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Multiple cell death modalities and their key features (Review)

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Abstract. Cell death, as a final cellular decision which is reached following complex communications, represents a critical process with which to maintain organismic homeostasis. Different classifications and nomenclatures have brought considerable confusion to cell death determination. In the present review article, the hallmarks of different cell death modes are systematically described and are fitted into a simple classification system, where the cell death entities are primarily categorized into programmed cell death (PCD) or non-PCD based on their signal dependency. PCD can be further categorized as apoptotic cell death or non-apoptotic cell death. Programmed apoptosis consists of apoptosis, as well as anoikis. Multiple mechanisms and phenotypes compose programmed non-apoptotic cell death, including vacuole-presenting cell death (autophagy, entosis, methuosis and paraptosis), mitochondrial-dependent cell death (mitoptosis and parthanatos), iron-dependent cell death (ferroptosis), immune-reactive cell death (pyroptosis and NETosis), as well as other types, such as necroptosis. Finally, necrosis represents a form of non-programmed cell death.

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Abbreviations: AIF, apoptosis-initiating factor; Arf6, ADP ribosylation factor 6; ATGs, autophagy-related proteins; ATP, adenosine triphosphate; BIM, BCL2-like 11; BMF, BCL-2 modifying factor; DAI, DNA-dependent activator of interferon; DRs, death receptors; ECM, extracellular matrix; ER, endoplasmic reticulum; GPX4, glutathione peroxidase 4; GSH, glutathione; IGF1R, insulin-like growth factor 1 receptor; JNK, c-JUN NH2-terminal kinase; LAMP1, lysosomal-associated membrane protein 1; LC3, microtubule-associated protein light chain 3; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; MAPKs, mitogen-activated protein kinases; MLKL, mixed lineage kinase domain-like protein; MOMP, mitochondrial outer membrane permeabilization; NET, neutrophil extracellular trap; NLR, NOD-like receptor; NOX4, NADPH oxidase 4; PAD4, peptidylarginine deiminase 4; PAR, poly (ADP-ribose); PARP, poly(ADP-ribose)-polymerase; PCD, programmed cell death; PI3K, class III phosphoinositide 3-kinase; Rac1, Rac family small GTPase 1; RIPKs, receptor-interacting protein kinases; ROCK, Rho associated coiled-coil containing protein kinase; ROS, reactive oxygen species; TCR, T-cell receptor; TIMM8a/DDP, translocase of inner mitochondrial membrane 8a; TLRs, Toll-like receptors

Key words: anoikis, apoptosis, autophagy, entosis, ferroptosis, methuosis, mitoptosis, necroptosis, necrosis, NETosis, paraptosis, parthanatos, pyroptosis

Contents

1. Introduction

Cell death, survival, proliferation and differentiation represent fundamental processes of life. Cell death plays a pivotal role in embryonic development, maintaining the homeostasis of the organism and eliminating damaged cells. Cell death was initially divided into three types (1): Type I cell death (apoptosis), type II cell death (autophagy) and type III cell death (necrosis). In recent years, multiple novel cell death modalities have been identified and characterized concerning their corresponding stimuli, molecular mechanisms and morphologies. Some of these modalities share overlapping, but not identical signal pathways and fail to be incorporated into the type I-III categories. In 2018, the Nomenclature Committee on Cell Death listed multiple cell death types in a molecule-oriented manner (2). Tang *et al* also provided historical origins of items used during cell death research development and a brief summary of molecular machinery involved in regulated cell death (3). However, the hierarchical association among different cell death types remained vague and the molecular interplays led to further confusion. Therefore, the present review article aims to provide a simpler classification system and key features of different cell death modalities are abstracted.

Cell death entities can be categorized into programmed or non-programmed cell death based on their signal dependency

(Fig. 1). Programmed cell death (PCD) is driven by tightly regulated intracellular signal transduction pathways. By contrast, accidental cell death is referred to as non-PCD as a result of unexpected cell injury. Given the morphological characteristics and molecular mechanisms, PCD can be further categorized into apoptotic cell death and non-apoptotic cell death. Apoptosis retains cell membrane integrity and occurs in a caspase-dependent manner. By contrast, non-apoptotic cell death is mostly characterized by membrane rupture and caspase-independency. For simplicity, the present review article focuses on the key features of the diverse cell death modes and their assessment methods commonly utilized in research (Table I), and refers the reader to specialized recent review articles describing the processes of each cell death mode in further detail (4-15).

