CELL DEATH

Mitochondria as multifaceted regulators of cell death

*Florian J. Bock and Stephen W. G. Tait**

Abstract | Through their many and varied metabolic functions, mitochondria power life. Paradoxically, mitochondria also have a central role in apoptotic cell death. Upon induction of mitochondrial apoptosis, mitochondrial outer membrane permeabilization (MOMP) usually commits a cell to die. Apoptotic signalling downstream of MOMP involves cytochrome *c* release from mitochondria and subsequent caspase activation. As such, targeting MOMP in order to manipulate cell death holds tremendous therapeutic potential across different diseases, including neurodegenerative diseases, autoimmune disorders and cancer. In this Review, we discuss new insights into how mitochondria regulate apoptotic cell death. Surprisingly, recent data demonstrate that besides eliciting caspase activation, MOMP engages various pro-inflammatory signalling functions. As we highlight, together with new findings demonstrating cell survival following MOMP, this pro-inflammatory role suggests that mitochondria-derived signalling downstream of pro-apoptotic cues may also have non-lethal functions. Finally, we discuss the importance and roles of mitochondria in other forms of regulated cell death, including necroptosis, ferroptosis and pyroptosis. Collectively, these new findings offer exciting, unexplored opportunities to target mitochondrial regulation of cell death for clinical benefit.

BH3-mimetics
Drugs modelled after the pro-apoptotic BH3 domain of BH3-only proteins that are used in cancer therapy.

Mitochondria are essential for life. Positioned at the heart of cellular metabolism, they serve a key role in ATP generation via oxidative phosphorylation. Beyond their many core metabolic functions, mitochondria are implicated in an expanding array of cellular processes, ranging from inflammation to regulation of stem cell generation[1,2]. In what may seem a paradox, mitochondria are often essential for cell death.

Regulated cell death underpins health; for example, inhibition of cell death promotes cancer and autoimmunity whereas excessive cell death contributes to neurodegenerative diseases, including Parkinson disease, Alzheimer disease, amyotrophic lateral sclerosis and Huntington disease. Consequently, considerable interest has centred upon targeting of mitochondria to manipulate cell death in disease. Validating this rationale, recently developed anticancer drugs called BH3-mimetics sensitize cells to mitochondria-dependent death, displaying potent antitumour activity[3,4]. The role of mitochondria in cell death is unequivocally established in apoptosis, where mitochondrial outer membrane permeabilization (MOMP) driven by effector pro-apoptotic members of the B cell lymphoma 2 (BCL-2) family of proteins (prominently BAX and BAK; BOX 1) initiates a signalling cascade that leads to cell death; although, as we have now come to appreciate, induction of MOMP is not synonymous with apoptosis and the commitment of a cell to die is not definitive downstream of MOMP. In addition, MOMP has other consequences beyond execution of cell death, including induction of pro-inflammatory signalling. Finally, while apoptosis is a major form of regulated cell death, it is by no means the only one. More recently described types of regulated cell death include necroptosis, pyroptosis and ferroptosis. Mitochondria have also been implicated in these additional modalities of regulated cell death, but their roles are still poorly defined and appear less conspicuous.

In this Review we discuss how mitochondria contribute to regulated cell death, placing this contribution in the context of health and disease. Specifically, we highlight new insights into how mitochondria initiate apoptosis, and discuss their parallel role in eliciting pro-inflammatory signalling activity with important consequences for physiology. Taken together with recent studies showing heterogeneity in MOMP between mitochondria within a cell treated with pro-apoptotic stimuli, we highlight that mitochondrial permeabilization can exert various non-lethal signalling functions. We then discuss the contribution of mitochondria to more recently described types of regulated cell death, highlighting mitochondria as a central nexus between different cell death modalities.

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Death-inducing signalling complex
(DISC). A complex consisting of death receptor, Fas-associated death domain (FADD) and caspase 8 that can mediate apoptosis.

SMAC
(Also known as DIABLO). A mitochondrial intermembrane space protein that upon mitochondrial outer membrane permeabilization binds to and inhibits XIAP.

Mechanisms of mitochondrial apoptosis

Apoptotic cell death is a major form of regulated cell death that has central roles in many processes ranging from embryonic development to immune homeostasis[5]. As we now discuss, in many instances, mitochondria are crucial for the initiation of apoptosis.

Apoptotic signalling. There are two main apoptotic signalling pathways: the extrinsic (also called death receptor) pathway of apoptosis and the intrinsic, or mitochondrial, pathway of apoptosis (FIG. 1). Both converge upon activation of caspase 3 and caspase 7. As proteases, these executioner caspases cleave hundreds of different proteins causing the biochemical and morphological hallmarks of apoptosis[6]. The extrinsic pathway is activated at the plasma membrane by death receptor ligands binding to their cognate receptors, leading to activation of caspase 8 (a component of a complex known as the death-inducing signalling complex (DISC))[7]. Active caspase 8 propagates apoptosis by cleaving pro-caspase 3 and pro-caspase 7, causing their activation (FIG. 1).

Diverse cellular stresses, for instance growth-factor deprivation or DNA damage, kill via the mitochondrial pathway of apoptosis. The mitochondrial pathway requires MOMP to release soluble proteins from the mitochondrial intermembrane space, leading to cell death (FIG. 1). Amongst these intermembrane space proteins, cytochrome *c* — an essential component of the electron transport chain — binds the adaptor molecule apoptotic peptidase activating factor 1 (APAF1), forming a complex called the apoptosome[8]. The apoptosome, in turn, binds to and activates the initiator caspase 9, which subsequently cleaves and activates the executioner caspases. MOMP also causes the release of proteins including SMAC and OMI that block the caspase inhibitor XIAP, facilitating apoptosis. The extrinsic apoptotic pathway crosstalks to the mitochondrial pathway by caspase 8-mediated cleavage of BID, a pro-apoptotic BH3-only member of the BCL-2 family (BOX 1), which generates tBID that potently induces MOMP (FIG. 1).

With some notable exceptions that we will later discuss, MOMP typically commits cells to death, even in the absence of caspase activity (this phenomenon is known as caspase-independent cell death). Thus, MOMP is considered a point of no return in apoptosis execution[9–11]. Consistent with MOMP being the point of commitment to cell death, mice deficient in caspase activity associated with the mitochondrial pathway of apoptosis (for example, *APAF1*$^{-/-}$ and *caspase-9*$^{-/-}$) display much milder developmental defects than MOMP-inhibited (*BAX*$^{-/-}$, *BAK*$^{-/-}$) mice[12–17]. The reason for MOMP being able to mediate caspase-independent cell death is overall metabolic catastrophe, related to the fact that often all mitochondria undergo MOMP during apoptosis[18] and their progressive dysfunction following MOMP causes widespread ATP loss[19]. Because MOMP serves to commit a cell to die, it is tightly regulated, primarily by members of the BCL-2 protein family (BOX 1).

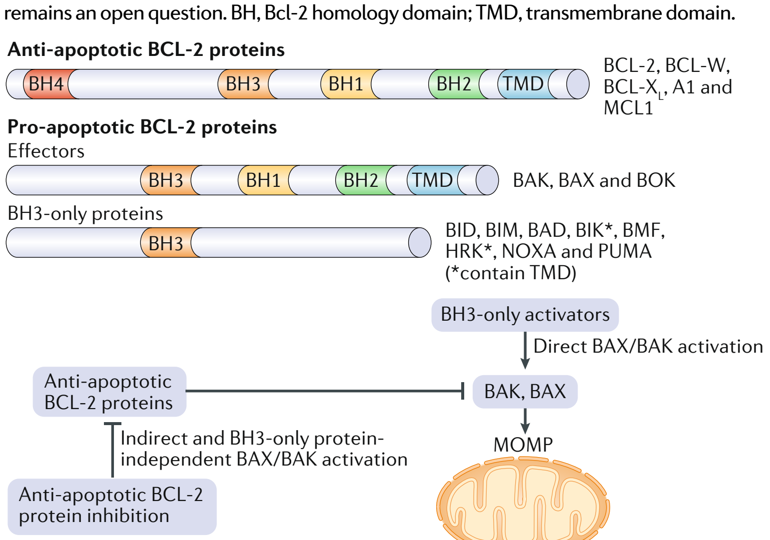
Mechanisms of MOMP. During mitochondrial apoptosis, activation of the pro-apoptotic effectors BAX and BAK is usually essential for MOMP and cell death[20]. BAX and BAK are largely considered redundant because only upon their combined loss are cells resistant to mitochondrial apoptosis and extensive developmental defects are observed[15,16,20]. Nevertheless, differences for BAX versus BAK in mitochondrial apoptosis have been reported in some studies[21,22]. For example, BAX and BAK display a differential requirement for the mitochondrial porin voltage-dependent anion-selective channel 2 (VDAC2) in their ability to induce apoptosis: while VDAC2 associates with both proteins, VDAC2 is required for BAX, but not BAK, to induce apoptosis[23–25]. Importantly, such differences in apoptotic requirement for BAX or BAK

Box 1 | **BCL-2 protein-mediated regulation of mitochondrial apoptosis**

B cell lymphoma 2 (BCL-2) protein-mediated regulation of cell death has recently been reviewed in depth elsewhere[158], therefore here we present only an overview. The BCL-2 protein family comprises three subsets: the anti-apoptotic proteins, pro-apoptotic effectors and pro-apoptotic BH3-only proteins (see the figure). Following an apoptotic stress, BH3-only proteins are activated in different ways, for instance, by transcriptional upregulation (for example, p53-mediated upregulation of PUMA) or by post-translational modification (for example, caspase 8-mediated cleavage of BID). They subsequently activate BAX and BAK, causing mitochondrial outer membrane permeabilization (MOMP) and apoptosis.

