cIAP1 Localizes to the Nuclear Compartment and Modulates the Cell Cycle

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Abstract

We explored the location and function of the human cIAP1 protein, a member of the inhibitor of apoptosis protein (IAP) family. Unlike family member X-linked IAP (XIAP), which was predominantly cytoplasmic, the cIAP1 protein localized almost exclusively to nuclei in cells, as determined by immunofluorescence microscopy and subcellular fractionation methods. Interestingly, apoptotic stimuli induced nuclear export of cIAP1, which was blocked by a chemical caspase inhibitor. In dividing cells, cIAP1 was released into the cytosol early in mitosis, then reaccumulated in nuclei in late anaphase and in telophase, with the exception of a pool of cIAP1 that associated with the midbody. Survivin, another IAP family member, and cIAP1 were both localized on midbody microtubules at telophase, and also interacted with each other during mitosis. Cells stably overexpressing cIAP1 accumulated in G2-M phase and grew slower than control-transfected cells. These cIAP1-overexpressing cells also exhibited cytokinesis defects over 10 times more often than control cells and displayed a mitotic checkpoint abnormality with production of polyploid cells when exposed to microtubule-targeting drugs nocodazole and paclitaxel (Taxol). Our findings demonstrate a role for Survivin in controlling a cell cycle checkpoint that results in cell cycle arrest. Survivin, the smallest IAP member with a single Bir domain, regulates both the cell cycle and apoptosis. Whereas the cell cycle function of Survivin is independent on its association with the chromosomal passenger proteins (INCENP, Aurora B, and Borealin) at the kinetochore (18-23), the apoptosis functions have been related to its interaction with cofactors such as HBXIP, which facilitates interactions with procaspase-9 (24), and to interactions with other IAPs that directly bind caspases (25). Survivin and procaspase-9 reportedly colocalize in mitotic cells (26), suggesting a role for Survivin in controlling a cell cycle checkpoint that results in cell cycle failure if improperly executed (27-29).

Despite the overall sequence and structural similarity among the IAP family members, the subcellular distribution of IAPs seems extremely variable. For example, Survivin in normal cells is predominantly a nuclear protein, the expression of which is cell cycle dependent, peaking at G2-M (30). On the other hand, most tumor cells show nuclear and cytoplasmic Survivin distribution, which is cell cycle independent (31). Another member of the IAP family, XIAP, is expressed mostly in the cytoplasm, but in the presence of the antagonist protein, XAF1, is sequestered in nuclei (32). Expression of cIAP1 has been suggested to be cytoplasmic, nuclear, or both (33-36). Surprisingly, cIAP2 expression has been detected also in mitochondrial fractions (33). The mechanisms regulating the distribution of IAPs into various subcellular compartments and the correlation of their subcellular location with protein function require further clarification.

In this study, we examined the intracellular distribution of cIAP1, the contribution of the Bir domains to localization of cIAP1, and explored whether certain stimuli alter cIAP1 localization within cells. Our findings indicate that cIAP1 is predominantly a nuclear protein, and its nuclear localization is mediated by the Bir domains. We also report that overexpression of cIAP1 induces aberrant cell division and accumulation of polyploid cells through defective cytokinesis. Moreover, a pool of cIAP1 concentrates at the midbody of telophase cells, suggesting a potential role for cIAP1 in the cell cycle. We also present evidence that cIAP1 interacts with Survivin, possibly linking cIAP1 overexpression to the cytokinesis phenotype produced by cIAP1 overexpression.

Materials and Methods

Antibodies and Reagents. Antibodies for immunoblotting and fluorescent microscopy were obtained from the following sources: cIAP1 and cIAP2 (R&D systems, Minneapolis, MN); cIAP1 monoclonal antibody, XIAP,
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and poly(ADP-ribose) polymerase (PARP, BD PharMingen, San Diego, CA); myc clone 9E10 (Santa Cruz Biotechnology, Santa Cruz, CA); p53 Ab-6 (Calbiochem, San Diego, CA); Flag-M2 and actin (Sigma, St. Louis, MO). The rabbit anti-Survivin antibody has been described (37). Other reagents purchased were etoposide (Calbiochem), staurosporine and nocodazole (Sigma), TNF-related apoptosis-inducing ligand (TRAIL) and TNF-α (Biomol, Plymouth Meeting, PA), and 4-(2-aminoethyl) benzenesulfonylfluoride (AEBSF, Roche Diagnostics, Mannheim, Germany). Alexa Fluor-conjugated secondary antibodies were purchased from Molecular Probes (Eugene, OR).

