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When cell death goes wrong

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REVIEW ARTICLE

When cell death goes wrong: inflammatory outcomes of failed apoptosis and mitotic cell death

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Apoptosis is a regulated cellular pathway that ensures that a cell dies in a structured fashion to prevent negative consequences for the tissue or the organism. Dysfunctional apoptosis is a hallmark of numerous pathologies, and treatments for various diseases are successful based on the induction of apoptosis. Under homeostatic conditions, apoptosis is a non-inflammatory event, as the activation of caspases ensures that inflammatory pathways are disabled. However, there is an increasing understanding that under specific conditions, such as caspase inhibition, apoptosis and the apoptotic machinery can be re-wired into a process which is inflammatory. In this review we discuss how the death receptor and mitochondrial pathways of apoptosis can activate inflammation. Furthermore, we will highlight how cell death due to mitotic stress might be a special case when it comes to cell death and the induction of inflammation.

FACTS

- Death receptors can signal both cell death and inflammation.
- Through release of mtDNA, mitochondrial cell death can be inflammatory.
- Problems during mitosis lead to cell death.
- Mitotic stress can also induce inflammation.

OPEN QUESTIONS

- Under which circumstances is physiological apoptosis inflammatory?
- What are the differences between death receptors with regards to inflammation?
- Why is mitotic stress inflammatory?

INTRODUCTION

Every day billions of cells die in our bodies. In order to maintain a healthy, well-functioning organism, these cells must be rapidly removed to prevent any unwanted immune responses. To cope with this constant turnover, cells have developed elegant pathways of programmed cell death which execute the cell's demise [1, 2]. The best studied of these is apoptosis, a complex but highly regulated form of cell death which serves to activate caspase proteases resulting in the demolition of the cell. Apoptosis is executed through two main pathways: the extrinsic pathway,

which is activated by death receptors on the cell membrane or in the cytosol, and the intrinsic pathway, which harnesses unique features of the mitochondria to initiate cell death. Other forms of regulated cell death, such as necroptosis and pyroptosis, are highly inflammatory, serving as powerful barriers against bacterial, viral, protozoan, and fungal infection [3]. In contrast, given the large number of cells which undergo apoptosis, apoptosis does not elicit an immune response. However, work in the last number of years has contradicted this, and in certain and specific situations apoptosis (or rather activation of the apoptotic machinery) can be highly inflammatory leading to a potent induction of various inflammatory pathways, such as NF-κB and type I interferons [4–6]. In this review we will summarise the recent evidence that apoptosis and activation of the extrinsic, intrinsic and mitotic apoptotic machinery can be pro-inflammatory.

THE APOPTOTIC MACHINERY: A BRIEF PRIMER

Broadly, apoptosis is activated through one of two mechanisms. The first of these is the death receptor, or extrinsic pathway. Binding of a cognate ligand to a plasma membrane-bound death receptor (such as death domain (DD)-containing TRAIL-R1, TRAIL-R2 or Fas/CD95) stimulates the trimerisation of the receptor which is then capable of recruiting FADD through homotypic DD interactions on the cytoplasmic portion of the death receptor forming the death-inducing signalling complex (DISC) [7]. FADD in turn recruits death effector domain (DED)-containing proteins such as caspase-8, which is activated and cleaves a number of substrates [8, 9]. These substrates include the effector caspases-3 and -7 (in type I cells) or BID, a pro-apoptotic BH3-only protein

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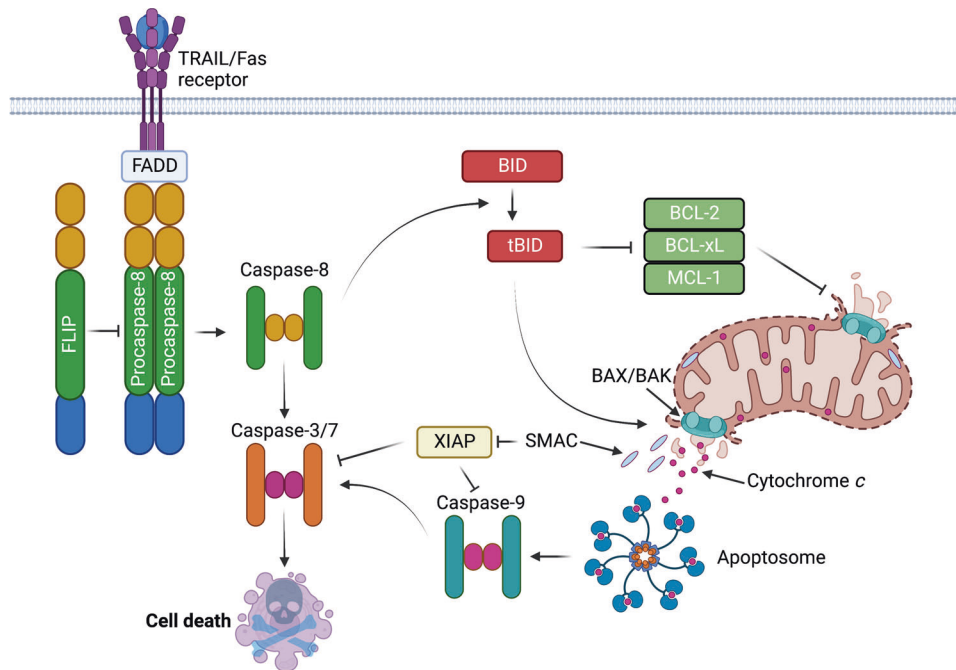
Fig. 1 Overview of the death receptor and mitochondrial pathways of apoptosis. Ligation of the TRAIL or Fas death receptor stimulates its trimerisation. FADD binds to the cytoplasmic death domain (DD) portion of the death receptor, which in turn recruits death effector domain-containing proteins such as caspase-8, cFLIP, and caspase-10 to form the death-inducing signalling complex (DISC). Activation of caspase-8 at the DISC forms a catalytically active dimer, which can cleave a limited number of substrates, but crucially including caspases −3 and −7 and BID. Cleavage and activation of the executioner caspases −3 and −7 drives cellular demolition.

which permeabilises the mitochondrial outer membrane (in type II cells) [10]. In addition, other DED proteins, notably c-FLIP, also form part of the DISC and depending on levels of expression can promote or inhibit apoptosis [11–15] (Fig. 1). Furthermore, DISCs can form intracellularly on autophagosomal membranes, not only at the plasma membrane [16].

