Caspase-9-induced Mitochondrial Disruption through Cleavage of Anti-apoptotic BCL-2 Family Members*

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Mitochondrial disruption during apoptosis results in the release of cytochrome c that forms apoptosomes with Apaf-1 and caspase-9. Activation of caspase-9 by dimerization in apoptosomes then triggers a caspase signaling cascade. In addition, other apoptosis signaling molecules released from the mitochondrion, such as apoptosis-inducing factor and endonuclease G, may induce caspase-9-independent apoptosis. To determine the signaling events induced by caspase-9, we used chemically induced dimerization for specific activation of caspase-9. We observed that caspase-9 dimerization resulted in the loss of mitochondrial membrane potential and the cleavage of anti-apoptotic Bcl-2, Bcl-xL, and Mcl-1. Moreover, cleavage-resistant Bcl-2, Bcl-xL, or Mcl-1 potently inhibited caspase-9-dependent loss of mitochondrial membrane potential and the release of cytochrome c. Our data suggest that a caspase-9 signaling cascade induces feedback disruption of the mitochondrion through cleavage of anti-apoptotic Bcl-2, Bcl-xL, and Mcl-1.

In mitochondrion-dependent apoptosis, exposure of cells to various stress signals triggers the disruption of the mitochondrion, leading to the release of cytochrome c into the cytosol (1–6). The formation of apoptosome containing cytochrome c, Apaf-1, and caspase-9 results in the activation of caspase-9 in apoptosome through dimerization (7–11). Caspase-9 then triggers a caspase signaling cascade to induce apoptosis. In addition to cytochrome c, the disruption of the mitochondrion also leads to the release of other factors. Apoptosis-inducing factor and endonuclease G released from the mitochondrion may induce caspase-9-independent apoptosis (12–15).

The Bcl-2 family proteins are important for regulating the integrity of the mitochondrion. Bcl-2 family members share the Bcl-2 homology (BH)3 domains and can be divided into three subfamilies (16–18), including the anti-apoptotic subfamily members, such as Bcl-2, Bcl-xL, and Mcl-1; the pro-apoptotic Bax- and Bak-like proteins; and the pro-apoptotic BH3-only subfamily members. In living cells, anti-apoptotic proteins bind to endogenous BH-3 proteins or directly activate pro-apoptotic Bax and Bak, thereby inhibiting apoptosis (19). BH3-only proteins are upstream apoptosis sensors for different apoptosis signaling in specific cell types. BH3-only proteins either inhibit the anti-apoptotic molecules or directly activate pro-apoptotic Bax or Bak to induce apoptosis (16, 17). Cleavage of Bcl-2 family members by caspases has been shown to regulate their functions. Caspase-8-dependent cleavage and activation of Bid can contribute to mitochondrial disruption (20–23). In addition, caspase-mediated cleavage of anti-apoptotic Bcl-2, Bcl-xL, and Mcl-1 may convert these anti-apoptotic molecules to nonfunctional or pro-apoptotic molecules (24–31), thereby facilitating the induction of apoptosis.

Caspase activities have been implicated in inducing mitochondrial damage during apoptosis (32–34). Caspase-2 may function upstream of the mitochondria to induce apoptosis (35–37). Knock-out of caspase-9 has been shown to delay the loss of mitochondrial membrane potential (38). In addition, double knock-outs of caspase-3 and caspase-7 also significantly delay the mitochondrial events during apoptosis (39). However, the molecular mechanisms for caspases-3, -7, and -9 in mediating the mitochondrial events have not been resolved.

In this study, we investigated whether specific engagement of caspase-9 could directly trigger the disruption of the mitochondrion. We utilized chemically induced dimerization for specific activation of caspase-9. We observed that dimerization of caspase-9 induced the loss of the mitochondrial membrane potential, as well as the cleavage of Bcl-2, Bcl-xL, and Mcl-1. Moreover, downstream effector caspases, including caspases-3, -6, and -7, may differentially regulate caspase-9-dependent cleavage of these anti-apoptotic molecules and mitochondrial events. Our data suggest a role for the caspase-9 signaling cascade in amplifying mitochondrial disruption through cleavage of anti-apoptotic Bcl-2 family proteins.

