



Cell death mechanisms in eukaryotes

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Abstract Like the organism they constitute, the cells also die in different ways. The death can be predetermined, programmed, and cleanly executed, as in the case of apoptosis, or it can be traumatic, inflammatory, and sudden as many types of necrosis exemplify. Nevertheless, there are a number of cell deaths—some of them bearing a resemblance to apoptosis and/or necrosis, and many, distinct from each—that serve a multitude of roles in either supporting or disrupting the homeostasis. Apoptosis is coordinated by death ligands, caspases, b-cell lymphoma-2 (Bcl-2) family proteins, and their downstream effectors. Events that can lead to apoptosis include mitotic catastrophe and anoikis. Necrosis, although it has been considered an abrupt and uncoordinated cell death, has many molecular events associated with it. There are cell death mechanisms that share some standard features with necrosis. These include methuosis, necroptosis, NETosis, pyronecrosis, and pyroptosis. Autophagy, generally a catabolic pathway that operates to ensure cell survival, can also kill the cell through mechanisms such as autosis. Other cell-death mechanisms include entosis, ferroptosis, lysosome-dependent cell death, and parthanatos.

Keywords Apoptosis · Autosis · Entosis · Ferroptosis · Methuosis · Necroptosis · Necrosis · NETosis · Parthanatos · Pyronecrosis · Pyroptosis

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Introduction

For the homeostatic well-being of eukaryotes, a fine balance between birth and death of its cells is necessary. The cells eliminate themselves through a number of dying mechanisms. From elegantly orchestrated apoptosis to abrupt and stochastic necrotic death, the cell meets its end either for the well-being or to the peril of the organism. There is a multitude of ways a cell can die via interrelated mechanisms, and specific guidelines have been evolved to identify these mechanisms of cell death (Kroemer et al. 2009). Understanding the mechanisms of cell death not only sheds light on the basic cellular biology but it also helps in devising novel therapeutic strategies against proliferative, degenerative, infectious, and autoimmune diseases.

This review focuses on some of the well-characterised cell death mechanisms. We have classified these mechanisms based on their mode of execution and end-result as apoptotic cell death (characterised by the involvement of apoptotic proteins), necrotic cell death (characterised by membrane rupture and inflammation), and other forms of cell death. Under apoptotic death mechanisms, *the extrinsic pathway*, initiated by the binding of death-inducing ligands onto specific receptors on the cells, *the intrinsic pathway*, whose initiation is centered in and around the mitochondria, and *the endoplasmic reticulum stress-mediated pathway* that is induced by excessive ER stress, are described. An apoptotic pathway designed to fight pathogens, called *the perforin/granzyme pathway*, has also been described. Under necrotic cell death mechanisms, *classical necrosis*, *mitochondrial permeability transition-mediated necrosis*, *methuosis*, *necroptosis*,

NETosis, *pyronecrosis*, and *pyroptosis* are described. Under other forms of cell death, *autosis*, *entosis*, *ferroptosis*, *lysosome-dependent cell death*, and *parthanatos* are described.

Apoptosis

Apoptosis (derived from the Greek word for ‘falling off’ of leaves from a tree) is a programmed cell death (PCD) that plays a crucial role in development and diseases (Green and Llamby 2015). The morphological manifestations of apoptosis include cell membrane blebbing, shrinkage of the cell, and formation of apoptotic bodies (see below). Apoptosis can generally be executed via three different pathways, namely, *the extrinsic pathway*, *the intrinsic pathway*, and *endoplasmic reticulum stress (ER stress)–induced pathway* (Fig. 1). These pathways can converge to bring forth the culminating face of apoptosis (Elmore 2007). These pathways involve the action of the cysteine proteases called *caspases* that cleave their target proteins after an aspartic acid residue.

The extrinsic pathway is triggered by the binding of ligands to the transmembrane death receptors, such as *tumour necrosis factor receptor 1* (TNFR1), and Fas receptor (FasR). The ligand binding causes clustering of the cytoplasmic domains of these receptors, which, in turn, recruits adapter proteins containing corresponding death domains (Fig. 1a). The death domains of these adapter proteins serve as a binding site for different upstream (initiator) procaspases (such as caspase-8 and caspase-10) to form a death-inducing signaling complex (DISC), which activates these caspases (Locksley et al. 2001). The activated upstream caspases then activate downstream (executioner) caspases (such as caspase-3, caspase-6, and caspase-7) to execute the cell death. A prominent feature of apoptosis is the absence of systemic or localised damage to other cells owing to lack of inflammation during its initiation, completion, and the clearance of the dead cells. The apoptotic cell achieves this by carefully wrapping the cell membrane around the now fragmented cell, forming *apoptotic bodies*. Nucleotides such as adenosine 5′-triphosphate (ATP) and uridine-5′-triphosphate (UTP), which are released by the apoptotic cell, serve as the ‘find-me’ signal for macrophages (Medina and Ravichandran 2016). Once a macrophage gain sufficient proximity, the apoptotic bodies enable the former to engulf them by displaying phosphatidyl serine (the ‘eat-me signal’) on their surface

(Nagata 2018). The process of engulfment of apoptotic cells by macrophages is called *efferocytosis*, a process bearing similarity to micropinocytosis. Apoptosis, in concordance with efferocytosis, is necessary for the well-being of the organism. If an apoptotic cell fails to undergo proper clearance via efferocytosis, it may undergo secondary necrosis. Inefficient efferocytosis can lead to diseases such as cystic fibrosis and rheumatoid arthritis (Vandivier et al. 2006).

The intrinsic pathway, also called the mitochondrial pathway, involves the release of cytochrome c from the mitochondria to the cytoplasm in response to cellular stress (Fig. 1a). The cytochrome c, in association with *apoptotic protease-activating factor-1* (Apaf-1), forms an *apoptosome*. Within apoptosome, procaspase-9 is activated to become caspase-9. Caspase-9, in turn, activates downstream effector caspases. The mitochondria further release proapoptotic proteins such as *apoptosis-inducing factor* (AIF) and endonuclease G. AIF translocates to the nucleus and facilitates DNA fragmentation and chromatin condensation. After this, ‘stage I’ chromatin condensation (also called *ring condensation*) (Toné et al. 2007) ensues the translocation of endonuclease G to the nucleus leading to further fragmentation of the DNA. Finally, the caspase-activated DNase (CAD) is translocated to the nucleus, leading to ‘stage II condensation’ (the *necklace condensation*) (Toné et al. 2007). The mitochondrial phase of apoptosis is regulated by the Bcl-2 family of proteins (Elmore 2007; Edlich 2018). The proapoptotic proteins of this family, such as Bax and Bak, create pores on the mitochondrial membrane in response to apoptotic stimuli, facilitating the release of cytochrome c. Meanwhile, other proapoptotic proteins, such as Bim, keep the anti-apoptotic protein, Bcl-2, inactive. The released cytochrome c, through its interaction with Apaf-1, activates caspase-9. Meanwhile, the mitochondria release a second molecule, aptly named, *second mitochondria-derived activator of caspase* (SMAC) that inhibits IAP (for *inhibitor of apoptosis*) proteins preventing the latter from deactivating caspase-9 (Edlich 2018). Caspase-9, thus fully enabled, carries out the cell death by activating downstream caspases, such as caspase-3.

Infections, hypoxia, starvation, chemicals such as tunicamycin, thapsigargin, Brefeldin A (Osłowski and Urano 2011), and non-homeostatic changes in secretory functions of the endoplasmic reticulum (ER) contribute to the accumulation of unfolded or misfolded proteins in it facilitating ER stress–induced cell death (Iurlaro and

