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Mini Review

The role of mitochondria in apoptosis

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Apoptosis (programmed cell death) is a cellular self-destruction mechanism that is essential for a variety of biological events, such as developmental sculpturing, tissue homeostasis, and the removal of unwanted cells. Mitochondria play a crucial role in regulating cell death. Ca^{2+} has long been recognized as a participant in apoptotic pathways. Mitochondria are known to modulate and synchronize Ca^{2+} signaling. Massive accumulation of Ca^{2+} in the mitochondria leads to apoptosis. The Ca^{2+} dynamics of ER and mitochondria appear to be modulated by the Bcl-2 family proteins, key factors involved in apoptosis. The number and morphology of mitochondria are precisely controlled through mitochondrial fusion and fission process by numerous mitochondria-shaping proteins. Mitochondrial fission accompanies apoptotic cell death and appears to be important for progression of the apoptotic pathway. Here, we highlight and discuss the role of mitochondrial calcium handling and mitochondrial fusion and fission machinery in apoptosis. [BMB reports 2008; 41(1): 11-22]

INTRODUCTION

Mitochondria are dynamic organelles that can change in number and morphology in healthy cells (1). The major and essential role of mitochondria is to provide a myriad of services to the cell, including energy production, calcium buffering and regulation of apoptosis (2). Despite their critical role, how these diverse functions are precisely coordinated in a cell is largely unknown.

The mitochondria act at the core of the apoptotic pathway by providing many important factors including those that induce caspase activation and chromosome fragmentation (3). Mitochondria also play a key role in a modulation of Ca^{2+} homeostasis and oxidative stress (4). Under certain conditions, mitochondria in mammalian cells can be physically interconnected (5). This mitochondrial network can effectively deliver energy, or channel calcium between different areas of the cell.

The number and morphology of mitochondria are precisely controlled through mitochondrial fusion and fission machinery by mitochondria-shaping proteins (6, 7), including the large GTPases mitofusins, Mfn1 and Mfn2 (8) and optic atrophy protein 1 (Opa1) (9). Mitochondrial fission machinery consists of the proteins Fis1 (10) and dynamin related protein 1 (Drp1) (11). Equilibrium between mitochondrial fusion and fission controls the morphology of the mitochondria. Whereas disruption of fusion fragments the normal, tubular network of mitochondria into short rods or spheres, disruption of fission generates elongated, interconnected tubules that often cluster perinuclearly (7). Mitochondrial fission accompanies several types of apoptotic cell death and appears to be associated with progression of the apoptotic pathway (12). In this review, we focused on the role of previously overlooked morphological changes, particularly mitochondrial fragmentation, in apoptosis of mammalian cells.

Mitochondrial regulation of apoptosis

Apoptosis (13), or programmed cell death, is a cellular self-destruction mechanism involved in a variety of biological events. Excessive or insufficient apoptosis contributes to various diseases related to ischemia, neurodegeneration, autoimmunity, and viral infections, and is involved in the growth and regression of tumors (14). There are multiple cellular pathways triggering apoptosis, two of which, the extrinsic and intrinsic pathways, are better characterized (15): intrinsic, for which mitochondrion is the central organelle governed by pro- and anti-apoptotic Bcl-2 family members, and extrinsic, consisting of cell surface TNF-related family of receptors (TNF receptor, CD95/Fas, and TRAIL death receptors, etc.), their inhibitory counterparts and cytoplasmic adapter or death inhibitory molecules (e.g., FADD or FLIP). The apoptotic process is executed by a family of cysteine proteases which specifically cleave their substrates at aspartic acid residues (3). These proteases, known as caspases, are activated through extrinsic and/or intrinsic pathways. The extrinsic pathway is activated by cell surface death receptors, while the intrinsic pathway is initiated by formation of the cytosolic apoptosome composed of Apaf-1, procaspase 9, and the cytochrome *c* released from mitochon-

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dria (16) (Fig. 1).

Mitochondria are known as an important intracellular organelle for producing energy, adenosine 5′-triphosphate (ATP). Recent studies have uncovered that mitochondria are the garden of cell death that plays a crucial role in regulating cell death pathways (17, 18). Dysfunction of mitochondria induced by DNA damage and other genotoxic factors leads to an irreversible event, apoptotic cell death (17). Mitochondrial fragmentation during apoptosis was connected with the collapse of the mitochondrial membrane potential ($\Delta\Psi m$) that was considered a point of no return (irreversible point) in the death cascade (19). Members of the Bcl-2 family of proteins may regulate outer mitochondrial membrane (OMM) integrity and function (20, 21). The Bcl-2 family can be divided into three different groups based on Bcl-2 homology (BH) domains and function. The anti-apoptotic members, such as Bcl-2 and Bcl-xL, typically have BH1 through BH4 domains. The pro-apoptotic members can be divided into two groups (22, 23): those with BH1, BH2 and BH3 domains, such as Bax and Bak, and those with only BH3 domains, such as Bad, Bid and Bim. Mitochondrial outer membrane permeabilization (MOMP) is one of the crucial events in the process linking lethal signals to controlled death of mammalian cells (15). During early stages of apoptosis, the pro-apoptotic proteins Bax translocates to the OMM and, almost instantly after translocation, concentrates into submitochondrial punctate foci (24). In addition, Bak co-