In healthy cells, anti-apoptotic BCL-2 proteins prevent MOMP by binding activated BAX and BAK effectors and BH3-only proteins[159]. This binding occurs via a hydrophobic groove, which interacts with the BH3 domain of pro-apoptotic BCL-2 proteins. Competitive disruption of this interaction forms the basis of the pro-apoptotic activity of BH3-mimetics. Of note, the efficiency of BH3-mimetics can be compromised by additional regulation of anti-apoptotic proteins, leading to drug resistance. For example, mitochondrial association of BCL-X_L can increase its affinity for BH3-only proteins[160], whereas BIM has been found to encode an additional carboxy-terminal site that binds to anti-apoptotic BCL-2 proteins in a manner that is resistant to displacement by BH3-mimetics[161].

How exactly BAX and BAK become activated has been contentious. Two prominent models have been proposed: the indirect activation model, in which inhibition of anti-apoptotic BCL-2 proteins activates BAX and BAK; and the direct model of activation, in which a subset of BH3-only proteins called direct activators (BID, BIM, PUMA) directly activate BAX and BAK. Distinguishing between these two models has proved challenging, in large part because direct activator BH3-only proteins also inhibit all anti-apoptotic BCL-2 proteins. Intriguingly, a recent study has found that in the absence of all known BH3-only proteins, inhibition of anti-apoptotic BCL-2 function using BH3-mimetics is sufficient to activate BAX and BAK, leading to apoptosis[162]. This demonstrates that BH3-only proteins are dispensable for the direct activation of BAX and BAK, but how BAX and BAK can acquire active conformations in the absence of BH3-only proteins remains an open question. BH, Bcl-2 homology domain; TMD, transmembrane domain.



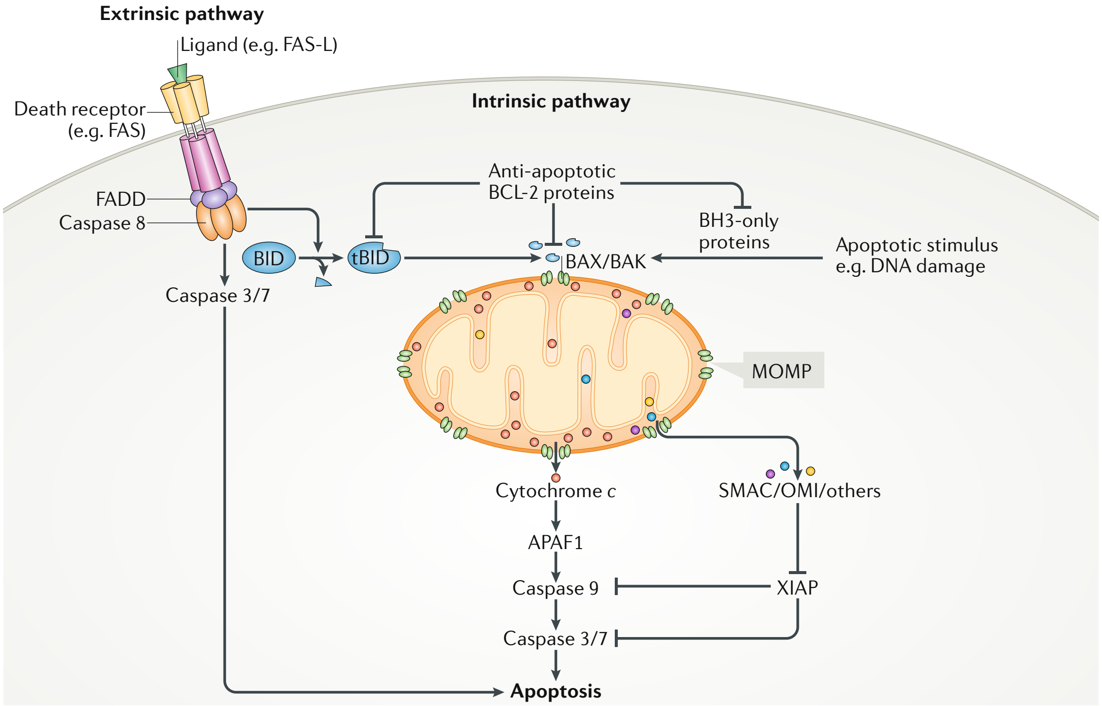


Fig. 1 | **Apoptotic signalling pathways.** Apoptosis can occur via two pathways: extrinsic and intrinsic. The extrinsic (also known as death receptor) apoptotic pathway involves the binding of a death receptor ligand to a member of the death receptor family (members of the tumour necrosis receptor superfamily). For example, Fas-ligand (FAS-L) binding to FAS initiates apoptosis by recruiting the adaptor molecule Fas-associated death domain (FADD). FADD binds to and induces dimerization of the initiator caspase 8, leading to its activation. Active caspase 8 cleaves and activates the executioner caspases 3 and 7, leading to wide-scale cleavage of cellular components and rapid cell death. The intrinsic (also known as mitochondrial) apoptotic pathway is induced by a vast number of different stimuli (including DNA damage, growth factor withdrawal and mitotic arrest), which cause activation of BH3-only members of the B cell lymphoma 2 (BCL-2) protein family. BH3-only proteins inhibit anti-apoptotic BCL-2 proteins and activate the effector pro-apoptotic BCL-2 proteins BAX and BAK, leading to mitochondrial outer membrane permeabilization (MOMP). This allows the release of mitochondrial intermembrane space proteins that activate caspases, most importantly cytochrome *c*. Cytochrome *c* binds to apoptotic peptidase activating factor 1 (APAF1), forming a heptameric structure called the apoptosome. This recruits and activates the initiator caspase 9, which cleaves and activates caspase 3 and caspase 7. MOMP also causes the release of proteins including SMAC and OMI that block the caspase inhibitor XIAP, facilitating apoptosis. Caspase 8-mediated cleavage and activation of BH3-only protein BID (to generate tBID) connects the extrinsic apoptotic pathway to the intrinsic pathway.

can govern the effectiveness of chemotherapy responses that often require mitochondrial apoptosis[21].

In healthy cells, BAX localizes to the cytoplasm and BAK to the mitochondria; however, both can shuttle between the mitochondria and the cytoplasm[26–28] (FIG. 2). Under basal conditions, BAX and BAK are inactive. Following activation, BAX accumulates at the mitochondria. BAX and BAK can be directly activated by binding a subclass of BH3-only proteins called direct activators (BID, PUMA and BIM)[29]. Structural studies have demonstrated that the direct activator BH3 domain binds within the hydrophobic groove of BAX and BAK, leading to extensive conformational changes, allowing activation[30–32]. This structural information has guided the development of modified BH3 peptides derived from BH3-only proteins that block BAK activation, providing a proof-of-concept for therapeutic targeting of this step to block cell death[33].

Experiments with chemically stabilized BH3 peptides also enabled the discovery of a second BH3-binding site in BAX[34]. This second BH3-binding site is distant from the BAX hydrophobic groove, located in the amino terminus of the protein, and promotes BAX activation through an allosteric conformational change[34,35]. Notably, BAX-activating small molecules that target this amino-terminal site and promote BAX activation display potent antitumour activity[36]. Reconciling a requirement for two activation sites, recent data support a sequential model of BAX activation in which BH3-proteins first bind the amino-terminal site, facilitating BH3 binding to the hydrophobic groove for full BAX activity[37]. Of note, there is evidence that BH3-only proteins are not absolutely essential for BAX and BAK activation (see BOX 1). During activation, BAX and BAK expose their BH3 domains, which can further propagate their own activity[35,38]. Once activated, BAX and BAK homodimerize and these dimers form higher-order oligomers that are essential for MOMP[39–43] (FIG. 2).

How do active BAX and BAK permeabilize the mitochondrial outer membrane, initiating cell death? Consensus about this long-standing question centres on activated BAX and BAK inducing lipidic (toroidal)

OMI
(Also known as HtrA2). A serine protease located within the mitochondrial intermembrane space that binds to and inhibits XIAP following mitochondrial outer membrane permeabilization.

XIAP
A protein that binds to and inhibits caspases 3, 7 and 9.

