

REVIEW ON CELL DEATH AND

DIFFERENTIATION

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**CELL DEATH**

INTRODUCTION:

Cell death is a natural endpoint of normal cell physiology that results in irreversible termination of cellular functions, including growth, division, and metabolic homeostasis. Recent discoveries have reshaped our understanding of the complex modes by which eukaryotic cells die during normal growth and development, in response to physiological injury, and during progression of disease.

Different types of cell death are often defined by morphological criteria, without a clear reference to precise biochemical mechanisms.

Cell death can be classified according to its morphological appearance (which may be apoptotic, necrotic, autophagic or associated with mitosis), enzymological criteria (with and without the involvement of nucleases or of distinct classes of proteases, such as caspases, calpains, cathepsins and transglutaminases), functional aspects (programmed or accidental, physiological or pathological) or immunological characteristics (immunogenic or non-immunogenic).

In this journal we elaborately discuss three different type of cell death:

1. Apoptosis
2. Necroptosis
3. Pyroptosis

**LITERATURE REVIEW**

**Apoptosis**

The term “apoptosis” is derived from the Greek words “απο” and “πτωσιζ” meaning “dropping off” and refers to the falling of leaves from trees in autumn. It is used, in contrast to necrosis, to describe the situation in which a cell actively pursues a course toward death upon receiving certain stimuli.

Understanding apoptosis in disease conditions is very important as it not only gives insights into the pathogenesis of a disease but may also leaves clues on how the disease can be treated.

Apoptosis occurs normally during development and aging and as a homeostatic mechanism to maintain cell populations in tissues. Apoptosis also occurs as a defense mechanism such as in immune reactions or when cells are damaged by disease or noxious agents (Norbury and Hickson, 2001). Although there are a wide variety of stimuli and conditions, both physiological and pathological, that can trigger apoptosis, not all cells will necessarily die in response to the same stimulus.

Apoptosis is an ordered and orchestrated cellular process that occurs in physiological and pathological conditions. An understanding of the underlying mechanism of apoptosis is important as it plays a pivotal role in the pathogenesis of many diseases. Cancer is one of the scenarios where too little apoptosis occurs, resulting in malignant cells that will not die.

<https://jeccr.biomedcentral.com/articles/10.1186/1756-9966-30-87>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2117903/>

Defects can occur at any point along these pathways, leading to malignant transformation of the affected cells, tumour metastasis and resistance to anticancer drugs. Despite being the cause of problem, apoptosis plays an important role in the treatment of cancer as it is a popular target of many treatment strategies.

One example is the downregulation of p53, a tumour suppressor gene, which results in reduced apoptosis and enhanced tumour growth and development and inactivation of p53, regardless of the mechanism, has been linked to many human cancers

Hence, apoptosis plays an important role in both carcinogenesis and cancer treatment.

Drugs or treatment strategies that can restore the apoptotic signalling pathways towards normality have the potential to eliminate cancer cells, which depend on these defects to stay alive. Many recent and important discoveries have opened new doors into potential new classes of anticancer drugs.

The alternative to apoptotic cell death is necrosis, which is considered to be a toxic process where the cell is a passive victim and follows an energy-independent mode of death. But since necrosis refers to the degradative processes that occur after cell death, it is considered by some to be an inappropriate term to describe a mechanism of cell death.

Whether a cell dies by necrosis or apoptosis depends in part on the nature of the cell death signal, the tissue type, the developmental stage of the tissue and the physiologic milieu.

Necrosis is an uncontrolled and passive process that usually affects large fields of cells whereas apoptosis is controlled and energy-dependent and can affect individual or clusters of cells.

Caspases are central to the mechanism of apoptosis as they are both the initiators and executioners. There are three pathways by which caspases can be activated. The two commonly described initiation pathways are the intrinsic (or mitochondrial) and extrinsic (or death receptor) pathways of apoptosis. Both pathways eventually lead to a common pathway or the execution phase of apoptosis. A third less well-known initiation pathway is the intrinsic endoplasmic reticulum pathway

