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Review

Crosstalk between apoptosis, necrosis and autophagy ☆

Vassiliki Nikoletopoulou, Maria Markaki, Konstantinos Palikaras, Nektarios Tavernarakis *

Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion 71110, Crete, Greece

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ABSTRACT

Apoptosis and necrosis are the two major modes of cell death, the molecular mechanisms of which have been extensively studied. Although initially thought to constitute mutually exclusive cellular states, recent findings reveal cellular contexts that require a balanced interplay between these two modes of cellular demise. Several death initiator and effector molecules, signaling pathways and subcellular sites have been identified as key mediators in both processes, either by constituting common modules or alternatively by functioning as a switch allowing cells to decide which route to take, depending on the specific situation. Importantly, autophagy, which is a predominantly cytoprotective process, has been linked to both types of cell death, serving either a pro-survival or pro-death function. Here we review the recent literature that highlights the intricate interplay between apoptosis, necrosis and autophagy, focusing on the relevance and impact of this crosstalk in normal development and in pathology. This article is part of a Special Section entitled: Cell Death Pathways. Guest Editors: Frank Madeo and Slaven Stekovic.

1. Introduction

Distinct modes of cell death were for a long time studied in isolation, as the prevailing model suggested that they represented mutually exclusive cellular states. However, the past decade has witnessed a steady accumulation of findings suggesting that apoptosis, necrosis and autophagy are often regulated by similar pathways, engage common sub-cellular sites and organelles, and even share initiator and effector molecules. Depending on the cellular context and death trigger, the two main modes of cell death often co-operate in a balanced interplay that involves autophagy, or they are employed by cells in a complementary fashion to facilitate cellular destruction.

Apoptosis has been intensively studied in the past two decades and is widely appreciated as a major mechanism of regulated death, employed not only upon cell damage or stress, but also during normal development and morphogenesis. For example, the peripheral nervous system of vertebrates is shaped by the apoptotic death of almost half of the new born peripheral neurons during development in order to regulate their number such that it matches to the need of their target tissues in the periphery [1]. Apoptosis may be triggered either by extrinsic stimuli through cell surface death receptors, such as TNFα (tumor necrosis factor-α), Fas (CD95/APO1) and TRAIL (TNF related apoptosis inducing ligand) receptors or by intrinsic stimuli via the mitochondrial signaling pathway [2,3]. In either case, activation of cysteine aspartyl proteases, called caspases, results in mitochondrial membrane permeabilization, chromatin condensation and DNA fragmentation, thereby leading to the destruction of the cell [4]. These events bestow the apoptotic cell a distinct and characteristic morphology (Fig. 1c) that includes the rounding up of the cell so that it appears pyknotic, the condensation of chromatin, the fragmentation of the nucleus and the shedding of apoptotic bodies, vacuoles containing cytoplasm and intact organelles.

Apoptosis has been classically contrasted to pathological necrosis, which for a long time was thought to represent a diametrically "opposite" mode of unordered and passive cellular explosion in response to acute and overwhelming trauma. Morphologically, necrotic cells are characterized by the swelling of organelles, such as the endoplasmic

Abbreviations: AIF, Apoptosis Inducing Factor; AMPK, Adenosine Monophosphate activated Kinase; APAF1, Apoptotic Protease Activating Factor 1; BCL-2, B-cell lymphoma 2; BCL-X_L, B-cell lymphoma extra large; BEC1, Beclin-1; BH, Bcl-2 homology; cIAP1, cellular inhibitor of apoptosis 1; cIAP2, cellular inhibitor of apoptosis 2; DAPK, death associated protein kinase; DRAM, Damage-Regulated Autophagy Modulator; FADD, FAS Associated Death Domain; FLICE, caspase 8; FLIP, FLICE-Like Inhibitory Protein; FOXO1, Forkhead Box Protein O1; HDGF, Hepatoma Derived Growth Factor; HMGB1, High Mobility Group protein B1; IGF1, Insulin Growth Factor 1; IL1β, interleukin 1β; LKB1, Liver Kinase B1; MEFs, mouse embryonic fibroblasts; MNNG, N-methyl-N0-nitrosoguanidine; MOMP, mitochondrial outer membrane permeabilization; mTOR, mammalian target of rapamycin; NEMO, NF-kB essential modulator; NF-kB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NLRP3, NOD-like receptor family pyrin domain containing 3; PAMP, Pathogen Associated Molecular Patterns; PARP1, Poly(ADP ribose) Polymerase 1; PCD, programmed cell death; PI3K, Phosphatidyl Inositol 3 Kinase; PRR, pathogen recognition receptor; PTP, permeability transition pore; PUMA, p53-Upregulated Modulator of Apoptosis; RIP1, Receptor Interacting Protein 1; RIP3, Receptor Interacting Protein 3; SIRT2, Sirtuin 2; tAIF, truncated Apoptosis Inducing Factor; TNFα, Tumor Necrosis Factor alpha; TNFR1, TNFα Receptor 1; TNFR2, TNFα receptor 2; TRADD, TNFR Associated Death Domain; TRAF2, TNFR Associated Factor 2; TRAF5, TNFR Associated Factor 5; TRAIL, TNF related apoptosis inducing ligand; TRAILR1, TRAIL Receptor 1; TRAILR2, TRAIL Receptor 2

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* Corresponding author at: Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, N. Plastira 100, Vassilika Vouton, P.O. Box 1385, Heraklion 70013, Crete, Greece. Tel.: +30 2810 391066; fax: +30 2810 391067.
E-mail address: tavernarakis@imbb.forth.gr (N. Tavernarakis).

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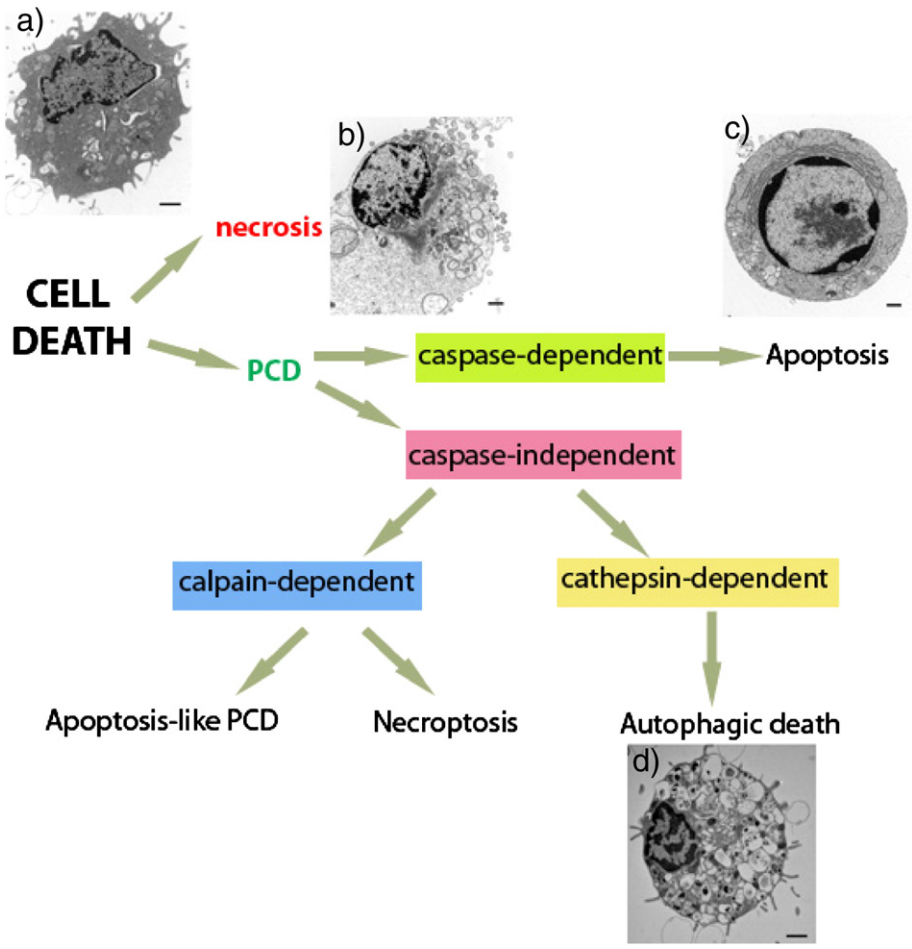