Plasmids. Generation of the full-length cIAP1 was described previously (14). Plasmids encoding individual fragments of cIAP1 corresponding to Bir1, Bir2, Bir3, and Bir3-RING were generated by PCR and cloned into pcDNA3, in-frame with an NH2 terminal myc epitope tag.

Cell Culture, Transfections, and Treatments. HeLa and HCT116 cells were grown in DMEM (Irvine Scientific, Santa Ana, CA) supplemented with 10% fetal bovine serum, glutamine, and penicillin/streptomycin. Du145 cells were grown in RPMI medium (Irvine Scientific) supplemented as above. Transfections were done using Lipofectamine 2000 (or Lipofectamine Plus reagents) and OptiMem reduced serum medium (Invitrogen, Carlsbad, CA), Lipofectamine transfection mix was removed after 4 hours. Independent clones of Du145-cIAP1 stable transfectants were selected with 600 μg/mL G418. For some experiments, transfected or untransfected HeLa cells were treated with TRAIL (200 ng/mL), TNF (20 ng/mL), staurosporine (0.7 μmol/L), or UV irradiation (0.12 J/cm2) from UV Stratalinker (Stratagene, La Jolla, CA). G1 synchronization of HeLa cells was achieved by treatment with 40 ng/mL nocodazole for 2 minutes at 4°C. The supernatant was transferred to a new tube for 4 minutes at 4°C. The final volume was adjusted to 10 μL for 20 minutes at 4°C. After 20 minutes at 4°C, the supernatant was removed and nuclear pellet was resuspended in modified radioimmunoprecipitation assay buffer [150 mmol/L KCl, 20 mmol/L HEPES (pH 7.4), 0.5 mmol/L EGTA, 0.5 mmol/L EDTA, 0.3% NP40, and “Complete Mini” protease inhibitor mixture (Roche Diagnostics)]. The cell suspension was incubated on ice for 10 minutes. Complete lysis was ensured by microscopy analysis of small aliquots of the suspension, allowing more time for lysis when necessary. The lysate was centrifuged at 800 × g for 2 minutes at 4°C, and the supernatant was carefully collected, and stored as cytosolic extract. The pellet was resuspended in 0.5 mL of hypotonic lysis buffer and washed in lysis solution, recovering nuclei by centrifugation at 800 × g for 4 minutes at 4°C. The supernatant was removed and nuclear pellet was resuspended in modified radioimmunoprecipitation assay buffer [150 mmol/L KCl, 20 mmol/L HEPES (pH 7.4), 1% Triton X-100, 0.5% deoxycholate, 0.3% NP40, and “Complete Mini” protease inhibitor mixture (Roche Diagnostics)]. The cell suspension was incubated on ice for 10 minutes. Complete lysis was ensured by microscopy analysis of small aliquots of the suspension, allowing more time for lysis when necessary. The lysate was centrifuged at 800 × g for 2 minutes at 4°C, and the supernatant was carefully collected, and stored as cytosolic extract. The pellet was resuspended in 0.5 mL of hypotonic lysis buffer and washed in lysis solution, recovering nuclei by centrifugation at 800 × g for 4 minutes at 4°C. The supernatant was removed and nuclear pellet was resuspended in modified radioimmunoprecipitation assay buffer [150 mmol/L KCl, 20 mmol/L HEPES (pH 7.4), 1% Triton X-100, 0.5% deoxycholate, 1% SDS, 5 mmol/L EDTA, and protease inhibitors], Fractions were normalized for cell number and analyzed by SDS-PAGE and immunoblotting.

