MEK Kinase 1 Induces Mitochondrial Permeability Transition Leading to Apoptosis Independent of Cytochrome c Release

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Induction of apoptosis often converges on the mitochondria to induce permeability transition and release of apoptotic proteins into the cytoplasm resulting in the biochemical and morphological alteration of apoptosis. Activation of a serine threonine kinase MEK kinase 1 (MEKK1) is involved in the induction of apoptosis. Expression of a kinase-inactive MEKK1 blocks genotoxic-induced apoptosis. Upon apoptotic stimulation, MEKK1 is cleaved into a 91-kDa kinase fragment that further induces an apoptotic response. Mutation of a consensus caspase 3 site in MEKK1 prevents its induction of apoptosis. The mechanism of MEKK1-induced apoptosis downstream of its cleavage, however, is unknown. Herein we demonstrate that full-length and cleaved MEKK1 leads to permeability transition in the mitochondria. This permeability transition occurs through opening of the permeability transition (PT) pore. Inhibiting PT pore opening and reactive oxygen species production effectively reduced MEKK1-induced apoptosis. Overexpression of MEKK1, however, failed to release cytochrome c from the mitochondria or activate caspase 9. Since Bcl2 regulates changes in mitochondria and blocks MEKK1-induced apoptosis, we determined that Bcl2 blocks MEKK1-induced apoptosis when targeted to the mitochondria. This occurs downstream of MEKK1 cleavage, since Bcl2 fails to block cleavage of MEKK1. In mouse embryonic fibroblast cells lacking caspase 3, the cleaved but not full-length MEKK1 induces apoptosis and permeability transition in the mitochondria. Overall, this suggests that cleaved MEKK1 leads to permeability transition contributing to MEKK1-induced apoptosis independent of cytochrome c release from the mitochondria.

Apoptotic signals often lead to changes in the mitochondria. These changes consist of mitochondrial permeability transition, production of reactive oxygen species (ROS), and release of proteins into the cytoplasm (1, 2). These events could cause blockage of ATP production, damage to membranes, DNA condensation, and activation of caspases leading to apoptosis (1–4). In response to apoptotic stimulation, the mitochondrial membrane opens to allow solutes and water to enter the mitochondria. This is controlled by a multiprotein complex found in the inner and outer membranes of the mitochondria known as the permeability transition (PT) pore (2, 5, 6). The PT pore consists of voltage-dependent anion channel/porin, adenine nucleotide translocator, cyclophilin D, creatine kinases, and other proteins (6, 7). Upon PT pore opening, the mitochondria loses its membrane potential (ΔΨm) across the inner membrane. This often occurs following apoptotic stimuli. This is associated with increased production of ROS that further damages proteins and membranes (6, 7). In addition, proteins are released from the mitochondria following apoptotic stimulus such as cytochrome c. When cytochrome c is released, it binds to an Apaf1 and caspase 9 complex (1, 4, 8). This leads to caspase 9 activation, causing cleavage of specific proteins and activation of other caspases. These mitochondrial events regulate the induction of apoptosis.

The Bcl2 family of proteins control mitochondrial apoptotic responses (9). Bcl2 family members consist of pro- and anti-apoptotic proteins (10, 11). Proapoptotic Bcl2 family members translocate to the outer membrane of the mitochondria from the cytosol following an apoptotic signal (11). This translocation results in release of cytochrome c into the cytosol and opening of the PT pore. The exact mechanism of how these proteins release mitochondrial cytochrome c or open the PT pore remains unclear. Anti-apoptotic Bcl2 family members such as Bcl2 itself can reverse the effects of the pro-apoptotic members (9). Bcl2 expression effectively blocks the PT pore opening and cytochrome c release. This is accomplished through Bcl2 binding to pro-apoptotic Bcl2 family members, forming heterodimers. This inhibits these pro-apoptotic proteins from opening the PT pore and releasing cytochrome c from the mitochondria (9, 11). This interplay between pro- and anti-apoptotic Bcl2 family members regulates the role of the mitochondria in apoptosis.