Fig. 1. Types of cell death and their morphological hallmarks. Diagrammatic classification of different types of cell death. PCD: programmed cell death. Morphological features of a) a healthy cell, b) a necrotic cell, c) an apoptotic cell and d) an autophagic cell. (Electron micrograph pictures adapted from ref. [150]. Scale bar: 1 mm.)

reticulum and mitochondria, the rupture of the plasma membrane and the lysis of the cell [5,6], while, unlike in apoptosis, the nucleus becomes distended and remains largely intact (Fig. 1b). Necrotic death is typically followed by inflammatory reactions [7]. Necrotic cells selectively release factors like HMGB1 and HDGF to evoke an inflammatory response [8] and are sensed by NLRP3, a core protein of the inflammasome, resulting in inflammasome activation and the subsequent release of the pro-inflammatory cytokine IL1β. NLRP3 inflammasome activation is triggered mainly through ATP produced by mitochondria released from damaged cells [9]. Mechanistically, necrosis is typically not associated with activation of caspases, and it is thought that it mediates cell demise in response to damage, or in pathology [10,11], but not during normal development. Despite this, it turns out that a programmed form of necrotic death (termed necroptosis) is very common in vivo, not only in physical traumas, but mainly in diverse forms of neurodegeneration, and death inflicted by ischemia or infection. In addition, progress in the field has revealed that unlike unordered necrosis, this more physiological and programmed type of necroptotic death shares several key processes with apoptosis, as discussed later.

A cellular process that has been involved in both main types of cell death mentioned above is macroautophagy (hereafter referred to as autophagy), a self-cannibalization mechanism that involves the engulfment of cytoplasmic material and intracellular organelles within double-membrane vesicles, called autophagosomes. Completion of the autophagosome is followed by fusion with a lysosome to form an autolysosome, where the captured material is degraded by specific acidic hydrolases [12]. Although it is primarily considered to have a cytoprotective function, autophagy can also promote cell death during normal development (reviewed in [13]), as well as, in disease (reviewed in [14]). A low level of constitutive autophagy has an important housekeeping role in the normal turnover of long-lived proteins and whole organelles, thereby being crucial for maintaining healthy cells. The homeostatic role of autophagy is particularly critical in post-mitotic differentiated cells, such as neurons and cardiomyocytes. Starvation and other environmental and hormonal cues such as nutrient deprivation, growth factor depletion and hypoxia are known to activate autophagy [15,16]. As a consequence, degradation of cytoplasmic components is enhanced in response to stress conditions, thereby promoting survival. However, excessive autophagy or activation of autophagy in the context of specific diseases may be harmful. Indeed, accumulating evidence, discussed below, reveals that autophagy is linked to cell death under certain circumstances. Here, we overview the recent literature on the interplay between cell death mechanisms and autophagy. Our aim is to highlight the cellular states, sub-cellular sites and signaling mechanisms that participate in, and are crucial for this interplay, the significance of which during normal development and disease is currently being explored.

2. Crosstalk between apoptosis and necrosis

2.1. Programmed necrosis: necroptosis

In the late 1980s it became clear that necrosis can also function as an alternative programmed mode of cell death, triggered by the same death signals that induce apoptosis. More specifically, it was shown that while in F17 cells TNFα treatment induced a classical form of

apoptosis, in a different cell type, the L-M cells, it induced a necrotic form of cell death [17]. The term "necroptosis" was later introduced to describe the cases where necrosis represented a regulated and programmed form of death, instead of an accidental death, and which is yet distinct from apoptosis. In addition to TNFR1, a number of death receptors, including FAS [18], TNFR2, TRAILR1 and TRAILR2 [19–21] that typically induce apoptosis, have also been clearly shown to induce necroptosis in different cell types. This is particularly the case when apoptosis has been blocked (for instance by the use of caspase inhibitors), as shown for T cells [22], or under low levels of ATP [7,23]. In addition to death receptors, necroptosis can also be initiated by members of the pathogen recognition receptor (PRR) family, that are expressed by cells of the innate immune system to sense pathogen-associated molecular patterns (PAMPs; reviewed in ref. [24]). However, the downstream death signaling events remain poorly characterized.

By contrast, the signaling cascade downstream of TNFα leading to necroptosis has been studied in detail [25–29] (Fig. 2). Briefly, in the absence of TNFα, TNFR1 subunits spontaneously trimerize at the plasma membrane. Upon ligand binding, these receptor trimers undergo a conformational change that allows their cytosolic tails to recruit multiple proteins, including TNFR-associated death domain (TRADD), RIP1, cellular inhibitor of apoptosis 1 (cIAP1), cIAP2, TNFR-associated factor 2 (TRAF2) and TRAF5. As a result, a large structure is generated proximally to the plasma membrane, termed complex I [30], which activates the pro survival NF-κB signaling pathway.

Binding of TNFα to TNFR1 leads to its internalization and the formation of a cytosolic death-inducing signaling complex (DISC), also known as complex II [24,31]. RIP1 polyubiquitylation not only affects NF-κB activation but also influences the transition from complex I to complex II. Deubiquitylated RIP1 (along with its cognate kinase RIP3) is recruited to a complex that includes TRADD, FAS-associated protein with a death domain (FADD) and caspase-8. In complex II, RIP1 and RIP3 are inactivated by proteolytic cleavage by caspase-8, thereby initiating the pro-apoptotic caspase activation cascade [28,32]. Inhibition of cIAPs prevents RIP1 ubiquitylation and favors the formation of complex II, thus sensitizing cells to RIP1-dependent activation of caspase-8 and apoptosis [33]. By contrast, when caspase-8 is deleted, depleted or inhibited, complex II cannot initiate the apoptotic program [34,35] and ligation of TNFR1 results in necroptosis (Fig. 2) [20] (reviewed in detail elsewhere [24]). Recent work performed in mouse embryonic fibroblasts indicates that in addition to RIP1 and RIP3, the adaptor protein