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—Inducible caspase-9 (iCasp9) with caspase-9 pro tease domain fused to a modified FK506-binding protein and a 5’ HA tag has been described previously (40). H9 (ATCC) and Jurkat cells (clone E6-1, ATCC) were transfected with pcDNA3-iCasp9 by electroporation and selected with 0.5 and 1 mg/ml G418 (Cellgro), respectively. Three independent H9 or Jurkat clones were selected by limiting dilution, and no significant differences in caspase-9 signaling were observed between these clones. One H9-iCasp9 and one Jurkat-iCasp9 clones were used for subsequent experiments.

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3 The abbreviations used are: BH, Bcl-2 homology; WT, wild type; HA, hemagglutinin; CID, chemical inducer of dimerization; DAPI, 4’6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; VDAC, voltage-dependent anion channel; iCasp9, inducible caspase-9.

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AP20187 (41), a dimeric FK506 analog (Ariad Pharmaceuticals) as the chemical inducer of dimerization (CID), was added to H9-iCasp9 or Jurkat-iCasp9 cells to induce dimerization and activation of caspase-9.

Apoptosis Assays—For apoptosis assays, H9-iCasp9 or Jurkat-iCasp9 cells (10^5/well) were incubated in 96-well plates with the indicated concentrations of AP20187 or 40 μM etoposide (Sigma). Cells were cultured for 20 h, and the percentage of cell loss was determined by propidium iodide exclusion assays as described (40). Alternatively, iCasp9 or Jurkat-iCasp9 cells were cultured with 100 nM AP20187 for indicated times, followed by staining with annexin V and analysis by flow cytometry.

RNA Interference—To make RNA interference constructs, heteroduplex oligonucleotides targeting different caspases were cloned into the pSUPERRetro vector (OligoEngine). The forward oligonucleotides contained a 19-nucleotide targeting sequence, and its antisense sequence was separated by a 9-nucleotide spacer. After annealing to their reverse complementary oligonucleotides and cloning into the pSUPERRetro vector, each pSUPER-RNAi vector was co-transfected with the corresponding caspase expression vector into 293T cells, followed by Western blot analysis to verify efficient suppression of targeted caspases. The pSUPER-RNAi vector was then transfected into H9-iCasp9 cells by electroporation, and caspase-9 was confirmed by Western blot analyses. The 19-nucleotide targeting sequences are as follows: caspase-2, ACAGCTGT-TGTTAGCCGAA; caspase-3, TGACATCTGGTTGTTAC; caspase-6, CATGACAGAAACAGATGCC (42); and caspase-7, TGACAGATTGCCAGGACC.

Dominant-negative Caspase-9 Clones—cDNA encoding a protease-deficient caspase-9 (casp9dn) with a cysteine to serine substitution at Cys-315 of the protease-active site (40) was cloned into a pSH1 vector containing a puromycin resistance gene. This expression construct was transfected into H9 or Jurkat cells by electroporation. Stable H9 and Jurkat transfectants expressing casp9dn were selected with 0.5 μg/ml puromycin (Sigma). Specific suppression of the targeted caspases in H9-iCasp9 cells was confirmed by Western blot analyses. The 19-nucleotide targeting sequences are as follows: caspase-2, ACAGCTGT-TGTTAGCCGAA; caspase-3, TGACATCTGGTTGTTAC; caspase-6, CATGACAGAAACAGATGCC (42); and caspase-7, TGACAGATTGCCAGGACC.

Western Blotting—Cells were lysed in lysis buffer (50 mM HEPES, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1× protease inhibitor mixture from Roche Applied Science, and 10 μM benzoxylcarbonyl-VAD) on ice for 30 min, followed by centrifugation at 16,000 × g for 10 min at 4 °C. The supernatants were collected, and protein concentrations were determined by the Bradford assay (Bio-Rad). Samples were then used for SDS-PAGE and transferred onto a polyvinylidene difluoride membrane. The membrane was then probed with various antibodies, followed by horseradish peroxidase-conjugated secondary antibodies (Southern Biotechnology), and developed with the Supersignal Dura substrate (Pierce). The following antibodies were used to probe different proteins in Western blot analyses: mouse monoclonal antibodies to caspase-2, caspase-7, caspase-9 (Cell Signaling), Bax, Bcl-2, cytochrome c (BD Biosciences), caspase-6 (Medical & Biological Laboratories), FLAG (Sigma), HA1.1 (Covance), and polyclonal rabbit antibodies to Bak (Upstate Biotechnology), caspase-3, Bcl-xL (Cell Signaling), and Mcl-1 (Santa Cruz Biotechnology). Some blots were also probed with monoclonal anti-α-tubulin (Santa Cruz Biotechnology) or the voltage-dependent anion channel (VDAC1) (Abcam) to ensure equal loading.