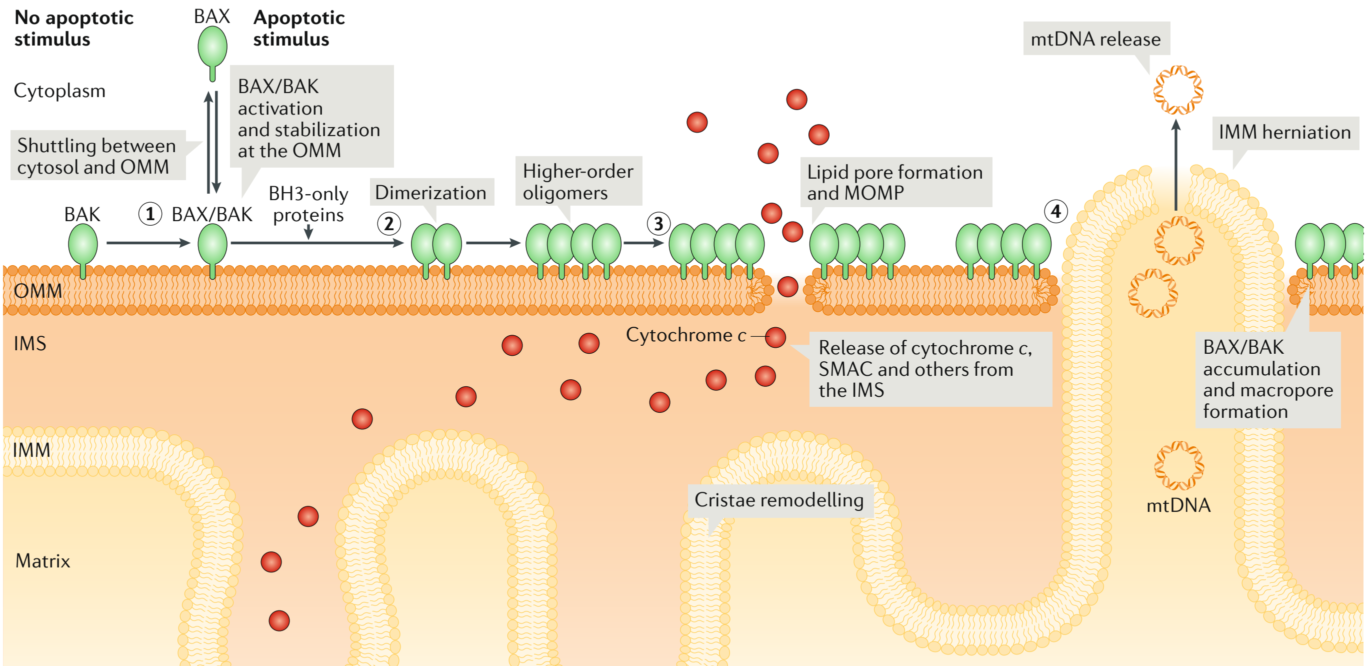


Fig. 2 | **BAX/BAK-mediated mitochondrial outer membrane permeabilization.** In healthy conditions, BAX and, to a lesser degree, BAK shuttle between the mitochondria and cytoplasm (step 1). During apoptosis, BAX and BAK can be directly activated by binding BH3-only proteins; this leads to their stabilization at the outer mitochondrial membrane (OMM) and their homodimerization (step 2). BAX/BAK dimers then further oligomerize, forming higher-order multimers that generate lipid pores within the OMM causing mitochondrial outer membrane permeabilization (MOMP); this leads to the non-selective release of soluble intermembrane space proteins, such as cytochrome *c*, from the intermembrane space (IMS); this release process can be further facilitated by inner mitochondrial membrane (IMM) remodelling that involves opening of the mitochondrial cristae to allow robust release of cytochrome *c* (step 3). Over time, BAX/BAK-mediated pores expand, forming macropores; this enables IMM extrusion through the OMM, whereupon the IMM herniates and ruptures allowing the release of mitochondrial DNA (mtDNA) (step 4). Although the exact mechanism of IMM herniation and rupture is not known, dilution of the mitochondrial matrix and the associated increased pressure may play a role.

pores in the mitochondrial outer membrane (FIG. 2). Such lipidic pores are formed by fusion of the inner and outer leaflets of membranes, which is promoted and stabilized by protein insertion. Indeed, studies using synthetic liposomes and mitochondrial outer membrane-derived vesicles demonstrate that BAX can induce large (>100 nm) membrane pores visible by cryo-electron microscopy that grow over time[44,45]. Moreover, BAX pores are tuneable in size dependent on the BAX concentration[45]. Importantly, super-resolution microscopy has enabled direct visualization of BAX-mediated pores in apoptotic cells[46,47]. On apoptotic mitochondria, BAX localizes in heterogeneous ring-like structures, roughly approximating in size to holes observed in mitochondrial outer membrane-derived vesicles. Formation of such rings on apoptotic mitochondria was associated with membrane permeabilization, further supporting permeabilization of the mitochondrial outer membrane via lipidic pore formation[46].

Extensive genetic and biochemical data firmly establish BAX and BAK as central effectors of MOMP. However, other proteins can also cause MOMP. Particular interest has focused on BOK, a BAX/BAK-like BCL-2 protein, since recent studies have demonstrated that BOK can induce MOMP and cell death in the absence of BAX and BAK[48,49]. Genetic support for this observation comes from the finding that BOK deficiency exacerbates the developmental defects observed in *Bax*$^{-/-}$*Bak*$^{-/-}$ double-knockout mice[15]. Nevertheless, BOK-induced MOMP differs from classical BAX/BAK-dependent MOMP in many ways. For instance, unlike BAX and BAK, the pro-apoptotic activity of BOK does not appear to be regulated by BCL-2 proteins in any way[48,50]. In vitro liposome and mitochondrial permeabilization assays demonstrate that BOK is inherently active[48,51]. This constitutive activity relates to the intrinsic instability of the BOK hydrophobic core such that it can mediate MOMP independently of BH3-only proteins[51]. Consistent with BOK having constitutive pro-apoptotic activity, in healthy cells BOK undergoes endoplasmic reticulum-associated degradation, which maintains the protein at low levels[48]. However, because BOK is expressed in many healthy tissues, additional regulatory mechanisms must exist to counter its pro-apoptotic activity[52].

Non-BCL-2 family proteins can also induce MOMP. Specific members of the gasdermin protein family exhibit pore-forming activity upon cleavage. As we will discuss later, cleavage of gasdermin D (GSDMD) is essential for an inflammatory type of cell death called pyroptosis. During mitochondrial apoptosis, caspase 3-mediated cleavage of gasdermin E (GSDME; also known as DFNA5) liberates a pore-forming amino-terminal

Endoplasmic reticulum-associated degradation
Pathway that serves to degrade misfolded endoplasmic reticulum (ER) proteins by the proteasome, mitigating ER stress.

fragment that can promote plasma membrane permeabilization during apoptotic cell death[53,54]. GSDME-mediated plasma membrane permeabilization induces a necrotic-like cell death that has been proposed to contribute to the chemotherapy-associated toxicity[53]. This GSDME amino-terminal cleavage fragment can also localize to the mitochondria and cause MOMP[55]. In this manner, GSDME is proposed to elicit a feedforward mechanism that enhances caspase activation during apoptosis. In an analogous manner, during pyroptosis, the GSDMD amino-terminal cleavage fragment can also induce MOMP[55] (see also below). Although requiring further investigation, given their established pore-forming properties, the amino-terminal fragments of gasdermins likely directly permeabilize mitochondria independently of BAX and BAK.

Dynamics of MOMP. Independent of apoptotic stress, MOMP is usually rapid and complete — all mitochondria undergo MOMP over a 10-min window[18,56]. Emphasizing an earlier point, the extensive nature of MOMP is likely central to it being a point of no return in apoptotic commitment. High-speed imaging of mitochondrial apoptosis has shown that MOMP can initiate in a discrete subpopulation of mitochondria, before progressing in a wave-like manner across all of the mitochondria in the cell[57–59]. Using frog egg extracts in vitro, MOMP has been found to propagate between mitochondria as a trigger wave, maintaining constant speed and amplitude over a long distance; this may facilitate the execution of apoptosis in large cells such as neurons[60].

Why is MOMP rapid and extensive? One model proposes that MOMP initiates a caspase-dependent feedforward loop, possibly by caspase-mediated BID cleavage that promotes further MOMP. However, while caspase activity supports MOMP trigger-wave propagation in vitro, blocking caspase activity following a mitochondrial apoptotic stimulus affects neither the kinetics nor the extent of MOMP in cells[18]. Furthermore, inhibiting caspase activity following a mitochondrial apoptotic stimulus usually does not protect against cell death. These findings argue against an important role for caspase activity in amplifying MOMP. Other proposed mechanisms include reactive oxygen species (ROS)-dependent feedforward propagation of MOMP, although how ROS promote this remains unclear[61]. Perhaps the most likely explanation centres on the ability of active BAX and BAK to activate other BAX and BAK molecules[35,38]. Akin to falling dominoes, this would be predicted to rapidly and extensively drive MOMP.

Inner mitochondrial membrane remodelling during apoptosis. Soluble mitochondrial intermembrane space proteins are released following MOMP irrespective of protein size[62]. However, some studies have shown that the release of cytochrome *c* can be further regulated even following MOMP, affecting caspase activation and apoptosis[63–67]. This is because the majority of cytochrome *c* resides within mitochondrial cristae — dynamic inner mitochondrial membrane folds that harbour electron transport chain components. Cristae accessibility to the intermembrane space is regulated by cristae junctions[68]. As such, cytochrome *c* has been proposed to be trapped within cristae in healthy cells, necessitating widening of the cristae junctions in order to allow efficient cytochrome *c* release. Indeed, following MOMP, extensive cristae remodelling has been observed. But how is this remodelling regulated? Mitochondria are dynamic organelles that constantly undergo cycles of fission and fusion. Immediately following MOMP, extensive mitochondrial fragmentation occurs at mitochondria–endoplasmic reticulum contact sites[69], which requires the mitochondrial fission protein dynamin related protein 1 (DRP1)[58,66]. Although dispensable for MOMP[70,71], DRP1 promotes cristae remodelling, which has been proposed to facilitate cytochrome *c* release. Several reports suggest that remodelling occurs via the effect of DRP1 on the GTPase OPA1. In the intermembrane space, OPA1 regulates inner mitochondrial membrane fusion and the cristae junction size: oligomers of OPA1 keep junctions narrow, whereas OPA1 oligomer disassembly widens the junctions[70]. Following MOMP, OPA1 is cleaved by different intermembrane space proteases including OMA1, leading to oligomer disassembly and junction opening[72–74]. During apoptosis, DRP1 is modified with the ubiquitin-like protein SUMO, leading to stabilization of the mitochondria–endoplasmic reticulum membrane contact sites. This promotes calcium influx into the mitochondria from the endoplasmic reticulum, which has been shown to be required for cristae remodelling[69]. However, it has also been shown that cristae remodelling mediated by DRP1 during apoptosis is independent of OPA1 and that OPA1 oligomers can disassemble even in the absence of DRP1 (REF.[75]).

Regardless of the exact mechanism, the importance of inner membrane remodelling for mitochondrial apoptosis is controversial. For instance, some studies have shown that inhibiting components of the cristae remodelling machinery (for example, DRP1) has minimal effect upon the release of cytochrome *c*, caspase activation and apoptosis[70,71]. Second, inner mitochondrial membrane remodelling has been reported to occur as a secondary consequence of caspase activation[76]. Irrespective of caspase activity, inner mitochondrial membrane remodelling occurs subsequent to MOMP. Thus, similar to caspase inhibition, blocking inner mitochondrial membrane remodelling would not be expected to prevent cell death unless cells can somehow survive MOMP — an area we now discuss.

Surviving MOMP

Although MOMP is considered the point of no return in mitochondrial apoptosis, some exceptions exist where, downstream of apoptotic stimuli, MOMP occurs to varying degrees with wide-ranging effects, beyond lethality. It is also now evident that cells are able to survive MOMP, which can have an important impact on physiology. Our discussion centres on how cells can survive MOMP in three distinct settings: widespread MOMP under caspase-inhibited conditions; limited MOMP; and widespread mitochondrial permeabilization accompanied by effector caspase activity.