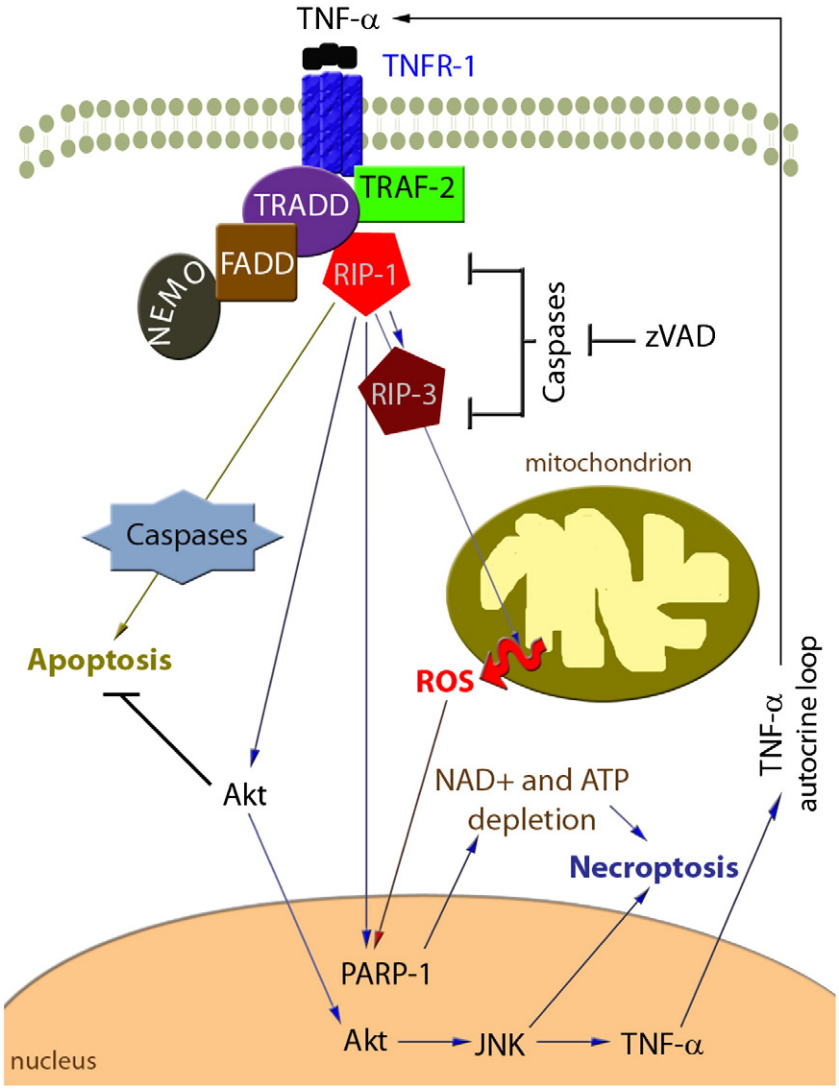

Fig. 2. TNFα induces both apoptosis and necroptosis via distinct signaling pathways. Schematic representation of TNFα signaling to apoptotic and necroptotic cell death, as it emerges from recent findings. Inhibition of caspases by zVAD is required in many experimental models for the facilitation of the necroptotic program downstream TNFR activation. PARP1 and AKT were recently shown to be directly activated by RIP1, contributing to the necroptotic phenotype by reducing ATP levels and activating JNK respectively. Moreover, NEMO functions as an essential component of the necroptosis-inducing complex II. See text for details.

FADD is also crucial for TNFα-induced necroptosis, while the formation of TNFα-induced RIP1-RIP3 necroptotic complex is independent of cytosolic Ca^{2+} [36]. Furthermore, the same study demonstrates that FADD–RIP1–RIP3-mediated mitochondrial malfunction is dependent on TNFα signaling molecule NEMO, a key mediator of cytokine-induced necroptosis. It is thus proposed that a FADD–RIP1–RIP3–NEMO complex induces BAX/BAK-dependent disintegration of mitochondrial bioenergetics to promote TNFα-driven necroptosis.

Recent work has also identified new components of the necrosome (reviewed in [37]). The mixed lineage kinase domain-like protein (MLKL) has also been identified as an interacting partner of RIP3 and thus a component of the necrosome [38]. Subsequent findings further supported the hypothesis that MLKL is indeed required for TNFa induced necroptosis [39], as MLKL mutants that cannot be phosphorylated at key residues prevented the activation of the necrosome despite still being able to incorporate into the necrosomal complex. Interestingly, PGAM5, a mitochondrial phosphoglyceratemutase, was also recently reported to associate with the necrosome, providing a potential point of convergence for increased mitochondrial fission and necroptosis ([40,41]; reviewed in [37]).

At the level of signaling, several recent studies have shed light on the close interplay between apoptosis and necroptosis. Analysis of FADD/RIP3 and FLIP/RIP3 double knockouts, revealed an intricate cross regulation of apoptosis and necrosis. Briefly, FLIP prevents the assembly of FADD-dependent, caspase-8 homodimers that mediate apoptosis, while at the same time the resulting caspase-8-FLIP heterodimers prevent the activation of RIP3 that mediates necrosis. In the absence of this heterocomplex RIP3 promotes necrosis, while in the presence of FADD but absence of FLIP, caspase-8 drives apoptosis. Deregulation of either pathway during development results in embryonic lethality [42].

In another study, using L929 cells, it was demonstrated that during necroptosis, AKT is activated in a RIP1 dependent fashion through its phosphorylation on Thr308. AKT activity, mediated in part through mTORC1, links RIP1 to JNK activation and autocrine production of TNFα. In other cell types, such as mouse lung fibroblasts and macrophages, AKT activation resulted in TNFα production without however contributing to cell death [43]. Activation of AKT appears to act as a switch, since in addition to facilitating the necroptotic response, it also acts to inhibit apoptosis. AKT ability to prevent apoptosis in some cells is established through phosphorylation and inhibition of pro-apoptotic mediators such as Bad and caspase-9 [44]. In other situations, AKT activates the transcription factor CREB, and the IκB kinase (IKK), a positive regulator of NF-κB, to regulate the expression of genes with anti-apoptotic activity [45,46].

Although there have been many reports indicating that necroptosis takes place upon death receptor activation in conditions where apoptosis is blocked, an important question is whether it also occurs in physiological conditions. Some progress has been made towards this end, especially in the immune system. Using cells infected with vaccinia virus, a natural condition was illustrated for the first time in 2003, in which apoptosis is inhibited and necrosis takes over (like many other viruses, vaccinia encodes an anti-apoptotic protein). Upon addition of the pro-inflammatory TNFα, also likely to be present during an anti-viral immune response, necrotic rather than apoptotic death is induced in infected cells. Although it has long been known that viruses encode anti-apoptotic proteins to prevent their host cells from killing themselves by apoptosis, this work also demonstrates that viruses harbor proteins that suppress programmed necrosis [19]. It remains to be firmly demonstrated whether these anti-necrotic proteins are necessary for viral pathogenicity. However, in support of this notion, it has been observed that mice lacking the TNFR2 protein (and therefore deficient in the necrotic response to vaccinia infection) exhibit both reduced inflammation in response to infection and decreased clearance of the virus. Thus, programmed necrosis might not simply be a backup when apoptosis fails, but might serve an important function in fighting microbial infection [19]. Moreover, it was recently demonstrated that RIP kinase-dependent necrosis drives TNF-induced systemic inflammatory response syndrome [47].

In addition, it was recently shown that acidic extracellular pH (pHe) switches TRAIL-induced apoptosis to regulated necrosis (or necroptosis) in human HT29 colon and HepG2 liver cancer cells. Knockdown of RIP1 or PARP1 or pretreatment with pharmacological inhibitors inhibited both TRAIL-induced necroptosis and PARP1-dependent intracellular ATP depletion demonstrating that RIP1 and RIP3 were involved upstream of PARP1 activation and ATP depletion. In line with this, in the mouse model of Con A-induced hepatitis, where death of mouse hepatocytes is dependent on TRAIL, PARP1 activity was positively correlated with liver injury and hepatitis [21]. These results underline the clinical relevance of the necroptotic pathway and the need for identifying components of this pathway that may be promising therapeutic targets for treatment of immunological disorders.

2.2. The role of ATP

Intracellular ATP levels have a determining role in the interplay between apoptosis and necrosis: high ATP levels typically enable a cell to undergo apoptosis, whereas low ATP levels favor necrosis. Thus, depletion of intracellular ATP levels switches the energy-requiring apoptotic cell death to necrosis [7,48]. It is worth noting, however, that necrosis still requires some levels of ATP, as complete ATP depletion triggers yet a different cellular demise that is morphologically and mechanistically distinct from both apoptosis and necrosis [23].

Given the central role of energy in the decision of a cell between the two modes of cell death, mitochondria are key organelles in this context. TNFα, the best characterized necrosis-inducing ligand, has a direct involvement on mitochondrial ATP production, as well as the generation of reactive oxygen species (ROS; [23]). TNFα also induces the activation of PARP1 (presumably via mitochondrial ROS, causing DNA-damage) leading to ATP depletion and subsequent necrosis [7]. PARP1 is a nuclear enzyme involved in DNA repair, DNA stability and transcriptional regulation, and becomes activated by DNA damage [5,7]. Its inhibition in cells exposed to genotoxic factors leads decreased rates of DNA repair and increased ROS [49,50]. PARP1 over-activation consumes large amounts of NAD+, resulting in a massive ATP depletion [51,52]. Therefore, PARP1 functions as a molecular switch between apoptosis and necroptosis by regulating ATP levels in the cell.