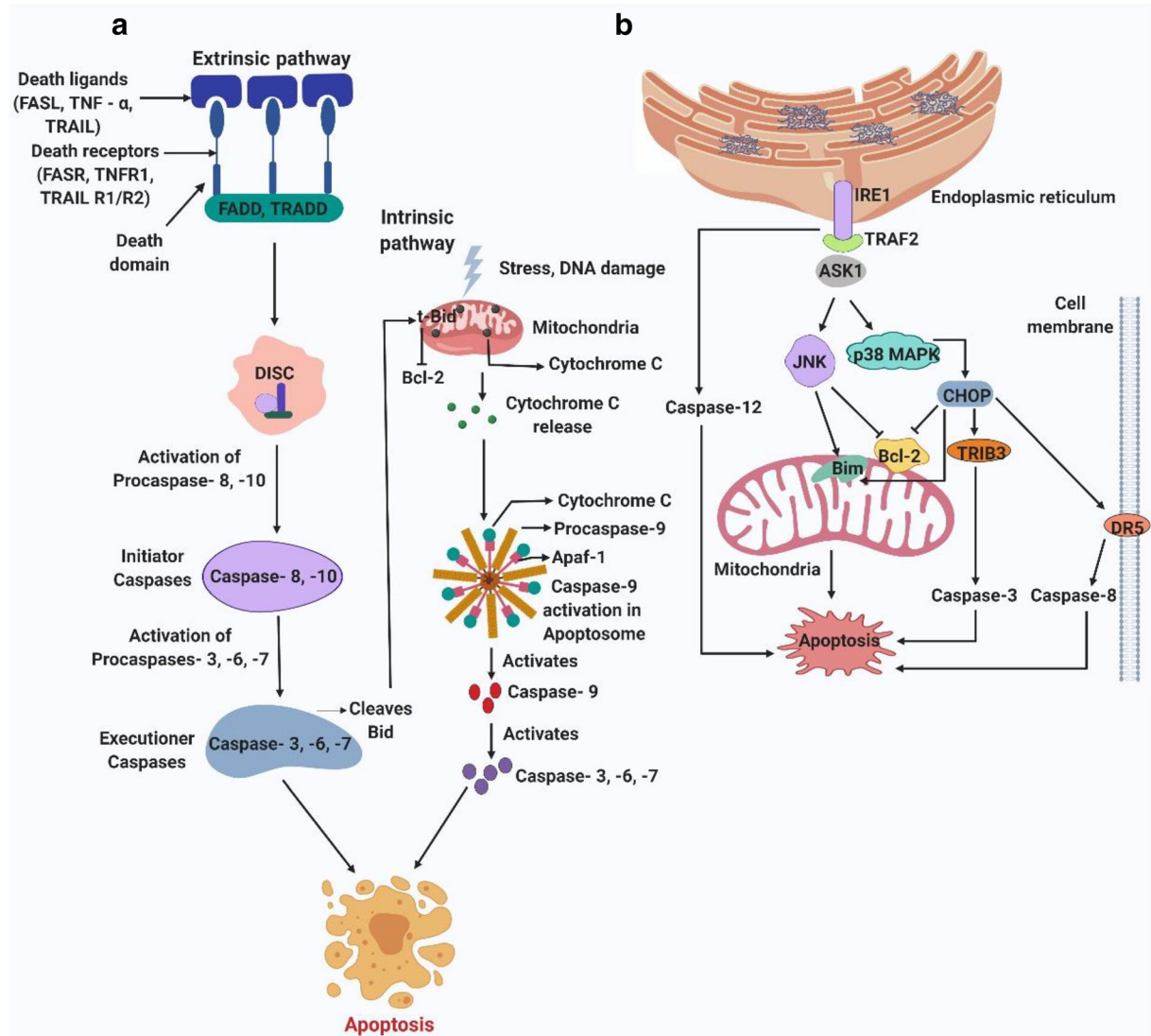


Fig. 1 Signalling pathways of apoptosis. (A) The extrinsic pathway of apoptosis is mediated by binding of death-inducing ligands to death receptors, such as TNFR1, FASR, and TRAIL R1/R2. The binding recruits adapter proteins and initiator procaspases (procaspase-8, procaspase-10) to the death domains of the receptors, TRADD and FADD, leading to the formation of death-inducing signalling complex (DISC). The induced proximity thus achieved by the procaspases activates them. The initiator caspases then activate executioner procaspases (procaspase-3, procaspase-6, and procaspase-7) leading to cell death. The intrinsic pathway of apoptosis is mediated by cellular stress results in the permeabilisation of mitochondrial membranes by the proapoptotic family of Bcl-2 proteins. Following apoptotic stimulation, cytochrome c, and subsequently, other apoptosis-promoting factors are

released from the mitochondria. In the cytosol, cytochrome c associates with Apaf-1 to form apoptosome, activating thus caspase-9, leading to the activation of downstream caspases resulting in apoptosis. (B) The ER stress-induced pathway is initiated. The major component of this pathway, IRE1, recruits TRAF2 and ASK1 and bring about the cell death through JNK-, and p38 MAPK-signalling cascades. IRE1 promotes apoptosis via caspase-12 as well. JNK pathway operates by activating Bim and inhibiting Bcl-2. The p38-MAPK facilitates cell death via activation of CHOP. CHOP facilitates the expression of proapoptotic proteins such as GADD 34, TRIB3, and DR5. CHOP also activates Bim and inhibits Bcl-2

Muñoz-Pinedo 2016) (Fig. 1b). When misfolding of proteins occurs in the ER, an unfolded protein response (UPR) pathway is initiated. The UPR then halts global

protein synthesis and focuses specifically on the replacement of the unfolded proteins with correctly folded proteins. Three major components of this pathway are

protein kinase RNA-like endoplasmic reticulum kinase (PERK), *inositol-requiring enzyme 1* (IRE1), and *activating transcription factor 6* (ATF6) that work in tandem to restore proteostasis in ER. However, if the cell's attempt to resolve the persistence of unfolded proteins fails, it chooses to initiate apoptosis. IRE1 plays a significant role in this apoptosis induction via a caspase-12-mediated pathway (Yoneda et al. 2001) and also via a *TNF receptor-associated factor 2-apoptosis signal-regulating kinase 1* (TRAF2-ASK1) complex-mediated, *c-Jun N-terminal kinase 1* (JNK), and p38-MAPK signaling cascade. The JNK pathway activation leads to apoptosis by inhibition of the anti-apoptotic protein, Bcl-2, and activation the proapoptotic protein, Bim. The p38-MAPK pathway facilitates the cell death via activation of a key protein located downstream of the PERK and ATF6 pathways, called *C/EBP homologous protein* (CHOP). CHOP facilitates the expression of many proapoptotic proteins such as the caspase-3 activator *tribbles homologue 3* (TRIB3) (Sano and Reed 2013), *growth arrest and DNA damage protein-34* (GADD-34), and the tumour necrosis factor (TNF) receptor protein, *death receptor 5* (DR5), promoting caspase-8-dependent apoptosis. Further, CHOP, like JNK, inhibits Bcl-2 transcription and promotes expression of Bim. The ER stress-induced apoptosis has been reported in diseases such as Alzheimer's and Parkinson's disease, retinitis pigmentosa, glaucoma, macular degeneration, inflammatory diseases, tumorigenesis, and metabolic diseases (Sano and Reed 2013). Drugs, such as Withaferin A, can induce ER stress-mediated apoptosis via activation of caspase-4, a functional homologue of caspase-12 (Hitomi et al. 2004). Bax inhibitor-1 (BI-1/Tmbim6) and Bcl-2/Bcl-XL are major inhibitors of ER stress-induced apoptosis (Sano and Reed 2013).

Two cellular phenomena that can induce apoptosis are *mitotic catastrophe* (MC) and *anoikis*. MC occurs when a cell fails to complete mitosis either due to DNA damage or to dysregulation of proteins that are involved in mitosis. MC can also occur when a cell enters the mitotic phase prematurely with incomplete DNA synthesis. Further, ionising radiation, physical or chemical stress, agents which disrupt the microtubule stability, and cell cycle checkpoints defect can lead to MC (Vakifahmetoglu et al. 2008). The cell death following MC could be a metaphase cell death, or multinucleation followed by cell death (Kroemer et al. 2009). Interestingly, MC can initiate either apoptosis or necrosis

depending on the severity of the damage. However, occasionally, the cells' attempt to survive and proliferate may lead to potentially carcinogenic aneuploidies. Some of such cells may also become able to reduce the ploidy by a reductive division (depolyplodisation) by utilising pathways that are similar to meiotic division (Ianzini et al. 2009). *Anoikis* (Greek for 'homelessness') occurs when a cell detaches from its neighbouring cells and extracellular matrix. This detachment from neighbouring cells triggers apoptosis (Gilmore 2005). *Anoikis* acts as a defence mechanism that prompts apoptosis in the wayward cell.

For addressing infections, cells execute a pathway that bears a resemblance to the apoptosis pathway, called the *perforin/granzyme pathway* (Fig. 2). Cytotoxic T-lymphocytes and natural killer cells, for example, directly contact pathogen-infected and transformed cells and eliminate them without harming non-infected cells. In this mode of cell death induction, perforin (a membrane-disrupting protein) and a specialised group of serine proteases, called granzymes, are exocytosed from the immune cells to initiate apoptosis in the infected cells. Granzyme A facilitates the caspase-independent pathway via the Fas-associated DNase complex called SET, leading to DNA fragmentation (Lieberman 2010). This pathway is insensitive to Bcl-2 proteins and is independent of the membrane integrity of the mitochondria. Granzyme B, on the other hand, works via caspase-9 and caspase-3, and elevates *reactive oxygen species* (ROS) levels to promote cell death (Martinalet 2015).