Immunoprecipitation. For immunoprecipitation experiments, loosely adherent mitotic cells were harvested from culture dishes by forceful washing with PBS and centrifuged at 300 × g for 10 minutes. The cell pellets were resuspended in 2× lysis buffer containing 2,500 μL/L Tris (pH 7.5), 50 μmol/L NaCl, 0.2% NP40, 10% glycerol, and protease inhibitor mixture containing 1% SDS and boiled for 5 minutes at 95°C. Any insoluble material was removed by centrifugation at 16,000 × g for 20 minutes at 4°C. The supernatant was transferred to a new tube and the final volume was adjusted to 10× lysis buffer containing 0.5% deoxycholate, 1% SDS, 5 mmol/L EDTA, and protease inhibitors. Fractions were normalized for cell number and analyzed by SDS-PAGE and immunoblotting.

In vitro Protein Interaction Assays. Glutathione S-transferase (GST) fusion proteins were produced from pGEX4T-1 plasmid in XL1-blue cells (Stratagene) and affinity purified using glutathione-Sepharose. In vitro translated proteins were produced using the TNT Quick Coupled Transcription System (Promega, Madison, WI). A total of 2.5 μL of 35S-labeled in vitro translated protein mix was incubated with purified proteins (0.5-1.0 μg) immobilized on 20 μL of glutathione-Sepharose beads in 0.1 mL of binding buffer [50 mmol/L Tris-HCl (pH 7.5), 5 mmol/L MgCl2, 10% glycerol, 0.5 mmol/L bovine serum albumin and 5 mmol/L 2-mercaptoethanol] at 4°C for 60 minutes. The beads were washed thrice with 1.0 mL binding buffer followed by boiling in 25 μL of SDS sample buffer, and proteins were analyzed by SDS-PAGE analysis (12%). Use of equivalent amounts of intact purified proteins and successful in vitro translation of all proteins was confirmed by SDS-PAGE analysis using Coomassie staining or autoradiography, respectively (data not shown).

Immunofluorescence/Confocal Microscopy. For indirect immunofluorescence microscopy, cells were grown on 22-mm-diameter coverslips. Cells transfected on coverslips were examined 24 hours after transfection. Cells were fixed on coverslips using freshly made 3.7% formaldehyde (containing 1-1.5% methanol) in PBS (pH 7.4) for 15 minutes, permeabilized with 0.1% Triton X-100 in PBS for 10 minutes, and preblocked with 2% bovine serum albumin in PBS for 1 hour. Primary antibodies were diluted in 1% bovine serum albumin and PBS and incubated for 1 hour. After washing, cells were incubated with secondary antibodies conjugated to Alexa Fluor 488 (green), or Alexa Fluor 594 (red) dyes (Molecular Probes). Actin cytoskeleton was stained with phallolidin conjugated to Alexa Fluor dyes (Molecular Probes). Finally, coverslips were mounted on microscope slides using Vectashield mounting medium containing 4′,6-diamidino-2-phenylindole (DAPI). Cells were visualized by laser-scanning confocal microscopy (Bio-Rad, Hercules, CA) or by using a Leica DMI 3000T microscope equipped with Z-focus motor and Simple PCI (Compu, Inc., Tualatin, OR) image capturing and analysis software (version 5.1.0.0110).

Flow Cytometry. Cells were harvested by trypsinization and washed once with PBS. Washed cells were resuspended in 0.3 mL PBS (pH 7.4), and fixed by addition of 100% ethanol while vortexing. Fixation proceeded for at least overnight at −20°C. Fixed cells were centrifuged, resuspended in propidium iodide solution (40 μg/mL propidium iodide, 320 μg/mL RNase A in PBS without calcium and magnesium), and incubated for 15 minutes at 37°C. Stained cells were passed through nylon-mesh sieve to remove cell clumps and analyzed by a FACScan flow cytometer and CellQuest analysis software (Becton Dickinson, San Jose, CA).

Carboxyfluorescein Diacetate Iodacetate Succinimidyl Ester Dilution Assay. Carboxyfluorescein diacetate iodacetate succinimidyl ester (CFDA-SE, also commonly called CFSE) was obtained from Molecular Probes. Du145 cells were plated at a density of 104 cells per well in six-well dishes. At 16 hours after seeding, cells were labeled with 10 μmol/L CFSE in PBS for 15 minutes at 37°C according to the manufacturer’s recommendations. After 15 minutes, the labeling solution was replaced with fresh, prewarmed medium. Cells were collected by trypsinization at various times and processed for flow cytometry as above.