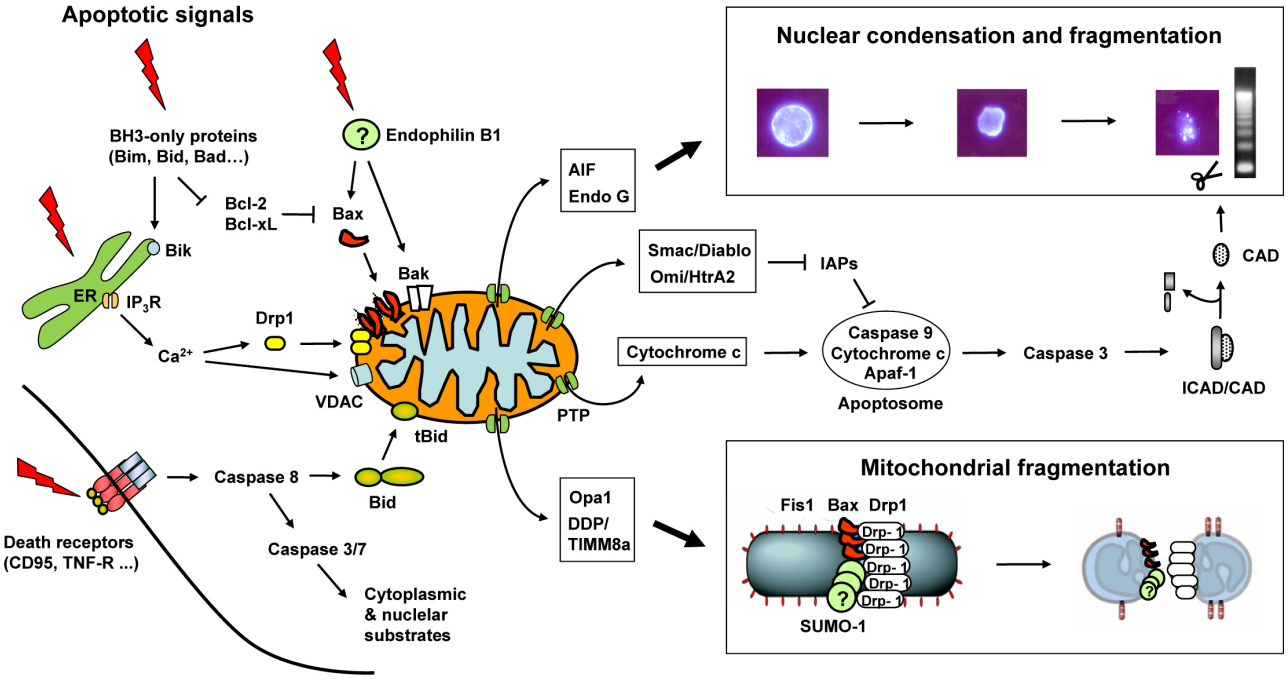

Fig. 1. Intrinsic and extrinsic apoptotic pathways in mammalian cells. Activation of the intrinsic or mitochondria- dependent pathway leads to cytochrome c release, apoptosome formation, and caspase activation. Extracellular ligand binding to death receptors (CD95, TNF-R, etc.) triggers the extrinsic pathway that directly activates caspases or translocates tBid to the mitochondria, which in turn leads to activation of the intrinsic pathway. Both apoptotic signaling pathways converge at the level of effector caspases, such as caspase-3 and caspase-7. Bcl-2 family members regulate apoptosis by modulating the release of cytochrome c. Bax and Bak are pro-apoptotic Bcl-2 family members, physically interact to form oligomers that can move onto the mitochondrial membrane. Bcl-2 and Bcl-xL are anti-apoptotic and can block the function of Bax and Bak. BH3-only member (Bim, Bid, Bad, etc.) are pro-apoptotic and can modulate the pro-apoptotic processes of Bax/Bak via inhibiting anti-apoptotic Bcl-2 members. Bik enhances Ca^{2+} release through the inositol 1,4,5-triphospate receptor (IP_3R) from endoplasmic reticulum (ER). This release promotes mitochondrial translocation of the mitochondria-shaping protein, DRP-1. Massive and/or a prolonged accumulation of Ca^{2+} in the mitochondria can lead to the opening of the permeability transition pore (PTP) in the mitochondrial inner and outer membranes. The PTP, a transmembrane channel between the inner and outer mitochondrial membranes, is formed by the interaction of the adenine nucleotide translocator (ANT) and the voltage-dependent anion channel (VDAC) during the process of swelling. The PTP has a role in regulating mitochondrial membrane potential and the release of cytochrome c. In the cytosol, released cytochrome c, Apaf1, and procaspase-9 from the mitochondria interact to form the apoptosome that drives the activation of caspase-3. AIF and endonuclease G are mitochondrially released proteins with nuclease activity that can translocate to the nucleus. The inhibitors of apoptosis (IAPs) are inhibited by mitochondrially derived Smac/Diablo, and Omi/HtrA2. Caspase-3 cleaves many substrate proteins, some of which are endonucleases including caspase-activated DNase (CAD) that translocates to the nucleus to cleave DNA into internucleosomal fragments. Release of mitochondrial membrane proteins Opa1 and DDP/TIMM8a and conjugation of small ubiquitin-like modifier-1 (SUMO-1) to Drp1 promotes mitochondrial cristae remodeling (fragmentation) and modulation of apoptosis.