MEK kinase 1 (MEKK1) is a serine threonine kinase that when overexpressed induces caspase activation and apoptosis (12, 13). Many apoptotic stimuli including genotoxic agents activate MEKK1 kinase activity (12, 14). Following genotoxic treatment, MEKK1 activation leads to increased expression of death receptors such as Fas and death receptors 4 and 5, presumably through activation of transcription factors such as NF-κB (15). This up-regulation of death receptors contributes to genotoxin-induced apoptosis. Following treatment with apoptotic stimuli, MEKK1 is cleaved by caspases into a 91-kDa fragment containing the kinase domain (13, 16). Overexpression of this cleaved product is more potent at activating caspases and inducing apoptosis than full-length MEKK1 (13). Cleavage of MEKK1 is dependent on caspase 3-like molecules,
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since MEKK1 contains a consensus site for caspase 3 cleavage, and caspase 3 inhibitors block MEKK1 cleavage. Mutation of this cleavage site prevents MEKK1-induced apoptosis following expression in cells (13). MEKK1-induced apoptosis is dependent on its kinase activity, since expression of a kinase-inactive form of MEKK1 fails to induce apoptosis (14, 16). Indeed, kinase inactive MEKK1 acts as a dominant negative protein blocking apoptosis following genotoxic treatment (14, 16). The downstream mechanism of MEKK1 induction of apoptosis following caspase cleavage, however, remains unknown.

Herein, we demonstrate that overexpression of MEKK1 suppresses mitochondrial ΔΨm mediated by opening of the PT pore. Inhibiting the PT pore and ROS formation effectively reduced MEKK1-induced apoptosis. Furthermore, full-length MEKK1 expression fails to suppress ΔΨm or induce apoptosis in mouse embryonic fibroblasts (MEFs) lacking caspase 3, whereas the 91-kDa kinase fragment still is capable of ΔΨm suppression and induction of apoptosis in these cells. However, MEKK1 fails to release cytochrome c from the mitochondria. This provides evidence that MEKK1 cleavage causes permeability transition, leading to apoptosis independent of cytochrome c release.

MATERIALS AND METHODS

Cell Culture—Human embryonic kidney (HEK) 293 cells, breast cancer cell line MCF-7, and human transformed cell line HELa were maintained in a humidified 5.0% CO2, 37 °C incubator in Dulbecco's modified medium supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin (Invitrogen). Medium for HEK 293 and MCF-7 cells was supplemented with 10% fetal bovine serum (Invitrogen). Primary MEF cells were maintained in minimum essential supplement medium with 10% fetal bovine serum (kind gift from Dr. Tak Mak, Amigen Institute). HEK 293 cells expressing vector alone, MEKK1 KM and Bcl2 proteins were under selection with 1 mg/ml G418 (kind gift from Dr. Charles Epstein, University). HEK 293 cells overexpressing superoxide dismutase were maintained in 0.2 mg/ml G418 (Invitrogen). MCF-7 cells were maintained in minimum essential medium supplemented with 10% fetal bovine serum, 10% fetal bovine serum, 1% EGM (Lonza) or 91-kDa MEKK1 (8 μg) using Geneporter as per the manufacturer's instructions. After transfection, these cells were washed four times in 1× HBSS, and vacuum grease was applied to the edges of the coverslip. The cells were then stained with 150 μM TMRM in 1× HBSS. The coverslip was washed four times with 1× HBSS and analysed on an Olympus IX70 inverted confocal laser microscope using Flouview 2.0 software.

Immunohistochemistry and Apoptosis Measurement—Transfected HEK 293 cells were untreated or treated with 20 μM cyclosporin A (CsA) or 5 μM 4,5-dihydroxy-1,3-benzene disulfonic acid (Tiron) for 24 h where indicated. Parental and superoxide dismutase-expressing HELa cells along with MCF-7 cells expressing Bcl2 variants were also transfected as described above. The coverslips were collected in 1× phosphate-buffered saline (PBS). They were then washed twice for 5 min with 500 μl in 1× PBS and 0.1% Nonidet P-40. The coverslips were then fixed and washed twice for 5 min with 500 μl in 1× PBS and 0.1% Nonidet P-40. The coverslips were then fixed in 3.7% formaldehyde in 1× phosphate-buffered saline (PBS). The cells were then washed twice for 5 min with 500 μl in 1× PBS and 0.1% Nonidet P-40.