Apoptosis can be detected by a variety of microscopic and flow cytometry methods, and by biochemical assays. Light microscopy, for example, can reveal some of the morphological changes of apoptosis. Changes at ultrastructural levels can be visualised by transmission electron microscopy (TEM) (Martinez et al. 2010). Scanning electron microscopy (SEM) can be used to identify membrane blebbing and cell shrinkage (Abdel Wahab et al. 2009). DNA-specific fluorochromes, such as Hoechst 33258 and *4',6-diamidino-2-phenylindole* (DAPI), are routinely used to examine chromatin condensation and fragmentation (Tinari et al. 2008). Multimodal holographic microscopy (MHM), which combines holographic microscopy and fluorescence detection techniques, has been shown to help in distinguishing apoptosis from other forms of cell deaths by allowing concurrent detection of apoptotic-specific cell morphology and cell surface markers using

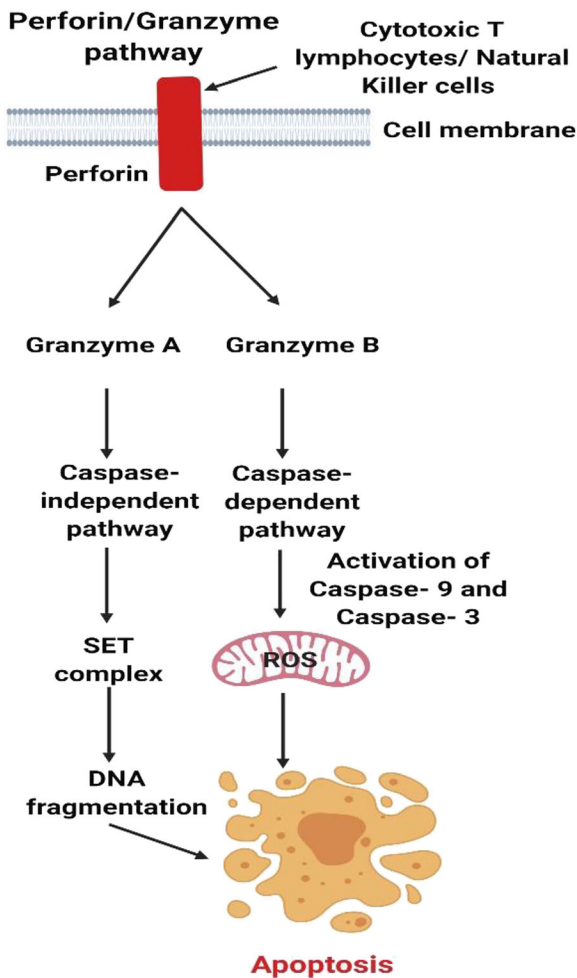


Fig. 2 In the granzyme pathway of apoptosis-like cell death, serine proteases such as granzyme A and granzyme B are released by cytotoxic T cells/natural killer cells to pathogen-infected cells. Subsequently, the cytotoxic T cells release perforin that makes pores on membrane of the infected cell. Through the perforin pores, the granzyme A and B enters the cell. Granzyme A forms the SET complex inducing DNA fragmentation and apoptosis via a caspase-independent pathway. Granzyme B mediates activation of procaspase-9 and procaspase-3, induction of ROS to promote apoptosis

immunohistofluorescence (Balvan et al. 2015). Apart from microscopy, techniques, such as flow cytometry using propidium iodide that quantifies the DNA content of a cell, can indicate the presence and prevalence of fragmented DNA (Martinez et al. 2010). Measurement of mitochondrial membrane potential (MMP) and the levels of ROS in the cell could suggest the initiation of apoptosis. However, since the loss of MMP and elevated ROS are associated with other forms of cell death as well, these cannot be considered as hallmarks of

apoptosis. The ladder-like pattern of fragmented DNA on the agarose gel has been used to describe apoptosis (Jamali et al. 2018). Annexin-V-FITC staining has been used to detect the presence of phosphatidylserine on the outer layer of the cell membrane. Acridine orange/ethidium bromide staining can be employed for checking the viable, early, and late apoptotic cells, and dead cells (Jamali et al. 2018). TdT-mediated dUTP nick-end-labelling (TUNEL) assay that probes the presence of fragmented DNA is another method for the detection of apoptosis (Gupta et al. 2007).

Necrosis

Necrosis is classified as an inflammatory form of cell death characterised by the rupture of the cell membrane (Green and Llambi 2015) (Fig. 3). Infection, high-dose radiation, electric, chemical or poison shock, high pressure, and asphyxiation can cause necrosis. While apoptosis is considered as ‘programmed cell death’, necrosis is designated as ‘unprogrammed or unregulated cell death’. During necrosis, the cell membrane integrity is broken down, and the cytoplasmic constituents ooze out into the extracellular space. Unlike apoptosis, necrosis does not follow ordered signal transduction pathways. Nevertheless, various molecular events are known to lead to necrosis, and multiple patterns for this form of cell death were reported in the scientific literature (Bernd and Rohrbach 2016). It must be noted that necrosis need not be confined to cells that are integrated into tissues. Cells in *in vitro* culture medium can also undergo necrosis. For example, ATP depletion has been shown to induce necrosis in primary cells cultured *in vitro* (Lieberthal et al. 2017). Similarly, drugs, such as tamoxifen, at high concentrations can induce necrosis in MCF-7 and HL-60 cells. Interestingly, at lower doses, tamoxifen promotes apoptosis, and, at its minimal dose, induces autophagy (Fröhwein et al. 2008). Arsenic trioxide (As_2O_3) is another example of necrosis-inducing drugs (Selvaraj et al. 2013). Although the pivotal switches that decide between apoptosis and necrosis are yet to be fully identified, elevated levels of intracellular ROS, inhibition of caspase activity, and reduced levels of ATP are known to trigger this form of cell death (Yousefi et al. 2006). DNA strand break caused by ROS, glutamate excitotoxicity, etc., can promote excessive activation of the DNA repair enzyme, *poly [ADP-ribose] polymerase-1* (PARP-1), leading to ATP

depletion, and, thereby, to necrotic cell death (Duprez et al. 2009). Similarly, an increase in the Ca^{2+} concentrations can lead to leaky mitochondria that further reduces ATP levels (Santulli et al. 2015). When apoptosis is blocked by caspase inhibition, the mode of cell death can shift to necrosis (Lin et al. 2004). Certain cell surface receptors, such as *RIG-I-like receptors* (RLRs), *Toll-like receptors* (TLRs), and *nucleotide oligomerisation domain (NOD)-like receptors* (NLRs) that are involved in pathogen recognition and innate immunity activation, can induce necrotic cell death by identifying double-stranded RNA (dsRNA), lipopolysaccharides (LPS), and flagellin found in pathogens. Further, synthetic dsRNA, on recognition by TLR, induces necrosis (Kalai et al. 2002).

Persistent opening of the high-conductance permeability transition pores of mitochondria, called *mitochondrial permeability transition* or MPT, can also drive a cell to necrosis. MPT-driven necrosis involves a rapid increase in the permeability of the mitochondrial membrane induced by elevated levels of intramitochondrial Ca^{2+} and oxidative stress (Takeyama et al. 1993). The elevated calcium levels, combined with the oxidative stress, promote the influx of several solute molecules into the mitochondria. The resultant influx of water into the mitochondrial matrix, loss of mitochondrial membrane potential, loss of ATP, the release of several mitochondrial proteins into the cytoplasm, and rupture of the outer mitochondrial membrane, culminate to the cell death (Galluzzi et al. 2018). It must be noted that mitochondrial rupture releases proapoptotic factors, such as cytochrome c, that can induce apoptotic cell death as well. However, the rapid loss of ATP can tilt the cell's destiny to MPT-driven necrosis (Bonora and Pinton 2014). Cyclophilin D, which is located in the mitochondrial matrix, has been shown to facilitate MPT-driven necrosis (Nakagawa et al. 2005). This type of necrosis can be inhibited by cyclophilin D inhibitors, such as cyclosporin A (Griffiths and Halestrap 1993), JW47, and sanglifhehrin A (Porter and Beutner 2018). In a pathophysiological perspective, these inhibitors can confer protection against ischemia/reperfusion in vivo (Griffiths and Halestrap 1993). Many of these inhibitors have been found to possess clinical potential against acute myocardial infarction (Piot et al. 2008). Electron microscopy, assays probing the levels of hexosaminidase (an enzyme whose presence indicates inflammation), and staining the cells with lipophilic dyes, such as calcein acetoxymethyl ester (calcein-AM),

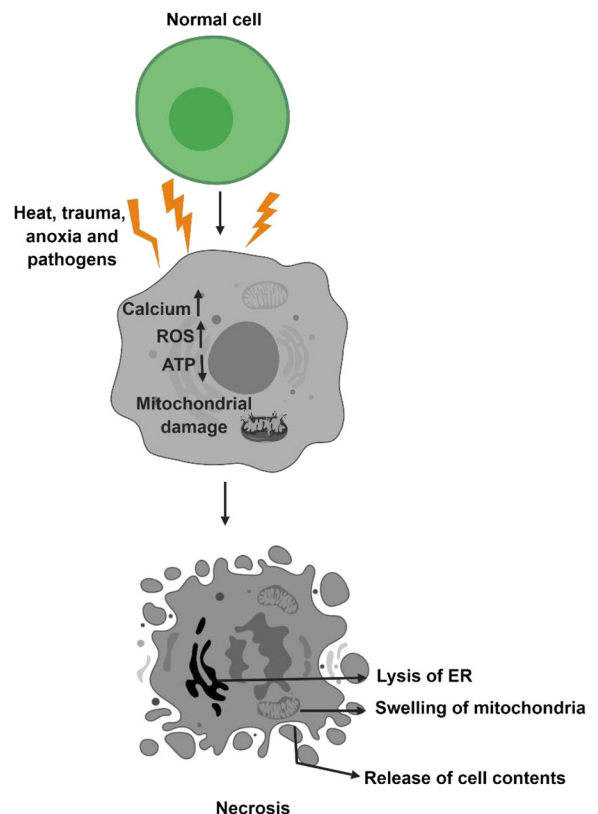


Fig. 3 Necrotic cell death. A cell can undergo necrosis as a result of exposure to heat, trauma, anoxia, and infection. Highly elevated levels of ROS, Ca^{2+} , and near-complete depletion of ATP promote necrosis which is characterised by lysis of the cell membrane and inflammation

have been used to detect necrosis (Vanden Berghe et al. 2013) (Vaseva et al. 2012).

