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REVIEW

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Role of mitochondria as the gardens of cell death

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Abstract Mitochondria play a crucial role in regulating cell death, which is mediated by outer membrane permeabilization in response to death triggers such as DNA damage and growth factor deprivation. Mitochondrial membrane permeabilization induces the release of cytochrome c, Smac/DIABLO, and AIF, which are regulated by proapoptotic and antiapoptotic proteins such as Bax/Bak and Bcl-2/xL in caspase-dependent and caspase-independent apoptosis pathways. Mitochondrial dysfunction is mediated in two ways. The first is by increased calcium in mitochondria derived from endoplasmic reticulum (ER); this calcium increase is regulated by Bcl-2 and Bax through the ER-mitochondria connection and the unfolded protein response in the ER. The second is by the lysosomal enzyme cathepsin, which activates Bid through lysosome–mitochondria cross-signaling. The genomic responses in intracellular organelles after DNA damage are controlled and amplified in the cross-signaling via mitochondria; such signals induce apoptosis, autophagy, and other cell death pathways. This review discusses the recent advancements in understanding the molecular mechanism of mitochondria-mediated cell death.

Keywords Mitochondria · Apoptosis · Autophagy · Endoplasmic reticulum · Lysosome

Abbreviations Smac: Second mitochondria-derived activator of caspase · DIABLO: Direct inhibitor of apoptosis-binding protein with low pI · AIF: Apoptosis-inducing factor · MMP: Mitochondrial membrane permeabilization · VDAC: Voltage-dependent anion channel · ANT: Adenine nucleotide translocator · ROS: Reactive oxygen species · ER: Endoplasmic reticulum · PCD: Programmed cell death · SERCA: Sarcoplasmic/ER Ca^{2+} ATPase · UPR: Unfolded protein response

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Introduction

Since the discovery of the morphological manifestation of apoptotic cell death by Kerr et al. in 1972 [57], the molecular mechanisms induced by various genotoxic factors, including anticancer drugs, irradiation, growth factor deprivation, oxidative stress, and FasL have been clarified, focusing on mitochondrial dysfunction mediated by mitochondrial membrane permeabilization (MMP) [36]. Mitochondria are important intracellular organelles for producing energy from adenosine 5'-triphosphate (ATP), and dysfunction induced by DNA damage and other genotoxic factors leads to an irreversible event, cell death: thus mitochondria are the gardens of cell death. Mitochondrial membrane permeabilization induces the membrane transition pore to release small molecules from the intermembrane space, including cytochrome c [60], Second mitochondria-derived activator of caspase (Smac)/Direct inhibitor of apoptosis-binding protein with low pI (DIABLO) [25], Omi/HtrA2 [42], apoptosis-inducing factor (AIF) [90], and endonuclease G [58], resulting in caspase-dependent and caspase-independent apoptotic cell death activities [24]. The membrane transition pore is regulated by proapoptoic and antiapoptotic Bcl-2 family proteins, such as Bax/Bak and Bcl-2/xL, through the composition of the voltage-dependent anion channel (VDAC) [86] and adenine nucleotide translocator (ANT) [98]. Cytochrome c plays an essential role in caspase-dependent apoptotic cell death: its release triggers apoptosome [1]

assembly from apoptotic protease-activating factor-1 (Apaf-1), ATP, and procaspase-9, which activates effecter caspase-3 and caspase-7, leading to oligonucleosomal DNA fragmentation [3]. In contrast, AIF serves in a caspase-independent pathway that produces large-scale DNA fragmentation leading to apoptotic cell death [21].