Assessment of Cytochrome c Release—HEK 293 cells were grown on glass coverslips. The coverslips were then fixed and washed twice for 5 min with 500 μl in 1× PBS and 0.1% Nonidet P-40. The coverslips were then fixed in 3.7% formaldehyde in 1× phosphate-buffered saline (PBS). The cells were then washed twice for 5 min with 500 μl in 1× PBS and 0.1% Nonidet P-40. The coverslips were then fixed and washed twice for 5 min with 500 μl in 1× PBS and 0.1% Nonidet P-40. The coverslips were then fixed in 3.7% formaldehyde in 1× phosphate-buffered saline (PBS). The cells were then washed twice for 5 min with 500 μl in 1× PBS and 0.1% Nonidet P-40. The coverslips were then fixed in 3.7% formaldehyde in 1× phosphate-buffered saline (PBS). The cells were then washed twice for 5 min with 500 μl in 1× PBS and 0.1% Nonidet P-40. The coverslips were then fixed and washed twice for 5 min with 500 μl in 1× PBS and 0.1% Nonidet P-40. The coverslips were then fixed and washed twice for 5 min with 500 μl in 1× PBS and 0.1% Nonidet P-40. The coverslips were then fixed and washed twice for 5 min with 500 μl in 1× PBS and 0.1% Nonidet P-40. The coverslips were then fixed and washed twice for 5 min with 500 μl in 1× PBS and 0.1% Nonidet P-40. The coverslips were then fixed and washed twice for 5 min with 500 μl in 1× PBS and 0.1% Nonidet P-40.
electrophoresis and Western blotted for cytochrome c (anti-cytochrome c; Santa Cruz Biotechnology).

**Immunoblots**—Cells were lysed in Nonidet P-40 lysis buffer (50 mM HEPES, pH 7.25, 150 mM NaCl, 50 mM ZnCl₂, 50 mM NaN₃, 2 mM EDTA, 1 mM sodium vanadate, 1.0% Nonidet P-40, 2 mM phenylmethylsulfonyl fluoride). Cell debris was removed by centrifugation at 8000 × g for 5 min, and protein concentration was determined by a Bradford assay. 200–400 µg of cell lysate protein was subject to SDS-polyacrylamide electrophoresis and transferred to nitrocellulose membranes. The membranes were blocked in Tris-buffered saline and 5% milk (w/v). Blots were incubated with the appropriate antibody concentration overnight (anti-MEKK1, anti-cytochrome c, and anti-HA were purchased from Santa Cruz Biotechnology; anti-caspase 9 was purchased from New England Biolabs), washed three times with 1× Tris-buffered saline, and incubated 1 h with the appropriate secondary antibody conjugated with alkaline phosphatase. Blots were visualized on x-ray film with enhanced chemiluminescence reagents (PerkinElmer Life Sciences).

**RESULTS**

**Overexpression of MEKK1 Suppresses Δψₘ in the Mitochondria**—HEK 293 cells were transiently transfected with GFP,
full-length MEKK1, and 91-kDa MEKK1. The cells were stained with the potentiometric dye TMRM, which detects $\Delta \phi_m$ levels in the mitochondria for 24 h as described under “Materials and Methods,” and then analyzed by flow cytometry. This showed that cells expressing GFP have 20% $\Delta \phi_m$ suppression (defined as the reduction in mitochondrial membrane potential) while cells expressing MEKK1 or 91-kDa MEKK1 have 40 and 49% $\Delta \phi_m$ suppression, respectively (Fig. 1A). Expression of the kinase-inactive form of MEKK1 (MEKK1 KM) that prevents apoptosis failed to suppress $\Delta \phi_m$ (21%; Fig. 1A) compared with cells expressing only GFP. MEKK1 suppression of $\Delta \phi_m$ was further demonstrated by comparing GFP-positive cells with TMRM-positive cells in a dot blot. In Fig. 1B, there were 98 events for GFP-positive and TMRM-negative cells (upper left quadrant), whereas 3535 events represented GFP-MEKK1- and TMRM-positive cells. In untransfected cells (lower quadrant of each dot blot), there were 48 and 78 events (lower left quadrant) for TMRM-negative cells, whereas 25,786 and 23,455 events (lower right quadrant) in TMRM-positive cells were observed (Fig. 1B). This indicates that expression of MEKK1 reduced the amount of TMRM-positive cells that represents loss of $\Delta \phi_m$. Loss of $\Delta \phi_m$ by MEKK1 was further confirmed by immunohistochemical staining of HEK 293 cells with TMRM. Cells expressing only GFP showed mitochondrial membrane potential as indicated by red fluorescence of TMRM staining, whereas cells expressing GFP fused to full-length MEKK1 showed $\Delta \phi_m$ suppression as indicated by reduced TMRM staining (Fig. 1C). Some cells expressing the GFP-MEKK1 fusion protein showed TMRM staining. This correlates to the ability of MEKK1 to suppress $\Delta \phi_m$ in 40% of cells (Fig. 1A) and induce apoptosis in 30% of cells after 24 h (data not shown). Expression of kinase-inactive MEKK1 effectively blocked genotoxic agent etoposide-induced apoptosis in HEK 293 cells (14). Treatment of HEK 293 cells expressing MEKK1 KM with etoposide also blocked $\Delta \phi_m$ suppression compared with vector alone cells over a 48-h time course (Fig. 1D).

