Nuclear Survivin Has Reduced Stability and Is Not Cytoprotective*

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Survivin is an essential mitotic protein that is overexpressed in many cancers, and its presence is correlated with increased resistance to radiation and chemotherapy. Here we demonstrate that sending survivin into the nucleus accelerates its degradation in a cdh1-dependent manner, abolishes the radioresistance normally conferred to cells by its overexpression, and prevents survivin from inhibiting apoptosis without affecting its mitotic localization. Our data suggest that targeting survivin to the nucleus provides an efficient means of eliminating it from the cell and may prove a novel strategy in cancer treatment, particularly in combination with radiotherapy.

EXPERIMENTAL PROCEDURES

Unless otherwise stated tissue culture reagents were from Invitrogen, and all other chemicals were from Sigma.

Cell Culture and Generation of Stable Lines—HeLa cells were maintained at 37 °C with 5% CO2 in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, penicillin/streptomycin, 500 μg/ml G418, and fungizone. Lines made specifically for the study were survivinNLS(LANA)-GFP, survivinNLS(SV40)-GFP, and GFPNLS-GFP. All other lines have been described previously (4, 9). Proteins of interest were expressed by FuGENE 6 (Roche Applied Science)-mediated transfection of pcDNA3.1 constructs and selected with G418 (500 μg/ml). The cells stably expressing proteins of interest were maintained similarly but were grown in the presence of G418. Prior to experimentation, the lines were sorted using an LSRII fluorescence-activated cell sorter (BD Biosciences) to ensure homogeneous populations and used within five passages of sorting.

Nuclear Cytoplasmic Fractionation—The cells were harvested, washed in phosphate-buffered saline then resuspended in ice-cold hypotonic buffer (10 mM HEPES, pH 7.9, 1.5 mM MgCl2, 10 mM KCl, 1 mM dithiothreitol, plus protease inhibitors). The cells were sheared by passage through a 25-gauge needle 15 times. The lysates were centrifuged at 11,000 × g for 20 min at 4 °C, and the supernatant was collected. The pellet was resuspended in 20 mM HEPES, pH 7.9, 1.5 mM MgCl2, 0.42 M NaCl, 0.2 mM EDTA, 25% (v/v) glycerol, 1 mM dithiothreitol, plus protease inhibitors. The lysates were centrifuged at 21,000 × g for 5 min to obtain nuclear fractions. Protein concentration was determined using a Bradford Reagent protein assay (Bio-Rad), and equal concentration of cytoplasmic and nuclear extracts were used for immunoblotting analyses.

Immunoblotting—Standard procedures were followed for SDS-PAGE, immunoblotting and enhanced chemiluminesence detection (GE Healthcare). The antibodies used were goat anti-survivin (1/500; R & D Systems), anti-Myc (9E10, 1/500), and survivin stability has not been addressed; such regulation

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3 The abbreviations used are: NES, nuclear exportation signal; GFP, green fluorescent protein; NLS, nuclear localization signal; FACS, fluorescence-activated cell sorter; LMB, leptomycin B; TRAIL, TNF-related apoptosis-inducing ligand; TNF, tumor necrosis factor.
Nuclear Survivin Is Rapidly Degraded

anti-tubulin (1/2000; B512), anti-GFP (1/500; 3E1; CR-UK), anti-XRCC1 (a gift from K. Caldecott), anti-aurora-B kinase (anti-ALM1, 1/250, Transduction Labs), and anti-cdh1 (AbCam). Horseradish peroxidase-conjugated secondary antibodies were from Dako Cytometers and were used at dilutions of 1/1000–1/5000.

