and undergo condensation, losing contact with neighboring cells. The nucleus of a dead cell becomes intensely basophilic.

A distinctive feature of apoptosis is pyknosis, in which nuclear chromatin condenses, forming one or more darkly stained masses against the nuclear membrane. The nuclear membrane dissolves, and an endonuclease cuts the DNA into short, evenly spaced fragments (karyorrhexis).[3]

Subsequently, the condensed cytoplasm and nucleus disintegrate into fragments called apoptotic bodies, which bud off from the cell. Macrophages then remove these apoptotic bodies in a process called efferocytosis. The cell membrane remains intact, without inflammation, unlike necrosis, pyroptosis, or ferroptosis, which typically involve cellular swelling and inflammation. Macrophages rapidly remove apoptotic cells, causing little or no inflammation in surrounding tissues. Thus, this mechanism is considered immunologically silent.[12]

Effective detection of apoptosis in clinical pathology requires the use of multiple methods, as its multi-step complexity cannot be fully captured by a single method. Below is an overview of key methods, organized by their specific detection objectives.

Apoptotic cells are characterized by specific cytomorphological changes, including chromatin condensation and membrane blebbing. Chromatin condensation can be visualized using fluorescent DNA dyes such as DAPI or Hoechst, which exhibit increased fluorescence intensity in condensed nuclei under microscopy. Membrane blebbing, observed by phase-contrast microscopy in living cells, is caused by caspase-mediated cleavage of proteins such as gelsolin and ROCK-1 kinase. Analysis of fixed cells using caspase substrate markers can lead to false-positive results.

Late apoptosis is characterized by fragmentation of deoxyribonucleic acid (DNA)—its cleavage into fragments of a certain length (180–200 base pairs). The TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) method, based on labeling the 3'-OH ends of DNA fragments with fluorescent labels, allows for the visualization of these fragments using microscopy or flow cytometry. An alternative, more accessible but less sensitive method is DNA electrophoresis in agarose gel, which allows for the detection of the "ladder" of fragments characteristic of apoptosis.

Activation of caspases, particularly caspases-3, -8, and -9, can be measured using fluorogenic substrates or fluorescent inhibitors. Mid-stage apoptosis is often confirmed by detecting poly(ADP-ribose) polymerase cleavage using Western blot. Furthermore, the balance between pro- and anti-apoptotic proteins, such as Bcl-2 and Bax, can be assessed using enzyme-linked immunosorbent assays to evaluate regulatory mechanisms.[14]

Early apoptosis is characterized by externalization of phosphatidylserine, which is detected using annexin V staining. This staining is often combined with propidium iodide to distinguish apoptotic cells from necrotic cells. In later stages, disruption of membrane integrity is monitored using dye exclusion methods.[15]

Apoptosis in embryos or tissues can be detected using acidophilic dyes such as acridine orange, Nile blue sulfate, and neutral red. However, these dyes require verification due to nonspecific staining of debris and have limitations such as toxicity and low penetrating ability. LysoTracker Red provides stable 3D imaging of thick tissues using confocal microscopy.[3]

To detect apoptosis, mitochondrial assays focus on key changes, including loss of mitochondrial membrane potential, which is determined using dyes such as JC-1, tetramethylrhodamine methyl ester, or tetramethylrhodamine ethyl ester [3]. Opening of the permeability transition pore is monitored using calcein fluorescence quenching. Additionally, cytochrome c release is monitored using immunofluorescence or subcellular fractionation. These assays are often combined with caspase activity measurements to improve specificity [16].

How do cell proliferation and death occur in all normal tissues of multicellular organisms? Normal cell death, vital for cell development and health, is called apoptosis and involves the following pathways. All of these pathways involve the activation of caspases in the final stage.

The intrinsic pathway is activated when a cell

experiences internal stress caused by various factors, such as DNA damage from X-ray or ultraviolet radiation, chemotherapy drugs, hypoxia, or the accumulation of misfolded proteins within the cell, which is observed in conditions such as Alzheimer's disease, Parkinson's disease, or Huntington's disease. When stressed, cytochrome c is released from the mitochondrial intermembrane space into the cytosol, leading to the activation of caspase-9. Regulation of this pathway is controlled by the Bcl-2 and TP53 genes.[17][18]

The Bcl-2 protein family plays a key role in the regulation of apoptosis (programmed cell death). It includes both anti-apoptotic and pro-apoptotic proteins. Pro-apoptotic family members recognize signals that initiate apoptosis and initiate a cascade of events leading to cell death. Conversely, anti-apoptotic proteins block this process. These evolutionarily conserved proteins can exert their function through a variety of mechanisms depending on the species. Their activity and localization are regulated by various cellular signals, creating a complex network of interactions that balances cell survival and death.

