The Researcher’s Guide to
Mechanisms of Cell Death
The Three Major Modes of Cell Death

Cell death is the process by which living cells cease to function and it can occur in many different ways for different reasons. Cells can die either during development, as a result of cellular stress and metabolic disruption, from pathogenic invasion, or due to physiological tissue damage.

Decades of research have allowed for the detailed characterization of the myriad ways that cells can self-destruct. Classically, cell death is categorized into three different types, based on morphological changes, triggers, and the biochemical pathways involved.

**Apoptosis**, or Type I cell death, is a tightly regulated form of programmed cell death (PCD) that triggers cells to self-destruct without any external influence. It is an essential part of life, particularly for multicellular organisms that must control the growth, development, and turnover of cells in order to maintain homeostasis. Apoptosis is critical for proper embryonic development, and a classic example of this process can be observed when cells between the digits on a hand apoptose in order to separate the fingers.

**Autophagy**, or Type II cell death, is also often categorized as a type of programmed cell death. However, over time it has become clear that it can promote cell survival as well as cell death, depending upon the circumstance. Autophagy is a process by which cellular organelles and other contents are devoured by lysosomes to clear away unnecessary or dysfunctional components. This critical mechanism allows for the systematic degradation and recycling of cellular materials. While autophagy is an important cellular mechanism, this guide will focus on other mechanisms of cell death, including apoptosis and regulated necrotic pathways.

**Necrosis**, or Type III cell death, has historically been thought of as a process that occurs accidentally due to extreme external physiological stress. In recent years, researchers have shed light on specific regulated molecular mechanisms that can be triggered when a cell is under stress and alternative methods of cell death are not available. This guide will describe some of these regulated necrotic cell death pathways, including necroptosis, pyroptosis, ferroptosis, and NETosis.

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One of the earliest and often-used methods of classifying different types of cell death is to identify the observable morphological differences between them. An apoptotic cell is morphologically characterized by cytoplasmic shrinkage, membrane blebbing, chromatin condensation, and nuclear fragmentation. During apoptosis, membrane blebbing, or the malformation of the plasma membrane, can also lead to the formation of small vesicles, termed apoptotic bodies, around the edge of the dying cell. It is important to note that the cell membrane remains intact during apoptosis. However, phosphatidylserine (PS) lipids within the membrane, which normally face the cytosol, flip to become exposed to the extracellular space. Their presence acts as a signal for neighboring phagocytes to remove the dead cell.

Its ability to bind to PS lipids on the cell membrane has caused Annexin V to be used traditionally as a marker of early apoptosis in combination with reagents that confirm the integrity of the cell membrane, such as propidium iodide. Whether the cell membrane remains intact or not is critical information to differentiate apoptosis from necrotic cell death pathways. However, the presence of Annexin V staining alone, indicating PS exposure, is not enough to differentiate between types of cell death.

In contrast, necrosis is often characterized by breakage of the cell membrane after cellular swelling and membrane blebbing, which leads to leakage of the cell contents that often induces inflammation of the affected tissue. This rupture may be caused by sudden overwhelming physiological forces applied to the cell or, alternatively, it may be the result of externally triggered and internally regulated cellular programming. Vacuoles and lysosomes are notably absent during necrotic cell death processes.

Within the category of necrotic cell death, regulated necrotic processes can display distinctive morphological features as well. During necroptosis, different stages of cell breakdown can be observed, including the swelling of organelles, rupture of the cell membrane, and eventually, the disintegration of the cytoplasm and nucleus. Cells that undergo pyroptosis display characteristics such as cell swelling, membrane blebbing, DNA fragmentation, and eventual cell lysis. However, the nucleus often remains intact, which differs from the nuclear destruction that can be observed during apoptosis and necroptosis. Ferroptosis results neither in the chromatin condensation observed during apoptosis nor in the loss of plasma membrane integrity that occurs during necrosis. Rather, it is characterized by the presence of condensed mitochondria, reduction in mitochondrial cristae, and increased membrane densities.