Loss of $\Delta \phi_m$ could be due to opening of the PT pore. To confirm if MEKK1 is opening the PT pore to suppress $\Delta \phi_m$, HEK 293 cells were stained with the mitochondrial CalceinAM fluorescence dye. CalceinAM is freely permeable across cellular membranes but becomes fluorescent (green) and impermeable upon cleavage by intracellular esterases. This prevents its exit from the mitochondria until the PT pore opens. Once the PT pore is open, CalceinAM is released into the cytoplasm from the mitochondria, where it is quenched by CoCl$_2$ (added to the cells along with CalceinAM). Using confocal laser microscopy, HEK 293 cells expressing RFP alone or in combination with MEKK1 or 91-kDa MEKK1 were analyzed for calcein fluorescence. In cells expressing RFP alone, calcein fluorescence was punctate, indicating that CalceinAM is localized in the mitochondria. However, in cells expressing MEKK1 or 91-kDa MEKK1, cal-
CalceinAM fluorescence was eliminated, indicating a release of calceinAM into the cytoplasm, where its fluorescence is quenched (Fig. 2A). Loss of \( \Delta \psi_{\text{m}} \) could be due to membrane rupture or other open channels in the mitochondria. To determine if MEKK1-mediated loss of \( \Delta \psi_{\text{m}} \) was due to PT pore opening, the PT pore opening inhibitor CsA was added to cells expressing MEKK1, and the amount of \( \Delta \psi_{\text{m}} \) suppression was determined. GFP alone expression failed to increase \( \Delta \psi_{\text{m}} \) suppression compared with cells expressing GFP fused to MEKK1 (12% versus 45%; Fig. 2B). In the presence of CsA, the amount of \( \Delta \psi_{\text{m}} \) suppression was reduced to 12% (Fig. 2B). CsA treatment failed to change the amount of \( \Delta \psi_{\text{m}} \) suppression in cells expressing GFP alone (data not shown). This indicates that the PT pore is open when MEKK1 is expressed, causing loss of \( \Delta \psi_{\text{m}} \).

MEKK1 Fails to Release Cytochrome c from the Mitochondria

In addition to changes in membrane potential, mitochondrial cytochrome c release occurs during apoptosis, leading to caspase activation (1). We investigated the ability of MEKK1 to release cytochrome c from the mitochondria. HEK 293 cells were transfected with vector alone, MEKK1, or 91-kDa MEKK1. The cells were then fixed and stained with Hoechst. The amount of apoptosis was determined by DNA condensation on an Olympus fluorescent microscope. B, HEK 293 cells were also transiently transfected with GFP or MEKK1 fused with GFP and at the same time treated with the free radical scavenger Tiron (5 mM) for 24 h. After 24-h incubation, the cells were fixed and stained with Hoechst. The amount of apoptosis was determined as in A. C, HeLa cells parental or expressing superoxide dismutase (SOD) were transiently transfected with GFP or MEKK1 fused to GFP. The cells were incubated for 24 h. The cells were then fixed and stained with Hoechst. The amount of apoptosis was determined as described above. S.D. values were determined by three independent experiments.
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Our data indicate that the overexpression of MEKK1 leads to Δψm suppression in the mitochondria mediated by PT pore opening. We further investigated if blockade of PT pore opening by treatment with CsA or blockade of reactive oxygen species production inhibits MEKK1-induced apoptosis. HEK 293 cells were transiently transfected with MEKK1 in the absence or presence of CsA. CsA effectively inhibited MEKK1-induced apoptosis (Fig. 4A). Δψm suppression also associated with increased production of ROS. Using a free radical scavenger, Tiron, HEK 293 cells were transiently transfected with MEKK1 in the presence and absence of Tiron. In the presence of Tiron, MEKK1-induced apoptosis was blocked (Fig. 4B). To further confirm the involvement of ROS in MEKK1 induction of apoptosis, HeLa cells overexpressing superoxide dismutase, which eliminates accumulation of ROS, were transiently transfected with MEKK1. As a control, parental HeLa cells were also transiently transfected with MEKK1. The cells were then stained for DNA and MEKK1, and the amount of apoptosis in MEKK1-expressing cells was determined by DNA condensation. When superoxide dismutase was overexpressed, MEKK1-induced apoptosis was also blocked as compared with parental cells (Fig. 4C). These results indicate that PT pore opening and production of ROS is involved in MEKK1-induced apoptosis.