Analysis of Mitochondrial Membrane Potential—iCasp9 cells were treated with CID or etoposide for different times, followed by incubation with 50 nM tetramethylrhodamine ethyl ester (Molecular Probes) at 37 °C for 20 min to measure mitochondrial membrane potential.

In some experiments, the cells were stained with Mitocasp dual sensor (Cell Technology) according to the manufacturer’s protocol. Briefly, after induction of apoptosis for various times, the cells were incubated with the mitochondrial membrane potential dye and FAM-DEVD-fluoromethyl ketone in culture medium at 37 °C for 30 min. The cells were then washed and analyzed by flow cytometry.

In immunocytochemistry analyses, the cells were stained with 100 nM Mitotracker Red CMXRos (Molecular Probes) at 37 °C for 20 min. The cells were then washed and added to polylysine-coated glass slides by cytopsin. After fixation with 4% paraformaldehyde and permeabilization with 0.1% Triton X-100, the slides were incubated with anti-cytochrome c (BD Biosciences) at 4 °C overnight, followed by incubation with FITC-conjugated goat anti-mouse IgG (Sigma). The cells were then counterstained with DAPI using VectaShield mounting medium containing DAPI (Vector Laboratories) and visualized under a fluorescence microscope.

Establishment of H9-iCasp9 Cells Expressing Wild Type or Cleavage-resistant Bcl-2, Bcl-xL, or Mcl-1—Cleavage-resistant Bcl-2, Bcl-xL, and Mcl-1 mutants were generated by site-directed mutagenesis (Stratagene). Mcl-1D/A mutant with Asp to Ala substitution at Asp-34 (D34A) and Bcl-xL D/A with D61A and D76A substitutions were generated by Dr. Patrick Auberger (29). The mutations were confirmed by DNA sequencing. Wild type or cleavage-resistant Bcl-2, Bcl-xL, or Mcl-1 was fused to HA or FLAG tag and cloned into pSH1 plasmid containing a puromycin resistance gene. The vectors were transfected into H9-iCasp9 cells, and stable cell lines were selected with 0.5 μg/ml puromycin. The transfected cells were treated with CID for 6 h and stained with Mitocasp (Cell Technology) followed by flow cytometry analysis. Percentage of the loss of Δψm+ cells was calculated as follows: (control − treated sample)/control × 100%. Statistical significances were analyzed by Student’s t test using GraphPad Prism version 4 for Macintosh. A p value of <0.05 was considered statistically significant.

Subcellular Fractionation—Fractionation of cytosol and particulate fractions containing mitochondria was performed according to an established protocol (43). Cells (10^6) were resuspended in 30 μl of homogenization buffer (75 mM KCl, 1 mM NaH2PO4, 8 mM Na2HPO4, 250 mM sucrose, 1 mM EDTA, and 50 μg/ml digitonin) and incubated on ice for 5 min, followed by centrifugation at 14,000 rpm for 10 min in an Eppendorf microcentrifuge 5417c. Supernatants were collected as

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4 M. Chen, A. D. Guerrero, L. Huang, Z. Shabier, M. Pan, T.-H. Tan, and J. Wang, unpublished data.
cytosol and the pellets as the particulate fractions. The samples were used for Western blot analysis of cytochrome c.

RESULTS

Activation of a Caspase Cascade by Dimerization of Caspase-9—Biochemical studies and crystal structural analyses suggest that caspase-9 is activated in apoptosomes through formation of caspase-9 dimers (7–11). We therefore constructed iCasp9 by fusing the caspase-9 protease domain to a mutated FK506-binding protein to induce caspase-9 dimerization (40). A dimeric FK506 analog, AP20187, was used as a CID to dimerize iCasp9 (41). Human leukemia H9 and Jurkat cells stably expressing iCasp9 were generated (Fig. 1A). Clones with the expression of iCasp9 at levels comparable with endogenous caspase-9 were selected (Fig. 1A). As expected, dimerization of iCasp9 induced rapid processing of iCasp9 and cell death in H9-iCasp9 and Jurkat-iCasp9 cells, but not in control H9 or Jurkat cells (Fig. 1A and B), suggesting that chemically induced dimerization is suitable for inducing caspase-9 activation and apoptosis in these cell lines.

