Enhanced Cytoprotective Effects of the Inhibitor of Apoptosis Protein Cellular IAP1 through Stabilization with TRAF2

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Inhibitor of apoptosis (IAP) proteins are key regulators of intracellular signaling that interact with tumor necrosis factor (TNF) receptor superfamily members as well as proapoptotic molecules such as Smac/DIABLO and caspases. Whereas the X-linked IAP is an established caspase inhibitor, the protective mechanisms utilized by the cellular IAP (c-IAP) proteins are less clear because c-IAPs bind to but do not inhibit the enzymatic activities of caspases. In this study, c-IAPs are shown to be highly unstable molecules that undergo autoubiquitination. The autoubiquitination of c-IAP1 is blocked upon coexpression with TNF receptor-associated factor (TRAF) 2, and this is achieved by inhibition of the E3 ubiquitin ligase activity intrinsic to the RING of c-IAP1. Consistent with these observations, loss of TRAF2 results in a decrease in c-IAP1 levels. Stabilized c-IAP1 was found to sequester and prevent Smac/DIABLO from antagonizing X-linked IAP and protect against cell death. Therefore, this study describes an intriguing cytoprotective mechanism utilized by c-IAP1 and provides critical insight into how IAP proteins function to alter the apoptotic threshold.

The inhibitors of apoptosis (IAPs) are an evolutionarily conserved gene family described originally as encoding cell death inhibitors. IAP proteins have subsequently been found to participate in a variety of additional intracellular signaling processes (1), and it has become evident that IAP proteins are versatile molecules playing numerous distinct roles within the cell. Although a more complete understanding of these additional functions for IAP proteins is emerging, the distinct mechanisms utilized by some IAP proteins to function in their originally defined roles as cell death inhibitors remain unclear.

Members of the IAP family are characterized by the presence of 1–3 tandem repeats of an ∼70-residue baculovirus IAP repeat domain (2). The baculovirus IAP repeat domains of many IAP proteins have been shown to be the region within IAP proteins that associates with caspases and other proapoptotic molecules (3, 4). IAP proteins have remarkably different apoptotic inhibitory abilities. For example, X-linked IAP (XIAP) is a highly potent cell death inhibitor (5) and is thought to be the only mammalian IAP protein that directly inhibits the enzymatic activities of caspases (2–4, 6). Although cellular IAP1 and -2 (c-IAP1 and c-IAP2) are anti-apoptotic proteins that can bind to caspase-7 and -9, they do not inhibit the enzymatic activities of these caspases (2, 6).

Many IAP proteins, including c-IAP1 and c-IAP2, contain a carboxyl-terminal RING domain that can function as an E3 ubiquitin ligase (7). The E3 ubiquitin ligase activity of the RING domain in c-IAP1 and c-IAP2 was previously shown to negatively regulate the apoptotic inhibitory properties of c-IAP proteins and to promote autoubiquitination and degradation of c-IAP1 (8, 9), thus hindering attempts to define the cellular properties of this protein.

A specialized property of the c-IAP proteins is their involvement in tumor necrosis family (TNF) signaling (10–12). Both c-IAP1 and c-IAP2 were discovered in a biochemical screen for factors associated with the type 2 TNF receptor. This association was found to be indirect and bridged by interactions with TNF receptor-associated factors (TRAFs), most notably TRAF1 and TRAF2 (11). Though the consequences of the association between TRAF2 and c-IAP1 on TNF-mediated signaling have been investigated (12), less is known about the functional significance of the association between TRAF2 and c-IAP1 on cell death inhibition. Because both c-IAP1 and TRAF2 possess E3 ubiquitin ligase activity in their respective RING domains, it seemed that the association between these molecules might impact the protective properties of c-IAP1 and alter the apoptotic threshold.