The other pathway of apoptosis is the intrinsic, or mitochondrial pathway. Upon encountering a cellular stress, such as ER stress, DNA damage, or most anti-cancer drugs, cells activate BH3-only proteins. They bind and inhibit members of the pro-survival BCL-2 family, such as BCL-2, BCL-xL and MCL-1, whose main function is to safeguard the integrity of the mitochondrial outer membrane by binding and inhibiting BAX and BAK [17] (Fig. 1). When pro-survival BCL-2 proteins are inhibited BAX and BAK are free to be activated by BH3-only proteins, and form pores in the mitochondrial outer membrane. This process is known as mitochondrial outer membrane permeabilisation, or MOMP, and is the defining hallmark of mitochondrial apoptosis. BAX/BAK pores allow for the efflux of a number of different intermembrane space proteins, such as cytochrome *c*, SMAC, and Omi [18]. Upon entering the cytoplasm, cytochrome *c* binds and facilitates the formation of the apoptosome, comprised of multiple copies of APAF-1, which acts as an activation platform for caspase-9 [19]. Active caspase-9 cleaves a number of proteins, but most importantly it cleaves and activates caspases −3 and −7, which together execute the demolition phase of apoptosis [20, 21].

INFLAMMATION ARISING FROM DEATH RECEPTORS
Although most studies of death receptors focus on death as the outcome, death is not always inevitable from death receptor activation. Amongst the many studies which focus on the death of a cell from death receptor activation, there are reports that both TRAIL and Fas receptors promote the production of pro-inflammatory chemokines and cytokines [22–25] and that cells can survive TRAIL treatment [26]. Moreover, ligation of TRAIL and

Fas death receptors can have many other outcomes, such as increased cell invasiveness [27], cancer metastasis [28–30], proliferation [31], activation of dendritic cells [32], and entosis [33]. Indeed, the founding member of the tumour necrosis factor (TNF) superfamily, TNF, despite its name rarely kills cells, and requires the blockade of NF-κB-mediated transcription to do so (see Box 1). Together, the observations that TRAIL or Fas ligation can have pro-survival roles in part explains the relative lack of success of TRAIL receptor agonists in the clinic, particularly given that TRAIL can actually promote tumourigenesis [34].

At the molecular level, complexes formed following TRAIL and Fas receptor ligation are more complicated than originally understood. Initially, TRAIL receptor activation leads to the formation of a plasma membrane-bound DISC complex, comprised of the adaptor protein FADD, caspase-8, the long and short isoforms of c-FLIP, TRAF2, and RIPK1, among others, termed complex I. Here, ubiquitination events determine the signalling outcomes, whether that be cell death or gene expression. For example, caspase-8 can be modified with K63 ubiquitin chains by the E3 ligase cullin-3 [35], promoting its activation, whereas TRAF2 can modify caspase-8 with K48 ubiquitin chains, which trigger the proteasomal destruction of caspase-8 [36]. In this way, temporal changes in ubiquitination can act as a molecular timer of caspase-8 activation. More recently, the linear ubiquitin chain assembly complex (LUBAC) has been shown to ubiquitinate caspase-8 and RIPK1 with linear chains whilst they are in complex I [37]. LUBAC-mediated ubiquitination of caspase-8 serves to limit caspase-8 activation and, through NEMO binding to linear ubiquitin chains, recruits the IKK complex. LUBAC also ubiquitinates caspase-8 in complex II, a complex which forms after complex I previously thought to be mainly responsible for gene activation [38]. However, it is now understood that both TRAIL complexes I and II can mediate apoptosis and cytokine production [37].

What is the biological significance of cytokine and chemokine production in response to TRAIL and Fas receptor signalling? This has perhaps been best studied in the context of cancer, especially

Tumour necrosis factor (TNF) gained its name due to the observation that it could shrink some tumours [137]. However, when administered to patients, TNF causes shock and cytokine storms, which was attributed to huge upregulation in gene expression [138]. More recent studies have shown this to actually be due to TNF-induced cell death [139].

TNF binds to both TNFR1 and TNFR2, however since only TNFR1 possesses a death domain (DD) it is the only receptor which can reliably be called a death receptor. Binding of TNF to TNFR1 is receptor trimiersation, similar to TRAIL and Fas death receptor ligation. Following trimerisation, TRADD and RIPK1 are recruited through homotypic DD interactions [140], and this complex initiates the formation of the TNF receptor signalling complex, or TNF-RSC. TRAF2 is then recruited to TRADD, which can then recruit cIAP1 and cIAP2, E3 ligases which ubiquitinate RIPK1 and other proteins in the TNF-RSC. Addition of ubiquitin chains allows the recruitment of the linear ubiquitin chain assembly complex (LUBAC), a multimeric protein which forms another E3 ligase capable of conjugating M1-linked linear ubiquitin chains to components of the TNF-RSC. The orchestrated concert of ubiquitin chains added to TNF-RSC components by cIAP1, cIAP2 and LUBAC are essential to recruit kinase-containing complexes such as IKKα/IKKβ/NEMO [141], TAK1/TAB2/TAB3 [142] and IKKε/TANK/TBK1 [143]. Together, this complex, known as complex I, blocks cell death and activates NF-κB-, JNK- and p38-dependent innate immune and pro-survival gene transcriptional programs [144]. However, under certain circumstances such as incomplete ubiquitination of RIPK1, complex I can dissociate from the membrane and form a cytosolic complex II comprised of FADD, caspase-8, c-FLIP, RIPK1, as well as RIPK3 if expressed. FADD and RIPK1 can assemble to activate caspase-8, which induces apoptosis (complex IIa/b), or, if caspase-8 activity is blocked the cell death can be converted to necroptosis, which requires both RIPK1 and RIPK3 (complex IIc) [145–147].