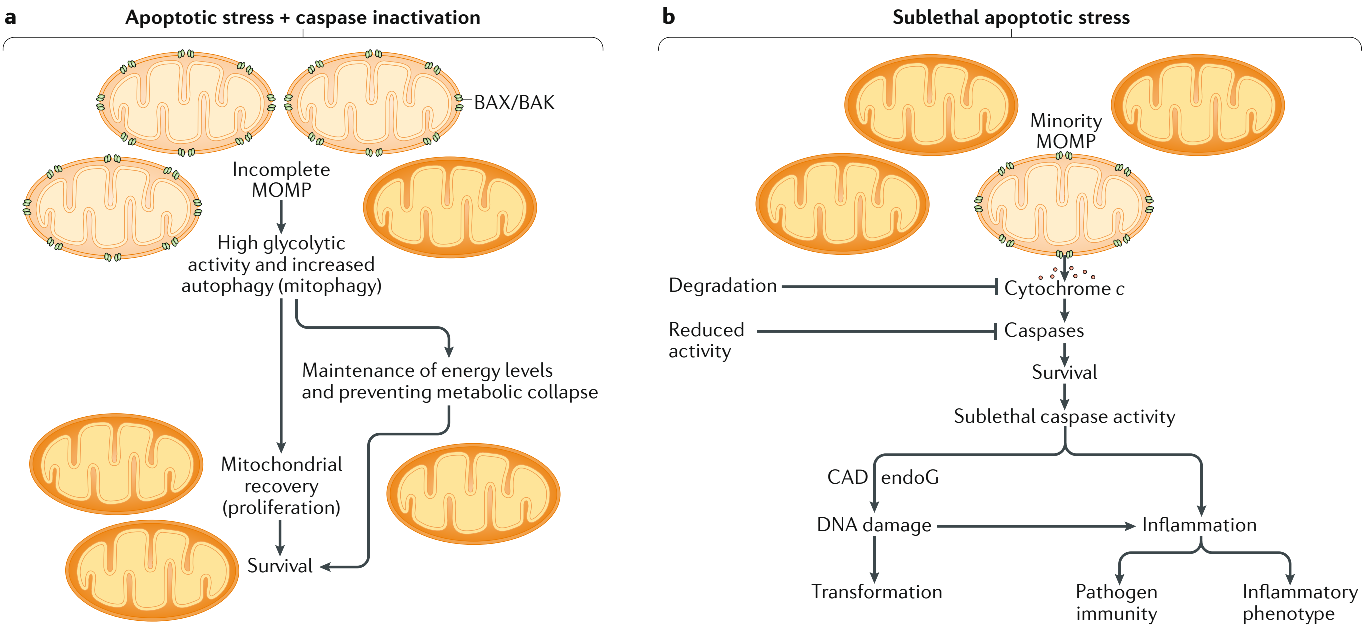


Fig. 3 | **Differential levels of mitochondrial outer membrane permeabilization permit cell survival and unmask signalling functions.** Apoptotic stresses can lead to incomplete mitochondrial outer membrane permeabilization (MOMP), which is compatible with cell survival. **a** | Cells induced to undergo apoptosis can survive under conditions of caspase inhibition. Cell survival in this context requires the presence of a subpopulation of intact mitochondria that did not undergo MOMP. Cell survival also depends on the expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which supports high glycolytic activity and autophagy, thereby generating energy to prevent metabolic catastrophe and removing dysfunctional mitochondria that could instigate further damage. Through these mechanisms, cells can survive long enough to allow the intact mitochondria to proliferate, enabling cell survival. **b** | Sublethal stresses, for instance, BH3-mimetic treatment, can cause only a subset of mitochondria to undergo MOMP — a condition known as minority MOMP. Minority MOMP can engage a limited, sublethal caspase activity, which is associated with DNA damage dependent on caspase-activated DNase (CAD) and caspase 3-dependent release of endonuclease G (endoG) from mitochondria. Such DNA damage can promote oncogenic transformation. Minority MOMP can also drive pro-inflammatory signalling in the absence of cell death, for instance, by inducing CAD-dependent DNA damage or by causing mtDNA release, both of which can drive pro-inflammatory signalling via cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) (see also FIG. 4).

MOMP can be heterogeneous, permitting survival and signalling functions. MOMP was originally defined as an all-or-nothing event. However, more recently, it has been shown that the cells can survive MOMP under caspase-inhibited conditions — when cleavage of cellular components is prevented — and the key to cell survival is the maintenance of metabolic activity. The glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH) can promote cell survival following MOMP, which is dependent on its well-established glycolytic role in ATP synthesis and its ability to transcriptionally stimulate autophagy to remove permeabilized and, hence, non-functional, mitochondria via mitophagy[77]. Survival under these conditions also tightly correlates with the presence of intact mitochondria that evaded MOMP, a condition termed incomplete MOMP[78]. These intact mitochondria serve as a crucial pool to re-establish a mitochondrial network in the cell, permitting cell survival (FIG. 3a).

Although further studies in this area are needed, it is likely that incomplete MOMP underpins survival in various cell contexts. In support of this, sympathetic neurons undergo MOMP following nerve growth factor (NGF) deprivation, but under conditions of caspase inhibition, NGF re-addition restores intact mitochondria in these neurons to enable cell survival[79,80].

Variable MOMP is also observed in response to sublethal apoptotic stresses triggered by low doses of cytotoxic drugs like BH3-mimetics or proteasome and mitotic inhibitors. However, in this case, only a small fraction of mitochondria undergoes MOMP without the execution of cell death, a condition called minority MOMP[81] (FIG. 3b). While minority MOMP does not kill cells, it still engages caspase activity. To permit survival, caspase activity is likely restrained by multiple mechanisms, including degradation of cytochrome *c* upon MOMP, leading to reduced caspase activity[82], lowered affinity of active (cleaved) caspase 9 for the apoptosome[83], restriction of caspase localization[84], caspase turnover[85] or expression of inhibitors to dampen caspase activity[86].

Minority MOMP-induced caspase activity likely has both positive and negative consequences. Apoptosis has well-established anticancer activity, for instance, the tumour suppressor p53 engages apoptosis to prevent cancer and anticancer treatments often kill cancer cells through apoptosis. Nevertheless, different studies argue that apoptotic signalling has pleiotropic oncogenic effects[87]. Along these lines, minority MOMP causes

Neoantigens
Newly generated antigens that, in cancer, usually arise from mutated genes.

caspase-dependent DNA damage and genomic instability, promoting cellular transformation[81]. The DNA-damaging effects of minority MOMP require activation of caspase-activated DNase (CAD)[81]. Following sublethal stress, caspase 3-dependent release of endonuclease G from the mitochondria can also cause DNA damage[88]. DNA-damaging effects of sublethal caspase activity have also been reported following diverse apoptotic stimuli, encompassing extrinsic and intrinsic apoptotic triggers[89–91]. By affecting genome integrity, minority MOMP might impact on cancer in different ways, for instance, by enhancing its initiation or by promoting the evolution of resistance to apoptosis-inducing therapies (FIG. 3b). However, tumour mutational load resulting from DNA damage is also responsible for the generation of so-called neoantigens, which correlate with the activation of anti-tumour immunity. As proposed elsewhere[92], potentially the DNA-damaging effects of minority MOMP could have beneficial effects in cancer therapy by increasing neoantigen generation.

At face value, effects of minority MOMP in cancer appear more of an unwanted glitch of the mitochondrial apoptotic pathway, but does minority MOMP have any physiological roles? Because it permits caspase activity without cell death, minority MOMP is ideally suited to initiate non-lethal caspase signalling, which has been implicated in wide-ranging cellular functions such as differentiation and proliferation[93]. Furthermore, as we discuss in more detail in the following section, MOMP is also a potent inductor of inflammatory signalling. In this context, a recent study has shown that minority MOMP can engage innate immune signalling pathways (both caspase dependent and independent) that inhibit the growth of diverse intracellular pathogens[94] (FIG. 3b). Dissecting the functions for minority MOMP remains a major challenge, primarily because it shares the same initiating machinery as mitochondrial apoptosis (centring on BAX/BAK activation). Because caspase substrates downstream of MOMP are dispensable for cell death, specific analysis of these substrates where relevant (for example, CAD in DNA damage) should allow genetic definition of minority MOMP functions in vivo.

Besides identifying physiological functions of mitochondrial heterogeneity in the event of MOMP, several key mechanistic questions remain to be answered. Most importantly, why do some mitochondria selectively permeabilize and how do these mitochondria differ compared with those that remain intact? Some level of regulation presumably exists, as exemplified by the physiological role of minority MOMP in pathogen defence. One observation is that in the context of incomplete MOMP, intact mitochondria had higher levels of anti-apoptotic BCL-2 proteins associated with them. Accordingly, neutralization of anti-apoptotic BCL-2 function (by BH3-mimetic treatment) converted incomplete MOMP into complete MOMP, thereby impeding cell survival[78].

Cell recovery via anastasis. To permit survival following extensive MOMP, ideally a cell would require prevention of caspase activation coupled to a means of generating (or retaining) non-permeabilized mitochondria. However, recovery from full-scale apoptosis has been described in mammalian HeLa cells exposed to ethanol and called anastasis (Greek for 'rising to life')[95]. Generally, ethanol induces MOMP and caspase activation. Intriguingly, removal of ethanol after caspase activation allowed recovery of intact mitochondria in some cells that enabled cell survival and proliferation. This recovery was rapid and within 24 h following removal of the apoptotic stimulus the entire mitochondrial population was reinstated. Survival under these conditions was associated with increased genomic instability, suggesting that anastasis may be oncogenic[95]. Anastasis was also associated with a specific transcriptional response programme that led to increased migratory capacity of recovered cells[96].