Bcl2 Targeted to the Mitochondria Prevents MEKK1-induced Apoptosis but Fails to Prevent Cleavage of MEKK1—We have previously shown that Bcl2 prevents MEKK1-induced apoptosis. Bcl2 has also been shown to block permeability transition in the mitochondria. Using the breast cancer cell line MCF-7 expressing vector alone, Bcl2 wild type (Bcl2-wt), and Bcl2 targeted to the mitochondria (Bcl2-mito) were transiently transfected with GFP, MEKK1, or 91-kDa MEKK1. The cells were then stained for DNA and MEKK1, and the amount of apoptosis in MEKK1-expressing cells was determined by DNA condensation. When superoxide dismutase was overexpressed, MEKK1-induced apoptosis was also blocked as compared with parental cells (data not shown). This indicates that Bcl2 blocks MEKK1-induced apoptosis at the mitochondria.

MEKK1 is cleaved following treatment with genotoxins such as etoposide and following overexpression in HEK 293 cells (12–14). Etoposide treatment of HEK 293 cells expressing Bcl2 leads to the cleavage of MEKK1 similar to HEK 293 cells expressing vector alone (Fig. 5B). Increased Bcl2 expression, however, was effective at inhibiting etoposide-induced apoptosis (data not shown). Overexpression of MEKK1 in HEK 293 cells expressing Bcl2 also failed to block MEKK1 cleavage (Fig. 5C). This suggests that Bcl2 blocks MEKK1-induced apoptosis downstream of MEKK1 cleavage.

Cleavage of MEKK1 by Caspase 3 Is Required for Δψm Suppression—MEKK1 is cleaved during apoptosis mediated by caspase 3-like molecules (12–14). To determine whether caspase 3 plays a role in MEKK1-induced apoptosis and Δψm suppression, we investigated primary MEFs lacking caspase 3 expression. MEF cells lacking caspase 3 were transiently transfected with GFP, MEKK1, or 91-kDa MEKK1. The cells were then stained for DNA and MEKK1 expression, and the amount of apoptotic cells was determined by DNA condensation. Expression of MEKK1 caused apoptosis in wild type MEF cells (33%) but was significantly reduced in MEF cells lacking caspase 3 (20%) as compared with GFP expression (9.5 and 15%, respec-
In contrast, 91-kDa MEKK1 expression was found to induce apoptosis in both wild type and caspase 3-lacking cells (49 and 43%, respectively; Fig. 6A). MEKK1 and 91-kDa MEKK1 induced Δψm suppression in wild type cells, but only 91-kDa MEKK1 induced Δψm suppression in MEF cells lacking caspase 3 (Fig. 6B). This correlates with the ability of 91-kDa MEKK1 to induce apoptosis in wild type and caspase 3-lacking MEF cells, whereas full-length MEKK1 only induces apoptosis in wild type MEF cells. Treatment of MEF cells lacking caspase 3 with etoposide revealed that MEKK1 failed to be cleaved, whereas MEF wild type cells treated with etoposide caused cleavage of MEKK1 (data not shown). This indicates that Δψm suppression in the mitochondria occurs downstream of MEKK1 cleavage.

**DISCUSSION**

Full-length MEKK1 is activated during apoptosis and has been implicated in the induction of apoptosis (13, 16, 21, 22). This is partially mediated by MEKK1 up-regulating proapoptotic genes such as death receptors (15, 21, 23). This up-regulation contributes to genotoxin-induced apoptosis. In contrast, activation of MEKK1 also occurs following treatment with nonapoptotic stimuli such as growth factors (24, 25). Furthermore, MEKK1 is activated by and might protect cells from microtubule toxin-induced apoptosis (14, 26). MEKK1, however, fails to be cleaved following treatment with growth factors or microtubule toxins. Thus, activation of full-length MEKK1 is not sufficient to induce an apoptotic response, suggesting that cleavage of MEKK1 is required for its pro-apoptotic function.