Although MMP is crucial for caspase-dependent and caspase-independent apoptotic cell death pathways, it can also be induced by other triggers such as calcium (Ca^{2+}) in the endoplasmic reticulum (ER) and cathepsins in lysosomes in response to ceramide and reactive oxygen species (ROS) [47]. Given that Bcl-2, Bax, and Bak are also localized in the ER, the ER serves as an important apoptotic control point that is mediated by the mitochondria–ER connection [10]. Overexpression of Bcl-2 prevented cell death by the passive release of ER Ca^{2+} when thapsigargin was used to block the sarcoplasmic/ER Ca^{2+} ATPase (SERCA) reuptake pump [40]. Either the overexpression of Bcl-2 or the loss of Bax/Bak leads to reduced resting ER Ca^{2+} concentrations and a secondary decrease in Ca^{2+} uptake in mitochondria. Cathepsin in lysosomes is one of the enzymes involved in cytoplasmic degradation in cell homeostasis [95]. Cathepsins B and D activated by DNA damage cross-signal with mitochondria at the mitochondrial–lysosomal axis and directly or indirectly activate the Bax/Bak-dependent caspase cascade leading to apoptosis [9]. Furthermore, the activation of cathepsin is also involved in autophagic cell death, the other caspase-independent pathway for the disposal of intracellular organelles in cell death. Autophagy is a dynamic process of membrane engulfment in which portions of the cytoplasm are sequestered within double-membrane vesicles called autophagosomes [102]. The induction of autophagy by nutrient starvation and DNA damage is prominently controlled by phosphoinositide 3-kinase (PI3K)/Akt, mammalian target of rapamycin (mTor), and the mammalian autophagy gene beclin 1, which interacts with Bcl-2 [59, 63]. Clear overlaps in signal transduction pathways between autophagy and apoptosis are suggested, because the PI3K/Akt/mTor pathway influences the shared intermediates in cell survival, such as p70S6-kinase [35]. Thus, mitochondria seem to serve as a central gate for cell death pathways including apoptosis, autophagy, and other cell death that share common pathway elements (Fig. 1). The present review discusses the role of mitochondria as the gardens of cell death induced by various genotoxic agents, in particular to explore potential targets for cancer therapy according to recent advances in the understanding of mitochondria-mediated cell death.

Programmed cell death

Programmed cell death (PCD) is a genetically encoded form of cell suicide that is central to the development and homeostasis of multicellular organisms. PCD shares the genes encoding the cell death elements and the morphological and biological features of apoptosis. Three types of PCD have been described [12, 52]. Type I is apoptotic cell death, which includes the morphological changes of cell shrinkage, membrane blebbing, and extensive chromatin condensation. The induction of apoptosis occurs by two distinct modes: signaling by death receptors in the plasma membrane (extrinsic) and disruption of intracellular homeostasis (intrinsic). Type II is autophagic cell death, characterized by the formation of autophagic vacuoles in the cytoplasm of dying cells. Type III is necrotic cell death, characterized by a rapid loss of plasma membrane integrity and spillage of the intracellular contents. The appearance of one distinct type of cell death may depend on the stimulus and the cellular context in damaged cells.

The role of the Bcl-2 family proteins and p53 in cell death

Members of the Bcl-2 family proteins are critical death regulators that reside upstream of mitochondria and may be proapoptotic or antiapoptotic [93]. They can be divided into three main subclasses, defined in part by the homology shared within four conserved regions known as the Bcl-2 homology (BH)1–4 domains, corresponding to the α-helices that describe the structure and function. The first subclass contains antiapoptotic members that conserve all four BH1-4 domains; members of this subclass include Bcl-2 [94], Bcl-xL [6], Mcl-1 [54], A1 [19], and Bcl-W [32]. In the second subclass, the structure of the BH1-3 domains in Bcl-xL creates a hydrophobic pocket that can accommodate the BH3 domain of a proapoptotic member. The third subclass contains multidomain proapoptoic members such as Bax [72] and Bak [18] that display sequence conservation in BH1-3 domains and the BH3-only proteins Bid [57, 62], Bad [101], Bim [68], Noxa [71], and Puma [103] that display sequence conservation only in the amphipathic α-helical BH3 region and require Bax and Bak to mediate cell death. Cells deficient for both Bax and Bak proapoptotic molecules demonstrated resistance to all tested intrinsic death pathway stimuli [100]. The BH3-only proteins reside upstream in the pathway and death signals trigger their transcriptional activation and post-translational modification to connect proximal signals with the core apoptotic pathway. Bax and Bak exist as monomers in viable cells; however, when death signals are received, Bax is inserted into the mitochondrial outer membrane as homo-oligomerized multimers [22]. Bak also resides in an inactive state in the mitochondria and undergoes an allosteric conformational activation in response to death signals, leading to its oligomerization and the permeabilization of the mitochondrial outer membrane for the release of small molecules from the intermembrane space [37].

Although the precise mechanism by which small molecules are released is still unclear, we have identified four possibilities. (1) The oligomerized Bax or Bak

