Cell death

(Released as an addition to the lectures from Molecular pathophysiology 2025-2026 for students of FM PJS Uni Kosice)

Cell death occurs in multiple forms, which are broadly classified as **programmed (regulated) or unregulated (accidental).** The main ones relevant to your query are apoptosis, necrosis, and necroptosis, with others like pyroptosis and ferroptosis also existing as regulated necrotic pathways

Cell death - comparisons

(1) Apoptosis (Programmed Cell Death, Type I)

Apoptosis also known as programmed cell death is a tightly regulated biological process that occurs in multicellular organisms. It is a fundamental mechanism involved in maintaining tissue homeostasis eliminating damaged or unnecessary cells and shaping various developmental processes. Apoptosis plays a crucial role in embryonic development immune system regulation and the prevention of cancerous growth.

- **Nature**: Highly regulated, genetically controlled . energy (ATP)-dependent process. It is essential for development, tissue homeostasis, and eliminating damaged or unnecessary cells without causing inflammation.
- **Triggers**: Intrinsic (e.g., DNA damage, mitochondrial stress) or extrinsic (e.g., death receptor ligands like TNF, FasL, TRAIL) pathways.
- **Key mechanisms**: Activation of caspases (especially caspase-3/7 as executioners). Involves mitochondrial outer membrane permeabilization (Bax/Bak), cytochrome c release, and apoptosome formation in the intrinsic pathway.
- **Morphology**:
  - Cell shrinkage.
  - Nuclear condensation and fragmentation (karyorrhexis).
  - Membrane blebbing.
  - Formation of apoptotic bodies.
  - Plasma membrane remains intact until late stages.
- **Inflammation**: Generally no-inflammation, i.e "silent" — apoptotic bodies are phagocytosed cleanly, preventing release of intracellular contents.
- **Markers**: Annexin V+ (phosphatidylserine exposure) / PI- (early), DNA laddering, caspase activation,

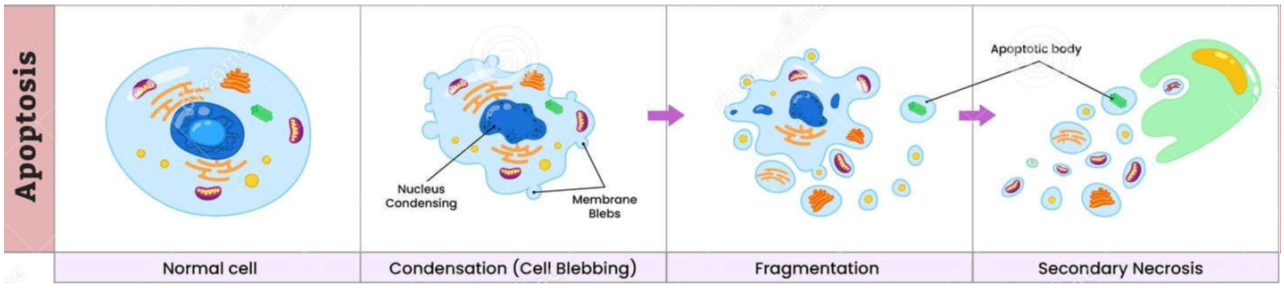


Fig. Stages of Apoptosis

(2) Necrosis (Accidental/Unregulated Cell Death)

Necrosis is a form of cell death that occurs as a result of cellular injury trauma or pathological conditions. It is typically characterized by uncontrolled cell death inflammation and the breakdown of the cellular components. Unlike apoptosis which is a regulated and programmed form of cell death necrosis is considered an accidental and unplanned process.

- **Nature**: Traditionally viewed as passive and uncontrolled, resulting from severe injury. It affects groups of cells and leads to tissue damage.
- **Triggers**: Extreme physical/chemical stress (e.g., ischemia, toxins, trauma, hyperthermia).
- **Key mechanisms**: No specific signaling pathway; loss of ion homeostasis, ATP depletion, organelle swelling, and

membrane rupture.

- **Morphology**:
  - Cell and organelle swelling (oncosis).
  - Plasma membrane rupture.
  - Leakage of cellular contents.
  - Inflammatory response due to release of DAMPs (damage-associated molecular patterns).
- **Inflammation**: Highly pro-inflammatory.
- **Distinction**: Rapid, no caspase involvement, no ordered DNA fragmentation.

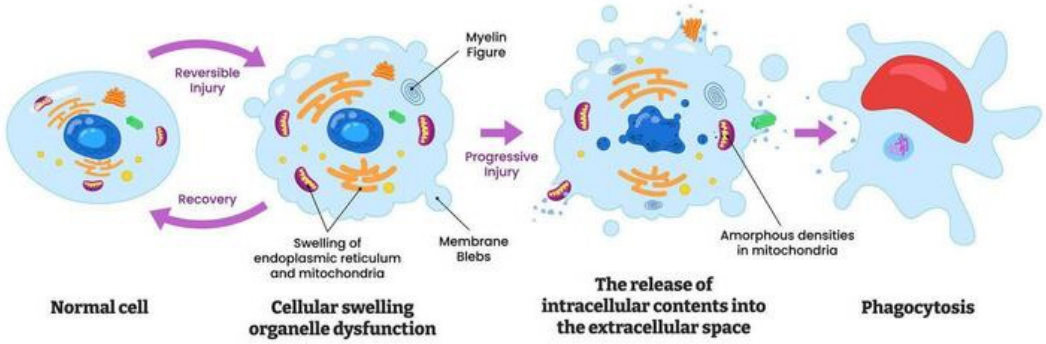


Fig.2 . Process of Necrosis.

Types of cell necrosis

Cell necrosis can be classified into the following types based on protein degeneration in tissues:

1. Coagulation necrosis: Coagulation necrosis refers to the dry state of local tissue cells after death, also known as ischemic necrosis, cells due to water loss, protein coagulation, and the formation of dry coagulation. There are also some special types of coagulation necrosis, such as caseous necrosis, often caused by tuberculosis bacillus infection, due to the role of tuberculosis bacilli, resulting in tissue necrosis after yellow, soft caseous state;

2. Liquefaction necrosis: it is a state of liquefaction after cell death, called liquefaction necrosis, which is more common in tissues with less protein, more fat or water. It is easy to dissolve and liquidate after necrosis. Fat necrosis is also a kind of liquefaction necrosis, which is mainly liquid due to the disintegration and necrosis of fat cells.

3. Fibrinoid necrosis: this kind of necrosis can only be seen under the medical microscope, which is manifested as small strips or small lumps of unstructured tissue after necrosis, like fibrin, so it is called fibrinoid necrosis;

4. Gangrene: a secondary bacterial infection of necrotic tissue cells, resulting in the black and dark green appearance of these necrotic tissues. Gangrene includes dry gangrene, which is coagulation necrosis, and wet gangrene, which is similar to liquefaction necrosis. Some gangrene will produce a large amount of gas, called gas gangrene, this gas gangrene is often formed on the basis of liquefaction necrosis, belonging to the type of special wet gangrene.