2.3. p53, apoptosis and necrosis

p53 has a pivotal role in sensing cellular stress as it responds to a wide range of signals including DNA damage, oxidative stress and ischemia. In turn, it controls programs of apoptosis typically by inducing the transcription of components of the death receptor and mitochondrial pathways such as CD95, PUMA, NOXA, BAX, and others, which act cooperatively to promote cell death [53,54]. Moreover, p53 can directly promote mitochondrial outer membrane permeabilization (MOMP) to trigger apoptosis by modulating the MOMP governing BCL-2 family [55,56]. A p53-orchestrated mitochondrial apoptotic program has been described by several groups. Upon stress, a cytoplasmic pool of p53 rapidly translocates to the mitochondrial surface, where it physically interacts with both anti- and pro-apoptotic BCL-2 family members to inhibit or activate their respective functions, leading to MOMP and apoptosis [57].

A clear case where interplay of apoptosis and necrosis takes place depending on the magnitude of the death insult is during ischemic brain infarction, where an apoptotic penumbra surrounds a necrotic center [8]. The long-standing paradigm had been that p53 controls apoptosis but plays no role in necrosis. Only recently the very first link between p53 and necrosis was reported. Upon etoposide-mediated DNA

damage *Bax*/*Bak* double knockout MEFs die by a necrotic mechanism, largely controlled by p53-mediated transcription of cathepsin Q in cooperation with DNA damage-induced ROS [58]. Notably, this transcriptional p53–cathepsin Q pathway is dispensable in the necrotic death of the corresponding wildtype MEFs. In contrast, H_2O_2-induced necrosis in double knockout MEFs was found to be completely transcription independent [58]. Necrosis in ischemic tissues depends on cyclophilin D (CypD), the key regulator of the mitochondrial permeability transition pore (PTP) at the inner membrane, whose opening leads to cell death [59]. As shown in four independent strains, CypD null mice are resistant to ischemia-induced necrosis in myocardial infarction and stroke, and CypD-deficient mitochondria and cells are resistant to Ca^{2+} and H_2O_2-induced cell death.

In line with these observations, a recent report provides compelling evidence that p53 has a novel role as a modulator of necrosis. Using H_2O_2 treatment of cultured cells to model necrosis in ischemic tissues, p53 was found to be required for necrotic cell death activation [56]. It does so by accumulating in the mitochondrial matrix, where it triggers PTP opening, mPT and necrosis by physical interaction with the critical PTP regulator CypD. This p53 action is transcription-independent and inhibited by the specific CypD inhibitor CsA, and by genetic CypD deletion or knockdown [56].

2.4. BCL-2 proteins in apoptosis and necroptosis

BCL-2 family members regulate the mitochondrial pathway of apoptosis by complex interactions that determine the integrity of the outer mitochondrial membrane [60,61]. As already mentioned, this pathway is initiated by MOMP, which allows soluble proteins (e.g., cytochrome c) in the mitochondrial intermembrane space to diffuse into the cytosol. Cytochrome c causes Apoptotic Protease Activating Factor 1 (APAF1) to oligomerize into a caspase activation complex, termed the apoptosome. This binds and promotes the activation of initiator caspase-9, which then activates executioner caspases-3 and -7 that cleave several substrates to elicit the apoptotic phenotype [62].

The BCL-2 family of proteins is subdivided into three groups depending on their function [60]. The first group consists of BCL-X_L, BCL-2, BCL-W and MCL-1. They contain three or four BCL-2 homology (BH) domains that are known to be necessary for their anti-apoptotic function. Through their interactions with other BCL-2 members, they negatively regulate the mitochondrial release of pro-apoptotic proteins. The second group includes pro-apoptotic proteins, such as BAX and BAK, which are able to form pores or associate with pore-forming proteins in the outer mitochondrial membrane. This process induces mitochondrial permeabilization and the release of cell death-promoting proteins. The third group consists of proteins that only have a short BH3 domain. These BH3-only proteins interact with both anti- and pro-apoptotic BCL-2 members to induce programmed cell death [60].

While BCL-2 family members have well established roles in apoptosis, their involvement in necroptosis is only recently beginning to unravel. An early indication for the involvement of BCL-2 in necrotic death came from studies on models of insulin-dependent diabetes mellitus, a chronic autoimmune disease resulting from progressive destruction of the insulin-producing β-cells in the pancreas [63]. It has been clearly demonstrated that β-cells die by apoptosis in response to a variety of stimuli [64,65] and that their death can be prevented by overexpression of BCL-2 [66–68]. However, necrosis is also thought to occur at the early phases of the disease when activated macrophages are drawn into the tissue. Using the rat insulin-producing β-cell line RINm5F, it was shown that both apoptosis and necrosis of these cells, induced by treatment with various cytokines, could be prevented by overexpression of BCL-2 [69]. Moreover, overexpression of BCL-2 was also found sufficient to prevent the necrotic death of neuronal [70] and non-neuronal cells [71].

Necroptosis is known to be induced by high doses of the alkylating DNA-damage agent. N-methyl-N0-nitro-N0-nitrosoguanidine (MNNG) [72]. This necroptotic process, which is also regulated by the kinase RIP1[73] is executed by the activation of PARP1, Ca^{2+}-dependent calpain Cys-proteases, and the pro-apoptotic BAX [74]. In MNNG-induced necroptosis, the key role of the two first subgroups of the BCL-2 family has been recently established: BCL-2 and BAX manage the mitochondrial release of tAIF [74]. Recent work revealed that BID regulates BAX activation and necroptosis. Cleaved into tBID via a calpain cleavage at Gly70, this BH3-only protein is indeed the link between calpains and BAX in MNNG-mediated necroptosis [75]. Along similar lines, the pro-cell-death BCL-2-family proteins BAX and BAK were shown to be required for mitochondrial dysfunction in response to necroptotic agonists, in mouse embryonic fibroblasts, while overexpression of BCL-X_L is protective [36].

2.5. Necroptosis and apoptosis-like death: the role of AIF and PARP1

The term "apoptosis-like" programmed cell death (Fig. 1) has been introduced to describe cases where death with apoptotic features, such as nuclear chromatin condensation morphology takes place in a caspase-independent manner [76]. This type of programmed cell death is controlled by mitochondria and its major effector is the mitochondrial protein AIF [77,78]. AIF is a flavoprotein, located in the inter-membrane space of mitochondria and embedded in the inner mitochondrial membrane. AIF is required for the maintenance of the mitochondrial respiratory complex I, and displays NADH oxidoreductase and peroxide scavenging activities [79]. In addition to this function, AIF has also been implicated as an inducer of apoptotic-like cell death in several experimental models [76]. This activity is associated with the cleavage of AIF into a soluble form (tAIF) by calpains or cathepsins, its release from mitochondria and translocation into the nucleus. This release is typically either upon increased levels of intracellular Ca^{2+} or extensive DNA damage. In the former case, increased intracellular Ca^{2+} levels trigger depolarization of mitochondrial membrane, loss of membrane potential, generation of reactive oxygen species (ROS) and AIF release.

Although initially considered specific to apoptotic-like cell death, AIF has also been implicated in necroptotic death induced by DNA damage, triggered by MMNG. In this case, AIF release from the mitochondrial inter-membrane space follows the over-activation of PARP1. Chemical inhibition or genetic ablation of PARP1, as well as of AIF prevent DNA-damage-induced death, [73,74,80] further demonstrating the absolute requirement of PARP1 and AIF in necroptosis. Therefore, these effectors appear both in programmed necrosis and apoptosis-like PCD.