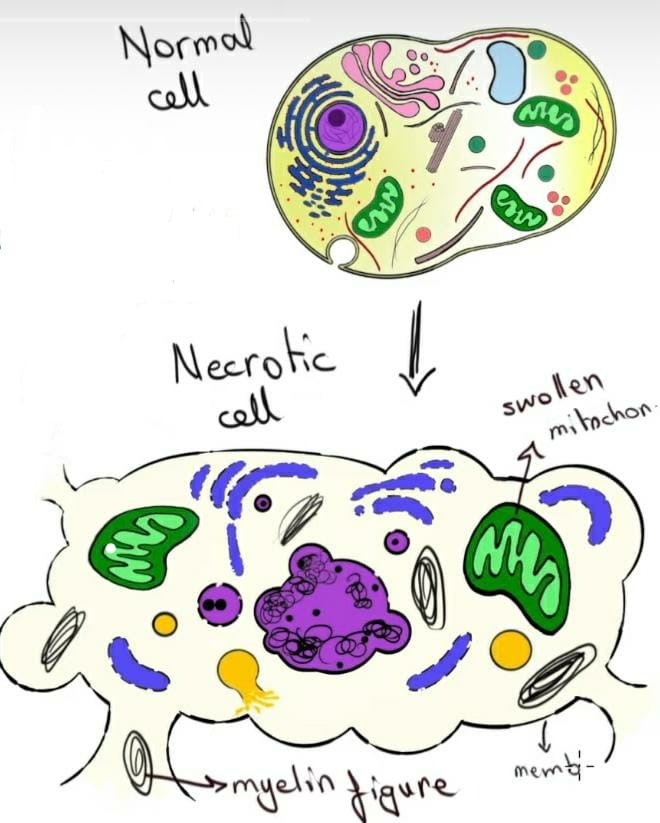
The upstream caspase for the intrinsic pathway is caspase 9 while that of the extrinsic pathway is caspase 8. The intrinsic and extrinsic pathways converge to caspase 3. Caspase 3 then cleaves the inhibitor of the caspase-activated deoxyribonuclease, which is responsible for nuclear apoptosis

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**Necroptosis**

Necroptosis, an emerging field closely related to apoptosis, is a non-caspase-dependent cell death that has been implicated in the pathological processes of various diseases. It is regulated by various genes that cause regular and ordered cell death. Through activating specific death signalling pathways, it shares typical characteristics of necrosis, including loss of metabolic function and subcellular changes ([1](https://www.spandidos-publications.com/10.3892/ijmm.2021.4922#b1-ijmm-47-05-04922),[2](https://www.spandidos-publications.com/10.3892/ijmm.2021.4922#b2-ijmm-47-05-04922)). Receptor-interacting protein kinase 1 (RIP1) was the first signalling molecule identified in the necrosome ([3](https://www.spandidos-publications.com/10.3892/ijmm.2021.4922#b3-ijmm-47-05-04922)). RIP1 and RIP3 interact with the receptor protein, transducing death signals, and further recruiting and phosphorylating mixed lineage kinase domain-like protein (MLKL) ([4](https://www.spandidos-publications.com/10.3892/ijmm.2021.4922#b4-ijmm-47-05-04922)-[7](https://www.spandidos-publications.com/10.3892/ijmm.2021.4922#b7-ijmm-47-05-04922)). Necroptosis can be involved in the regulation of several signalling pathways, including the caspase-8-dependent apoptotic pathway, the mitogen-activated protein (MAP) kinase cascade, and activation of the nuclear factor-κB (NF-κB) pathway.

Necroptosis is a programmed form of necrosis. It can be initiated by different stimuli, such as tumor necrosis factor (TNF), TNF-related apoptosis-inducing ligand (TRAIL), Fas ligand (FasL), interferon (IFN), LPS, viral DNA or RNA, DNA-damage agent and requires the kinase activity of receptor-interacting protein 1 (RIPK1) and RIPK3.



**Difference of the key characteristics between apoptosis and necroptosis**

Although necroptosis is characterized by caspase independence, the molecular pathway involved is similar to and shares features of apoptosis. However, the immunological and morphological consequences of necroptosis are vastly different. Necroptosis shares the major morphological features of necrosis, such as the swelling of organelles, gradually translucent cytoplasm, and rupture of the cellular membrane. By contrast, apoptosis is characterized by membrane blebbing, cell shrinkage, nuclear fragmentation, and chromatin concentration. The rupture of the cellular membrane results in the release of cellular contents, leading to the exposure of damage-associated molecular patterns (DAMPs), triggering a strong inflammatory response in necroptosis, suggesting necroptotic cells are more immunogenic than apoptotic cells, which is relatively intact, with DAMP restricted to the plasma membrane, or encapsulated in the apoptotic bodies. It has also been shown that necroptosis was associated with maintenance of T-cell homeostasis, as it has been found to be able to clear excess and abnormal T cells in the absence of caspase-8, which can prevent abnormal proliferation of lymphocytes.

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4035222/>

HOW TO IDENTIFY NECROTOPSIS?