localizes with Bax in these foci. Bax and Bak induce cell death via MOMP that leads to the release of small pro-apoptotic molecules such as cytochrome *c* (25), second mitochondria-derived activator of caspase/direct IAP (inhibitor-of apoptosis) binding protein with low pI (Smac/Diablo) (26), Omi/HtrA2 (27), apoptosis-inducing factor (AIF) (28), and endonuclease G (EndoG) (29) from the mitochondrial intermembrane space to the cytosol and subsequent activation of the caspase-activating pathways in caspase-dependent and caspase-independent apoptotic cell death pathways (30) (Fig. 1). cytochrome *c* plays an essential role in mitochondria-dependent apoptotic cell death. Its release triggers apoptosome (31) assembly from apoptotic protease-activating factor-1 (Apaf-1), ATP, and procaspase-9, which activates effector caspase-3 and caspase-7, leading to oligonucleosomal DNA fragmentation (32).

The membrane permeability transition pore (PTP) is regulated by pro-apoptoic and anti-apoptotic Bcl-2 family proteins, such as Bax, Bak, Bcl-2 and Bcl-xL, through the composition of the voltage-dependent anion channel (VDAC) (33) and adenine nucleotide translocator (ANT) (34). The BH3-only proteins are widely suggested to affect mitochondria directly or via interaction with Bax (and possibly Bak) (35). The BH3-only proteins appear to activate Bax and Bak indirectly, by engaging and neutralizing pro-survival Bcl-2 family member, which otherwise constrain Bax and Bak from permeabilizing mitochondria. Recent data showed that inhibition of anti-apoptotic Bcl-2 proteins is not sufficient for apoptosis initiation and requires Bid or Bim-mediated activation of Bax/Bak (36). Furthermore, oligomers of tBid (truncated Bid) are reportedly able to trigger apoptosis without inducing the dimerization of Bax or Bak (37). The tBid can be further modified by N-terminal myristoylation, allowing its interaction with Bax or Bak, induction of Bax conformational change and Bax/Bak oligomerization. Although BH3-only proteins are essential for mitochondrial apoptosis, the precise mechanism underlying both Bax and Bak activation remains to be determined.

Mitochondrial permeability transition (MPT) is a mitochondrial state in which the proton-motive force is disrupted (38, 39). This disruption involves the mitochondrial PTP. The PTP is a transmembrane channel formed at the contact sites between the inner mitochondrial membrane (IMM) and the outer mitochondrial membrane (OMM). The components of the PTP are the VDAC in the outer mitochondrial membrane and the ANT in the inner mitochondrial membrane (38). During normal mitochondrial function, the intermembrane space separates the inner and outer mitochondrial membranes and the VDAC and the ANT do not interact. When mitochondrial permeability is activated by the formation of the PTP, the inner mitochondrial membrane loses its integrity, and oxidative phosphorylation is uncoupled. When this occurs, oxidation of metabolites by O_2 proceeds with electron flux not coupled to proton pumping, resulting in dissipation of the transmembrane proton gradient and ATP production and the production of reactive oxygen species (ROS) (39). Bcl-2 family members can modulate mitochondrial permeability transition.

Bif-1 (Endophilin B1), a member of the fatty acyl transferase endophilin B protein family, has been proposed as a novel Bax/Bak activator (40). Inhibition of endogenous Bif-1 expression in HeLa cells by RNA interference (RNAi) abrogated the conformational change of Bax and Bak, cytochrome *c* release, and caspase 3 activation induced by various intrinsic death signals. The interaction of Bif-1 with Bax in mammalian cells appears to be specifically enhanced by apoptotic stimulation, such as interleukin 3 (IL-3) withdrawal or microtubule damage, which is accompanied by a conformational change in the Bax protein (41).

Calcium signaling from ER and cytosol to mitochondria during apoptosis

Ca^{2+} is one of the key regulators of not only cell survival but also cell death in response to a variety of cellular signals (42). The pro-apoptotic effects of Ca^{2+} are mediated by a diverse range of Ca^{2+} sensitive factors that are compartmentalized in various intracellular organelles including the ER, cytoplasm, and mitochondria (43). The mitochondria and endoplasmic reticulum (ER) are interconnected both physically and physiologically, affecting mitochondrial metabolism and complex cellular processes (44, 45). A key aspect of the mitochondria and ER relationship is the modulation of Ca^{2+} signaling during cell activation, which thus affects a variety of physiological processes (46).