In addition to complexes that form from the plasma membrane, intracellular death complexes can form independently of death receptor ligation. For example, the Ripoptosome forms in response to genotoxic stress and depletion of IAPs. In fact, this complex is identical to complex IIb, but since it forms independently of death receptor ligation it is termed the Ripoptosome [148, 149]. It can also contain additional proteins, such as caspase-10, c-FLIP$_L$, or RIPK3, depending on cell type, but importantly is capable of inducing apoptosis and inflammation [150].

since cytokines and chemokines have been shown to be important in the tumour microenvironment [39]. Cancer cells treated with TRAIL secrete a vast array of proteins, including the cytokines and chemokines CXCL1, CXCL4, CCL2, IL-8, and NAMPT [40]. Importantly, these cytokines and chemokines are secreted by cells which survive TRAIL treatment, rather than those which die, and strictly require the presence of FADD and caspase-8 (although studies differ as to whether TRADD is required) [40–42]. However, it does not require the caspase activity of caspase-8, as treatment with the pan-caspase inhibitor QVD does not alter the inflammatory outcome [42]. In line with this, Henry & Martin show that TRAIL treatment results in the production of cytokines and chemokines in HeLa cells, which can be uncoupled from cell death [42]. While caspase-8 activity is dispensable for this cytokine production, it requires FADD, RIPK1 and caspase-8 protein expression. Cytokine and chemokine secretion also occurs when cells are treated with doses of TRAIL insufficient to cause cell death. In this scenario, procaspase-8 aids the assembly of a cytoplasmic "FADDosome" complex consisting of FADD, RIPK1 and caspase-8, also known as complex II to draw parallels to TNF signalling (Fig. 2). Formation of the "FADDosome"/complex II drives NF-κB-dependent inflammatory gene expression. Additionally, LUBAC is recruited to both TRAIL complex I and II promoting gene expression and preventing apoptosis [37]. What is the function of this in cancer models? Non-small cell lung cancer (NSCLC) patients with high levels of *FADD* mRNA have poorer survival than patients with low *FADD* mRNA [43], leading to the tempting suggestion that the FADDosome might form downstream of TRAIL receptor ligation in cancers, driving inflammatory gene expression. Accordingly, deletion of FADD in a mouse model of NSCLC significantly decreased tumour burden as well as levels of IL-8, CXCL1 and CCL2 [40]. Furthermore, secretion of TRAIL/TRAIL receptor-dependent CCL2 produces a tumour-supportive microenvironment in which cancers can thrive. The FADDosome also forms in vivo following activation of the Fas receptor in mice lacking MLKL (crucial for necroptosis) and harbouring a non-

cleavable caspase-8 ($Casp8^{DA/DA}Mlkl^{-/-}$), and deletion of one allele of *Fasl*, *Fadd* or *Ripk1* was sufficient to abrogate FADDosome-dependent inflammation, highlighting the important role for this complex in vivo [44]. Interestingly, selective expression of different head and neck cancer associated-caspase-8 mutations show different capacities to mediate inflammation [45]. For example, the D303G mutation appears to increase chemokine and cytokine production in cells and in xenograft in vivo models. It will be interesting to see if these findings can be confirmed in more sophisticated mouse models of cancer.

Taxanes are a group of microtubule poisons that induce mitotic arrest and cell death via various cell death modalities [46] and are commonly used in the clinic for the treatment of lung, breast, and ovarian cancers. There is extensive evidence in the literature showing that taxanes invoke inflammatory responses in cancers, facilitating cancer progression and resistance [47–51]. However, in addition to being microtubule poisons that induce cell death, they also robustly induce endoplasmic reticulum (ER) stress, which is known to cause ligand-independent TRAIL receptor activation, although this remains the topic of some debate [52–55]. How TRAIL receptors can activate and signal without a ligand was a mystery. Whilst others have reported that misfolded proteins can directly bind and activate TRAIL-R2 to invoke cell death [56], Sullivan *et al* showed that ER stress leads to the transcriptional upregulation of TRAIL receptors, which can then stimulate the formation of the FADDosome complex, activating NF-κB and expression of cytokines and chemokines [57] (Fig. 2). This induction of inflammation does not fit within the usual pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) paradigm [58], and so a new term has been coined: stress-associated molecular patterns (SAMPs), which could potentially form in response to a wide variety of stressors.

Similar to TRAIL/TRAIL-R signalling, binding of Fas ligand to the Fas receptor stimulates the formation of a FADD/caspase-8/c-FLIP DISC which is capable of activating caspase-8 and inducing rapid cell death. Until recently, apoptosis was thought to be an immune-silent event, which does not invoke an immune response. However, work in the last decade has shown that dying cells secrete a number of "find-me" signals that attract phagocytic cells to the dying cell to aid in the removal of the corpse. These "find-me" signals include ATP [59], lysophosphatidylcholine (LPC) [60], and IL-8, among others [61]. More recently, it was found that upon stimulation with Fas ligand, multiple cell types can secrete more conventional cytokines and chemokines, such as IL-6, CCL2, CXCL1, and sICAM-1 in a manner that can be uncoupled from cell death [62]. Importantly, this appears to be dependent on inhibitor of apoptosis (IAP) proteins, as treatment with BV6 (an IAP antagonist) blocks the production of cytokines as well as RIPK1 [62].