Overall, anastasis defies the dogma that MOMP and extensive caspase activity commits a cell to die. While fascinating, it also poses several challenging questions. First, how can a cell withstand such extensive caspase activity, causing widespread cleavage of subcellular substrates, yet survive? Second, why is the persistence of initiating apoptotic stimulus (in this case ethanol) required for death even following MOMP initiation of caspase activity? Third, how does the mitochondrial population recover so quickly following MOMP? Given the rapidity of mitochondrial recovery and a requirement to remove MOMP-inducing stimulus to enable cell survival, does this suggest that MOMP may be reversible in some situations? Further supporting a reversible nature of MOMP, a recent study reported a chemical inhibitor of mitochondrial apoptosis called compound A that blocks cell death downstream of BAX activation[97]. Compound A exerts a cytoprotective function by targeting succinate dehydrogenase subunit B (SDHB), a critical component of complex II in the electron transport chain. This cytoprotective effect is related to the inner mitochondrial membrane remodelling discussed above. By binding SDHB, compound A maintains electron transport chain function following BAX activation, which is proposed to inhibit OMA1 protease activity by preventing generation of ROS, which could activate OMA1. In doing so, compound A blocks OPA1 processing, inner mitochondrial membrane remodelling and extensive cytochrome *c* release. However, an alternative explanation may be that compound A prevents MOMP from initially occurring downstream of activated BAX. Irrespective of its cytoprotective mechanism, in vivo administration of compound A displayed beneficial effects in a rat model of Parkinson disease: it reduced the death of dopaminergic neurons and prevented the onset of Parkinson-like behaviour, implying that neuronal functionality, at least in the short term, is maintained[97]. Compound A may represent a basis to develop therapeutic inhibitors of the mitochondrial apoptotic pathway.

MOMP and inflammation

The textbook view of apoptosis is that it is a non-inflammatory, silent form of cell death[98]. Intuitively, this makes perfect sense — billions of cells in our bodies undergo mitochondrial apoptosis on a daily basis[99]. Despite this common view, recent research has shown that the apoptosis-initiating event, MOMP, is inherently pro-inflammatory (FIG. 4).

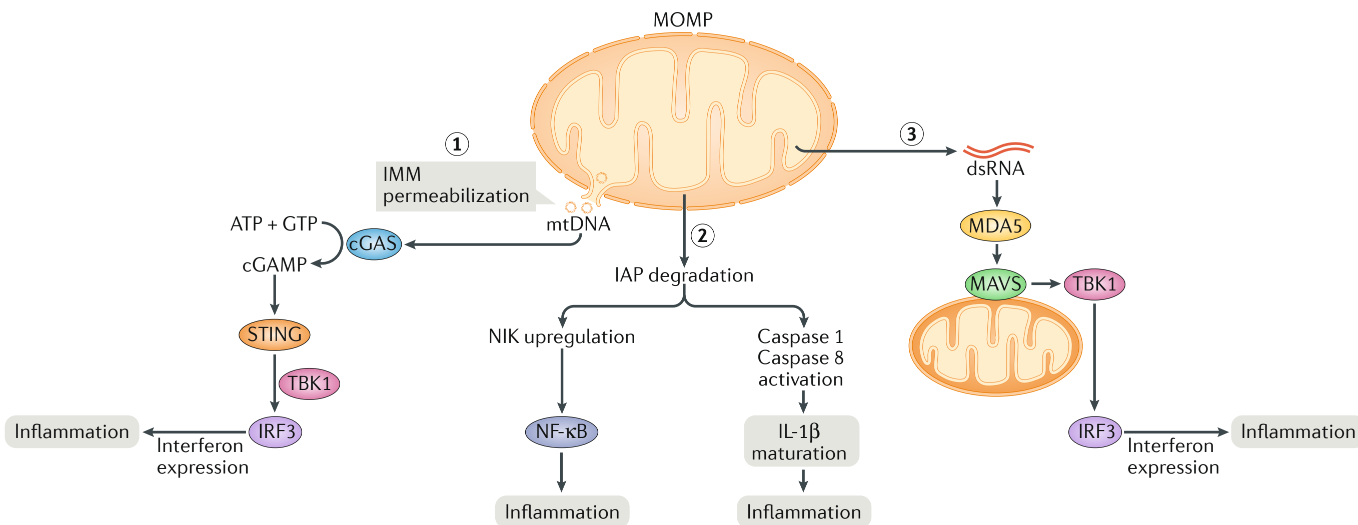


Fig. 4 | **Pro-inflammatory effects of mitochondrial outer membrane permeabilization.** Mitochondrial outer membrane permeabilization (MOMP) can induce inflammation in multiple ways. Following MOMP, the outer membrane pores progressively widen, enabling inner mitochondrial membrane (IMM) extrusion and rupture (step 1). This allows mitochondrial DNA (mtDNA) release into the cytosol whereupon it can engage cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) signalling, leading to pro-inflammatory interferon signalling. MOMP causes the proteasomal degradation of inhibitors of apoptosis proteins (IAPs), which leads to upregulation of nuclear factor-κB-inducing kinase (NIK) causing pro-inflammatory nuclear factor-κB (NF-κB) signalling and activation of caspase 8, in turn causing maturation of pro-inflammatory IL-1β (step 2). Under conditions of defective degradation of mitochondrial double-stranded RNA (dsRNA), such as knockdown of RNA degradosome components, dsRNA is released via an ill-defined mechanism from the mitochondria in a BAX/BAK-dependent manner (step 3). In the cytosol, dsRNA can bind adaptor protein MDA5, which then binds mitochondrial antiviral signalling protein (MAVS), which subsequently oligomerizes and activates NF-κB and interferon regulatory factor 3 (IRF3) to induce an interferon response. cGAMP, cyclic guanosine monophosphate–adenosine monophosphate; TBK1, TANK binding kinase 1.

Mechanisms and consequences of MOMP-driven inflammatory signalling. Pro-inflammatory effects of MOMP were first observed under conditions of caspase 9 deficiency, most likely because these cells show delayed death allowing inflammation to be detected[100,101]. A consequence of increased inflammation in caspase 9-deficient mice was that these mice displayed enhanced resistance to viral infection and impaired haematopoietic stem cell function[100,101]. Both phenotypes are associated with a type I interferon response that is induced by cyclic GMP–AMP synthase (cGAS)–stimulator of interferon genes (STING) signalling. The cGAS–STING signalling pathway is a key innate immune pathway that senses double-stranded DNA — mostly foreign, coming from bacteria or DNA viruses — to drive inflammation[102]. Upon DNA binding, cGAS catalyses the reaction of ATP and GTP to generate the secondary messenger, cyclic guanosine monophosphate–adenosine monophosphate (cGAMP). cGAMP binds to and activates the adaptor protein STING, which subsequently activates TANK binding kinase 1 (TBK1). TBK1 phosphorylates and activates the transcription factor interferon regulatory factor 3 (IRF3) as well as nuclear factor-κB (NF-κB), leading to a type I interferon expression.

BAX and BAK were found to be required for MOMP-induced cGAS–STING activity but, surprisingly, so was mitochondrial DNA (mtDNA), suggesting that mtDNA is recognized by cGAS–STING in the context of apoptosis, providing the basis for inflammatory signalling. This was unexpected because cGAS and STING reside outside the mitochondria, whereas mtDNA localizes to the mitochondrial matrix, and the inner mitochondrial membrane was thought to remain intact during apoptosis. Various studies employing different imaging approaches in murine embryonic fibroblasts as well as various cancer cell lines have addressed how mtDNA could be exposed to cGAS–STING[103–105]. Super-resolution imaging of cells undergoing mitochondrial apoptosis demonstrated that MOMP induction is followed, over time, by the formation of expanding pores on the mitochondrial outer membrane. These large pores, called macropores, were decorated with activated BAX at their edges[103,104], suggesting that BAX-mediated membrane permeabilization progresses over time, causing widening of these outer mitochondrial membrane pores. Similar BAX/BAK-dependent progressive membrane permeabilization has been previously reported in liposomes[45]. These macropores allowed extrusion of the inner mitochondrial membrane, which in some cases was associated with permeabilization of the membrane at such extrusions; this would allow mtDNA release and cGAS–STING activation (FIG. 2). Whether inner mitochondrial membrane permeabilization is regulated remains unclear. Although the underlying mechanism remains unknown, we know that it is independent of DRP1-mediated mitochondrial fission[103,104]. Furthermore, compared with healthy mitochondria, the matrix of apoptotic mitochondria is more dilute[105]. Potentially, the extra pressure associated with the increased volume of a more dilute matrix may

Type I interferon
Class of cytokines mediating inflammation.

be an important driver of both macropore expansion and inner mitochondrial membrane extrusion and subsequent rupture.

By allowing mtDNA release, inner mitochondrial membrane permeabilization may be an important initiator of inflammation in different areas of health and disease. One such example is Parkinson disease, which is associated with defective mitochondrial clearance through a selective autophagy process called mitophagy. Early-onset Parkinson disease is often caused by the loss of mitophagy regulators: the E3 ubiquitin ligase Parkin or its upstream mitochondrial kinase, PTEN induced putative kinase 1 (PINK1). Loss of PINK1 or Parkin has been found to activate cGAS–STING signalling, most likely by mtDNA released from defective mitochondria that are not cleared by mitophagy, leading to an inflammatory phenotype[106]. Underscoring the functional importance of this inflammatory response, deletion of STING prevents inflammation in Parkin-deficient mice, inhibiting the death of dopaminergic neurons and Parkinson-like behavioural defects[106]. Beyond driving Parkinson disease, cytosolic mtDNA has various other documented roles in inflammation and immunity, although how mtDNA is released into the cytoplasm in those different contexts remains unclear[107–109]. In many of these instances, mtDNA-dependent activation of inflammation occurs without cell death; it is possible that damaged mitochondria promote the activation of BAX/BAK, leading to inner mitochondrial membrane permeabilization downstream of MOMP as discussed above. Should BAX or BAK be required for mtDNA release in these circumstances, it must occur under conditions of minority MOMP. Relating this to our earlier discussion, the ability of minority MOMP to mediate pathogen clearance is, in part, due to mtDNA-dependent activation of cGAS–STING[94].