Radiolabeling and Immunoprecipitation—In vivo labeling was carried out by incubating 10⁶ cells with 50 mCi/ml [³⁵S]methionine. To determine the rate of protein turnover, the cells were pulsed as above and chased for up to 16 h in the presence of an excess of unlabeled amino acids. After radiolabeling cells were lysed for 30 min on ice in 500 μl of radiimmune precipitation assay buffer (20 mM Tris, pH 8, 137 mM NaCl, 0.5 mM EDTA, 10% glycerol, 1% Nonidet P-40, 0.1% SDS, 1% deoxycholate with 1 mM β-glycerophosphate, and 1 μg/ml each of the protease inhibitors 4-(2-aminoethyl) benzensulfonyl fluoride (AEBSF), chymostatin, leupeptin, antipain, pepstatin A), containing 2 mM MgCl₂ and 25 units/ml benzonase (VWR). The lysates were then cleared, and the supernatants were incubated for 1.5 h at 4 °C with 2 μg of a polyclonal anti-survivin (Novus) antibody. Protein G-Sepharose beads were then added (40 μl of a 50% slurry in lysis buffer), and the samples were incubated for a further 2 h at 4 °C. The samples were then washed and separated by SDS-PAGE, and band intensities were quantified from the dried gel using a Storm 860 PhosphorImager (GE Healthcare). cDNA for exogenous survivin-GFP (and variants) was annealed to the 5’-end of a GFP open reading frame and a reverse primer, which annealed at 40 °C. The samples were then washed with phosphate-buffered saline and resuspended in 200 μl of propidium iodide solution containing 50 μg/ml propidium iodide and 100 μg/ml RNase A (MP Biomedicals, UK). Propidium iodide-stained cells were analyzed with a FACScan cytometer using CellQuest software (Becton Dickinson).

Analysis of APC/C Modulators—To overexpress cdc20 and cdh1, pcDNA-cdc20-Myc and pcDNA-cdh1-Myc (gifts from Dr. Katya Ravid, University of Massachusetts, Boston, MA) were transiently transfected into HeLa cells using FuGENE 6 (Roche Applied Science) and expression assessed 24 h later by immunoblotting using anti-Myc antibodies (9E10).

To deplete cdh1 predesigned cdh1 small interfering RNA oligonucleotides (Ambion, ABI Biosystems) were transfected into HeLa cells using Hyperfect (Qiagen). Depletion was assessed by immunoblotting with anti-cdh1 antibodies (AbCam), 24 h post-transfection.

X-irradiation and Clonogenic Survival—The cells were seeded at low density (500–1000 cells/dish) in 9-cm² Petri dishes and allowed 2 h to attach, before exposure to X-irradiation using an Hs-X-Ray System (A.G.O. Installations Ltd., Reading, UK). Seven days post-irradiation, the colonies were stained with methylene blue (1 h at room temperature), dried, and then rinsed with H₂O, and colonies of 50 cells or more were counted.

Apoptosis Assays—To induce apoptosis by the extrinsic caspase-8/caspase-3 pathway, exponentially growing cells were treated with 250 μg/ml recombinant human TRAIL (Pepro Tech EC Ltd) for 60 or 90 min. The cells were lysed (45 min at room temperature) in mammalian protein extraction buffer, MPER (Pierce), supplemented with 1 mM EDTA, 1 μg/ml pepstatin A, and 1 mM 4-(2-aminoethyl) benzensulfonyl fluoride (AEBSF), at a concentration of 10⁶ cell equivalents/ml. The lysates were then cleared, snap frozen in liquid nitrogen, and stored at −80 °C.

To determine apoptotic activity, tetrapeptide cleavage assays were performed in a 96-well plate. Briefly, 5 ng/ml of the caspase-3-specific tetrapeptide substrate (DEVD-AMC; Biomol) was incubated at 37 °C for 1 h with 20–50 μl of whole cell lysate prepared in MPER (Pierce) in 20 mM HEPES, pH 7.5, with 10% glycerol and 1 mM dithiothreitol. Relative fluorescence release was measured using a Spectramax Gemini fluorimeter (Molecular Devices) with excitation set at 380 nm and emission at 440 nm.

Cell Viability Assay—The cells were seeded at a density of 10⁵/well in a 24 well dish and then irradiated at the doses indicated. Seven days later, the cells were incubated with 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, and cell viability was assessed using a Spectramax Gemini fluorimeter (Molecular Devices).
RESULTS

Endogenous Survivin Is Preferentially Degraded in the Nucleus—It has previously been shown that survivin is subject to proteasome-mediated degradation, because levels of endogenous survivin increase after treatment with MG132 (17). This increase is not due to accelerated synthesis because immunoprecipitation of endogenous survivin pulse-labeled with \(^{35}\)S-methionine for 2 h actually showed decreased incorporation of \(^{35}\)S (thus decreased synthesis) in the presence of MG132 (data not shown). To investigate any dependence of survivin stability on subcellular compartmentalization, we have fractionated asynchronous HeLa cells following MG132 treatment and analyzed the level of endogenous survivin in nuclear and cytoplasmic fractions (Fig. 1A). Using tubulin and XRCC1 as markers of cytoplasmic and nuclear fractions, respectively, we observed a selective increase in nuclear levels of endogenous survivin following MG132 treatment. Consistent with this finding, when endogenous survivin was sequestered in the nucleus by treatment with the exportin inhibitor LMB, its expression was reduced (Fig. 1B) by \(-30\%\). Together, these data suggest that nuclear survivin may be less stable than survivin localized to the cytoplasm.