Cell deaths that are similar to necrosis

While necrosis is an unprogrammed form of cell death, there are related cell deaths with cell membrane rupture as the common denominator. These include methuosis, necroptosis, NETosis, pyronecrosis, and pyroptosis. Such 'necrosis-like' cell deaths are, nevertheless, regulated by different signalling pathways and external or internal inducers and mediators.

Methuosis

Methuosis is characterised by the accumulation of fluid-filled cytoplasmic vacuoles in cells and eventual detachment of the cell from the neighbouring cells. This Ras-

induced, caspase-independent cell death (Fig. 4a) was first observed in malignant glioma and gastric cancer cell lines (Chi et al. 1999). Unlike the autophagosomes associated with autophagy, the vacuoles in methuosis are bound by a single membrane and are derived from macropinosomes (Chi et al. 1999). Although the precise mechanism behind this vacuolisation is yet to be fully comprehended, deviations in the trafficking of clathrin-independent endocytic vesicles along with elevated micropinocytosis are thought to induce the formation of these large vacuoles (Chi et al. 1999). The vacuolisation can lead, eventually, to the rupture of the cell membrane (Chi et al. 1999). At a molecular level, the process of methuosis involves the induction of a Ras GTPase pathway that is independent of the Ras-associated proliferative pathway (the Ras-Raf-MEK-ERK1/2 pathway (Maltese and Overmeyer 2014a)). The Ras GTPase here activates *Ras-related C3 botulinum toxin substrate 1* (Rac1) GTPase, facilitating the formation of macropinosome. Further, Rac1 interacts with an adapter protein, GIT1, that inhibits a GTPase (Arf-6 GTPase) involved in the recycling of macropinosomes to the plasma membrane, leading to the accumulation the macropinosomes in the cell (Maltese and Overmeyer 2014a). The macropinosomes then join together to form large vacuoles that resemble late endosomes (Overmeyer et al. 2011). Compounds, such as chalcones and their derivatives, have been shown to promote methuosis via disruption of vesicular trafficking and procathepsin processing (Mbah et al. 2017). Similarly, an anticancer agent, silmitasertib, can induce methuosis in cholangiocarcinoma (Lertsuwan et al. 2018) and colorectal cancer cell lines (Silva-Pavez et al. 2019a). Interestingly, the same drug-induced autophagic cell death in pancreatic cancer cells and apoptosis in non-small cell lung cancer cells (Hwang et al. 2017). Electron microscopy, time-lapse fluorescence microscopy, visualisation of the vacuoles using fluorescent dyes, and metabolic flux analyses can be used for detecting methuosis (Overmeyer et al. 2011).

Necroptosis

Upon pathogenic stimulation or in the presence of drugs such as shikonin (Wada et al. 2015), a TNFR1-associated receptor-interacting protein kinase, called *receptor-interacting protein kinase-1* (RIPK1), induces necroptosis (Mishra et al. 2018), a type of cell death whose features bears resemblance to apoptosis and

necrosis (Fig. 4b). Binding of the ligand to TNFR1 leads first to the formation of a complex at the cytosolic side of the TNFR1 that consists of proteins such as TRAFF2/5, RIPK1, and *tumor necrosis factor receptor type 1-associated death domain* (TRADD). From this point, the cell may choose one of the three fates. If ubiquitination of RIPK1, mediated by *cellular inhibitor of apoptosis proteins* (cIAPs) and TRAFF2/5, prevails, cell death is aborted. If inhibition of cIAP in concert with deubiquitination of RIPK1 triumphs, caspase-8 activation ensues, resulting in apoptosis. However, when caspase-8 activity is inhibited at this point, two other proteins, *receptor-interacting protein kinase-3* (RIPK3) and RIPK1, form the necrosome complex which then phosphorylates *mixed-lineage kinase domain-like protein* (MLKL) (Fig. 4b). The phosphorylated MLKL then substantially increases mitochondria-dependent ROS production. Further, MLKL translocates to the plasma membrane to disrupt the membrane integrity, leading to the cell death. Unlike apoptosis, necroptosis induces inflammation (necroinflammation), which is seen prevalently in neurodegenerative diseases (Heckmann et al. 2019). In further contrast to apoptosis, necroptotic cells have randomly degraded DNA (Xu et al. 2018). On a therapeutic perspective, it has been shown that cancer metastasis can be restricted through the induction of necroptosis (Mishra et al. 2018). RIPK1 and MLKL inhibitors, such as necrostatin-1, can block necroptotic cell death (Kearney et al. 2014). Flow cytometry, Western blotting, and immunohistochemistry techniques probing the levels of proteins such as MLKL, RIPK3, and RIPK1 give indications of the presence of necroptosis (Vanden Berghe et al. 2013).

NETosis

NETosis is a form of cell death exhibited by neutrophils as a defence against infectious pathogens (Fig. 4c). When encountering pathogens, neutrophils release *neutrophil extracellular traps* (NETs), consisting mostly of DNA fibres, to capture and kill the pathogens (Yipp and Kubes 2013). This NADPH oxidase-dependent cell death (Yipp and Kubes 2013) progresses with substantial elevation of ROS, leading to the activation of an arginine deiminase, called *protein-arginine deiminase 4* (PAD4). PAD4 then citrullinates (converts arginine to citrulline via deimination) the histones, facilitating decondensation of the nuclear chromatin. Besides, enzymes, such as elastase and myeloperoxidase, which are

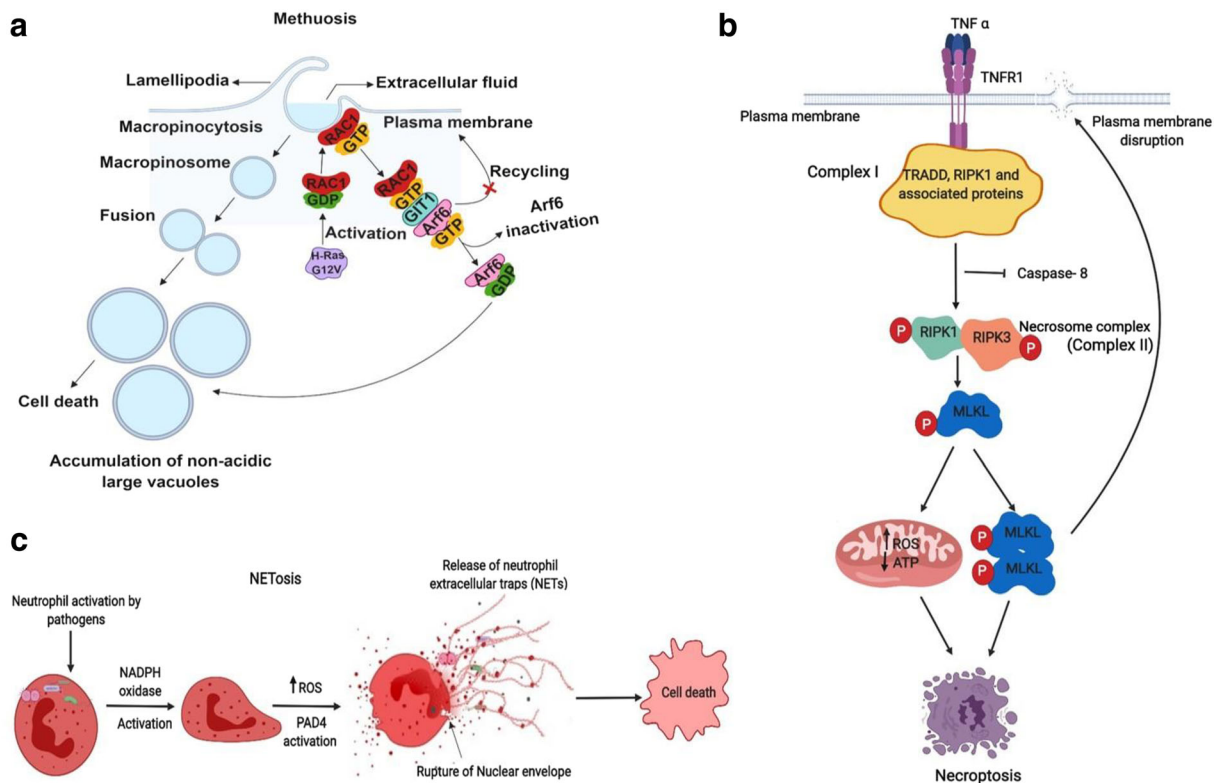


Fig. 4 Necrotic-like cell deaths. (A) Methuosis: This non-apoptotic cell death is characterised by extreme macropinosome-derived vacuolisation. In response to the overexpression of H-Ras oncoprotein, RAC1 promotes the formation of macropinosomes. RAC1 then suppresses the macropinosome recycling activity of Arf6 via GIT1. Consequently, the macropinosomes accumulate and fuse together to form large fluid-filled non-acidic vacuoles, leading to the death of the cell. (B) Necroptosis: Binding of TNF- α to TNFR1 leads to the formation of a signalling complex (complex I) containing TRADD, RIPK1, and other associated proteins whose natural result is caspase-8-mediated apoptosis. However, in the absence of active caspase-8, RIPK1 destabilises complex I via deubiquitination. The deubiquitinated RIPK1 and RIPK3 get phosphorylated leading to the formation of necrosome (complex

