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Identification of Omi/HtrA2 as a Mitochondrial Apoptotic Serine Protease That Disrupts Inhibitor of Apoptosis Protein-Caspase Interaction

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To identify human proteins that bind to the Smac and caspase-9 binding pocket on the baculoviral inhibitor of apoptosis protein (IAP) repeat 3 (BIR3) domain of human XIAP, we used BIR3 as an affinity reagent, followed by elution with the BIR3 binding peptide AVPIA, microsequencing, and mass spectrometry. The mature serine protease Omi (also known as HtrA2) was identified as a mitochondrial direct BIR3-binding protein and a caspase activator. Like mature Smac (also known as Diablo), mature Omi contains a conserved IAP-binding motif (AVPS) at its N terminus, which is exposed after processing of its N-terminal mitochondrial targeting sequence upon import into the mitochondria. Mature Omi is released together with mature Smac from the mitochondria into the cytosol upon disruption of the outer mitochondrial membrane during apoptosis. Finally, mature Omi can induce apoptosis in human cells in a caspase-independent manner through its protease activity and in a caspase-dependent manner via its ability to disrupt caspase-IAP interaction. Our results provide clear evidence for the involvement of a mitochondrial serine protease in the apoptotic pathway, emphasizing the critical role of the mitochondria in cell death.

The IAP1 proteins were first identified from baculoviruses as proteins that function to suppress host cell death upon viral infection (1). More recently, IAPs were found in mammals, insects, nematodes, and yeast (1–3). All IAPs share one or more signature motifs, referred to as BIRs, that are essential for the antiapoptotic activity associated with these proteins. The BIR motifs have been shown to bind directly to caspases and to inhibit their activity (2, 4). In the insect Drosophila melanogaster, three proteins known as Reaper, Hid, and Grim have been identified as direct IAP-binding proteins that promote caspase activation by binding to the BIR domain of IAPs and disrupting IAP-caspase interaction (5–7). Although Smac/Diablo does not share sequence homology with these proteins except for the first four N-terminal residues, which constitute its IAP-binding motif (8, 9), it is the only known mammalian functional homolog of these proteins with a similar mode of action. Smac promotes caspase activation and apoptosis by binding to the BIR3 and BIR2 domains of XIAP and disrupting its interaction with caspase-9 and the effector caspases (caspase-3 and -7) (8–10). Since the mechanism of IAP inhibition of caspases is conserved in mammals and insects, it is expected that other mammalian IAP-binding proteins are still undiscovered. In this report, we identified the serine protease Omi/HtrA2 as a mitochondrial direct IAP-binding protein, which is released from the mitochondria upon induction of apoptosis by apoptotic stimuli. Like Smac, the mature Omi protein contains a conserved IAP-binding motif (AVPS) at its N terminus. We demonstrate that deregulated expression of Omi in the cytoplasm of mammalian cells induces apoptosis in these cells, indicating that Omi could participate in the mitochondrial apoptotic pathway.

EXPERIMENTAL PROCEDURES

Affinity Purification of Omi from Human 293 Cells—5 ml of 293 cell pellet was washed once in phosphate-buffered saline (10 mM phosphate, pH 7.4, containing 150 mM NaCl) and centrifuged, and the pellet was resuspended in 10 ml of cell lysis buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 1 mM EGTA supplemented with protease inhibitor mix (Sigma P-8340) containing 4-(2-aminoethyl)-benzenesulfonyl fluoride, peptatin A, E-64, bestatin, leupeptin, and aprotinin). Cells were homogenized and centrifuged once at 20,000 × g, and the supernatant was then subjected to centrifugation at 100,000 × g. The resultant supernatant was precleared with glutathione-Sepharose beads and then incubated with GST-BIR3 bound to glutathione-Sepharose beads for 12 h at 4 °C. The mixture was centrifuged at 500 rpm for 3 min, and the GST-BIR3 bead pellet was washed three times in the lysis buffer. The GST-BIR3-bound proteins were eluted with 200 μM AVPIA-Smac peptide at 37 °C for 1 h and then analyzed by Far Western blotting with 35S-labeled in vitro translated XIAP as described below.

