CELL DEATH AND AUTOPHAGY — OPINION

Regulated necrosis: the expanding network of non-apoptotic cell death pathways

Tom Vanden Berghe, Andreas Linkermann, Sandrine Jouan-Lanhouet, Henning Walczak and Peter Vandenabeele

Abstract | Cell death research was revitalized by the understanding that necrosis can occur in a highly regulated and genetically controlled manner. Although RIPK1 (receptor-interacting protein kinase 1)- and RIPK3–MLKL (mixed lineage kinase domain-like)-mediated necroptosis is the most understood form of regulated necrosis, other examples of this process are emerging, including cell death mechanisms known as parthanatos, oxytosis, ferroptosis, NETosis, pyronecrosis and pyroptosis. Elucidating how these pathways of regulated necrosis are interconnected at the molecular level should enable this process to be therapeutically targeted.

The development and homeostasis of organisms depends on the balance between cell survival and cell death — simply put, there is no life without death. In 1842, Karl Vogt, while studying the metamorphosis of amphibians, realized that the resorption of the notochord and its replacement by vertebrae involved physiological cell death[1]. The concept of 'programmed cell death' was conceived more than a century later, in 1964, when Lockshin and Williams described regulated cell death during insect metamorphosis[2]. Schweichel and Merker were subsequently the first to report the presence of three distinct cell death morphologies in rat embryos, after exposure to toxins, which also occur with very low frequency in the developing mouse: type I cell death was associated with heterophagy ('eating of another'); type II cell death was associated with autophagy ('eating of itself'); and type III cell death did not involve digestion[3]. Today, these cell death modes are referred to as apoptosis, cell death associated with autophagy and necrosis, respectively. Pioneering work in *Caenorhabditis elegans* revealed the genetic programme of apoptosis[4], whereas its biochemical mechanisms have been elucidated in various animal models[5,6]. We currently understand cell death as a fundamental process that is regulated by multiple interconnected signalling pathways, and we are starting to decipher how cell death influences processes in addition to development, such as chemotaxis, phagocytosis, regeneration and immunogenicity[7].

For two decades, apoptosis was considered to be the standard cell death form during development, homeostasis, infection and pathogenesis[6,8], whereas necrosis was mostly considered to be an 'accidental' cell death that occurred in response to physicochemical insults. Recent genetic evidence[9–16], as well as the discovery of chemical inhibitors of necrosis[10,17,18], have greatly changed this view, and revealed the existence of multiple pathways of regulated necrosis. Regulated necrosis is defined as a genetically controlled cell death process that eventually results in cellular leakage, and it is morphologically characterized by cytoplasmic granulation, as well as organelle and/or cellular swelling ('oncosis'). Multiple modes of cell death share these morphological hallmarks, and they now need to be examined for common or distinct underlying signalling pathways. Attempts to define and classify forms of cell death and their underlying pathways have resulted in multiple neologisms, such as necroptosis, parthanatos, oxytosis, ferroptosis, ETosis, NETosis, pyronecrosis and pyroptosis; all of these processes are characterized by a particular aspect of the cell death process. We propose that these alternative types of cell death are considered as forms of regulated necrosis. In an attempt to integrate the different subroutines of regulated necrosis schematically, we have classified the mechanistic steps of each into four levels (FIG. 1): a trigger (level 1) activates an initiator mechanism (level 2). This subsequently activates several mediators that propagate the signal (level 3) and ultimately relay it to overlapping biochemical mechanisms (executioners) that cause necrotic cell death (level 4). In this Opinion article we first describe necroptosis, the form of regulated necrosis that is best understood at the molecular level, and then consider the emerging modes of regulated necrosis with a focus on levels 1 and 2 in the molecular cascade. We then integrate the signalling pathways of regulated necrosis by mapping the connections and overlaps between them, with the aim of identifying common mediators (level 3) and executioner mechanisms (level 4). Finally, we explore how the expanding network of regulated necrosis pathways could be therapeutically targeted.

Necroptosis: the prototype

Although tumour necrosis factor (TNF)-induced necrotic cell death[19,20] and its negative regulation by caspases[21] have been studied since the late 1980s, the broad interest in regulated necrosis was prompted by the discovery that receptor-interacting protein kinase 1 (RIPK1)[9,16,18,22] and RIPK3 (REFS 12–15) are crucial kinases in TNF-induced regulated necrosis[9]. Since then, an assortment of necroptosis triggers have been identified (BOX 1). This molecular revelation, and the evidence that necrosis is genetically regulated, led to the term necroptosis to define regulated necrosis that is dependent on RIPK1 and/or RIPK3 activity[18,23]. Necrostatin 1 (NEC1) was identified as a potent inhibitor of necroptosis owing to its ability to block the kinase activity of RIPK1 (REFS 16,18).

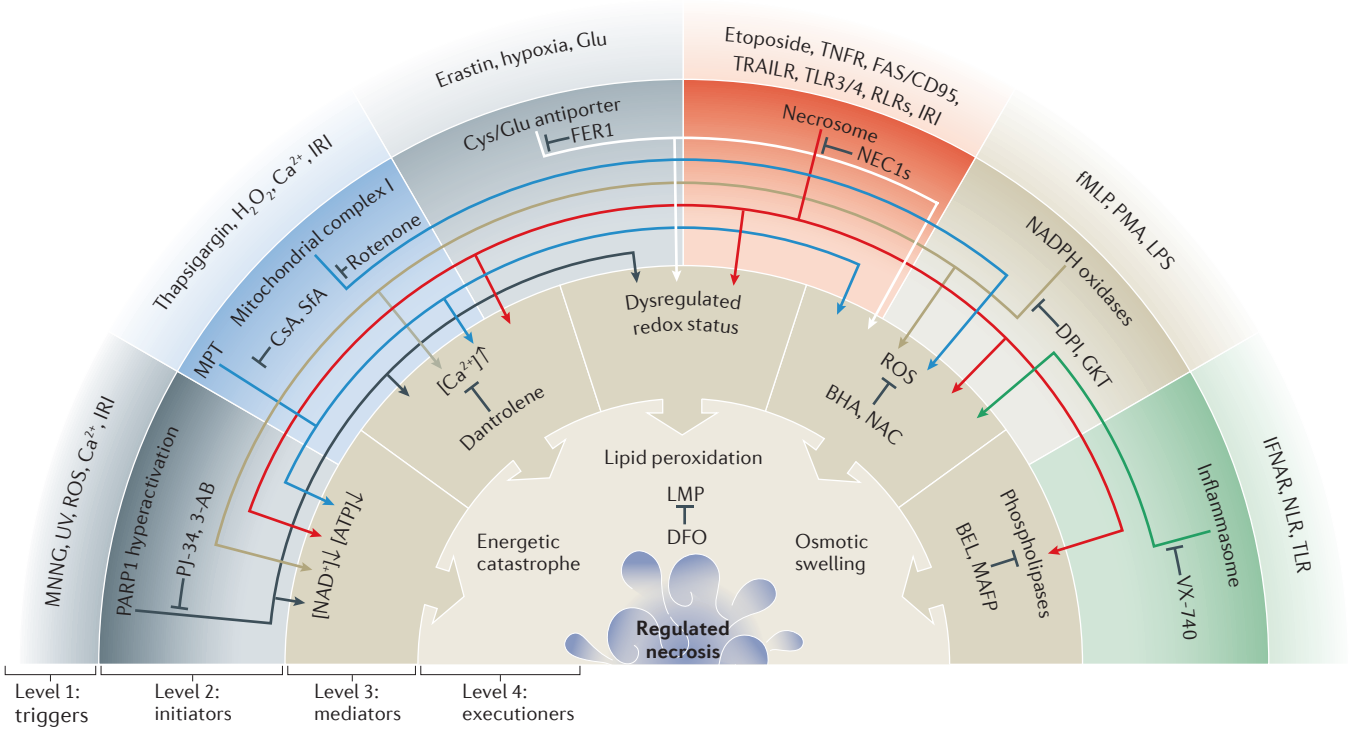


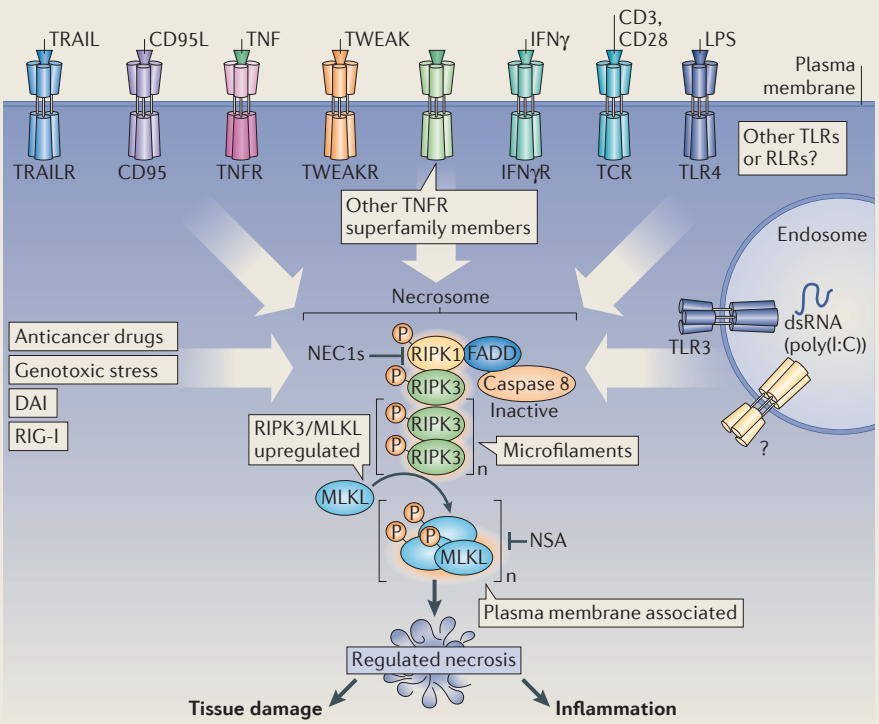
Figure 1 | **An integrated view of the emerging modes of regulated necrosis.** Regulated necrosis can be induced by poly(ADP-ribose) polymerase 1 (PARP1) hyperactivation, mitochondrial permeability transition (MPT), mitochondrial complex I, theCys/Glu antiporter, the necrosome, NADPH oxidases and the inflammasome. Diverse pathophysiological stimuli can trigger (level 1) each of these initiators (level 2), which can be blocked by the listed specific inhibitors. The coloured arrows indicate the established links between the initiator signals and various common intracellular mechanisms that mediate regulated necrosis (level 3), such as NAD^+ and ATP-depletion, Ca^{2+} overload, dysregulation of the redox status, increased production of reactive oxygen species (ROS) and the activity of phospholipases. All of these factors are mediators of regulated necrosis, and even at this level, inhibitors such as dantrolene, BHA (butylated hydroxyl anisole), NAC (*N*-acetyl-Cys), BEL (bromoenol lactone) and MAFP (methyl-arachidonyl fluorophosphonate) may interfere with necrotic signalling. Importantly, similar mediators can act downstream of various initiators, through different mechanisms. The complex interconnected effects of the mediators on cellular organelles and membranes results in the activation of processes that execute regulated necrosis (level 4), including cellular osmotic swelling, bioenergetic breakdown that results in energetic catastrophe, lipid peroxidation and the loss of lysosomal membrane integrity through lysosomal membrane permeabilization (LMP). Note that feedback loops are not included for simplicity. 3-AB, 3-aminobenzamide; CsA, cyclosporin A; DFO, deferoxamine; DPI, diphenylen iodonium; FER1, ferrostatin 1; fMLP, *N*-formylated methionyl-leucyl-Phe; GKT, GKT137831 (an NADPH oxidase 1 and NADPH-oxidase 4 inhibitor; Genkyotex); IFNAR, IFNα/β receptor; IRI, ischaemia–reperfusion injury; LPS, lipopolysaccharide; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; NEC1s, more specific and stable variant of necrostatin 1; NLR, NOD-like receptor; PJ-34, an inhibitor of poly(ADP-ribose) polymerase; PMA, phorbol-12-myristate-13-acetate; RIG-I, retinoic acid-inducible gene 1; RLR, SfA, sanglifehrin A; TLR, Toll-like receptor; TNFR, tumour necrosis factor receptor; TRAILR, TNF-related apoptosis-inducing ligand receptor; UV, ultraviolet light; VX-740, a caspase 1 inhibitor.