**Summary of the different morphologies, mechanisms, and outcomes of the three forms of cell death (apoptosis, pyroptosis, and necroptosis)**

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Apoptosis

Apoptosis is a tightly controlled pattern of programmed cell death characterized by distinct morphological changes along with the activation of specific caspases and mitochondrial control pathways. It can be triggered via intrinsic or extrinsic pathways. The intrinsic pathway can be activated by cell stress, DNA damage, developmental cues, or withdrawal of survival factors. The extrinsic pathway is triggered via the detection of extracellular death signals from other cells.

The intrinsic apoptotic pathway involves many conserved signaling proteins and depends upon the integrity of the mitochondrial membrane. This process is tightly regulated by a balance between the activities of proteins in the Bcl-2 family, which consists of pro-apoptotic and anti-apoptotic members. Apoptosis is triggered when pro-apoptotic family members with a BH3 domain, such as BAD, BID, and BIM, are activated during cellular stress via changes in expression or through post-translational modifications. Then pro-apoptotic proteins, BAX and BAK, induce changes in the mitochondrial outer membrane permeability (MOMP), leading to the release of cytochrome c from the organelle’s intermembrane space. Free cytochrome c then forms a complex with Apaf-1, called the apoptosome, that activates caspase-9 and triggers a cascade of apoptotic events.

Anti-apoptotic family members, such as Bcl-2, Bcl-XL, and MCL-1, can bind to and inhibit pro-apoptotic proteins to prevent apoptosis. These proteins are often active in cancer cells and are therefore attractive targets for the development of cancer therapeutics. Translational research has focused on targeting these proto-oncogenic proteins in different ways; one strategy has been to design compounds to mimic BH3-containing pro-apoptotic proteins that induce apoptosis by competing with the interaction between pro- and anti-apoptotic family members.

Both the intrinsic and extrinsic apoptotic pathways are ultimately dependent upon the protease activity of specific members of the caspase family. These caspases fall into two main categories. The "initiator" caspases-2, -8, -9, -10, -12 are coupled to upstream, pro-apoptotic signals and act by cleaving the downstream "executor" caspases-3, -6, and -7, which modify proteins ultimately responsible for the disassembly of the cell. Cleavage-specific antibodies targeting these caspases or their substrates serve as important tools to identify cell death. For example, targets such as PARP and lamin A/C, are cleaved by executioner caspases and are useful markers of apoptosis. Additionally, proteins in the Inhibitors of Apoptosis (IAP) family, such as XIAP, block the activity of different caspases to prevent apoptosis, and their expression is an indicator of enhanced cell survival.

Caspases can be also activated through the extrinsic pathway upon activation from extracellular ligands that trigger cell surface death receptors. The death receptors consist of members of the TNFR family and their associated ligands include TNF-α, FasL, TRAIL, and TWEAK. Receptor activation leads to the formation of a Death Inducing Signaling Complex (DISC) that activates a pro-caspase. Members of the complex include adaptor proteins, FADD and TRADD, that recruit and activate the initiator caspase-8 and the executioner caspase-3. Interestingly, the death receptor pathway can also lead to cell survival through TNFR2-mediated signaling to NF-κB, which induces expression of pro-survival genes, Bcl-2, FLIP, and XIAP.
Regulation of Apoptosis

- Casp-2, Casp-9
- TAK1, ASK1
- NIK, TRAF2
- NF-κB, IκBα
- IKKα, IKKβ, IKKγ
- IKK
- RIP
- TRADD, FADD
- Casp-8,10
- p53
- Bad, Bcl-2, Bax, Bak
- NF-κB
- IκBα
- XIAP, FLIP, CYLD
- p90RSK, Erk1/2, Akt
- JNK
- PI3K
- p53, c-Jun
- p14-3-3
- APP, DFF40, DFF45
- Smac/Diablo, AIF, Endo G
- Apaf-1, Cyto c, AIF, tBid
- HtrA2, SirT2, Mcl-1, HECTH9
- Bcl-xL, Bad, Bax, Bak
- Bad
- ROCK, ATM/ATR, Chk1/2
- DNA fragmentation
- DNA damage
- PARP
- Calpain, XIAP, FLIP
- ER stress
- Cell shrinking
- Membrane blebbing