cDNA Cloning and Expression of Recombinant Proteins—The full-length human Omi cDNA clone was obtained from the IMAGE consortia (GenBank™ accession number A1979237). Constructs encoding full-length Omi or truncated mutants were generated by PCR using modified complementary PCR adapter primers. C-terminal FLAG epitope tagging was done by cloning the PCR-generated Omi cDNAs in frame into FLAG-C-pCDNA. Plasmids encoding GFP fusion proteins were constructed using pEGFP-N1 (CLONTECH). Full-length XIAP and its BIR3-RING (residues 243–497) domain were overexpressed in E. coli strain DH5α as N-terminally GST-tagged proteins using a pGEX 4T vector (Amersham Biosciences, Inc.). Full-length XIAP, CIA1, and...
cIAP2 were in vitro translated in the presence of [35S]methionine in reticulocyte lysates using MYC-pcDNA3 constructs. Mature Smac or Omi and its mutants were overexpressed in *Escherichia coli* strain BL21(DE3) as C-terminally GST- or His<sub>6</sub>-tagged proteins using a pET-28-GST or pET-28 vector, respectively.

**Subcellular and Submitochondrial Fractionation**—Cells were homogenized in buffer A (20 mM Hepes, pH 7.5, 10 mM KCl, 1.5 mM MgCl<sub>2</sub>, 1 mM EDTA, 1 mM EGTA, 250 mM sucrose, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, with protease inhibitor mix). The homogenate was centrifuged at 800 *g* of empty vector, or a construct encoding C-terminally FLAG-tagged Omi precursor using the Lipo-fectAMINE™ method. Cells were treated with increasing concentrations of staurosporine (0–2.5 µM) for 7 h. Cells were stained with 4',6-diamidino-2-phenylindole–propidium iodide and 4',6-diamidino-2-phenylindole stains. Normal and apoptotic GFP-expressing cells were counted using fluorescence microscopy. The percentage of apoptotic cells in each experiment was expressed as the mean percentage of apoptotic cells as a fraction of the total number of GFP-expressing cells. The ability of mature Omi to potentiate TRAIL-induced apoptosis (Fig. 6j) was assayed by transfecting human MCF-7 cells (1 × 10<sup>6</sup> cells/well) in six-well plates with 1.2 µg of GFP-IETD-Omi expression construct or pEGFP-N1 plasmid 24 h after transfection, cells were treated with TRAIL (0.25 µg/ml) for 16 h, and the percentages of GFP-positive, 4',6-diamidino-2-phenylindole-positive apoptotic cells were determined as above. Similar assays were performed to determine the ability of M-Omi-GFP proteins to induce apoptosis in MCF-7 cells or Apaf-1−/− or caspase-9−/− mouse embryonic fibroblasts.

**Immunofluorescence Confocal Microscopy**—Cells were grown on coverslips and then stained with a polyclonal antibody raised against pure mature recombinant Omi protein and a mouse anti-Smac monoclonal antibody after fixing the cells with 4% paraformaldehyde. Fluorescein isothiocyanate-conjugated anti-rabbit and rhodamine-conjugated anti-mouse antibodies were used as secondary antibodies. After staining, the coverslips were mounted on slides and observed using confocal microscopy.

**In Vitro Interaction Assays**—All in vitro interactions were performed as described recently (8–10).

**RESULTS AND DISCUSSION**

To identify novel IAP binding proteins, we used a GST-BIR3 fusion protein as an affinity reagent to purify new IAP-binding proteins from extracts of human 293 cells and mouse tissues. After binding the extracts to the GST-BIR3 protein, the bound proteins were eluted with the IAP-binding motif peptide AVPIA and analyzed by Far Western blotting with <sup>35</sup>S-labeled protein A and analyzed as in a. The antibody-bound proteins (Ab pellet) were also fractionated and subjected to the same analyses. Last lane, 293T cells were transfected with an Omi precursor expression construct (Omi lane) or depleted with Smac antibody (Depleted lane), and the extracts were bound to GST-BIR3 fusion protein and analyzed as in a. An asterisk indicates nonspecific bands. An arrow indicates the IgG band.
XIAP. Three proteins, migrating as 23-, 38-, and 80-kDa bands, that specifically interact with the AVPS motif, were detected in the AVPIA peptide eluates from the human 293 cells and the mouse tissues (Fig. 1a). A large scale affinity purification was then fractionated on an SDS-polycrylamide gel (Fig. 1b), and the three bands were cut and subjected to mass sequencing. Due to the low level of the 80-kDa protein in this preparation, limited N-terminal sequence information was obtained, which was not sufficient for protein identification. The N-terminal sequence information of the other two proteins revealed that the 23-kDa protein is mature Smac/Diablo (13, 14), whereas the 38-kDa band is a previously described serine protease named Omi or HtrA2 (15, 16) (GenBank™ accession number XM_002750). Mass spectrometric peptide mass fingerprinting of these two protein bands confirmed the mass sequencing results. Furthermore, Western blot analysis of the AVPIA peptide eluates using Smac and Omi antibodies confirmed that the 23-kDa band represents Smac and the 38-kDa band represents Omi (Fig. 1a and c).