The molecular pathway of necroptosis. The induction of necroptosis can be exemplified by the TNF signalling pathway. Upon stimulation of TNFR1 (TNF receptor 1) by TNF, a RIPK1- and TRADD (TNFR1-associated death domain)-dependent receptor-bound complex I is formed, which is pivotal for the activation of NF-κB (nuclear factor-κB) and the resulting upregulation of anti-apoptotic genes such as *A20* and *Flip*$_L$ (FLICE-like inhibitory protein long isoform) (FIG. 2a). In a negative feedback loop, the deubiquitylating activity of A20 is believed to restrict TNF-induced NF-κB signalling by removing Lys63-linked polyubiquitin chains from RIPK1 (REF. 24). Moreover, cylindromatosis (CYLD) also removes polyubiquitin chains from RIPK1, which results in the dissociation of RIPK1 from TNFR1 and the formation of a cytosolic death-inducing signalling complex (DISC) (FIG. 2b). FAS-associated death domain (FADD), RIPK3, FLIPs and pro-caspase 8 are members of this newly identified complex, which is called complex IIa and is dependent on TRADD for its formation[25]. It is believed that FLIP$_L$, which is upregulated by NF-κB, and pro-caspase 8 form heterodimers that cleave and inactivate RIPK1, RIPK3 and CYLD to prevent necroptosis. Pro-caspase 8 homodimers are thought to undergo rapid autoproteolysis that results in caspase 8 activation, and its dissociation from TRADD-dependent complex IIa; caspase 8 then activates the executioner caspases caspase 3 and caspase 7 to execute apoptosis. However, when caspase 8 is inhibited by caspase inhibitors or virally expressed proteins, RIPK1 and RIPK3 associate, autophosphorylate and transphosphorylate each other[12,13,15], and eventually aggregate in microfilament-like complexes[26] that are referred to as necrosomes[11]. Under these caspase-inhibitory conditions, CYLD deubiquitylates RIPK1 in the necrosome to facilitate kinase activation and regulated necrosis[27] (FIG. 2c). The phosphorylation of human RIPK3 at Ser227 or mouse RIPK3 at Ser232 is essential to recruit MLKL (mixed lineage kinase domain-like)[10,28–30]. MLKL is subsequently phosphorylated at Thr357 and Ser358 by human RIPK3 (REF. 10) or at Ser345, Ser347, Ser352 and Thr349 by mouse RIPK3 (REF. 30). This results in a late wave of JUN N-terminal kinase (JNK) activation[31], reactive oxygen species (ROS) production[31,32] and the induction of necroptosis. Mitochondria seem to promote

necroptosis by generating ROS, probably by aiding in the translocation of the necrosome to mitochondria-associated membranes[28]. For example, it was recently proposed that the RIPK1-mediated phosphorylation of STAT3 (signal transducer and activator of transcription 3) induces its interaction with GRIM19 (gene associated with retinoic and interferon-induced mortality 19; a subunit of mitochondrial complex I), which causes STAT3 to translocate to the mitochondria, where it triggers an increase in ROS production and cell death[32] (FIG. 2c). Note that although complex I-mediated ROS formation contributes to the progression of TNF-mediated necroptosis[33], it was recently found that depletion of mitochondria by PARK2 (parkin RBR E3 ubiquitin protein ligase)- and/or CCCP (carbonyl cyanide m-chlorophenylhydrazone)-induced mitophagy activation omits this dependency, which suggests that the requirement for mitochondrial ROS in TNF-zVAD.fmk (*N*-benzlyoxycarbonyl-valyl-alanyl-aspartyl-fluoromethylketone)- and dimerized RIPK3-mediated necroptosis can be bypassed under these conditions[34]. The structures of MLKL[30,35] and of the kinase-like domain of MLKL bound to the kinase domain of RIPK3 were recently solved and clarified some of the activation events[30]. Oligomerized MLKL translocates to the plasma membrane, where it mediates TNF-induced necroptosis in a calcium influx-dependent way[184,185]. Mice deficient in MLKL are viable and display no haematopoietic anomalies or other obvious pathology, but as is the case for RIPK3-deficient mice, they are protected against cerulean-induced acute pancreatitis[35,36], which underscores the crucial role of MLKL in necroptosis.

Importantly, when cIAPs (cellular inhibitor of apoptosis proteins) are degraded (for example, in the presence of SMAC (second mitochondria-derived activator of caspase; also known as DIABLO) mimetics), a slightly different signalling cascade occurs. SMAC mimetics activate the E3 ubiquitin ligase activity of cIAP1 and cIAP2 by binding to their baculovirus IAP repeat (BIR) domains, which causes their autodegradation[37–41]. The degradation of cIAPs reduces canonical NF-κB pathway activation[40,42,43] and facilitates non-canonical NF-κB signalling[38,39,44]. The transition of TNFR1 complex I to RIPK1-dependent complex II (also referred to as the ripoptosome or complex IIb) is increased[25], resulting in non-canonical NF-κB activation and increased cell death (FIG. 2d). Similar to TRADD-dependent complex IIa, RIPK1-dependent complex IIb can induce both necroptosis (FIG. 2c) and apoptosis (FIG. 2e), which is dependent on the absence or presence of caspase 8 activity, respectively. A more detailed description of signalling during necroptosis has been reviewed elsewhere[11,45–48].

Box 1 | Triggers of necroptosis and its links to tissue damage and inflammation



Many triggers of necroptosis have been identified (see the figure). These include TNF (tumour necrosis factor)[9], CD95L (also known as FASL and APO-1L)[9], TRAIL (TNF-related apoptosis-inducing ligand; also known as APO-2L)[9], TWEAK (TNF-related weak inducer of apoptosis)[157], genotoxic stress[158,159], polyclonal stimulation of TCR (T cell receptor)[160], virus-mediated activation of DNA-dependent activator of IFN-regulatory factors (DAI)[50] and anticancer drugs such as shikonin[162,163] and GX15-070 (obatoclax, Cephalon)[164]. Necroptosis can also be triggered by PAMPs (pathogen-associated molecular patterns) such as: polycytidylic acid (poly(I:C))-mediated activation of Toll-like receptor 3 (TLR3)[22,58]; retinoic acid inducible gene I (RIG-I)-like receptors (RLRs), such as RIG-I or MDA5 (melanoma differentiation-associated protein 5)[161]; or LPS (lipopolysaccharide)-mediated activation of TLR4 (REF. 12). Recently, IFNs (interferons) were also shown to induce necroptosis, in a manner dependent on the STAT (signal transducer and activator of transcription)-dependent expression of PKR (protein kinase R), in cells that were transiently deficient in apoptosis owing to phosphorylated FADD (FAS-associated death domain) during mitosis[165]. Considering that PKR is activated by viral RNA during acute virus infections to block translation[166], this could imply that PKR may also act as a platform to induce the lytic cycle via regulated necrosis.

Typically, necroptosis is induced upon caspase 8 inhibition, as was originally observed *in vitro*[21,167] and confirmed in caspase 8-deficient mice[168,169] and FADD-deficient mice[170,171]. Of note, necroptotic damage can be reversed by receptor-interacting protein kinase 3 (RIPK3) deficiency in caspase 8-knockout mice[168,169], and by RIPK3 (REF. 172) or RIPK1 (REF. 171) deficiency in FADD-deficient mice. The pharmacological inhibition of necroptosis — for example, by using necrostatin 1 (NEC1) — or the genetic ablation of RIPK3 or mixed lineage kinase domain-like (MLKL) has shown that necroptosis can also occur in the absence of caspase inhibition in various pathological settings, such as ischaemic or traumatic brain injury[18,173], myocardial infarction[174], retinal ischaemia[175], photoreceptor cell loss[176,177], renal ischaemia–reperfusion injury[178], experimental pancreatitis[15,34], skin necroptosis[179], *Salmonella enterica* infection[180], TNF-induced systemic inflammatory response syndrome[181] and atherosclerosis[182]. Finally, low levels of MLKL were recently found to be associated with decreased overall survival in patients with early-stage resected pancreatic adenocarcinoma[183], which indicates a correlation between chemo-sensitivity and the induction of regulated necrosis.

NSA (necrosulfonamide) only acts on human MLKL and cannot be used to study necroptosis in mouse models. The question mark indicates possible other, still unknown receptors. dsRNA, double-stranded RNA; TNFR, TNF receptor; TWEAKR, TWEAK receptor.

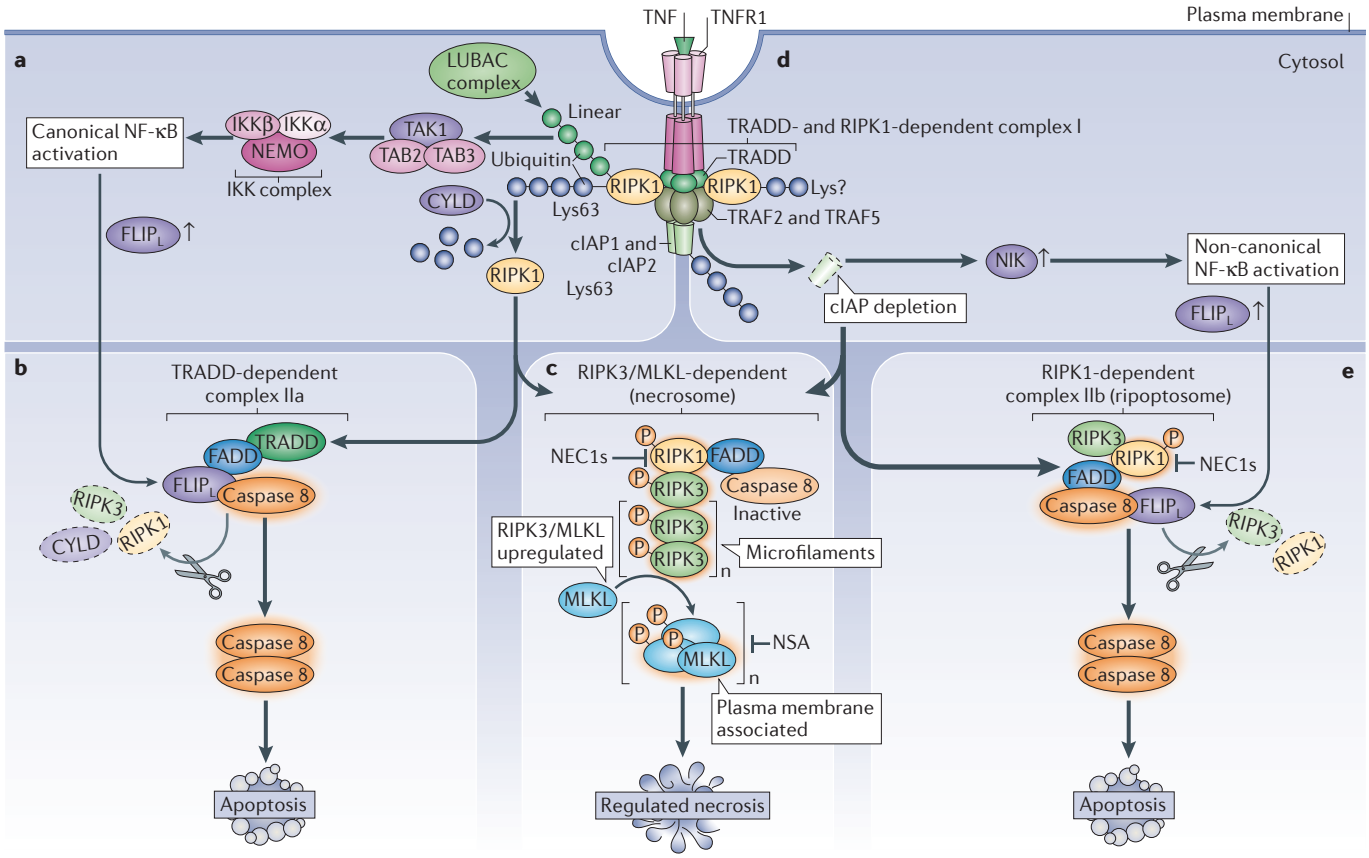


Figure 2 | **TNFR1-mediated necroptosis: the prototype of regulated necrosis.** **a** | Upon stimulation with tumour necrosis factor (TNF), TNF receptor 1 (TNFR1) recruits TNF receptor-associated death domain (TRADD), which in turn attracts receptor-interacting protein kinase 1 (RIPK1), cellular inhibitor of apoptosis protein 1 (cIAP1), cIAP2, TNF receptor-associated factor 2 (TRAF2) and TRAF5. RIPK1 is then subject to Lys63-linked polyubiquitylation by cIAP1 and cIAP2, which allows docking of TAK1 (transforming growth factor-β-activated kinase 1) in complex with TAB2 (TAK1 binding protein 2) or TAB3, as well as of the IKK (inhibitor of NF-κB kinase) complex. The assembly of the IKK complex activates the NF-κB (nuclear factor-κB) pathway, which is enhanced by the recruitment of the LUBAC (linear ubiquitin chain assembly complex) through the linear ubiquitin chains on RIPK1. **b** | Subsequently, cylindromatosis (CYLD) removes Lys63-linked polyubiquitins from RIPK1, rendering complex I unstable and allowing RIPK1 to dissociate from the plasma membrane and to interact withTRADD, FAS-associated death domain (FADD), pro-caspase 8 and FLICE-like inhibitory proteins (FLIPs). The long isoform of FLIP ($FLIP_L$) and pro-caspase 8 form a heterodimeric caspase that cleaves and inactivates RIPK1 and RIPK3, as well as CYLD, to prevent necroptosis (dashed ovals indicate cleaved proteins). This TRADD-dependent complex IIa also allows caspase 8 homodimerization and activation, which activates the executioner caspases caspase 3 and caspase 7, resulting in apoptosis. **c** | However, when caspase 8 is inhibited by chemical caspase inhibitors or virally encoded proteins such as CrmA (cytokine response modifier protein A) or vIRA (viral inhibitor of RIP activation), the RHIM (RIP homotypic interaction motif) domains of RIPK1 and RIPK3 associate in microfilament-like complexes called necrosomes. The auto- and transphosphorylation of RIPK1 and RIPK3 and the recruitment of mixed lineage kinase domain-like (MLKL) initiate necroptosis. Several events could mediate necroptosis, including GRIM19 (gene associated with retinoic and interferon-induced mortality 19)-mediated ROS (reactive oxygen species) production, JUN N-terminal kinase (JNK) activation or the translocation of the necrosome to mitochondria-associated membranes. **d** | When cells are depleted of cIAPs — for example, by SMAC (second mitochondria-derived activator of caspase) mimetics — and RIPK1 is thus not ubiquitylated (indicated by a question mark), the formation of complex I leads to the upregulation of NF-κB-inducing kinase (NIK) and the activation of the non-canonical NF-κB pathway. **e** | Moreover, in the absence of cIAPs, a large TRADD-independent cytosolic complex is formed between RIPK1, RIPK3, FADD and the $FLIP_L$–caspase 8 heterodimer, which is referred to as RIPK1-dependent complex IIb or the 'ripoptosome'. As is the case in complex IIa, RIPK1 and RIPK3 are inactivated through cleavage that is mediated by caspase 8–$FLIP_L$ heterodimers, apoptosis is induced by the release of caspase 8 homodimers and necroptosis is induced when the function or recruitment of caspase 8 is defective. NSA (necrosulfonamide) only acts on human MLKL (**c**). NEC1s, more specific and stable variant of necrostatin 1; NEMO, NF-κB essential modulator.