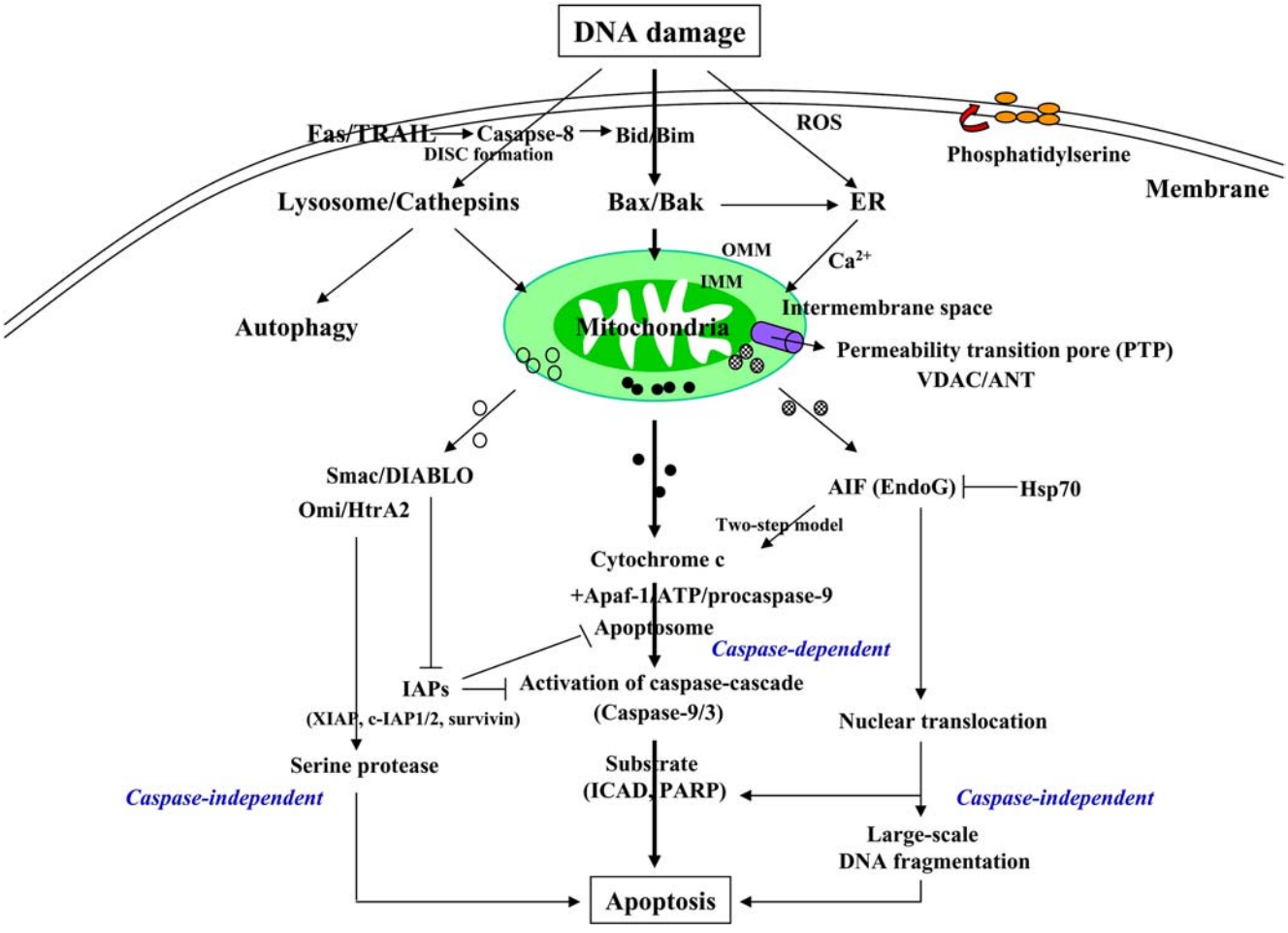

Fig. 1 The features of the mitochondria-mediated cell death pathway activated in response to DNA damage. The activation of a BH3-only protein such as Bid by caspase-8 through Fas/TRAIL in an extrinsic pathway or by cathepsin in an intrinsic pathway promotes the oligomerization of Bax or Bak in the outer mitochondrial membrane (OMM), and induces *MMP*, resulting in the release of proapoptotic small molecules of cytochrome c, *Smac*)/*DIABLO*, *Omi*/*HtrA2*, *AIF*, and *EndoG* in caspase-dependent and caspase-independent apoptotic cell death pathways. The MMT triggers death signals that are exchanged with the *ER*, triggering the release of calcium by Bax/Bak in the ER and cathepsin that produces autophagic cell death by lysosomes. Phosphatidylserine was externalized in the outer membrane in apoptotic cells. *IAPs* inhibitor of apoptosis proteins; *ICAD* inhibitor of caspase-activated DNase; *PARP* poly (ADP-ribose) polymerase