2. Non-programmed cell death

Non-programmed necrosis. Non-programmed necrosis is stimulated by a number of external factors, e.g., infection, toxins and physical injury, which lead to morphological alterations, such as cytoplasmic swelling [oncosis, pre-lethal phase caused by the disruption of ionic pumps such as Ca^+ influx (16)], plasma membrane rupture and the subsequent loss of intracellular organelles without severe chromatin condensation, but randomly degraded DNA (17) (Fig. 2). Non-programmed necrosis is often observed in ischemia, trauma and possibly some forms of neurodegeneration. It is commonly considered as a passive process, which does not require *de novo* macromolecular synthesis, but minimal energy (4).

Based on the morphological features of necrosis, a number of methods, including lactate dehydrogenase (LDH) activity detection and cell-impermeable DNA binding dye, are commonly used to certify the cellular leakage and membrane permeability (Table I).

3. Programmed apoptotic cell death

Apoptosis. Apoptosis involves a series of tightly controlled events and is characterized by cell shrinkage, membrane blebbing, positional organelle loss, DNA condensation and fragmentation (Fig. 2). Three signaling pathways are known to trigger apoptotic cell death: The extrinsic (death receptors) pathway, the intrinsic (mitochondrial) pathway and the perforin/granzyme pathway (Fig. 3) (5).

Anoikis is a particular type of apoptosis, which essentially shares identical pathways as with apoptosis; however, is triggered by inadequate or inappropriate cell-matrix interactions (18) (Fig. 3). The architectural state of the cytoskeleton is expected to interfere with the function of integrin, a pro-survival effector (6). However, the connection between cell architecture alteration and apoptosis remains poorly identified. It has recently been indicated that c-JUN NH2-terminal kinase (JNK) signaling is required for efficient anoikis through a BAK/BAX-dependent manner by increasing BCL2-like 11 (BIM) expression and BCL-2 modifying factor (BMF) phosphorylation (19).

Apoptosis assessment methods have been rapidly developed over the past years (Table I). Terminal deoxynucleotidyl transferase dUPT nick-end labeling (TUNEL) assay and comet assay are able to detect the presence of fragmented DNA. Annexin V in combination with cell-impermeable DNA staining dye is used to detect the outwards exposed phosphatidylserine on cell membrane and cellular integrity. Alternatively, some assays evaluate the intermediate modulators, e.g., caspase assay and poly-ADP ribose polymerase (PARP) cleavage assay (20). Furthermore, specific apoptosis inhibitors, such as the pan-caspase inhibitor, zVAD-fmk, can also shed some light on the presence of apoptosis.

4. Programmed non-apoptotic cell death

Vacuole-presenting cell death

Autophagy. Autophagic cell death is characterized by the appearance of large intracellular vesicles, plasma membrane blebbing, enlarged organelles and the depletion of cytoplasmic organelles in the absence of chromatin condensation (21) (Fig. 2). Noticeably, it functions as a lever in the cell process. Autophagy is initiated upon cellular stress as a protective response. Once the cellular stress is irreversible, the cell will be committed to death also through excessive levels of autophagy. There are three forms of autophagy: Macro-autophagy (Fig. 3), micro-autophagy and chaperone-mediated autophagy (7). The macro-autophagic process has been well documented (22-24) (Fig. 3). In micro-autophagy, the cytoplasmic components are directly sequestrated into the lysosomes, where acidic hydrolases further mediate the degradation. Chaperone-mediated autophagy selectively targets KFERQ motif (Lys-Phe-Glu-Arg-Gln)-containing proteins. These proteins can be recognized by chaperones, are subsequently hijacked into lysosomes and eventually degraded (25). The specific degradation of the mitochondria is referred to as mitophagy. The selective autophagy of foreign pathogens is coined as xenophagy. There are also some other selective autophagy forms, such as lipophagy, aggrephagy and lysophagy (26).

The detection methods are mostly developed for macro-autophagy embodying direct measurement of autophagic activity (e.g., turnover of long-lived proteins and LDH sequestration) and indirect analysis with autophagy specific antibodies through western blot-based assay, fluorescence microscopy-based assay and flow cytometry-based assay (27) (Table I).

Entosis. Entosis (or cannibalism) is characterized by cell-in-cell formation (Fig. 2). Upon internalization, the entotic cells remain viable for a short period of time. This process is frequently followed by lysosome-mediated degradation and non-apoptotic cell death, while a fraction of the internalized cells can also extricate themselves or are expelled from the host cell (28). Entosis is believed to be triggered by integrin-extracellular matrix (ECM) detachment (29). Unlike phagocytosis, the engulfment of entotic cells represents a self-control process through RhoA and the Rho-associated coiled-coil containing protein kinases (ROCK). The entotic cell and the host cell interact with each other through the E-cadherin and α-catenin cell junction interface. RhoA and ROCK in entotic cells lead to specific accumulation of actin and myosin complex (actomyosin) at the cell cortex opposite to the junctional interface, which generates the unbalanced contractile force driving cell-in-cell formation. However, entosis is also observed in matrix-attached epithelial cells.