INFLAMMATION ARISING FROM THE MITOCHONDRIAL CELL DEATH MACHINERY

The large majority of apoptosis is executed via the mitochondrial cell death pathway. In a healthy cell, the mitochondrial outer membrane is kept under constant guard by a delicate balance of pro- and anti-apoptotic proteins, which together maintain membrane integrity. Upon encountering a cellular insult, the balance of these proteins is disrupted in such a way that it tips in favour of pro-apoptotic proteins, allowing BAX/BAK to form pores on the mitochondrial outer membrane [17] (Fig. 1). Through these pores flow a number of different proteins, such as cytochrome *c*, SMAC and Omi, and when they enter the cytoplasm they activate caspases [17]. For example, when cytochrome *c* enters the cytoplasm it triggers the formation of the apoptosome, a multimeric complex of APAF-1 at which the initiator procaspase-9 is activated. This triggers a caspase cascade which activates caspases −3 and −7 which ultimately demolish the cell by
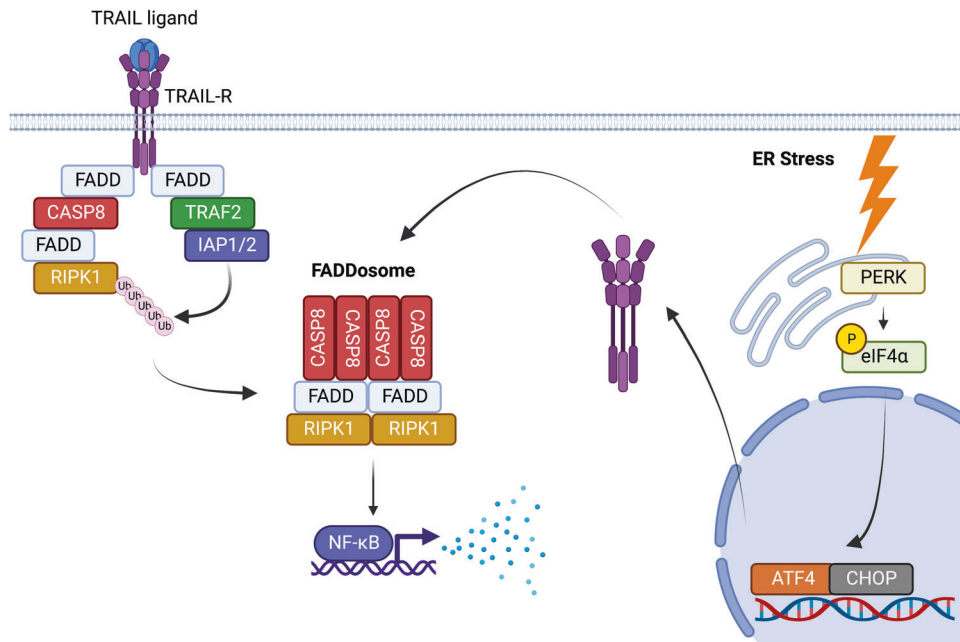
Fig. 2   Death receptor apoptotic machinery can be proinflammatory. Ligation of the TRAIL receptor stimulates the formation of the death-inducing signalling complex (DISC). FADD is the first to be recruited, which allows the recruitment of multiple copies of caspase-8 through death-effector domain (DED) interactions. RIPK1 is also recruited, and can be modified with ubiquitin chains by cIAP1/2. This complex can then dissociate from the plasma membrane, forming the FADDosome which can activate NF-κB-dependent gene transcription and cytokine and chemokine production.

cleaving a large array of substrates. Similarly, when SMAC enters the cytoplasm it binds and neutralizes members of the IAP family, cellular inhibitors of caspases. Importantly, mitochondrial permeabilisation is a point-of-no-return, and once it has occurred, the cell will die regardless of caspase activation.

In contrast to other forms of cell death, such as necrosis, apoptosis has been considered a largely regulated and "immune-silent" form of cell death. However, work from the past number of years has shown that mitochondrial apoptosis in particular is far from immune-silent and in fact has the potential to trigger potent immune responses under specific conditions, such as when caspases are inhibited. One of the major drivers of this is due to the origins of mitochondria, which arrived into a host cell related to Asgard archaea as an endosymbiotic alphaproteobacterium, and over time have become adapted to the host through significant proteome rewiring [63, 64]. However, due to this bacterial origin they have maintained their own independent genome, mitochondrial DNA (mtDNA). Usually mtDNA is kept inside the mitochondria and away from innate immune sensors in the cytoplasm. But what happens if the mitochondrial membranes are permeabilised? A number of studies in 2014 were the first to show that pharmacological or genetic blockade of caspases was sufficient to drive a cGAS-STING type I interferon response in cells [5, 6]. Further investigation revealed that mtDNA was being released from the mitochondria into the cytoplasm. Importantly, the activity of apoptotic caspases (such as caspase-9 and caspase-3/7) was enough to block this inflammatory response, rendering apoptosis immunologically silent. Using super-resolution microscopy, we and others have shown that BAX/BAK pores during the initial stage of MOMP are large enough to only permit the release of small proteins, such as cytochrome *c*. However, these initial pores rapidly unite to form large BAX/BAK "macropores" through which the mitochondrial inner membrane herniates, ruptures, and allows the release of mtDNA into the cytoplasm [65–67]. Once in the cytoplasm, it is detected by cGAS, which catalyses production of a second messenger cGAMP, which then activates STING. STING subsequently activates TBK1, which phosphorylates IRF3

allowing the expression of type I interferon genes [68] (Fig. 3). Importantly, it should be noted that for MOMP-induced inflammation to proceed, caspases must be inhibited. One of the reasons for this is that proteins in innate immune pathways, such as cGAS, IRF3 and MAVS are rapidly cleaved and inactivated by caspases, helping to maintain the immuno-silent nature of apoptosis [69].