Necroptosis in pathology. Mice deficient for RIPK3 or MLKL, the central inducers of necroptosis, did not show defects in development or during homeostasis[36,49], which illustrates that necroptosis is not required during these processes. Interestingly, viruses can interfere with necroptosis[50], highlighting its physiological relevance as an antiviral mechanism, as is demonstrated by the fact that RIPK3-deficient mice die after challenge with vaccinia virus[13]. Some viruses, such as cytomegalovirus, have developed a strategy to interfere with this host antiviral response. For example, the viral inhibitor of RIPK1 activation (vIRA) and the viral inhibitor of caspase 8 activation (vICA) prevent the activation of both necroptosis[51] and apoptosis[52], respectively by TNF. In many settings necroptosis dysregulation contributes to tissue damage and inflammation (BOX 1), and has thus been found to contribute to several pathologies. An important milestone in necroptosis research was the discovery of the RIPK1 inhibitor NEC1, which blocks necroptosis and, under certain conditions, also apoptosis[53] (FIG. 2). Therefore, data generated using NEC1 should be interpreted

with caution. In addition, NEC1 targets the immunomodulatory enzyme indoleamine 2,3-dioxygenase (IDO), and this may markedly influence immune responses[54,55]. The use of the NEC-1 analogue NEC1s (also known as 7-Cl-O-NEC1 or NEC1 stable)[18,56], which is more selective for RIPK1 and more stable *in vivo*, or the use of RIPK1 or RIPK3 kinase-dead (catalytically inactive) knock-in mice is required to assess the unique role of RIPK1 and RIPK3 kinase activity in disease models. Recently, another potent NEC1 analogue (NEC21) was reported to show an improved off-target profile, which will be useful in further dissecting the potential clinical relevance of targeting RIPK1 kinase activity[57]. RIPK3 inhibitors[58] or allosteric inhibitors of MLKL[10] might be more specific than NEC1s given that RIPK1 kinase activity can also contribute to apoptosis[53,59], inflammasome activation[60] and interleukin-1α (IL-1α) release[61]. All of these data suggest that the *in vivo* efficacy of RIPK1 inhibition may be because it targets multiple processes beyond necroptosis.

Emerging mechanisms

This section describes the molecular events that initiate emerging pathways of regulated necrosis, including ferroptosis, oxytosis, ETosis, NETosis, cyclophilin D (CYPD)-mediated regulated necrosis, parthanatos, pyroptosis and pyronecrosis, with a focus on the triggers and initiators of these pathways (FIG. 1). As these necrotic-like cell death processes are consistent with the morphological definition of regulated necrosis, but are either reported to occur independently of RIPK1 or RIPK3, or have been shown to occur in the presence of RIPK1 or RIPK3 inhibitors[58], they could be considered as specialized forms of regulated necrosis. Basically, all of these cell death processes represent a form of genetically controlled cellular explosion following a stage of oncosis. The different names for regulated necrosis refer to this process occurring in different physiological conditions, such as cellular stress, pathogen infection or ischaemia–reperfusion injury (IRI), or in particular cell types. These neologisms may therefore only reflect different pathways towards a conserved pool of mediators and executioners of regulated necrosis that results in a similar outcome.

Ferroptosis and oxytosis. It was recently shown that RAS-transformed tumour cells, treated with a lethal small molecule called erastin, undergo regulated necrosis that seems to be partially mediated through inhibiting the system X_c^- Cys/Glu antiporter, which allows the exchange of extracellular L-Cys and intracellular L-Glu across the plasma membrane (FIG. 3a). This type of cell death was defined as ferroptosis because it crucially depends on intracellular iron metabolism and its chemical inhibitor was dubbed ferrostatin 1 (REF. 62), but the mechanism of action for this compound still remains unclear. It is believed that ROS generated by Fenton-type reactions, rather than the mitochondrial electron transport chain, are the main drivers of ferroptosis. Glutathione (GSH) peroxidase 4 (GPX4) is a crucial inhibitor of ferroptosis, and its activity relies on GSH levels. Therefore, GSH depletion typically leads to loss-of-function of GPX4, resulting in ROS-mediated lipid peroxidation[63]. In addition to ferroptosis, Glu-[62] and oxidative stress-induced[64,65] cell death are inhibited by iron chelation. In line with this, iron-dependent neuronal cell death is blocked by metal protein-attenuating compounds (for example, clioquinol) and iron chelators (for example, desferrioxamine), which are being used in clinical trials for treating neurodegenerative diseases[45].

Originally, cell death induced when the system X_c^- Cys/Glu antiporter is inhibited by an excess of the neurotransmitter Glu was classified as oxytosis[66], or excitotoxicity in neuronal cells[67]. Inhibition of the antiporter reduces the level of intracellular L-Cys required for GSH synthesis. This results in GSH depletion, which activates 12-lipoxygenase (LOX12) and LOX15 to initiate mitochondrial ROS production and an increase in cyclic GMP (cGMP)[66]. cGMP opens cGMP-gated channels on the plasma membrane, allowing calcium influx[68] (FIG. 3a). The 'calpain–cathepsin cascade'[69] occurs downstream of this calcium-wave and involves the activation of calpains by calcium, which triggers lysosomal membrane permeabilization (LMP) to induce regulated necrosis; this seems to be conserved from nematodes to primates[70]. Despite a clear mechanistic overlap between oxytosis and ferroptosis, including the dependence on inhibition of the system X_c^- Cys/Glu antiporter, a decrease in GSH levels and the presence of lipid peroxidation, ferroptosis seems to depend mainly on iron instead of calcium signalling[62]. The negative control of lipid peroxidation by GPX4, at least in a neuronal context, was previously shown and supported by the observation that inducible *Gpx4* inactivation in neurons also results in neurodegeneration[71]. In summary, ferroptosis and/or oxytosis are typically observed in the context of cancer and neurodegeneration, which may reflect their high demand for iron and calcium metabolism, respectively.

NETosis and ETosis. Whereas most sources of ROS are generated as metabolic byproducts, the NADPH oxidase (NOX) family constitutes the only known enzyme family with the sole function of producing ROS in physiological settings[72]. The contribution of NOX activity to cell death is especially clear in neurodegeneration[73], brain injury[74] and heart failure[75], conditions in which regulated necrosis has been implicated. Future research is required to determine how NOX-induced regulated necrosis might contribute to other emerging forms of this cell death mode. NOX1-mediated necrosis was shown to contribute to ferroptosis[62], and NOX1 is also recruited to the TNFR1 by riboflavin kinase[60,76] (FIG. 3b) to contribute to necroptosis. Recently, IL-1β release was shown to be dependent on NOX-derived ROS in human neutrophils[77], whereas caspase 1-mediated NOX2 activation was shown to be crucial for phagosome function[78], which illustrates that NOX2 can also be activated during pyroptosis (see below).

The best studied model of NOX-induced cell death is probably NETosis[79], a neutrophil specific form of ETosis. NETosis is a specialized form of neutrophil death that releases neutrophil extracellular traps (NETs)[80]. These are composed of chromatin and histones, and enable neutrophils to immobilize and kill bacteria. As NETosis is also characterized by the controlled release of the intracellular content of neutrophils, it is also a form of regulated necrosis. In contrast to apoptosis, DNA decondenses during NETosis to let the chromatin unfold in the extracellular space upon plasma membrane rupture[81]. Although NOX contributes to NETosis, RIPK1 kinase activity is not implicated in NETosis[81]. In addition to neutrophils, NETosis was described for eosinophils and mast cells[79]. Therefore, the more generalized term 'ETosis' has been introduced[82]. Although the occurrence of NET formation *in vivo* was questioned, Gram-positive bacteria that cause skin infections were recently shown to rapidly induce NETs[83]. In humans, the importance of NOX is illustrated by congenital defects that result in chronic granulomatous disease, which is characterized by aberrant immune responses and recurrent life-threatening infections by a narrow set of microorganisms[84]. Taken together, the evidence suggests that NETosis could be considered as a form of regulated necrosis that is typically observed in neutrophils as an antibacterial immune defence mechanism.