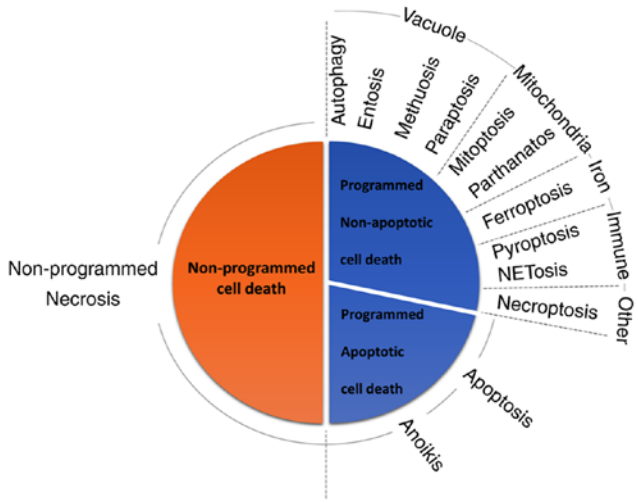

Figure 1. Cell death classification. The cell death entities are categorized according to their signal-dependency, morphological characteristics and molecular mechanisms. The pie area in the figure does not represent the frequency of occurrence of each cell death.

Wan *et al* proposed that the overactivation of myosin or unbalanced myosin activation through regulatory polarity proteins between the contacting cells acted as the driving force for entosis in matrix-attached epithelial cells (30). The engulfment is followed by lysosome-mediated degradation, which differs from autophagic cell death (31). The autophagic protein, microtubule-associated protein light chain 3 (LC3), does not participate to form the autophagosome. Instead, LC3 is directed to the single-membrane vacuole in the host cell that harbors the engulfed cell through lipidation with the help of autophagy-related protein (ATG)5, ATG7 and Vps34, and promotes lysosome fusion followed by lysosome-mediated degradation (8) (Fig. 3).

However, there is as yet no specific assay available for the detection of entosis, at least to the best of our knowledge. The presence of entosis is deduced from its typical cell-in-cell structure, as detected by fluorescence imaging and electron microscopy (32,33) (Table I).

Methuosis. Methuosis represents a type of cell death characterized by the presence of the massive accumulation of large fluid-filled single membrane vacuoles derived from macropinosomes, which is specifically accompanied with Ras hyper-activation and apoptosis impairment. Intriguingly, methuosis is not associated with the conventional Ras-Raf-MEK-ERK axis or class III phosphoinositide 3-kinase (PI3K) signaling (34). The consequent morphology resembles necrosis in the manner of cell swelling and plasma membrane integrity loss. In methuosis, activated Ras stimulates micropinocytosis through the downstream activation of Rac family small GTPase 1 (Rac1). Coincidently, the reduction of ADP ribosylation factor 6-GTP (Arf6-GTP) impedes macropinosome recycling (35). The abnormal coalescence of nascent macropinosomes gives rise to massive cytoplasmic vacuolization. The vacuoles formed in the early stages of methuosis are decorated with late endosomal markers [e.g., lysosomal-associated membrane protein 1 (LAMP1) and Rab7] (9). The massive vacuoles, which are not able to be recycled or merged with lysosomes, will finally lead to cell death. Methuosis with its typical morphology, is often assessed by electron microscopy in research (36-38) (Table I).

Paraptosis. The hallmark of paraptosis is the extensive cytoplasmic vacuolization derived from the dilated endoplasmic reticulum (ER) or the mitochondria (39) (Fig. 2). It has been reported that the activation of insulin-like growth factor 1 receptor (IGF1R) and its downstream signaling incorporating mitogen-activated protein kinases (MAPKs) and JNK pathways can induce paraptosis, despite the fact that IGF1R is commonly considered as a pro-survival modulator (40). A number of studies have indicated that paraptosis is associated with reactive oxygen species (ROS) generation and the accumulation of misfolded proteins in the ER, as well as mitochondrial Ca^{2+} overload (10,41-43), which exert an osmotic force to distend the ER lumen and mitochondria for vacuolization. In spite of the current available evidence, the molecular mechanisms underlying paraptosis have not yet been fully addressed.