(3) Necroptosis (Programmed Necrosis)

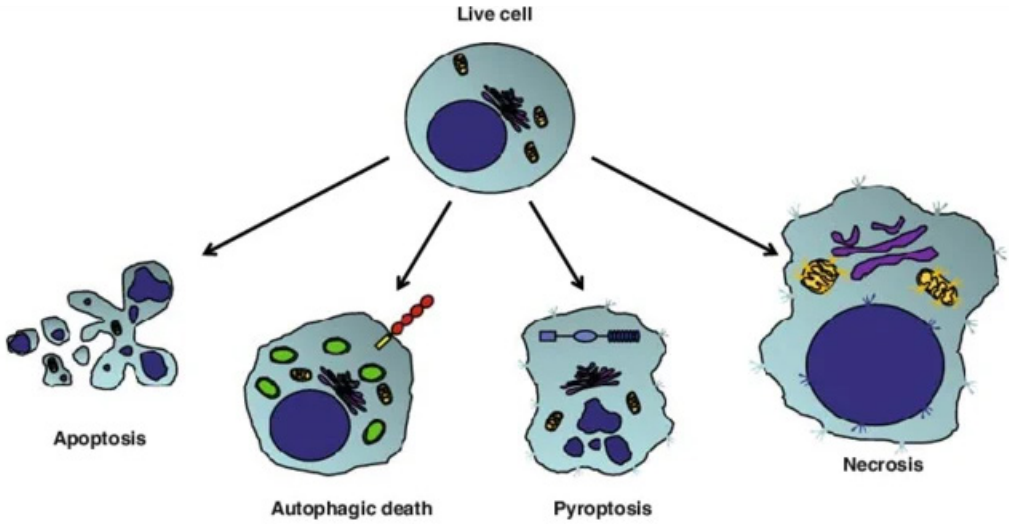
- **Nature**: A regulated form of necrotic cell death that occurs when apoptosis is blocked (e.g., by caspase inhibitors). It has a necrotic morphology but is molecularly controlled. Apoptosis often inhibits necroptosis (via caspase-8 cleaving RIPK1/3).
- **Triggers**: Death receptor signaling (e.g., TNF when caspases are inhibited), viral infections, or intracellular cues.
- **Key mechanisms**: Involves RIPK1 and RIPK3 kinases forming the necrosome, which phosphorylates MLKL. Phosphorylated MLKL oligomerizes and forms pores in the plasma membrane, leading to rupture. It is caspase-independent. When caspase-8 is inhibited, RIPK1 and RIPK3 interact via RHIM domains to form the necrosome. RIPK3 phosphorylates MLKL, causing its oligomerization and translocation to the plasma membrane, where it forms cation channels leading to osmotic lysis and DAMP release
- **Morphology**: Similar to necrosis — cell swelling, membrane rupture, organelle breakdown. No apoptotic bodies or nuclear fragmentation like in apoptosis.
- **Inflammation**: Pro-inflammatory due to DAMP release (similar to necrosis).
- **Inhibitors**: Necrostatin-1 (targets RIPK1) can block it.

Feature	Apoptosis	Necrosis (Accidental)	Necroptosis
Regulation	Highly programmed	Unregulated	Programmed (regulated necrosis)
Energy requirement	ATP-dependent	ATP-independent (depletion)	ATP-dependent (early)

Feature	Apoptosis	Necrosis (Accidental)	Necroptosis
Caspase involvement	Yes (executioner caspases)	No	No (inhibited by caspases)
Morphology	Shrinkage, blebbing, apoptotic bodies	Swelling, rupture	Swelling, rupture
Membrane integrity	Preserved until late	Early rupture	Early rupture
DNA fragmentation	Ordered (laddering)	Random	Random
Inflammation	Low/none	High (DAMPs)	High (DAMPs)
Physiological role	Development, homeostasis	Injury response	Host defense (e.g., when apoptosis blocked)
Key molecules	Caspases, Bcl-2 family	None specific	RIPK1/3, MLKL

Necroptosis and pyroptosis are both forms of programmed lytic (necrotic) cell death. They result in cell swelling, plasma membrane rupture, and the release of intracellular contents (including DAMPs), triggering inflammation—unlike the non-inflammatory, non-lytic apoptosis.
They serve as host defense mechanisms, especially against pathogens that inhibit apoptosis, but can contribute to excessive inflammation and tissue damage in diseases.

- **Lytic morphology**: Both cause cell swelling (oncosis), organelle breakdown, and plasma membrane rupture, leading to pro-inflammatory outcomes.
- **Inflammatory response**: Release of DAMPs (e.g., HMGB1) and cytokines, recruiting immune cells.
- **Programmed/regulated**: Genetically controlled, not accidental like necrosis.
- **Membrane pore formation**: Both involve pore-forming proteins that disrupt membrane integrity.
- **Physiological/pathological roles**: Important in infection defense, but dysregulation linked to inflammatory diseases, ischemia-reperfusion injury, neurodegeneration, and cancer.
-



F**ig.3. Different forms of cell death.** Apoptosis and necrosis apparently are the most distinct forms of cells death. Apoptosis is a strictly regulated process of cell death in which cells undergo some modifications that permits their silent removal by adjacent cells. These modifications include reduction of cell volume and packaging of cellular contents, DNA and nuclear fragmentation, formation of cytoplasmic and membrane blebs, and phosphatidylserine externalization. In contrast, necrotic cells increase their volume and lose the cell membrane integrity leading to the release of their cellular contents and consequent inflammation. Note that necrotic cells preserve the uncondensed DNA content. Autophagy and pyroptosis could be seen as another polarized scheme of cell death, since they have opposite consequences to the immune response. Like necrosis, pyroptosis is a highly inflammatory process in which cells

Feature	Necroptosis	Pyroptosis
Primary Triggers	Death receptors (e.g., TNFR1 by TNF), viral infection, or when apoptosis is blocked (caspase-8 inhibition). Also via TLRs, ZBP1/DAI.	Pathogen-associated molecular patterns (PAMPs) or DAMPs via inflammasomes (e.g., NLRP3, AIM2). Microbial infections, toxins, chemotherapy.
Key Pathway	RIPK1/RIPK3 **necrosome** → phosphorylation of MLKL.	**Inflammasome activation** → inflammatory caspases (caspase-1 canonical; caspase-4/5/11 non-canonical in humans).
Executioner Protein	MLKL (mixed lineage kinase domain-like pseudokinase): Oligomerizes to form pores/channels.	Gasdermin D (GSDMD): N-terminal fragment forms large pores (~10-20 nm). Other gasdermins possible.

Feature	Necroptosis	Pyroptosis
Caspase Involvement	Caspase-independent (caspases, especially caspase-8, inhibit it).	Caspase-dependent (inflammatory caspases cleave GSDMD and process IL-1β/IL-18).
Cytokine Release	Indirect via DAMP release; can activate pyroptosis secondarily.	Direct and **robust**: Active IL-1β and IL-18 released through GSDMD pores before full lysis.
Cell Type Preference	Broader (many cell types).	Prominent in innate immune cells (macrophages, monocytes, dendritic cells).
Morphological Nuances	Swelling, membrane rupture; nuclear changes milder.	Rapid swelling, pore formation (distinct pore size/morphology from MLKL), nuclear condensation but often intact initially; "pyroptotic bodies."
Inhibitors	Necrostatin-1 (targets RIPK1).	Caspase-1 inhibitors, GSDMD inhibitors.
Primary Role	Backup when apoptosis fails (e.g., viral evasion).	Primary innate immune response to infection; amplifies inflammation.

In infections, pyroptosis acts as a frontline defense in myeloid cells, while necroptosis provides a fail-safe rescue. These pathways are highly relevant in research for therapies: inhibiting them may help in inflammatory/autoimmune diseases, while inducing them could benefit cancer treatment.
Experimental distinction often uses specific inhibitors, Western blots for cleaved GSDMD vs. phosphorylated MLKL, cytokine ELISAs, and morphology via microscopy or flow cytometry (e.g., Annexin V/PI staining with caveats).

As a type of active end of life of cells, apoptosis plays a very important role in the process of maintaining the stability of the body. Its occurrence is caused by the gradual activation of the apoptotic pathway under the control of the program and does not produce inflammation. Cell necrosis is often the type of acute end of life of cells, and cell contents will be released to the outside of the cell, leading to inflammation. They are all modes of cell death, but there are great differences in the causes, mechanisms, and morphology of each part of the cell.