Results
cIAP1 Localizes to Nuclei. To establish the subcellular distribution of cIAP1, we did cell fractionation experiments using HeLa cells. Examination of the fractions for cIAP1 and other endogenous apoptosis-related proteins showed that cIAP1 and the control protein, PARP, localized to the nuclear fractions, whereas XIAP, Smac, and pro-caspase 3 localized to cytosolic fractions (Fig. 1A). To complement the fractionation experiments, we did immunofluorescence staining for endogenous cIAP1, cIAP2, XIAP, Survivin, and p53 in HeLa cells, using the DNA-binding fluorochrome DAPI, phallolidin, or anti-tubulin antibody as counterstains with indirect immunofluorescence detection. Fluorescence microscopy confirmed the results from subcellular fractionation studies, indicating that cIAP1 is a primarily nuclear protein (Fig. 1B and C). As shown in Fig. 1A and C, cIAP2 localized to both cytoplasmic and nuclear compartments, whereas XIAP was mostly cytoplasmic. Survivin and p53 localized to the nuclear compartment.
The Bir Domains of cIAP1 Mediate Nuclear Localization. We analyzed the domains in cIAP1 responsible for its nuclear targeting. Direct examination of the cIAP1 sequence revealed no amino acid sequence motifs meeting criteria for potential nuclear localization signal sequences. Therefore, to determine which domains of cIAP1 confer nuclear localization to the protein, we expressed fragments of the cIAP1 protein, with NH2-terminal myc tags, representing the Bir1, Bir2, Bir3, and Bir3-RING domains, as well as the full-length protein. Immunofluorescence microscopy was then used in conjunction with an antibody directed against the myc tag to localize these cIAP1 fragments in transfected HeLa cells (Fig. 2). At 12 hours post transfection, the expressed proteins were mostly cytoplasmic (Fig. 2A). However, after 24 hours, all Bir domains of cIAP1 localized to nuclei (Fig. 2B and data not shown). We also observed that expression of the Bir3-RING fragment of cIAP1 resulted in diffuse staining of the expressed protein, where the Bir3-RING protein was detected in both the cytoplasmic and nuclear compartments. The full-length protein, however, was entirely nuclear, indicating the probable dominance of the Bir domains on the overall distribution of cIAP1 (Fig. 2C). To ensure that the nuclear localization of the ectopically expressed proteins was not influenced by the myc tag, we also expressed Flag-tagged full-length cIAP1 in HCT116 cells and analyzed the localization of the expressed protein in cytosolic-nuclear fractions by immunoblotting. As shown in Fig. 2D, the Flag-tagged cIAP1 was also exclusively detected in the nuclear fractions. The purity of the fractions was controlled by immunoblotting for Smac and PARP proteins, which were localized to the cytosolic and nuclear fractions, respectively. These results indicate that both endogenous and ectopically expressed cIAP1 target to nuclei.

Inducible Relocalization of cIAP1. Because most of the known functions of cIAP1 require cIAP1 to be in the cytoplasm, we examined whether the subcellular location of cIAP1 changes in response to various apoptotic stimuli. To this end, we treated HeLa cells with various apoptotic agents that trigger cell death through either the death receptor-mediated pathway (e.g., TRAIL) or the mitochondria-dependent pathway (e.g., DNA-damaging agents, such as UV irradiation, or the broad-spectrum kinase inhibitor, staurosporine. The location of cIAP1 was then examined by subcellular fractionation (Fig. 3A and B).
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We found that several agents that induced either the extrinsic or intrinsic apoptotic pathways promoted the redistribution of cIAP1 from the cytoplasmic compartment to the nuclear compartment, including TNF, TRAIL, staurosporine, and UV irradiation (Fig. 3A-C). Under the same conditions, PARP was always detected in the nuclear fractions, whereas Smac remained cytoplasmic. The process of cIAP1 redistribution correlated with caspase activation, as evidenced by PARP cleavage (Fig. 3A and B). Therefore, to examine if inhibition of caspases would block the redistribution of cIAP1, HeLa cells were treated with TRAIL in the absence or presence of the broad-spectrum caspase inhibitor z-VAD-fmk or the serine protease inhibitor AEBSF. Then, cytosolic and nuclear fractions were analyzed for cIAP1, Survivin, PARP, and Smac proteins. As shown in Fig. 3B, the nuclear-cytoplasmic redistribution of cIAP1 was suppressed by inhibition of caspases but not by inhibition of serine proteases. Moreover, z-VAD-fmk but not AEBSF inhibited the cleavage of PARP. Neither z-VAD-fmk nor AEBSF affected the subcellular distribution of Survivin, PARP, and Smac in TRAIL-treated HeLa cells.