3. Crosstalk between autophagy and apoptosis

Both autophagy and apoptosis are well-controlled biological processes that play essential roles in development, tissue homeostasis and disease. Interactions among components of the two pathways indicate a complex cross-talk, which is often induced by similar stimuli. For example, studies show that both apoptosis and autophagy are activated in response to metabolic stress. Growth factor deprivation, limitation of nutrients and energy metabolism, activate the LKB1–AMPK pathway, thereby increasing stability of cyclin-dependent kinase inhibitor $p27^{kip1}$ and thus promoting cell survival through induction of autophagy. Conversely, knockdown of $p27^{kip1}$ under these conditions activates apoptosis [81].

Moreover, autophagy is induced as an adaptive response against endoplasmic reticulum (ER) stress. Interestingly, perturbation of either ER calcium homeostasis or ER function increases autophagy and apoptotic cell death. The impact of autophagy on cell survival in the ER stress depends on the tissue type. In colon and prostate cancer cells, ER-induced autophagy has an important role in disposing of unwanted polyubiquitinated protein aggregates, thus protecting against cell death. However, in normal human colon cells and in

non-transformed murine embryonic fibroblasts, autophagy does not alleviate ER stress but rather contributes to ER-induced apoptosis [82]. Accumulating evidence reveals that autophagy and apoptosis can cooperate, antagonize or assist each other, thus influencing differentially the fate of the cell. Recent studies have delineated several pathways that mediate the complex interplay between autophagy and apoptosis providing mechanistic insight into the network that regulates both processes.

3.1. The TOR kinase pathway in autophagy and apoptosis

The mammalian target of rapamycin (mTOR) kinase integrates signals from nutrients, growth factors, energy and stress to regulate growth during early development, and ageing during adulthood. Low insulin–IGF1 signaling, nutrient or energy limitation and stress converge to downregulate the activity of TOR [83]. TOR inhibition results in reduction of mRNA translation rates, as well as, in induction of autophagy. Under starvation conditions, TOR is rapidly inhibited and this activates autophagy [84]. Intriguingly, a recent study suggests that mTOR signaling is inhibited during autophagy initiation, but reactivated upon prolonged starvation. As a consequence, reactivated mTOR attenuates autophagy and generates proto-lysosomal tubules and vesicles that extrude from autolysosomes. These mature into functional lysosomes, thereby restoring lysosome homeostasis [85]. This negative feedback mechanism ensures the reversion of autophagy upon nutrient replenishment and as such prevents excess cytoplasmic vacuolization, which could lead to autophagic cell death [86–88].

mTOR has been reported to have pleiotropic effects on apoptosis that are most likely dependent on the cellular context as well as diverse downstream targets, such as p53, BAD and BCL-2 proteins, among others [89]. Recently, two new mTOR interactors have been identified, the proline-rich AKT substrate (PRAS40) and the protein Q6MZQ0/FLJ14213/CAE45978, which appear to be implicated in the regulation of apoptosis, thereby controlling the balance between cell growth and cell death [90]. A more recent study has shown that the anti-apoptotic BCL-2 homolog MCL1 acts as a stress sensor that coordinately controls autophagy and apoptosis. The final outcome is determined by the interplay between BAX and Beclin 1 activation downstream of MCL1 degradation. Consistent with the potential role for TOR in modulating both autophagy and apoptosis, mTOR inhibition following nutrient deprivation has been suggested to cause MCL1 degradation [91]. Despite the well-established role of TOR in positively and negatively regulating several anabolic and catabolic processes respectively, further investigation is required for a better understanding of the molecular mechanisms that underlie the action of TOR as apoptosis inducer or effective inhibitor.

3.2. Beclin 1: at the crossroads between autophagy and apoptosis

Beclin 1, the mammalian ortholog of yeast Atg6, has a critical role in autophagosome formation as a component of a multiprotein class III phosphatidyl inositol-3 kinase (PI3K) complex, which also includes VPS34 and VPS15, among others (Fig. 3; [92,93]). Beclin 1 is expressed in many human and murine tissues. Similarly, *bec-1*, the *Caenorhabditis elegans* ortholog of mammalian *beclin 1*, is expressed in all tissues that are remodeled during dauer larval formation (a stage of developmental arrest). *bec-1* is essential during embryogenesis and is required for normal dauer development and adult lifespan [94]. Moreover, loss of *beclin 1* contributes to embryonic lethality in mice and to increased tumor incidence in *beclin 1*+/− mice. These phenotypes are tightly associated with defects in autophagy with no apparent defect in apoptotic cell death [95]. It is well-documented that the crosstalk between autophagy and apoptosis is mediated at least in part by the functional and structural interaction between Beclin 1 and the anti-apoptotic proteins BCL-2 and BCL-X_L [96,97]. The Beclin 1/BCL-2 interaction appears to be evolutionarily conserved. In *C. elegans*, BEC-1 forms two functionally distinct complexes with the BCL-2 homolog CED-9 and with LET-512/VPS34. BEC-1-depleted larvae lack the lipid product PtdIns 3-phosphate of LET-512/VPS34 supporting the view that BEC-1 is required for the function of LET-512/VPS34, which has essential roles in autophagy, membrane trafficking and endocytosis. Moreover, inactivation of *bec-1* triggers apoptosis as evidenced by the increased number of apoptotic cell corpses in somatic tissues and in the germline of animals [97]. Together these findings suggest that BEC-1 acts as a crucial regulator of both autophagy and apoptosis. The structural basis of Beclin 1/BCL-2/BCL-X_L interaction has recently been elucidated. Beclin 1 possesses a BCL-2 homology (BH) 3 region, which physically interacts with BCL-2/BCL-X_L proteins [98]. BH3 domains can bind to BH3 receptors and inhibit the anti-apoptotic BCL-2 proteins, such as BCL-2 and BCL-X_L, or activate the pro-apoptotic BCL-2 family members, such as BAX and BAK [99]. Interestingly, mutations in BH3 domain of Beclin 1 or the BH3 receptor domain of BCL-X_L disrupt the physical interaction between Beclin 1 and BCL-X_L, thereby abolishing the BCL-X_L-mediated inhibition of autophagy. Notably, BCL-2 targeted specifically to the ER and not to mitochondria can effectively inhibit starvation-induced autophagy in yeast and mammalian cells and in the heart muscle of transgenic mice co-expressing cardiac BCL-2 and the fluorescent autophagy marker GFP-LC3 [96]. It has been shown that siRNA-mediated depletion or deletion of the BH3-only protein Bad reduces starvation-induced autophagy, whereas transfection-enforced overexpression of BAD or addition of the pharmacological BH3-mimetics is sufficient to induce autophagy in human cells. Similarly, EGL-1 deficiency, the sole BH3-only protein from *C. elegans*, compromises starvation-induced autophagy, while gain-of-function mutation of EGL-1 stimulates autophagy [98]. Together, these findings indicate that BH3-only proteins or BH3 mimetics act not only as cell death inducers, but also as autophagy regulators. Intriguingly, although the binding of BCL-2 to Beclin 1 reduces the capacity of Beclin 1 to activate autophagy, and despite the fact that Beclin 1 contains a BH3-only motif typical of pro-apoptotic proteins, Beclin 1 fails to modulate the anti-apoptotic potential of BCL-2 and thus, to induce apoptosis. The fact that binding of Beclin 1 to BCL-2 does not modify apoptosis even in autophagy-deficient *Atg5*−/− mouse embryonic fibroblasts (MEF) argues against a protective role for Beclin 1-mediated autophagy [100].