(4) Pyroptosis

Pyroptosis is a highly **inflammatory form of programmed lytic (necrotic) cell death**. It serves as a critical innate immune defense mechanism, particularly against intracellular pathogens, but excessive activation contributes to tissue damage and chronic inflammation. Important in infection defense. In infections, pyroptosis acts as a frontline defense in myeloid cells, while necroptosis provides a fail-safe. Necroptosis can trigger secondary pyroptosis through DAMPs activating inflammasomes. Both can intersect with other pathways like ferroptosis or panoptosis:

Inflammasomes (multiprotein complexes) sense danger signals and activate caspase-1, which cleaves GSDMD. The N-terminal GSDMD inserts into the membrane to form pores, allowing ion flux, water influx, cytokine release (IL-1β, IL-18), and eventual cell rupture (gasdermin pores, inflammasome/caspase-1/4/5/11).. Non-canonical pathways directly activate caspase-4/5/11 by intracellular LPS(gasdermin pores, inflammasome/caspase-1/4/5/11)..

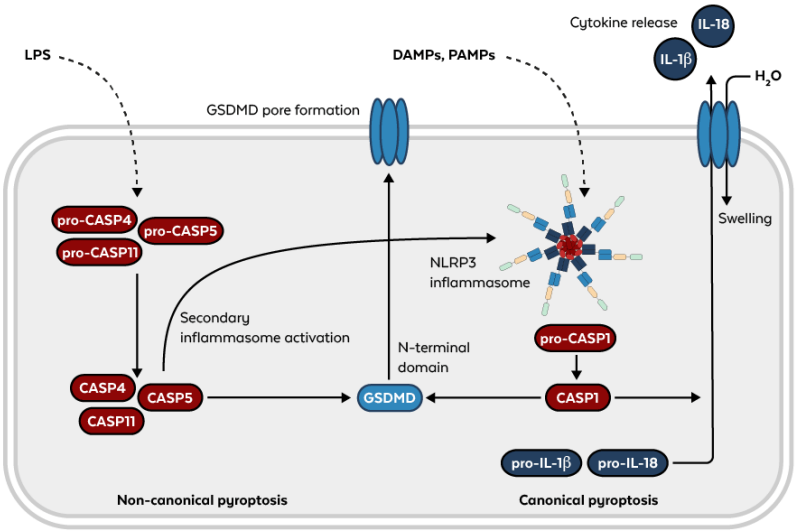


Fig. Pytoptosis simplified.

Nature.

- **Programmed but lytic**: Unlike non-inflammatory apoptosis, pyroptosis involves regulated cell swelling, membrane rupture, and release of intracellular contents (DAMPs, cytokines).

- **Caspase-dependent** (inflammatory caspases) and **gasdermin-dependent** (pore-forming proteins).
- Primarily occurs in myeloid cells (macrophages, monocytes) but also in epithelial cells, endothelial cells, and others.
- Dual role: Protective in infection clearance; pathological when dysregulated.

Triggers

- **Pathogen-associated molecular patterns (PAMPs)**: Bacterial components (e.g., LPS, flagellin, toxins), viruses, fungi.
- **Damage-associated molecular patterns (DAMPs)**: ATP, uric acid crystals, amyloid-β, etc.
- Microbial infections (e.g., *Salmonella*, *Legionella*, *Francisella*), toxins, chemotherapy drugs, ischemia, and metabolic stress.

Mechanisms. There are two main pathways, with additional variants:

a) Canonical pathway (caspase-1 dependent):

- Inflammasome assembly (e.g., NLRP3, NLRP1, NLRC4, AIM2, Pyrin) upon sensing danger signals.
- Recruitment of ASC (adaptor) and pro-caspase-1 → active caspase-1.
- Caspase-1 cleaves: (1) Gasdermin D (GSDMD) → N-terminal fragment (GSDMD-N) forms pores; (2) pro-IL-1β and pro-IL-18 → mature cytokines.

b) Non-canonical pathway (caspase-4/5/11 dependent):

- Direct binding of intracellular LPS (from Gram-negative bacteria) to caspase-4/5 (human) or caspase-11 (mouse).
- These caspases cleave GSDMD → pore formation.
- Often leads to secondary NLRP3 activation and IL-1β/IL-18 release.

Other pathways: Caspase-3/8 or granzyme-mediated cleavage of gasdermins (e.g., GSDME) in certain contexts.

Execution: GSDMD-N (or other gasdermins like GSDMA, GSDMB, GSDME) oligomerizes and inserts into the plasma membrane, forming ~10–20 nm pores. This causes ion/water influx, swelling, lysis, and release of contents.

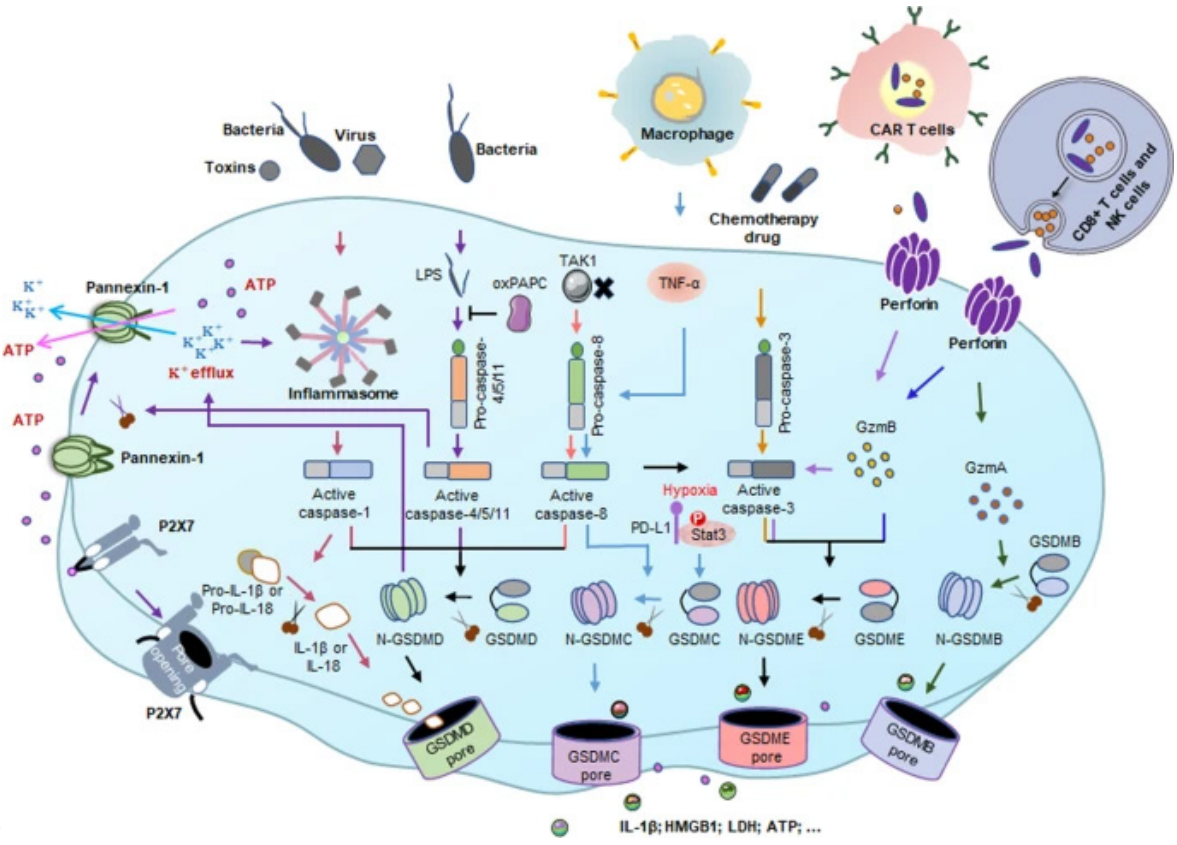


Fig. Molecular mechanism of pyroptosis .In the **canonical pathway,** PAMPs and DAMPs receive intracellular signaling molecule stimulation and assemble with pro-caspase-1 and ASC to form inflammasomes and active caspase-1. Cleaved-caspase-1 cleaves GSDMD and pro-IL-1β/18. N-GSDMD perforates the cell membrane by forming nonselective pores, further causing water influx, lysis, and death. In addition, IL-1β and IL-18 are secreted from the pores formed by N-GSDMD. In the noncanonical pathway, cytosolic LPS activates caspase-4/5 and caspase-11, triggering pyroptosis by cleaving GSDMD. However, oxPAPC competes with LPS to bind caspase-4/1, thus inhibiting pyroptosis. In addition, the cleavage of GSDMD results in efflux of K+, ultimately mediating the assembly of NLRP3 inflammasome, resulting in the cleavage of pro-IL-1β and pro-IL-18. The activated caspase-11