Cellular Stress

- TNF, FasL, TRAIL
- PIDD, RAIDD, TRADD, FADD
- c-IAP, TRAF2
- p53, Noxa, Bim
- PKC, Erk1/2, PI3K, Akt
- p53, c-Jun
- JNK
- FOXO1
- NF-κB
- IκBα
- IKKα, IKKβ, IKKγ
- IKK
- RIP
- TRADD, FADD
- Casp-8,10
- p53
- Bad, Bcl-2, Bax, Bak
- NF-κB
- IκBα
- XIAP, FLIP, CYLD
- p90RSK, Erk1/2, Akt
- JNK
- PI3K
- p53, c-Jun
- p14-3-3
- APP, DFF40, DFF45
- Smac/Diablo, AIF, Endo G
- Apaf-1, Cyto c, AIF, tBid
- HtrA2, SirT2, Mcl-1, HECTH9
- Bcl-xL, Bad, Bax, Bak
- Bad
- ROCK, ATM/ATR, Chk1/2
- DNA fragmentation
- DNA damage
- PARP
- Calpain, XIAP, FLIP
- ER stress
- Cell shrinking
- Membrane blebbing

Cell Cycle

- TNF, FasL, TRAIL
- PIDD, RAIDD, TRADD, FADD
- c-IAP, TRAF2
- p53, Noxa, Bim
- PKC, Erk1/2, PI3K, Akt
- p53, c-Jun
- JNK
- FOXO1
- NF-κB
- IκBα
- IKKα, IKKβ, IKKγ
- IKK
- RIP
- TRADD, FADD
- Casp-8,10
- p53
- Bad, Bcl-2, Bax, Bak
- NF-κB
- IκBα
- XIAP, FLIP, CYLD
- p90RSK, Erk1/2, Akt
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- Bcl-xL, Bad, Bax, Bak
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- ROCK, ATM/ATR, Chk1/2
- DNA fragmentation
- DNA damage
- PARP
- Calpain, XIAP, FLIP
- ER stress
- Cell shrinking
- Membrane blebbing

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Necrosis has been classically defined as unprogrammed cell death that occurs in response to overwhelming chemical or physical insult. External forces that may lead to this accidental cell death include extreme physical temperature, pressure, chemical stress, or osmotic shock. Rupture of the cell, which is characteristic of necrosis, leads to leakage of the cellular contents into the extracellular space. This causes the release of biomolecules, called damage-associated molecular patterns (DAMPs), that can be recognized by immune cells and trigger an inflammatory response. However, recent studies have uncovered several regulated pathways that can also trigger necrotic cell death. These programmed necrosis pathways include necroptosis, pyroptosis, and ferroptosis.
Necroptosis

Necroptosis is a process of cellular self-destruction that is activated via extracellular signals (death receptor-ligand binding) or intracellular cues (microbial nucleic acids) when apoptosis is otherwise prevented. Necroptosis is highly immunogenic in nature and is useful as a host defense mechanism against pathogens. It is also activated in inflammatory diseases, neurodegeneration, and cancer.

Necroptosis is a distinct form of programmed necrotic cell death that progresses independently of caspase activity. Instead, it requires the RIPK3-dependent phosphorylation of MLKL (Ser356 in human, Ser345 in mouse). This phosphorylation event allows MLKL to generate a pore complex in the plasma membrane leading to the secretion of DAMPs, cell swelling, and membrane rupture.