The ER is a complex organelle composed of membrane sheets that enclose the nuclear envelope and an elaborate interconnected tubular network in the cytosol (47). The highly dynamic and elaborate structure of ER is maintained by a constant remodeling process that involves the formation of new tubules (47). The ER can be an initiator of apoptosis when accumulation of unfolded proteins or inhibition of the ER-Golgi transport results in the ER stress response (48). Ca^{2+}-dependent stimuli that require Bax and Bak at the ER induce apoptosis through a mitochondrial pathway including mitochondrial dysfunction, cytochrome *c* release and caspase activation (49). Bcl-2 was revealed to also localize at the ER and that it can modulate Ca^{2+} fluxes during the course of cell death (50). Overexpressed Bcl-2 reduces resting ER Ca^{2+} concentration and the extent of capacitative Ca^{2+} entry, pointing to a specific role of Bcl-2 at the ER in the control of cell death (50).

Mitochondria also modulate and synchronize Ca^{2+} signaling (51). Within mitochondria, Ca^{2+} modulates mitochondrial dehydrogenase activity and thus ATP production. Ca^{2+} from ER and/or cytosol primarily moves to the MOM through VDAC (52) (Fig. 1). Mitochondrial Ca^{2+} uptake regulates the spatiotemporal pattern of the cytosolic Ca^{2+} signal and many Ca^{2+}-dependent cellular processes. When large quantities of Ca^{2+} are accumulated in the mitochondrial matrix, Ca^{2+} interacts with cyclophilin D to induce opening of the mitochondrial PTP and this large membrane pore in the inner mitochondrial

membrane can lead to matrix swelling, causing the rupture of the outer mitochondrial membrane and release of cytochrome *c* (53, 54). Furthermore, the rise in mitochondrial Ca^{2+} stimulates the generation of factors, including ROS and free fatty acids, which also promote the opening of the PTP. Opening of the PTP causes dissipation of the $\Delta\Psi m$ and release of Ca^{2+} (44).

Stimuli that generate inositol 1,4,5-trisphosphate (IP_3) cause release of Ca^{2+} from the ER, which is rapidly taken up by closely juxtaposed mitochondria (55). The existence of mutual interactions between the two organelles, ER and mitochondria, is supported by the finding that Bik, a BH3-only member of the Bcl-2 family, is anchored on the ER surface and induces the recruitment of Drp1 to mitochondria (56, 57). Bik stimulates Ca^{2+} release from ER and Drp1 recruitment to the mitochondria, which in turn induces cristae remodeling in a manner dependent on the transmission of Ca^{2+} to the mitochondria (56) (Fig. 1). Another data showed that Bik regulates Bax/Bak-dependent release of Ca^{2+} from ER stores and mitochondrial apoptosis during stress-induced cell death (57).

Recent study have demonstrated that the regulated process of mitochondrial fusion and fission controls the spatiotemporal properties of mitochondrial Ca^{2+} responses and, thus, physiological and pathological consequences of cellular Ca^{2+} (58). Two proteins involved in the mitochondrial fission machinery, Drp1 and hFis1, have an antagonistic effect on Bcl-2 (59). Drp1, with the assistance of hFis1, sensitizes cells to MPT by reducing the mitochondrial Ca^{2+} retention capacity. Further, recruitment of Drp1 to mitochondria seems to be stimulated by Ca^{2+} uptake into the organelles (60), whereas fragmentation of the mitochondrial network reduces the global amplitude of mitochondrial Ca^{2+} responses (58). Over-production of the ER-associated protein p20, a cleavage product of the Bcl-2 interacting protein BAP31, stimulates Ca^{2+} release from the ER and the subsequent recruitment of the fission protein Drp1 to mitochondria (60). Massive accumulation of Ca^{2+} in the mitochondria can lead to the opening of the PTP in the MIM and swelling of the organelle or, acting on mitochondria-shaping proteins (Drp1, Fis1, Opa1, mitofusins 1 and 2), to mitochondrial cristae remodeling and modulation of apoptosis.

Mitochondrial morphology dynamics

Mitochondria are dynamic organelles that continuously move, fuse and divide (1, 7, 61). Mitochondria form an interconnected tubular network, whose steady-state morphology is precisely controlled by the frequent fission and fusion events (6, 7). The dynamics of these organelles draws a tremendous attention because of its importance in development and disease (62). The OMM surrounds an IMM which encloses a protein-rich matrix containing the mitochondrial DNA. The large surface area of the IMM in relation to that of the OMM necessitates organization of the IMM into numerous invaginations, or cristae (63, 64). Mammalian cells maintain the overall shape of their mitochondria by balancing the opposing processes of mitochondrial fusion and fission (1, 7). Unbalanced fission and fusion result in mitochondrial fragmentation and elongation, respectively. Frequent fusion and fission within a dynamic network may be an efficient means of intermitochondrial DNA complementation through exchange of genomes between fusing mitochondria (65).

Recent studies have revealed that the mitochondrial morphology is an important determinant of mitochondrial function (2). The mitochondrial reticulum can be rapidly remodeled by dynamic fission and fusion events in response to the physiological requirements of a cell (1, 66, 67). In mammalian cells, morphological changes in mitochondria have been shown to require numerous mitochondria-shaping proteins (7). Mitochondrial fusion is regulated by the large GTPases mitofusins, Mfn1 and Mfn2 (8, 68) and optic atrophy protein 1 (Opa1) (9, 69) and mitochondrial fission is controlled by proteins of Fis1 (10) and dynamin related protein 1 (Drp1) (11) along with endophilin B1/Bif-1 (40, 70), MTP18 (71), GDAP1 (72), DAP3 (73), and potentially other known and unknown proteins (7) (Table 1).