In this study, the role of TRAF2 in c-IAP1 stability and how the association of TRAF2 with c-IAP1 affects the apoptotic inhibitory properties of c-IAP1 were examined. The presence of TRAF2 greatly enhanced the stability of c-IAP1, and these data suggest that the interaction between TRAF2 and c-IAP1 inhibits the E3 ubiquitin ligase activity intrinsic to the RING domain of c-IAP1. Using stabilized c-IAP1, the anti-apoptotic activity of c-IAP1 was characterized, and it was found that c-IAP1 suppresses apoptosis to a degree comparable with
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XIAP. Furthermore, we show that c-IAP1 functions to prevent the IAP antagonist, Smac/DIABLO (13, 14), from interfering with XIAP inhibition of caspases. Together, this study demonstrates that although c-IAP1 does not directly inhibit caspase activity, stabilized c-IAP1 can sequester Smac/DIABLO, prevent Smac/DIABLO from antagonizing XIAP, and inhibit cell death.

EXPERIMENTAL PROCEDURES

Materials—Reagents were obtained from the following sources: MG-132 (Sigma); protein G-coupled agarose, L-glutamine, and phosphate-buffered saline (Invitrogen); DMSO (Sigma); Dulbecco’s modified Eagle’s medium and fetal bovine serum (Mediatech, Inc.); small interfering RNA (siRNA) oligonucleotides (Xeragon/Qiagen); caspase assay kit (Biosource); DEVD-AFC (BioMol); protease inhibitor mixture tablets (Complete mini; Roche Applied Science); and a QuikChange site-directed mutagenesis kit (Stratagene). Dr. John Silke (La Trobe University, Melbourne, Australia) kindly provided mouse, anti-rabbit, and anti-rat (GE Healthcare). Dr. Tak Mak (University of Toronto) kindly provided TRAF2-deficient murine embryonic-derived fibroblasts (MEFs) (15). Antibodies were obtained from the following sources: hemagglutinin (HA; Covance), β-actin- and peroxidase-conjugated anti-HA (Sigma), TRAF2 (BD Pharmingen), XIAP (BD Transduction Laboratories), Smac/DIABLO (Calbiochem), cleaved caspase-3 (Cell Signaling), and peroxidase-conjugated antimouse, anti-rabbit, and anti-rat (GE Healthcare). Dr. John Silke (La Trobe University, Melbourne, Australia) kindly provided anti-c-IAP1 (16).

Plasmids—pEBB HA-c-IAP1 and pEBB HA-c-IAP2 were subcloned from pEBG c-IAP1 and pEBG c-IAP2, respectively (17). Site-directed mutagenesis of c-IAP1 and c-IAP2 to generate c-IAP1 H588A, c-IAP1 E64A/R65A, and c-IAP2 H574A was performed using the QuikChange site-directed mutagenesis kit (Stratagene). To generate c-IAP1ΔBIR1, an internal BamHI site was used to subclone the c-IAP1 coding sequence lacking nucleotides 1–363 (corresponding to residues 1–121) into pEBB-HA. Unless otherwise noted, plasmids and siRNA oligonucleotides used for this study have been described previously (17–19).

Cell Culture, Transfections, and MG-132 Treatment—HEK293 cells and MEFs were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 2 mM L-glutamine and maintained at 37 °C in an atmosphere of 95% air and 5% CO2. Cells were transfected with plasmids and siRNA oligonucleotides using a standard calcium phosphate transfection protocol. HEK293 cells were treated with 35 siRNA oligonucleotides using a standard calcium phosphate transfection protocol. HEK293 cells were treated with 35 siRNA oligonucleotides using a standard calcium phosphate transfection protocol. HEK293 cells were treated with 35 siRNA oligonucleotides using a standard calcium phosphate transfection protocol. HEK293 cells were treated with 35 siRNA oligonucleotides using a standard calcium phosphate transfection protocol. HEK293 cells were treated with 35 siRNA oligonucleotides using a standard calcium phosphate transfection protocol. HEK293 cells were treated with 35 siRNA oligonucleotides using a standard calcium phosphate transfection protocol. HEK293 cells were treated with 35 siRNA oligonucleotides using a standard calcium phosphate transfection protocol. HEK293 cells were treated with 35 siRNA oligonucleotides using a standard calcium phosphate transfection protocol.