To corroborate the results from cell fractionation experiments, we undertook immunomicroscopy-based localization of cIAP1 using HeLa cells exposed to TNF. cIAP1-transfected or untransfected cells were treated with TNF-α with or without cycloheximide. Cells were treated 24 hours after transfection when the overexpressed protein was found predominantly in nuclei. After TNF treatment, the proportion of cells displaying nuclear and cytoplasmic staining for cIAP1 was quantified. As shown in Fig. 3C and D, a significant amount of cIAP1 was found in the cytoplasm after TNF treatment. Interestingly, whereas the subcellular redistribution of exogenously expressed cIAP1 was readily induced by TNF-α without requirement for cycloheximide (~49 ± 14%), the endogenous cIAP1 protein translocated efficiently only after treatment with the combination of TNF-α and cycloheximide (~83 ± 7% TNF plus cycloheximide versus 10 ± 4% TNF only).

Localization of cIAP1 Protein in Dividing Cells. To date, Survivin is the only IAP member shown to directly regulate the cell cycle through interaction with chromosomal passenger proteins (reviewed in refs. 19, 38). We determined the location of cIAP1 during the cell cycle, making comparisons with Survivin. We used indirect immunofluorescence microscopy for these experiments, staining replicate cultures of HeLa cells to detect endogenous cIAP1 or Survivin (Fig. 4A). Parallel staining was done because both primary antibodies for immunostaining were raised in rabbits. These antibodies were tested from among multiple commercially available and self-produced reagents and shown to be monospecific for the intended proteins (37).

Immunofluorescence staining of HeLa cells at different phases of the cell cycle revealed that although endogenous cIAP1 is nuclear during interphase, it becomes diffusely distributed throughout the cell from prophase to anaphase, and then recovers during anaphase/telophase, when nuclei reassemble. Interestingly, a pool of cIAP1 also localized to the midbody (Fig. 4A and B), a structure that holds recently replicated cells together until the final stages of cytokinesis split them into two cells. Use of control antibody confirmed the specificity of this midbody staining in telophase cells (Fig. 4B). Western blot examination of cIAP1 protein levels using extracts made from thymidine-synchronized cells revealed no remarkable differences in the steady-state levels of cIAP1 during the cell cycle (data not shown).
Similar to cIAP1, Survivin was found diffusely through nuclei in interphase cells. Unlike cIAP1, however, after nuclear envelop breakdown, Survivin localized to kinetochores of cells in metaphase, then distributed to the midzone microtubules in anaphase. At telophase, Survivin was localized exclusively to the midbody (Fig. 4C), consistent with previous reports (21, 39). Thus, cIAP1 and Survivin are both found associated with the midbody in telophase cells, and are both found diffusely in the nucleus of interphase cells, but were not detectably colocalized during metaphase and anaphase.

Recently, it was reported that XIAP can bind Survivin via a Bir-Bir interaction (25). Given that cIAP1 colocalizes with Survivin during metaphase and anaphase (21), it is likely that XIAP and cIAP1 compete with one another during these stages for Survivin binding. However, we did not observe a decrease in cIAP1 signal at the midbody in telophase cells, suggesting that other proteins may be responsible for Survivin localization at this stage of the cell cycle.

To further study cIAP1 function during the cell cycle, we generated stable transfectants of diploid Du145 human prostate carcinoma cells overexpressing cIAP1. Among a total of nine positive clones obtained (Fig. 5A), two clones (3 and 4) were randomly selected for subsequent studies. Both clones displayed similar phenotypes, although data are presented here only for clone 3. Note that the cIAP1-overexpressing clones were examined for only 8 to 10 tissue culture passages because the phenotype became progressively more aberrant, then cIAP1 detection declined with increasing passage number.

We studied the cell growth characteristics of cIAP1 and vector control Du145 cells. In cultures of cIAP1-overexpressing cells, cells at the periphery of expanding colonies often displayed cytokinesis defects (Fig. 5B). Such aberrant cell divisions were rarely seen in cultures of vector-transfected cells (0.7 ± 0.2% versus 8.4 ± 2% in cIAP1 cells).