Mitochondrial dynamics are dictated by the equilibrium between fusion and fission (division) of mitochondria (74). These two processes occur in normal conditions. Mitochondrial fusion and fission rely on the function of multiple proteins that mediate the remodeling of the outer and inner mitochondrial membrane (75). Mitochondrial fusion is a complex regulation process involving multiple proteins that fuse both the outer and inner membranes (76). Recently, key components of the mitochondrial fusion machinery, Opa1, Mfn 1 and Mfn2, have been identified. Opa1 is a 120 kDa GTPase located on the inner mitochondrial membrane facing the intermembrane space (77). Opa1 regulates remodeling of the cristae but not for mitochondrial docking (9). Cristae remodeling conversely requires presenilin-associated rhomboid-like (Parl) (78). Parl itself is regulated by proteolysis to generate a cleaved form, which in turn modulates the shape of the mitochondrial reticulum. Mitofusin 1 (Mfn1) shows similar topology and high sequence homology with Mfn2, and both proteins result necessary for apposing mitochondria during fusion process (68, 79). Both proteins are anchored in the OMM via two transmembrane domains connected by an IMS loop, with the N-terminal GTPase domain and the C-terminal coiled coil domain facing the cytosol (76).

Recent results indicate that mitochondrial fission has important physiological functions in cellular functions, especially in apoptosis (80, 81). The increase of fission leads to mitochondrial fragmentation, which appears to be important for execution of apoptotic death programs including neuronal cell death (82). Drp1 is a cytosolic protein, with an N-terminal GTPase domain and a C-terminal GTPase effector domain (GED), both of which are necessary for fission (10). During fission process, Drp1 recruitment to the OMM is thought to occur via transient interaction with the TPR motifs of hFis1 (10,

Table 1. Proteins and their regulators involved in mitochondrial fusion and fission machinery in mammalian cells [Reproduced from the review by Cerveny et al. (7)]

Function	Protein	Regulator	Role of regulator	Reference
Fusion	Mfn1 & 2	Bax and Bak	Assembly of Mfn2-containing complexes	(106)
		Bcl-xL	Unknown	(107)
		MARCH-V/MITOL	Possible ubiquitination and degradation	(108)
		MIB	Unknown	(109)
		Stomatin-like protein 2	Unkonwn	(110)
	Opa1	Parl	Proteolytic processing	(9)
		Paraplegin	Proteolytic processing	(111)
		Yme1L	Proteolytic processing	(112)
Fission	Drp1	Cyclin B-CDK	Phosphorylation during cell cycle	(113)
		cAMP-dependent protein kinase	Inhibition of assembly and GTPase activitiy by phosphorylation	(114)
		SUMO-1	Sumoylaiton	(88)
		SENP5	Removal of SUMO	(115)
		Bax and Bak	Mitochondrial association of Drp1	(89)
		Fis1	Mitochondrial association of Drp1	(10)
		MARCH-V/MITOL	Ubiquitination and degradation	(108)
	Fis1	MARCH-V/MITOL	Ubiquitination and degradation	(116)
Fission-related	Bif-1	Drp1	Unknown	(70)
	MTP18	-	-	(71)
	GDAP1	-	-	(72)
	DAP3	-	-	(73)

83, 84). The TPR motifs are known to facilitate specific protein-protein interactions (85). Recent findings have suggested that mitochondrial fission may be controlled in response to a variety of cellular events, including cell division, metabolic flux, cell death and differentiation (7). Most of these processes seem to regulate the localization, dynamics and activity of Drp1 including phosphorylation (86), ubiquitinylation (87) and sumoylation of Drp1 (88, 89). Another fission inducing protein, Fis1 (also termed as hFis1 (human Fis1) in order to distinguish human Fis1 from yeast Fis1, Fis1p), consists of a flexible N-terminal tail and six α-helices connected with short loops construct a single core domain. The C-terminal tail containing a transmembrane segment appears to be disordered. In the core domain, each of two sequentially adjacent helices forms a hairpin-like conformation, resulting in a six helix assembly forming a slightly twisted slab similar to that of a tandem array of tetratrico-peptide repeat (TPR) motif folds (90).

Roles of mitochondrial fusion and fission machinery in apoptosis

Many studies have demonstrated that apoptosis induced by various stimuli is closely associated with fragmentation of the mitochondrial network (64, 74, 81, 91). Dramatic alteration in mitochondrial morphology is observed during the early stages of apoptotic cell death, fragmentation of the network and remodeling of the cristae (92, 93). Drp1 recruitment to mitochondria and foci formation at scission sites and tips has been observed during apoptosis (94). Interestingly, Bax colocalization with Bak (24) and Drp1 (94), but not with hFis, has been observed in the scission foci. This observation supports a model wherein a transient interaction of hFis with Drp1 is followed by stable Drp1 association with the OMM. In addition, Mfn2 which is involved in regulating the mitochondrial fusion colocalize with Bax in fission foci in different cell types treated with various inducers of stress-dependent apoptosis (94), suggesting that the mitochondrial fusion as well as fission is closely related with apoptosis.