To confirm that Omi associates directly with the BIR3 domain of XIAP but not with the Smac protein, we depleted Smac from the 293 extracts with a Smac monoclonal antibody, bound the depleted extracts to the GST-BIR3 protein, and then analyzed the bound proteins by Far Western (Fig. 1d). Moreover, Omi, Smac, and the 80-kDa protein did not bind to the GST-BIR3-E314S mutant (Fig. 1d, last lanes). This mutation has been shown to abolish binding of Smac and caspase-9 to BIR3 (8, 9, 17). These results indicate that Omi, Smac, and the 80-kDa protein associate directly with the Smac binding pocket on the BIR3 of XIAP.

The BIR3 affinity-purified Omi starts with an amino-terminal AVPS sequence, which represents a conserved IAP-binding motif (Fig. 2A). Based on the deduced amino acid sequence of the cloned Omi protein (15, 16), this motif is located at residue 134, suggesting that Omi is made as a precursor protein that undergoes proteolytic processing at residue 133 to remove the N-terminal leader sequence. To determine whether a recombinant Omi lacking the first 133 residues (mature Omi) can interact with the GST-BIR3 fusion protein, we expressed this protein with a C-terminal His6 tag in bacteria and purified it to apparent homogeneity (Fig. 2A). N-terminal amino acid sequencing of the recombinant proteins indicated that the initiator methionine is removed. Both recombinant mature Omi and Smac were able to interact equally with the wild type GST-BIR3 protein but not with the GST-BIR3-E314S mutant (Fig. 2B).

Mutational analysis of the active site Ser306 to Ala of mature Omi did not affect its interaction with GST-BIR3. However, deletion of the AVPS motif of mature Omi completely abolished its interaction with the GST-BIR3 fusion protein. Mature Omi can also interact with endogenous XIAP in transfected 293 cells (Fig. 2D). This interaction requires the AVPS motif, since mutation of this motif to AAAS abolishes its interaction with the endogenous XIAP. Interestingly, mutation of the AVPS motif to AAAS did not affect the processing and removal of the N-terminal leader sequence of the transfected Omi precursor but completely prevented the interaction with endogenous XIAP (Fig. 2D).
interact with cIAP1 and cIAP2 (Fig. 2c). The above data indicate that Omi is a bona fide IAP-binding protein with an N-terminal IAP binding motif essential for its ability to bind IAPs.

Computer analysis using the PSORT program revealed that the N-terminal leader sequence of the Omi precursor contains within its first 60 residues a typical mitochondrial targeting sequence. This sequence might be removed by mitochondrial processing peptidases upon import into the mitochondria. To examine whether the endogenous mature Omi protein is indeed localized in the mitochondria, we subfractionated 293 cells into cytosolic, mitochondrial, microsomal, and nuclear fractions and bound each fraction to the GST-BIR3 protein. As shown in Fig. 3a, Far Western and Western blot analyses with 35S-XIAP and Smac and Omi antibodies, respectively, showed the majority of Omi and Smac to be localized in the mitochondrial membrane. The fractions were analyzed by immunoblotting with Omi and Smac antibodies. Both Omi and Smac proteins were predominantly present in the intermembrane space fraction (Fig. 3b).

Immunofluorescence confocal microscopy of MCF-7 cells stained with Omi- and Smac-specific antibodies revealed that the endogenous Omi and Smac proteins colocalize with each other and exhibit punctate perinuclear staining characteristic of mitochondrial localization (Fig. 3c). To confirm the mitochondrial localization of Omi, we transfected MCF-7 cells with a C-terminal GFP-tagged Omi precursor construct. The ectopically transfected Omi precursor exhibited punctate mitochondrial fluorescence (Fig. 3d, right panel). The removal of the mitochondrial targeting sequence of Omi resulted in the expression of mature Omi (residues 134–458) in the cytoplasm (Fig. 3d, middle panel). The first 60 residues of the Omi precursor, which harbors the MTS, was sufficient for targeting GFP to the mitochondria when expressed as a fusion protein with GFP (Fig. 3e). Taken together, the above data indicate that Omi colocalizes with Smac in the intermembrane membrane space.