Caspase Activity Assays—Adherent and floating cells were harvested, and immunoblot analysis was performed as described above.

Viability Experiments—Cells were transfected with pEBB-GFP, pcDNA3-Bax, and other indicated plasmids and incubated at 37 °C. Sixteen h following transfection, cell viability was determined by observing green fluorescent protein (GFP) fluorescent cells using a Leica DM IRB inverted microscope.

Caspase Activity Assays—Adherent and floating cells were harvested, and caspase-3 assays were performed using a caspase assay kit (Biosource) according to the manufacturer’s instructions. AFC released was measured over time at 37 °C using a CytoFluor 4000 multi-well plate reader (Applied Biosystems). A total of 20 measurements at 90-s intervals were taken for AFC release with an excitation wavelength of 400 nm and an emission wavelength of 508 nm.

Cell Lysate Preparation and Immunoblot Analysis—Cell lysates were prepared with radiolabeled protein precipitation assay lysis buffer (phosphate-buffered saline containing 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS) supplemented with protease inhibitors for 30 min on ice to ensure complete lysis unless noted otherwise. Protein quantification was determined by Bradford assay (Bio-Rad). Radiolabeled protein precipitation assay lysis buffer cell lysates of equal protein concentrations were prepared in lithium dodecyl sulfate sample buffer (Invitrogen NuPAGE LDS) separated on denaturing NuPAGE 4–12% polyacrylamide gradient gels, and transferred to 0.45-μm nitrocellulose membranes (Invitrogen). Membranes were blocked in 5% milk in Tris-buffered saline with 0.02–0.2% Tween 20 (Bio-Rad) depending on the antibody requirements, followed by incubation with the indicated antibodies in 2.5% milk for 1 h at room temperature or overnight at 4 °C. After washing with Tris-buffered saline containing 0.02–0.2% Tween 20 (Bio-Rad) depending on the antibody requirements, followed by incubation with the indicated antibodies in 2.5% milk for 1 h at room temperature. Enhanced chemiluminescence (GE Healthcare) was used to visualize the blots on Kodak XAR film.

Immunoprecipitations—Cell lysates were prepared in radiolabeled protein precipitation assay lysis buffer, normalized for protein content, and incubated with HA or XIAP antibodies for 2 h at 4 °C. Protein G-coupled agarose beads were then added to the lysates and incubated for an additional 1 h. Centrifugation was performed to recover agarose beads, followed by washing in radiolabeled protein precipitation assay lysis buffer. Precipitated proteins were eluted by adding lithium dodecyl sulfate sample buffer (Invitrogen) and heating the samples for 10 min at 95 °C. Recovered proteins were subsequently separated by electrophoresis, and immunoblot analysis was performed as described above.

Real-time Reverse Transcription-PCR—HEK293 cells were transfected with plasmids, and 24 h following transfection, total RNA was isolated using the RNeasy mini kit (Qiagen) according to the manufacturer’s instructions. Reverse transcription with random hexamer primers and MultiScribe™ reverse transcriptase (Applied Biosystems) was performed on 100 ng of total RNA. The indicated target assays were performed on 1 μl of resulting cDNA. Each target assay was performed in triplicate and normalized to glyceraldehyde 3-phosphate dehydrogenase.

RESULTS

C-IAP1 Levels Are Reduced in TRAF2-deficient Cells—Previous studies have shown that the cellular signaling regulators c-IAP1 and c-IAP2 can interact with TNF receptor superfamily members and that an association with TRAF proteins, primarily TRAF1 and TRAF2, mediates this interaction (10–12). Although the interaction between c-IAP1 and TRAF2 was subsequently described to result in c-IAP1-mediated ubiquitination and degradation of TRAF2 (12), less is known about the functional consequences of the TRAF2-c-IAP1 interaction on c-IAP1 expression and cellular properties of c-IAP1.