To measure the rate of population doubling, we plated equivalent numbers of cIAP1 and vector control cells and followed the population growth by counting the number of cells in culture on subsequent days. As shown in Fig. 5B (right), Du145-cIAP1 cells had a substantially lower rate of cell expansion.

Because we saw no increase in cell death to account for the reduced growth rate, we hypothesized that cell division defects were responsible for the apparent slow growth of Du145-cIAP1 cells. To test this hypothesis, we measured growth of Du145-cIAP1 and vector control cells by comparing the rate of decline of incorporated CFSE during cell division (Fig. 5C). Such aberrant cell divisions were rarely seen in cultures of vector-transfected cells (0.7 ± 0.2% versus 8.4 ± 2% in cIAP1 cells).

To measure the rate of cell division, we used the Cytocounter to enumerate the number of cells in culture. As shown in Fig. 5B (right), Du145-cIAP1 cells had a substantially lower rate of cell expansion.

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This result confirmed that cIAP1 Du145 cells had pronounced cell division defects. Taken together, these observations suggest that the slow growth of cells might be associated with defective cell cycle or mitotic exit.

We therefore assessed the cell cycle status of vector control–transfected and cIAP1-transfected cells by DNA content analysis, using flow cytometry. cIAP1-overexpressing cells had significantly higher proportions of cells in the G2-M phase of the cell cycle (4n DNA content), and the DNA content per cell was remarkably polymorphic, suggesting defective cytokinesis, which might have led to nuclear fusion and polyploidy (data not shown, and Figs. 5 and 6).

DNA content analysis of Du145-cIAP1 cells suggested that the proportion of cells in the G2-M phase increased with increasing passage, reaching a plateau of 60% to 66% cells with ≥4n DNA content between the 7th and 8th passages (Fig. 5D). Du145-cIAP1 cells also had a reduced percentage of cells in S phase at later passages compared to the vector control–transfected cells (not shown). In contrast, the percentage of cells with ≥4n DNA content did not exceed 31 ± 4% in cultures of vector control Du145 cells. Thus, cIAP1-overexpressing cells may have difficulties in completing mitosis. Moreover, treatment of Du145-neo and -cIAP1 cells with microtubule-targeting agents nocodazole and paclitaxel (Taxol) led to significantly higher proportion of cIAP1 cells entering 8n state compared to vector control cells (Fig. 6), further suggesting defective control of mitotic arrest in Du145-cIAP1 cells.

Discussion

In this report, we show that IAPs localize to different subcellular compartments, presenting data on the nuclear localization and subsequent export of cIAP1, as well as the dynamics of intracellular cIAP1 targeting during the cell cycle. We also show that each of the Bir domains of cIAP1 is capable of independently localizing to the nucleus. Previous analysis of the Bir domains of viral IAPs had
suggested that Bir domains are capable of binding metal ions and nucleic acids (40). Although there is no evidence that Bir domains can directly bind DNA, our report suggests that the nuclear localization of cIAP1 is a function of its Bir domains and not the RING or CARD domain. While this article was under preparation, two independent reports (36, 41) also showed evidence of cIAP1 targeting to nuclei, and mapped putative Nuclear Export Sequences to the CARD and the region between Bir2 and Bir3. Interestingly, Plenchette et al. (36) further showed that cIAP1 translocates from the nucleus to the Golgi apparatus in cells undergoing differentiation.