Besides mtDNA-dependent activation of cGAS–STING, MOMP engages additional pro-inflammatory signalling pathways (FIG. 4). Under caspase deficiency, MOMP caused downregulation of inhibitors of apoptosis proteins (IAPs), such as cIAP1 and cIAP2. This, in turn, upregulated NF-κB-inducing kinase (NIK), leading to NF-κB activation[110]. This mechanism is analogous to that previously observed with SMAC-mimetic compounds[111,112]. Like SMAC mimetics, MOMP can trigger NF-κB-dependent production of tumour necrosis factor that, coincidentally, can trigger an alternative form of cell death called necroptosis (discussed later) following MOMP[110]. Nevertheless, how MOMP triggers IAP depletion is unclear. While it requires the ability of cIAP1 to bind to SMAC-like proteins, combined genetic deletion of SMAC and OMI (another IAP binding protein) does not prevent cIAP degradation following MOMP. IAP degradation independent of SMAC and OMI may be due to redundancy with other mitochondrial IAP binding proteins[113,114]. Interestingly, MOMP in macrophages also causes IAP depletion but engages a different pro-inflammatory signalling pathway[115,116]. In macrophages, MOMP-dependent depletion of IAPs activated caspase 8 (REFS [117,118]). Caspase 8 activity promoted the maturation of the pro-inflammatory cytokine IL-1β[115,116]. By demonstrating caspase 8 activation downstream of MOMP, these studies also reveal a novel means of crosstalk between the intrinsic and extrinsic apoptotic signalling pathways. In parallel, the NLRP3 inflammasome is also activated downstream of MOMP, causing caspase 1-dependent IL-1β maturation[115,116]. In this context, the NOD-, LRR- and pyrin domain-containing 3 (NLRP3) inflammasome is activated by apoptotic caspase-dependent potassium efflux[119].

A final aspect of MOMP-induced inflammation relates to its recently described role in the release of mitochondrial double-stranded RNA (dsRNA), a potent trigger of an antiviral interferon response[120]. Because of its circular structure, bidirectional transcription of the mtDNA genome generates long dsRNAs. Normally, these dsRNAs are degraded by a protein complex called the RNA degradosome. Inhibition of RNA degradosome components causes accumulation of cytosolic dsRNAs that bind the adaptor molecule MDA5. MDA5 then activates the mitochondria-bound mitochondrial antiviral signalling protein (MAVS), which subsequently oligomerizes and activates NF-κB and IRF3 to induce an interferon response. Supporting the relevance of this pathway in vivo, patients bearing a hypomorphic mutation in polyribonucleotide nucleotidyl transferase 1 (PNPT1), an exoribonuclease involved in mitochondrial dsRNA breakdown and an RNA degradosome component, display increased markers of immune activation. Mitochondrial release of dsRNA requires either BAX or BAK, possibly engaging the same macropore-based mechanism as described for mtDNA[120].

Counteracting MOMP-induced inflammation. Although MOMP can engage a plethora of inflammatory signalling pathways, in most cases mitochondrial apoptosis is non-inflammatory. Trying to reconcile this observation, the likely main reason is that MOMP simultaneously activates apoptotic caspases to effectively quench inflammation (FIG. 5). Apoptotic caspase function inhibits inflammation at multiple levels. First, inflammatory signalling components including MAVS, cGAS and IRF3 are directly cleaved (and inactivated) by apoptotic caspases[121]. Second, apoptotic caspase function inhibits many processes, including protein translation and canonical protein secretory pathways, to prevent the production and release of inflammatory cytokines and thereby suppress inflammation[6]. Finally, caspase activity causes rapid cell death that is coupled with caspase-dependent generation of 'find-me' and 'eat-me' signals[122]. These signals recruit phagocytic cells to engulf and remove dying apoptotic cells before they can release any pro-inflammatory molecules. Nevertheless, caspase activity may not be absolutely essential to curb MOMP-driven inflammation. For instance, on some genetic backgrounds *Caspase-3*$^{-/-}$ or *Apaf1*$^{-/-}$ mice can survive to adulthood without an obvious hyper-inflammatory phenotype[123,124]. A potential explanation for lack of inflammation is that MOMP also engages additional caspase-independent anti-inflammatory mechanisms. One means is through MOMP-dependent release of PNPT1 from the mitochondrial intermembrane space, which causes global mRNA degradation

SMAC-mimetic compounds
Chemicals that were designed to phenocopy the inhibitor of apoptosis protein-binding and inhibitory properties of SMAC.

NLRP3 inflammasome
A protein complex containing NOD-, LRR- and pyrin domain-containing 3 (NLRP3) and caspase 1 that processes and activates inflammatory cytokines such as IL-1β and IL-18.

RNA degradasome
A multiprotein complex present in bacteria and mitochondria that degrades RNA.

'Find-me' and 'eat-me' signals
Molecular signals used by dying cells to attract phagocytes; examples of find-me signals include ATP and lysophosphatidylcholine, and the best-characterized eat-me signal is phosphatidylserine.

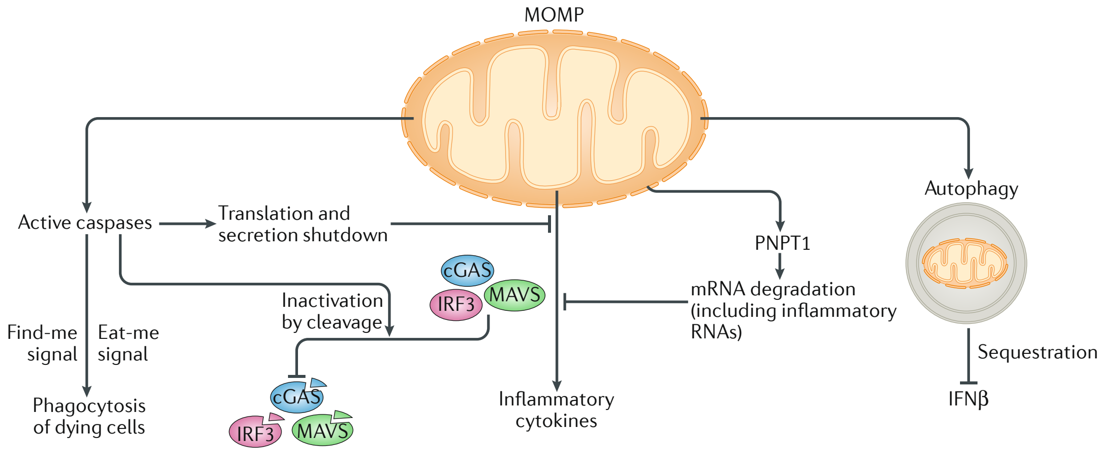


Fig. 5 | **Inhibition of mitochondrial outer membrane permeabilization-induced inflammation.** Inflammatory signalling downstream of mitochondrial outer membrane permeabilization (MOMP) is regulated in multiple ways. First, caspases inhibit multiple processes required for pro-inflammatory cytokine synthesis and secretion. This includes general downregulation of protein translation and canonical protein secretion to prevent the production and release of inflammatory cytokines. Caspases also directly cleave and inactivate various pro-inflammatory signalling molecules including cyclic GMP–AMP synthase (cGAS), mitochondrial antiviral signalling protein (MAVS) and interferon regulatory factor 3 (IRF3). Caspase activity also promotes the quick death and phagocytic removal of dying cells by invoking 'find-me' and 'eat-me' signals, limiting the time in which the dying cells can produce pro-inflammatory signalling molecules. Beyond the role of caspases, MOMP is associated with the release of RNA degradasome component polyribonucleotide nucleotidyl transferase 1 (PNPT1), which can cause global mRNA degradation, likely causing downregulation of inflammatory gene transcripts. MOMP also activates autophagy, which sequesters permeabilized mitochondria and inhibits the release of pro-inflammatory IFNβ.

and likely includes degradation of inflammatory transcripts[125]. Finally, MOMP engages autophagy, which supports autophagic sequestration of defective, permeabilized mitochondria. Autophagy also inhibits the secretion of specific pro-inflammatory cytokines such as IFNβ[126] (FIG. 5).

Because MOMP normally engages anti-inflammatory caspase activity, when the inflammatory consequences of MOMP would manifest is unclear. Tracking back to our discussion of minority MOMP and pathogen immunity, minority MOMP has been shown to trigger inflammation under caspase-proficient conditions; in this setting, MOMP-induced inflammation overrides anti-inflammatory signals associated with caspase activity[94]. This implies that MOMP has wide potential to drive inflammation, in particular in cell types exhibiting limited potential to engage caspase activity, such as cardiomyocytes (which show reduced APAF1 expression) or sympathetic neurons (which are characterized by increased expression of the caspase inhibitor XIAP)[127,128]. Mitochondrial apoptosis in these cells may thus potentially have deleterious consequences. In line with this, recent studies have shown that inflammatory cGAS–STING signalling contributes to pathology observed during cardiac infarction[129]. Whether MOMP drives this inflammatory phenotype is not known, but, in support of this idea, myocardia-specific deletion of anti-apoptotic protein MCL1 — leading to increased apoptotic potential — has previously been shown to cause heart failure associated with inflammation[130].

In cancer therapy, intense interest surrounds making cancer cell death immunogenic in order to engage antitumour immunity[131]. Cell death is typically immunogenic through two distinct, although not mutually exclusive, means: release of inflammatory molecules (for example, ATP, DNA), collectively referred to as damage-associated molecular patterns (DAMPs) from dying cells, or active engagement of pro-inflammatory signalling in the dying cell[132]. Unleashing pro-inflammatory effects of apoptosis can be achieved by caspase inhibition, resulting in caspase-independent cell death. As shown in cancer cells, this immunogenic type of cell death requires NF-κB activation in the dying cell[110]. Direct comparison of therapeutically inducing caspase-independent cell death versus canonical apoptosis demonstrated that, by engaging antitumour immunity, caspase-independent cell death is much more effective than apoptosis in clearing cancer cells, often leading to tumour regression. This suggests that inhibiting apoptotic caspase function may be beneficial in cancer treatment[110]. Supporting this idea, previous reports have shown that caspase inhibitors can have antitumour effects[133,134]. By eliciting an interferon response, targeting mitochondrial apoptotic caspase activity may also have antiviral activity. Indeed, genetic inhibition of caspase function enhances antiviral immunity that requires interferon signalling[100,121]. Moreover, emricasan, a clinically applicable pan-caspase inhibitor, was recently found to inhibit Zika virus infection, potentially by eliciting an interferon response[135].