also cleaves Pannexin-1, inducing ATP release and P2X7R-related pyroptotic cell death. In the caspase-3-mediated pathway, active caspase-3 cleaves GSDME to form N-GSDME, inducing pyroptosis. In the caspase-8-mediated pathway, inhibiting TAK1 induces the activation of caspase-8, which cleaves GSDMD, resulting in pyroptosis. In addition, under hypoxia conditions, PD-L1 is transferred to the nucleus and regulates the transcription of GSDMC together with p-Stat3, resulting in the conversion of apoptosis to pyroptosis after TNFα-activated caspase-8. In the granzyme-mediated pathway, CAR T cells rapidly activate caspase-3 in target cells by releasing GzmB, and then GSDME- was activated, causing extensive pyroptosis. In adittion, GzmA and GzmB in cytotoxic lymphocytes enter target cells through perforin and induce pyroptosis. GzmA hydrolyzes GSDMB, and GzmB directly activates GSDME

Morphology

- Cell swelling (oncosis) and osmotic lysis.
- Plasma membrane pore formation and rupture.
- Chromatin condensation, DNA fragmentation (but nucleus often remains relatively intact initially).
- Release of "pyroptotic bodies" or cellular contents.
- Distinct from apoptosis (no apoptotic bodies, membrane blebbing less prominent) and similar to necroptosis but with specific pore morphology.

Inflammation

- **Highly pro-inflammatory** ("fiery death" — from Greek *pyro* = fire).
- Direct release of mature IL-1β and IL-18 through GSDMD pores (before full lysis).
- Massive DAMP release (e.g., HMGB1, ATP, LDH, nucleic acids) activates neighboring immune cells.
- Amplifies inflammation, recruits neutrophils/macrophages, and can lead to cytokine storm if uncontrolled.

Inhibitors

- **Caspase-1 inhibitors**: VX-765 (belnacasan), Ac-YVAD-CMK.
- **NLRP3 inhibitors**: MCC950 (CRID3), and derivatives.
- **GSDMD inhibitors**: Disulfiram (repurposed), necrosulfonamide (NSA), LDC7559, dimethyl fumarate (DMF), Ac-FLTD-CMK (peptide inhibitor of GSDMD cleavage).
- Others: Some natural compounds (e.g., baicalein) and experimental drugs targeting upstream signals.

Examples

- Macrophage response to *Salmonella* or *Shigella* infection.
- Abortive HIV infection in T cells.
- NLRP3-driven response to monosodium urate crystals in gout.
- Chemotherapy-induced pyroptosis in cancer cells (can enhance anti-tumor immunity).

Diseases

Pyroptosis is implicated in many conditions (often protective in acute infection, harmful in chronic states):

- **Infectious**: Sepsis, bacterial/viral infections (e.g., COVID-19 hyperinflammation).
- **Inflammatory/Autoimmune**: Rheumatoid arthritis, inflammatory bowel disease, gout.
- **Neurodegenerative**: Alzheimer's (amyloid-β → NLRP3), Parkinson's, multiple sclerosis, stroke.
- **Metabolic/Cardiovascular**: Atherosclerosis, type 2 diabetes, ischemia-reperfusion injury.
- **Cancer**: Context-dependent (inducing pyroptosis can kill tumor cells and boost immunity; inhibition may reduce inflammation-driven progression).
- Others: Acute lung injury, liver diseases.

Markers (for detection/experimental use)

- Cleaved (active) GSDMD (N-terminal fragment) by Western blot.
- Phosphorylated or cleaved inflammatory caspases (caspase-1 p20/p10, caspase-4/5/11).
- Mature IL-1β and IL-18 secretion (ELISA).
- LDH release (cell lysis).
- ASC specks (inflammasome assembly).
- Propidium iodide (PI) uptake with Annexin V (flow cytometry; note overlap with other deaths).
- Morphology: Swelling/rupture via microscopy.

Pyroptosis research is rapidly evolving, with therapeutic interest in both inducing it (e.g., cancer) and inhibiting it (e.g., inflammatory/neurodegenerative diseases). Crosstalk exists with apoptosis, necroptosis, and ferroptosis (sometimes termed PANoptosis).

Feature	**Apoptosis**	**Pyroptosis**
Nature	Non-lytic, "silent" programmed cell death	Lytic (necrotic-like), highly inflammatory programmed cell death

Feature	Apoptosis	Pyroptosis
Primary Role	Development, tissue homeostasis, elimination of damaged/unwanted cells	Innate immune defense against pathogens (PAMPs); amplifies inflammation
Triggers	Intrinsic (DNA damage, stress, mitochondrial signals); Extrinsic (death receptors: Fas, TNF)	Microbial infections (bacteria, viruses), PAMPs/DAMPs, inflammasome activators, toxins, some chemotherapies
Key Caspases	Initiator (caspase-8/9/10); Executioner (caspase-3/6/7)	Inflammatory (caspase-1 canonical; caspase-4/5/11 non-canonical)
Executioner Proteins	None (caspase-mediated proteolysis)	Gasdermins (mainly GSDMD; also GSDME etc.) — form membrane pores
Morphology	Cell shrinkage, chromatin condensation (pyknosis), nuclear fragmentation (karyorrhexis), membrane blebbing, apoptotic bodies	Cell swelling (oncosis), plasma membrane pore formation (~10–20 nm) and rupture, chromatin condensation (milder), pyroptotic bodies
Membrane Integrity	Maintained until late stages; apoptotic bodies phagocytosed	Early rupture due to pores; release of contents
DNA Fragmentation	Ordered (internucleosomal laddering)	Random, less intense; nucleus often remains relatively intact initially
Inflammation	Low/none — anti-inflammatory (promotes tolerance)	Highly pro-inflammatory — releases IL-1β, IL18, and DAMPs
Energy Requirement	ATP-dependent	ATP-dependent (early stages)
Cell Types	Most cell types	Prominent in myeloid cells (macrophages, monocytes); also epithelial/endothelial cells
Physiological Outcome	Clean removal without immune activation	Immune cell recruitment, cytokine storm potential

(5) Panoptosis

a hybrid involving elements of pyroptosis, apoptosis, and necroptosis.

Panoptosis is a unique, innate immune-driven, lytic, and highly inflammatory form of programmed cell death (PCD) that integrates key features of **pyroptosis (P)**, **apoptosis (A)**, and **necroptosis (N)**. It cannot be fully explained by any single pathway alone and is regulated by multiprotein complexes called **PANoptosomes**.

It was conceptualized in 2019 by the Kanneganti lab, initially observed in macrophages during influenza A virus infection. It serves as a robust host defense mechanism but can drive excessive inflammation and tissue damage when dysregulated.

Nature

- **Inflammatory and lytic**: Leads to cell membrane rupture, release of damage-associated molecular patterns (DAMPs), cytokines (e.g., IL-1β, IL-18), and other inflammatory mediators.
- **Programmed and coordinated**: Driven by caspases and receptor-interacting protein kinases (RIPKs) via PANoptosome scaffolds.
- Distinct from individual pathways: Blocking one (e.g., only apoptosis or only pyroptosis) often fails to fully prevent it.