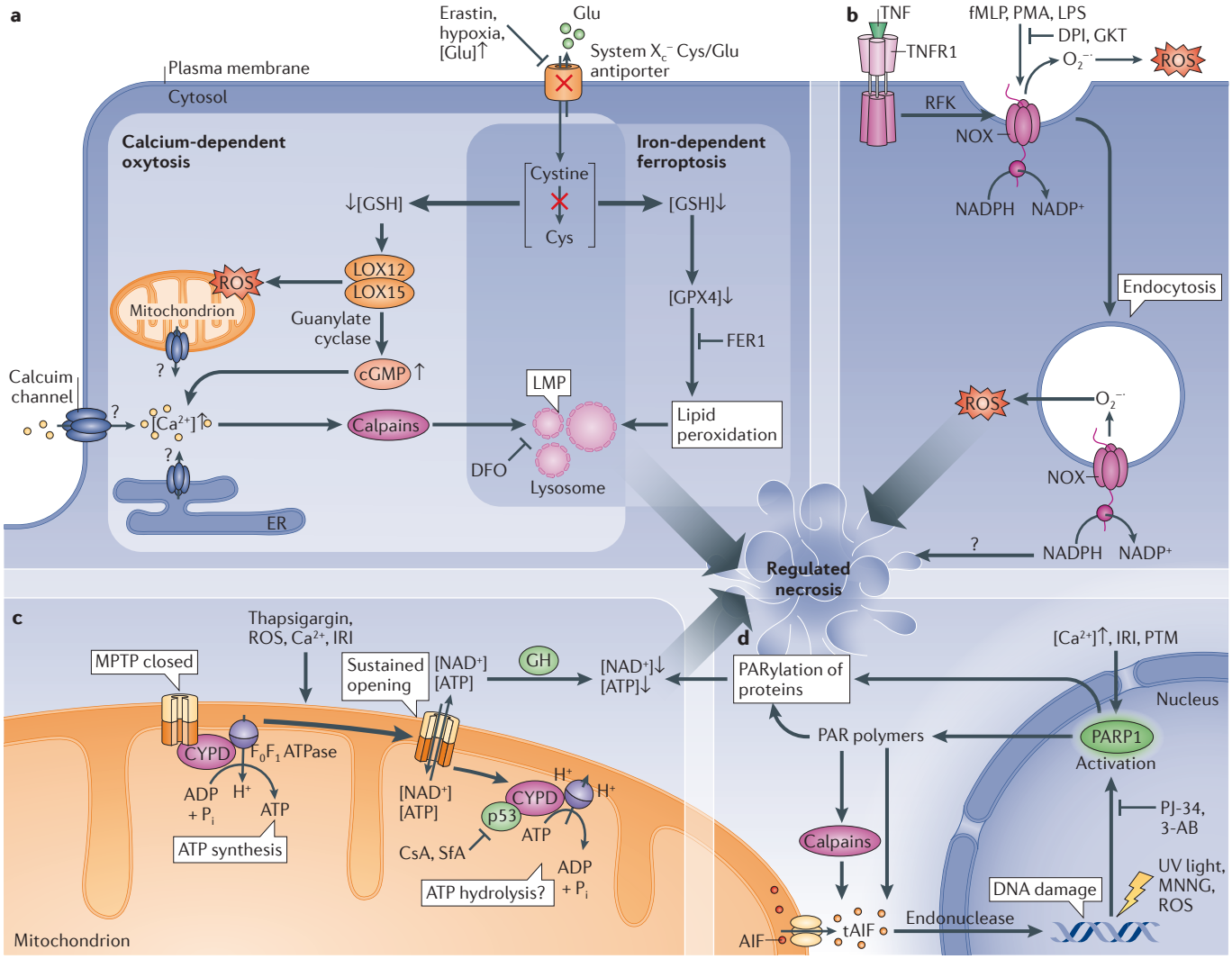


Figure 3 | **Emerging modes of regulated necrosis involving the cellular redox metabolome. a** | The system X_C^- Cys/Glu antiporter mediates the exchange of extracellular L-Cys and intracellular L-Glu across the cell plasma membrane. Inhibition of the antiporter during oxytosis reduces the level of intracellular L-Cys, an amino acid that is required for the synthesis of glutathione (GSH). This results in GSH depletion, which activates 12-lipoxygenase (LOX12) and LOX15 to initiate mitochondrial reactive oxygen species (ROS) production and an increase in cyclic GMP (cGMP). cGMP opens cGMP-gated channels on the plasma membrane, allowing Ca^{2+} influx. Calpains are activated by high intracellular Ca^{2+} levels and facilitate lysosomal membrane permeabilization (LMP), which results in regulated necrosis. The small molecule erastin triggers ferroptosis by inhibiting the system X_C^- Cys/Glu antiporter, which blocks the reduction of cystine to Cys, resulting in GSH depletion-induced loss-of-function of GSH peroxidase 4 (GPX4), high intracellular levels of H_2O_2 and lipid peroxidation. Iron chelators scavenge accumulated iron in lysosomes to inhibit Fenton-type reactions and ferroptosis. Ferrostatin 1 (FER1) is a chemical inhibitor of ferroptosis, but its target is still unknown. **b** | NOX (NADPH oxidase) can be activated by stimuli such as fMLP (*N*-formylated methionyl-leucyl-Phe), PMA (phorbol-12-myristate-13-acetate) and LPS (lipopolysaccharide), which leads to ROS production with NADPH as a cofactor and regulated necrosis as a result. NOX also contributes to ferroptosis and tumour necrosis factor (TNF)-induced necroptosis, the latter as a result of TNF receptor 1 (TNFR1) activating riboflavin kinase (RFK). NOX can be inhibited by diphenylen iodonium (DPI) or GKT (GKT137831; a NOX1 and NOX4 inhibitor; Genkyotex). **c** | The matrix protein cyclophilin D (CYPD) has peptidyl prolyl isomerase activity and is thought to aid the opening of the mitochondrial permeability transition pore (MPTP). This pore opens upon persistent stimulation by thapsigargin, Ca^{2+}, ROS or following ischaemia–reperfusion injury (IRI), which results in the translocation of NAD^+ from the mitochondrial matrix to the cytosol and loss of mitochondrial potential. An inverse activity of F_0F_1 ATPase (ATP hydrolysis) aims to restore the mitochondrial potential. Subsequently, cellular NAD^+ pools are further depleted by NAD^+ glycohydrolases (GH). This, combined with ATP depletion, leads to regulated necrosis. CYPD–MPT-mediated necrosis can be blocked by immunosuppressive drugs such as cyclosporin A (CsA) or sanglifehrin A (SfA). The MPTP may include the F_0F_1 ATPase and might be regulated by p53. **d** | Poly(ADP-ribose) polymerase 1 (PARP1) is activated by DNA damage (induced by UV (ultraviolet) light), ROS or alkylating agents such as MNNG (*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine)), an increase in Ca^{2+} concentration or post-translational modifications (PTMs) of PARP1 (for example, phosphorylation, acetylation and poly(ADP-ribosyl)ation (PARylation)). In MNNG-induced parthanatos, PARP1 hyperactivation causes the accumulation of PAR polymers, which are redistributed to the cytosol and to the mitochondria. In the cytosol these activate calpains, which cleave apoptosis-inducing factor (AIF) at the inner mitochondrial membrane to release the active truncated form (tAIF) into the cytoplasm. PAR polymers can also directly induce AIF release from the outer mitochondrial membrane. It is unknown how tAIF induces regulated necrosis, but an undefined endonuclease seems to cleave DNA to induce PARP1 activation in a positive feedback loop. PAR polymers also PARylate several proteins, which leads to the depletion of NAD^+ and, consequently, ATP. PARP1 inhibitors such as PJ-34 or 3-AB (3-aminobenzamide) prevent PARylation. Question marks indicate unknown pathways or proteins. DFO, deferoxamine; ER, endoplasmic reticulum; P_i, inorganic phosphate.

CYPD-dependent regulated necrosis. Mitochondrial permeability transition (MPT) is characterized by the opening of an outer mitochondrial membrane–inner mitochondrial membrane-spanning channel that is permeable to solutes of 1,500 Da or lower[85]. The molecular composition of the MPT pore (MPTP) is elusive, probably because the MPTP is a highly dynamic entity with a large interactome[86]. One crucial regulator of the MPTP is CYPD[87], which promotes the opening of this channel and is inhibited by cyclosporin A or sanglifehrin A, two drugs with potent immunosuppressive properties and protective effects on IRI[88]. CYPD interacts with the adjacent adenine-nucleotide transporters on its matrix-exposed domain[89] as well as with the lateral stalk of the F_0F_1 ATP synthase[90,91] (FIG. 3c). The fact that CYPD mediates the opening of the MPTP, presumably leading to the translocation of NAD^+ from the mitochondrial matrix to the cytosol, has been concluded from the finding that mitochondria isolated from CYPD-null mice require twice the calcium load for MPTP opening compared with littermates[87,92,93]. Furthermore, CYPD-deficient mouse embryonic fibroblasts are protected from regulated necrosis that is induced by calcium release from the endoplasmic reticulum (ER)[87] and H_2O_2 treatment[92]. CYPD seems to be crucial for MPTP-induced cytotoxicity, as deficiency in CYPD, but not voltage-dependent anion channels or adenine-nucleotide transporters [87,94], exerts neuro-, cardio- and renoprotective effects in mice subjected to ischaemic injury[87,89,92,95]. Preventing the MPTP from opening by pharmacologically inhibiting CYPD might provide a certain degree of protection to patients following myocardial infarction[96]. Thus, CYPD-mediated regulated necrosis is typically observed in the context of IRI-linked pathologies.

Despite these documented protective effects, the inducers of MPT are still poorly characterized but seem to involve activation by ions (including phosphates, H^+, Ca^{2+} and Mg^{2+}), ROS, adenine nucleotides, ubiquinones and other factors[86]. Recently, p53 was proposed to regulate MPT-mediated necrosis[97,98], although the precise mechanism is still under debate[99]. Future work is needed to clarify the importance of MPT in regulated necrosis beyond IRI-linked pathologies. Phenotypic characterization of mice deficient in both CYPD and another emerging factor that induces regulated necrosis could clarify the potential overlap with other regulated necrosis pathways. Recent data have indicated an additive protective effect for mice that are deficient in both CYPD and RIPK3 in renal IRI, an effect that is largely phenocopied by the combined application of NEC1 and sanglifehrin A[100]. These data point to a clear separation of necroptosis from CYPD-mediated regulated necrosis, but emphasize the therapeutic potential of targeting different pathways of regulated necrosis simultaneously in a setting of IRI-linked pathologies.

Parthanatos. Poly(ADP-ribose) polymerase (PARP) proteins are ADP-ribosyl transferase enzymes that transfer ADP-ribose groups from NAD^+ to their targets. By causing poly(ADP-ribosyl)ation (PARylation) of target proteins, PARPs control a wide array of cellular processes[101]. Typically, PARPs such as PARP1 can be activated by DNA breaks induced by ultraviolet light, ROS or alkylating agents[102], the Ca^{2+} signalling pathway or posttranslational modifications such as phosphorylation, acetylation or ADP ribosylation[103] (FIG. 3d). Although PARP activation restores cellular homeostasis, overactivation of PARP1 results in regulated necrosis that has been named parthanatos[104]. Although PARP1 is proteolytically inactivated during apoptosis[105], it seems to be activated in multiple pathways of regulated necrosis, in line with the observation that *Parp1*-knockout mice are resistant to multiple regulated necrosis-linked pathologies[103,106]. The PARylation of proteins is thought to deplete cells of NAD^+ (and consequently ATP) to cause regulated necrosis, and PAR polymers also induce the release of active, truncated apoptosis-inducing factor (tAIF) from the outer mitochondrial membrane, although it is unknown how tAIF induces regulated necrosis[103,107]. PARP inhibitors are in clinical trials to treat cancer by increasing cell death through blocking DNA repair in combination with chemotherapeutic agents[108]. Multiple lines of preclinical evidence suggest that PARP inhibition may also be suitable for the treatment of vascular or neurodegenerative injuries[109].

The mutual linkage or overlap between parthanatos and necroptosis or CYPD-mediated regulated necrosis remains elusive. A role for PARP1 activation in the initiation of TNF signalling was initially observed in L929 fibrosarcoma cells[105], and it was later shown to have a role in promoting TRAIL (TNF-related apoptosis-inducing ligand)-induced necroptosis downstream of RIPK1 and RIPK3 (REF. 110). However, inhibition of PARP function did not affect TNF-induced necroptosis in Jurkat and 3T3 cells[18]. Conversely, the addition of NEC1, or the depletion of RIPK3, failed to block PARP1 activation and cell death induced by MNNG (*N*-methyl-*N*′-nitro-*N*-nitrosoguanidine)[111,112]. Phenotyping PARP1-deficient mice and mice that are deficient in both RIPK3 and PARP1 in response to TNF or DNA damaging agents could help to sharpen our view on the interaction between both modes of regulated necrosis.

Pyroptosis and pyronecrosis. The term pyroptosis was introduced to describe the atypical death of macrophages infected with *Salmonella enterica* subsp. *enterica* serova Typhimurium[113], but this type of cell death is not restricted to bacterial infections[114]. Pyroptosis is thought to result from osmotic pressure generated by the caspase 1-dependent formation of plasma membrane pores that dissipate cellular ionic gradients, and it is characterized by the rapid release of cytosolic contents[115]. A unique feature is the caspase 1-dependent active release of IL-1β and IL-18 from cells, which causes inflammation and fever[116] (FIG. 4a).

Interestingly, in many cases, the innate restriction of bacterial replication is independent of IL-1β and IL-18 (REFS 117–119). Therefore, pyroptosis might eliminate the niche exploited by intracellular pathogens for replication[120,121]. A crucial role for caspase 1 in pyroptosis was deduced from the use of caspase 1-deficient mice, which do not undergo pyroptosis. However, as these mice have recently been shown to also carry an inactivating passenger mutation in the gene encoding caspase 11 (REF. 122), these conclusions need to be revisited. This is especially important given that caspase 11 (often called caspase 4 for its human orthologue) is also involved in pyroptosis[122,123], innate immunity against cytosolic bacteria[123–125] and increasing caspase 1 activation during infection with Gram-negative bacteria[126], which illustrates that both caspase 1 and caspase 11 can initiate pyroptosis. Thus, pyroptosis and NETosis are typically observed in macrophages and neutrophils, respectively, and are part of the antibacterial innate immune defence mechanism. Recently, the *in vivo* relevance of pyroptosis has further been underlined by the demonstration that $CD4^+$ T cells die by pyroptosis upon infection with HIV[186].

In addition to pyroptosis, another necrosis-like cell death process named pyronecrosis has been described in