As there is currently no specific marker for necroptosis, multiple methods are usually required to identify necroptosis . In cultured cells, transmission electron microscopy can be used to identify necroptotic cells . Detection of key molecular, including RIP1, RIP3 and MLKL activation, necrosome formation, MLKL oligomerization, and membrane translocation can also be used to identify necroptosis. Activation of RIP3 and MLKL can be monitored by western blot analysis to assess phosphorylation status. Phosphorylation of MLKL at Ser358 and Thr357 and RIP3 at S227 indicates the activation of necroptosis.

**Pyroptosis**

Pyroptosis is composed of “pyro” and “ptosis”. “Pyro” means fire, indicating the properties of inflammation of pyroptosis, but “ptosis” means falling, which is consistent with other forms of programmed cell death.

Currently, pyroptosis has received more and more attention because of its association with innate immunity and disease. The research scope of pyroptosis has expanded with the discovery of the gasdermin family. Pyroptosis is a form of inflammatory programmed cell death pathway activated by human and mouse caspase-1, human caspase-4 and caspase-5, or mouse caspase-11. These inflammatory caspases are used by the host to control bacterial, viral, fungal or protozoan pathogens. Pyroptosis, or caspase 1-dependent cell death, is inherently inflammatory, is triggered by various pathological stimuli, such as stroke, heart attack or cancer, and is crucial for controlling microbial infections.

Pyroptosis was defined as gasdermin-mediated programmed death in 2015. The gasdermin superfamily is composed of gasdermin A/B/C/D (GSDMA/B/C/D), gasdermin E (GSDME, also referred to as DFNA5) and DFNB59 (Pejvakin, PJVK) in human (Gsdma1-3, Gsdmc1-4, Gsdmd, Dfna5, and Dfnb59 in mice) Among these conserved proteins, GSDMD and DFNA5 are most deeply studied in pyroptotic death.

<https://www.nature.com/articles/s41392-021-00507-5>

There are some similarities between pyroptosis and apoptosis, such as DNA damage and chromatin condensation. Interestingly, pyroptotic cells emerged swelling and a lot of bubble-like protrusions appear on the surface of the cellular membrane before its rupture. Similarly, membrane blebbing also occurs during apoptosis, and caspase-3 is necessary for this process. However, the unique morphological characteristics of pyroptosis are obviously different from those of apoptosis. It is generally believed that apoptosis is a safe form of death, but pyroptosis can cause inflammation, activated by extracellular or intracellular stimulation, such as bacterial, viral, toxin, and chemotherapy drugs. In fact, unlike the explosive rupture associated with necrosis, pyroptosis causes flattening of the cytoplasm due to plasma membrane leakage. In addition, caspases activation or release of granzymes results in the N-terminal of gasdermin oligomerization and pore formation (1–2 μm in diameter) in the plasma membrane, which allows mature IL-1β/IL-18 with a diameter of 4.5 nm and caspase-1 with a diameter of 7.5 nm to pass through, respectively.

## **Similarities b/w Pyroptosis and Apoptosis**

## Characteristics Pyroptosis Apoptosis

## Programmed cell death Yes Yes

## PS exposure Yes Yes

## Annexin V staining Yes Yes

## 

## TUNEL staining Yes Yes

## DNA damage Yes Yes

## Chromatin condensation Yes Yes

## Membrane blebbing Yes Yes

## The diameters of pyroptotic bodies and

## apoptotic bodies (1–5 µm) Yes Yes

## Caspase-3 activation **Yes** Yes

## Caspase-6 activation Yes Yes

## Caspase-8 activation Yes Yes

## Caspase-9 activation Yes Yes

<https://www.nature.com/articles/nrmicro2070>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5416822/>

**METHODOLOGY**

We referred to few journals and articles:

# Classification of cell death

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2744427/>

# Current translational potential and underlying molecular mechanisms of necroptosis

<https://www.nature.com/articles/s41419-019-2094-z>

# Necroptosis: a crucial pathogenic mediator of human disease

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6693822/>

# Apoptosis in cancer: from pathogenesis to treatment

<https://jeccr.biomedcentral.com/articles/10.1186/1756-9966-30-87>

# Apoptosis: A Review of Programmed Cell Death

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2117903/>

# Pyroptosis: mechanisms and diseases

<https://www.nature.com/articles/s41392-021-00507-5>

# Necroptosis

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4035222/>

**FUTURE WORK**

1. **To study potential role of necroptosis in clinical diseases.**
2. **Role of pyroptosis in diseases.**
3. **To study apoptosis in cancer.**
4. **To elaborate role of caspase 6 in apoptosis pathways**
5. **Comparing and contrasting the three types of cell death**

* **NECROPTOSIS**
* **APOPTOSIS**
* **PYROPTOSIS**

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