To further characterize the interaction between c-IAP1 and TRAF2, we examined how loss of TRAF2 might affect c-IAP1
expression. Interestingly, in TRAF2-deficient MEFs, a notable and specific reduction in endogenous c-IAP1 protein levels was observed, whereas levels of other proteins, including XIAP, remained unchanged (Fig. 1A). This finding suggested that TRAF2 might play a role in the regulation of c-IAP1 protein levels.

Given the observed reduction in endogenous c-IAP1 protein levels in TRAF2-deficient fibroblasts, we sought to determine whether a reduction of TRAF2 in human cells would affect c-IAP1 using an RNA interference approach. Two siRNA oligonucleotides for TRAF2 were used individually or in combination to suppress TRAF2 protein levels in HEK293 cells (Fig. 1B) and in MDA-MB-231 breast cancer cells (Fig. 1C). Examination of c-IAP1 protein levels in the TRAF2 suppressed cells also revealed a reduction in endogenous c-IAP1 levels. Other IAP proteins, such as XIAP, were unaffected by a reduction in TRAF2 levels (Fig. 1, B and C). These data suggest that the interaction between c-IAP1 and TRAF2 alters the regulation of c-IAP1 protein levels.

c-IAP1 and c-IAP2 Undergo Continuous Basal Autoubiquitination—Because the stability of c-IAP1 appeared to be dependent on TRAF2, we sought to compare the relative expression levels of several IAP proteins. Plasmids encoding XIAP, c-IAP1, and c-IAP2 with the same parental vector and epitope tag with no extraneous 5’ or 3’ sequences were transfected into HEK293 cells. Upon examination of cell lysates normalized for protein content, XIAP expression was consistently found to be significantly greater than c-IAP1 or c-IAP2 expression (Fig. 2A), suggesting that c-IAP1 and c-IAP2 were unstable proteins.

Because c-IAP1 and c-IAP2 were poorly expressed under these conditions, we examined whether these proteins might be undergoing ubiquitination and proteasome-mediated degradation. HEK293 cells were treated with MG-132 to inhibit proteasomal activity and to determine whether c-IAP proteins undergo ubiquitination and degradation. c-IAP1 and c-IAP2 protein levels were increased after MG-132 treatment in comparison with untreated cells that were transfected with c-IAP1 or c-IAP2 (Fig. 2B), suggesting that the c-IAP proteins were sensitive to ubiquitination and proteasomal degradation.

To determine whether the ubiquitination and degradation of c-IAP1 and c-IAP2 was because of the E3 ubiquitin ligase activity in their RING domains, site-directed mutagenesis was performed to replace critical histidines within their RING domains (H588A for c-IAP1 and H574A for c-IAP2). Interestingly, the expression of c-IAP1 and c-IAP2 variants deficient in their E3 ubiquitin ligase activities was greatly enhanced relative to their wild type counterparts and was comparable with that of wild type XIAP (Fig. 2C). Although loss of the E3 ubiquitin ligase activity of the RING domains resulted in stable forms of c-IAP1 and c-IAP2, modification of the corresponding critical histidine in the RING domain of XIAP did not result in a change in the expression level of XIAP H467A compared with wild type XIAP. Collectively, these data indicate that when expressed alone in culture, both c-IAP1 and c-IAP2 undergo RING-dependent autoubiquitination and degradation.