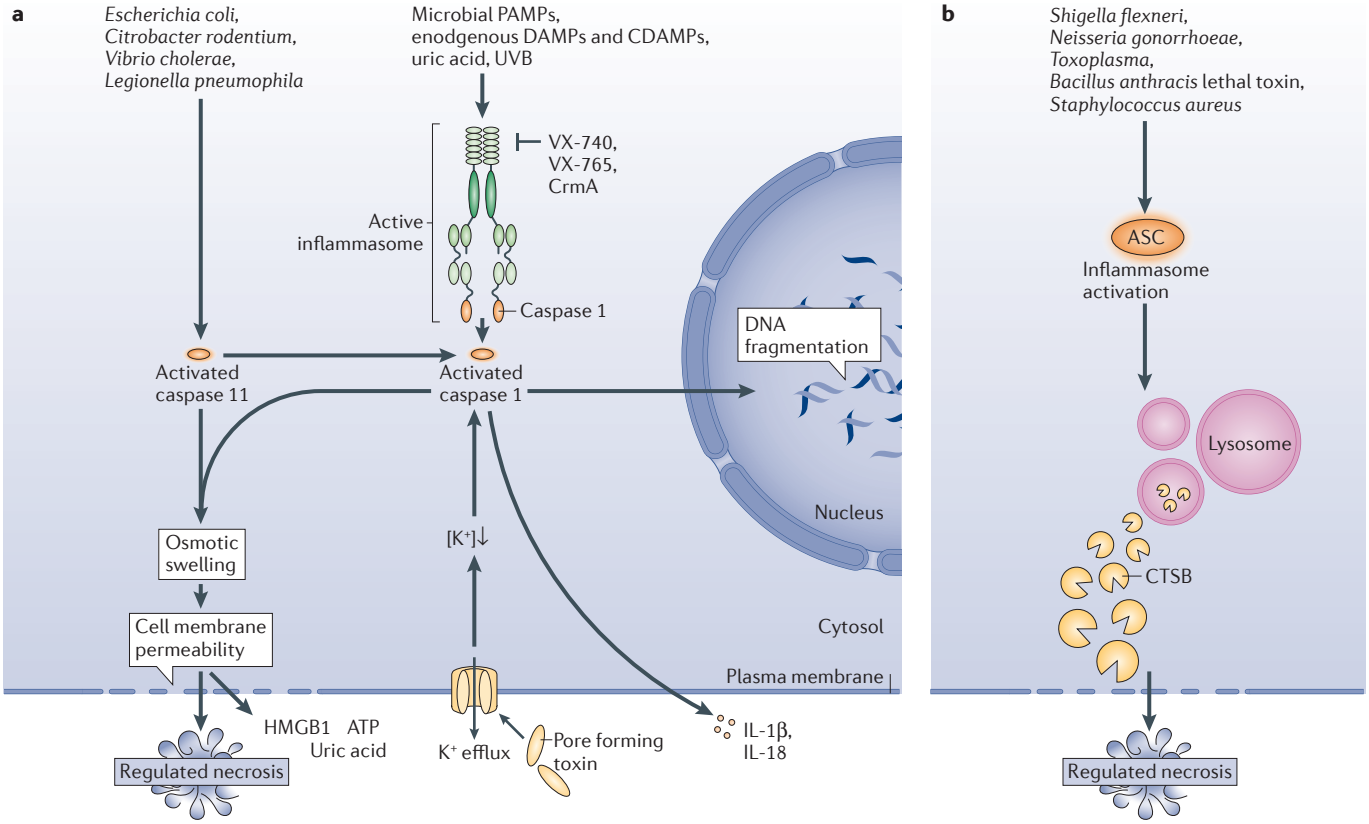


Figure 4 | **Regulated necrosis mediated by inflammasomes. a** | Caspase 1 is typically activated in inflammasome complexes upon exposure to microbial pathogen-associated molecular patterns (PAMPs), endogenous damage-associated molecular patterns (DAMPs), pore forming toxins, uric acid crystals, ultraviolet B (UVB) irradiation or cell death-associated molecular patterns (CDAMPs). Subsequently, caspase 1 activates its two major substrates (interleukin-1β (IL-1β) and IL-18) by cleavage to allow their cellular release and induces DNA fragmentation (through a still unknown mechanism) and osmotic swelling, which results in cell membrane rupture (pyroptosis). Pyroptosis is therefore considered to be the prototype of a highly pro-inflammatory form of regulated necrosis. Caspase 11 is also involved in pyroptosis[122,123], innate immunity against cytosolic bacteria[123,124] and increasing caspase 1 activation during infection with Gram-negative bacteria (*Escherichia coli*, *Citrobacter rodentium*, *Vibrio cholerae* and *Legionella pneumophila*)[126]. Pyroptosis can be inhibited by chemical caspase inhibitors such as VX-740 or with virus-derived proteins such as cytokine response modifier A (CrmA). **b** | Another necrosis-like cell death process, pyronecrosis, has been described in response to *Shigella flexneri*, *Neisseria gonorrhoeae*, *Toxoplasma gondii parasitophorous*, *Bacillus anthracis* lethal toxin and *Staphylococcus aureus*. Pyronecrosis occurs independently of caspase 1, but depends on the inflammasome-adaptor ASC (apoptosis-associated speck-like protein containing a CARD) and the lysosomal protein CTSB (cathepsin B). HMGB1, high mobility group box 1.

response to *Shigella flexneri*. Pyronecrosis is independent of caspase 1 and caspase 11, but dependent on the inflammasome-component ASC (apoptosis-associated speck-like protein containing a CARD) and the lysosomal protein CTSB (cathepsin B), and results in the secretion of the pro-inflammatory mediator HMGB1 (high mobility group box 1) from cells[127] (FIG. 4b). Later, *Neisseria gonorrhoeae*[128], *Toxoplasma gondii parasitophorous*[129], *Bacillus anthracis* lethal toxin[130] and *Staphylococcus aureus*[131] were reported to induce cell death that might be interpreted as pyronecrosis. Systematic studies using conditional knockout models will help to clarify the relative contribution of inflammatory caspases, RIPK1, RIPK3 and CTSB in response to different pathogen-associated molecular patterns in these types of regulated necrosis.

Connections between pathways

As described above, it has been proposed that regulated necrosis can be induced by several molecular pathways. The key question is whether these individual pathways are part of an interacting network that impinges on common execution mechanisms to result in a similar morphology, or whether multiple programmes of regulated necrosis have evolved separately in relation to specific stimuli resulting from processes such as infection and cellular stress. We consider the recurring mechanisms that occur downstream in the pathways of regulated necrosis (FIG. 1, level 2 and level 3) and how these are connected with upstream activators. The evidence suggests that common executioner mechanisms were selected during evolution. The common mechanisms involve redox metabolism and bioenergetics that impinge on physicochemical processes that regulate osmotic swelling, the loss of plasma and lysosomal and mitochondrial membrane integrity.

Role of ATP- and NAD+-depleting pathways. In addition to ATP, pyridine nucleotides such as NAD^+ are energy intermediates that act as signal transducers, in particular in regulated necrosis[132]. Loss of NAD^+ is one of the most detrimental outcomes of MPT[133], as NAD^+ is an essential cofactor in most enzymatic reactions that support mitochondrial function. In the absence of NAD^+, such reactions actively consume ATP in an attempt to maintain electrochemical gradients across the inner mitochondrial membrane[133]. This breaks down the cellular bioenergetic equilibrium and eventually results in regulated necrosis. A prominent

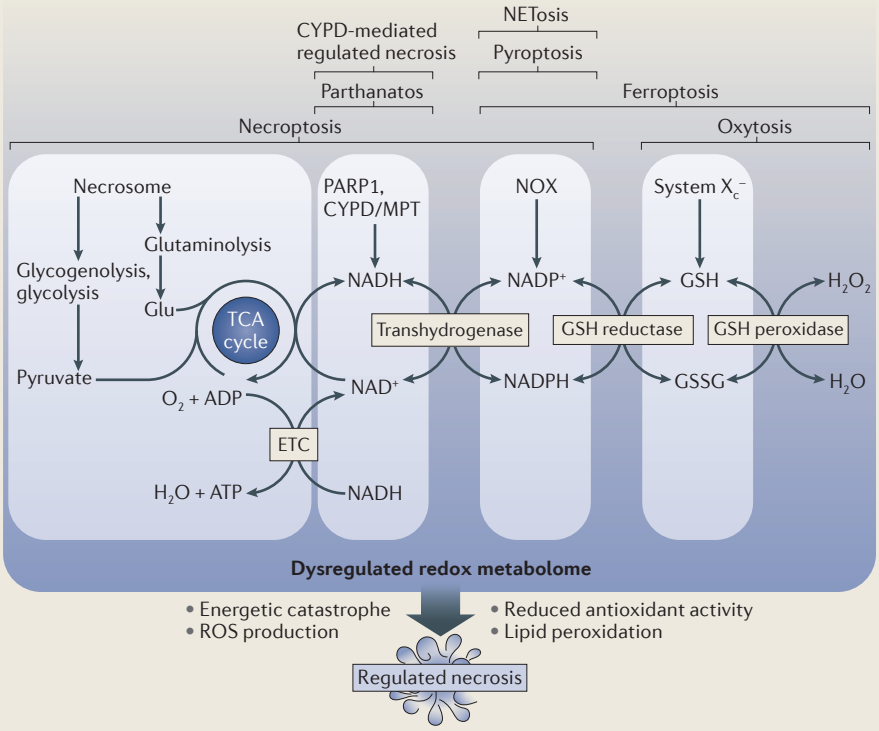
role for NAD^+ catabolism in cell death mechanisms is supported by the observation that *in vivo* models of brain ischaemia, epilepsy and Alzheimer's disease exhibit a decrease in total cellular NAD^+ levels before neuronal cell death[133,134]. The consumption of NAD^+ due to the overactivation of PARP1 (FIG. 3d) was proposed to block glycolysis, resulting in metabolic breakdown. This effect was increased by ATP consumption resulting from ATP-dependent NAD^+ synthesis[107]. Thus, NAD^+ depletion is a central mediator of regulated necrosis, at least in response to IRI and DNA damage. Considering that other emerging forms of regulated necrosis also affect the cellular redox metabolome (BOX 2), it is tempting to speculate that this dysregulation is also causally linked to the progression of regulated necrosis. As bioenergetics are crucial for cellular functioning, imbalances may be sensed by the basic processes that regulate membrane integrity.

Role of Ca^{2+} in regulated necrosis. In neurons, overactivation of Glu receptors allows high Ca^{2+} influx, followed by a second influx of Ca^{2+} from the ER and/or the extracellular space, which leads to regulated necrosis[135] (FIG. 3a). Mitochondrial Ca^{2+} overload and subsequent MPT triggers regulated necrosis[87,92,93]. In primates, excessive Ca^{2+} levels resulting from ischaemia induce the calpain-mediated release of cathepsin from lysosomes, which, in turn, leads to regulated necrosis[69,136]. In addition to contributing to LMP, calpains cleave the plasma membrane Na^+/Ca^{2+} antiporter during brain ischaemia, and their inhibition could prevent Ca^{2+} overload and rescue neurons from excitotoxic death[137]. Finally, the pharmacological inhibitor dantrolene, which prevents Ca^{2+} release from the ER, was previously shown to be protective in a series of *in vivo* models, many of which have been associated with necroptosis. These include cerulein-induced pancreatitis[138], hypoxic neurons[139,140], a transgenic mouse model of Huntington's disease[141], IRI[142,143] and heatstroke-induced regulated necrosis in *C. elegans*[144].

Role of LMP in regulated necrosis. The release of hydrolytic enzymes as result of LMP is detrimental to the cell. Considering the role of LMP in cell death, it is important to determine whether LMP is the cause or consequence of regulated necrosis[65] downstream of mediators such as sphingosines, calpains, phospholipase A_2 and ROS-mediated lipid peroxidation[45]. We therefore do not interpret LMP as an isolated entity of regulated necrosis[23]. On the basis of live cell imaging that illustrates the occurrence of LMP in different cell death modalities[65] and the recurring role of lysosomal biogenesis in regulated necrosis in several model organisms[45,145,146], we consider LMP as a proteolytic amplifier that contributes to the final executioner phase of regulated necrosis (FIG. 1). Nevertheless, some reports point towards an initiator role for LMP in regulated necrosis: for example, in response to lysomotropic agents[147], oxidative stress[65] or toxins such as cobra venum[148], algal yessotoxin[149] and *B. anthracis* lethal toxin[130]. Recently it was found that, during involution, lysosomes in the mammary epithelium undergo widespread LMP, which requires the upregulation of CTSB and CTSL, and the downregulation of their endogenous Ser protease inhibitor A3G (serpin A3G, also known as Spi2A)[150]. Thus, despite the downstream role of LMP in several cell death modalities, LMP ought to be interpreted as a primary inducer of regulated necrosis in certain situations.

Box 2 | Cellular redox metabolome signalling in regulated necrosis

Glycogenolysis, glycolysis and glutaminolysis are increased in necroptosis following receptor-interacting protein kinase 3 (RIPK3) activation[12], which consequently results in increased NADH production (see the figure). This might put pressure on the electron transport chain (ETC), and in particular on NADH dehydrogenase (mitochondrial complex I of ETC), resulting in enhanced reactive oxygen species (ROS) production. Other emerging modes of regulated necrosis also seem to affect the cellular redox metabolome. Indeed, parthanatos and cyclophilin D (CYPD)-mediated regulated necrosis result in a decrease in in NAD^+ and ATP levels. Ferroptosis, NETosis, pyroptosis and necroptosis result in NADPH consumption due to NADPH oxidase (NOX) activation. Both ferroptosis and oxytosis result in decreased levels of glutathione (GSH) owing to inhibition of the system X_C^- Cys/Glu antiporter. Interestingly, these redox couples are all interlinked: for example, NADH and NADPH are interconverted by the nicotinamide nucleotide transhydrogenase, GSSG (oxidized glutathione disulphide) is reduced using NADPH by the GSH reductase, and GSSG is formed by glutathione peroxidase as a result of scavenging H_2O_2. Finally, the dysregulation of the redox metabolome results in reduced antioxidant activity, enhanced ROS production and lipid peroxidation, energetic catastrophe and regulated necrosis.



MPT, mitochondrial permeability transition; PARP1, poly(ADP-ribose) polymerase 1; TCA, tricarboxylic acid.

Conclusions and perspectives

To understand the complexity of life and death, scientists try to simplify their observations in models and label them with neologisms. Unfortunately, this approach is prone to data misinterpretation, and often these labels are superseded by more recent

discoveries. This is illustrated by a retrospective view of the definition of necroptosis. In 2005, necroptosis was defined as regulated necrosis induced by TNF when caspases are inhibited[18]. In 2008, this process was redefined as being dependent on RIPK1 kinase activity[16], but immediately challenged by the finding that the kinase activity of RIPK1 also contributes to apoptosis in particular conditions[53], undermining the restrictive biochemical definition. In 2009, the concerted action between RIPK1 and RIPK3 (as part of the necrosome) was proposed[11]. Later on, RIPK3 was classified as the major initiator of necroptosis, as it can trigger it independently of RIPK1 (REFS 12,151,152). In 2012, MLKL was proposed as the crucial mediator of necroptosis downstream of RIPK3 (REFS 10,31). Now, it has been reported that RIPK3 and MLKL may also trigger inflammasome activation in epithelial and monocytic cells, even in a cell death-independent manner[60,153]. Hence, the pharmacological inhibition of RIPK1 activity or the use of RIPK3- or MLKL-deficient mice is not sufficient to prove that necroptosis is causative for a given phenotype. Another example that illustrates the difficulty of classifying distinct cell death processes is the phenomenon of 'autoschizis'[154], a necrotic cell death process characterized by membrane damage, progressive loss of cytoplasm, nuclear fragmentation, karyolysis and formation of cellular fragments. Autoschizis shares features of both apoptosis (blebbing-like morphology, nuclear fragmentation and DNA degradation) and necrosis (ROS-mediated lipid peroxidation, involvement of cathepsins and plasma membrane permeabilization), and it has been distinguished form the 'standard' form of apoptosis or necrosis[155]. Similarly, autophagy-inducing peptides, such as Tat (transactivating transcriptional activator)–Beclin 1, were shown to induce autophagy-dependent cell death named 'autosis', which is characterized by the rapid shrinkage of the nucleus with a portion of its surface becoming concave, followed by focal plasma membrane rupture and extracellular extrusion of the cytoplasmic contents[156]. This exemplifies the difficulty of labelling cell death processes before they have been fully characterized, and underscores the need to also study the relative contribution of emerging mechanisms of regulated necrosis as well as necroptosis. Moreover, exploring in more detail the contribution of common mediator and executioner mechanisms in differently induced forms of regulated necrosis could validate, challenge and integrate our current view of the different modes of regulated necrosis.