The release of mtDNA during cell death has now been noted in a variety of different pathological settings [70]. In cancer, inducing mitochondrial cell death with BH3 mimetics while simultaneously inhibiting caspase activity may seem counterintuitive, but has actually been shown to be a more effective means of controlling tumour growth. Using a variety of in vitro and in vivo models of cancer, Giampazolias et al. showed that deleting APAF-1 or pharmacological caspase inhibition not only activated NF-κB-dependent inflammation (through the release of SMAC and activation of NIK), but also cGAS-STING-dependent type I interferon responses (through the release of mtDNA into the cytoplasm) [4]. In a mouse model of breast cancer, irradiation causes MOMP, leading to the release of mtDNA through BAX/BAK pores [71]. In this scenario, if autophagy is inhibited mtDNA accumulates in the cytoplasm, triggering cGAS-STING-dependent type I interferon responses, a process that bears resemblance to mtDNA in heart failure [72]. In mouse models of colorectal cancer, inhibiting apoptotic caspase activity using FDA-approved emricasan/IDN-6556 combined with radiation enhances anti-tumour immunity, resulting in better tumour control [73]. Together, this strongly suggests that targeting MOMP while simultaneously inhibiting caspases could be a new strategic approach to treating a variety of different cancers.

The cGAS-STING axis is not the only immune pathway activated by mtDNA release into the cytoplasm. Some viruses, such as severe fever with thrombocytopenia syndrome virus (SFTSV) also release mtDNA in a BAX/BAK-dependent mechanism (though the precise mechanism is unclear); however the mtDNA is not recognised by cGAS but rather by the NLRP3 inflammasome, a multiprotein cytosolic sensor of DNA [74] (Fig. 3). Caspase-1 is
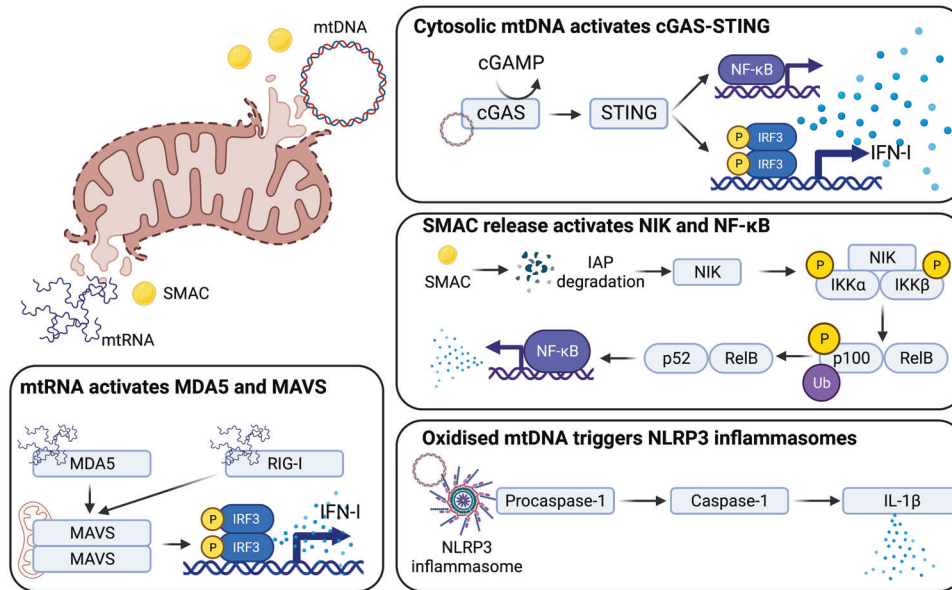
Fig. 3 Mitochondrial apoptosis is pro-inflammatory via BAX/BAK pores. Permeabilisation of the mitochondrial outer membrane by BAX/BAK can trigger inflammation in a number of different ways. Firstly, following MOMP, the inner membrane herniates and permeabilises, allowing the efflux of mtDNA into the cytoplasm where it activates cGAS-STING-dependent inflammatory responses, such as activation of type I interferon responses and NF-κB. SMAC release during MOMP triggers IAP degradation and NIK activation, leading to NF-κB activation. During infection with viruses, such as SFTSV, BAX/BAK pores facilitate the release of oxidized mtDNA, which can bind and activate the NLRP3 inflammasome, triggering NF-κB responses. Finally, mitochondrial RNA can be released via BAX/BAK pores, which are detected by MDA5 and MAVS in the cytoplasm, triggering a type I interferon response.

recruited to, and activated at, the NLRP3 inflammasome, which enables the cleavage and secretion of IL-1β. IL-1β is secreted by cells as part of an innate anti-microbial defence system. Interestingly, cells exposed to IL-1β are known to activate cGAS-STING signalling, which is now understood to be due to mtDNA release [75]. While not formally tested, the authors speculate that this might be due to BAX/BAK pore formation, since FOXO3 is activated following IL-1β stimulation, and there is evidence, albeit limited, suggesting that FOXO3 is linked to a "transient MOMP" [76, 77]. Similarly, TNF treatment induces the release of mtDNA into the cytoplasm in a model of arthritis, and again, although not formally tested, it seems plausible that this could be due to BAX/BAK pore formation [78]. Although this observation may be completely unrelated to apoptosis since there appears to be no loss of cell viability, it is intriguing that pharmacological caspase inhibition with zVAD-fmk enhances interferon production. Thus, it is tempting to speculate that there may be some degree of non-lethal "minority MOMP" (see below), although this should be formally tested.

