ABSTRACT

Bax is a crucial mediator of the mitochondrial pathway for apoptosis, and loss of this proapoptotic Bcl-2 family protein contributes to drug resistance in human cancers. We report here that the endoplasmic reticulum Ca\(^{2+}\)-ATPase inhibitor thapsigargin (THG) induces apoptosis of human colon cancer HCT116 cells through a Bax-dependent signaling pathway controlling the cytosolic release of mitochondrial apoptogenic proteins. Treating HCT116 cells with THG results in caspase-8 activation; Bid cleavage; Bax conformational change and mitochondrial translocation; the release of cytochrome c, Smac/Diablo, and Omi/HtrA2 into the cytosol; caspase-3 activation; and apoptosis. In contrast, knockout of Bax completely abrogates the full processing/activation of caspase-3 but has no effect on the processing of caspase-8 and the initial cleavage of caspase-3 to p24 fragment after THG treatment. The caspase-8-specific inhibitor z-IETD-fmk, as well as pan-caspase inhibitor z-VAD-fmk, but not the calpain inhibitor E-64d, prevents Bid cleavage, Bax conformational change, and subsequent caspase-3 processing and apoptosis. Caspase-8 processing is dependent on de novo protein synthesis; DR5 expression is strongly up-regulated by THG treatment. Moreover, the absence of Bax blocks THG-induced Omi and Smac release from mitochondria, and expression of cytosolic Omi (GFP-IETD-Omi) or Smac (GFP-IETD-Smac) restores the sensitivity of Bax-knockout HCT116 cells to apoptosis in response to THG treatment. Taken together, our results indicate that Bax-dependent Smac and Omi release plays an essential role in caspase-3 activation and apoptosis induced by THG in human colon cancer HCT116 cells.

INTRODUCTION

Apoptotic signal is regulated by an orchestrated cascade of cysteine proteases known as caspases whose activation can be mainly controlled by two distinct pathways, involving either death receptor (extrinsic) or mitochondria (intrinsic; Ref. 1). In the extrinsic pathway, stimulation of death receptors, such as Fas, tumor necrosis factor receptor 1, DR3, DR4, or DR5, with their cognate ligands leads to formation of a death-inducing signaling complex containing the death receptor, adapter proteins, such as Fas-associated death domain, and certain initiator caspases, such as pro-caspase-8 (2). Consequently, pro-caspase-8 becomes activated by autoproteolytic processing, and the resulting active caspase-8 cleaves and activates downstream effectors caspases, such as caspase-3, -6, -7. In the mitochondrial pathway, a variety of death signals triggers the release of several pro-apoptotic proteins, such as cytochrome c, Smac/Diablo, Omi/HtrA2, apoptosis-inducing factor, and endonuclease G, from mitochondria to the cytosol or nucleus, where they are actively involved in the process of cell death (3). Once released, e.g., cytochrome c forms a complex with Apaf-1 and pro-caspase-9 in the presence of dATP or ATP, resulting in processing and activation of this initiator caspase (4). In contrast, Smac/Diablo or Omi/HtrA2 promotes caspase activation by direct binding to and inhibiting the IAP family caspase inhibitors (3, 4).

The Bcl-2 family of proteins plays a critical role in the mitochondrial pathway of apoptosis (1, 5). The antiapoptotic Bcl-2 family proteins like Bcl-2 and Bcl-XL possess four conserved domains (BH1, 2, 3, and 4) and inhibit the release of certain pro-apoptotic factors from mitochondria. In contrast, pro-apoptotic Bcl-2 family molecules, which can be further divided into two subgroups, BH3 only and multidomain BH1–3 proteins, induce the release of mitochondrial apoptogenic molecules into the cytosol. Genetic studies show that the multidomain pro-apoptotic proteins Bax and Bak are absolutely required for apoptosis induction by diverse intrinsic death stimuli (6). Bak is a mitochondrial protein, whereas Bax is predominantly located in the cytoplasm of healthy cells (7). Apoptotic signals induce a conformational change in the Bax protein, which is intimately associated with its mitochondrial integration and pro-apoptotic activity (8, 9). The BH3-only proteins, such as Bid and Bim, have been shown to activate the multidomain proapoptotic proteins for apoptosis induction (10–12). However, the pro-apoptotic activities of BH3-only proteins are regulated by a variety of post-translational modifications, such as phosphorylation and proteolysis, or transcriptional control (1).