3.3. Caspases as modulators of autophagy

Recent findings provide new insights into the mechanisms underlying the molecular interplay between autophagy and apoptosis. Withdrawal of the obligatory interleukin 3 (IL3) growth factor from murine hematopoietic cell line induces autophagy as a pro-survival mechanism followed by apoptotic cell death if deprivation is sustained. Induction of apoptosis upon growth factor depletion is associated with caspase-mediated cleavage of Beclin 1 and PI3K. This event impairs the autophagic function of Beclin 1. Furthermore, cleavage of Beclin 1 and PI3K occurs independently of the cell type and the apoptotic trigger either intrinsic (through release of mitochondrial pro death factors) or extrinsic (death receptor-dependent). Importantly, the C-terminal fragment of Beclin 1 generated by caspase-mediated cleavage localizes to mitochondria and sensitizes cells to apoptosis, probably through release of pro-apoptotic factors (Fig. 3) [101]. The finding that the pro-apoptotic protein BAX reduces autophagy by enhancing caspase-mediated cleavage of Beclin 1 at D149 and the fact that non-caspase cleavable Beclin 1, as well as, BCL-X_L can rescue BAX-induced autophagy, indicates that apoptosis can suppress autophagy [102]. Further supporting the link between autophagy and apoptosis, accumulating evidence reveals that other autophagy proteins are also substrates for caspase-induced apoptosis. Caspase-3 cleaves human ATG4D, a cysteine protease that in turn cleaves the C-terminus of newly synthesised ATG8 (called LC3 in

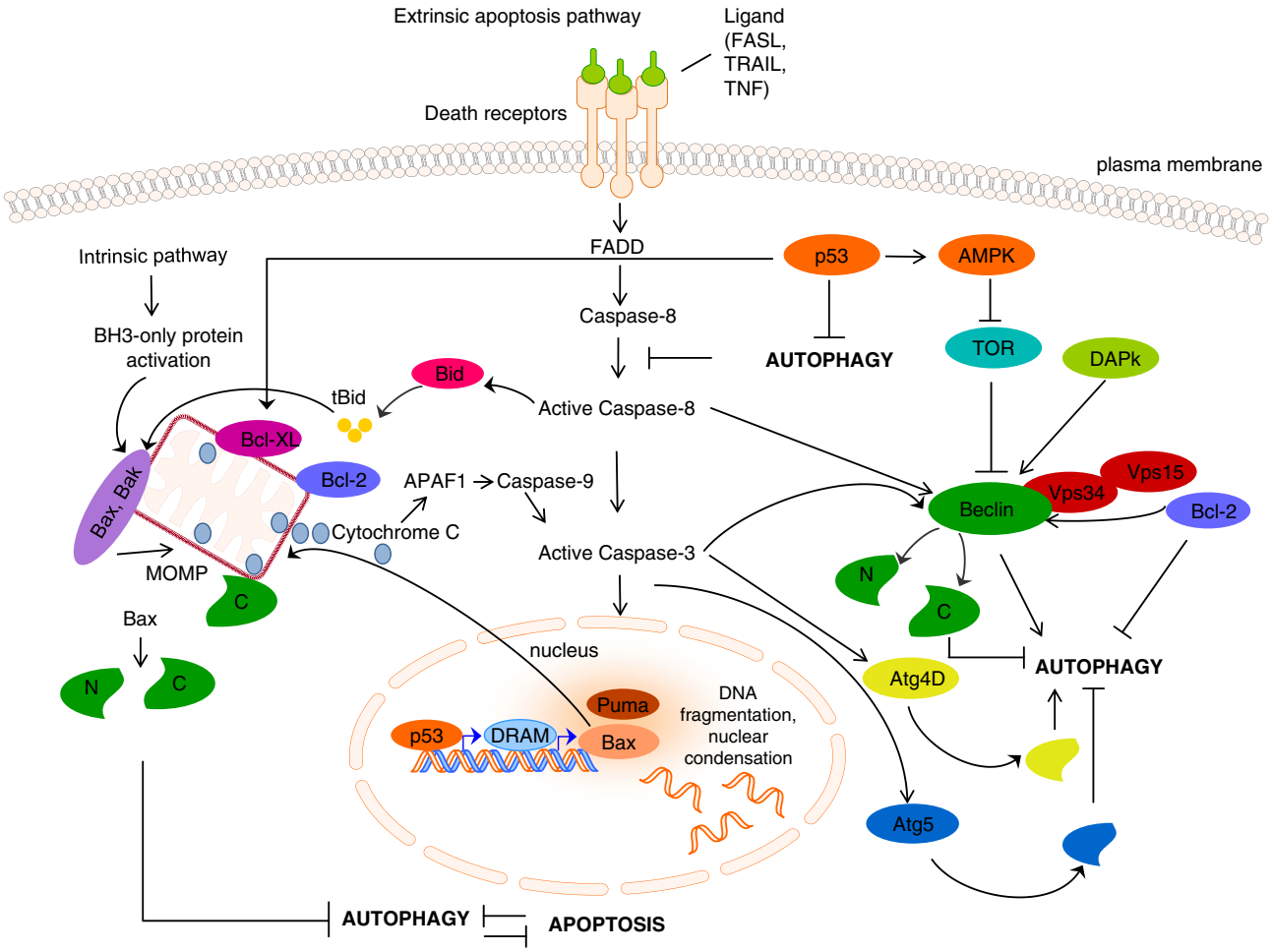

Fig. 3. The complex interplay between apoptosis and autophagy. Apoptosis can be triggered either by external receptor-dependent stimuli or internal mitochondria-mediated signaling. The extrinsic pathway is initiated by the ligation of death receptors with their cognate ligands, such as FASL, TRAIL or TNF. As a consequence, an adaptor molecule, FADD (FAS-associated death domain protein), couples death receptors and subsequently activates caspase-8. Activated caspase-8 can directly cleave and activate caspase-7 and caspase-3, thus promoting apoptosis. The intrinsic pathway is modulated by the activation of BH3-only proteins sensing different types of cell stress, such as DNA damage or ER stress, and then activating BAX/BAK at mitochondrial outer membrane (MOM). MOM permeabilization (MOMP) leads to release of different apoptosis-mediating molecules, such as cytochrome c, which activates caspase-9. In turn, caspase-9 cleaves and activates caspase-3 and caspase-7, thus triggering apoptotic cell death. Both pathways interface at the point of caspase-3 activation. Autophagosome formation requires Beclin 1 acting as a component of a multiprotein (PI3K) complex together with VPS34 and VPS15, among others. The crosstalk between autophagy and apoptosis is mediated at least in part by the functional and structural interaction between Beclin 1 and the anti-apoptotic proteins BCL-2 and BCL-X_L. Diverse apoptotic stimuli either intrinsic or extrinsic can lead to caspase-mediated cleavage of Beclin 1. As a consequence, Beclin 1 loses its ability to induce autophagy. Instead, its C-terminal fragment translocates to mitochondria, thereby sensitizing cells to apoptosis. In contrast to Beclin 1 and ATG5, which lose their ability to induce autophagy upon cleavage, caspase-cleaved ATG4D acquires enhanced autophagy activity. p53 has essential roles in both apoptosis and autophagy. At the transcriptional level, p53 upregulates BAX, PUMA and BID or reduces the expression of BCL-2, which antagonizes BAX. In addition to apoptosis, p53 can also induce autophagy through TOR inhibition and also through transcriptional activation of DRAM. Intriguingly, cytoplasmic p53 inhibits autophagy indicating a complex relationship between p53 and autophagy/survival pathways. DAPK, a calcium/calmodulin regulated Ser/Thr kinase, has also been linked to both apoptosis and autophagic cell death. Arrows indicate stimulatory inputs. Bars indicate inhibitory interactions. For clarity, some of the signaling connections between autophagy and apoptosis are not shown. See text for details.

mammals) (Fig. 3). Caspase-cleaved ATG4D acquires increased priming and delipidation activities against the yeast Atg8 paralog GABARAP-L1 (γ-aminobutyric acid receptor-associated protein-like 1). Silencing ATG4D expression suppresses autophagy and sensitizes cells to starvation and staurosporine-induced cell death. This supports the hypothesis that caspases stimulate ATG4D-mediated autophagy to promote the survival of starved cells. Interestingly, overexpression of caspase-cleaved ATG4D induces apoptosis in human cells that is preceded by the rapid recruitment of ATG4D to mitochondria [103]. ATG5, which is required for the formation of autophagosomes, also enhances susceptibility to apoptotic stimuli upon cleavage by caspase. Truncated ATG5 translocates to mitochondria, where it modulates the mitochondrial apoptotic pathway [104]. Noteworthy, caspase-mediated cleavage of ATG5 and Beclin 1 switches autophagy to apoptosis, while cleavage of ATG4D results in a truncated product with increased autophagic activity (Fig. 3). Together, these findings bear important implications for disease, in particular cancer pathogenesis and treatment.