Similar to entosis and methuosis, there is no specific assay available for the detection of paraptosis, at least to the best of our knowledge. It is mostly defined by the appearance of multiple single-membraned cytoplasmic vacuoles, as detected by electron microscopy (44) (Table I).

Mitochondrial-dependent cell death

Mitoptosis. Unlike mitophagy (autophagic degradation of mitochondria), mitoptosis, also known as mitochondrial suicide, represents a process of programmed fission and fusion of the mitochondria with the concomitant disruption of the adenosine triphosphate (ATP) supply. As a consequence, mitoptosis can be associated with both apoptosis (45) and autophagy (46). The degraded mitochondria either become autophagosomes or mitoptotic bodies, which are extruded from the cell. In this sense, mitoptosis itself is not a cell death pathway, but a mitochondrial death pathway. However, the extensive mitochondrial fragmentation through elevated fission finally leads to cell death (47). Mechanically speaking, mitochondrial outer membrane permeabilization (MOMP) induced by BAX/BAK triggers the release of a mitochondrial intermembrane space protein termed translocase of inner mitochondrial membrane 8a (TIMM8a/DDP). DDP subsequently binds to DRP1 in the cytoplasm. The interaction between DDP and DRP1 leads to the recruitment of DRP1 and retention in the mitochondria, which induces mitochondrial fission and finally, mitoptosis (48). Nevertheless, the process remains poorly understood and is described mostly by its morphological features.

As a manner of mitochondrial suicide, the visualization of fragmented mitochondria with mitochondria-specific dyes (e.g., MitoTracker Green®) by utilizing fluorescence microscopy and a close observation with electron microscopy provide certain clues on the presence of mitoptosis (45). Moreover, specific antibodies against cytochrome *c* and TIMM8a/DDP are also utilized in research (48) (Table I).

Parthanatos. Parthanatos represents a mitochondrial-linked, but caspase-independent cell death and is characterized by the hyperactivation of PARP. PARP mediates the synthesis of poly(ADP-ribose) (PAR), which further shuttles from the nucleus to the cytoplasm and binds to specific mitochondrial proteins followed by apoptosis-inducing factor

Table I. Cell death modalities, their features and common detection methods.

Classification	Cell death modality	Key molecules	Key morphology	Detection methods
Non-PCD	Necrosis	None	Cell swelling; membrane rupture; loss of organelle	Lactate dehydrogenase activity detection; visualizing membrane integrity loss by cell-impermeable DNA binding dye
PCD-apoptotic	Apoptosis/anoikis	DRs and their ligands, Bax, Bak, AIF, caspase-8, caspase-3, caspase-9	Cell shrinkage; membrane blebbing; loss of positional organization of organelles in the cytoplasm; DNA condensation and fragmentation; nuclear membrane rupture	Chromosome condensation detection; TUNEL assay; Annexin V assay; caspase assay; PARP cleavage assay; applying apoptosis inhibitors
PCD-vacuole presenting	Autophagy	UKL1, PI3KIII, ATGs, LC3	Large intracellular vesicles; membrane blebbing; enlarged organelles; depletion of cytoplasmic organelles	Turnover of long-lived proteins; LDH sequestration; western blot analysis with autophagy specific antibodies
	Entosis	RhoA, ROCKI/II, E-cadherin, α-catenin, actomyosin, LC3, ATGs	Cell-in-cell formation	Morphology observation with fluorescence imaging and electron microscopy
	Methuosis	Ras, Rac1, Arf6, LAMP1, Rab7	Accumulation of large fluid-filled single membrane vacuoles; cell swelling; membrane rupture	Morphology observation with electron microscopy
	Paraptosis	Unclear	Accumulation of large fluid-filled single membrane vacuoles; dilation of ER or mitochondria	Morphology observation with electron microscopy
PCD-mitochondria-dependent	Mitoptosis	Bax, Bak, TIMM8a(DDP), Drp1	Mitochondria disappearance; decomposition of the mitochondrial reticulum to small spherical organelles	Morphology observation with fluorescence microscopy and electron microscopy; western blot analysis with mitoptosis-specific antibodies
	Parthanatos	PARP, AIF	Membrane rupture; mitochondrial outer membrane permeabilization; chromatin condensation; DNA large-scale fragmentation	Western blot analysis with parthanatos specific antibodies; Mitochondrial depolarization detection with fluorescent probe
PCD-iron dependent	Ferroptosis	System XC−, GPX4, Lipid ROS	Diminutive mitochondria with decreased cristae and collapsed and ruptured membrane	Applying ferroptosis inhibitors; measuring lipid peroxides e.g. malondialhyde and 4-hydroxynonenal quantification
PCD-immune reactive	Pyroptosis	NLRs, ALRs, caspase-1, caspase-11	Cell swelling; membrane rupture; DNA condensation and fragmentation	Quantification of cytoplasmic LDH; visualizing membrane integrity loss by fluorescence microscopy; western blot analysis with pyroptosis-specific antibodies
	NETosis	NOX4, PAD4	Chromatin decondensation; membrane rupture	Morphology observation with fluorescence microscopy; free-cell DNA and DNA-neutrophil derived protein complex detection with fluorescent probe and immunoblot

Table I. Continued.