THG\(^{3}\) is an inhibitor of the ER Ca\(^{2+}\)-ATPase and induces proliferation-independent apoptosis in a variety of cell types (13–16). THG treatment elevates intracellular Ca\(^{2+}\) levels and induces ER stress by perturbation of Ca\(^{2+}\) levels in ER (13, 17). Elevation of intracellular Ca\(^{2+}\) activates several pro-apoptotic proteins, such as the protein phosphatase calcineurin and the Ca\(^{2+}\)-dependent protease calpain. Calcineurin dephosphorylates and activates the BH3-only protein Bad (16, 18–20). However, calpain is possibly involved in apoptosis by cleavage of caspase-12, XIAP, Bcl-XL, or Bid (21–23). In addition, prolonged ER stress activates the ER transmembrane serine/threonine kinase Ire1, and the activated Ire1 in turn recruits TRAF2, which interacts with ASK1 or caspase-12, resulting in c-Jun-NH2-terminal kinase or caspase-12 activation (24, 25).

In the present study, we demonstrate that THG induces apoptosis of HCT116 cells by activating Bax through a caspase-8-dependent pathway, leading to the release of Smac and Omi into the cytosol to further amplify the caspase-8 signal for the full processing/activation of caspase-3 and apoptosis.

MATERIALS AND METHODS

Reagents. THG, staurosporine, etoposide, Taxol, E-64d, CHX, and anti-Bax 6A7 monoclonal antibody were purchased from Sigma. Epothilone B analogue (BMS-247550) was kindly provided by Bristol-Myers Squibb. Anti-Bax (N-20) polyclonal antibody was purchased from Santa Cruz Biotechnol-
ogy. Anticaspase-3, caspase-7, and Bid polyclonal antibodies, as well as anticytochrome c monoclonal antibody, were purchased from BD PharMingen. Anti-COX IV monoclonal antibody was purchased from Molecular Probes. Anticaspase-8 monoclonal antibody and caspase inhibitors were purchased from Calbiochem. Anti-Omi antibody as well as pEGFP-IETD-Smac and pEGFP-IETD-Omi plasmids were kindly provided by Dr. Emad Alnemri (Kimmel Cancer Institute, Philadelphia, PA).

**Cell Culture and Transfection.** HCT116 Bax+/− and Bax−/− cells were kindly provided by Dr. Bert Vogelstein (John Hopkins University, Baltimore, MD) and sustained in McCoy’s 5A medium, supplemented with 10% FCS and 1% penicillin/streptomycin. These cells were transfected with TransIT-LT-1 transfection reagent (Mirus) according to the manufacturer’s protocol.

**Detection of DNA Fragmentation.** Cells (2 × 10⁶) were cultured for the indicated periods with 1 μM THG and lysed in 100 μl of lysis buffer (10 mM Tris-HCl (pH 8.0), 10 mM EDTA, 0.5% Triton X-100, and 0.1 mg/ml RNase A). After incubation for 1 h at 37°C, the lysate was further incubated for 30 min at 50°C in the presence of 0.2 mg/ml Protease K. Fragmented DNA was precipitated by adding 20 μl of 5 M NaCl and 120 μl of isopropanol, dissolved in 10 mM Tris-HCl (pH 8) and 1 mM EDTA, and electrophoresed on 1.5% agarose gel.

**Caspase Activity Assay.** The caspase fluorescent assay kits specific for caspase-3 (Sigma) or caspase-9 (Clontech) were used to detect caspase activation by measuring for the cleavage of a synthetic fluorescent substrate. In brief, cells were cultured in 60-mm dishes and treated with 1 μM THG for the indicated periods. Cell lysates were prepared in lysis buffer from each assay kit, normalized for protein content with bicinchoninic acid assay kit (Pierce), and 500 μg of total proteins were incubated with 2 μl of anti-Bax 6A7 antibody for 2 h at 4°C, followed by the addition of 20 μl of protein G agarose to precipitate the conformationally changed Bax protein. After extensive wash, the resulting immune complexes were subjected to immunoblot analysis with anti-Bax N20 polyclonal antibody.