3.4. Anti-apoptotic FLIPs as anti-autophagic mediators

In support of the complex crosstalk between the autophagic and apoptotic machinery, a recent study has revealed a novel function of the anti-apoptotic protein FLIP (Flice inhibitory protein), as a negative regulator of autophagy. The cellular and viral orthologs of the FADD-like interleukin-1 beta-converting enzyme (FLICE)-like inhibitor proteins (c-FLIP and v-FLIP, respectively) are known inhibitors of apoptosis triggered by death receptors of the TNF/NGF (tumor necrosis/nerve growth factor) family. Compelling evidence suggests that FLIPs compete with LC3 for binding with ATG3, an E2-like enzyme, under normal conditions. As a consequence, FLIPs prevent the ATG3-mediated elongation of autophagosomes, thereby decreasing the levels of autophagy. However, under conditions of stress, FLIPs allow ATG3–LC3 interaction, thus inducing autophagy. Collectively, FLIPs act not only as anti-apoptotic factors but also as suppressors of autophagy through their inhibitory interaction with ATG3[105].

3.5. The death-associated protein kinase (DAPK) family in apoptosis and autophagy

DAPK is a calcium/calmodulin regulated Ser/Thr kinase that mediates cell death induced by diverse death signals. DAPK acts as a tumor suppressor, whose expression is lost in many tumor types, mainly owing to DNA methylation (reviewed in ref. [106]). DAPK has been linked to both apoptosis and autophagic cell death. DAPK becomes activated during ER stress, which in turn triggers a mixed apoptotic and autophagic cell death response. Moreover, DAPK knockout confers protection against ER stress both in vitro in isolated fibroblasts and in vivo in a mouse kidney toxicity model. The finding that both apoptosis and autophagy are attenuated in these experimental settings, supports the view that DAPK integrates signals from apoptotic and autophagy pathways to induce cell death during ER stress [107]. The role of DAPK in the regulation of autophagy has been established in mammalian cell culture [108] and in *C. elegans*, where downregulation of *dapk-1*, the worm ortholog of DAPK, by mutation or RNAi knockdown reduces starvation-induced autophagy in the pharyngeal muscles [109]. However, the mechanisms through which DAPK promotes autophagy are not entirely clear. A recent study, which addresses this issue, has identified Beclin 1 as a target of DAPK (Fig. 3). DAPK-mediated phosphorylation of Beclin 1 on Thr119 at the BH3 domain promotes the dissociation of Beclin 1 from its inhibitor BCL-2-family members, thereby activating Beclin 1 to induce autophagy [110].

3.6. The role of p53 in autophagy and apoptosis

As already mentioned earlier, p53 is activated by a broad range of stressful conditions in the cell, such as DNA damage, hypoxia, or aberrant oncogene expression to promote cell-cycle checkpoints, DNA repair, cellular senescence, and apoptosis [111]. The role of p53 as regulator of apoptosis in both the extrinsic and the intrinsic pathways has been extensively studied. p53 functions to integrate distress signals and induce apoptosis through either the transcriptional activation of the 'multidomain' BCL-2 family member BAX, the 'BH3-only' members PUMA and BID or the attenuation of BCL-2 expression, which antagonizes BAX (Fig. 3). Notably, p53-induced apoptosis is often context dependent. For example, *Bax* deficiency compromises p53-mediated apoptosis in oncogenically transformed fibroblasts, but has no obvious effect on p53-mediated apoptosis in normal thymocytes (reviewed in ref. [112]). In addition to controlling transcription of proapoptotic members of the BCL-2 family, p53 can also transactivate components of the core apoptotic machinery, such as the gene encoding APAF1, which acts as a co-activator of caspase-9. Moreover, p53 can transcriptionally regulate the expression of the effector caspase-6. Although p53 can also activate the transcription of genes acting in the extrinsic apoptotic pathway, its action in apoptosis proceeds primarily through the intrinsic program. Surprisingly, beyond its transactivation functions, p53 has transrepression abilities as well, that may contribute to apoptosis [112]. While most studies have focused on the apoptotic activity of p53, several recent reports have highlighted the functional link between p53 and autophagy. p53 can stimulate autophagy by inhibiting mTOR via activation of the AMP kinase [113] or through the transactivation of DRAM (damage-regulated autophagy modulator), a gene encoding a lysosomal protein that induces autophagy (Fig. 3). Induction of autophagy by p53 via DRAM contributes to apoptotic cell death in response to genotoxic stress. Therefore, DRAM appears to be a critical component of the network that controls p53-mediated apoptosis and autophagy [114]. Interestingly, emerging findings unravel additional activities of p53 in the cytoplasm, where it triggers apoptosis and blocks autophagy. Deletion, depletion or pharmacological inhibition of p53 induces autophagy in human, mouse and nematode cells. Enhanced autophagy improves the survival of p53-deficient cancer cells under conditions of hypoxia and nutrient depletion, suggesting that autophagy induced by p53 inhibition serves a cytoprotective function. Notably, cytoplasmic, but not nuclear, p53 can repress the enhanced autophagy of p53-deficient cells, implying a complex role for p53 in regulation of autophagy. Compelling evidence suggests that the AMP kinase (AMPK) is activated, while the mammalian target of rapamycin (mTOR) nutrient sensing kinase is inhibited in $p53^{-/-}$ cells. Given the crucial role that AMPK and mTOR play in the regulation of autophagy, it is possible that p53 inhibition controls autophagy through the AMPK/mTOR-dependent pathway [115]. Overall, these findings indicate that p53 modulates autophagy depending on its subcellular localization [55]. Collectively, p53 links autophagy and apoptosis in a complex, context-dependent manner, aiming to restore cellular and organismal homeostasis.

3.7. Mitoptosis

Mitoptosis is a mitochondrial suicide process, which occurs primarily as a consequence of mitochondrial outer membrane permeabilization (MOMP) and subsequent potential loss. A recent study has shown that after Bax/Bak-mediated MOMP, a mitochondrial intermembrane space (IMS) protein, namely DDP/TIMM8a, is released into the cytoplasm where it binds to Drp1. This interaction activates Drp1-mediated mitochondrial fission and subsequently mitoptosis [116]. Mitochondrial dysfunction and the production of ROS are major factors triggering mitoptosis. Interestingly, accumulating evidence suggests that programmed destruction of mitochondria can lead to induction of autophagy. Indeed, recent studies have indicated that elimination of malfunctioning mitochondria may occur either through autophagosome formation via selective mitochondrial autophagy (mitophagy) [117] or through the formation of mitoptotic bodies, which are then released into the extracellular space via atypical exocytosis [118]. Lastly, it was reported that proteosomal inhibition by a prototypic proteasome inhibitor, MG132, in epithelial cancer cells activates a BAX/BAK-independent mitoptosis [119]. However, further characterization of the molecular mechanisms underlying mitoptosis is needed to unravel its pathophysiological significance.

4. Crosstalk between autophagy and necrosis

The interplay between autophagy and necrosis is rather complex. These processes can be activated in parallel or sequentially, and have either common or opposite objectives. The ability of autophagy to suppress various forms of necrotic cell death is considered to be one of the most important pro-survival functions of autophagy that is achieved either by blocking apoptosis or suppressing necrotic cell death.