In another example of inflammatory signals flowing from permeabilised mitochondria, SMAC release via BAX/BAK pores can trigger NLRP3 inflammasome activation in macrophages. Here, released SMAC binds and facilitates the degradation of IAPs, licensing formation of the Ripoptosome and caspase-8 activation which enables IL-1β secretion [79] (Fig. 3). Alternatively, SMAC release can also lead to IAP depletion allowing NLRP3 inflammasome formation which can likewise lead to IL-1β secretion, in a manner independent of gasdermin proteins [80].

It should be noted that not all mtDNA release occurs as a result of BAX/BAK-mediated permeabilisation of the mitochondrial outer membrane. Amyotrophic lateral sclerosis (ALS) is characterised by cytoplasmic accumulation of TDP-43, a nuclear DNA/RNA binding protein, which can enter the mitochondria [81–83]. TDP-43 expression in cells triggers the release of mtDNA into the cytoplasm through the mitochondrial permeability transition pore (mPTP) where it is detected by cGAS, activating a STING-dependent inflammatory response [84]. In a mouse model of

the autoimmune disease lupus, voltage-dependent anion channels (VDACs) can form pores in the mitochondrial outer membrane in response to oxidative stress, leading to mtDNA expulsion and cGAS-STING-dependent inflammation [85]. VDAC channels have also been implicated in mtDNA release observed in skin and lung samples from patients infected with SARS-CoV-2 [86]. Given the intimate relationship between BAX/BAK and VDACs, it is possible BAX/BAK may have a role [87], though the requirement for VDACs in mitochondrial permeabilisation is still a subject of debate [88, 89]. Finally, gasdermins D and E (GSDMD, GSDME) are pore forming proteins more recognised for their roles in permeabilising the plasma membrane during pyroptosis. However, a number of laboratories have shown that GSDMD and GSDME can both permeabilise the mitochondrial outer membrane [90, 91], and in the case of NLRP3 inflammasome activation, GSDMD can permeabilise the mitochondria to allow the efflux of mtDNA [91].

In addition to mtDNA, mitochondria also contain mtRNA. Under homeostatic conditions, this mtRNA is degraded by the degradosome, principally comprised of the mitochondrial RNA helicase SUV3 and polynucleotide phosphorylase PNPase. When this is disrupted (for example in patients with mutations in PNPase) mtRNA accumulates. In such pathogenic conditions, mtRNA is released through BAX/BAK pores on the mitochondrial outer membrane and into the cytoplasm where it is detected by the RNA sensor MDA5, which through MAVS elicits a type I interferon response [92] (Fig. 3). Similarly, mitochondrial-targeted transcription activator-like effector nuclease (TALEN)-mediated induction of mtDNA damage or radiation activates BAX/BAK pore formation on the mitochondrial outer membrane, facilitating the release of mtRNA which is detected by RIG-I (and presumably MDA5), triggering a type I interferon response [93] (Fig. 3). Notably, BAX/BAK pore formation following mtDNA damage induced by TALENs is not apoptotic, and thus does not cause caspase activation. How BAX/BAK can form pores on the mitochondrial outer membrane that does not result in apoptotic signalling is intriguing, but as of yet unclear.

Mitochondrial permeabilisation as an immune alarm

Permeabilisation of the mitochondrial outer membrane has always been thought of as an all-or-nothing event, in that within a single cell all mitochondria undergo MOMP and the cell rapidly dies [94, 95]. However, the general idea that all cells treated with a cell death-inducing stimulus die has been challenged: in 2010, Lovric & Hawkins noted that in a given population of cells treated with TRAIL some cells die, but not all [26]. Although they did not test a role for mitochondria, the cells which survive often have mutations dependent on caspase-8 activation, showing that surviving cell death creates mutations in the cells that do not die. Subsequently, we and others showed that treating cells with sub-lethal doses of drugs which invoke cellular stresses or anti-cancer drugs was able to permeabilise only a few mitochondria within a cell, challenging the notion that MOMP occurs in all mitochondria in a cell. Importantly, in cells which undergo "minority MOMP", we were able to detect a small, but significant, degree of caspase activation, enough to cleave the inhibitor of caspase-activated DNase (ICAD), releasing active CAD which induces DNA damage and genomic instability [96, 97]. We also recently defined the underlying basis of this process, showing that minority MOMP induced by BCL-2 antagonism occurs preferentially on dysfunctional, fragmented mitochondria, which possess higher levels of anti-apoptotic BCL-2 expression and higher BAX due to reduced retrotranslocation, rendering them more primed towards minority MOMP [98].