Direct activation of caspase-9 by dimerization would bypass the requirement for the disruption of mitochondria and the release of cytochrome c to activate caspase-9. Consequently, dimerization of caspase-9 would lead to rapid activation of downstream caspases and apoptosis. Indeed, apoptosis manifestation could be detected by annexin V staining in H9-iCasp9 and Jurkat-iCasp9 cells after dimerization of caspase-9 for 2 h (Fig. 2A). Consistently, the activation of downstream effector caspases, including caspases-3, -6, and -7, as well as caspase-2, was detectable 1 h after dimerization of caspase-9 (Fig. 2B). We also expressed a caspase-9 with cysteine to serine mutation at the protease-active site as a dominant-negative mutant (casp9dn) to inhibit the signaling of endogenous caspase-9. As expected, treatments with etoposide induced the activation of caspases-2, -3, -6, and -7 in control H9 or Jurkat cells (Fig. 2C). In contrast, the activation of these caspases was inhibited by casp9dn (Fig. 2C). This suggests that the activation of caspases-2, -3, -6, and -7 was dependent on endogenous caspase-9. These results also indicate that chemically
induced dimerization of caspase-9 mimics endogenous caspase-9 in inducing the activation of downstream caspases.

A Caspase-9 to Caspase-3 Cascade in the Disruption of Mitochondrial Membrane Potential ($\Delta \psi_m$)—Caspases have been shown to contribute to mitochondrial disruption during apoptosis (32–39). We therefore tested whether caspase-9 was involved in this process. We observed that dimerization of caspase-9 induced rapid loss of $\Delta \psi_m$ in H9-iCasp9 cells, but not in H9 controls, by staining with a mitochondrial membrane potential dye, tetramethylrhodamine ethyl ester (Molecular Probes) (Fig. 3). In contrast, the loss of $\Delta \psi_m$ was induced by etoposide in both H9-iCasp9 cells and H9 controls (Fig. 3). Similar results were observed in Jurkat-iCasp9 cells (Fig. 3). These results indicate that dimerization of caspase-9 could independently trigger the loss of $\Delta \psi_m$.

We also examined $\Delta \psi_m$ in H9-iCasp9 cells with Mitotracker Red (Molecular Probes) in immunocytochemistry analyses. Consistent with the above studies, we observed that the staining of mitochondria with Mitotracker was decreased in H9-iCasp9 cells after dimerization of caspase-9 (Fig. 4A). In the untreated H9-iCasp9 cells, cytochrome c co-localized with Mitotracker staining (Fig. 4A). After treatment with CID, cytochrome c staining became diffused and did not co-localize with Mitotracker staining (Fig. 4A). Subcellular fractionation studies showed that cytochrome c was released into the cytosol after dimerization of caspase-9 (Fig. 4B). In addition, the disintegration of the nucleus was evident in H9-iCasp9 cells after dimerization of caspase-9 (Fig. 4A). By contrast, silencing of caspase-3 in H9-iCasp9 cells inhibited the loss of Mitotracker staining (Fig. 4A and Fig. 5A). Although caspase-9-induced diffusion of cytochrome c was observed in H9-iCasp9 cells with silencing of caspase-3, co-localization of cytochrome c with the mitochondria was still observed 8 h after caspase-9 dimerization (Fig. 4A).

Consistently, significant inhibition of cytochrome c release into the cytosol by silencing of caspase-3 was observed in subcellular fractionation analyses (Fig. 4B). Also, caspase-9-induced
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nuclear disintegration was inhibited by silencing of caspase-3 (Fig. 4A). This suggests that a caspase-9 to caspase-3 signaling cascade mediates the loss of Δψm and the release of cytochrome c into the cytosol in H9-iCasp9 cells.

We also quantitated Δψm with MitoCasp dual sensor containing the mitochondrial membrane potential dye to measure

Δψm and DEVD-fluoromethyl ketone to detect caspase activation (Cell Technology). The mitochondrial membrane potential dye contains a cationic mitochondrial dye that accumulates in intact mitochondria to emit red fluorescence (Cell Technology). Consistently, dimerization of caspase-9 induced the loss of Δψm in control H9-iCasp9 cells (Fig. 5B). This was accompanied by an increase in caspase activities shown by increased cleavage of DEVD-fluoromethyl ketone (Fig. 5B). Consistent with immunocytochemistry studies, silencing of caspase-3 significantly inhibited caspase-9-mediated loss of Δψm and caspase activation (Fig. 5, A and B). This suggests that caspase-3 is essential for caspase-9-induced loss of Δψm.