Mitochondria beyond apoptosis

Mitochondria are central initiators of the intrinsic pathway of apoptosis, but they may also contribute to other forms of programmed cell death (FIG. 6). However, in these cases, their participation is less defined and not necessarily essential.

Ischaemic injury
Hypoxia-mediated injury due to diminished blood flow.

Mitochondria can support necroptotic signalling. Necroptosis is a regulated caspase-independent form of cell death that shares morphological and inflammatory characteristics with an unregulated, passive form of cell death called necrosis[136]. Aberrant levels of necroptosis have been implicated in various inflammatory diseases and ischaemic injury, making this cell death modality an important therapeutic target. Various stimuli, including

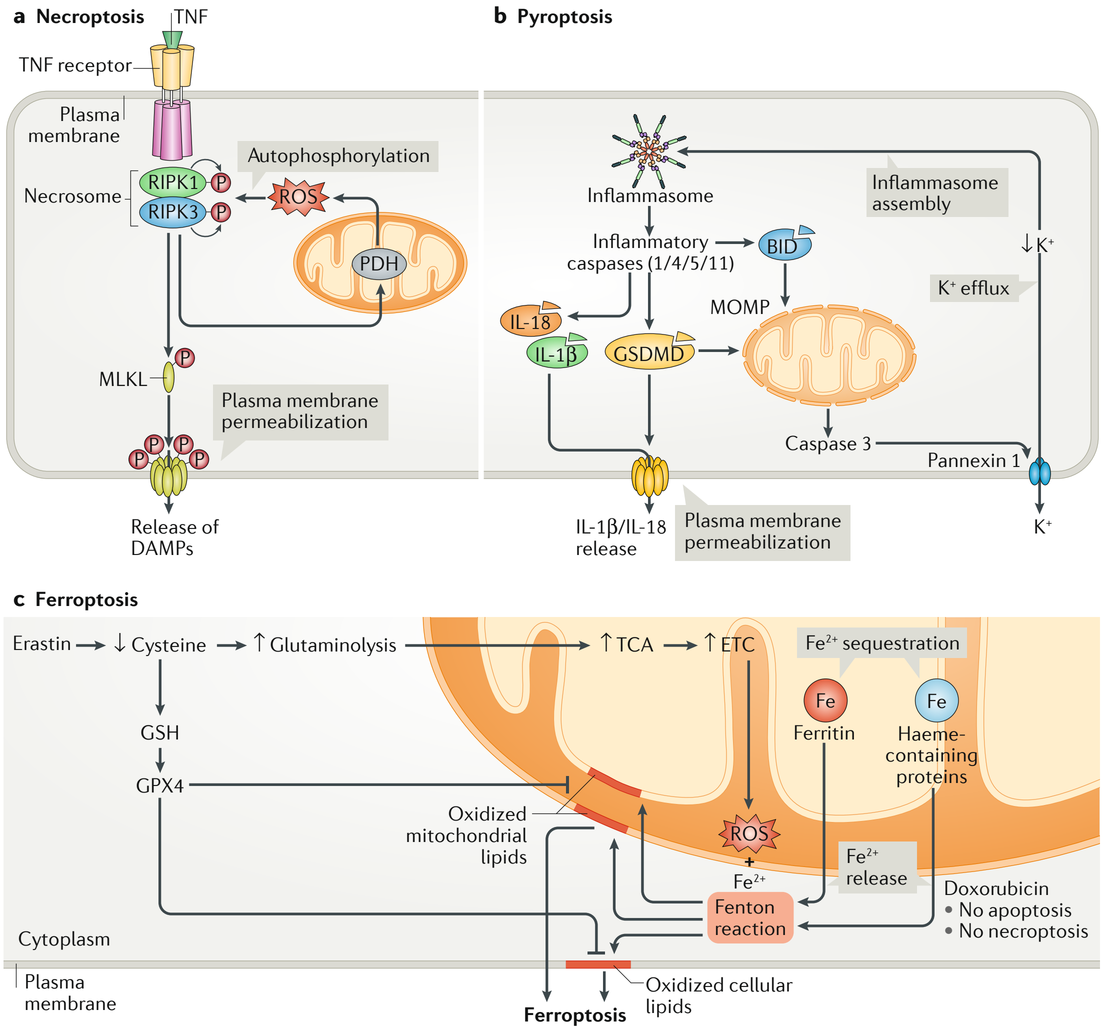


Fig. 6 | **Mitochondria and non-apoptotic cell death. a** | Necroptosis is a pro-inflammatory mode of cell death associated with the release of damage-associated molecular patterns (DAMPs). Various treatments can trigger necroptosis, which is best characterized following tumour necrosis factor (TNF) treatment. Under caspase inhibition, TNF treatment leads to sequential phosphorylation (P) and activation of receptor interacting protein kinase 1 (RIPK1) and RIPK3 and necrosome formation. The necrosome then phosphorylates and activates the pseudokinase mixed-lineage kinase domain-like pseudokinase (MLKL), which translocates to and permeabilizes the plasma membrane, killing the cell. RIPK3 also activates the mitochondrial pyruvate dehydrogenase (PDH) complex, causing enhanced aerobic respiration and increased generation of reactive oxygen species (ROS). These mitochondria-derived ROS can feedforward to enhance necrosome assembly and RIPK3 activity. **b** | Activation of inflammasome complexes, for instance by intracellular pathogens, causes activation of inflammatory caspases, which cleave pro-inflammatory cytokines IL-1β and IL-18, leading to their maturation. Inflammatory caspases also cleave and activate gasdermin D (GSDMD). Active GSDMD forms pores and permeabilizes the plasma membrane, leading to pyroptotic cell death. Active GSDMD can also cause mitochondrial outer membrane permeabilization (MOMP). Additionally, inflammasome activity promotes MOMP through cleavage and activation of the BH3-only protein BID. Downstream of MOMP, activation of caspase 3 leads to cleavage-dependent activation of the potassium channel-forming glycoprotein pannexin 1. This causes potassium efflux from the cell, which promotes inflammasome assembly. **c** | Ferroptosis is triggered by oxidized lipids in reactions catalysed with the help of iron and ROS (Fenton reaction). Defence against this reaction is provided by glutathione peroxidase 4 (GPX4), which inactivates harmful lipid peroxides. One means of ferroptosis induction is via treatment with erastin, which blocks import of cysteine and interferes with GPX4 activity or via cysteine deprivation. Beyond affecting GPX4, cysteine deprivation also causes increased glutaminolysis, which feeds the mitochondrial tricarboxylic acid (TCA) cycle, thereby increasing mitochondrial respiration and, in consequence, augmenting levels of mitochondrial ROS. Iron is stored in various iron-binding proteins including ferritin and haeme-containing proteins, and mitochondria contribute to this storage. These iron-storing proteins are degraded under certain cell death-inducing conditions, leading to iron release. Proximity of mitochondrial membranes to such sources of free iron and ROS makes them an important target for lipid oxidation associated with ferroptosis. ETC, electron transport chain; GSH, glutathione.

Toll receptor
A class of protein receptors that serve a key role in innate immunity by sensing conserved molecules derived from microorganisms.

Necrosome
A protein complex containing receptor interacting protein kinase 1 (RIPK1) and RIPK3 that promotes necroptotic cell death.

Fenton reaction
The reaction of peroxides with iron to yield free radicals.

Glutathione
A key cellular antioxidant that scavenges reactive oxygen species through reduction.

Ferritin
An iron-binding protein that plays important roles in the storage and transport of iron throughout the body.

Haeme
An iron-containing coordination complex present in haemoproteins such as haemoglobin, catalases and cytochrome c.

viral infection and Toll receptor signalling, can induce necroptosis, but it is best characterized in the context of tumour necrosis factor signalling. In a simplified model, under caspase 8 deficiency, tumour necrosis factor receptor engagement leads to activation of receptor interacting protein kinase 1 (RIPK1) and RIPK3, causing formation of the necrosome. RIPK3 phosphorylates mixed-lineage kinase domain-like pseudokinase (MLKL), leading to its activation[136]. Active, oligomerized MLKL permeabilizes the plasma membrane, killing the cell.

Do mitochondria have a role in necroptosis? Using a method of enforced mitophagy to deplete mitochondria, forced activation of RIPK3 by chemically induced dimerization has shown that necroptosis executes with the same kinetics, irrespective of mitochondria, consistent with activation of MLKL being the executioner mechanism of necroptosis[137] (FIG. 6). Nevertheless, at least in some cell types, mitochondrial ROS facilitate the initiation of necroptosis by promoting RIPK1 autophosphorylation, leading to its activation and necrosome formation[138,139]. In a feedforward manner, RIPK3 kinase activates the pyruvate dehydrogenase complex, leading to enhanced aerobic respiration and associated increased ROS generation[140] (FIG. 6). Because levels of ROS may be an important determinant as to whether a cell initiates necroptosis, progressive mitochondrial dysfunction — for example, that observed during ageing — may increase the propensity of cells to undergo necroptosis.

Interplay between mitochondrial apoptosis and pyroptosis. Pyroptosis is an inflammatory type of regulated cell death driven by the inflammatory caspases 1, 4, 5 and 11 (REF.[141]). Primarily serving as an innate immune response to intracellular pathogens, pyroptosis is executed by caspase-dependent cleavage of GSDMD[142,143]. Initiation of pyroptosis requires inflammatory caspase activation, which occurs on various signalling platforms that are collectively referred to as inflammasomes. During pyroptosis, the amino-terminal GSDMD cleavage fragment permeabilizes the plasma membrane, leading to the release of pro-inflammatory cytokines including IL-1β and IL-18.