II). The necrosome phosphorylates MLKL leading to its oligomerisation. The oligomerised MLKL1 promotes pore formation in the plasma membrane leading to loss of membrane integrity. The necrosomes also induce ATP depletion and elevate ROS levels. (C) NETosis: NETosis is an immune defence mechanism employed by neutrophils to trap and neutralise pathogens. During NETosis, NADPH oxidase complex is activated, leading to elevated production of ROS and decondensation of the DNA aided by myeloperoxidase and neutrophil elastase. Besides, PAD4 protein citrullinates histones and thereby assists the DNA decondensation. The decondensed chromatin is then released into the cytoplasm. The granular and cytosolic proteins finally associate with the decondensed chromatin networks and are released through the ruptured cell membrane

released by the neutrophil granules, translocate to the nucleus facilitating the unfolding of the chromatin. Disruption of the nuclear envelope follows, releasing the chromatin into the cytosol. Onto this decondensed chromatin, many cytosolic proteins are added. Finally, the outer membrane of the neutrophil disintegrates and the NET is released (Yang et al. 2015). Mast cells and eosinophils also show NETosis and is termed as ‘ETosis’ (Yipp and Kubes 2013). NETosis can be detected by electron microscope and imaging flow cytometry (Masuda et al. 2016). Western blot, probing the expression levels of PAD4, has also been employed for the detection (Perdomo et al. 2019).

Pyronecrosis

Pyronecrosis is a necrosis-like cell death (Fig. 5a) stimulated by pathogens, such as *Shigella flexneri* (Khalili and Radosevich 2018). It operates chiefly through the *NLR family pyrin domain containing 3* (NLRP3) receptor (a receptor belonging to *nucleotide oligomerisation domain-like receptor-family*), which induces the formation of an inflammasome complex. Specifically, upon stimulation by molecules of the pathogen, NLRP3 oligomerises and induces oligomerisation of another protein, ASC (for *apoptosis-associated speck-like protein containing a caspase-recruitment domain*), creating

the inflammasome complex. The complex induces the production of interleukin-1 β (IL-1 β). Further, a proinflammatory factor, HMGB1 (for *high-mobility group box 1*) is released (Fig. 5a). Finally, the cell membrane ruptures releasing intracellular components. It may be noted that cells that have already initiated apoptosis will not release HMGB1 even when they initiate secondary necrosis later (Scaffidi et al. 2002). Western blot immunodetection of HMGB1 has been used for recognising pyronecrosis (Willingham et al. 2009).

Pyroptosis

Pyroptosis is a form of regulated necrosis stimulated by factors such as microbial infection (Fig. 5b). Pyroptosis plays a major role in immune defence against infections (Sato et al. 2013). Nevertheless, other factors, such as stroke, can also lead to pyroptosis. When a cell is damaged, biomolecules such as *damage-associated molecular patterns* (DAMPs) initiate the inflammatory response by binding to DAMPs receptors, such as TLRs. With infections, microbial molecules having PAMPs initiate the response by binding to the respective *pattern-recognition receptors* (PRRs). The inflammasome complex consisting of sensory proteins, ASC (see *pyronecrosis*) and procaspase-1 triggers inflammation via activation of caspase-1, and subsequent activation of Gasdermin D. Gasdermin D makes pores on the plasma membrane. Inflammatory cytokines also play a significant role in assisting pyroptosis (Shi et al. 2017). The cell swells and ultimately ruptures its membrane resulting in the release of the proinflammatory contents. Western blot analysis using antibodies against Gasdermin D, IL-1 β , and measurement of the uptake of propidium iodide by the cells (de Oliveira et al. 2018) can be employed for the detection of pyroptosis.

Other forms of cell death

Autosis

Autosis is a form of autophagy-associated cell death (Liu and Levine 2015)(Fig. 6a). Therefore, we shall begin its description after giving an overview of autophagy. In eukaryotes, the term autophagy denotes the adaptive cellular pathway involving degradation of the damaged organelles or unused/misfolded proteins. Specialised double-membrane vesicles, called the

autophagosomes, encapsulate the damaged components and subsequently sequester them via lysosomes. Thus, autophagy reduces the levels of defective organelles and protein aggregates, which may otherwise abate efficient functioning and survival of the cell (Lin and Baehrecke 2015). In various cancers, autophagy plays an intricate role in either inhibiting or inducing proliferation (Marinković et al. 2018).

Autosis, a term used for the first time by Liu and Levine in 2015, describes it as an autophagy gene-dependent non-apoptotic cell death with distinct morphological features. A viral Tat (for *transactivator of transcription*, a cell-penetrating peptide) peptide fused to a small peptide sequence of Beclin-1 (TAT-Beclin 1; Beclin-1 is a critical regulator autophagosome formation) was found to induce this form of cell death. In autosis, the plasma membrane gets ruptured at the focal points. Mild condensation of the chromatin and presence of abundant autolysosomes and autophagosomes (and their disappearance at the later stages), swollen, electron-rich mitochondria, and fragmented, swollen ER are additional indicators (Fig. 6a). It has also been demonstrated that this form of cell death depends on Na⁺,K⁺-ATPase. Cerebral hypoxia-ischemia and hepatocyte injury associated with severe *anorexia nervosa* are known to promote autosis (Liu et al. 2013) (Kheloufi et al. 2015). Pharmacological agents, such as cardiac glycosides (neriifolin, digoxin, and digitoxigenin) that inhibit Na⁺,K⁺-ATPase, and knockdown of α -subunit of Na⁺,K⁺-ATPase inhibited autosis confirming the role of this pump in its initiation and progression (Liu et al. 2013) (Kheloufi et al. 2015). Ginger extract has been shown to induce autosis in cultured pancreatic cancer cells (PANC1) as indicated by an increase in the LC3-II/LC3-I ratio (an indicator of the formation and prevalence of autophagosomes) and the vacuolisation of the cytoplasm (Akimoto et al. 2015). Although multiple factors are found to be involved in autosis, sequential events that lead to autosis are yet to be fully understood. Nevertheless, the selective induction of autosis may be a potential therapeutic strategy. For example, as mentioned, ginger extract can eliminate cancer cells by causing autotic cell death without being considerably toxic to non-cancerous cell lines (Akimoto et al. 2015). Phycocyanin, one of the major pigment constituents of *Spirulina*, can induce autotic cell death in PANC-1 cells (Gao et al. 2016). Drugs such as neriifolin, digoxin, and digitoxigenin can inhibit autosis (Liu et al. 2013). Sytox Green staining, electron microscopy, and measurement