**Subcellular Fractionation.** Cells were lysed in mitochondria lysis buffer (12) with a Dounce homogenizer and subjected to centrifugation at 1,000× g to pellet nuclei. The postnuclear supernatant was centrifuged at 10,000× g to pellet mitochondria-enriched heavy membrane fraction, and the resulting supernatant was further centrifuged at 100,000× g to obtain cytosolic fraction. Total proteins (50 μg) from each fraction were subjected to immunoblot analysis.

**Immunofluorescence Analysis.** Cells were grown on coverslips and treated with or without 1 μM THG for 30 h. After incubation with 25 nM MitoTracker CMTMRos (Molecular Probes) for 30 min, cells were fixed with 3.7% formaldehyde for 5 min and permeabilized with 0.5% Triton X-100 in PBS for 3 min. After 30-min blocking with 3% BSA, cells were incubated with anti-Smac (1:200) or anti-Omi (1:500) for 2 h at 37°C, followed by incubation at 37°C for 1 h with FITC-conjugated secondary antibody. The coverslips were then mounted with DAPI-containing mount media (Vector Laboratories, Inc.) and analyzed by fluorescence microscopy.

**RESULTS**

**Bax Is Required for THG-induced Caspase-3 Activation and Apoptosis.** To determine the involvement of Bax in apoptosis induced by a variety of cytotoxic agents, we used HCT116 human colon cancer cells with one allele of bax gene (Bax−/−) versus Bax knockout HCT116 (Bax+/−) cells in which bax gene was inactivated by homologous recombination (27). These cells were exposed to DNA damage (etoposide), ER stress (THG), microtubule damage (Taxol and epothilone B), or mitochondrial stress (staurosporine) for different...
periods, and cell viability was determined by trypan blue exclusion assay. As shown in Fig. 1A, the deficiency of Bax affected cell death most remarkably in response to ER stress caused by the ER Ca\(^{2+}\)-ATPase inhibitor THG. The requirement of Bax for THG-induced cell death in HCT116 cells was confirmed by extending the treatment with 1\(\mu\)M THG for 72 h (Fig. 1B). In both Bax\(^{+/−}\) and Bax\(^{−/−}\) cells, the protein level of Bip/GRP78, a molecular chaperon elevated in response to ER stress, was induced by THG treatment at 24 h, indicating that ER stress occurs independently of Bax (Fig. 1D). However, DNA fragmentation (one of the characteristics of apoptosis) was observed only in Bax\(^{+/−}\) cells after THG treatment (Fig. 1C). Moreover, caspase-3 was processed to active forms (p20/17) in Bax\(^{+/−}\) cells, but it was not fully processed and presented as an inactive p24 form (28) in Bax\(^{−/−}\) cells (Fig. 1D). These results indicate that THG-induced full processing of caspase-3 and apoptosis in HCT116 cells are Bax dependent, but the initial processing of caspase-3 does not require the presence of Bax.

It has been shown that apoptotic stimuli trigger Bax conformational change and translocation to mitochondria where active Bax causes cytochrome \(c\) release for subsequent activation of caspase-9 and certain downstream effector caspases (29). To examine Bax activation in response to THG treatment, we performed immunoprecipitation experiments with anti-Bax 6A7 antibody. As shown in Fig. 2A, Bax

![Fig. 2. THG triggers Bax conformational change and translocation to mitochondria for cytochrome \(c\) release and caspase activation. HCT116 Bax\(^{+/−}\) or Bax\(^{−/−}\) cells were treated with 1\(\mu\)M THG for the indicated periods and subjected to the following assays. In A, Bax conformational change was detected by immunoprecipitation with anti-Bax 6A7 antibody. In B, mitochondrial translocation of Bax and cytochrome \(c\) release were assessed by subcellular fractionation/immunoblot analysis with antibodies specific for Bax, cytochrome \(c\) (Cyt \(C\)), or cytochrome \(c\) oxidase complex IV (COX IV). In C, the proteolytic processing of caspase-3 and -7 was examined by immunoblot analysis with antibodies specific for caspase-3 or -7. In D, caspase-3 and -7-like activities were determined by using synthetic fluorescent substrates DEVD-AMC or LEHD-AMC, respectively.

![Fig. 3. Caspase inhibitor z-VAD-fmk but not calpain inhibitor E-64d prevents THG-induced Bax conformational change and caspase-3 processing. HCT116 Bax\(^{+/−}\) and Bax\(^{−/−}\) cells were treated with 1\(\mu\)M THG in the presence of z-VAD-fmk (50 \(\mu\)M), E-64d (20 \(\mu\)M), or DMSO for the indicated periods and subjected to the following assays. In A, caspase-3 processing was examined by immunoblot analysis with anticaspase-3 polyclonal antibody. In B, Bax conformational change was assessed by immunoprecipitation with anti-Bax 6A7 monoclonal antibody.]