In the canonical pathway, RIPK3 is activated via phosphorylation at Ser227 (Thr231/Ser232 in mouse) after forming a complex with RIP1. RIPK1 activity can be monitored by examining autophosphorylation sites, such as Ser166 and Ser14, using phosphorylation-specific antibodies. It is important to note that RIPK1 is highly regulated and plays a role in complexes that initiate NF-κB signaling and survival, apoptosis, or necroptosis. Thus, analyzing the phosphorylation states of the key proteins RIPK1, RIPK3, and MLKL is an important step in identifying necroptosis. Alternate triggers of this pathway include pathogen activation of TRIF and ZBP-1 that subsequently interact with and activate RIPK3.

Necroptosis can be inhibited in various ways, including via the use of small molecules called necrostatins that directly inhibit RIPK1 activity. Additionally, active caspase-8 can inhibit necroptosis through cleavage of RIPK1 and RIPK3. In this way, the caspase-driven apoptotic pathway inhibits necroptosis. Conversely, activation of the protein FLIP, which is a catalytically inactive homolog of caspase-8, can prevent RIPK1 cleavage and drive necroptosis.

**KEY PRODUCTS FOR STUDYING NECROPTOSIS**

**RIP (E8S70) XP® Rabbit mAb #37333**
Necroptosis is initiated by the formation of a complex called the necrosome, which includes RIP kinase (RIPK1) and RIPK3.

**Phospho-RIP (Ser166) (E8S7U) XP® Rabbit mAb #65746**
Autophosphorylation of RIPK1 at Ser166 is induced by the necrosome signals Z-VAD, SM164, and TNF; and is inhibited by necrostatins.

**Phospho-RIP3 (Ser227) (D6W2T) Rabbit mAb #93654**
RIPK3 phosphorylation at Ser227, which is induced by interactions with RHIM domain proteins like RIPK1, is required for necroptosis.

**Phospho-MLKL (Ser345) (D6E3G) Rabbit mAb #37333**
MLKL phosphorylation, which is induced by RIPK3, triggers pore formation and is required for necroptosis.

**ANTIBODY SAMPLER KITS FOR STUDYING NECROPTOSIS**

**Necroptosis Antibody Sampler Kit #98110**
RIP (D9H4C12) XP® Rabbit mAb #3493, Phospho-RIP (Ser166) (D1L3S) Rabbit mAb #65746, MLKL (D2KIN) Rabbit mAb #14169, Phospho-MLKL (Ser356) (D6H3V) Rabbit mAb #19689, RIP3 (E1Z1D) Rabbit mAb #3526, Phospho-RIP3 (Ser227) (D6W2T) Rabbit mAb #3654, Anti-rabbit IgG, HRP-linked Antibody #7074

**Mouse Reactive Necroptosis Antibody Sampler Kit #47826**
Phospho-RIP (Ser166) (E7G6D) Rabbit mAb #55286, RIP (D9H4C12) XP® Rabbit mAb #3493, Phospho-RIP3 (Thr231/Ser232) (E7S1F) Rabbit mAb #91702, RIP3 (E8L3L) Rabbit mAb #15828, Phospho-MLKL (Ser345) (D6E3G) Rabbit mAb #37333, MLKL (D6W1K) Rabbit mAb (Mouse Specific) #37705, Anti-rabbit IgG, HRP-linked Antibody #7074

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Pyroptosis is a type of cell death that is activated upon intracellular infections from bacteria, viruses, fungi, and protozoa in the presence of pathogen-associated molecular patterns (PAMPs) or cell-derived DAMPs. It is typically induced in cells of the innate immune system, such as monocytes, macrophages, and dendritic cells.

Pyroptosis is characterized by the N-terminal cleavage of gasdermin D (GSDMD), which results in its oligomerization to form a lytic pore in the plasma membrane. This cleavage process relies on the activity of inflammatory caspases 1, 4, 5, and 11. Monitoring cleavage of GSDMD and its related family members, as well as these inflammatory caspases, are key to studying pyroptosis.