TRAF2 Enhances Expression of c-IAP1 Post-translationally—Because a reduction in endogenous c-IAP1 protein levels was observed that corresponded to reduced TRAF2 protein levels, and c-IAP proteins were observed to undergo autoubiquitination mediated by their E3 ubiquitin ligase activity when
expressed alone in culture, we sought to determine whether overexpression of any TRAF proteins might alter IAP protein levels. As shown in Fig. 3, c-IAP1, c-IAP2, and XIAP. Twenty-four h following transfection, cells were left untreated or treated with 35 μM MG-132 for 4 h. Following treatment with MG-132, cells were harvested and immunoblotted for HA (c-IAP1, c-IAP2, and XIAP). C. site-directed mutagenesis was performed to convert the coding sequence corresponding to histidine residues 588 in c-IAP1, 574 in c-IAP2, and 467 in XIAP located within the RING domains of these IAP proteins to alamines to generate E3 ubiquitin ligase deficient proteins. HEK293 cells were then transfected with plasmids encoding HA-c-IAP1, HA-c-IAP2, and XIAP or the corresponding HA-tagged RING mutants. Twenty-four h following transfection, cell lysates were prepared, and immunoblot analysis was performed. Equivalent protein loading was verified in A, B, and C by immunoblotting for β-actin.

The data thus far indicated that TRAF2 was capable of stabilizing c-IAP1. We therefore examined whether the enhanced expression of c-IAP1 in the presence of each of the six TRAFs. Twenty-four h following transfection, cell lysates were prepared, and immunoblot analysis was performed for HA-c-IAP1. B. HEK293 cells were transfected with a plasmid encoding HA-c-IAP1 in the presence each of the six TRAFs. Twenty-four h following transfection, cell lysates were prepared, and immunoblot analysis was performed for HA-c-IAP2. C. HEK293 cells were transfected with a plasmid encoding HA-XIAP in the presence each of the six TRAFs. Twenty-four h following transfection, cell lysates were prepared, and immunoblot analysis was performed for HA-XIAP. Immunoblot analysis was performed to confirm equivalent expression of all six TRAF proteins in A–C, and equivalent protein loading was verified in A–C by immunoblotting for β-actin.

FIGURE 2. c-IAP proteins undergo autoubiquitination mediated by their RING domains. A, HEK293 cells were transfected with amino-terminally HA epitope tagged c-IAP1, c-IAP2, and XIAP. Twenty-four h following transfection, cells were lysed and immunoblotted for HA (c-IAP1, c-IAP2, and XIAP). B, HEK293 cells were transfected with plasmids encoding HA-c-IAP1 and HA-c-IAP2. Eighteen h after transfection, cells were left untreated or treated with 35 μM MG-132 for 4 h. Following treatment with MG-132, cells were harvested and immunoblotted for HA (c-IAP1, c-IAP2, and XIAP). C, site-directed mutagenesis was performed to convert the coding sequence corresponding to histidine residues 588 in c-IAP1, 574 in c-IAP2, and 467 in XIAP located within the RING domains of these IAP proteins to alamines to generate E3 ubiquitin ligase deficient proteins. HEK293 cells were then transfected with plasmids encoding HA-c-IAP1, HA-c-IAP2, and XIAP or the corresponding HA-tagged RING mutants. Twenty-four h following transfection, cell lysates were prepared, and immunoblot analysis was performed. Equivalent protein loading was verified in A, B, and C by immunoblotting for β-actin.

Constructs.
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Stabilized c-IAP1 Is Cytoprotective—Because either coexpression of c-IAP1 with TRAF2 or mutation of the RING domain within c-IAP1 produced a stabilized c-IAP1, we sought to determine whether stabilized c-IAP1 might be capable of conferring a cytoprotective effect. To activate a well described, caspase-dependent apoptotic program, Bax was ectopically expressed in HEK293 cells (23). Prior studies have shown that XIAP can suppress this Bax-induced apoptotic process, which normally results in the cleavage and activation of caspase-3 in this cell type (4). As shown in Fig. 5A, c-IAP1, stabilized by TRAF2 was cytoprotective against Bax-mediated apoptosis. A significant reduction in caspase-3 processing (Fig. 5B) and activity (Fig. 5C) in comparison to Bax expressed alone or c-IAP1 expressed without TRAF2 was observed with TRAF2-stabilized c-IAP1. The level of protection by c-IAP1 stabilized by TRAF2 was comparable with the level of protection observed for XIAP. The ubiquitin ligase-deficient variant of c-IAP1 (H588A) was also capable of robustly protecting cells from undergoing Bax-induced apoptosis, as shown in Fig. 5D. Furthermore, a significant decrease in caspase-3 processing (Fig. 5E) and activity (Fig. 5F) was also observed with H588A c-IAP1. Additionally, c-IAP1 stabilized by the ΔRING TRAF2 variant was capable of protecting against Bax-induced cell death to a level comparable with c-IAP1 stabilized by TRAF2 (data not shown), suggesting that TRAF2 was not providing protection from cell death by activating the NF-κB signaling pathways. Taken together, these data suggest that stabilized c-IAP1 is a potent inhibitor of cell death capable of protecting cells to a level observed with XIAP.