Assuming that the diversity or redundancy in the molecular mechanisms of regulated necrosis is higher at the trigger and initiation levels than at mediator and execution levels (FIG. 1) would imply that regulated necrosis can be inhibited more efficiently downstream. This is exemplified by the need to inhibit both RIPK1 and CYPD, and thus parallel pathways of regulated necrosis, to protect against renal IRI[100], whereas compounds interfering with downstream nodes such as LMP (for example, metal protein-attenuating compounds) or Ca^{2+} signalling (for example, dantrolene) have more potential as a single treatment[45,138–144]. This model suggests three major lines for future research. First, we should experimentally challenge the current convergent nodes in regulated necrosis and try to identify new ones. Second, the contribution of the emerging forms of regulated necrosis must be clearly ascribed and linked to each pathophysiological model, because varying the pathophysiological conditions or cell types involved may result in different forms of regulated necrosis. Third, new inhibitors targeting the emerging forms of regulated necrosis and their convergent downstream signalling nodes should be identified. Other future challenges will include unravelling common cellular disintegration mechanisms in regulated necrosis, studying how regulated necrosis connects to other biological processes, such as immunological responses and regeneration processes, identifying defined biomarkers for selected regulated necrosis pathways, and, most importantly, understanding the contribution of regulated necrosis pathways, and the relationship between them, to cell death-associated diseases.

Finally, we would like to convey the following 'take-home' message: scientists studying the contribution of regulated necrosis in inflammatory and degenerative diseases should take into account the vast knowledge that has been built up on forms of regulated necrosis beyond necroptosis. In the past, the cell death research community has been largely biased towards apoptosis, and consequently apoptosis was supposedly observed everywhere and therefore thought to be responsible for many physiological and pathophysiological situations. However, as we now realize, regulated necrosis may actually be the more prominent mode of cell death, but we should avoid narrowing down regulated necrosis to necroptosis — life and death love variation, redundancy and pleiotropy.

Tom Vanden Berghe, Sandrine Jouan-Lanhouet and Peter Vandenabeele are at the Molecular Signaling and Cell Death Unit, Inflammation Research Center, Flanders Institute for Biotechnology (VIB), Ghent University, 9052 Ghent, Belgium.

Andreas Linkermann is at the Division of Nephrology and Hypertension, Christian-Albrechts-University, 24105 Kiel, Germany.

Henning Walczak is at the Centre for Cell Death, Cancer and Inflammation (CCCI), UCL Cancer Institute, University College London, 72 Huntley Street, London WC1E 6BT, UK.

T.V.B. and A.L. contributed equally to this work.

Correspondence to P.V.
email: Peter.Vandenabeele@irc.vib-ugent.be

doi:10.1038/nrm3737

1. Vogt, C. *Untersuchungen über die Entwicklungsgeschichte der Geburtshelferkröte (Alytes obstetricans)*. (Solothurn: Jent und Gassmann, 1842).
2. Lockshin, R. A. & Williams, C. M. Programmed cell death. II. Endocrine potentiation of the breakdown of the intersegmental muscles of silkmoths. *J. Insect. Physiol.* **10**, 643–649 (1964).
3. Schweichel, J. U. & Merker, H. J. The morphology of various types of cell death in prenatal tissues. *Teratology* **7**, 253–266 (1973).
4. Ellis, H. M. & Horvitz, H. R. Genetic control of programmed cell death in the nematode *C. elegans*. *Cell* **44**, 817–829 (1986).
5. Crawford, E. D. & Wells, J. A. Caspase substrates and cellular remodeling. *Annu. Rev. Biochem.* **80**, 1055–1087 (2011).
6. Suzanne, M. & Steller, H. Shaping organisms with apoptosis. *Cell Death Differ.* **20**, 669–675 (2013).
7. Kaczmarek, A., Vandenabeele, P. & Krysko, D. Necroptosis: the release of damage-associated molecular patterns and its physiological relevance. *Immunity* **38**, 209–223 (2013).
8. Taylor, R., Cullen, S. & Martin, S. Apoptosis: controlled demolition at the cellular level. *Nature Rev. Mol. Cell Biol.* **9**, 231–241 (2008).
9. Holler, N. *et al.* Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. *Nature Immunol.* **1**, 489–495 (2000).
10. Sun, L. *et al.* Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell* **148**, 213–227 (2012).
11. Vandenabeele, P., Galluzzi, L., Vanden Berghe, T. & Kroemer, G. Molecular mechanisms of necroptosis: an ordered cellular explosion. *Nature Rev. Mol. Cell Biol.* **11**, 700–714 (2010).
12. Zhang, D. W. *et al.* RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. *Science* **325**, 332–336 (2009).
13. Cho, Y. S. *et al.* Phosphorylation-driven assembly of the RIP1–RIP3 complex regulates programmed necrosis and virus-induced inflammation. *Cell* **137**, 1112–1123 (2009).
14. Feng, S. *et al.* Cleavage of RIP3 inactivates its caspase-independent apoptosis pathway by removal of kinase domain. *Cell Signal.* **19**, 2056–2067 (2007).
15. He, S. *et al.* Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-α. *Cell* **137**, 1100–1111 (2009).
16. Degterev, A. *et al.* Identification of RIP1 kinase as a specific cellular target of necrostatins. *Nature Chem. Biol.* **4**, 313–321 (2008).
17. Teng, X. *et al.* Structure–activity relationship study of novel necroptosis inhibitors. *Bioorg. Med. Chem. Lett.* **15**, 5039–5044 (2005).
18. Degterev, A. *et al.* Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nature Chem. Biol.* **1**, 112–119 (2005).
19. Laster, S., Wood, J. & Gooding, L. Tumor necrosis factor can induce both apoptic and necrotic forms of cell lysis. *J. Immunol.* **141**, 2629–2634 (1988).
20. Grooten, J., Goossens, V., Vanhaesebroeck, B. & Fiers, W. Cell membrane permeabilization and cellular collapse, followed by loss of dehydrogenase activity: early events in tumour necrosis factor-induced cytotoxicity. *Cytokine* **5**, 546–555 (1993).