generates pores sufficient for the release of cytochrome c and other proapoptotic proteins; this hypothesis is supported by the findings that the structure of Bcl-2 family molecules is similar to that of the pore-forming helices of bacterial toxins, and that Bax can form channels in artificial membranes and release cytochrome c from liposomes with large complexes containing Bax and Bak, seen by electron microscopy analysis [75]. (2) Proapoptotic family members can form complexes with mitochondrial membrane proteins such as VDAC or ANT to serve pores sufficient for the release of cytochrome c; this hypothesis is supported by the findings that microinjection of VDAC antibodies into the entrance of the channel inhibited the release of cytochrome c and prevented apoptosis [85, 96]. (3) In a new model, permeability transition pores are regulated by misfolded proteins and chaperones such as Hsp25; permeability transition pores are formed by aggregates of misfolded membrane proteins as the result of damaged proteins that are often targeted by the ANT in the inner membrane [41]. (4) The loss of mitochondrial membrane potential, causes swelling of the matrix and the inner membrane, which leads to a distortion of the cristae structure and rupture of the outer membrane for release of cytochrome c and the nonspecific release of proapoptotic small molecules residing in the intermembrane space [55].

The BH-3-only members serve as upstream triggers responding to death signals; modifications ranging from dephosphorylation to cleavage result in the activation of BH3-only proteins and their translocation to mitochondria, where they exert their biological function [78]. The efficiency of translocation is enhanced by modifications such as the *N*-terminal myristoylation of Bid followed by its cleavage by caspase-8 [104]. Four mechanisms by which BH3-only proteins exert their functional roles have been proposed. (1) Activation of Bax and Bak mediates cytochrome c [53]. (2) Interac-

tions inhibit members of the antiapoptotic Bcl-2 family [7]. (3) Interaction with intrinsic mitochondrial proteins such as VDAC and ANT for mitochondrial dysfunction result in the release of cytochrome c [89]. (4) Autonomous induction of cytochrome c release by BH3-only proteins occurs without the requirement of Bcl-2 family proteins, but with a requirement for negatively charged lipids [33]. Nevertheless, from the evidence of the lack of cytochrome c release and apoptosis in Bax/Bak double-knockout cells, it is likely that all BH3-only proteins require Bax and Bak to exert their mitochondrial proapoptotic activity [100]. Given that most BH3-only proteins such as Bad and Bim have a preference for binding to the antiapoptotic proteins Bcl-2 and Bcl-xL, Bad promotes cell death by binding with Bcl-2 or Bcl-xL [17]. The BH3 domains of Bid and Bim activate Bax and Bak when they are added to mitochondria, whereas Bad and Bik cannot directly activate Bax and Bak but preferentially bind to Bcl-2 and Bcl-xL as sensitizers [56]. In turn, Bad-like BH3 domains act on the antiapoptotic members, dissociating Bax and Bak from Bcl-2 and Bcl-xL; the dissociated Bax and Bak can then be activated by Bid-like activators. Similarly, Bid and Bim promote apoptosis by their release from Bcl-2 and Bcl-xL. Bim also activates and oligomerizes Bak residing in the mitochondrial outer membrane [74]. When Bid and Bim activate Bax in that matter, a conformational change is induced, triggering their oligomerization. Thus, it is likely that BH3-only proteins require the multidomain proapoptotic proteins to induce cytochrome c release and cell death.

The induction of apoptosis is central to the tumor-suppressor activity of p53 [30]. The p53 promotes the expression of a number of genes involved in apoptosis, including death receptors and proapoptotic members of Bcl-2 family, in response to DNA damage or oncogene-induced signaling [16]. The p53-induced apoptosis proceeds through the release of cytochrome c from mitochondria, which leads to activation of the caspase cascade [97]. The p53 is attributed with inducing apoptosis as a transcription factor. On the other hand, transcriptional-independent induction of apoptosis by p53 requires Bax and involves cytochrome c release and caspase activation in the absence of a nucleus, suggesting that p53 has the ability to induce apoptosis directly from the cytoplasm [15, 16]. In an experiment with $p53^{-/-}$ mouse embryonic fibroblasts (MEFs) [16], ultraviolet light (UV) induced apoptotic cell death exclusively in the cytoplasm in the presence of an inhibitor of nuclear transport. In contrast, $Bax^{-/-}$ MEFs were resistant to UV-induced apoptosis in the presence of inhibitor of nuclear transport, suggesting that cytoplasmic p53 cooperates with Bax to induce apoptosis without p53-transcriptional activation [16]. In addition, UV-induced apoptosis in $Bax^{-/-}$ MEFs without inhibitor of nuclear transport suggests that p53-dependent activation of Noxa and Puma is required for cell death. Apoptosis induced by p53 is dependent on Bax and Bak, given that p53 has been reported to bind to Bcl-2 and Bcl-xL, which might allow for activation of Bax, Bak, Bid, and Bim by their release from these inhibitors [4, 26, 64]. Although p53 does not directly release cytochrome c from mitochondria, it is capable of performing in a function analogous to that of BH3-only proteins in a transcription-independent fashion. The targeting of mitochondria by p53 is supported by the finding that wild-type p53 translocates to mitochondria following γ-irradiation in murine thymocytes [64]. Furthermore, endogenous Bcl-2 and Bcl-xL directly bind to wild-type p53 that target mitochondria, and these interactions are dependent on the DNA binding domain of the p53, specifically amino acids 239–248, because p53 without amino acids 239–248 is no longer able to bind to Bcl-xL [14, 64]. The mitochondria-targeted effect of p53 is inhibited by Bcl-2, as demonstrated by the finding that siRNA introduced to Bcl-2 caused p53-dependent apoptosis in the absence of genotoxic stress [48]. Thus, p53 is a tumor-suppressor gene that functions as a transcription factor to regulate genes involved in DNA repair, cell cycle arrest, and apoptosis, and it can dictate a cell's fate through direct caspase-activating functions in the cytoplasm, in coupling or uncoupling of its transcriptional effects.