In contrast, silencing of caspase-2 did not affect the caspase-9-mediated loss of Δψm or caspase activation (Fig. 5, A and B), whereas silencing of caspase-6 or caspase-7 partially inhibited the caspase-9-induced loss of Δψm as well as caspase activation (Fig. 5, A and B). This indicates that caspases-6 and -7 are involved in caspase-9-mediated disruption of the mitochondria and caspase activation, whereas caspase-2 is not required.

A Critical Role for Caspase-3 in Caspase-9-induced Cleavage of Bcl-2, Bcl-xL, and Mcl-1—Anti-apoptotic Bcl-2, Bcl-xL, and Mcl-1 have been shown to undergo caspase-dependent cleavage that inactivates their anti-apoptotic activities (24–31). We therefore investigated whether caspase-9 signaling could also mediate the cleavage of these anti-apoptotic molecules. We observed that dimerization of caspase-9 induced the cleavage of Bcl-2, Bcl-xL, and Mcl-1 in H9-iCasp9 cells (Fig. 6A). In contrast, pro-apoptotic Bak or Bax was not cleaved in H9-iCasp9 cells (Fig. 6A). Consistently, Bcl-2, Bcl-xL, and Mcl-1 were also processed in Jurkat-iCasp9 cells after dimerization of caspase-9 (Fig. 6A). The Jurkat-iCasp9 clone was derived from a Jurkat clone E6-1 (ATCC) that appeared to be deficient in Bax (Fig. 6A). Nevertheless, Bak was not processed in Jurkat-iCasp9 cells after dimerization of caspase-9 (Fig. 6A). These observations with H9-iCasp9 and Jurkat-iCasp9 cells suggest that dimerization of caspase-9 induces the processing of anti-apoptotic Bcl-2, Bcl-xL, and Mcl-1. Because these anti-apoptotic molecules are important for protecting the integrity of the mitochondria, cleavage-induced inactivation of their anti-apoptotic functions potentially contributes to caspase-9-mediated loss of Δψm.

Because silencing of downstream caspases differentially affected caspase-9-dependent loss of Δψm (Fig. 5), we examined whether these caspases were involved in the processing of Bcl-2, Bcl-xL, and Mcl-1. Silencing of caspase-3 significantly inhibited the cleavage of Bcl-2, Bcl-xL, and Mcl-1 after dimerization of caspase-9 (Fig. 6B), suggesting an essential role for the
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In this study, we observed that chemically induced dimerization of caspase-9 mimicked the activation of endogenous caspase-9 in inducing a downstream caspase cascade. Direct activation of caspase-9 by chemically induced dimerization would bypass the requirement for the disruption of the mitochondrion; however, a loss of $\Delta \psi m$ was observed after dimerization of caspase-9. This suggests that caspase-9 could mediate feedback disruption of the mitochondrion. Caspase-9-induced loss of $\Delta \psi m$ was accompanied by the cleavage of Bcl-2, Bcl-xL, and Mcl-1 in H9-iCasp9 cells (Fig. 8C). Although wild type Bcl-2, Bcl-xL, and Mcl-1 also reduced caspase-9-induced loss of $\Delta \psi m$, the effects were weaker than those of their cleavage-resistant mutants (Fig. 8C). In addition, Bcl-2D/A, Bcl-xLD/A, and Mcl-1D/A were more efficient than their wild type counterparts in attenuating caspase-9-induced release of cytochrome c into the cytosol (Fig. 8D), as well as apoptosis (Fig. 8, E and F). This suggests that caspase-9-induced loss of $\Delta \psi m$, the release of cytochrome c, as well as cell death involve the cleavage of anti-apoptotic Bcl-2, Bcl-xL, and Mcl-1.