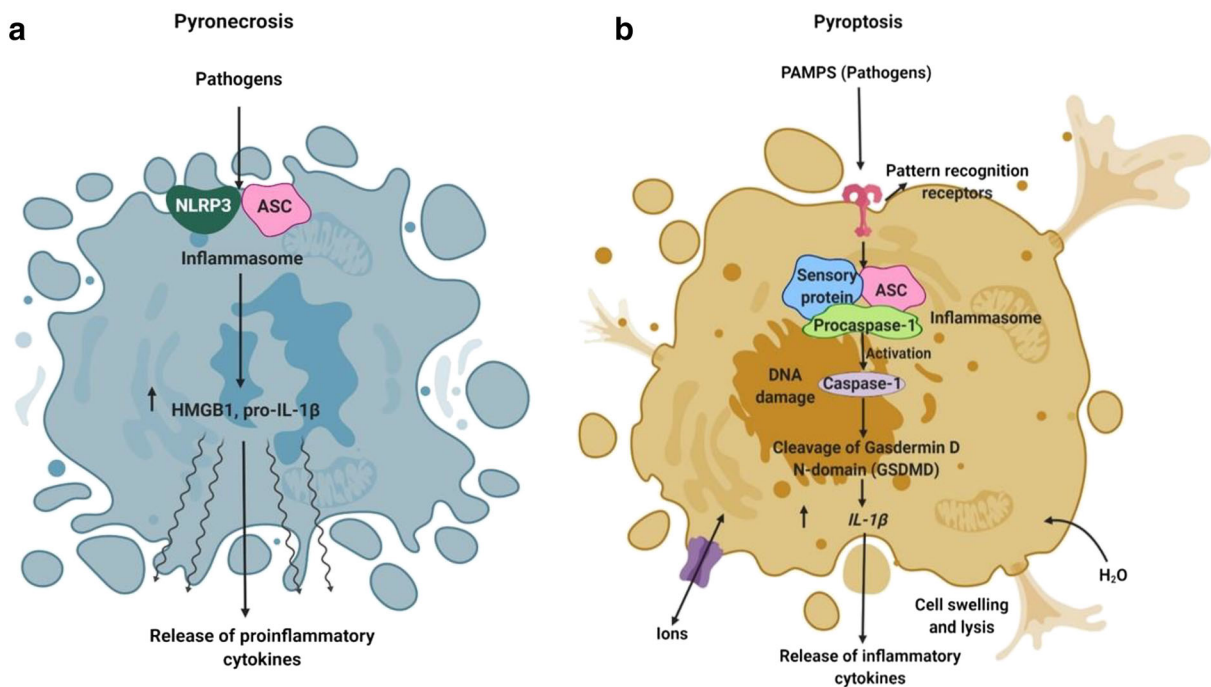


Fig. 5 Necrotic-like cell deaths. (A) Pyronecrosis: Pyronecrosis is a proinflammatory cell death. Induced mostly by pathogens, it is mediated via an inflammasome complex containing an oligomerised form of NLRP3 and ASC. The inflammasome promotes the release of HMGB1 and proinflammatory cytokines resulting in cell swelling and lysis. (B) Pyroptosis: In pyroptosis, pattern-recognition receptors such as Nod-like receptors (NLR)

and Toll-like receptors (TLR) on cell surface recognise PAMPs (pathogen-associated molecular patterns) of different pathogens and activate procaspase-1 via a molecular complex comprising of sensory proteins and ASC. Activated caspase-1 cleaves gasdermin D leading, eventually, to plasma membrane rupture and release of inflammatory cytokines IL-1β

of Na⁺,K⁺-ATPase can be used for detecting autosis (Liu et al. 2013)

Entosis

There are some interesting mechanisms with which live cells are engulfed by other live cells, and are, in most cases, eventually killed. One such cannibalistic mechanism that is prevalent in many epithelial tumour cells is entosis (Fig. 6b) in which one cell enters into another cell (Overholtzer et al. 2007). Glucose starvation is a known inducer of entosis where the starved cell engulfs another cell to obtain necessary nutrients. Although the exact mechanism is poorly understood, E-cadherin is known to assist the cell-to-cell interactions and subsequent engulfment (Wang et al. 2015). More recent studies have demonstrated the role of actin and myosin during the engulfment (Garanina et al. 2017). Microtubules, microfilaments, and Golgi apparatus are also involved in this form of cell death. The cell thus entered is then eaten up via lysosomal activation (Fig. 6b).

Interestingly, there are cases where the engulfed cell survives and divides within its host (Krishna and Overholtzer 2016). The engulfed cell, at times, can escape and survive (Krishna and Overholtzer 2016). Entosis differs from phagocytosis by the formation of cell-in-cell configuration. Entosis can be inhibited by Y27632 (a ROCK1/II inhibitor) and cytochalasin (an actin-assembly-inhibitor). AMPK-FRET measurements, immunoblotting, and immunohistochemistry have been used for detecting entosis (Hamann et al. 2017) (Garanina et al. 2017).

Ferroptosis

Ferroptosis is an iron-dependent cell death initiated and progressed by the accumulation of lipid peroxides (Fig. 7a). It is also presented in various degenerative diseases like Parkinson's disease and ischemia/reperfusion injury (Mishra et al. 2018). Ferroptotic cells display smaller mitochondria with ruptured outer membrane (Viswanathan et al. 2014).

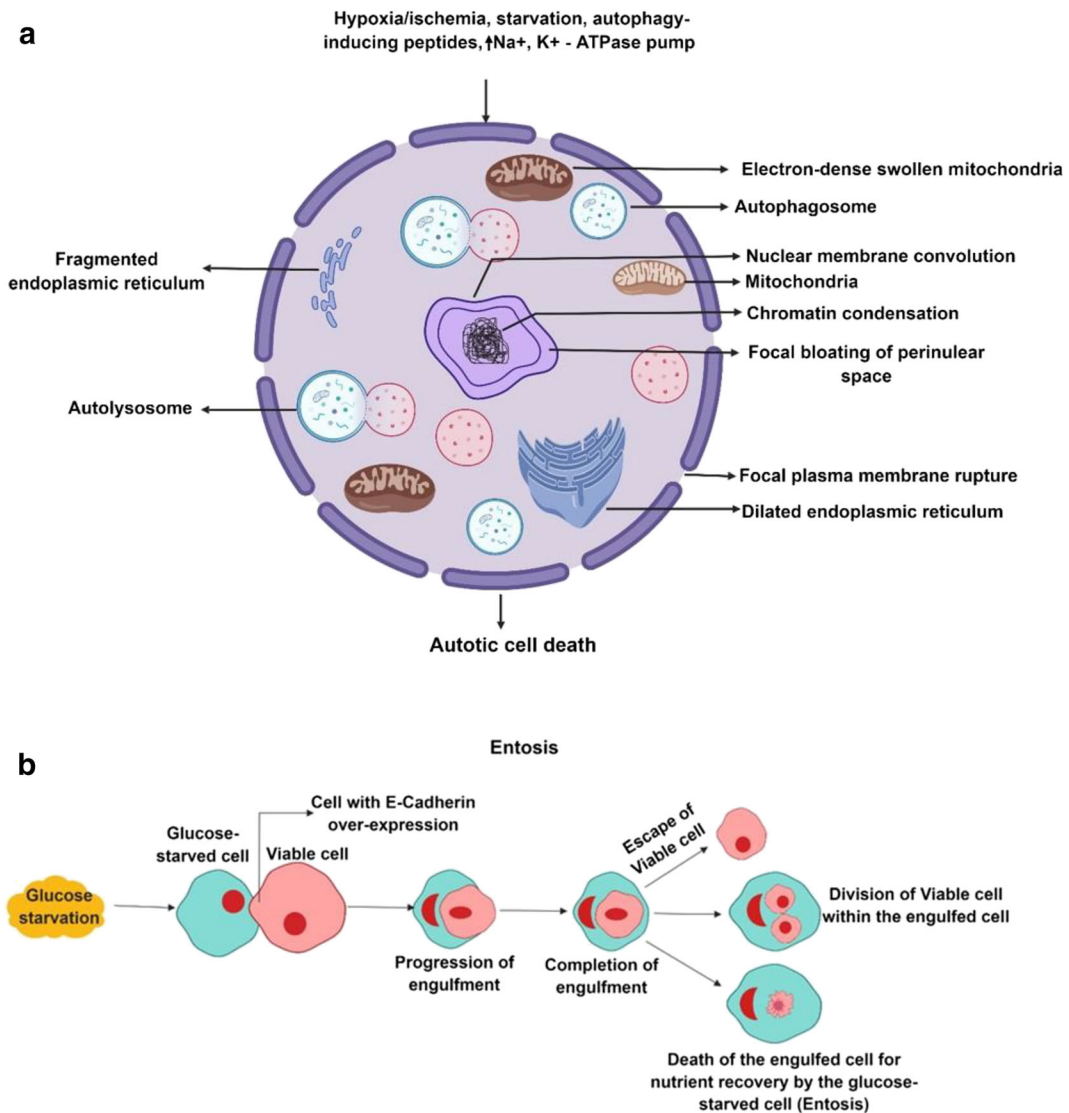


Fig. 6 Other forms of cell death. (A) Autosis: Autosis is a Na^+, K^+ -ATPase pump-mediated, non-apoptotic cell death. It is characterised by focal plasma membrane rupture, swollen mitochondria, fragmented endoplasmic reticulum, and excessive autophagy. (B) Entosis: Glucose starvation and matrix detachment of adherent cells trigger entosis (cell cannibalism). During entosis, the glucose-starved cell first attaches itself to a viable cell via E-

cadherin-mediated anchorage. Subsequently, the viable cell is completely engulfed by an actin and myosin-mediated mechanism. After the engulfment, the engulfed cell has multiple fates. It may escape the predator cell by a poorly understood process, it may divide within the cell, or it would die donating its nutrients to the host cell

This form of cell death starts with the accumulation of iron in cells that results in the generation of hydroxyl radicals, and it progresses rapidly when the plasma membrane system x_c^- (x_c^- system is a cystine/glutamate antiporter that is crucial for glutathione production and oxidative protection) becomes dysfunctional. As a result, the cells lose their antioxidant defence mechanisms. Although the complete

pathway leading to ferroptosis is yet to be comprehended, several proteins have been attributed as key players in its initiation and progression. Beclin-1, for example, promotes ferroptosis (Song et al. 2018) by inhibiting the plasma membrane system x_c^- . Specifically, during ferroptosis, 5' AMP-activated protein kinase (AMPK) phosphorylates beclin-1 (Song et al. 2018) which translocates to the