Mitochondrial fusion and fission machinery controls mitochondrial shape and physiology including mitochondrial remodeling in apoptosis (80). During the fission process, mitochondria undergo ultrastructural changes that include opening of the narrow tubular cristae junction and fusion of individual cristae. This results in increased availability of the cytochrome *c* release across the outer membrane (95). Changes in mitochondrial morphology were associated with apoptotic

sensibility in mammalian cells. Increased fission leads to mitochondrial fragmentation, whereas reduced mitochondrial fission causes elongation and increased connectivity of mitochondria (74, 80, 96). Mitochondrial fragmentation was not affected by caspase inhibitors but inhibited by ectopic expression of the dominant-negative mutant of Drp1, Drp1^{K38A} (92) and also by down regulation of Drp1 using RNAi (97). Taken together, these studies provide extensive evidence in support of a role for mitochondrial fission proteins in the regulation of apoptotic cell death.

Depletion of hFis1 also leads to inhibition of mitochondrial fragmentation (mitochondrial elongation) and cytochrome *c* release following apoptotic stimuli, but acts prior to Bax recruitment (97). This result indicates that blocking of mitochondrial fission inhibits apoptotic fragmentation of mitochondria and also inhibits or delays cytochrome *c* release, the decrease in $\Delta\Psi$m and fragmentation of nuclear DNA that are landmarks of apoptosis, but does not affect translocation/activation of Bax. Depletion of hFis1 or Drp1 by RNAi induces mitochondrial fusion and inhibits induction of apoptosis (97) (Fig. 2). Interestingly, loss of hFis1 inhibited Bax translocation, whereas loss of Drp1 did not, explaining the reason why the Drp1 depletion cells represent a moderate apoptotic inhibition compared to the hFis1 depletion cells (97). These results suggest that hFis1not Drp1 may be involved in Bax translocation in a step of the apoptosis pathway. More interestingly, loss of Drp1 prevented cytochrome *c* release even in cells where Bax has translocated to mitochondria. A large proportion of the cells become Bax translocation positive and cytochrome *c* release negative, showing a new stage of apoptosis inhibition. Drp1 seems to function subsequent to Bax translocation in the cytochrome *c* release process that occurs during apoptosis (80, 97).

Contrary to fission inhibition, inactivation of the core components of the mitochondrial fusion machinery, Mfn1 and 2 (98, 99) and Opa1 (77, 97), resulted in the increased mitochondrial fragmentation and increased sensitivity to apoptotic stimuli. When Opa1 was depleted by RNAi, the mitochondria were extensively fragmented and cells became highly susceptible to apoptosis, and further correlating mitochondrial fragmentation with sensitivity to apoptosis (77, 97) (Fig. 2). Surprisingly, cells have fragmented mitochondria in the depletion of both hFis1 and Opa1 showed strong resistance to apoptosis. These results indicate that although mitochondrial morphology per se may not be directly correlated to apoptosis, the components of the mitochondrial fission-fusion machinery, mitochondria-shaping proteins, can positively and negatively regulate apoptosis (Fig. 2). One explanation is that the molecules that play pivotal roles in the regulation of