Stabilization of c-IAP1 by TRAF2 Does Not Require Direct Interaction—To further examine the structural requirements of the stabilization effects of TRAF2 on c-IAP1, mutant versions of c-IAP1 were generated that have previously been shown to impair the interaction with TRAF2 (24, 25). As shown in Fig. 6A, a c-IAP1 double mutant (E64A/R65A) that appears to be abrogated in its ability to bind TRAF2 was still found to be stabilized by coexpression of TRAF2. Interestingly, a deletion construct lacking the entire BIR1 domain was robustly expressed, even in the absence of TRAF2, and in coprecipitation studies was found to be unable to associate with TRAF2 (Fig. 6A), suggesting that separate components within BIR1 participate in TRAF binding and destabilization. Taken together, these observations suggest that the ability of TRAF2 to stabilize c-IAP1 does not require a direct interaction but rather might involve a mechanism by which TRAF2 competes for a common factor, such as a component of the ubiquitination machinery.

Stabilized c-IAP1 Suppresses Apoptosis Induced by Both Intrinsic and Extrinsic Signals—To evaluate the specificity of the protective effects of TRAF2-stabilized c-IAP1, the protec-
The effects of c-IAP1/TRAF2 combinations were examined using two different proapoptotic stimuli induced by ectopic expression. Caspase-3 activation induced by an intrinsic apoptotic stimulus (Bax; Fig. 6B) or an extrinsic/receptor-mediated stimulus (26) (Fas/CD95; Fig. 6C), were both blocked by c-IAP1 expression when stabilized by TRAF2, and the E64A/R65A c-IAP1 mutant incapable of interacting with TRAF2 was similarly capable of suppressing caspase-3 levels.

**c-IAP1 Competitively Inhibits Interaction between Smac/DIABLO and XIAP**—Prior reports suggest that c-IAP proteins function to inhibit cell death without directly inhibiting the enzymatic activity of caspases (2, 6, 27). Based on
these findings and our observations that stabilized c-IAP1 can protect against Bax induced cell death, the binding interaction between c-IAP1 and Smac/DIABLO was examined to determine whether c-IAP1 was capable of protecting cells indirectly, for example by sequestering Smac/DIABLO away from endogenous XIAP. This hypothesis was tested by transfecting increasing amounts of a plasmid encoding c-IAP1 H588A, immunoprecipitating either XIAP or c-IAP1 from cell lysates, and immunoblotting for Smac/DIABLO in precipitated complexes (Fig. 7). Interestingly, as the concentration of plasmid encoding c-IAP1 was increased, the amount of endogenous Smac/DIABLO that was precipitated with c-IAP1 was increased. At the same time, the amount of endogenous Smac/DIABLO that was precipitated with endogenous XIAP was reduced (Fig. 7). These data indicate that stabilized c-IAP1 can competitively inhibit the interaction between Smac/DIABLO and XIAP.