Interaction of mitochondria and endoplasmic reticulum in cell death

Mitochondria play a pivotal role in amplifying apoptotic signals; however, other organelles, including the ER, have also been implicated. The ER can be an initiator of apoptosis, when unfolded protein accumulates or the ER–Golgi transport is inhibited, leading to the ER stress response [81]. The ER appears to be a regulator of apoptosis because (1) ER is a main intracellular store of Ca^{2+}, an important second messenger for cellular functions that are physiologically connected with Ca^{2+}-mediated signaling [69], and (2) the stimulation of inositol 1,4,5-triphosphate (IP3) causes the release of Ca^{2+} from ER, and this calcium is rapidly taken up by closely juxtaposed mitochondria [70]. After the discharge of the intracellular Ca^{2+} stores, the clearance of cytoplasmic Ca^{2+} by local mitochondria also modulates the opening of the channels in the plasma membrane. An increase in mitochondrial matrix Ca^{2+} regulates metabolism and Ca^{2+} also modulates the mitochondrial permeability transition, which is controlled by permeability transition pores [88]. Permeability transition pores have been implicated in both apoptotic and necrotic cell death pathways following death signals. Ca^{2+} is involved in signal transduction in different apoptotic pathways, and Ca^{2+}-dependent endonuclease induces DNA fragmentation in apoptosis [8]. Several apoptotic stimuli, including staurosporine, ceramide, and growth factor deprivation, increased cytoplasmic Ca^{2+} in the early phase of apoptosis.

Given that the Bcl-2 family of proapoptotic and antiapoptotic proteins serves as a critical death regulator

that resides upstream of mitochondria, Bcl-2 also localizes at the ER, which can modulate Ca^{2+} fluxes during cell death [79]. Transient overexpression of Bax results in the release of ER Ca^{2+}, with a subsequent increase in mitochondrial Ca^{2+} and enhanced release of cytochrome c [67]. In contrast, overexpression of Bcl-2 reduces resting ER Ca^{2+} and the extent of capacitative Ca^{2+} entry [84]. The mitochondria in Bax- and Bak-deficient MEFs (DKO cells) do not display any intrinsic defect in Ca^{2+} uptake and they respond with reduced Ca^{2+} expression to stimuli that release Ca^{2+} from the ER [23]. The reduced steady-state ER Ca^{2+} levels are a secondary phenomenon. In addition, the reconstitution experiment with SERCA or mitochondrial Bax (mtBax) in DKO cells indicates that different apoptotic stimuli are primarily controlled by either ER Ca^{2+} levels or mitochondrial-based Bax and Bak [23, 70]. Regulating the steady state of ER Ca^{2+} appears to be a crucial checkpoint for Ca^{2+}-dependent apoptotic cell death. The ablation of proapoptotic members or the overexpression of antiapoptotic members results in reduced steady-state ER Ca^{2+} levels and decreased mitochondrial Ca^{2+} uptake that is consistent with a rheostat model, in which the ratio of proapoptotic to antiapoptotic Bcl-2 members determines the susceptibility to death signals [13, 23, 77]. Ca^{2+}-dependent apoptotic stimuli that require Bax and Bak at the ER induce cell death through a mitochondrial pathway, which is characterized by mitochondrial dysfunction with cytochrome c and caspase activation. Thus, the ER is a checkpoint of mitochondrial Ca^{2+} uptake, which is controlled by proapoptotic and antiapoptotic Bcl-2 members upstream of mitochondria for Ca^{2+}-dependent cell death by various apoptotic stimuli.