Triggers

Common triggers include:

- **Microbial infections** — Viruses (e.g., influenza A, HSV1, SARS-CoV-2), bacteria (e.g., *Francisella novicida*, *Yersinia*, *Salmonella*), fungi (e.g., *Candida*, *Aspergillus*).
- **Pathogen-associated molecular patterns (PAMPs)**, damage-associated molecular patterns (DAMPs), cytokines (e.g., TNF + IFN-γ), heme, NAD+ depletion, heat stress/fever.
- **Altered cellular homeostasis** — Oxidative stress, metabolic changes, ischemia-reperfusion, certain chemotherapies, radiation, or combinations of stressors.

Mechanisms

PANoptosis is orchestrated by **PANoptosomes** — dynamic multiprotein complexes that integrate sensors, adapters, and effectors. Key examples:

- **ZBP1-PANoptosome** (e.g., in IAV): ZBP1, NLRP3, ASC, caspase-1/6/8, RIPK1/3.
- **AIM2-PANoptosome** (e.g., in HSV1 or *F. novicida*): AIM2, ASC, caspase-1/8, RIPK1/3, etc.
- Others: RIPK1-, NLRP12-, NLRC5-, NLRP3-PANoptosomes.

Downstream execution:

- Pyroptosis: Caspase-1 cleaves GSDMD (and sometimes GSDME) → pore formation.
- Apoptosis: Caspase-8/3/7 activation → substrate cleavage.
- Necroptosis: RIPK1/3 phosphorylation of MLKL → pore formation.
- Additional executors: NINJ1, etc.

This leads to simultaneous activation and amplified inflammation. Sensors detect triggers (e.g., Z-DNA/RNA by ZBP1, dsDNA by AIM2), recruit adapters (ASC, FADD), and activate effectors.

Morphology

Panoptosis shows hybrid features (lytic with elements of all three pathways):

- Cell swelling and membrane rupture (like pyroptosis/necroptosis).
- Pore formation (GSDMD/MLKL pores).
- Chromatin condensation, DNA fragmentation, apoptotic body-like features (apoptosis elements).
- Organelle damage and release of intracellular contents/DAMPs.

It is morphologically distinct from "pure" apoptosis (non-lytic, blebbing, apoptotic bodies without strong inflammation).

Inflammation and Inhibitors

Panoptosis is strongly pro-inflammatory due to DAMP/cytokine release and can contribute to cytokine storms. Excessive activation is detrimental in conditions like sepsis, ARDS, or severe COVID-19.

Inhibitors/Modulators (often target key components; no single specific "panoptosis inhibitor" exists yet):

- **Caspase inhibitors** (e.g., Z-VAD-FMK for broad caspases, including caspase-8/1).
- **RIPK inhibitors** (e.g., necrostatin-1 for RIPK1, RIPK3 inhibitors like GSK'872 or HS-1371).
- **NLRP3 inhibitors** (e.g., MCC950).
- **ZBP1 or AIM2 pathway blockers**.
- Combinations (e.g., triple inhibition of apoptosis/pyroptosis/necroptosis) are more effective than single agents.
- Other compounds: Some flavonoids (e.g., scutellarin), small molecules targeting oxidative stress/glycolysis, or existing drugs repurposed (e.g., certain kinase inhibitors).

In cancer, **inducers** (e.g., TNF + IFN-γ, certain chemotherapies, selinexor + IFN) are explored to promote antitumor effects.

Examples and Diseases

- **Infections**: Protective in many (clears infected cells) but pathogenic in others (e.g., contributes to severe COVID-19 via ZBP1 when combined with IFN).
- **Cancer**: Can suppress tumors via immunogenic cell death and immune activation; PANoptosis signatures are prognostic. Inducing it is a therapeutic strategy.
- **Inflammatory/Autoimmune**: Rheumatoid arthritis, SLE, ulcerative colitis, cytokine storms.
- **Neurological**: Contributes to neuroinflammation and damage in various disorders.
- **Cardiovascular, kidney, lung (ALI/ARDS), ischemia-reperfusion, hemolytic diseases**.

Markers

- **Molecular/Protein**: Upregulation/activation of PANoptosome components (ZBP1, AIM2, NLRP3, RIPK1/3, ASC, caspase-1/8, etc.); cleaved GSDMD/GSDME, cleaved caspases (3/7), phosphorylated MLKL.
- **Detection methods**: Western blot for cleaved/activated forms; immunofluorescence for colocalization in PANoptosomes; TUNEL (apoptosis), PI/EthD-III (membrane integrity), specific pathway inhibitors in functional assays.
- **Gene signatures**: PANoptosis-related gene panels for prognosis (e.g., in cancer).
- **Functional**: Release of IL-1β, IL-18, HMGB1, and other DAMPs.

Panoptosis represents a promising therapeutic target. Modulation (inhibition in inflammatory diseases, induction in cancer) is under active research, but context-dependency (beneficial vs. harmful) requires careful consideration. For clinical applications, consult current literature or specialists, as the field is rapidly evolving.

Aspect	**Pyroptosis**	**Panoptosis**
Definition	Inflammatory, lytic PCD primarily driven by inflammasomes and gasdermins.	Inflammatory, lytic PCD integrating features of pyroptosis (P), apoptosis (A), and necroptosis (N); cannot be fully explained by any single pathway.
Year Introduced	~2001	2019 (Kanneganti lab)
Nature	Primarily caspase-1/4/5/11 dependent.	Coordinated activation of multiple pathways via PANoptosomes.
Lytic?	Yes (membrane rupture)	Yes (strong lytic features)
Inflammatory?	Highly (IL-1β, IL18, DAMPs)	Highly (often amplified due to combined pathways)
Morphology	Cell swelling, large pores (GSDMD), membrane rupture, content release.	Hybrid: swelling/rupture (like pyroptosis/necroptosis) + chromatin condensation/DNA fragmentation (apoptosis-like).

(6) Ferroptosis

Ferroptosis is an **iron-dependent form of regulated cell death** characterized by uncontrolled **lipid peroxidation** of polyunsaturated fatty acids (PUFAs) in cell membranes, leading to membrane damage and cell rupture. It is distinct from apoptosis, necrosis, necroptosis, and pyroptosis. : Iron-dependent, lipid peroxidation-driven regulated necrosis. Inhibited by antioxidants like ferrostatin-1.

Nature

- **Regulated necrotic-like cell death**: Iron- and reactive oxygen species (ROS)-driven, non-apoptotic.
- **Key features**: Accumulation of lethal lipid peroxides, glutathione (GSH) depletion, and inactivation of antioxidant defenses (especially GPX4).
- It can be a backup mechanism in tumor suppression and antimicrobial defense but contributes to tissue damage in many diseases.

Triggers

- **System xc^- inhibition** (e.g., erastin): Blocks cystine import → GSH depletion.
- **GPX4 inhibition** (e.g., RSL3): Directly blocks lipid peroxide detoxification.
- Iron overload, oxidative stress, certain chemotherapies, ionizing radiation, and metabolic stresses.
- Inflammatory signals, ischemia-reperfusion, and depletion of antioxidants (e.g., vitamin E, coenzyme Q10).

Mechanisms

Ferroptosis occurs via two main routes (extrinsic/transporter-dependent and intrinsic/enzyme-regulated) converging on lipid peroxidation:

1. **Iron metabolism**: Labile Fe^{2+} catalyzes the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH$), generating hydroxyl radicals that initiate lipid peroxidation.
2. **Lipid metabolism**: PUFAs are incorporated into membrane phospholipids (via ACSL4 and LPCAT3). These are oxidized by lipoxygenases (LOXs) or via autoxidation, producing lipid hydroperoxides.
3. **Antioxidant failure**:
   - Cyst(e)ine/GSH/GPX4 axis (primary): GPX4 uses GSH to reduce lipid peroxides to alcohols.
   - Parallel pathways: FSP1-CoQ10, GCH1-BH4, and others.
4. **Execution**: Accumulation of lipid peroxides damages membranes, leading to rupture.