**DISCUSSION**

In this study, we investigated the mechanisms by which c-IAP proteins are regulated and found that TRAF2 stabilized c-IAP1 (Fig. 3). We demonstrated that stabilized c-IAP1 was capable of inhibiting cell death to a level comparable with the potent cell death inhibitor XIAP (Fig. 5). Whereas XIAP is considered the major mammalian cell death inhibitor because it is capable of binding to and inhibiting caspases, c-IAP1 and c-IAP2 were shown to bind but not inhibit the enzymatic activity of caspases (2, 6). Therefore, it has been hypothesized that c-IAP1 might instead interfere with the binding of the XIAP antagonist, Smac/DIABLO, and function to sequester Smac/DIABLO from neutralizing the apoptotic inhibition of XIAP (2, 6). Using stabilized c-IAP1, the anti-apoptotic properties of c-IAP1 were examined. As demonstrated here, c-IAP1 can sequester Smac/DIABLO and competitively interfere with Smac/DIABLO and XIAP.

**FIGURE 6. Stabilization of c-IAP1 does not require direct interaction with TRAF2.** A, HEK293T cells were transfected with the indicated c-IAP1 plasmids in the absence or presence of TRAF2. Two days following transfection, cells were harvested, and c-IAP1 was immunoprecipitated with an antibody against the amino-terminal HA tag. The presence of TRAF2 in the immunoprecipitate was determined by immunoblot analysis. B, HEK293T cells were transfected with Bax and the indicated c-IAP1 plasmids in the absence or presence of TRAF2. Sixteen h following transfection, caspase-3 activity was evaluated by incubation with the fluorogenic substrate DEVD-AFC. C, HEK293T cells were transfected with Fas and the indicated c-IAP1 plasmids in the absence or presence of TRAF2. Twenty-four h after transfection, caspase-3 activity was evaluated as in B. The data shown are derived from multiple independent samples and are representative of at least two independent experiments.
DIABLO binding to XIAP, releasing XIAP to inhibit caspases (Fig. 7). Therefore, these findings provide evidence that stabilized c-IAP1 is capable of protecting against cell death; however, the mechanism by which stabilized c-IAP1 protects against cell death is distinct from the protective mechanism utilized by XIAP. Our studies describe a mechanism by which c-IAP1 inhibits cell death and clarify how IAP proteins function to promote cell survival.

Although c-IAP1 and c-IAP2 are members of the IAP family because they both contain three baculovirus IAP repeat domains, the structural motif used to define IAP proteins, it is now evident that these molecules participate in additional signaling pathways. The observation that c-IAP1 can function as a putative oncogene through synergizing with c-Myc (28) and the presence of c-IAP1 in an amplicon associated with squamous cell carcinoma and hepatocellular carcinoma, where c-IAP1 expression correlates with resistance to radiotherapy (29, 30), provide important indications of the significant but not yet fully defined cellular functions of c-IAP1. Recently, much interest has been placed on understanding the nonapoptotic role of c-IAP proteins in TNF receptor signaling to NF-κB because of their association with TRAFs (16, 31–33). c-IAP1 and c-IAP2 play an important role in TNF receptor-mediated cellular proliferation through the activation of NF-κB (16, 31–34). Furthermore, the discoveries of compounds that selectively target c-IAP proteins are being thoroughly investigated because of their potential therapeutic effects (16, 31–33).

Because it is clear that c-IAP proteins may be an important target for cancer therapeutics, it is critical that all cellular roles of c-IAP proteins, both nonapoptotic and anti-apoptotic, are thoroughly investigated because it is evident that c-IAP1 and c-IAP2 are multifunctional proteins that play important roles in cell survival and proliferation, as well as other potential signaling cascades. Given that c-IAP1 and c-IAP2 have the potential to regulate signaling to NF-κB through interactions with TRAFs, as well as associate with Smac/DIABLO, which can regulate caspase-mediated apoptosis and caspase-independent signaling, it is not surprising that numerous forms of cancer may utilize c-IAP1 to drive oncogenesis. Whereas the anti-apoptotic and NF-κB mediated cell proliferation roles for c-IAP1 and c-IAP2 are now more defined, it will be imperative to differentiate which of these roles are critical for the oncogenic function.

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