Regulation of MEKK1 induction of apoptosis involves the cleavage of MEKK1 into a 91-kDa kinase fragment (13). This fragment is a potent inducer of apoptosis, and blockage of the cleavage site in MEKK1 that produces the 91-kDa fragment fails to induce apoptosis (13). The mechanism of 91-kDa MEKK1 induction of apoptosis was, however, unknown. We have demonstrated that cleaved MEKK1 leads to permeability transition in the mitochondria. Blockage of PT pore opening inhibits MEKK1-induced apoptosis. The importance of cleavage of MEKK1 was demonstrated in MEF cells lacking caspase 3. This showed that only 91-kDa MEKK1 induces apoptosis and permeability transition in these cells. In contrast, MCF-7 cells that lack caspase 3 expression undergo apoptosis following overexpression of MEKK1. MEKK1 is also cleaved following etoposide treatment in MCF-7 cells. In addition, treatment of these cells with doxorubicin induces apoptosis where doxorubicin fails to induce apoptosis in MEF cells lacking caspase 3 (27, 28). This suggests that MCF-7 cells have bypassed the requirement of caspase 3 to induce apoptosis and illustrates potential differences between primary cells (MEF cells) and transformed cell lines (MCF-7 cells) in inducing apoptosis. Overall, we have demonstrated that MEKK1 induction of apoptosis involves cleavage into its 91-kDa form that leads to Δψm suppression in the mitochondria.

MEKK1, unlike other proapoptotic proteins, fails to release cytochrome c from the mitochondria. This suggests that

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2 E. M. Gibson, E. S. Henson, J. Onio, and S. B. Gibson, unpublished data.
MEKK1-mediated caspase activation is independent of mitochondrial cytochrome c release, and loss of Δψm is not sufficient to release cytochrome c. Full-length MEKK1 up-regulates death receptors that could activate caspases independent of the mitochondria (15, 29). In addition, caspase 3 activation is required for full-length MEKK1-induced loss of Δψm and apoptosis, suggesting that caspase activation occurs prior to MEKK1-induced mitochondria permeability transition. Alternatively, cleavage of MEKK1 could also lead to the activation of signaling pathways that further activate caspases independent of mitochondrial cytochrome c release. Nevertheless, MEKK1 induces Δψm suppression independent of cytochrome c release, indicating that cytochrome c release is not involved in MEKK1-mediated caspase activation.

Bcl2 family members regulate mitochondrial permeability transition (11). We have demonstrated that Bcl2 negatively regulates MEKK1-induced apoptosis, and this is downstream of MEKK1 cleavage and occurs at the mitochondria. This suggests that pro-apoptotic Bcl2 family members are involved in MEKK1-induced apoptosis. Indeed, these proteins are translocated from the cytoplasm to the mitochondria during the induction of apoptosis (11). It has been reported that expression of the kinase domain MEKK1 translocates pro-apoptotic Bcl2 family member Bak to the mitochondria. The kinase-inactive form of MEKK1 kinase domain blocks this translocation and prevents cisplatin-induced apoptosis (30). Most of these pro-apoptotic proteins including Bak cause release of cytochrome c from the mitochondria (11), but MEKK1 overexpression fails to release cytochrome c, and the significance of pro-apoptotic Bcl2 family members such as Bak in regulating MEKK1-induced apoptosis is unclear. A new Bcl2 family member BNIP3 has been shown to localize to the mitochondria to the mitochondria in regulating MEKK1-induced apoptosis (34, 35). This could explain the changes in mitochondrial permeability transition remains unknown and will be the focus of further investigation.

Overall, these results indicate that MEKK1 apoptosis signaling involves mitochondria permeability transition but is independent of cytochrome c release. This occurs downstream of MEKK1 cleavage, committing cells to undergo apoptosis. By pharmacologically targeting the cleavage of MEKK1, MEKK1-induced apoptosis could be effectively regulated without affecting the other functions of full-length MEKK1. This could be used in the treatment of various diseases such as cancer.

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