21. Vercammen, D. *et al.* Inhibition of caspases increases the sensitivity of L929 cells to necrosis mediated by tumor necrosis factor. *J. Exp. Med.* **187**, 1477–1485 (1998).
22. Kalai, M. *et al.* Tipping the balance between necrosis and apoptosis in human and murine cells treated with interferon and dsRNA. *Cell Death Differ.* **9**, 981–994 (2002).
23. Galluzzi, L. *et al.* Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death Differ.* **19**, 107–120 (2012).
24. Wertz, I. E. *et al.* De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-κB signalling. *Nature* **430**, 694–699 (2004).
25. Wilson, N. S., Dixit, V. & Ashkenazi, A. Death receptor signal transducers: nodes of coordination in immune signaling networks. *Nature Immunol.* **10**, 348–355 (2009).
26. Li, J. *et al.* The RIP1/RIP3 necrosome forms a functional amyloid signaling complex required for programmed necrosis. *Cell* **150**, 339–350 (2012).
27. Moquin, D., McQuade, T. & Chan, F. CYLD deubiquitinates RIP1 in the TNFα-induced necrosome to facilitate kinase activation and programmed necrosis. *PloS ONE* **8**, e76841 (2013).
28. Chen, W. *et al.* Diverse sequence determinants control human and mouse receptor interacting protein 3 (RIP3) and mixed lineage kinase domain-like (MLKL) interaction in necroptotic signaling. *J. Biol. Chem.* **288**, 16247–16261 (2013).
29. McQuade, T., Cho, Y. & Chan, F. positive and negative phosphorylation regulates RIP1 and RIP3-induced programmed necrosis. *Biochem. J.* **456**, 409–415 (2013).
30. Xie, T. *et al.* Structural insights into RIP3-mediated necroptotic signaling. *Cell Rep.* **5**, 70–78 (2013).
31. Zhao, J. *et al.* Mixed lineage kinase domain-like is a key receptor interacting protein 3 downstream component of TNF-induced necrosis. *Proc. Natl Acad. Sci. USA* **109**, 5322–5327 (2012).
32. Shulga, N. & Pastorino, J. GRIM-19-mediated translocation of STAT3 to mitochondria is necessary for TNF-induced necroptosis. *J. Cell Sci.* **125**, 2995–3003 (2012).
33. Schulze-Osthoff, K. *et al.* Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. Evidence for the involvement of mitochondrial radical generation. *J. Biol. Chem.* **267**, 5317–5323 (1992).
34. Tait, Stephen, W. G. *et al.* Widespread mitochondrial depletion via mitophagy does not compromise necroptosis. *Cell Rep.* **5**, 878–885 (2013).
35. Murphy, J. *et al.* The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism. *Immunity* **39**, 443–453 (2013).
36. Wu, J. *et al.* Mlkl knockout mice demonstrate the indispensable role of Mlkl in necroptosis. *Cell Res.* **23**, 994–1006 (2013).
37. Du, C., Fang, M., Li, Y., Li, L. & Wang, X. Smac, a mitochondrial protein that promotes cytochrome *c*-dependent caspase activation by eliminating IAP inhibition. *Cell* **102**, 33–42 (2000).
38. Varfolomeev, E. *et al.* IAP antagonists induce autoubiquitination of c-IAPs, NF-kB activation, and TNF-dependent apoptosis. *Cell* **131**, 669–681 (2007).
39. Vince, J. E. *et al.* IAP antagonists target cIAP1 to induce TNFα-dependent apoptosis. *Cell* **131**, 682–693 (2007).
40. Bertrand, M. J. *et al.* cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination. *Mol. Cell* **30**, 689–700 (2008).
41. Yang, Q.-H. & Du, C. Smac/DIABLO selectively reduces the levels of c-IAP1 and c-IAP2 but not that of XIAP and livin in HeLa cells. *J. Biol. Chem.* **279**, 16963–16970 (2004).
42. Varfolomeev, E. *et al.* c-IAP1 and c-IAP2 are critical mediators of tumor necrosis factor α (TNFα)-induced NF-κB activation. *J. Biol. Chem.* **283**, 24295–24299 (2008).
43. Mahoney, D. J. *et al.* Both cIAP1 and cIAP2 regulate TNFα-mediated NF-κB activation. *Proc. Natl Acad. Sci. USA* **105**, 11778–11783 (2008).
44. Zarnegar, B. J. *et al.* Noncanonical NF-κB activation requires coordinated assembly of a regulatory complex of the adaptors cIAP1, cIAP2, TRAF2 and TRAF3 and the kinase NIK. *Nature Immunol.* **9**, 1371–1378 (2008).
45. Vanlangenakker, N., Vanden Berghe, T., Krysko, D. V., Festjens, N. & Vandenabeele, P. Molecular mechanisms and pathophysiology of necrotic cell death. *Curr. Mol. Med.* **8**, 207–220 (2008).
46. Galluzzi, L. *et al.* Programmed necrosis from molecules to health and disease. *Int. Rev. Cell. Mol. Biol.* **289**, 1–35 (2011).
47. Moriwaki, K. & Chan, F. RIP3: a molecular switch for necrosis and inflammation. *Genes Dev.* **27**, 1640–1649 (2013).
48. Zhou, Z., Han, V. & Han, J. New components of the necroptotic pathway. *Protein Cell* **3**, 811–817 (2012).
49. Newton, K., Sun, X. & Dixit, V. M. Kinase RIP3 is dispensable for normal NF-κBs, signaling by the B-cell and T-cell receptors, tumor necrosis factor receptor 1, and Toll-like receptors 2 and 4. *Mol. Cell Biol.* **24**, 1464–1469 (2004).
50. Kaiser, W., Upton, J. & Mocarski, E. Viral modulation of programmed necrosis. *Curr. Opin. Virol.* **3**, 296–306 (2013).
51. Mack, C., Sickmann, A., Lembo, D. & Brune, W. Inhibition of proinflammatory and innate immune signaling pathways by a cytomegalovirus RIP1-interacting protein. *Proc. Natl Acad. Sci. USA* **105**, 3094–3099 (2008).
52. Skaletskaya, A. *et al.* A cytomegalovirus-encoded inhibitor of apoptosis that suppresses caspase-8 activation. *Proc. Natl Acad. Sci. USA* **98**, 7829–7834 (2001).
53. Wang, L., Du, F. & Wang, X. TNF-α induces two distinct caspase-8 activation pathways. *Cell* **133**, 693–703 (2008).
54. Takahashi, N. *et al.* Necrostatin-1 analogues: critical issues on the specificity, activity and in vivo use in experimental disease models. *Cell Death Dis.* **3**, e437 (2012).
55. Degterev, A., Maki, J. & Yuan, J. Activity and specificity of necrostatin-1, small-molecule inhibitor of RIP1 kinase. *Cell Death Differ.* **20**, 366 (2013).
56. Zheng, W., Degterev, A., Hsu, E., Yuan, J. & Yuan, C. Structure–activity relationship study of a novel necroptosis inhibitor, necrostatin-7. *Bioorg. Med. Chem. Lett.* **18**, 4932–4935 (2008).
57. Wu, Z., Li, Y., Cai, Y., Yuan, J. & Yuan, C. A novel necroptosis inhibitor-necrostatin-21 and its SAR study. *Bioorg. Med. Chem. Lett.* **23**, 4903–4906 (2013).
58. Kaiser, W. *et al.* Toll-like receptor 3-mediated necrosis via TRIF, RIP3 and MLKL. *J. Biol. Chem.* **288**, 31268–31279 (2013).
59. Morgan, M., Kim, Y.-S. & Liu, Z.-G. Membrane-bound Fas ligand requires RIP1 for efficient activation of caspase-8 within the death-inducing signaling complex. *J. Immunol.* **183**, 3278–3284 (2009).
60. Kang, T.-B., Yang, S.-H., Toth, B., Kovalenko, A. & Wallach, D. Caspase-8 blocks kinase RIPK3-mediated activation of the NLRP3 inflammasome. *Immunity* **38**, 27–40 (2013).
61. Lukens, J. *et al.* RIP1-driven autoinflammation targets IL-1α independently of inflammasomes and RIP3. *Nature* **498**, 224–227 (2013).
62. Dixon, S. J. *et al.* Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* **149**, 1060–1072 (2012).
63. Yang, W. S. *et al.* Regulation of ferroptotic cancer cell death by GPX4. *Cell* http://dx.doi.org/10.1016/j.cell.2013.12.010 (2014).
64. Kurz, T., Gustafsson, B. & Brunk, U. T. Intralysosomal iron chelation protects against oxidative stress-induced cellular damage. *FEBS J.* **273**, 3106–3117 (2006).
65. Vanden Berghe, T. *et al.* Necroptosis, necrosis and secondary necrosis converge on similar cellular disintegration features. *Cell Death Differ.* **17**, 922–930 (2010).
66. Tan, S., Schubert, D. & Maher, P. Oxytosis: novel form of programmed cell death. *Curr. Top. Med. Chem.* **1**, 497–506 (2001).
67. Pérez-De La Cruz, V., Carrillo-Mora, P. & Santamaría, A. Quinolinic acid, an endogenous molecule combining excitotoxicity, oxidative stress and other toxic mechanisms. *Int. J. Tryptophan Res.* **5**, 1–8 (2012).
68. Henke, N. *et al.* The plasma membrane channel ORAI1 mediates detrimental calcium influx caused by endogenous oxidative stress. *Cell Death Dis.* **4**, e470 (2013).
69. Yamashima, T. Ca^{2+}-dependent proteases in ischemic neuronal death: a conserved 'calpain–cathepsin cascade' from nematodes to primates. *Cell Calcium* **36**, 285–293 (2004).
70. Syntichaki, P., Xu, K., Driscoll, M. & Tavernarakis, N. Specific aspartyl and calpain proteases are required for neurodegeneration in *C. elegans*. *Nature* **419**, 939–944 (2002).
71. Seiler, A. *et al.* Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. *Cell Metab.* **8**, 237–248 (2008).
72. Kleikers, P. *et al.* NADPH oxidases as a source of oxidative stress and molecular target in ischemia/reperfusion injury. *J. Mol. Med.* **90**, 1391–1406 (2012).
73. Valencia, A. *et al.* Elevated NADPH oxidase activity contributes to oxidative stress and cell death in Huntington's disease. *Hum. Mol. Genet.* **22**, 1112–1131 (2013).
74. Song, S. X. *et al.* Attenuation of brain edema and spatial learning de fi cits by the inhibition of NADPH oxidase activity using apocynin following diffuse traumatic brain injury in rats. *Mol. Med. Rep.* **7**, 327–331 (2013).
75. Zhang, M., Perino, A., Ghigo, A., Hirsch, E. & Shah, A. NADPH oxidases in heart failure: poachers or gamekeepers? *Antioxid. Redox Signal.* **18**, 1024–1041 (2013).
76. Yazdanpanah, B. *et al.* Riboflavin kinase couples TNF receptor 1 to NADPH oxidase. *Nature* **460**, 1159–1163 (2009).
77. Gabelloni, M. L. *et al.* NADPH oxidase derived reactive oxygen species are involved in human neutrophil IL-1β secretion but not in inflammasome activation. *Eur. J. Immunol.* **43**, 3324–3335 (2013).
78. Sokolovska, A. *et al.* Activation of caspase-1 by the NLRP3 inflammasome regulates the NADPH oxidase NOX2 to control phagosome function. *Nature Immunol.* **14**, 543–553 (2013).
79. Remijsen, Q. *et al.* Dying for a cause: NETosis, mechanisms behind an antimicrobial cell death modality. *Cell Death Differ.* **18**, 581–588 (2011).
80. Brinkmann, V. *et al.* Neutrophil extracellular traps kill bacteria. *Science* **303**, 1532–1535 (2004).
81. Remijsen, Q. *et al.* Neutrophil extracellular trap cell death requires both autophagy and superoxide generation. *Cell Res.* **21**, 290–304 (2011).
82. Wartha, F. & Henriques-Normark, B. ETosis: a novel cell death pathway. *Sci. Signal.* **1**, pe25 (2008).
83. Yipp, B. *et al.* Infection-induced NETosis is a dynamic process involving neutrophil multitasking *in vivo*. *Nature Med.* **18**, 1386–1393 (2012).
84. van den Berg, J. *et al.* Chronic granulomatous disease: the European experience. *PloS ONE* **4**, e5234 (2009).
85. Elrod, J. & Molkentin, J. Physiologic functions of cyclophilin d and the mitochondrial permeability transition pore. *Circ. J.* **77**, 1111–1122 (2013).
86. Javadov, S. & Kuznetsov, A. Mitochondrial permeability transition and cell death: the role of cyclophilin D. *Front. Physiol.* **4**, 76 (2013).
87. Baines, C. P. *et al.* Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death. *Nature* **434**, 658–662 (2005).
88. Clarke, S., McStay, G. & Halestrap, A. Sanglifehrin A acts as a potent inhibitor of the mitochondrial permeability transition and reperfusion injury of the heart by binding to cyclophilin-D at a different site from cyclosporin A. *J. Biol. Chem.* **277**, 34793–34799 (2002).
89. Schinzel, A. C. *et al.* Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia. *Proc. Natl Acad. Sci. USA* **102**, 12005–12010 (2005).
90. Bonora, M. *et al.* Role of the c subunit of the FO ATP synthase in mitochondrial permeability transition. *Cell Cycle* **12**, 674–683 (2013).
91. Giorgio, V. *et al.* Dimers of mitochondrial ATP synthase form the permeability transition pore. *Proc. Natl Acad. Sci. USA* **110**, 5887–5892 (2013).
92. Nakagawa, T. *et al.* Cyclophilin D-dependent mitochondrial permeability transition regulates some necrotic but not apoptotic cell death. *Nature* **434**, 652–658 (2005).
93. Basso, E. *et al.* Properties of the permeability transition pore in mitochondria devoid of Cyclophilin D. *J. Biol. Chem.* **280**, 18558–18561 (2005).
94. Kokoszka, J. E. *et al.* The ADP/ATP translocator is not essential for the mitochondrial permeability transition pore. *Nature* **427**, 461–465 (2004).
95. Devalaraja-Narashimha, K., Diener, A. & Padanilam, B. Cyclophilin D gene ablation protects mice from ischemic renal injury. *Am. J. Physiol. Renal Physiol.* **297**, 59 (2009).