Classification	Cell death modality	Key molecules	Key morphology	Detection methods
Other type	Necroptosis	DRs, TLRs, TCR, RIPKs, MLMK	Cell swelling; membrane rupture; loss of organelle; mitochondria swelling	Visualizing membrane integrity loss; mitochondrial depolarization detection; applying necroptosis specific inhibitors; western blot analysis with necroptosis-specific antibodies

PCD, programmed cell death; DRs, death receptors; PARP, poly(ADP-ribose)-polymerase; ULK1, unc-51 like autophagy activating kinase 1; PI3K, class III phosphoinositide 3-kinase; ATGs, autophagy-related proteins; LC3, microtubule-associated protein light chain 3; ROCK, Rho associated coiled-coil containing protein kinase; Rac1, Rac family small GTPase 1; Arf6, ADP ribosylation factor 6; LAMP1, lysosomal-associated membrane protein 1; ER, endoplasmic reticulum; TIMM8a/DDP, translocase of inner mitochondrial membrane 8a; AIF, apoptosis-initiating factor; ROS, reactive oxygen species; NLRs, NOD-like receptors; ALRs, AIM2-like receptors; NOX4, NADPH oxidase 4; PAD4, peptidylarginine deiminase 4; TLRs, toll-like receptors; TCR, T-cell receptor; RIPKs, receptor-interacting protein kinases; MLKL, mixed lineage kinase domain-like protein.

(AIF) release. Free AIF is translocated from the mitochondria into the nucleus. In the nucleus, AIF induces chromatin condensation and DNA breakage (49). Compared to the apoptotic process, intact PARP and its activation is required, rather than PARP cleavage. Moreover, parthanatos cannot be inhibited by broad-spectrum caspase inhibitors (50), which proves its independency of caspases. Parthanatos does not involve the formation of apoptotic bodies. Furthermore, the DNA fragmentation is large-scale rather than small-to-moderate scale, as typically observed in apoptosis (11) (Fig. 2).

PAR accumulation, PARP-1 activation and nuclear AIF are practically used as biomarkers of parthanatos. The process can be further confirmed with mitochondrial depolarization, as detected with fluorescent probe staining (Table I).

Iron-dependent cell death

Ferroptosis. Ferroptosis is normally associated with a normal-appearing morphology, with an intact cell membrane without blebbing and normal-sized nucleus free of chromatin condensation, although with diminutive mitochondria with decreased cristae and collapsed and ruptured membranes (51) (Fig. 2). It is initiated by the failure of the glutathione-dependent antioxidant defense through defects in system X_C^- or glutathione peroxidase 4 (GPX4) (12). System X_C^- transports extracellular cystine into the cell, which is then transformed into cysteine for glutathione (GSH) synthesis. GPX4 can directly catalyze the reaction between glutathione and lipid hydroperoxides to reduce the cellular level of lipid peroxidation. Either the depletion of GSH or the inhibition of GPX4 results in lipid hydroperoxide accumulation. Free iron interacts with lipid hydroperoxides through the Fenton reaction and forms lipid ROS (Fig. 3). Excessive lipid ROS generation finally leads to the cell death.

The induction of ferroptosis can be confirmed by applying ferroptosis inhibitors (e.g., ferrostatin-1 and liproxstatin-1) and by measuring lipid peroxides (e.g., malondialhyde quantification and 4-hydroxynonenal quantification) (Table I).

Immune-reactive cell death

Pyroptosis. Pyroptosis is an inflammatory form of programmed cell death that commonly occurs upon the recognition of intracellular pathogens in immune cells. The inflammation sensors [e.g., NOD-like receptors (NLRs)] of infected macrophages recognize the flagellin components of pathogens and initiate the formation of multi-protein complex inflammasomes, which subsequently activate caspase-1 (13) (Fig. 3). Upon activation, caspase-1 mediates the membrane pore formation through the cleavage of gasdermin D, allowing the rupture of the cell membrane (52). The process is also accompanied by DNA condensation and fragmentation (Fig. 2). Moreover, caspase-11 can be directly activated by bacterial lipopolysaccharide (LPS) and induces pyroptosis (53).