The canonical pathway is often described as occurring through a two-step process. In the first “priming signal” step, NF-κB is activated to induce the expression of a number of proteins that will become part of a complex called the inflammasome. Inflammasomes typically consist of a cytosolic-pattern recognition receptor (PRR; such as NLRP3, or AIM2-like family members), an adaptor protein, such as ASC/TMS1, and pro-caspase-1. Analyzing the activation of the inflammasome via the detection of NLRP3 and visualization of ASC speckling is another useful technique to monitor pyroptosis.

In the second activation step, caspase-1 is proteolytically activated before cleaving gasdermin D and the cytokines, pro-IL1β and pro-IL18, creating their pro-inflammatory forms, IL1β and IL18, which are secreted from the dying cell. Visualizing the presence of these important cytokines can be achieved through the use of specific antibodies. Once GSDMD is cleaved by caspase-1 to form a membrane pore, cytokines are secreted, and the influx of water eventually leads to cell rupture.

Alternatively, pyroptosis can be induced via the non-canonical pathway by the intracellular detection of Gram-negative bacterial LPS, which activates caspase-4, -5 (human) and caspase-11 (mouse) to cleave GSDMD.

**KEY PRODUCTS FOR STUDYING PYROPTOSIS**

- **Cleaved Caspase-1 (Asp296) (E2G2I) Rabbit mAb #89332**: WB analysis of cell extracts from the cells or media from mouse bone marrow-derived macrophages (mMDM), untreated (-) or treated with Lipopolysaccharides (LPS) #14011 (50 ng/ml, 4 hr) followed by Nigericin (15 μM, 45 min) (+), using #89332 (upper), or Caspase-1 (E2Z1C) Rabbit mAb #24232 (lower).

**Cleaved Gasdermin D (Asp275) (E7H9G) Rabbit mAb #36425**: WB analysis of extracts from THP-1, Jurkat, Balb/3T3, mouse bone marrow-derived dendritic cells (BMDC) and various cell lines using #15101 (upper) and β-Actin (D6A8) Rabbit mAb #8457 (lower).

**ASC/TMS1 (D2W8U) Rabbit mAb (Mouse Specific) #67824**: Confocal I-F analysis of mouse TgC576 brain which overexpresses mutant human APP995. Sections were first labeled with #67824 (green) and APPβ-Amyloid (NAB228) Mouse mAb #2450 (yellow). After blocking free secondary binding sites with TPA #4174 (50 ng/ml, overnight) and then treated with LPS #14011 (5 μg/ml, indicated times), using #36425 (upper), total Gasdermin D (GSDMD) Antibody #93709 (middle), or β-Actin (D6A8) Rabbit mAb #8457 (lower).

**NLRP3 (D4D8T) Rabbit mAb #15101**: WB analysis of extracts from mouse bone marrow-derived dendritic cells (BMDC) and various cell lines using #15101 (upper) and β-Actin (D6A8) Rabbit mAb #8457 (lower).

**ASC/TMS1 (D2W8U) Rabbit mAb #67824**: Confocal I-F analysis of mouse TgC576 brain which overexpresses mutant human APP995. Sections were first labeled with #67824 (green) and APPβ-Amyloid (NAB228) Mouse mAb #2450 (yellow). After blocking free secondary binding sites with Mouse (G3A1) mAb IgG1 Isotype Control #5415, sections were incubated with GFAP (GA5) Mouse mAb #2450 (yellow). After blocking free secondary binding sites with TPA #4174 (50 ng/ml, overnight) and then treated with LPS #14011 (5 μg/ml, indicated times), using #36425 (upper), total Gasdermin D (GSDMD) Antibody #93709 (middle), or β-Actin (D6A8) Rabbit mAb #8457 (lower).
Ferroptosis is a recently described form of programmed cell death with distinct morphological and biochemical characteristics. It is an iron-dependent cell death that results in an increase in lipid peroxides. As such, it is largely regulated by pathways that contribute to iron homeostasis and oxidative stress. Iron homeostasis is controlled, in part, by ferritin, which is a large protein complex that is responsible for the transport of iron in its non-toxic form. Ferritin levels can be regulated by a selective autophagy process targeting ferritin, termed ferritinophagy. This pathway is mediated by a selective cargo receptor for ferritin called nuclear receptor coactivator 4 (NCOA4). Ferroptosis has been shown to be inhibited with the use of some common iron chelators, such as deferoxamine.