Mitochondria often play a central role, with increased ROS production.

Morphology

- **Distinct mitochondrial changes**: Shrinking mitochondria, increased mitochondrial membrane density, reduced or absent cristae, outer mitochondrial membrane rupture.
- Plasma membrane rupture (necrotic-like) in late stages.
- No nuclear condensation/fragmentation (unlike apoptosis), no massive swelling (unlike classical necrosis), and intact nucleus initially.
- Rounded cell shape before death.

Inflammation

- **Pro-inflammatory**: Releases damage-associated molecular patterns (DAMPs) such as HMGB1, ATP, and lipid peroxidation products, activating immune responses and potentially driving sterile inflammation or cytokine storms.
- Can amplify necroinflammation in tissues.

Inhibitors

- **Iron chelators**: Deferoxamine (DFO), deferiprone.
- **Lipid peroxidation inhibitors**: Ferrostatin-1 (Fer-1), liproxstatin-1 (potent and commonly used).
- **Antioxidants**: Vitamin E (α-

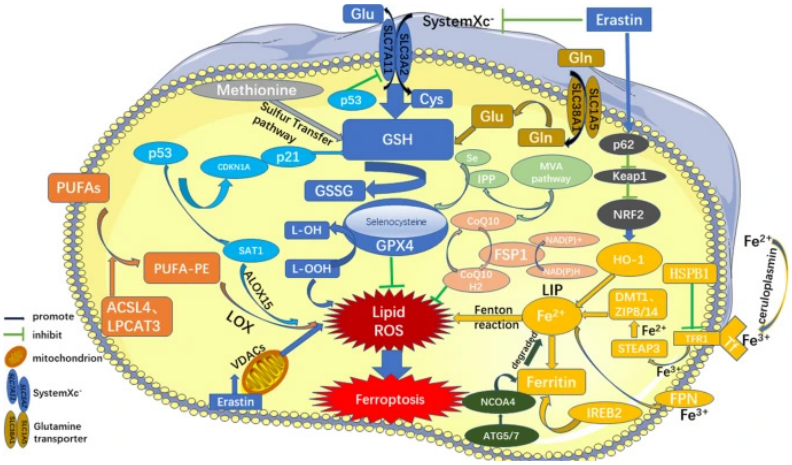


Fig.. Regulatory pathways of ferroptosis. These can be which can be roughly divided into three categories. The first one is regulated by GSH/GPX4 pathway, such as inhibition of system Xc-, sulfur transfer pathway, MVA pathway, glutamine pathway, and p53 regulatory axis. Second, the regulation mechanism of iron metabolism, such as the regulation of ATG5-ATG7-NCOA4 pathway and IREB2 related to ferritin metabolism, and the regulatory pathways of p62-Keap1-NRF2 and HSPB1 all have effects on iron. The third category is related pathways around lipid metabolism, such as P53-SAT1-ALOX15, ACSL4, LPCAT3, etc., which have effects on lipid regulation and ferroptosis. In addition, Erastin acts on mitochondria to induce ferroptosis. Also, recent studies have shown that the FSP1-CoQ10- NAD(P)H pathway exists as an independent parallel system that works cooperatively with GPX4 and glutathione to inhibit phospholipid peroxidation and ferroptosis.

tocopherol), coenzyme Q10, N-acetylcysteine (NAC, boosts GSH).
- **GPX4 activators or system xc⁻ agonists**: Certain compounds under study.
- Others: Specific pathway inhibitors (e.g., targeting ACSL4).

Examples
- Erastin- or RSL3-induced death in cancer cell lines.
- Ischemia-reperfusion injury in heart, brain, or kidney.
- Neurodegeneration models (e.g., glutamate toxicity in neurons).
- Tumor suppression by $CD8^+$ T cells via IFN-γ.

Diseases

Ferroptosis is implicated in both protective and pathological roles:
- **Neurodegenerative**: Alzheimer's, Parkinson's, Huntington's, stroke.
- **Ischemia-reperfusion**: Myocardial infarction, acute kidney injury, stroke.

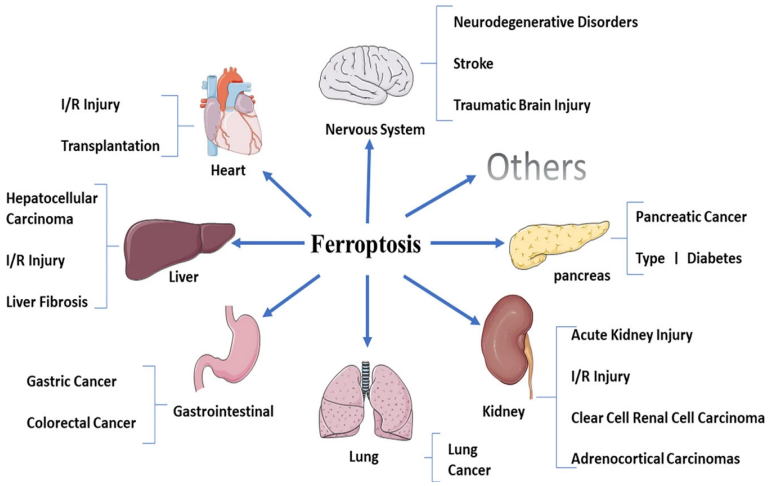


Fig. Ferroptosis plays important roles in multiple system diseases, such as nervous system diseases, heart diseases, liver diseases, gastrointestinal diseases, lung diseases, kidney diseases, pancreatic diseases, and so on.

- **Cancer**: Tumor suppression (induction is therapeutic); resistance in some cancers.
- **Inflammatory/Autoimmune**: Atherosclerosis, inflammatory bowel disease, osteoarthritis.
- **Others**: Acute lung injury, liver fibrosis, sepsis, viral infections, and aging-related damage.

Markers (for experimental detection)
- **Lipid peroxidation**: Malondialdehyde (MDA), 4-HNE (4-hydroxynonenal), BODIPY-C11 or Liperfluo probes (fluorescent shift).
- **Iron**: Increased labile Fe^{2+} (calcein-AM quenching or FerroFarRed), Prussian blue staining, upregulated TFRC (transferrin receptor).
- **GSH/GPX4**: Decreased GSH levels, GPX4 inactivation/depletion.
- **Gene/protein**: Upregulated *PTGS2* (COX-2), *CHAC1*; ACSL4 expression (sensitivity marker).
- **Morphological**: Transmission electron microscopy (TEM) for shrunken mitochondria with cristae loss.
- **Other**: LDH release (lysis), ROS (DCFH-DA), lipid ROS-specific probes.

Ferroptosis research is rapidly expanding, with therapeutic interest in **inducing** it for cancer treatment and **inhibiting** it for degenerative and ischemic diseases. It often crosstalks with apoptosis, necroptosis, and pyroptosis (sometimes in PANoptosis complexes). Experimental confirmation typically combines morphology, specific probes, inhibitors (rescue with Fer-1 or DFO), and molecular markers.