Pyroptosis can be evaluated through the quantification of released cytoplasmic LDH, the visualization of membrane integrity loss by fluorescence microscopy, the detection of interleukin (IL)-1β, caspase activation and gasdermin D cleavage by western blot analysis (54) (Table I).

Neutrophil extracellular trap-associated cell death (NETosis). NETosis, a unique form of cell death, is initiated

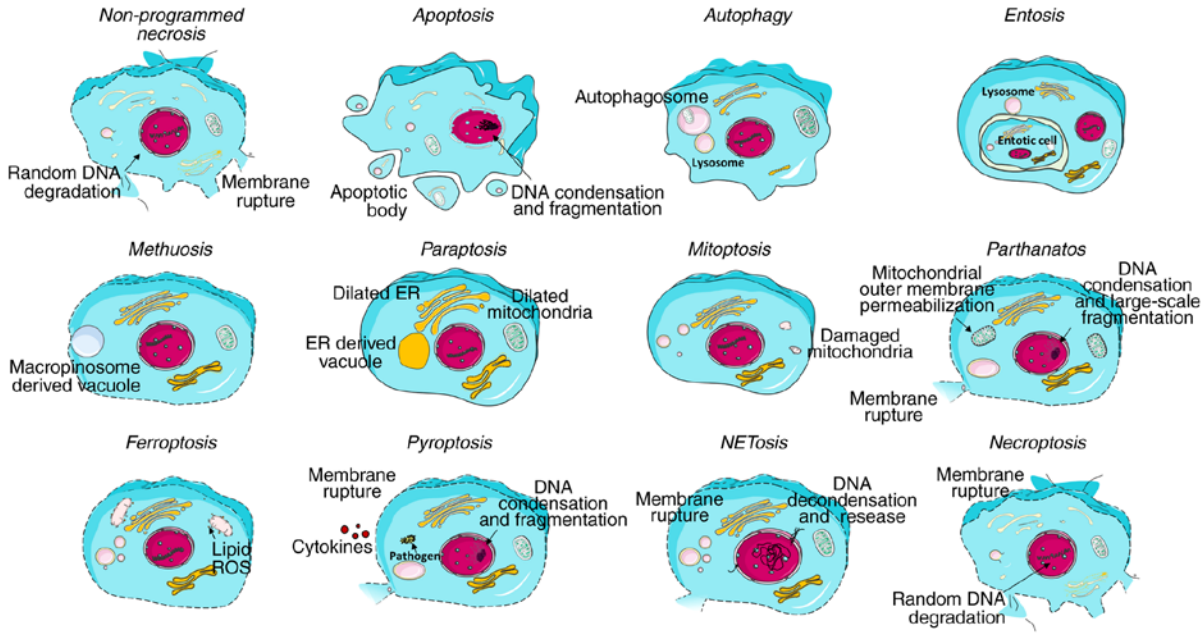

Figure 2. Typical morphology of each cell death. The morphological alteration focuses on cell size, membrane integrity, chromatin density, organelle arrangement and presence of vacuoles.