Due to their fundamental role in regulating apoptosis induced by multiple factors, Bcl-2 family proteins are essential for embryonic development and maintenance of homeostasis in the adult organism.

Unlike the intrinsic pathway, the extrinsic pathway is activated when the cell receives death signals from other cells. This pathway is associated with receptors, and ligands from other cells bind to these death receptors on the cell surface, activating apoptosis. This process involves subsequent cell surface receptors and their corresponding ligands, ultimately activating caspase-8, a key regulator of this cascade.

TNF- α : TNF- α is a cytokine produced by macrophages and is the primary extrinsic mediator of apoptosis. TNF- α binds to the TNFR1 receptor, activating caspases.

Fas: T cells generate a surface receptor, Fas, whose production increases during infection. After several days, activated T cells release Fas ligands. When Fas binds to these ligands on the same or different cells, apoptosis is triggered through the activation of caspases. The Fas receptor, a transmembrane protein of the TNF family, interacts with FasL, activating caspases. Apoptosis promotes the removal of activated T cells after the infection has resolved.

Bcl-2 genes: These anti-apoptotic genes are located on chromosome 18 and encode the Bcl-2 protein. Bcl-2 binds to APAF-1 and inhibits it, preventing the release of cytochrome c from mitochondria.

Cytochrome c is located between the inner and outer mitochondrial membranes. The release of cytochrome c leads to its binding to APAF-1, activating procaspase-9.

TP53 tumor suppressor gene: This gene encodes a protein that regulates the cell cycle and contributes to the suppression of tumor growth. Suppose DNA is damaged by ionizing radiation, chemotherapeutic drugs, or hypoxia. In this case, TP53 arrests the cell in the G1 phase of the cell cycle, preventing the proliferation of cells with damaged DNA and promoting DNA repair. Severe DNA damage triggers apoptosis by activating the BAX apoptotic genes. BAX gene products inactivate the anti-apoptotic gene Bcl-2.[21] This process is regulated by the balance between pro-apoptotic and anti-apoptotic genes (see image. DNA repair and apoptosis).

Cytotoxic CD8+ T cell pathway: CD8+ T cells secrete perforins, creating holes in target cells. Subsequently, CD8+ T cells secrete granzymes, which penetrate the target cells through these holes and activate caspases.

Caspases: Caspases are a group of protease-like enzymes. These enzymes exist in the cell in an inactive form and require proteolytic cleavage for activation. As described above, these enzymes are the main effectors of apoptotic responses, activated by several regulators.[22]

Initiator caspases include caspases 2, 8, 9, and 10. When activated, initiator caspases activate effector caspases. Effector caspases include caspases 3, 6, and 7. Active effector caspases cleave several proteins within the cell, leading to cell death and, ultimately, phagocytosis and removal of cellular debris.

Of all the caspases, caspase-3 is the most frequently activated, catalyzing the cleavage of essential cellular proteins and chromatin condensation. Caspase also activates DNase enzymes, which cause DNA fragmentation followed by internucleosomal fragmentation.[23][24]

The execution phase, the final step of apoptosis, destroys the cell through effector caspases such as caspases 3, 6, and 7, which are activated by upstream caspases of both the extrinsic and intrinsic pathways. These enzymes disrupt the nuclear envelope, block DNA repair, and fragment DNA into a specific pattern through activated nuclease, marking irreversible disintegration. The cell shrinks, alters its membrane to allow access to phagocytic signals, and forms debris-filled vesicles that are removed by phagocytes to prevent inflammation. Pro-apoptotic and anti-apoptotic proteins tightly regulate this orderly process.[25]

Following primary cell death, several danger-associated molecular patterns and pathogen-associated molecular patterns are released from killed cells, which, depending on the type of cell death and the presence of other mechanisms, signal the appearance of additional inflammatory mediators. Therefore, whether apoptosis is entirely immunologically silent remains a matter of debate.[5] Apoptotic proteins are thought to be inhibited in a number of pathological conditions, particularly cancer, in which apoptosis is normally suppressed. These modulators represent a family of anti-apoptotic proteins called inhibitors of apoptosis proteins.[26] Cathepsin D is thought to trigger apoptosis, particularly during tissue remodeling.[27]

Apoptosis, or programmed cell death, is a necessary process for the formation of a number of structures. Specifically, it enables the formation of fingers and toes by removing interdigital tissue.