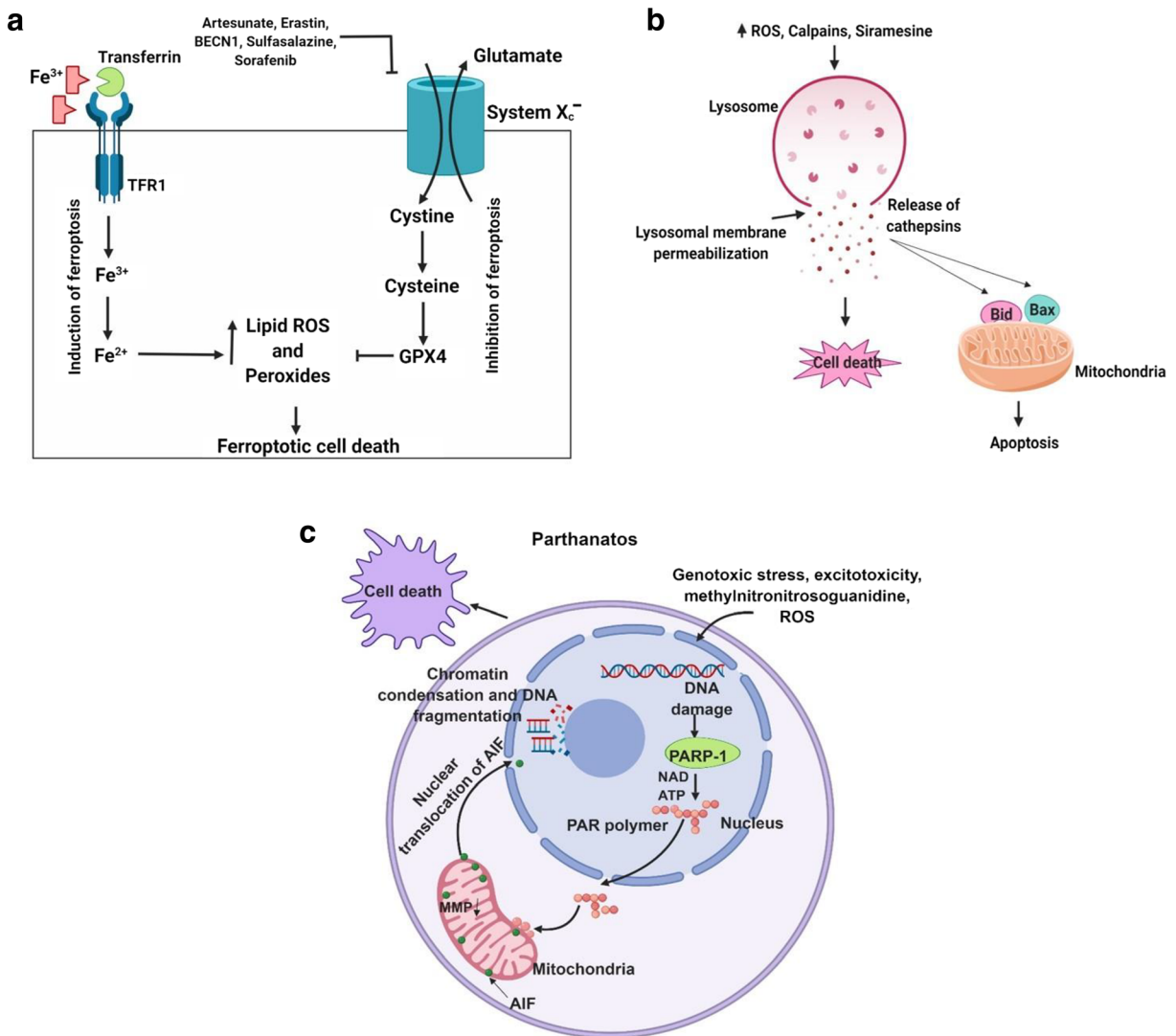


Fig. 7 Other forms of cell death. (A) Ferroptosis: Ferroptosis is iron-dependent cell death, activated by excess iron (Fe^{3+}) in the cell. Transferrin takes the iron to the TFR1, which in turn transports the iron into the cell. In the cell, Fe^{3+} is reduced to Fe^{2+} , and this process generates hydroxyl radicals. Subsequently, the hydroxyl radicals attack the lipid membranes producing lipid peroxides. The lipid peroxides further elevate ROS production. GPX4, an effective eliminator of intracellular lipid ROS, can inhibit ferroptosis under normal circumstances. Glutathione (a cofactor of GPX4), when depleted due to malfunction of the antiporter, the system x_c^- , inactivates GPX4, inducing ferroptosis. (B) Lysosome-dependent cell death: Lysosomal membrane permeabilisation

induces LDCD, facilitated by the release of lysosomal proteases like cathepsins into the cytoplasm and thereby acidifying the latter, leading, eventually, to cell death. Cathepsins can also activate proapoptotic proteins such as Bid and Bax to promote apoptosis. (C) Parthanatos: Excess ROS, genotoxic stress, or excitotoxicity can lead to excess production of PARP1. The PARP1 then generates PAR oligomers and polymers. Subsequently, the PAR oligomers translocate from the nucleus to the mitochondrion resulting in loss of mitochondrial membrane potential. This triggers the translocation of AIF from mitochondria to the nucleus to promote cell death

plasma membrane, promoting pore formation. A combination of tannic acid and Fe^{3+} was shown to induce ferroptosis in 4T1 breast cancer cells. Here, the reduction of Fe^{3+} led to lipid peroxidation, indirect inhibition of GPX4 (for *glutathione peroxidase*

4), and finally, induction of cell death (Liu et al. 2018). Erastin, sulfasalazine, sorafenib, and artesunate are also inducers of this type of cell death (Cao and Dixon 2016). Ferroptosis can be inhibited by GPX4 (Imai et al. 2017), and by chemical agents,

Table 1 A comparison of different cell death mechanisms in eukaryotes

Cell death mechanism	Features	Significance	Common methods for detection	Inhibitors	References
Apoptosis	Cell shrinkage, cell membrane blebbing, chromatin condensation, DNA fragmentation. Almost all eukaryotic cells can undergo apoptosis and it involves caspase-activation via: 1. Extrinsic pathway induced by TNF, FAS, or TRAIL 2. Intrinsic (mitochondrial) pathway mediated by proapoptotic Bcl-2 family proteins 3. ER stress pathway mediated by IRE1 and CHOP	Required for development. Maintenance of cell number, removal of damaged, infected, or stressed cells. May lead to degenerative diseases	Annexin-V assay, electron microscopy, in situ end labelling (ISEL), immunoblotting, immunohistochemistry, detection of ER stress markers by RT-PCR	BAX inhibitor-1, BAX inhibiting peptide V5, BI-6C9, bongkrekic acid, NS3694, pifithrin- α	(Elmore 2007) (Banfalvi 2017) (Siegel and Lenardo 2002) (Kumaraswamy et al. 2013) (Krajam et al. 2015) (Han et al. 2019) (Sano and Reed 2013) (Iurlaro and Muñoz-Pinedo 2016)
Necrosis	Cell swelling and rupture, organelle damage, inflammation. Cells that are exposed to trauma, pathogens, nutrient starvation, AIP depletion, and mitochondrial permeability transition can undergo necrosis. Reported for most of the eukaryotic cells	Can disrupt homeostasis, may lead to pathological conditions. Necrotic cells can elicit immune response	Time-lapse microscopy, electron microscopy, hexosaminidase assay, calcein-AM release assay.	Geldanamycin, radicicol, cyclosporin A, JW47, sanglifohrin A	(Escobar et al. 2015) (Vanden Berghe et al. 2013) (Griffiths and Halestrap 1993) (Warne et al. 2016) (Clarke et al. 2002) (Galluzzi et al. 2014) (Galluzzi et al. 2018) (Takeyama et al. 1993) (Vaseva et al. 2012) (Uribe et al. 2018)
Methuosis	Large, fluid-filled cytoplasmic vacuoles formed due to suppressed recycling of macropinosomes, organelle swelling and rupture of the cell. Chiefly operated via Ras-Rac-1 signalling pathway. Reported in glioblastoma and gastric cancer cells	Biological significance is not clear. Can be utilised therapeutically against apoptosis-resistant cells	Electron microscopy, time-lapse and fluorescence microscopy, visualisation of the vacuoles with fluorescent dyes (LysoTracker, Lucifer yellow), metabolic flux analysis	EHT 1864, bafilomycin A1	(Overmeyer et al. 2011) (Maltese and Overmeyer 2014b) (Li et al. 2019b) (Silva-Pavez et al. 2019b)
Necroptosis	Cell membrane swelling and rupture. Displays features of both apoptosis and necrosis. Operated by TNFR1-RIPK1/RIPK3 (necrosome)-MLKL pathway. Reported in many types of epithelial and cancer cells	Stimulates inflammatory response, targeted necroptosis can impede cancer metastasis.	Imaging flow cytometry, immunoblotting	Necrostatin-1, necrosulfonamide, pazopanib, ponatinib	(Dhuriya and Sharma 2018) (Bellizario et al. 2015) (Lin and Padilla 2017) (Galluzzi et al. 2016) (Vanden Berghe et al. 2013) (Webster et al. 2018)
NETosis	Disruption of the plasma membrane, decondensation of nuclear chromatin, the release of extracellular 'nets' comprising of chromatin fibres and many cytosolic proteins. NADPH oxidase-and Ros--dependent.	Host defence against infection	Light and electron microscopy, immunostaining, fluorescence microscopy, imaging flow cytometry, ELISA	Staurosporine, diphenyliodonium, vanilloids	(Barbu et al. 2018) (Masuda et al. 2016) (Sondo et al. 2019) (Martinez et al. 2017) (Healy et al. 2017)