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Our data suggest that caspase-9-mediated disruption of the mitochondrion involves the cleavage of Bcl-2, Bcl-xL, and Mcl-1. Consistent with the loss of Δψm induced by dimerization of caspase-9, the use of a protease-deficient caspase-9 as a dominant-negative mutant also inhibited the loss of Δψm in H9 or Jurkat cells after treatments with etoposide. Moreover, the protease-deficient caspase-9 also suppressed the processing of Bcl-2, Bcl-xL, and Mcl-1 after treatments of cells with etoposide, and this is consistent with the role for caspase-9 in inducing mitochondrial disruption through cleavage of Bcl-2, Bcl-xL, and Mcl-1.

To determine the roles of downstream caspases in caspase-9 signaling, we silenced individual effector caspases in H9-iCasp9 cells. Silencing of caspase-3 virtually blocked caspase-9-induced loss of Δψm, as well as the cleavage of Bcl-2, Bcl-xL, and Mcl-1. Silencing of caspase-6 or caspase-7 also partially inhibited caspase-9-induced loss of Δψm and the cleavage of Bcl-2 and Bcl-xL. This suggests that caspase-3 plays an essential role in mediating caspase-9-induced loss of Δψm through cleavage of anti-apoptotic molecules, whereas caspases-6 and -7 also contribute to the loss of Δψm and promote the cleavage of Bcl-2 and Bcl-xL. Although caspase-2 was activated after dimerization of caspase-9, silencing of caspase-2 did not affect caspase-9 in inducing the loss of Δψm or cleavage of Bcl-2, Bcl-xL, or Mcl-1. Therefore, caspase-2 does not appear to be required for caspase-9-mediated mitochondrial events. Although caspase-3 deficiency inhibited the loss of Δψm during apoptosis, additional caspase-7 deficiency led to more severe defects in the loss of Δψm (39). This suggests that caspase-7 may play a nonredundant role in mediating mitochondrial disruption. However, unique molecular

FIGURE 8. Cleavage-resistant Bcl-2, Bcl-xL, and Mcl-1 in inhibiting the loss of Δψm. A, H9-iCasp9 were transfected with wild type (WT) or cleavage-resistant mutants (D/A) of Bcl-2, Bcl-xL with an HA tag, or Mcl-1 with a FLAG tag. Stable transfectants were selected with 0.5 μg/ml puromycin. Western blot for HA or FLAG expression in the transfectants is shown. C, control. B, transfectants were treated with 100 nM CID for 0–3 h, and the transfected wild type protein and their cleavage-resistant mutants as well as the cleaved products were then detected by Western blot of HA or FLAG. FL, full-length protein. Asterisks represent potential degradation products of Mcl-1-FLAG or background bands. C, transfectants were treated with 100 nM CID, followed by culturing for 0, 3, or 6 h and staining with MitoCasp. Δψm cells were quantitated by flow cytometry, and the percentage of CID-induced loss of Δψm cells was calculated (mean ± S.D.). The p values are as follows: Bcl-2 D/A versus Bcl-2 WT, 0.035 (3 h), 0.018 (6 h); Bcl-xL D/A versus Bcl-xL WT, 0.038 (3 h), 0.003 (6 h); Mcl-1 D/A versus Mcl-1 WT, 0.044 (3 h), 0.023 (6 h). D, H9-iCasp9 transfectants were treated with 100 nM CID for various times, followed by preparation of cytosol and the particulate fraction containing mitochondria. The samples were used for Western blot analysis of cytochrome c. E, transfectants were also probed for tubulin or VDAC as indicated to ensure equal loading. F, transfectants were treated with CID or etoposide (Eto) for 6 h, followed by staining with FITC-annexin V and analysis by flow cytometry. F, transfectants were treated with CID or etoposide for 20 h. The cells were stained with propidium iodide, followed by flow cytometry. The percentage of cell loss (mean ± S.D.) was quantitated as described (40). The p values are as follows: Bcl-2 D/A versus WT, 0.0019 (CID), 0.013 (etoposide); Bcl-xL D/A versus WT, 0.0005 (CID), 0.0005 (etoposide); Mcl-1 D/A versus WT, 0.021 (CID), 0.026 (etoposide).
targets for caspase-7 in mediating the mitochondrial events remain to be identified.

It has been well established that caspase-9 is an initiator caspase to trigger the activation of effector caspases for the execution of apoptosis. Our data indicate that caspase-9 may also serve as an amplifier of apoptosis by promoting feedback disruption of the mitochondrion. Effector caspases activated by caspase-9, including caspases-3, -6, and -7, may mediate such feedback regulation by cleaving anti-apoptotic Bcl-2, Bcl-xL, and Mcl-1.

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