96. Piot, C. *et al.* Effect of cyclosporine on reperfusion injury in acute myocardial infarction. *New Engl. J. Med.* **359**, 473–481 (2008).
97. Vaseva, A. *et al.* p53 opens the mitochondrial permeability transition pore to trigger necrosis. *Cell* **149**, 1536–1548 (2012).
98. Baumann, K. Cell death: multitasking p53 promotes necrosis. Nature reviews. *Mol. Cell Biol.* **13**, 480–481 (2012).
99. Karch, J. & Molkentin, J. Is p53 the long-sought molecular trigger for cyclophilin D-regulated mitochondrial permeability transition pore formation and necrosis? *Circul. Res.* **111**, 1258–1260 (2012).
100. Linkermann, A. *et al.* Two independent pathways of regulated necrosis mediate ischemia-reperfusion injury. *Proc. Natl Acad. Sci. USA* **110**, 12024–12029 (2013).
101. Gibson, B. & Kraus, W. New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. *Nature Rev. Mol. Cell Biol.* **13**, 411–424 (2012).
102. Lonskaya, I. *et al.* Regulation of poly(ADP-ribose) polymerase-1 by DNA structure-specific binding. *J. Biol. Chem.* **280**, 17076–17083 (2005).
103. Bürkle, A. & Virág, L. Poly(ADP-ribose): PARadigms and PARadoxes. *Mol. Aspects Med.* **34**, 1046–1065 (2013).
104. Andrabi, S., Dawson, T. & Dawson, V. Mitochondrial and nuclear cross talk in cell death: parthanatos. *Ann. NY Acad. Sci.* **1147**, 233–241 (2008).
105. Los, M. *et al.* Activation and caspase-mediated inhibition of PARP: a molecular switch between fibroblast necrosis and apoptosis in death receptor signaling. *Mol. Biol. Cell* **13**, 978–988 (2002).
106. Simbulan-Rosenthal, C. M. *et al.* Inhibition of poly(ADP-ribose) polymerase activity is insufficient to induce tetraploidy. *Nucleic Acids Res.* **29**, 841–849 (2001).
107. Virag, L., Robaszkiewicz, A., Vargas, J. M. & Javier Oliver, F. Poly(ADP-ribose) signaling in cell death. *Mol. Aspects Med.* **34**, 1153–1167 (2013).
108. Jagtap, P. & Szabó, C. Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. *Nature Rev. Drug Discov.* **4**, 421–440 (2005).
109. Curtin, N. & Szabo, C. Therapeutic applications of PARP inhibitors: anticancer therapy and beyond. *Mol. Aspects Med.* **34**, 1217–56 (2013).
110. Jouan-Lanhouet, S. *et al.* TRAIL induces necroptosis involving RIPK1/RIPK3-dependent PARP-1 activation. *Cell Death Differ.* **19**, 2003–2014 (2012).
111. Sosna, J. *et al.* TNF-induced necroptosis and PARP-1-mediated necrosis represent distinct routes to programmed necrotic cell death. *Cell Mol. Life Sci.* http://dx.doi.org/10.1007/s00018-013-1381-6 (2013)
112. Xu, X. *et al.* The role of PARP activation in glutamate-induced necroptosis in HT-22 cells. *Brain Res.* **1343**, 206–212 (2010).
113. Cookson, B. & Brennan, M. Pro-inflammatory programmed cell death. *Trends Microbiol.* **9**, 113–114 (2001).
114. von Moltke, J., Ayres, J. S., Kofoed, E. M., Chavarria-Smith, J. & Vance, R. E. Recognition of bacteria by inflammasomes. *Annu. Rev. Immunol.* **31**, 73–106 (2013).
115. Fink, S. & Cookson, B. Caspase-1-dependent pore formation during pyroptosis leads to osmotic lysis of infected host macrophages. *Cell. Microbiol.* **8**, 1812–1825 (2006).
116. Brennan, M. & Cookson, B. *Salmonella* induces macrophage death by caspase-1-dependent necrosis. *Mol. Microbiol.* **38**, 31–40 (2000).
117. Miao, E. *et al.* Caspase-1-induced pyroptosis is an innate immune effector mechanism against intracellular bacteria. *Nature Immunol.* **11**, 1136–1142 (2010).
118. Sauer, J.-D. *et al. Listeria monocytogenes* engineered to activate the Nlrc4 inflammasome are severely attenuated and are poor inducers of protective immunity. *Proc. Natl Acad. Sci. USA* **108**, 12419–12424 (2011).
119. Case, C., Shin, S. & Roy, C. Asc and Ipaf inflammasomes direct distinct pathways for caspase-1 activation in response to *Legionella pneumophila*. *Infection Immun.* **77**, 1981–1991 (2009).
120. Zamboni, D. *et al.* The Birc1e cytosolic pattern-recognition receptor contributes to the detection and control of *Legionella pneumophila* infection. *Nature Immunol.* **7**, 318–325 (2006).
121. Derré, I. & Isberg, R. Macrophages from mice with the restrictive *Lgn1* allele exhibit multifactorial resistance to *Legionella pneumophila*. *Infection Immun.* **72**, 6221–6229 (2004).
122. Kayagaki, N. *et al.* Non-canonical inflammasome activation targets caspase-11. *Nature* **479**, 117–121 (2011).
123. Case, C. *et al.* Caspase-11 stimulates rapid flagellin-independent pyroptosis in response to *Legionella pneumophila*. *Proc. Natl Acad. Sci. USA* **110**, 1851–1856 (2013).
124. Aachoui, Y. *et al.* Caspase-11 protects against bacteria that escape the vacuole. *Science* **339**, 975–978 (2013).
125. Hagar, J., Powell, D., Aachoui, Y., Ernst, R. & Miao, E. Cytoplasmic LPS activates caspase-11: implications in TLR4-independent endotoxic shock. *Science* **341**, 1250–1253 (2013).
126. Rathinam, V. *et al.* TRIF licenses caspase-11-dependent NLRP3 inflammasome activation by gram-negative bacteria. *Cell* **150**, 606–619 (2012).
127. Willingham, S. *et al.* Microbial pathogen-induced necrotic cell death mediated by the inflammasome components CIAS1/cryopyrin/NLRP3 and ASC. *Cell Host Microbe* **2**, 147–159 (2007).
128. Duncan, J. *et al. Neisseria gonorrhoeae* activates the proteinase cathepsin B to mediate the signaling activities of the NLRP3 and ASC-containing inflammasome. *J. Immunol.* **182**, 6460–6469 (2009).
129. Zhao, Y., Khaminets, A., Hunn, J. & Howard, J. Disruption of the *Toxoplasma gondii* parasitophorous vacuole by IFNγ-inducible immunity-related GTPases (IRG proteins) triggers necrotic cell death. *PLoS Pathog.* **5**, e1000288 (2009).
130. Averette, K. *et al.* Anthrax lethal toxin induced lysosomal membrane permeabilization and cytosolic cathepsin release is Nlrp1b/Nalp1b-dependent. *PloS ONE* **4**, e7913 (2009).
131. Holzinger, D. *et al. Staphylococcus aureus* Panton-Valentine leukocidin induces an inflammatory response in human phagocytes via the NLRP3 inflammasome. *J. Leukocyte Biol.* **92**, 1069–1081 (2012).
132. Oka, S.-I., Hsu, C.-P. & Sadoshima, J. Regulation of cell survival and death by pyridine nucleotides. *Circul. Res.* **111**, 611–627 (2012).
133. Kristian, T., Balan, I., Schuh, R. & Onken, M. Mitochondrial dysfunction and nicotinamide dinucleotide catabolism as mechanisms of cell death and promising targets for neuroprotection. *J. Neurosci. Res.* **89**, 1946–1955 (2011).
134. Belenky, P., Bogan, K. & Brenner, C. NAD^+ metabolism in health and disease. *Trends Biochem. Sci.* **32**, 12–19 (2007).
135. Sattler, R. & Tymianski, M. Molecular mechanisms of glutamate receptor-mediated excitotoxic neuronal cell death. *Mol. Neurobiol.* **24**, 107–129 (2001).
136. Yoshida, M. *et al.* Primate neurons show different vulnerability to transient ischemia and response to cathepsin inhibition. *Acta Neuropathol.* **104**, 267–272 (2002).
137. Bano, D. *et al.* Cleavage of the plasma membrane Na^+/Ca^{2+} exchanger in excitotoxicity. *Cell* **120**, 275–285 (2005).
138. Orabi, A. *et al.* Dantrolene mitigates caerulein-induced pancreatitis *in vivo* in mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* **299**, G196–G204 (2010).
139. Staats, K. *et al.* Dantrolene is neuroprotective *in vitro*, but does not affect survival in $SOD1^{G93A}$ mice. *Neuroscience* **220**, 26–31 (2012).
140. Mattson, M., Zhu, H., Yu, J. & Kindy, M. Presenilin-1 mutation increases neuronal vulnerability to focal ischemia *in vivo* and to hypoxia and glucose deprivation in cell culture: involvement of perturbed calcium homeostasis. *J. Neurosci.* **20**, 1358–1364 (2000).
141. Chen, X. *et al.* Dantrolene is neuroprotective in Huntington's disease transgenic mouse model. *Mol. Neurodegener.* **6**, 81 (2011).
142. Yu, G., Zucchi, R., Ronca-Testoni, S. & Ronca, G. Protection of ischemic rat heart by dantrolene, an antagonist of the sarcoplasmic reticulum calcium release channel. *Bas. Res. Cardiol* **95**, 137–143 (2000).
143. Javadov, S. A. *et al.* Protection of hearts from reperfusion injury by propofol is associated with inhibition of the mitochondrial permeability transition. *Cardiovasc. Res.* **45**, 360–369 (2000).
144. Kourtis, N., Nikoletopoulou, V. & Tavernarakis, N. Small heat-shock proteins protect from heat-stroke-associated neurodegeneration. *Nature* **490**, 213–218 (2012).
145. Artal-Sanz, M., Samara, C., Syntichaki, P. & Tavernarakis, N. Lysosomal biogenesis and function is critical for necrotic cell death in *Caenorhabditis elegans*. *J. Cell Biol.* **173**, 231–239 (2006).
146. Aits, S. & Jäättelä, M. Lysosomal cell death at a glance. *J. Cell Sci.* **126**, 1905–1912 (2013).
147. Kagedal, K., Zhao, M., Svensson, I. & Brunk, U. T. Sphingosine-induced apoptosis is dependent on lysosomal proteases. *Biochem. J.* **359**, 335–343 (2001).
148. Feofanov, A. *et al.* Cancer cell injury by cytotoxins from cobra venom is mediated through lysosomal damage. *Biochem. J.* **390**, 11–18 (2005).
149. Malagoli, D., Marchesini, E. & Ottaviani, E. Lysosomes as the target of yessotoxin in invertebrate and vertebrate cell lines. *Toxicol. Lett.* **167**, 75–83 (2006).
150. Kreuzaler, P. *et al.* Stat3 controls lysosomal-mediated cell death *in vivo*. *Nature Cell Biol.* **13**, 303–309 (2011).
151. Vanlangenakker, N., Bertrand, M., Bogaert, P., Vandenabeele, P. & Vanden Berghe, T. TNF-induced necroptosis in L929 cells is tightly regulated by multiple TNFR1 complex I and II members. *Cell Death Dis.* **2**, e230 (2011).
152. Upton, J., Kaiser, W. & Mocarski, E. Virus inhibition of RIP3-dependent necrosis. *Cell Host Microbe* **7**, 302–313 (2010).
153. Vince, J. *et al.* Inhibitor of apoptosis proteins limit RIP3 kinase-dependent interleukin-1 activation. *Immunity* **36**, 215–227 (2012).
154. Jamison, J., Gilloteaux, J., Taper, H., Calderon, P. & Summers, J. Autoschizis: a novel cell death. *Biochem. Pharmacol.* **63**, 1773–1783 (2002).
155. Gilloteaux, J. *et al.* Cell damage and death by autoschizis in human bladder (RT4) carcinoma cells resulting from treatment with ascorbate and menadione. *Ultrastruct. Pathol.* **34**, 140–160 (2010).
156. Liu, Y. *et al.* Autosis is a Na^+,K^+-ATPase-regulated form of cell death triggered by autophagy-inducing peptides, starvation, and hypoxia-ischemia. *Proc. Natl Acad. Sci. USA* http://dx.doi.org/10.1073/pnas.1319661110 (2013).
157. Wilson, C. & Browning, J. Death of HT29 adenocarcinoma cells induced by TNF family receptor activation is caspase-independent and displays features of both apoptosis and necrosis. *Cell Death Differ.* **9**, 1321–1333 (2002).
158. Tenev, T. *et al.* The ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs. *Mol. Cell* **43**, 432–448 (2011).
159. Feoktistova, M. *et al.* cIAPs block ripoptosome formation, a RIP1/caspase-8 containing intracellular cell death complex differentially regulated by cFLIP isoforms. *Mol. Cell* **43**, 449–463 (2011).
160. Ch'en, I. L. *et al.* Antigen-mediated T cell expansion regulated by parallel pathways of death. *Proc. Natl Acad. Sci. USA* **105**, 17463–17468 (2008).
161. Zou, J. *et al.* Poly IC triggers a cathepsin D- and IPS-1-dependent pathway to enhance cytokine production and mediate dendritic cell necroptosis. *Immunity* **38**, 717–728 (2013).
162. Han, W. *et al.* Shikonin circumvents cancer drug resistance by induction of a necroptotic death. *Mol. Cancer Ther.* **6**, 1641–1649 (2007).
163. Huang, C. *et al.* Shikonin kills glioma cells through necroptosis mediated by RIP-1. *PLoS ONE* **8**, e66326 (2013).
164. Basit, F., Cristofanon, S. & Fulda, S. Obatoclax (GX15-070) triggers necroptosis by promoting the assembly of the necrosome on autophagosomal membranes. *Cell Death Differ.* **20**, 1161–1173 (2013).
165. Thapa, R. *et al.* Interferon-induced RIP1/RIP3-mediated necrosis requires PKR and is licensed by FADD and caspases. *Proc. Natl Acad. Sci. USA* **110**, 18 (2013).
166. Williams, B. PKR; a sentinel kinase for cellular stress. *Oncogene* **18**, 6112–6120 (1999).
167. Matsumura, H. *et al.* Necrotic death pathway in fas receptor signaling. *J. Cell Biol.* **151**, 1247–1256 (2000).
168. Kaiser, W. J. *et al.* RIP3 mediates the embryonic lethality of caspase-8-deficient mice. *Nature* **471**, 368–372 (2011).
169. Oberst, A. *et al.* Catalytic activity of the caspase-8–$FLIP_L$ complex inhibits RIPK3-dependent necrosis. *Nature* **471**, 363–367 (2011).
170. Welz, P. S. *et al.* FADD prevents RIP3-mediated epithelial cell necrosis and chronic intestinal inflammation. *Nature* **477**, 330–334 (2011).
171. Zhang, H. *et al.* Functional complementation between FADD and RIP1 in embryos and lymphocytes. *Nature* **471**, 373–376 (2011).

172. Dillon, C. *et al.* Survival function of the FADD–CASPASE-8–cFLIP$_L$ complex. *Cell Rep.* **1**, 401–407 (2012).
173. You, Z. *et al.* Necrostatin-1 reduces histopathology and improves functional outcome after controlled cortical impact in mice. *J. Cereb. Blood Flow Metab.* **28**, 1564–1573 (2008).
174. Lim, S. Y., Davidson, S. M., Mocanu, M. M., Yellon, D. M. & Smith, C. C. The cardioprotective effect of necrostatin requires the cyclophilin-D component of the mitochondrial permeability transition pore. *Cardiovasc. Drugs Ther.* **21**, 467–469 (2007).
175. Rosenbaum, D. M. *et al.* Necroptosis, a novel form of caspase-independent cell death, contributes to neuronal damage in a retinal ischemia-reperfusion injury model. *J. Neurosci. Res.* **88**, 1569–1576 (2010).
176. Trichonas, G. *et al.* Receptor interacting protein kinases mediate retinal detachment-induced photoreceptor necrosis and compensate for inhibition of apoptosis. *Proc. Natl Acad. Sci. USA* **107**, 21695–21700 (2010).
177. Murakami, Y. *et al.* Receptor interacting protein kinase mediates necrotic cone but not rod cell death in a mouse model of inherited degeneration. *Proc. Natl Acad. Sci. USA* **109**, 14598–14603 (2012).
178. Linkermann, A. *et al.* Rip1 (receptor-interacting protein kinase 1) mediates necroptosis and contributes to renal ischemia/reperfusion injury. *Kidney Int.* **81**, 751–761 (2012).
179. Bonnet, M. C. *et al.* The adaptor protein FADD protects epidermal keratinocytes from necroptosis *in vivo* and prevents skin inflammation. *Immunity* **35**, 572–582 (2011).
180. Robinson, N. *et al.* Type I interferon induces necroptosis in macrophages during infection with *Salmonella enterica* serovar Typhimurium. *Nature Immunol.* **13**, 954–962 (2012).
181. Duprez, L. *et al.* RIP kinase-dependent necrosis drives lethal systemic inflammatory response syndrome. *Immunity* **35**, 908–918 (2011).
182. Lin, J. *et al.* A role of RIP3-mediated macrophage necrosis in atherosclerosis development. *Cell Rep.* **3**, 200–210 (2013).
183. Colbert, L. *et al.* Pronecrotic mixed lineage kinase domain-like protein expression is a prognostic biomarker in patients with early-stage resected pancreatic adenocarcinoma. *Cancer* **119**, 3148–3155 (2013).
184. Cai, Z. *et al.* Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis. *Nature Cell Biol.* **16**, 55–65 (2014).
185. Chen, X. *et al*. Translocation of mixed lineage kinase domain-like protein to plasma membrane leads to necrotic cell death. *Cell Research* **24**,105–121 (2014).
186. Doitsh, G. *et al*. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature* http://dx.doi.org/10.1038/nature12940 (2013).

Acknowledgements
The authors apologize to colleagues whose work was not discussed in this Opinion owing to space limitations. Research in the group of P.V. is supported by Belgian grants (Interuniversity Attraction Poles, IAP 7/32), Flemish grants (Research Foundation Flanders (FWO) G.0875.11, FWO G.0973.11, FWO G.0A45.12N, FWO G.0787.13N, Methusalem grant - BOF09/01M00709), Ghent University grants (MRP, GROUP-ID consortium, Belgium), a grant from the Foundation against Cancer, F94 and grants from the Flanders Institute for Biotechnology (VIB). H.W. is funded by a Cancer Research UK programme grant, an European Research Council (ERC) Advanced Grant and a Wellcome Trust Senior Investigator Award, UK. T.V.B. holds a postdoctoral fellowship from the FWO. A.L. is funded by the German Society for Nephrology, Novartis, Pfizer, Fresenius and the Hans-Werner Jackstädt Stiftung. S.J.L. is an omics@vib postdoctoral fellow at the VIB, co-funded by the Marie Curie COFUND initiative.

Competing interests statement
The authors declare competing interests: see Web version for details.