Table 1 (continued)

Cell death mechanism	Features	Significance	Common methods for detection	Inhibitors	References
Pyronecrosis	Characterised by histone citrullination by PAD4. Commonly seen in neutrophils. Membrane rupture and cell lysis. Operated via an NLRP3- and cathepsin-dependent, caspase-independent pathway. Commonly seen in monocytes, macrophages, and mast cells	Plays a role in autoinflammatory diseases and host immune defence	Flow cytometry, measurement of <i>IL-1β</i> , ELISA, lactate dehydrogenase assay, electron microscopy, fluorescent microscopy	CA-074Me, AMF1-5	(Motiani et al. 2011) (Satoh et al. 2013) (Velin et al. 2011) (Zeng et al. 2006) (Edwan et al. 2015)
Pyroptosis	Cell swelling, rupture of the cell membrane, and release of <i>IL-1β</i> . ASC-mediated inflammasome formation, followed by caspase-1 dependent activation of gasdermin D that create pores in cell membrane. Many types of eukaryotic cells undergo pyroptosis	Triggers inflammation.. Immune defence against microbial infections	Annexin-V and TUNEL assays, ELISA, lactate dehydrogenase assay, immunofluorescence, immunohistochemistry	Necrosulfonamide, strychnine, brucine	(Man et al. 2017) (Rathkey et al. 2018) (McKenzie et al. 2018) (Loomis et al. 2019)
Autosis	Focal enlargement of the perinuclear space, abundant autolysosomes and autophagosomes, swollen, electron-rich mitochondria, swollen endoplasmic reticulum. Na ⁺ /K ⁺ -ATPase pump plays a major role. Seen in many eukaryotic cells, including neurons and liver cells. Can be induced by Tat-beclin-1 peptide	Can occur during cerebral hypoxia-ischemia and during anorexia nervosa	Sytox Green staining, electron microscopy, immunofluorescence, immunohistochemistry, immunoblotting	Nerifolin, digoxin, and digitoxigenin	(Wang et al. 2012) (Liu et al. 2013) (Liu and Levine 2015) (Munoz-Pinedo and Martin 2014) (Zhang et al. 2018)
Entosis	Cellular cannibalism and the formation of cell-in-cell configuration. Induced by glucose starvation. Assisted by E-cadherin. Facilitated by RhoA/ROCK1/2 signalling pathway. Seen in cancer cells including prostate cancer cells	A survival response of the cells under glucose starvation	Immunoblotting, immunofluorescence, immunohistochemistry, AMPK-FRET measurements	ROCK/II inhibitor (Y27632) cytochalasin B	(Liu et al. 2019) (Liu et al. 2019) (Hamann et al. 2017) (Garamina et al. 2017)
Ferroptosis	Iron-dependent cell death, initiated by lipid peroxides. Membrane rupture. Smaller mitochondria with the ruptured outer membrane. Signalling pathway involves AMPK-mediated phosphorylation of beclin-1. Seen in many types of eukaryotic cells	Suppression of tumorigenesis. May promote neurodegenerative diseases	Assessment of mitochondrial lipid peroxides	Deferoxamine, desferrioxamine mesylate, ferrostatin-1, liproxstatin-1	(Xie et al. 2016) (Mou et al. 2019) (Huang et al. 2019) (Van Camp et al. 2018) (Conrad et al. 2018) (Mou et al. 2019)
Lysosomal membrane permeabilisation and	Implicated in neurodegeneration, autoimmune diseases and cancer	Electron microscopy, immunoblotting,	cystatins, serpin	(Domaglia et al. 2018) (Serrano-Puebla and Boya 2018)	

Table 1 (continued)

Cell death mechanism	Features	Significance	Common methods for detection	Inhibitors	References
Lysosome-dependent cell death	translocation of cathepsins into the cytoplasm. Seen in many types of eukaryotic cells		immunostaining, flow cytometry. Assessment of mitochondrial permeability transition		(Wang et al. 2018) (Michelet et al. 2018) (Li et al. 2018)
Parthanatos	Caspase-independent cell death induced by PARP-1 overactivation. Depolarised mitochondrial membrane, DNA fragmentation. Seen in several type eukaryotic cells.	Implicated in pathological conditions, such as Parkinson's disease and stroke	Measurement of ROS, immunofluorescence, annexin-V/PI staining, immunoblotting, lactate dehydrogenase (LDH) assay	INO-1001, benzamide, 3-aminobenzamide, cilostazol	(Fatokun et al. 2014) (Jiang et al. 2018) (Li et al. 2019a) (Zhong et al. 2018)

such as ferrostatin-1 and liproxstatin-1 (Zilka et al. 2017). Ferroptosis can be examined by quantifying lipid peroxides (by staining the cells with C11-BODIPY) coupled with flow cytometry (Van Camp et al. 2018) .

Lysosome-dependent cell death

Lysosome-dependent cell death (LDCD) is characterised by the lysosomal membrane permeabilisation (LMP) and the resultant effusion of lysosomal proteases, such as cathepsins, into the cytoplasm (Fig. 7b). Elevated levels of ROS, non-lysosomal proteases such as calpains, and lysosomotropic drugs (such as siramesine) can induce LMP to trigger a cascade of events leading to acidified cytoplasm and eventually to the death of the cell (Serrano-Puebla and Boya 2018). Cathepsins released by the lysosomes play pivotal roles in inducing the cell death (Gómez-Sintes et al. 2016). Cathepsins, especially, cathepsin B, has been shown to activate proapoptotic proteins such as Bid and Bax as well (Aits and Jaattela 2013). LDCD plays vital roles in conditions such as neurodegeneration, inflammation, host immune response, and cardiovascular disorders. Cystatins and serpin can block cathepsin and thereby inhibits LDCD (Wang et al. 2018). LDCD can be detected using assessment of MPT, electron microscopy, and flow cytometry (Michelet et al. 2018).

Parthanatos

Parthanatos depends on the activation of PARP1 enzyme (Fig. 7c). Hyperactivation of PARP1 in response to extreme genomic stress, hypoxia, and hypoglycemia leads to ATP depletion, triggering parthanatos (Virág et al. 2013). Specifically, after DNA damage, PARP1 activity shoots up nearly 500-fold, generating *poly (ADP-ribose)* (PAR) polymers. The PAR polymers in the cytoplasm induce the release of AIF. AIF translocates to the nucleus where it fragments the DNA, leading, eventually, to cell death (Fig. 7c) (Baek et al. 2013). Parthanatos is independent of caspases, and although it is associated with loss of membrane potential, it does not involve membrane swelling and rupture like that of necrotic cell death. INO-1001 and cilostazol are inhibitors of parthanatos. This mode of cell death can be detected by lactate dehydrogenase assay, annexin-V

staining, measurement of the ROS levels, and immunofluorescence (Zhong et al. 2018) (Li et al. 2019a).

Conclusion

There are multiple mechanisms and causes for a cell to end its existence. A summary of the key features, significance, detection methods, and inhibitors of major cell death mechanisms is provided in Table 1. With their ability to self-destruct, many cell types orchestrate the development and sustenance of the organism. Depending on the need and type of the cell, it may kill itself to fight infection, to prevent hyperproliferative disorders, to mould organs and limbs, to heal wounds, and to deal with metabolic deficiencies. Nevertheless, programmed cell death, if it goes out of homeostatic control, could lead to degenerative diseases. Cell death caused/ followed by inflammation and membrane rupture (necrotic type cell death) may also help the host organism to survive. However, cells that die due to severe injury, anoxia, electric shock, high heat, or pressure would contribute little to the reestablishment of the homeostasis and thereby the survival of the host. On a therapeutic perspective, chemical induction of apoptosis and many other forms of cell death is one of the effective treatment modalities for many types of tumours. As the mechanisms of different types of cell death are unravelled, novel therapeutics may emerge that can kill cancer cells without affecting healthy cells.

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