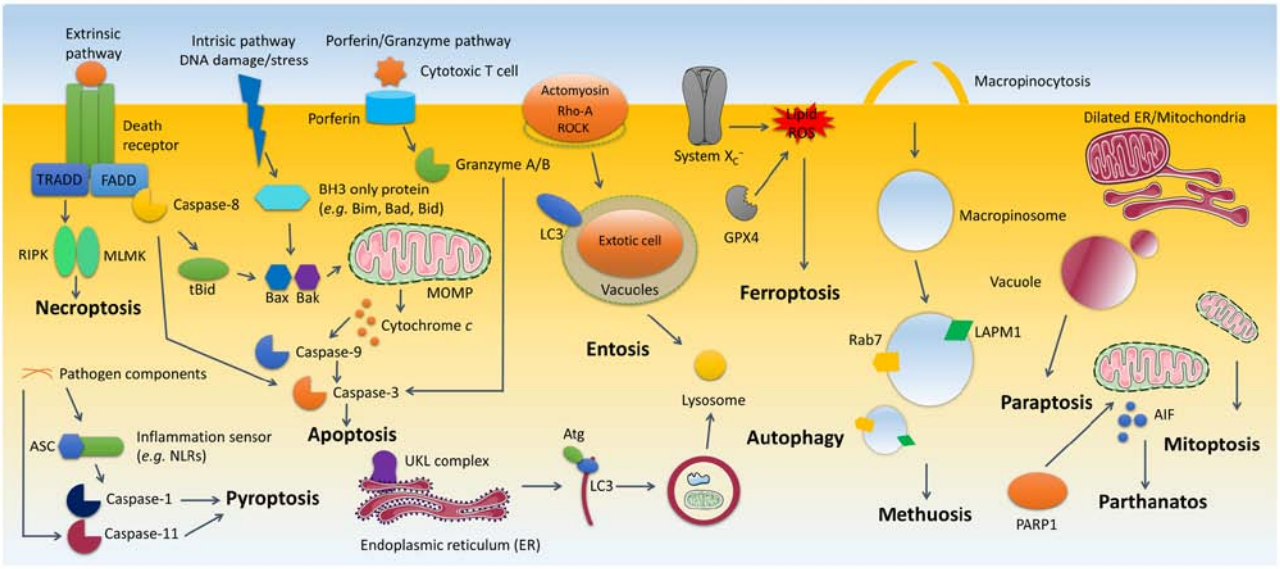

Figure 3. Synopsis of cell death processes. Ten cell death modalities (apoptosis, autophagy, entosis, methuosis, paraptosis, mitoptosis, parthanatos, ferroptosis, pyroptosis and necroptosis) are presented. Anoikis shares identical signaling pathways as apoptosis, apart from the fact that it is stimulated by inadequate or inappropriate cell-matrix interactions. The cell death modalities (necrosis and NETosis) without elucidative mechanism were not included. Grey color indicates non-functional molecules. Arrow direction indicates the causal association. RIPK, receptor-interacting protein kinase; MLKL, mixed lineage kinase domain-like protein; NLRs, NOD-like receptors; MOMP, mitochondrial outer membrane permeabilization; LC3, microtubule-associated protein light chain 3; ROCK, Rho associated coiled-coil containing protein kinase; GPX4, glutathione peroxidase 4; ROS, reactive oxygen species; UKL complex, UKL1 in a complex with FIP200, ATG13 and ATG101.

by the presence of pathogens or their components and mostly occurs in immune cells, particularly neutrophils. Upon the recognition of pathogens within neutrophils, the cells undergo histone modification, chromatin decondensation and neutrophil extracellular trap [NET, comprising chromatin and antimicrobial components including myeloperoxidase, neutrophil elastase, cathepsin G, lysozyme and defensins (55)] release and this eventually leads to cell death. The process is promoted through superoxide generated by NADPH oxidase 4 (NOX4), autophagy and peptidylarginine deiminase 4 (PAD4)-dependent histone citrullination (56,57). However, further research is expected to provide a clear molecular elucidation.

The staining of co-localized neutrophil-derived proteins and extracellular DNA, as well as citrullinated histones is utilized to evaluate NETosis. Moreover, cell-free DNA and

DNA-neutrophil derived protein complexes can be detected by PicoGreen® and ELISA. Both morphology and cell-appendant NETosis components can be detected through flow cytometry (58) (Table I).

Other types

Necroptosis. Necroptosis, also known as programmed necrosis, is characterized by the activation of receptor-interacting protein kinases (RIPKs) through several signaling pathways (15). RIPKs are activated upon recruitment to macromolecular complexes from various cell-surface receptors: Death receptors (DRs), Toll-like receptors (TLRs), and the T-cell receptor (TCR) (Fig. 3) (59,60). RIPK1 and RIPK3 function as the key components of necrosome (61). RIPK3 further activates downstream molecule mixed lineage kinase domain-like protein (MLKL) through phosphorylation (62,63), which leads to MLKL oligomerization. The oligomerized MLKL inserts into and permeabilizes cellular membrane, which finally gives rise to cell death (64). Moreover, RIP3-dependent necroptosis is also triggered by the cytosolic DNA sensor, DNA-dependent activator of interferon (DAI) regulatory factors, following viral infection or the presence of double-stranded viral DNA (65). Necroptosis reveals the necrotic morphology with membrane rupture and loss of organelles (Fig. 2).

Necroptosis can be assessed by the loss of plasma membrane integrity by utilizing cell-impermeable DNA binding dyes, the release of cellular contents, including LDH, high mobility group box 1 protein (HMGB1) and cyclophilin A by western blot analysis, mitochondrial potential by fluorescent probes and morphology by electron microscopy. The utilization of necroptosis specific inhibitors, such as necrostatin-1 and measuring key proteins in the pathway represent alternative strategies (66) (Table I).