The ER–mitochondria connection enables Ca^{2+} signals not only to stimulate cellular metabolism, but also to modulate the ability of mitochondria to undergo apoptosis. Despite the fact that Bax, Bak, and Bcl-2 interfere with the ER Ca^{2+} load in a manner that is clearly dependent on the ER–mitochondria connection, the transfer of Ca^{2+} from the ER to the mitochondria is required for initiation of cell death by some, but not all, apoptotic signals [84]. In the previous studies, Bax/Bak DKO cells exhibited no intrinsic defects in the handling of mitochondrial Ca^{2+}, yet Ca^{2+} delivery to the mitochondria was insufficient due to the low ER Ca^{2+} concentration [69, 84]. However, the killing ability of Ca^{2+}-increasing apoptotic agents was restored by the overexpression of SERCA that conducts the ER Ca^{2+} load. These findings indicate that Ca^{2+} is capable of acting on mitochondria independently of Bax or Bak and that expression of mitochondria-targeted Bax, but not Ca^{2+} transfer to the mitochondria, was restored by Bid. In turn, the restoration of apoptosis in the DKO cells by expression of organ-targeted proteins is independently controlled by ER Ca^{2+} and mitochondrial Bax. Therefore, the cell death pathway of the ER–mitochondria connection in response to apoptotic signals may be explained with three patterns: (1) direct engagement of the ER Ca^{2+}-dependent pathway by ceramide and oxidative stress for an increase in mitochondrial Ca^{2+} that is dependent on ER Ca^{2+}, but independent of Bax/Bak action [91]; (2) tBid in cooperation with Bax or Bak, without requiring the engagement of mitochondrial and ER Ca^{2+} [31]; or (3) the engagement of both pathways of Ca^{2+} and Bax/Bak action in mitochondria regulated by ER Ca^{2+} in balance with Bcl-2 and Bax/Bak in cell death induced by anticancer drug such as etoposide. Although the mechanism by which the lack of Bax/Bak function decreases ER Ca^{2+} is still uncertain, several candidates have been suggested: the IP3 receptor, the Ca^{2+} release channel of the ER, SERCA, and Bcl-2. Many chaperone proteins in the ER regulate and maintain proteins in a folded state to prevent protein folding from aggregating in cellular functions [83]. Upon response to various stimuli that disrupt ER function, such as Ca^{2+} depletion from the ER lumen or overexpression of some proteins, protein misfolding occurs and unfolded proteins accumulate and aggregate in the ER. The misfolded and aggregated proteins trigger the unfolded protein response (UPR) that is able to respond to the magnitude of ER stress [41]. When the threshold of cell death has not yet been reached, UPR reduces general protein synthesis and activates selective proteins that facilitate the chaperone functions [80]. However, in severe ER stress beyond the threshold of cell death, UPR triggers apoptosis, in association with the proapoptotic transcription factor increasing CCAAT/enhancer-binding protein (C/EBP)-homologous protein/growth arrest and DNA damage-inducible protein 153 (CHOP/Gadd153) [99], which may lead to the activation of the caspase cascade involved in the activation of caspase-12 in the ER [73]. ER stress derived from glucose deprivation and hypoxia in the tumor microenvironment can also disrupt protein folding in the ER, resulting in the accumulation of unfolded protein in the ER to activate the UPR. The induction of resistance to chemotherapy is associated with the induction of the UPR-targeting genes, such as glucose-regulated protein 78 (GRP 78) and GRP 94 that are involved in the antiapoptotic function as ER chaperones [28, 34].

Interaction of lysosomes and mitochondria in cell death

Lysosomal proteases, cathepsins, translocate from the lysosomal lumen to the cytosol in response to various death stimuli, such as tumor necrosis factor (TNF) and TNF-related apoptosis-inducing ligand (TRAIL) [27], growth factor deprivation [44], and staurosporin [49]. Cathepsins can cleave and activate Bid, which may function as one of the links connecting to MMP, which may also trigger mitochondrial function in a Bid- and Bcl-2-independent manner [20]. Hydroxide produced as a second messenger in mitochondria by ROS diffuses into lipofuscin-loaded lysosomes, and hydroxide causes damage to lysosomal membranes that causes the leak of lysosomal enzyme. Lysosomal enzyme, directly or indi-