Feature	**Ferroptosis**	**Pyroptosis**
Nature	Iron-dependent regulated necrosis driven by lipid peroxidation	Caspase- and gasdermin-dependent inflammatory lytic cell death
Primary Triggers	Oxidative stress, iron overload, GSH depletion, GPX4 inhibition, certain drugs (erastin, RSL3)	PAMPs/DAMPs (e.g., bacterial toxins, LPS, crystals), inflammasome activators, infections
Key Pathway	Failure of lipid peroxide detoxification (GPX4/GSH axis) + iron-catalyzed Fenton reaction	Inflammasome assembly → inflammatory caspases (1/4/5/11)
Executioner	Lipid hydroperoxides (accumulated in membranes)	Gasdermin D (GSDMD) pores (or other gasdermins)
Morphology	Shrunken mitochondria with cristae loss & dense membrane; plasma membrane rupture; minimal nuclear changes	Cell swelling (oncosis), large GSDMD pores (~10–20 nm), plasma membrane rupture, chromatin condensation
Inflammation	Pro-inflammatory via DAMPs and oxidized lipids	Highly pro-inflammatory (direct IL-1β/IL18 release + DAMPs)
Caspase Involvement	No (caspase-independent)	Yes (inflammatory caspases essential)
Iron Dependence	Yes (central)	No
Energy Requirement	ATP-dependent (early)	ATP-dependent (early)
Cell Types	Broad (cancer cells, neurons, endothelial cells, etc.)	Especially myeloid cells (macrophages, monocytes); also epithelial cells
Physiological Role	Tumor suppression, antimicrobial (limited), tissue damage	Host defense against intracellular pathogens; amplifies innate

Feature	Ferroptosis	Pyroptosis
	in ischemia	imunity

(7) Autophagy (programmed cell death type II)

Autophagy (from Greek: "self-eating") is a highly conserved, lysosome-dependent cellular degradation and recycling process essential for maintaining homeostasis, removing damaged components, and responding to stress. It is primarily a **pro-survival mechanism** but can contribute to cell death in certain contexts (autophagic cell death). Autophagy is the phagocytosis of damaged organelles or aging proteins by intracellular lysosomes, which is a kind of self-protection of cells and usually triggers inflammation. but is primarily a survival mechanism.
There are three main types:

- **Macroautophagy** (the most studied, often just called autophagy): Involves formation of double-membrane vesicles.
  - **Microautophagy**: Direct engulfment by lysosomes.
  - **Chaperone-mediated autophagy (CMA)**: Selective, receptor-mediated translocation of proteins.

Nature

- **Bulk or selective**: Can non-specifically degrade cytoplasm or selectively target organelles (mitophagy), proteins (aggrephagy), pathogens (xenophagy), lipids (lipophagy), etc.
- **Dynamic flux**: Involves initiation, autophagosome formation, fusion with lysosomes (autolysosome), degradation, and nutrient recycling.
- Regulated by nutrient/energy sensors: Inhibited by mTOR (under nutrient-rich conditions) and activated by AMPK (energy depletion).

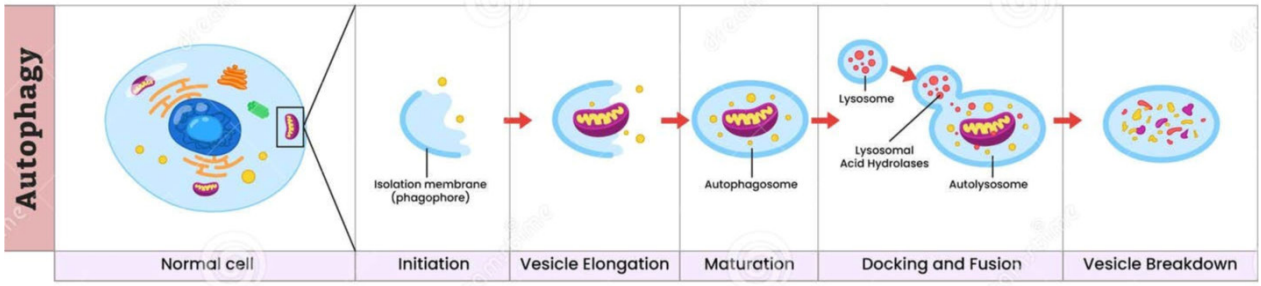


Fig. Autophagy

Triggers

Common inducers include:

- Nutrient/amino acid/glucose starvation or growth factor withdrawal.
- Energy depletion (low ATP).
- Cellular stresses: Hypoxia, oxidative stress, ER stress, DNA damage, protein aggregates, damaged organelles.
- Infections (pathogens), exercise, caloric restriction, certain drugs (e.g., rapamycin).
- Developmental cues and immune signaling.

Mechanisms (Macroautophagy)

1. **Initiation**: ULK1/2 complex (activated when mTOR is inhibited or AMPK is active) phosphorylates downstream targets.
2. **Nucleation**: Beclin-1-VPS34 (PI3K) complex generates PI3P at the phagophore (isolation membrane), often from ER or other sources.
3. **Elongation & Closure**: Two ubiquitin-like conjugation systems — ATG12-ATG5-ATG16L1 and LC3 (ATG8) lipidation (LC3-I to LC3-II) — expand and close the autophagosome.
4. **Maturation & Fusion**: Autophagosome fuses with lysosome (via SNAREs, Rab proteins) to form autolysosome.
5. **Degradation**: Lysosomal hydrolases break down contents; nutrients (amino acids, lipids) are released.

Selective autophagy uses receptors (e.g., p62/SQSTM1, NBR1, OPTN) that link cargo to LC3.

Morphology

- **Phagophore**: Cup-shaped double-membrane structure.
- **Autophagosome**: Double-membrane vesicle (0.5–1.5 μm) containing cytoplasmic material/organelles.
- **Autolysosome**: Single-membrane vesicle after fusion, with degraded contents.
- Electron microscopy classically shows double-membrane structures; fluorescence shows LC3 puncta.

Relation to Inflammation and Inhibitors
Autophagy generally **suppresses inflammation** by:
- Removing damaged mitochondria (reducing ROS and NLRP3 inflammasome activation).
- Degrading pro-inflammatory proteins/aggregates.
- Regulating immune cell function and cytokine release.
- Promoting resolution of inflammation.

Dysregulated autophagy can promote chronic inflammation. It has complex crosstalk with inflammasomes and other PCD pathways (e.g., can inhibit or be inhibited by them).
Common pharmacological modulators:
- **Inducers**: Rapamycin (mTOR inhibitor), starvation, metformin (AMPK), spermidine.
- **Inhibitors**:
  - Early stage: 3-Methyladenine (3-MA, PI3K inhibitor), ULK1 inhibitors.
  - Late stage: Chloroquine/hydroxychloroquine (raises lysosomal pH, blocks fusion/degradation), Bafilomycin A1 (V-ATPase inhibitor, blocks acidification and fusion).

Examples
- **Mitophagy**: Removal of damaged mitochondria (PINK1/Parkin pathway).
- **Xenophagy**: Clearance of intracellular bacteria/viruses.
- **Lipophagy**: Lipid droplet breakdown.
- **Reticulophagy, pexophagy**, etc.

Diseases
Autophagy has **context-dependent dual roles** (protective or pathogenic):
- **Neurodegenerative** (Alzheimer's, Parkinson's, Huntington's, ALS): Often impaired; leads to protein aggregate accumulation (e.g., amyloid, tau, α-synuclein). Enhancing autophagy is therapeutic.
- **Cancer**: Tumor-suppressive in early stages (prevents genomic instability); pro-survival in established tumors (helps cancer cells survive stress/chemotherapy). Autophagy modulation is explored in therapy.
- **Infectious/Inflammatory**: Protective against pathogens; defects linked to Crohn's disease (e.g., ATG16L1 mutations). Dysregulation in sepsis, autoimmune diseases.
- **Metabolic**: Type 2 diabetes, obesity, NAFLD (impaired lipophagy).
- **Aging and cardiovascular/renal diseases**: Decline in autophagy contributes to pathology.

Markers
Commonly used to monitor autophagy (note: flux assessment — e.g., with/without inhibitors — is crucial, as increased autophagosomes can mean induction or blocked degradation):
- **LC3-II** (lipidated form): Increased in autophagosomes (Western blot, puncta by IF); gold standard.
- **p62/SQSTM1**: Decreased when autophagy is active (degraded with cargo); accumulates if flux is blocked.
- **Beclin-1**: Upregulation often indicates initiation.
- Others: ATG proteins, ULK1 phosphorylation, autophagic flux assays (tandem LC3 reporters like mRFP-GFP-LC3), electron microscopy.