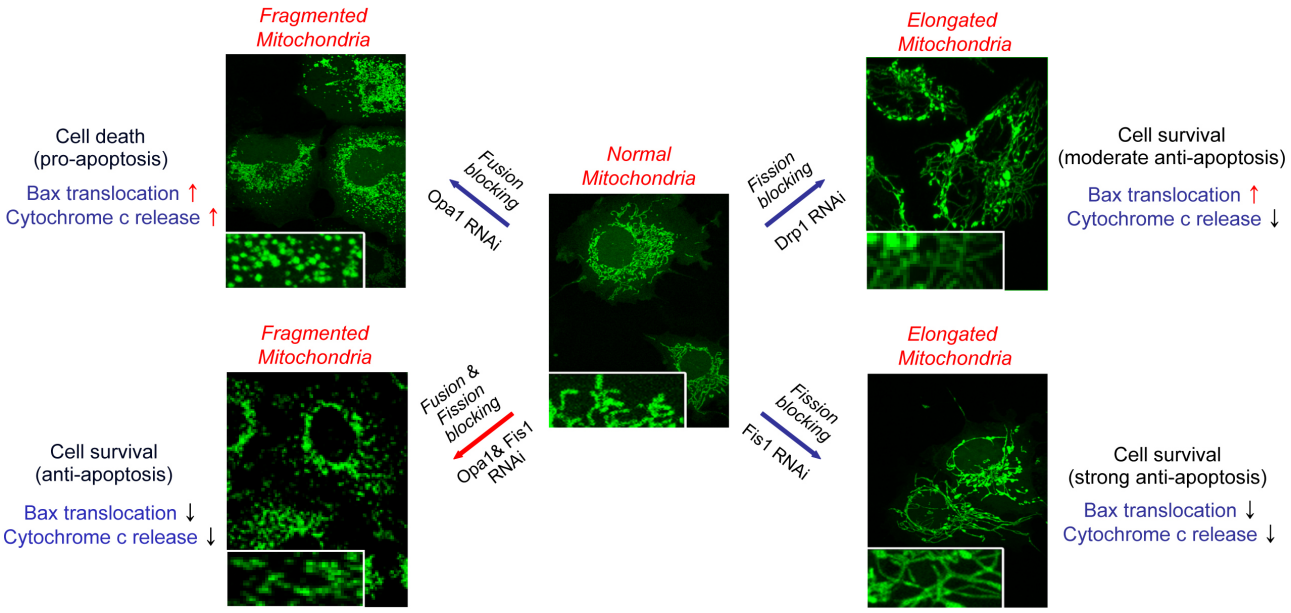

Fig. 2. Correlation between mitochondrial morphology and apoptotic sensibility. Blocking of mitochondrial fission by knockdown (RNAi) of Fis1 or Drp1 expression induces mitochondrial fusion. HeLa cells with the elongated and interconnected mitochondria show apoptosis resistance. Fis1 RNAi cells show stronger anti-apoptotic phenotype than Drp1 RNAi cells. Elongated mitochondria in the Fis1 RNAi cells inhibit both Bax translocation to mitochondria and cytochrome *c* release from mitochondria. Elongated mitochondria, however, in the cells depleted of Drp1 inhibit cytochrome *c* release but not Bax translocation. In contrast to hFis1 and Drp1 RNAi cells, Opa1 RNAi cells having fragmented mitochondria are more sensitive to apoptosis induced by various stimuli compared to the control cells. Fragmented mitochondria in the cells promote both Bax translocation and cytochrome *c* release. In the cells depleted of both hFis1 and Opa1, despite of mitochondria are extensively fragmented, the cells show apoptosis resistance.

fission may have dual functions and may participate in cell death signaling independent of their roles in mitochondrial fission (97). Fis1 is thought to be essential for Drp1 recruitment to the OMM (10, 83, 84). However, judging from the result that mitochondrial fission undergoes without hFis1 in the double hFis1/Opa1 RNAi cells, hFis1 does not seem to require for the recruitment of Drp1 to the mitochondrial membrane (97).

Although many data support an important role of mitochondria shaping-proteins in the execution of cell death, some recent data showed that hFis1 and/or Drp1 modulate mitochondrial fission but not cell death (100, 101). Fission inhibition by down-regulating of either Drp1 or Fis1 (97), prevented the fragmentation of the mitochondrial network and partially inhibited the release of cytochrome *c* from the mitochondria but fails to block the efflux of Smac/Diablo (26). In addition, preventing mitochondrial fragmentation does not inhibit cell death induced by Bax/Bak-dependent death stimuli, in contrast to the effects of Bcl-xL or caspase inhibition (100). Consistent with the results of hFis1 and Opa1 double RNAi (97), these results indicate that although fission is required for cell death, fission itself was not sufficient for cell death initiation. One possible explanation for these results is that mitochondrial fission may participate in cell death by modifying the lipid microenvironment or deform lipid bilayers for Bax/Bak induced MOMP (102). Because that Drp1, Mfn2, and Bax co-localize at fission sites during apoptosis (94), fission proteins may interact with Bax during apoptosis.

Except some studies, most data have demonstrated that mitochondrial fusion and fission is closely related to apoptotic cell death. From the accumulating data, alternative four scenarios have been proposed as to how mitochondrial fission might participate in the execution of apoptosis (64, 74, 96) (Fig. 3):

(1) Both Bax translocation and Drp1-dependent mitochondrial fission are responsible for apoptosis, but these two machinery works in tandem. Apoptotic signals activate Bax translocation and Drp1-dependent mitochondrial fission at the same. Active Bax forms channels across the outer mitochondrial membrane and also participates in the activation of Drp1-dependent fission of the mitochondria. Activated Bax forms pores across the MOM that contribute to MOMP. Bax-mediated MOMP and Drp1-mediated mitochondrial fission promote apoptosis independently. Several studies have reported that preventing mitochondrial fission during apoptosis leads to a partial inhibition of cytochrome *c* release (60, 70, 92). This suggests that mitochondrial fragmentation or components of the mitochondrial fission/fusion machinery could be involved in the translocation and/or the activation of Bax/Bak at the MOM.

(2) Drp1-dependent mitochondrial fission promoted by Bax translocation is responsible for MOMP. Apoptotic signals activate Endophilin B1 due to translocation of Bax to mitochondria. Active Bax participates in the activation of Drp1-mediated fission of mitochondria. Fission of mitochondria is essential for