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underwent a conformational change at ~12 h (Fig. 2A), preceding the full processing of caspase-3 and -7, which was detectable ~24 h after THG treatment (Fig. 2C). Subcellular fractionation/immunoblot analysis confirmed Bax translocation to mitochondria and cytochrome c release in Bax+/− cells treated with THG (Fig. 2B). Moreover, caspase activity assays revealed that caspase-3 and -9-like activities began to increase at 12 h after THG treatment in Bax+/− cells (Fig. 2D). In contrast, THG failed to induce cytochrome c release and activation of caspase-3, -7, and -9 in Bax knockout HCT116 cells (Fig. 2B–D).

**Caspase-8 but not Calpain Is Involved in THG-induced Bax Activation, Caspase-3 Processing, and Apoptosis.** THG has been reported to activate calpain that subsequently activates ER-localized caspase-12 or other caspases in cells (21). Calpain also cleaves the BH3-only protein Bid, which in turn causes mitochondrial dysfunction and cytochrome c release (22, 30). To determine whether calpain is involved in THG-induced Bax conformational change and caspase-3 processing, we treated HCT116 cells with 1 μM THG in the presence or absence of 20 μM caspase-8 inhibitor (z-IETD-fmk) for 30 h and subjected to immunoprecipitation with anti-Bax 6A7 antibody to determine Bax conformational change as well as immunoblot analysis with antibodies specific for caspase-8, Bid, or α-tubulin. In E, HCT116 Bax+/− cells were treated with 1 μM THG together with either 20 μM z-VAD-fmk, 20 μM z-IETD-fmk, or DMSO as control for various times and subjected to trypan blue dye exclusion assay. Half of the medium was replaced every day with fresh medium containing the indicated drugs.

To identify the caspase involved in the initiation of THG-induced apoptosis, we treated HCT116 cells with a variety of caspase inhibitors and subjected them to an analysis of cell viability and caspase-3 processing. Among the specific inhibitors, treating cells with

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**Fig. 4. Caspase-8 is the initiator caspase for THG-induced Bax conformational change and apoptosis.** In A and B, HCT116 Bax+/− cells were treated with 1 μM THG in the presence of the indicated caspase inhibitors (10 μM) for 30 h and subjected to trypan blue exclusion assay (A) and immunoblot analysis (B) with anticaspase-3 antibody. In C, HCT116 Bax+/− and Bax−/− cells were treated with 1 μM THG for the indicated times before immunoblot analysis with anticaspase-8 or anti-Bid antibodies. In D, HCT116 Bax+/− cells were exposed to 1 μM THG with or without 20 μM caspase-8 inhibitor (z-IETD-fmk) for 30 h and subjected to immunoprecipitation with anti-Bax 6A7 antibody to determine Bax conformational change as well as immunoblot analysis with antibodies specific for caspase-8, Bid, or α-tubulin. In E, HCT116 Bax+/− cells were exposed to 1 μM THG with or without 20 μM caspase-8 inhibitor (z-IETD-fmk) for 30 h and subjected to immunoblot analysis with antibodies specific for caspase-8, Bid, or α-tubulin.

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**Fig. 5. THG-induced caspase activation requires de novo protein synthesis, and THG up-regulates DR5 expression in HCT116 cells.** In A, HCT116 Bax+/− and Bax−/− cells were treated with 1 μM THG in the presence (+) or absence (−) of 50 μg/ml CHX for 24 h. Caspase processing was assessed by immunoblot analysis with anticaspase-3 or -8 antibodies. In B, HCT116 Bax+/− and Bax−/− cells were exposed to 1 μM THG for various times before immunoblot analysis with anti-DR5 antibody.