To determine whether Omi is released together with Smac and cytochrome c from the mitochondria to the cytoplasm during apoptosis, we treated Jurkat and HL-60 cells with staurosporine, which is known to induce the mitochondrial apoptotic pathway, and analyzed their cytosolic extracts by immunoblot analysis. As shown in Fig. 4a, cytochrome c, Smac, and Omi accumulated in the cytosol of these cells in a time-dependent manner after treatment with staurosporine. Processing of procaspase-3 also followed a similar time course, with maximum processing observed at 6–8 h after treatment. Very little or no Omi or Smac proteins nor processing of procaspase-3 was detected in the extracts at zero time points. Similar results were obtained after treatment of Jurkat cells with TRAIL (Fig. 4b). Immunofluorescence confocal microscopy of staurosporine-treated HeLa cells or TRAIL-treated MCF-7 cells showed diffused Immunofluorescence staining of Omi, whereas in the untreated control cells it is mostly punctate and perinuclear (Fig. 4c). The above data clearly show that Omi is released together with cytochrome c and Smac during apoptosis.

**Fig. 3. Omi is localized in the mitochondria.** a, 293T cells were fractionated into nuclear, mitochondrial, microsomal, and cytosolic fractions. The nuclear, mitochondrial, and microsomal fractions were lysed in a hypotonic buffer, and the soluble proteins bound to GST-BIR3 fusion protein and were analyzed as in the legend to Fig. 1a. The same Far Western blot (upper panel) was then probed with Omi and Smac antibodies (second and third panels, respectively). Total subcellular fractions were also immunoblotted with antibodies against cytochrome c oxidase (fourth panel), poly(ADP-ribose) polymerase (fifth panel), and β-actin (sixth panel). b, purified mitochondria from 293 cells were treated with a buffer containing 1.2% digitonin, and the supernatant and mitoplasts (pellet) were then analyzed by Western blotting with Omi, Smac, and cytochrome c antibodies. c, immunofluorescence confocal microscopy of MCF-7 cells stained with Omi-specific (middle panel, green) and Smac-specific (left panel, red) antibodies. d, confocal micrographs of MCF-7 cells transfected with GFP (left panel) or C-terminal GFP-tagged mature Omi (Omi134–458-GFP; middle panel) or Omi precursor (Omi-GFP; right panel). e, confocal micrographs of MCF-7 cells transfected with C-terminal RFP-tagged Omi1–60 (Omi-MTS-RFP; left panel) and pEYFP-Mito marker (middle panel). The right panels in c and e represent merged micrographs.
293 mitochondria were incubated with or without purified tBid (350 nM) treated cells and the diffused staining in the treated cells. a specific antibody. Note the punctate perinuclear staining in the un-H9262 and HL-60 cells were treated with staurosporine (2 α from the mitochondria during apoptosis.

The ability of TRAIL to induce release of Omi from the mitochondria of MCF-7 and Jurkat cells suggests that tBid, which is generated by active caspase-8 after TRAIL-receptor ligation, is responsible for the release of Omi. To test this hypothesis, we incubated isolated mitochondria with a physiological amount of tBid. As shown in Fig. 4d, tBid induced release of Omi, Smac, and cytochrome c from the mitochondria into the supernatants. Combined, the above data clearly show that Omi is released from the mitochondria together with cytochrome c and Smac during apoptosis and after stimulation of mitochondria with tBid.