Mitochondria lose function prior to GSDMD-dependent plasma membrane rupture, but there is little evidence that they play an important role in pyroptosis[144]. Nevertheless, extensive crosstalk exists between pyroptosis and mitochondrial apoptosis (FIG. 6). First, as discussed previously, the inflammasome-generated GSDMD amino-terminal cleavage fragment can induce MOMP, causing caspase 3 activation[55]. Second, in cells expressing low amounts of GSDMD, rather than pyroptosis, caspase 1 activation leads to mitochondrial apoptosis[145], which is, at least in part, due to caspase 1-dependent cleavage and activation of the BH3-only protein BID. Finally, mitochondrial apoptosis has also been shown to initiate activation of the NLRP3 inflammasome, leading to caspase 1 activity[145]. This requires caspase 3-dependent cleavage of a potassium channel-forming glycoprotein, pannexin 1, which activates the channel and causes potassium efflux from the cell, which promotes inflammasome assembly (FIG. 6). Although the physiological significance of crosstalk between different cell death modalities is currently unclear, this emphasizes that individual types of cell death cannot be viewed in isolation.

Mitochondria, ROS and membrane peroxidation in ferroptosis. Ferroptosis is another pro-inflammatory cell death modality, which is triggered by lipid peroxides that kill the cell by attacking lipid membranes, leading to loss of cell integrity[146,147]. As the name suggests, iron plays a crucial role in this process, as it is required for the Fenton reaction responsible for lipid peroxidation. Under normal circumstances, peroxidized lipids are converted into lipid alcohols by glutathione peroxidase 4 (GPX4), which inactivates these harmful peroxides. GPX4 requires glutathione as a cofactor to convert peroxidized lipids into lipid alcohols, and glutathione, in turn, requires cysteine. Transport of cysteine (via cystine, an oxidized cysteine dimer) into the cells is driven by the export of glutamate via System X_c^-, a mechanism that can be inhibited by a small-molecule inhibitor called erastin. Blocking System X_c^- with erastin therefore leads to decreased levels of glutathione, and subsequently impaired neutralization of lipid peroxides by GPX4 (REF.[148]).

A role for mitochondria in regulating ferroptosis is contentious. For instance, ferroptosis sensitivity has been found to be unaffected by loss of mtDNA or, indeed, removal of mitochondria[147,149]. Nevertheless, in some instances mitochondria can contribute to ferroptosis, which is mainly related to the generation of ROS (FIG. 6). For example, mitochondrial (as well as cytosolic) ferritin chelates iron and therefore prevents accumulation of free iron and iron-dependent lipid peroxidation by the Fenton reaction[150]. Along similar lines, the increase in free iron — as a result of haeme degradation — was shown to drive ferroptosis in vivo in mice, in apoptosis and/or necroptosis-deficient cardiomyocytes exposed to the DNA-damaging agent doxorubicin or ischaemia–reperfusion[151]. In this case, the excess free iron accumulated in mitochondria and caused lipid peroxidation of their membranes (FIG. 6). Another route to lipid peroxide accumulation in the mitochondria is cysteine deprivation, which promotes glutaminolysis, and therefore potently enhances mitochondrial respiration (by stimulating the activity of the tricarboxylic acid cycle). This leads to mitochondrial hyperpolarization and increased production of ROS, which was shown to promote lipid peroxidation and the induction of ferroptosis[152].

Conclusions and perspectives

In this Review we have discussed the central role of mitochondria in apoptotic cell death. Beyond discussing the well-established roles in the execution of cell dismantling via apoptotic signalling, we aimed to highlight the surprising new role of mitochondria as pro-inflammatory signalling hubs during apoptosis. Together with recent findings that cells can tolerate limited MOMP, this emerging role suggests that apoptotic signalling may have non-lethal functions.

Going forward, a key area of research will be to define the occurrence and roles of MOMP-induced inflammation in health and disease. This will require further understanding of how MOMP engages both pro-inflammatory and

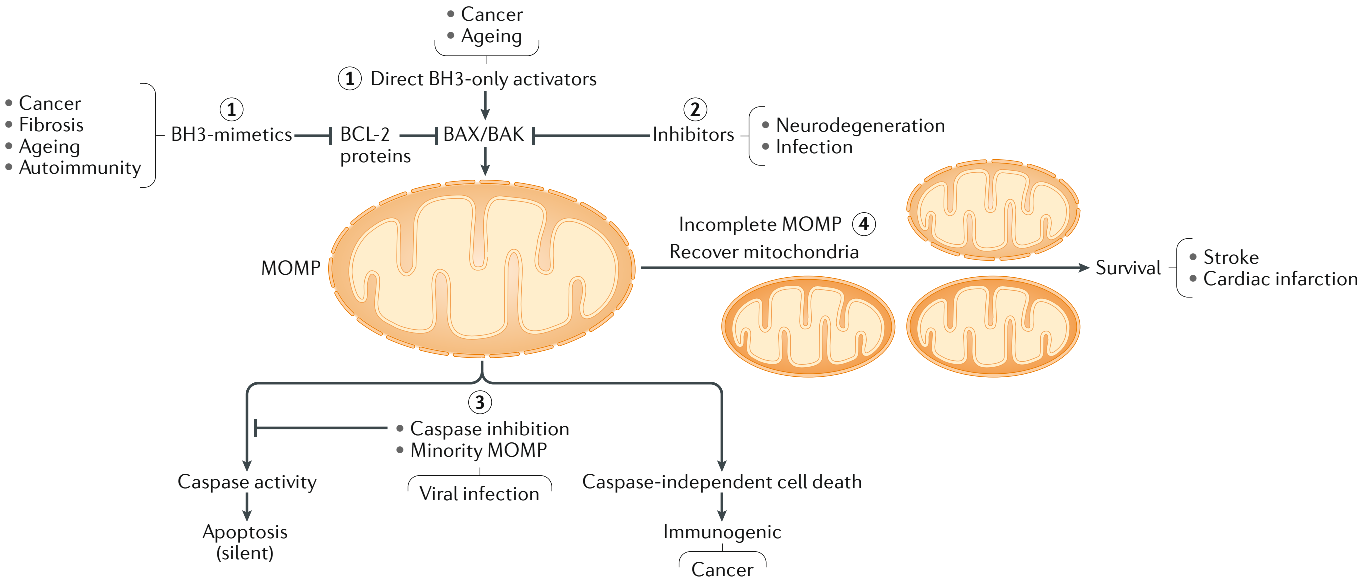


Fig. 7 | **Strategies to target mitochondrial apoptosis in disease.** Apoptosis can be activated either through inhibition of anti-apoptotic B cell lymphoma 2 (BCL-2) proteins (with BH3-mimetics) or by directly activating BAX/BAK (for example, with small molecules) (step 1). Such approaches have a proven use in oncology and have a clinical potential in the treatment of autoimmunity, fibrosis and ageing. Efficient inhibition of mitochondrial apoptosis can be achieved via blocking BAX and BAK (for example, with small molecules), which has a potential use in counteracting pathological cell loss, for instance, in the context of neurodegenerative diseases or infection (step 2). Inhibition of caspase function following mitochondrial outer membrane permeabilization (MOMP; see also FIG. 3) has the potential to turn apoptosis into an immunogenic type of cell death, which could be used to boost immune responses in antitumour and antiviral therapies (step 3). Better understanding of the heterogeneity of MOMP and mechanisms of mitochondrial network recovery in the absence of cell death following MOMP could be used to promote cell survival in the context of cell loss in response to various insults, such as stroke or infarction (step 4).

anti-inflammatory effects and how they interplay with each other. It will be interesting to address why these two opposing effects of MOMP coexist. One possibility is that the pro-inflammatory effects of MOMP evolved specifically to support innate immune responses to pathogen invasion. For instance, viruses can encode caspase inhibitors, and, in this scenario, induction of mitochondrial apoptosis by viruses could serve to elicit an antiviral interferon response.

The finding that MOMP can occur in the absence of cell death opens further research questions. As we have discussed, there is support for non-lethal apoptotic signalling; nevertheless, this evidence comes from in vitro experiments and the significance of non-lethal apoptotic signalling in vivo is currently lacking. Key to investigating this problem will be designing a way to mark mammalian cells in vivo that have undergone minority MOMP resulting in sublethal caspase activity using genetically tractable reporter systems, similar to analogous approaches in *Drosophila melanogaster*[153]. On a mechanistic level, a crucial question will be to understand why some mitochondria selectively undergo MOMP, since the mechanisms underlying this heterogeneity in MOMP are completely unknown at present.

Therapeutic targeting of mitochondrial apoptosis has great clinical potential in various diseases, best evidenced by the development of BH3-mimetics in oncology. We now have effective ways to sensitize cells to mitochondrial apoptosis (FIG. 7). Promoting mitochondrial apoptosis, using BH3-mimetics and possibly other approaches (for example, small-molecule BAX activators) may have utility in various settings including, but not limited to, cancer[3], fibrosis[154] and ageing[155]. Although our ability to therapeutically inhibit mitochondrial apoptosis trails behind the approaches to induce apoptosis, progress is being made with inhibitors of BAX/BAK-dependent apoptotic activity recently being described[156,157] that can promote neuroprotection in the context of neurodegenerative disease (FIG. 7). Recent discoveries that the outcome of apoptotic cell death (inflammatory versus non-inflammatory) can be modulated following MOMP, for example, by caspase inhibition, also opens new ways to think about therapeutically targeting the mitochondrial apoptotic pathway to promote immune responses against malignant, infected or otherwise dysfunctional cells (FIG. 7).

Finally, as we have discussed, mitochondria have also been implicated in other forms of regulated cell death including necroptosis, pyroptosis and ferroptosis, although their role in these types of cell death appears less crucial, or at least context dependent. Nevertheless, it is increasingly apparent that these different cell death modalities crosstalk with one another and that this crosstalk involves mitochondria. Given that some forms of cell death can be more inflammatory than others, how death is initiated, propagated and finally executed can have important consequences in cellular homeostasis as well as in the various disease settings involving deregulation of cell death.

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This study shows that in the absence of all known BH3-only proteins, inhibition of anti-apoptotic BCL-2 proteins is sufficient to activate BAX and BAK.

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