In male embryos, apoptosis also plays a key role in sexual differentiation. Under the influence of duct-like inhibitory factor (DCI), synthesized by Sertoli cells in the testes, the destruction of the DCI structures, which are the precursors of the female reproductive organs, occurs.

The shedding of the uterine endometrium that occurs at the end of the menstrual cycle when estrogen and progesterone levels decrease is an example of apoptosis.

Apoptosis plays a key role in maintaining a healthy immune system:

- * Destruction of infected cells: Cytotoxic T cells destroy cells infected with viruses by causing them to undergo apoptosis.

- * Removal of cells with damaged DNA: Cells whose DNA has been damaged by radiation or chemotherapy are arrested in the G1 phase of the cell cycle for repair. The p53 gene, which plays a tumor suppressor role, is activated during this process. Mutations in the p53 gene can inhibit apoptosis, leading to the survival of abnormal cells and the development of cancer.

- * Elimination of autoreactive T cells: Apoptosis is also used to eliminate autoreactive T cells in the thymus, preventing the development of autoimmune diseases.

After the pathogen is eliminated, apoptosis promotes the removal of acute inflammatory cells (eg, neutrophils) from the healing site.

- * Lymphocyte regulation: Apoptosis is involved in controlling the number of B and T lymphocytes by removing cells that react to the body's own

components.

Thus, apoptosis is a fundamental process that ensures the proper functioning of the menstrual cycle and the immune system.

Apoptosis removes misfolded proteins, such as amyloids and proteins found in prion diseases. This eliminates some of the formations that can lead to neurodegenerative diseases.

Fig. Mechanism of apoptosis. When DNA repair is impaired, apoptosis is triggered, destroying the damaged cell without inflammation. The initiated cell evades tumor suppressor genes and activates constitutive proliferation (autoproliferation). Subsequently, the initiated cell is supported by the acquisition of additional mutations. This mutation makes the cell immortal, bypassing immune checkpoints. Additional mutations are acquired, facilitating distant tissue invasion.

Reduced apoptosis leads to increased cell survival, which contributes to cancer development. In follicular lymphoma, a translocation event moves the Bcl-2 gene from chromosome 18 to chromosome 14, leading to excessive transcription and elevated Bcl-2 levels. Elevated Bcl-2 levels inhibit APAF-1, which inactivates caspases and apoptosis, leading to the development of follicular lymphoma. Mutations or deletions in the p53 gene increase the risk of tumor formation by allowing cells with damaged DNA to divide uncontrollably.[31]

Factors such as exposure to chemicals, radiation, and viruses can damage the p53 gene. People with Li-Fraumeni syndrome have only one functional copy of p53, making them more likely to develop tumors in early adulthood. When DNA repair mechanisms fail to remove damaged, translocated, or deleted DNA, cells begin to bypass cell cycle checkpoints, leading to apoptosis (see image: Mechanism of apoptosis).[32][33]

Decreased apoptosis of autoreactive immune cells may lead to the development of autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and autoimmune lymphoproliferative syndrome.[34] Recently, the role of mitochondria in regulating cell death has been linked to the development of diabetes due to the destruction of β -cells.[35]

Cell death is also associated with many neurodegenerative diseases. Necrosis and apoptosis are observed in neurological diseases such as acute ischemic syndrome, as well as in chronic neurodegenerative diseases such as Parkinson's disease. In Sanz's disease, Alzheimer's disease, and

Huntington's disease, neuronal death occurs predominantly through apoptosis and is considered one of the factors influencing development [36].

For a long time, necrosis was considered the sole cause of myocardial infarction. However, recent studies have shown that apoptosis also occurs, primarily during the reperfusion phase after acute infarction, leading to further myocardial damage. The staging of atherosclerotic plaques and their rupture correlates with apoptosis, particularly macrophage death.[10][37]

In light of the close relationship between physiological and pathological processes, the identified factors regulating both intrinsic and extrinsic apoptosis represent promising targets for immunotherapy. [37] A striking example is cancer treatment methods based on Bcl-2 inhibition. [38] The complexity and multi-level nature of apoptotic pathways make it difficult to develop a single standard of therapeutic intervention.

Recently, the TUNEL assay has been successfully used to quantify apoptotic cell death, which is particularly relevant for staging pathological tissue samples.[11] A promising direction for treatment development is the use of inhibitors specific to antibodies targeting malignant tumor antigens, which will allow for a higher degree of therapeutic specificity.[9]

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