Next, we determined whether Omi, like Smac, promotes caspase-9 activity in HEK293 S100 extracts in the presence of XIAP. To measure the caspase-9 activity in these extracts, we added [35S]-labeled procaspase-3 to the S100 extracts and stim-
sis, we transfected human HeLa cells with the Omi precursor and stimulated the cells with different doses of staurosporine. We reasoned that treatment with staurosporine should release the overexpressed Omi from the mitochondria, thereby enhancing apoptosis. As shown in Fig. 5b, transiently expressed wild type Omi did not induce significant apoptosis in the absence of staurosporine. However, at 500 nM staurosporine, ~48% of the Omi-transfected cells showed signs of apoptosis compared with ~20% in the case of the vector-transfected cells. With higher concentrations of staurosporine, increased apoptosis was observed in the Omi-transfected cells compared with the vector-transfected cells. No apoptosis potentiation was observed with those cells to apoptosis.

activities, we wanted to determine the contribution of each of these activities separately toward its overall proapoptotic activity. To determine the contribution of the serine protease activity of Omi toward its proapoptotic activity, it was necessary to express cytosolic protease-active and -inactive forms of Omi that do not bind IAPs in cells. This was achieved by expressing active and inactive (S306A) Omi-GFP fusion proteins without the MTS (M-Omi-GFP and M-Omi-S/A-GFP, respectively) in MCF-7 cells (Fig. 6a). We found that these forms of Omi do not bind XIAP-BIR3 (Fig. 6c, first lane) because the initiator methionine before the AVPS motif is not removed in transfected human cells (not shown). Nevertheless, only the serine protease-active M-Omi-GFP, but not the inactive M-Omi-S/A-GFP, was able to induce cell death in MCF-7 cells (Fig. 6b). Similar results were obtained with cytosolic Omi variants lacking the AVPS motif (data not shown). Of note, the Omi-killing activity was independent of the cellular caspase activity, since inhibition of cellular caspases with zVAD-FMK, XIAP, XIAP-BIR3, or caspase-9-DN did not block the ability of M-Omi-GFP to kill these cells. Consistent with these results, M-Omi-GFP was also able to induce cell death in Apaf-1−/− and caspase-9−/− mouse embryo fibroblasts (Fig. 6c). These results indicate that Omi can induce cell death in mammalian cells independent of caspases, Apaf-1, or IAPs via its serine protease activity.

FIG. 6. Dual role of Omi in cell death. a, schematic diagrams of the Omi-GFP and GFP-IETD-Omi constructs used to assay the ability of Omi to induce apoptosis or to enhance apoptosis by other stimuli. The Omi fusion proteins that induce or enhance apoptosis are marked with a plus sign, and those that do not are marked with a minus sign. b, MCF-7 cells were transfected with M-Omi/S/A-GFP or pEGFP-N1 alone or M-Omi-GFP together with empty vector or expression construct encoding XIAP, XIAP-BIR3, or caspase-9-DN. The cells were also transfected with the M-Omi-GFP expression construct in the presence of zVAD-FMK (20 μM). 36 h after transfection, the percentages of apoptotic cells were determined as described under “Experimental Procedures.” c, Apaf-1−/− or caspase-9−/− mouse embryo fibroblasts were transfected with pEGFP-N1, M-Omi-GFP, or M-Omi-S/A-GFP. 36 h after transfection, the percentages of apoptotic cells were determined. d, MCF-7 and 293T cells were transfected with pEGFP or M-Omi-Flag, GFP-IETD-APV (AVP), or GFP-IETD-SSA (SSA) expression constructs in the presence or absence of zVAD-FMK (20 μM). 24 h after transfection, the cells were harvested, and their extracts were fractionated and analyzed by immunoblotting with FLAG-horseradish peroxidase antibody. M-Omi-FLAG is similar to the M-Omi-GFP shown in a, except that the GFP tag was replaced with Flag tag. e, 293T cells were transfected with the indicated expression constructs. 24 h after transfection, the cells were harvested, and their extracts were precipitated with GST-BIR3. The extracts and the precipitated proteins were immunoblotted with FLAG-horseradish peroxidase antibody. pOmi-FLAG, C-terminal FLAG-tagged Omi precursor (only the mature Omi can be seen); MCF-7 cells were transfected with pOmi-FLAG. f, MCF-7 cells were transfected with the indicated expression constructs. 24 h after transfection, the cells were left untreated (−) or treated with TRAIL (+) for 16 h. The percentage of round apoptotic cells was determined.