Nuclear localization and export of cIAP1 are particularly interesting because cIAP1 has thus far been described to function in regulating the cytosolic activities of caspases and Smac and because it also modulates TNF signaling in extranuclear compartments. We observed that a pool of cIAP1 redistributes into the cytosolic compartment when cells are exposed to certain apoptotic stimuli, including agents that trigger the extrinsic pathway (e.g., TNF, TRAIL) or intrinsic pathway (e.g., UV irradiation, staurosporine). The export of cIAP1 from the nucleus seems to depend on caspase activation, as the broad-spectrum caspase inhibitor z-VAD-fmk blocked the cytoplasmic redistribution of cIAP1. At this stage, we can only hypothesize how caspase activation might promote export of cIAP1. Caspase activity could be required to compromise nuclear membrane integrity, allowing the protein to readily leave the nucleus, or, alternatively, caspases may cleave yet unknown cIAP1-anchoring proteins that normally keep it sequestered in the nucleus. It should also be noted that because others have observed nuclear export of cIAP1 during cell differentiation (36), the possibility exists of both caspase-dependent
and-independent mechanisms for triggering export of this protein. However, the process of differentiation has been shown to involve moderate caspase activation in some cellular contexts (reviewed in ref. 42), differentiation-associated nuclear export of cIAP1 could also involve caspases. Because activated caspases cleave nuclear substrates, it is possible that under physiologic conditions cIAP1 regulates apoptotic signaling in the nucleus. IAPs are believed to restrain basal caspase activity in cells (43). Therefore, the intranuclear localization of some IAPs could be part of a regulatory mechanism whereby any active caspases in the nucleus are checked. However, it is also likely that the intranuclear IAPs have functions different from inhibition of caspases. For example, Survivin, an IAP family member, localizes to the nucleus of normal cells and is known to function both as a regulator of apoptosis and the cell cycle (28, 29).

As reported here, cIAP1 localization changes during mitosis, and that cIAP1 overexpression leads to cell cycle aberrations. Moreover, cIAP1 is capable of interacting with Survivin in vitro, and they also colocalized in vivo in mitotic cells. These data suggest novel functions for cIAP1 in the cell cycle. We speculate that the cytogenesis defects seen in cIAP1-overexpressing cells are due to interference with Survivin functions required for proper chromosome segregation and cytokinesis. The ability of cIAP1 overexpression to interfere with Survivin may also explain why it has been so difficult for investigators to establish cell lines stably expressing cIAP1.

Because cIAP1 has an E3-ubiquitin ligase function via its RING domain, it is possible that some cell cycle components are targeted by cIAP1 for ubiquitination and subsequent degradation. In dividing cells at telophase, we found that cIAP1 concentrates both in the re-formed nuclei and at the midbody. It is now well established that the ubiquitin-mediated degradation of several mitotic proteins, including Survivin, BubR1, and Cyclin B1, is essential for proper mitotic exit and cytokinesis (44–46). The intrinsic ubiquitin ligase functions of cIAP1 and its localization to the midbody during telophase raise the intriguing possibility that cIAP1 could be an integral component of a ubiquitin-ligase system operating at a precise time and intracellular location for completion of cytokinesis. However, because cIAP1 knockout mice are born normally and have no overt phenotype, it is unlikely that cIAP1 is uniquely required for midbody degradation. The recent report that cIAP1 is exported from the nuclei of differentiating cells also has interesting implications because upon terminal differentiation and cessation of cell division competency, a nuclear role for cIAP1 in regulating cell cycle may no longer be necessary.

In a recent study that used cDNA microarray technology to analyze HeLa cells for cell cycle–based periodicity of expressed genes, the expression patterns for cIAP1 (BIRC2) and Survivin (BIRC5) were found to be cell cycle–dependent at the mRNA level (47). Whereas the cell cycle–dependent expression of Survivin has already been established, the pattern for cIAP1 was described for the first time in the report. However, a change in cIAP1 protein levels with cell cycle was not confirmed in our study by using immunoblotting analysis.

Overexpression of cIAP1 is one of the hallmarks of prostate cancer progression (37), and overexpression of cIAP1 has also been reported in ovarian cancer (48). Our finding of an effect of cIAP1 on cell growth and cytokinesis suggests that overexpression of this protein may contribute to genetic instability associated with cell cycle and checkpoint perturbations, in addition to impacting apoptosis resistance. Thus, cIAP1 may exert several functions that are relevant to tumor biology.

In conclusion, we present evidence showing that cIAP1 is a nuclear protein and it translocates to the cytosol in response to apoptotic signals that activate caspases. The nuclear localization of cIAP1 is dependent on the Bir domains. Our results also indicate that cIAP1 modulates the cell cycle, and overexpression of cIAP1 causes genomic defects because of defective cell division, possibly through interference with Survivin. The mechanisms regulating cIAP1 trafficking into and out of the nucleus and the relevance of cIAP1 to cell cycle regulation remain to be elucidated.

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