Pathways regulating cellular defense against oxidative stress are critical to mitigate ferroptosis. Small-molecule lipophilic free radical scavengers, like ferrostatin-1 and liproxstatin-1, can inhibit ferroptosis. The glutathione pathway, in particular, has been identified as a key antioxidant defense pathway. A central player in this process is the metabolic protein glutathione peroxidase 4 (GPX4), which converts GSH into oxidized glutathione (GSSG), thus protecting cells against ferroptosis by limiting cytotoxic lipid peroxidation. The glutathione peroxidase pathway is further regulated by System Xc-, an amino acid antiporter consisting of a heterodimer of SLC7A11 and SLC3A2, which is critical for glutathione (GSH) synthesis. Importantly, one way to induce ferroptosis is to use the compound erastin that directly inhibits the function of System Xc-.

Regulation of genes involved in oxidative stress, including GPX4, are largely controlled by the transcription factor NRF2. This serves as a critical defense against ferroptosis. Under normal conditions, expression of NRF2 is inhibited through interaction with KEAP1. Following oxidative or electrophilic stress, NRF2 is released from KEAP1, thereby allowing this transcriptional activator to translocate to the nucleus and regulate oxidative stress response genes. KEAP1 (D6B12) Rabbit mAb #8047

Ferritin (FTH) is a ubiquitous and highly conserved protein that plays a major role in iron homeostasis by sequestering and storing iron. Ferritin levels can be regulated by a selective autophagy process targeting ferritin, termed ferritinophagy. This pathway is mediated by a selective cargo receptor for ferritin called nuclear receptor coactivator 4 (NCOA4). Ferroptosis has been shown to be inhibited with the use of some common iron chelators, such as deferoxamine.

Tools to monitor ferroptosis may involve multiple approaches including pharmacological sensitivity (such as iron chelators, antioxidants), changes in expression of targets (SPX4, SCL7A11, ferratin, NRF2, etc.), monitoring reactive oxygen and lipid peroxidation, and glutathione assays.

**KEY PRODUCTS FOR STUDYING FERROPTOSIS**

**Cellular Glutathione Detection Assay Kit #13859**
The presence of reduced glutathione is important for preventing cellular damage caused by reactive oxygen species. Depletion of glutathione is an indicator of the progression of cell death.

**FTH1 (D1D4) Rabbit mAb #4393**
Ferritin (FT1) is a ubiquitous and highly conserved protein that plays a major role in iron homeostasis by sequestering and storing iron.

**GPX4 Antibody #52455**
Glutathione peroxidase 4 (GPX4) protects cells against ferroptosis by limiting cytotoxic lipid peroxidation.

**KEAP1 (D6B12) Rabbit mAb #8047**
Regulation of the genes controlling oxidative stress serves as a critical defense against ferroptosis. Under normal conditions, expression of NRF2 is inhibited through interaction with KEAP1.

**NCOA4 (E8H8Z) Rabbit mAb #66849**
Nuclear receptor coactivator 4 (NCOA4) is a selective cargo receptor that mediates ferritin levels and is involved in the process of ferritinophagy.

**NRF2 (D129C) XP® Rabbit mAb #12721**
Following oxidative or electrophilic stress, NRF2 is released from KEAP1, thereby allowing this transcriptional activator to translocate to the nucleus and regulate oxidative stress response genes.

**NRF2 (D9J1B) Rat mAb (IF Specific) #14596**
This NRF2 antibody is specifically designed for use with immunofluorescence protocols to visualize the localization of NRF2 within cells.