4.1. Autophagy and necroptosis

Autophagy and necroptosis are intricately interlinked processes. The molecular underpinnings of this relationship remain largely elusive and somewhat controversial; autophagy has been shown to either promote [120], suppress [121,122] or not link with necroptosis [123].

Several studies converge to demonstrate that in response to TNFa, antigen stimulation and starvation, autophagy is activated to block necroptosis in several cell lines, such as L929 cells, lymphocytes and cancer cells [121,122]. zVAD, a short peptide that acts as a general caspase inhibitor preventing apoptosis, triggers necroptosis in response to TNFa treatment [124]. The fact that zVAD can robustly induce necroptosis and prevent autophagy through its inhibitory effect on lysosomal cathepsins, underscores the pro-survival function of autophagy against necroptosis. The pro-survival function of autophagy in zVAD-induced necroptosis is also highlighted by the observation that inhibition of autophagy via the mTOR signaling pathway enhances necroptosis, while starvation protects against zVAD-induced necroptotic cell death [86,124–126].

Sirtuins are NAD^+-dependent protein deacetylases, involved in the regulation of a number of processes including transcription, apoptosis, stress resistance and ageing [127]. Accumulating evidence implicates sirtuins both in autophagy and necroptosis. Indeed, Sirt1 forms a complex with, and deacetylates several autophagy components, such as ATG5, ATG7 and ATG8, to induce autophagy [128]. In addition, the FOXO1 transcription factor has been shown to dissociate from SIRT2 in response to oxidative stress or starvation, in human cancer cells. In turn, FOXO1 becomes acetylated and binds to ATG7, inducing autophagy [129]. Recent findings indicate that SIRT2 is also involved in the regulation of necroptosis [130]. SIRT2 associates with RIP3 and mediates RIP1 deacetylation in response to TNFα stimulation. As a consequence, RIP1 and RIP3 directly interact and form complex II, triggering necroptosis [130]. These early observations suggest a functional link between autophagy and necroptosis. However, much still remains to be uncovered about the molecular mechanisms underlying the complex interplay between these two processes.

4.2. Autophagy and PARP-mediated necrosis

As already mentioned earlier, PARP1 belongs to a family of nuclear enzymes, modulating DNA repair, transcriptional regulation, chromatin modification and genomic stability through polyADP-ribosylation [131,132]. PARP1 over-activation leads to ATP depletion, thereby inducing necrotic cell death and preventing energy-dependent apoptosis [7,28,133]. Interestingly, PARP1 activation interfaces with signaling pathways known to promote autophagy. AMPK (AMP-activated kinase) functions as a cellular energy biosensor, activated upon ATP depletion [134]. AMPK promotes autophagy either through inhibition of the mTOR signaling pathway (Fig. 3; [135,136]), or through activation of the ULK1 complex [137,138]. PARP1 activation in response to DNA damage can lead sequentially to ATP depletion, AMPK activation, inhibition of mTOR and autophagy induction [139–141]. DNA damage-induced autophagy acts as a pro-survival mechanism to protect against necrotic cell death resulting from PARP1 activation. Together these findings indicate that PAPR1 elicits opposing functions upon activation; induction of necrosis due to ATP depletion and at the same time protection against cell death through induction of autophagy. The final life-or-death outcome depends on the fine balance between autophagy and necrosis. In this setting, pro-survival induction of autophagy serves as the last resort before cell death under extreme stress conditions.

4.3. Autophagy and necrosis: the involvement of the DAPK–PKD pathway

PKD (Protein Kinase D1) is a serine/threonine kinase involved in many cellular processes such as cell proliferation, cell motility and cell death [142], and is activated upon oxidative stress [143,144]. Accumulation of large amounts of ROS (reactive oxygen species) eventually causes cellular damage and activates several cellular responses including autophagy, apoptosis and necrosis. Stimulation of autophagy promotes cell survival through elimination of damaged proteins and organelles preserving cellular homeostasis [145,146]. Recent findings reveal that PKD acts as a novel regulator of autophagy under oxidative stress [147], whereby it phosphorylates VSP34 to promote autophagosomal formation (Fig. 4). The DAPK (death-associated protein kinase), which also responds to oxidative stress and is known to induce autophagy through phosphorylation of Beclin1, releasing it from BCL-X_L (Fig. 3; [110]), activates PKD through phosphorylation [148]. Interestingly, PKD acts downstream of DAPK, with both of them required to induce autophagy under oxidative stress [147]. Thus, DAPK coordinates the induction of autophagy through two distinct mechanisms under stress conditions: First, through phosphorylation of Beclin1 and second, through phosphorylation of PKD, which in turn phosphorylates VSP34 to activate autophagy (Fig. 4).

5. Outlook

Recent findings have linked the three major routes to cell death, apoptosis, necrosis and autophagy in various ways, placing the field on a new and exciting base. With the development of ever better imaging technologies and more specific pharmacological inhibitors, our understanding of cell death processes is set to expand. New sub-modes of cellular demise are likely to appear as we are progressively more able to characterize the death pathways in different cell types and upon diverse death triggers. One such example is a recent study investigating the death of mouse embryonic stem cells upon treatment with etoposide, a topoisomerase-II DNA damaging agent. While cell death was both caspase- and necroptosis-independent, it was partially dependent on the activity of lysosomal proteases. A role for autophagy in the cell death process was eliminated, suggesting that etoposide induces a novel form of programmed cell death in this cellular context. Inhibition of p53 either as a transcription factor by pifithrin α or in its mitochondrial role by pifithrin μ significantly ameliorated cell death. Finally, EndoG was identified as a novel protease participating in the DNA fragmentation observed in this process. The authors of the report coined the term "charontosis" after Charon, the ferryman of the dead in Greek mythology, to describe this distinct form of program cell death in mouse embryonic stem cells [149].

The upcoming challenge will be to understand how these novel emerging relationships are related to developmental processes and pathological conditions, as opposed to models created in vitro. Although some progress towards this end has been made, especially in the field of immunology, there is a long way ahead and numerous diseases to investigate. The results of such efforts are eagerly anticipated as they are likely to form the basis for novel and context-specific pharmacological interventions.

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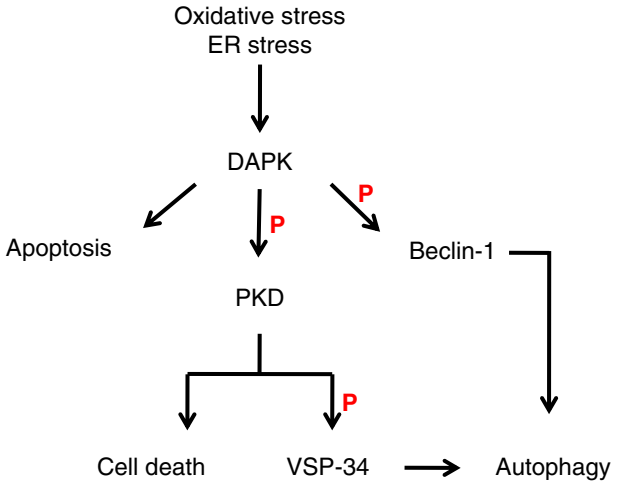

Fig. 4. The DAPK–PKD pathway coordinates the induction of apoptosis, necrotic cell death and autophagy. Under harsh stimuli DAPK functions as a signal mediator that coordinates the induction of apoptosis, necrosis and autophagy. DAPK becomes activated under stress conditions such as oxidative and ER stress. In turn, DAPK activates PKD through phosphorylation. PKD activation induces either caspase-independent cell death or autophagy. PKD binds and directly phosphorylates VSP34. VSP34 is involved in the formation of the autophagosomal membrane. DAPK also phosphorylates Beclin 1. Upon phosphorylation, Beclin 1 is released from inhibitory BCL-2 association and autophagy commences.

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