rectly through activation of Bid and Bax, permeabilizes mitochondrial membranes [5], resulting in the release of cytochrome c, AIF, and Smac/DIABLO, triggering cell death. Cathepsins can also mediate the MMP-independent and caspase-independent cell death pathway of autophagy [11]. In the process of autophagy, large portions of cytoplasm, often containing organelles, are sequestered in an autophagosome, a vacuolar structure, by a specific double membrane [11, 65]. Autophagosomes are then fused with lysosomes. Autophagy is a conserved lysosomal pathway involved in the turnover of long-lived proteins and organelles, and it is constitutive in all cell types containing a lysosomal compartment. Although autophagy is considered to be a programmed process, selective sequestration of organelles can be observed in various pathophysiological situations and specific cases of DNA damage [87]. Despite the fact that selective sequestration of mitochondria has been proposed to be instrumental during the early phase of apoptosis, and that the molecular basis for the selective process is unclear, mitochondria with decreased membrane potential and activated production of ROS are very sensitive to autophagic sequestration. Autophagy is stimulated in the part of the ER where misfolded proteins accumulate, which contributes to the elimination of misfolded proteins accumulated in and out of the ER. Further studies on the interaction of apoptosis and autophagy in triggering cell death are required.

In relation to excessive production of ROS induced by anticancer drug, direct effect on mitochondrial membrane potential that leads to depolarization of the membrane potential followed by MMP has been reported in the cell death triggered by arsenic trioxide (ATO, As_2O_3) [46] and other anticancer drugs, such as anthracyclines and cisplatin [76]. ATO rapidly induced