Since mtDNA can be extruded from mitochondria during cell death, and that it is possible that only a limited subset of mitochondria can undergo MOMP in a given cell, Brokatzky et al set out to test the possibility that pathogens could trigger minority MOMP. Indeed, they were able to show that sub-lethal doses of BH3 mimetics was sufficient to trigger IL-6 release in a BAX/BAK-, BCL-xL- and STING-dependent manner [99]. Furthermore, when screening various of bacterial, viral and protozoan pathogens they were able to show that infection also causes minority MOMP and DNA damage. In cells, the function of this is likely to induce MOMP to trigger mtDNA release (or release of other proteins) to activate cGAS-STING and elicit a type I interferon response. In this manner, the infected cell becomes an "alarm" for the surrounding cells, perhaps to warn them of a potential threat. Similarly, infection with *Helicobacter pylori* also induces sub-lethal caspase activation and inflammatory signalling. In contrast to other pathogens however, *Hp* infection seems to only permit the release of SMAC from BAX/BAK pores, and not cytochrome *c*. How SMAC can be preferentially released from BAX/BAK pores remains to be elucidated, but it is in line with other data [100, 101]. One tempting hypothesis may be drawn from recent data from the Garcia-Saéz laboratory, who showed that BAX and BAK form different-sized pores at divergent rates. It has been known for a number of years that BAX and BAK can form pores that resemble lines, arcs and rings [102, 103]; however we now know that BAK can oligomerise and form pores faster, but at a smaller size than BAX [104]. Crucially, this means cells which predominantly express BAK release mtDNA faster than cells which only express BAX. The mitochondrial protein ERA G-protein-like 1 (ERAL1) is also released from the mitochondrial matrix through BAX/BAK pores following infection with RNA viruses such as VSV and Sendai virus [105]. Translocation of ERAL1 into the cytoplasm appears to promote ubiquitination of RIG-I and MDA5, which promote oligomerisation of MAVS on the mitochondrial outer membrane, resulting in anti-viral responses [105]. However, it is not clear from this study how cells remain alive, as formation of the BAX/BAK pore would usually result in a rapid and complete cell death, so it is possible that other processes, such as minority MOMP and/or caspase inhibition could play a role.

INFLAMMATION ARISING FROM MITOTIC CELL DEATH

The correct and error-free execution of cell division is essential for an organism to prevent the introduction or propagation of damaged or dangerous cells [106]. Errors introduced during mitosis can have a profoundly negative impact if passed on to daughter cells. Cells have therefore developed several strict checkpoints during the cell cycle that have to be passed before they can progress [106]. Failure to fulfill these checkpoint requirements can lead to a stall in mitosis to remedy the underlying reason of the failure to pass the checkpoint. If this stall cannot be overcome, e.g., if the sustained damage is too severe, the cell undergoes cell death. While cell death due to errors in mitosis often uses much of the same machinery as the intrinsic pathway of apoptosis described previously, particularly in the execution of the death, how it is specifically induced is still a matter of active research and not yet understood in detail.

Recent publications suggest that activation of caspase-2 in a complex called the PIDDosome promotes cleavage and inactivation of MDM2, which under basal conditions promotes degradation of p53 [107–110]. Activation of p53 can then push cells towards cell cycle arrest, senescence, or apoptosis, the distinction between those different outcomes believed to be due to the dynamics and sustained levels of increased p53 [111–113]. In some cancer cells, active nuclear caspase-8 cleaves the deubiquitinase USP28 in response to DNA damage. The cleaved USP28 is therefore incapable of stabilizing p53, enabling the cell to progress in mitosis instead of undergoing p53-mediated apoptosis [114].

Like apoptosis triggered by other stresses, mitotic cell death relies on activation of pro-apoptotic BH3-only proteins and concomitant inactivation of pro-survival BCL-2 family members [115]. Several of these pro- and anti-apoptotic proteins are modified by components of the cell cycle machinery. While in reality the picture might be more complicated and is still to be elucidated, current models suggest that the interplay between mitotic progression and induction of apoptosis ultimately decides the fate of a cell encountering problems during mitosis.

In addition to components of the extrinsic pathway, components of the extrinsic pathway interface with the regulation of mitosis. A non-apoptotic and non-inflammatory "Ripoptosome" (see Box 1) forms during mitosis to ensure that chromosomes are segregated correctly [116]. Furthermore, phosphorylation of procaspase-8 by Cdk1/cyclin B1 and polo-like kinase 1 (Plk1) prevents its activation during mitosis [117], while phosphorylation of FADD by casein kinase 1α is required for the progression of mitosis [118].