Summary: Autophagy is a vital housekeeping and stress-response pathway. Its dysregulation is implicated in numerous diseases, making it a major therapeutic target (induction for neurodegeneration/aging, inhibition for some cancers). Context (cell type, stage, stimulus) determines beneficial vs. detrimental effects. The field is active, with ongoing research into selective autophagy and clinical modulators. For specific contexts or experimental guidance, provide more details.

Programmed cell death: Molecular pathophysiology (2008 -2022) commmentary to the lecture Cell cycle and Apopsosis ans necrosisi

Apoptosis

A controlled sequence of cells dies through a process known as **apoptosis** without releasing toxic chemicals into the surrounding region. Cell suicide or planned cell death are other names for it. The most well-understood type of programmed cell death is this one. Even when a cell is killed, the organism gains from it. The cell's contents are systematically packed into little membrane packets for the immune cells to gather waste. Removing cells that are in the process of developing, as well as possibly malignant and virus-infected cells, aids in maintaining the body's equilibrium.

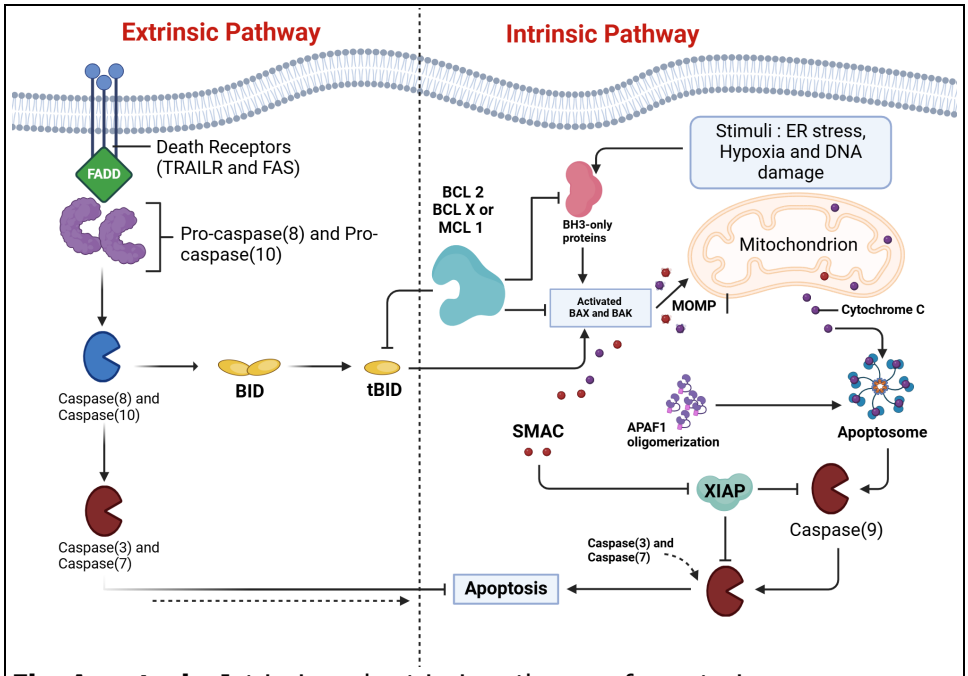


Fig. Apoptosis. Intrinsic and extrinsic pathways of apoptosis.

The process is known as apoptosis when a cell "decides" to die on purpose in a multicellular organism. This frequently happens for the benefit of the entire organism, for example, when a cell's DNA has been broken and it may become cancer.

Mechanism of both Intrinsic and Extrinsic Pathways

There are two main categories of apoptosis pathways:

a) Intrinsic Pathway: The mitochondrial pathway is another term for this process. When DNA damage is detected, one of the cell's own genes or proteins sends a signal to the cell to kill itself. This process is also called the mitochondrial pathway.

b) Extrinsic Pathway: In this case, other cells in the body provide a signal to a cell to begin apoptosis. When a cell has reached the end of its functional life or is no longer a wise investment for the organism to sustain, this occurs. This is also called the death receptor pathway.

Apoptosis Steps

- The decrease of cell volume is a common feature of programmed cell death, and Cell Shrink is seen in all forms of apoptosis.
- The DNA in the cell's nucleus condenses and fragments into pieces of uniform size in the Cell Fragments step.
- Cytoskeleton Collapses - At this point, the cytoskeleton of the young organism collapses.
- Disassembling the Nuclear Envelope: This stage involves disassembling the nuclear envelope.
- In the stage of Cells Release of Apoptotic bodies, cells go through morphological changes that include membrane damage, the creation of thin membrane protrusions, and the production of discrete apoptotic bodies

Function of Apoptosis

Apoptosis provides the following functions for the organism and is essential for planning.

- Rebuild some defective and older cells with fresh ones that do the same function, maintaining the health of the body. This is crucial in the case of the body's defense cells, which may acquire a tendency to wrongly target healthy areas.
- Getting elimination of atypical cells that have small, abnormal, virus- or DNA-damaged, or atypical characteristics during birth.
- Participate in the early development of the organism during critical moments, such as the embryonic stage, when tissue must be shed or divided. For instance, a membrane holds the fingers together as they develop.
- The membrane's cells need to be set up to die in order to separate the fingers. During menstruation, the uterine endometrium experiences the same thing.

ignificance of Apoptosis

- Apoptosis causes about one lakh cells in the human body to die every single second, while mitosis results in a comparable number of new cells.
- Cell development depends on programmed cell death.
- In typical youngsters between the ages of 8 and 14, between 29 and 30 billion cells each day perish.
- Our bodies undergo a full epithelial lining change called apoptosis every 23 days.
- Apoptosis eliminates the harmful T-lymphocytes.
- Apoptosis keeps the number of cells in an organism constant.
- Apoptosis causes undesirable cells to die and leave the body.
- This results in the overproduction of cells that eventually experience programmed cell death, shaping various or-

gans and tissues throughout development.

Difference Between Apoptosis And Necrosis: Apoptosis and Necrosis are two distinct processes of cell death that play crucial roles in various physiological and pathological conditions. While both processes involve the demise of cells. they differ significantly in their underlying mechanisms and consequences. This article aims to explore and elucidate the dissimilarities between apoptosis and necrosis shedding light on their distinctive characteristics and implications.

Feature	Apoptosis	Necrosis
Cell death mechanism	The Programmed cell death	The Unprogrammed cell death
Trigger	Controlled signaling pathways	External factors, injury, or infection
Energy requirement	Requires ATP	Does not require ATP
Morphological changes	The Cell shrinkage, membrane blebbing, nuclear fragmentation	The Cell swelling, membrane rupture, and organelle damage
Inflammation	Usually no inflammation	Frequently associated with the inflammation
Cell membrane integrity	Maintained until late stages	Early loss of the cell membrane integrity
Immunological response	Phagocytosis of the apoptotic bodies by neighboring cells	Inflammatory response to cell rupture
Tissue consequences	The Minimal impact on surrounding tissue	The Inflammatory response may damage nearby tissue
Physiological role	The Normal development, tissue homeostasis, removal of damaged cells	The Pathological conditions, injury, infection
Examples	Developmental processes, immune system regulation	Trauma, ischemia, infections

References

Li, J., Cao, F., Yin, Hl. et al. Ferroptosis: past, present and future. Cell Death Dis 11, 88 (2020). https://doi.org/10.1038/s41419-020-2298-2

Yu, P., Zhang, X., Liu, N. *et al.* Pyroptosis: mechanisms and diseases. *Sig Transduct Target Ther* **6**, 128 (2021). https://doi.org/10.1038/s41392-021-00507-5