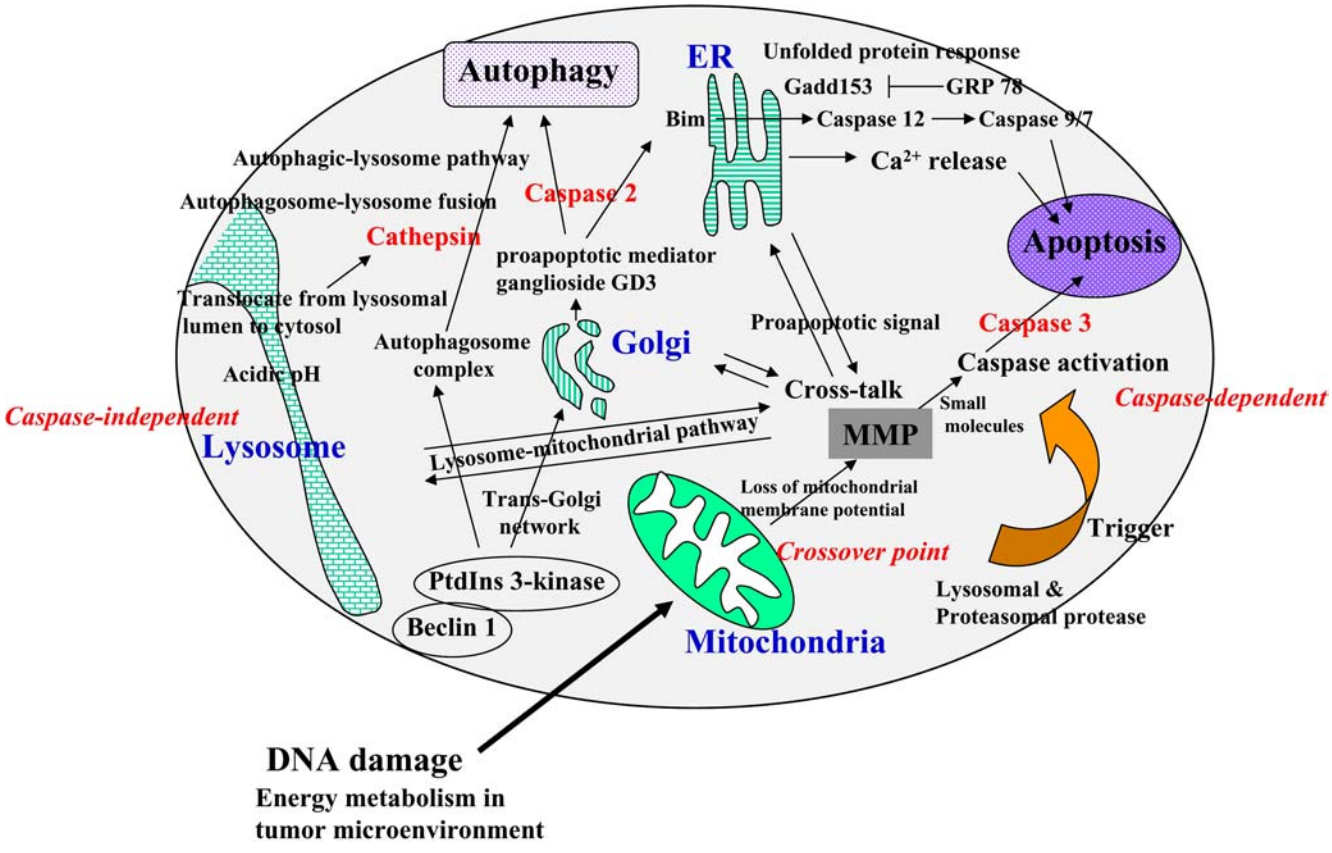

Fig. 2 Activation of several sensors in intracellular organelles that are networked with mitochondrial dysfunction in response to DNA damage. The induction of *MMP* releases small molecules in caspase-dependent and caspase-independent cell death pathways. The *MMP* is shared by cross-signals with the cell death pathways through *lysosomes*, *Golgi complex*, and *ER*. The mammalian autophagic protein, beclin-1, is composed of class III PI3-kinase, which induces the formation of autophagosomes that are fused with lysosomes in autophagic cell death. The lysosomal proteases, cathepsins, are activated in acidic pH conditions. In addition, the beclin 1/*PI3K* complex activates the trans-Golgi network, resulting in the activation of caspase-2 through the proapoptotic mediator ganglioside GD3 in autophagic cell death. Bim in the ER activates *caspase-12* leading to activation of the caspase cascade. The ER releases Ca^{2+}, which is regulated by Bcl-2 and Bax/Bak, resulting in increased cytoplasmic calcium that leads to an increase in mitochondria for apoptosis. The increased unfolded protein response in the *ER* serves both a proapoptotic and an antiapoptotic function: in the proapoptotic function, the increased unfolded protein response induces *CCAAT/enhancer-binding protein* (*C/EBP*)-homologous protein/growth arrest and DNA damage-inducible protein 153 (*CHOP/Gadd153*), whereas in the antiapoptotic function it induces *GRP78*, depending on the magnitude of DNA damage

TRAIL, activation of caspase-8, cleavage of BID, depolarization of mitochondrial membrane, and release of cytochrome c and AIF from mitochondria in a Bcl-2 independent fashion [2]. Treatment with the pancaspase inhibitor z-VAD-fmk cannot block ATO-induced cell death, and also the cell death cannot be inhibited in caspase-$9^{-/-}$ and caspase-$2^{-/-}$ cells [82] even though ATO-induced apoptosis is inhibited under some circumstances [43, 45]. Despite the fact that release of cytochrome c activates caspase cascades, inhibition of caspase cascades facilitates switching to caspase-independent cell death, such as necrotic and autophagic cell death via death receptor-dependent or lysosome-mediated pathway, indicating potential interplay of apoptosis and autophagy [50]. It is likely that determination in induction of either apoptotic or nonapoptotic cell death is dependent on magnitude of the production of ROS by anticancer drug [66], and that loss of mitochondrial membrane potential involves MMP. In addition, although the proteasome inhibitor PS-341 inhibits NF-κB activation, it also induces UPR and ROS, which are associated with mitochondrial dysfunction mediated by loss of membrane potential and MMP [29]. Thus, direct effect on mitochondria with excessive production of ROS by arsenic trioxide and other anticancer drugs is involved in interaction of apoptotic and nonapoptotic cell death pathway through mitochondria membrane depolarization.

Concluding remarks

Although apoptotic cell death and the activation of caspases dominate the current understanding of cell death, different types of cell death are found to overlap. Nevertheless, mitochondrial dysfunction mediated by MMP plays a crucial role in triggering cell death by various death stimuli, in cooperation or compensation with the function of the ER, lysosomes, and other intracellular organelles (Fig. 2). Cell death due to the lack of proapoptotic or overexpression of antiapoptotic proteins is involved in tumorgenesis [92], tumor progression [38], and resistance to anticancer treatment [61]. A better understanding of cell death systems through mitochondria as the gardens of cell death may provide new targets to explore for cancer therapy and chemoprevention. The various cross-signals in genomic response to DNA damage may encode the directions for cytoprotective or cytocidal selection in damaged cells to maintain cellular homeostasis. Given that the regulation of the dual selection of cell death or survival in damaged cells can be maintained and that the susceptibility for cell death is determined depending on individual genetic alterations of death-related genes, the means for inducing a cell death pathway even in tumors remains to be explored. Inducing cell death with the intent to study the interaction of mitochondria and other important organelles in different cell death pathways may improve the therapeutic efficacy of anticancer treatments in individual tumor cells.

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