**xCT/SLC7A11 (D2M7A) Rabbit mAb #12691**
xCTSLC7A11 is part of the heterodimeric amino acid transport system x(c)- that regulates intracellular glutathione (GSH) synthesis. Importantly, one way to induce ferroptosis is to use the compound erastin that directly inhibits the function of System Xc-.

**ANTIBODY SAMPLER KITS FOR STUDYING FERROPTOSIS**

**Ferroptosis Antibody Sampler Kit #29650**
GPX4 Antibody #52455, NCOA4 (E8H8Z) Rabbit mAb #66849, KEAP1 (D6B12) Rabbit mAb #8047, NRF2 (D129C) XP® Rabbit mAb #12721, 4F2hc/CD98 (D3F9D) XP® Rabbit mAb #47213, FTH1 (D1D4) Rabbit mAb #4393, xCT/SLC7A11 (D2M7A) Rabbit mAb #12691, DMT1/SLC3A2 (D3963) Rabbit mAb #15083, Anti-rabbit IgG, HRP-linked Antibody #7074

**Redox Homeostasis and Signaling Antibody Sampler Kit #16815**
GPX1 (C24A) Rabbit mAb #52455, Thioredoxin 1 (C63C6) Rabbit mAb #2429, NRF2 (D9J1B) Rat mAb (IF Specific) #14596

**NRF2 (D9J1B) Rat mAb (IF Specific) #14596**
Confocal IF analysis of MEF NRF2 wild-type cells, untreated (left) or treated with MG-132 #2194 (10 μM, 8 hr; center) and MEF NRF2 knock-out cells treated with MG-132 #2194 (10 μM, 8 hr; right), using #14596 (green pseudocolor).

**Keap1 (D6B12) Rabbit mAb #8047**
Confocal F analysis of OVCAR cells using #8047 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

**GPX4 Antibody #52455**
Western blot analysis of extracts from various cell lines using #52455.
NETosis

NETosis is a unique form of regulated cell death that is characterized by membrane rupture and the extrusion of chromatin, histones, and granular and cytoplasmic components into a web-like structure called neutrophil extracellular traps (NETs). NETosis was identified as a response to bacterial infection and can be activated by lipopolysaccharides (LPS) as well as inflammatory pathway activators like phorbol 12-myristate 13-acetate (PMA). NETosis can occur via multiple pathways, but several key players have emerged. The calcium dependent enzyme protein arginine deiminase 4 (PAD4) catalyzes hypercitrullination of histones that contributes to chromatin decondensation. In addition, release of neutrophil elastase (ELANE) and myeloperoxidase (MPO) from cytoplasmic granules leads to disassembly of cytoskeletal structures and degradation of histones. Activation of NETosis may also occur following accumulation of reactive oxygen species (ROS). The pathological significance and potential therapeutic role of NETosis continues to be studied, but has been associated with host defense against pathogens as well as a number of disease states, including autoimmune diseases, thrombosis, cardiovascular diseases, and tumor progression.

KEY PRODUCTS FOR STUDYING NETOSIS

Myeloperoxidase (E1E7I) XP® Rabbit mAb #14569
Release of neutrophil elastase and myeloperoxidase from cytoplasmic granules leads to disassembly of cytoskeletal structures and degradation of histones.

Neutrophil Elastase (E9C9L) XP® Rabbit mAb #89241
Neutrophils play a significant role in mediating the inflammatory response through the release of neutrophil elastase, which activates pro-inflammatory cytokines and degrades components of the extracellular matrix.