5. Implications of cell death in human diseases

The dysregulation of cell death processes is highly relevant to tumorigenesis, as well as to the pathogenesis of a number of other diseases, such as degenerative, cardiovascular and autoimmune diseases. The association between cell death and cancer is complex. The complexity is attributed to several factors: On the one hand, there is more than one type of cell death endogenously engaged in cancer. On the other hand, some types of cell death have dual and even opposing effects on tumorigenesis. Firstly, apoptosis is involved in cancer. Cancerous cells can evade apoptosis by downregulating or blocking apoptosis signaling (67). Unexpectedly, apoptosis can also drive tumor formation by promoting cell proliferation as a compensation for cell loss (68). Secondly, necrosis is commonly observed in tumors due to hypoxic microenvironments (67). Thirdly, cancerous cells with defects in apoptosis tend to utilize autophagy as a pro-survival mechanism. Paradoxically, impeded autophagy is also associated with tumorigenesis (69). Fourthly, entosis represents tumor suppressive activity in pancreatic cancer, whereas it promotes tumor progression in most other situations (70,71). Although the other cell death types are much less endogenously involved in cancer development, they are mostly utilized as anti-cancer defense strategies of the body and defects in their signaling plays an important role in drug resistance and clinical failures.

As for neurodegenerative diseases, the initial phase of cell death in ischemia represents necrotic cell death, while delayed cell death is apoptotic in nature due to the fact that the ischemic core tends to be necrotic and the penumbra region apoptotic (72). Autophagic cell death and parthanatos are linked to ischemia (11,73). In Parkinson's disease, apoptosis contributes to the loss of nigral neurons due to the fact that almost every Lewy body-containing neuron (as a pathological feature of Parkinson's disease) is positive for pro-apoptotic modulator staining (74). Another study demonstrated that necrostatin-1, an inhibitor of necroptosis, ameliorated neuronal loss in a model of Parkinson's disease (75), indicating that necroptosis may also play a role in Parkinson's disease. There is also evidence suggesting the role of apoptosis in Huntington's disease. However, its role in Alzheimer's disease remains under debate (76).

Cell death modes, such as apoptosis, necrosis and autophagy in cardiac myocytes have been frequently reported to affect a variety of cardiovascular diseases, including myocardial infarction, diabetic cardiomyopathy, ischemic cardiomyocyte and congestive heart failure (77-79). In addition, ferroptosis, pyroptosis, as well as parthanatos are also documented to contribute to ischemia/reperfusion injury (80). The other cell death types have been studied to a much lesser extent as compared to cardiovascular diseases. Likewise, apoptosis and secondary necrosis are considered as major modes of cell death in systemic autoimmune diseases. Recent evidence indicates that NETosis accounts for certain immunological features in systemic lupus erythematosus (81).

6. Conclusions and perspectives

The cell death modes presented in the present review article are mostly distinguished by stimuli, molecules and morphologies. Apart from non-programmed necrosis, the other cell death modes are regulated in a signal-dependent manner, despite the fact that a number of the pathways have not yet been fully addressed. Some cell death modes are intensively interacting with others. For instance, the activation of tumor necrosis factor receptor (TNFR) can stimulate both apoptosis and necroptosis; however, compromised apoptosis can shift the downstream pathway to necroptosis (82) and vice versa (83). Some processes during cell death are connected; for instance, the occurrence of mitoptosis can turn out as autophagic cell death or apoptotic cell death. In general, necrosis-like cell death is associated with membrane rupture. The consequent release of intracellular inflammatory factors can give rise to inflammation as observed in necrosis, necroptosis, NETosis and pyroptosis. By contrast, apoptotic cells do not stimulate inflammation, since they are rapidly eliminated by phagocytes. However, if apoptotic cells are not properly processed, they can develop secondary necrosis. These mutual connections indicate that different cell death types are not isolated from each other. The molecular links await to be unveiled in greater detail. Their implications on diverse diseases are expected to be unraveled in the near future, since current studies on cell death modes involved in diseases are mostly confined to the more classical cell death categories. Green (84) also addressed five quite interesting and inspiring questions about the balance and context of cell death. In fact, much is still unknown. Noticeably, this review article has primarily focused

on the features of pathological cell death and is limited to the animal kingdom. However, there also exist physiologic cell death such as cornification (85) to form termination differentiation and some cell death types are also similarly present in the plant kingdom (e.g., apoptosis-like cell death) (86).

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Authors' contributions

GY was responsible for the drafting of the manuscript and cell death information collection. ME was responsible for information presentesst and figure construction. TE was responsible for the initial conception of the study and for the revision of the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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