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**ROLE OF BAX IN ER STRESS-INDUCED APOPTOSIS**

**Fig. 5. THG-induced caspase activation requires de novo protein synthesis, and THG up-regulates DR5 expression in HCT116 cells.** In A, HCT116 Bax+/− and Bax−/− cells were treated with 1 μM THG in the presence (+) or absence (−) of 50 μg/ml CHX for 24 h. Caspase processing was assessed by immunoblot analysis with anticaspase-3 or -8 antibodies. In B, HCT116 Bax+/− and Bax−/− cells were exposed to 1 μM THG for various times before immunoblot analysis with anti-DR5 antibody.
caspase-8 inhibitor distinguishably prevented THG-induced cell death (Fig. 4A) and processing of caspase-3 (Fig. 4B). In contrast, caspase-9 inhibitor had a little effect on THG-induced cell death (Fig. 4A) and caspase-3 activation (Fig. 4B), suggesting that THG induces apoptosis via a caspase-8-dependent but cytochrome c/Apaf-1/caspase-9 (apoptosome)-independent pathway in HCT116 cells.

It has been reported that the BH3-only protein Bid is a substrate of caspase-8 (31, 32) and that the tBid can trigger Bax and Bak activation (10, 33). As shown in Fig. 4C, THG-induced caspase-8 activation and Bid proteolysis could occur independently of Bax in HCT116 cells. However, treating cells with z-IETD-fmk (a specific inhibitor of caspase-8) blocked Bid cleavage (Fig. 4D), Bax conformational change (Fig. 4D), and apoptosis (Fig. 4E), indicating that caspase-8 is the initiator caspase responsible for THG-induced Bax activation and apoptosis in HCT116 cells.

To examine whether THG-induced caspase activation requires new protein synthesis, HCT116 cells were exposed to 1 μM THG in the presence or absence of 50 μg/ml CHX for 24 h and subjected to immunoblot analysis with anticaspase-8 or caspase-3 antibody. As shown in Fig. 5A, both caspase-8 and -3 processing were completely inhibited in the presence of CHX, suggesting that de novo protein synthesis is involved in THG-induced apoptosis of HCT116 cells. Moreover, it has been reported recently that THG induces DR5 expression in several cancer cells (34). Thus, we examined DR5 expression in HCT116 Bax+/− and Bax−/− cells in response to THG treatment. As shown in Fig. 5B, DR5 was strongly induced by THG treatment, suggesting a possibility that THG induces Bax activation and apoptosis through DR5-mediated caspase-8 activation.

Bax-dependent Smac and Omi Release from Mitochondria Is Essential for THG-induced Cell Death. It has been shown that caspase-8 cleaves pro-caspase-3 to produce the p12 small subunit and a p24 intermediate that undergoes autoproteolytic processing to generate the p20/p17 forms of the large subunit and that the IAP family proteins inhibit this process (28, 35). Early during apoptosis, Smac/Diablo and Omi/HtrA2 are released from mitochondria into the cytosol where they bind to and inhibit the IAP family proteins (3). To investigate whether the release of Smac and Omi from mitochondria is dependent of Bax, we performed immunofluorescence analysis with specific antibodies for Smac or Omi in HCT116 Bax+/− versus Bax−/− cells treated with or without 1 μM THG for 30 h. As shown...

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**Fig. 6.** Bax-mediated Smac and Omi release plays an essential role in THG-induced apoptosis. In A, HCT116 Bax+/− or Bax−/− cells were treated with 1 μM THG for 30 h and stained with anti-Smac or Omi antibodies followed by FITC-conjugated secondary antibody. Nuclei and mitochondria were stained with DAPI and MitoTracker, respectively. In B, HCT116 Bax−/− cells were transfected with pEGFP-IETD-Smac or pEGFP-IETD-Omi plasmid DNA for 24 h and treated with 1 μM THG or DMSO for an additional 24 h. Cells were fixed with 3.7% formaldehyde, stained with DAPI, and analyzed by fluorescence microscopy. In C, cells with fragmented or condensed DNA in B were counted as apoptosis (mean ± SD, n = 3). D, a model of THG-induced apoptosis.
in Fig. 6A, both Smac and Omi colocalized with mitochondria in control DMSO-treated cells. In contrast, THG treatment resulted in a diffuse distribution of Smac and Omi in Bax+/− but not Bax−/− cells, indicating that THG-induced Smac and Omi release requires the presence of Bax.

To determine whether Bax-mediated Smac and/or Omi release is critical for THG-induced apoptosis, we transfected Bax−/− HCT116 cells with either GFP-IETD-Smac or GFP-IETD-Omi expression plasmids to produce cytosolic mature Smac or Omi after caspase-8 cleavage (36, 37). As shown in Fig. 6, B and C, overexpression of cytosolic Smac or Omi restored the sensitivity of Bax-null HCT116 cells to apoptosis induced by THG treatment.