While canonical apoptosis is considered immunologically silent and not able to trigger an inflammatory response, does the same hold true for mitotic cell death? While clear evidence that mitotic cell death itself is inflammatory is unavailable, some features leading to its induction make it more likely to induce an inflammatory response. One trigger of mitotic cell death is DNA damage, induced by radiation or genotoxic drugs. Additionally, in a pathway reminiscent of minority MOMP (see above), prolonged mitotic arrest can lead to limited activation of caspases. After caspase-mediated cleavage of ICAD, activated CAD can then cleave genomic DNA and thereby induce DNA damage [119]. Even though it has been known for a while that DNA damage can induce inflammation, this activation seems to be independent of mitosis and rather mediated by the canonical DNA damage response [120, 121]. DNA damage occurred during mitosis can lead to DNA double-strand breaks and the formation of micronuclei, fragments of chromosomes encapsulated by nuclear membranes outside the nucleus. These micronuclei can then be detected by cGAS, leading to activation of an interferon response and inflammation [122, 123]. A similar effect has also been described during senescence: here cytoplasmic chromatin fragments (CCF) translocate from the nucleus to the cytoplasm, where they are subsequently detected by cGAS and induce inflammation [124–126]. These data are in line with the general perception that cGAS is a sensor of "out of place" dsDNA, for example from pathogens or mitochondria. Chromatin fragments, micronuclei or other sources of nuclear DNA in the cytoplasm are detected by
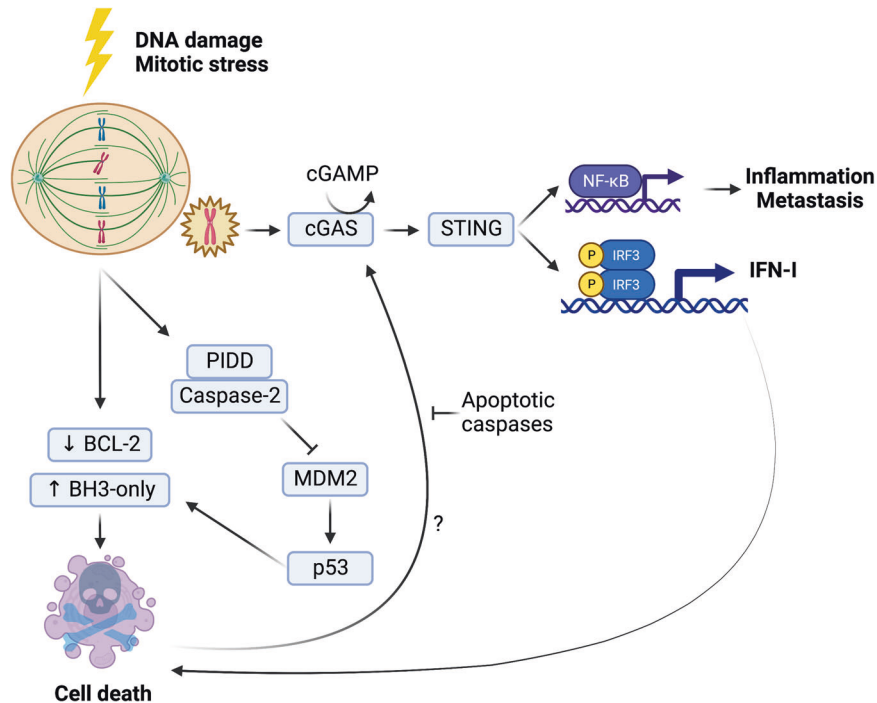
Fig. 4 Stress during mitosis activates apoptosis and inflammation. DNA damage or other mitotic stresses can lead to defects in mitosis, including chromosomal instability and micronuclei. These issues are detected by various surveillance mechanisms to prevent the propagation of potentially mutated or otherwise harmful cells. Micronuclei or cytoplasmic chromatin fragments are detected by the cGAS-STING pathway, leading to the activation of type I interferon, NF-κB signaling, and subsequently inflammation, but also metastasis or cell death. Supernumerary centrosomes promote the activation of the PIDDosome, which cleaves and inhibits MDM2. Therefore, p53 is stabilized and can promote apoptosis, cell cycle arrest or senescence. Mitotic stress also shifts the balance of pro-survival BCL-2 proteins and proapoptotic BH3-only proteins to promote apoptosis.

cGAS and constitute signs that necessitate an inflammatory response. Several mechanisms exist to prevent accidental activation of cGAS, which can also be found in the nucleus, such as its tight tethering to nucleosomes [127]. Inhibition of cGAS in this way also prevents its activation during mitosis, when the nuclear envelope breaks down and nuclear DNA is exposed to cGAS.

Surprisingly, activation of the cGAS-STING pathway itself can eventually lead to the induction of mitotic cell death [128]. Here, prolonged mitotic arrest leads to low-level activation of IRF3 by cGAS. Interestingly, while this IRF3 activation is insufficient to induce an inflammatory response, by a yet undefined mechanism this cGAS-STING-IRF3 axis promotes MOMP via inactivation of BCL-xL [128] (Fig. 4).

In cancer, activation of cGAS due to chromosomal instability and micronuclei can also have negative effects. Mediated by STING, tumor cells with chromosomal instability induce NF-κB signaling and inflammation, promoting metastasis [129]. These results are in accordance with previous research showing heightened carcinogenesis due to STING-dependent inflammation [130]. Remarkably, tumour cells have even developed mechanisms to counteract anti-tumour immunity evoked by STING in chromosomally instable cells. By expressing the ectonucleotidase ENPP1, they degrade the cGAMP that is produced by cGAS and excreted to the extracellular space [131]. Therefore, cGAMP cannot be recognised by surrounding immune cells and activate an anti-tumour response in them. In this way, the tumor can become resistant to elimination by the host immune system.

CONCLUDING REMARKS
It is now evident that in addition to being immuno-silent, apoptosis can be, under certain circumstances, an inflammatory event. Active areas of current research include uncovering in which physiological settings apoptosis can be inflammatory, independent of pharmacological or genetic inhibition of caspases, as well as how it can be exploited therapeutically. For example, cardiomyocytes show a decreased expression of APAF-1 [132, 133], rendering them functionally deficient in the activation of caspase-9 and thus intrinsic apoptosis. Whether this deficiency results in increased inflammation of the heart when cardiomyocytes undergo mitochondrial permeabilisation is an interesting avenue for future research, particularly since heart disease is the leading cause of death in the developed world.

Another interesting potential role of apoptosis-induced inflammation mediated by caspase inhibition is during cellular infection with viruses, which often express proteins that inhibit caspases [134]. These inhibitors, therefore, prevent apoptosis of the infected cell and give the virus more time to replicate. One can speculate that inflammation induced by the induction of apoptosis triggered by viral infection, in combination with viral caspase inhibition, is an additional cellular mechanism to boost inflammation to fight the virus.

Clinically, some chemotherapeutic drugs like doxorubicin have been shown to induce an inflammatory response even under caspase-proficient conditions [135]. However, it is believed that these and some other drugs initiate a specific form of cell death termed "immunogenic cell death" [136], characterised by the release of several DAMPs which activate the immune system.

While there is an increasing number of reports suggesting an inflammatory outcome of apoptosis, so far they are limited to fairly specific situations. It remains to be seen whether apoptosis is potentially inflammatory, or if this controversial role is merely a byproduct of the circumstances.

8

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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