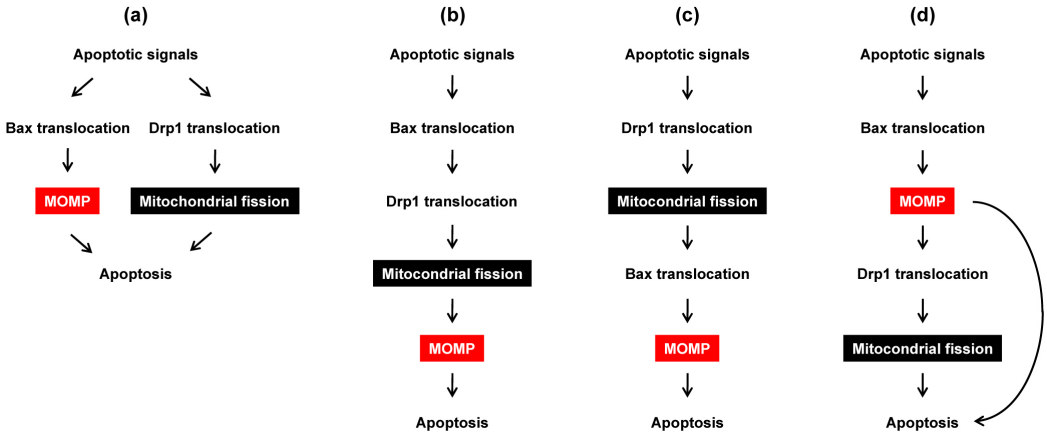

Fig. 3. Four alternative models for explaining the connections between mitochondrial fission and apoptosis. **(a)** Both Bax translocation and Drp1-dependent mitochondrial fission are responsible for apoptosis. Apoptotic signals activate Endophilin B1 due to translocation of Bax to mitochondria and at the same time trigger Ca^{2+} efflux from ER due to translocation of Drp1 to mitochondria. Active Bax forms channels across the outer mitochondrial membrane, resulting in MOMP. Bax-mediated MOMP and Drp1-mediated mitochondrial fission promote apoptosis independently. **(b)** Drp1-dependent mitochondrial fission promoted by Bax translocation is responsible for MOMP. Apoptotic signals activate Endophilin B1 due to translocation of Bax to mitochondria. Active Bax participates in the activation of Drp1-mediated fission of mitochondria. Fission of mitochondria is essential for MOMP. **(c)** Mitochondrial fission is essential step for MOMP. Apoptotic signals induce release of ER calcium at first, resulting in the translocation of Drp1 to mitochondria and induction of fragmentation. Next, Bax translocation and the formation of Bax-containing channels trigger MOMP. **(d)** Mitochondrial fission lies downstream of MOMP or does not necessary for MOMP. Apoptotic signals trigger the translocation of Bax to mitochondria. Active Bax forms channels that induce MOMP and the release of some of the soluble proteins localized in the intermembrane space such as some of the cytochrome *c* and OPA1, and DDP/TIMM8a. Activating Drp1-mediated fission synergistically induce prominent mitochondrial fragmentation and MOMP.

MOMP. Data showed that Bax translocation occurs, without Drp1, but Bax translocation alone is not sufficient for cytochrome *c* release, suggesting that Drp1 seems to function subsequent to Bax translocation in the cytochrome *c* release process that occurs during apoptosis (80, 97).

(3) Mitochondrial fission is essential step for MOMP. Apoptotic signals induce the release of ER calcium at first. This triggers translocation of Drp1 to mitochondria and induction of fragmentation. Mitochondrial fission induces Bax translocation leads to MOMP. Recent data showed that the mitochondrial fission machinery acts upstream of the Bcl-2 family of proteins in neurons challenged with nitrosative stress (103). Mitochondrial fission induced by nitrosative stress is an early event and is followed by Bax foci formation on mitochondria. Mfn1, or $Drp1^{K38A}$ inhibits mitochondrial fission, Bax foci formation, and neuronal cell death, but anti-apoptotic Bcl-xL cannot block mitochondrial fission and only prevents Bax clustering on mitochondria and neuronal cell death. The result suggesting that Bax foci formation occurs after mitochondrial fission (103).

(4) Mitochondrial fission lies downstream of MOMP or does not be necessary for MOMP. Apoptotic signals trigger the translocation of Bax to mitochondria. Opa1 and DDP/TIMM8a are both released from the mitochondria after MOMP during Bax/Bak-dependent cell death (104, 105) (Fig. 1). The release of Opa1 could potentially explain the inhibition of mitochondrial fusion occurring during apoptosis, and DDP/TIMM8a may bind to Drp1 and promotes its recruitment to the mitochondria, thus providing a mechanism for how mitochondrial fission is activated during apoptosis. Some data support this model that mitochondrial fission is not directly related to apoptosis (97, 100, 101).

These scenarios suggest that mitochondrial fission may participate in apoptosis with different ways according to cell types and/or apoptotic signal characteristics. However, the accurate mechanisms remain to be clarified convincingly.

Concluding remarks

During recent years, mitochondria have been known to represent much more than just an energy power house of the cell. Mitochondria play a crucial role in regulating cell death pathways that are mediated by Bcl-2 family proteins. Calcium signaling from ER and/or cytosol to mitochondria is one of the crucial cellular processes during apoptosis in response to a variety of cell death signals. Mitochondrial fusion and fission machinery controls mitochondrial shape and physiology including mitochondrial remodeling during apoptosis. Mitochondrial fragmentation occurs in most forms of apoptosis and is due to activation of mitochondrial fission machinery together with or without the inhibition of fusion. However, whether two groups of regulator, Bcl-2 family member proteins and mitochondrial-shaping proteins, participate in this process dependently or independently is just beginning to be understood.

Here, we have discussed the importance of mitochondrial calcium signaling and mitochondrial fusion and fission machinery in apoptosis. This review will provide insight into how mitochondria communicate one another as well as to ER to response the apoptotic cell death signals.

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