**DISCUSSION**

Loss of the pro-apoptotic multidomain Bcl-2 family member Bax contributes to drug resistance in cancer (27). Bax can be activated by a variety of death signals (6), but it seems to be cell type dependent. THG is an inhibitor of Ca2+–ATPase of the ER and induces proliferation-independent apoptosis in a variety of cell types. THG analogues coupled with a synthetic peptide specifically cleaved by prostate-specific antigen have been developed recently (38). This promising prodrug can be targeted to specific cancer for induction of apoptosis. The precise biochemical mechanisms by which THG induces apoptosis of cancer cells, however, are still unclear, although several theories have been advanced, including regulation of calcineurin, calpain, caspase-12, ASK1, transglutaminase, and Gadd153 (21, 24, 39–42).

In the present study, we showed that THG up-regulates DR5 expression and triggers caspase-8 activation independently of Bax in human colon cancer HCT116 cells. Moreover, de novo protein synthesis is required for the processing and activation of this initiator caspase, which is particularly involved in the death receptor-mediated pathway for apoptosis, suggesting that up-regulation of DR5 could be the mechanism for THG-induced caspase-8 activation in HCT116 cells. In support of this model, recent evidence provided by He et al. (34) indicates that ER stress indeed induces apoptosis through activation of the DR5 pathway.

In most of cancer cells, caspase-8 activation cannot achieve the full processing of caspase-3; thus, the apoptotic signal needs to be amplified through a mitochondrial pathway (4). The BH3-only proapoptotic protein Bid undergoes proteolysis by caspase-8 during apoptosis, and the resulting tBid contributes to amplification of the apoptotic signal from death receptors by activating Bax. Consistently, our results show that inhibition of caspase-8 activity blocks Bid proteolysis and Bax conformational change induced by THG treatment (Fig. 4D). Although Bax is not required for THG-induced caspase-8 activation and Bid cleavage, it plays an important role in the release of apoptogenic molecules, such as cytochrome c. Smac/Diablo, and Omi/HtrA2, from mitochondria induced by THG in HCT116 cells (Figs. 2B and 6A). However, caspase-9 activity is not essential for THG-induced caspase-3 activation and apoptosis of HCT116 cells (Fig. 4, A and B), implying the importance of other mitochondrial molecules besides cytochrome c for achieving the full caspase-3 processing and apoptosis induction by ER stress.

It has been shown that Smac/Diablo and Omi/HtrA2 promote the processing and activation of caspase-3 by antagonizing the IAP family proteins once released from mitochondria (3). Moreover, transfecting cells with cytosolic Smac or Omi expression plasmids or synthetic Smac peptide sensitizes tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis (36, 37, 43–45). We found that overexpression of GFP-IETD-Smac or GFP-IETD-Omi, which is converted to mature cytosolic Smac or Omi after caspase-8 cleavage, restored the sensitivity of Bax-knockout HCT116 cells to apoptosis after THG treatment (Fig. 6, B and C). Compared with Smac, Omi is more potent to induce cell death of HCT116 cells (Fig. 6, B and C), probably because of its serine protease activity (37, 46). These findings strongly argue that Bax-dependent release of Smac and/or Omi plays a critical role in ER stress-induced apoptosis.

Taken together, our findings reported here suggest a possible pathway for ER stress-mediated apoptosis in human colon cancer cells (Fig. 6D). In response to THG treatment, caspase-8 becomes activated through the death receptor, such as DR5, pathway, to trigger the initial processing of caspase-3/7. However, the full processing/activation of these executor caspsases is blocked by the IAP family proteins. To achieve apoptosis, caspase-8 cleaves the BH3-only protein Bid to generate active tBid that induces Bax conformational change and translocation to mitochondria, causing the release of apoptogenic molecules, such as Smac and Omi, into the cytosol, where they antagonize the IAP family proteins. Therefore, the efforts to manipulate the activity of Bcl-2 family or IAP family proteins in malignant cells may one day become an important part of the armamentarium used to destroy chemoresistant cancer cells.

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Bax Plays a Pivotal Role in Thapsigargin-induced Apoptosis of Human Colon Cancer HCT116 Cells by Controlling Smac/Diablo and Omi/HtrA2 Release from Mitochondria

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