CELL DEATH PATHWAY CROSSTALK

Although different cell death pathways utilize distinct proteins and methods of cellular destruction, crosstalk between the pathways can occur. Both apoptosis and necroptosis can be driven by the activation of TNF receptors. Similarly, RIPK1 can be involved in the progression of either of these forms of cell death. Additionally, the molecular machinery between apoptosis and necroptosis has been shown to be linked in ways that make them mutually exclusive processes that regulate each other. Caspase-8, which is an “initiator” protein during apoptosis, not only cleaves proteins to drive apoptosis forward, but also cleaves RIPK1 and RIPK3 to prevent the progression of necroptosis. Importantly, while caspases are often thought of as markers of apoptosis, caspase-1, -4, and -5 are active during pyroptosis and play an important role in the innate immune system. Caspase-3 is also active during both apoptosis and pyroptosis. This enzyme can cleave gasdermin D to prevent pore formation and the progression of pyroptosis. Alternatively, recent evidence suggests that caspase-3 can also cleave gasdermin E to activate a pore complex, which may allow pyroptosis to progress with variable cleavage machinery.

The complex nature of each pathway along with what we know about crosstalk between these processes highlights how important it is to examine multiple readouts when identifying forms of cell death in experiments. Investigating more than one molecular pathway and cell death marker is imperative to obtain a clear picture of how cells die under different circumstances.
The importance of characterizing different cell death pathways has become clearer as studies elucidate specific cell death mechanisms and identify targeted therapeutics. Loss of apoptosis has long been known to be a hallmark of cancer. Cancer cells evade apoptosis through overexpression of anti-apoptotic proteins, including Bcl-2 and IAP family members, as well as through the suppression of pro-apoptotic signals, such as p53. Small molecule therapies that restore function to mutant p53 or inhibit its interactions with oncogenic proteins have been shown to be effective against tumor cells. Drugs that target and inhibit IAPs, including mimetics of the protein SMAC, have shown promise for their ability to induce apoptosis. Additionally, therapeutic agents, including BH3 mimetics, that inhibit or prevent the expression of anti-apoptotic proteins, have been shown to be effective against lung cancer and lymphoma in clinical trials. These targets continue to be studied to develop new potential therapeutic treatments for cancer.

Forms of necrotic cell death, particularly the programmed processes, have been implicated in the pathology of many diseases, including inflammatory autoimmune diseases, fibrosis, and neurodegenerative-related diseases, such as Alzheimer’s Disease (AD), multiple sclerosis (MS), and Amyotrophic Lateral Sclerosis (ALS). Necroptosis, in particular, has been shown to play a role in neurodegenerative diseases. Research from clinical trials has shown that treatment with Necrostatin-1, a RIPK1 inhibitor, improved cell viability in Alzheimer’s and Parkinson’s diseases. Studies have also identified inhibitors that directly target RIPK3 and MLKL.

Similarly, pyroptosis has been shown to be involved in neurodegenerative and autoimmune diseases, as well as acute tissue injury, such as ischemia-reperfusion damage. Currently, gasdermin D inhibitors are being investigated for their therapeutic potential to treat autoimmune and inflammatory diseases. Also, therapeutic strategies targeting IL-1β and its receptor have been developed for the treatment of arthritis and other inflammatory disorders. However, since these approaches can lead to general immune suppression, strategies to inhibit the inflammasome by targeting NLRs and inflammatory caspases, may yield additional therapeutics.

Ferroptosis inhibitors show potential for the treatment of neurodegenerative diseases as an increase in iron is often associated with the induction of ferroptotic cell death. Conditions, such as stroke, ischemia-reperfusion damage, fibrosis, and inflammatory disease, all display cell death that has been successfully suppressed by ferroptosis inhibitors. Conversely, a ferroptosis inducer, like erastin, may prove to be useful in combating cancer. Tumor cells have already been shown to be susceptible to ferroptosis, so erastin, and compounds like it, may enhance cell death caused by chemotherapeutic agents. The role of ferroptosis in tumor progression and the potential for targeted treatments is a fascinating area of ongoing research.

Importantly, components of many cell death pathways can serve as key biomarkers as well as potential therapeutic targets. Together, protein targets in these cell death pathways offer tremendous promise for translational research and the treatment of multiple diseases.

References
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