Since Omi possesses both serine protease and IAP binding
We next tested the contribution of the IAP binding activity of Omi to its killing activity. To do that, we replaced the MTS of Omi with a GFP-IETD sequence and mutated its active site Ser306 to Ala (Fig. 6a, GFP-IETD-APV). Of note, the presence of the IETD sequence between GFP and Omi-S/A allowed caspase-dependent cleavage at the IETD site after the Asp residue and release of mature Omi-S/A in the cytosol of the transfected 293 and MCF-7 cells (Fig. 6d). This mature Omi-S/A was able to bind the GST-BIR3 fusion protein (Fig. 6e), indicating that it is correctly processed to expose its AVPS motif. Consistent with this result, very little or no processing at the IETD site was observed in the cytosolic extracts of these cells in the presence of the pancaspase inhibitor zVAD-FMK (Fig. 6d). As shown in Fig. 6f, expression of the GFP-IETD-ADV fusion protein in MCF-7 cells enhanced TRAIL-induced apoptosis in these cells severalfold above the GFP-transfected control cells. This activity was dependent on the AVPS motif, since mutation of the AVPS motif to an SSAS motif (GFP-IETD-SSA) abolished the severalfold above the GFP-transfected control cells. This activity was dependent on the AVPS motif, since mutation of the AVPS motif to an SSAS motif (GFP-IETD-SSA) abolished the apoptotic enhancement activity of Omi-S/A and the ability to bind to BIR3. These results indicate that the IAP binding activity of Omi represented by its AVPS motif plays a significant role in its ability to potentiate apoptosis independent of its protease activity. Taken together, Omi not only can induce cell death through its serine protease activity but also via its ability to bind and neutralize IAPs.

Our observations reveal a new level of regulation of apoptosis by the mitochondria. The identification of Omi as a mitochondrial serine protease that can also bind and neutralize IAPs adds to the list of proapoptotic proteins such as cytochrome c, apoptosis-inducing factor, Smac, and endonuclease G (18–21), which are released from the mitochondria during apoptosis, and emphasizes the importance of the mitochondria in the cell death pathway of eukaryotic cells. Because Omi is a serine protease, its sequestration in the mitochondria protects healthy cells from its proteolytic activity. However, when cells become damaged, they release Omi, together with other inter-mitochondrial membrane space proteins, into the cytoplasm, where it could cause irreparable proteolytic damage to important structural and regulatory components of the cell. In addition, mature Omi could play a role as an IAP inhibitor in the caspase-dependent apoptotic pathway by binding to the BIR3 domain of XIAP and disrupting its interaction with the activated caspase-9. This activity may complement the activity of Smac by neutralizing more IAPs in the cytoplasm, resulting in more caspase activation in cells undergoing apoptosis.

While the importance of caspases in cell death is well established, a large body of evidence suggests that other types of proteases might also be important (22, 23). For example, inhibitors of serine proteases such as diisopropyl fluorophosphate, 1-chloro-3-tosylamido-2-phenylethyl chloromethyl ketone, and L-1-tosyl-amido-2-phenylethyl chloromethyl ketone can delay or fully inhibit distinct steps of the cell death pathway (22, 24). Moreover, inhibition of caspases generally delays but does not completely block cell death by some cell death stimuli. Thus, the serine protease activity of Omi could be responsible for the noncaspase-dependent cell death observed in many model systems.

Although there is now clear evidence that cytochrome c, apoptosis-inducing factor, and endonuclease G contribute to the demise of the cell when released from the mitochondria into the cytoplasm or nucleus, the real physiological function of these proteins is far from being killer proteins. In fact, these proteins are essential for normal mitochondrial function and cell survival. This is most likely true for Omi. Omi belongs to the heat shock response serine proteases (HtrAs), which have been shown in bacteria to be essential for cell survival at high temperature (25). Omi and its related family members could control protein stability and turnover under thermal, osmotic, pH, and other stress conditions. Interestingly, an Omi-related protease has been identified in the chloroplast of Arabidopsis thaliana, suggesting that these proteases play important roles in organelar biogenesis and function (26). Although our data clearly show that Omi is localized predominantly in the mitochondria, recent data indicated that Omi is also localized in the nucleus of stressed cells (16), suggesting that Omi perhaps could translocate from mitochondria to the nucleus after stress.

In conclusion, the release of Omi from the mitochondria might represent a novel cell death pathway that has not yet been fully appreciated. Omi could bind and inhibit the cellular IAPs, thus potentiating the caspase-dependent apoptotic pathway. More importantly, Omi could induce cell death independent of caspases via its serine protease activity. The targeted disruption of the Omi gene in the future should allow the determination of the precise role of Omi in cell death and survival.

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