Generation of Stable Lines Expressing Survivin\(_{\text{NLS}}\)-GFP—Survivin is a nuclear cytoplasmic shuttling protein that is primarily cytoplasmic when overexpressed. To further investigate post-translational regulation of survivin levels, we sought to send survivin to the nucleus. To this end we fused full-length human survivin to two separate NLS sequences, the bipartite LANA sequence RRHERPTTRIRHRKLR (10) and the monopartite SV40 T-antigen NLS sequence PKKKRKV (11), hereinafter referred to as survivin\(_{\text{NLS(LANA)}}\)-GFP and survivin\(_{\text{NLS(SV40)}}\)-GFP, respectively. Because these survivin constructs are expressed from the cytomegalovirus promoter, they are not subject to transcriptional regulation; thus they enable us to investigate changes in protein level attributed solely to post-translational regulation. HeLa cell lines were generated that stably overexpressed these versions of survivin and were FACS-sorted to homogeneity prior to use. As shown in Fig. 2A, survivin-GFP was predominantly cytoplasmic, whereas both survivin\(_{\text{NLS(LANA)}}\)-GFP and survivin\(_{\text{NLS(SV40)}}\)-GFP were retained in the nucleus (see Fig. 2, B and C). Lines were also generated that expressed GFP or GFP\(_{\text{NLS}}\)-GFP, for use as controls (data not shown). Importantly, the presence of an NLS on survivin did not alter its localization in mitosis, where both constructs were found at the same locations as survivin-GFP: the centromeres, midzone, and midbody, during prometaphase, anaphase, and cytokinesis, respectively, (Fig. 2, D–F).

FIGURE 1. Endogenous survivin is preferentially degraded in the nucleus. A, nuclear (lanes N) and cytoplasmic (lanes C) fractionation was carried out on HeLa cells that had been incubated in the absence (−) or presence (+) of MG132 (50 \(\mu\)M, 6 h). An increase in endogenous survivin was apparent upon proteasome inhibition in the nuclear (compare lanes 2 and 4) but not the cytoplasmic fraction (lanes 1 and 3). B, HeLa cells were incubated in the absence (−) or presence (+) 6 ng/ml LMB for 12 h to inhibit exportation of survivin from the nucleus. This treatment alone caused a 30\% reduction in survivin expression.

FIGURE 2. Expression of survivin-GFP and survivin\(_{\text{NLS}}\)-GFP constructs in HeLa cells. A–C, Interphase cells stably expressing the constructs indicated were probed with anti-lamin B antibodies (red) to show the nuclear margins, and 4′,6′-diamino-2-phenylindole to visualize the DNA (blue). The right panels show a representative field of cells from each population. D–F, Mitotic cells as above were probed with anti-tubulin antibodies (red) and 4′,6′-diamino-2-phenylindole (blue). NLS fusion caused nuclear sequestration of survivin-GFP in interphase but did not alter localization during mitosis.
Survivin-NLS-GFP Is Degraded More Rapidly than Survivin-GFP—From our low magnification fluorescence data in Fig. 2 (A–C, right panels), we noted that the level of expression of survivin-NLS-GFP in both lines appeared lower than for the line expressing survivin-GFP. By reverse transcription-PCR, we confirmed that the transcripts to these forms were present (Fig. 3A), and therefore mRNA was still being expressed. We also ascertained that these forms did not have different rates of protein synthesis (Fig. 3G). Thus we reasoned that the differential expression was due to differences in the rates of protein turnover. To examine this, we immunobotted whole cell extracts from asynchronous populations of cells expressing survivin-

**FIGURE 3.** Survivin-NLS-GFP is degraded more rapidly than survivin-GFP. A, reverse transcription-PCR was performed on cells expressing survivin-GFP (lane 1), survivin-NLS(LANA)-GFP (lane 2), and survivin-NLS(SV40)-GFP (lane 3) and confirmed that mRNA was expressed in each line. B, lysates were prepared from cell lines expressing survivin-GFP or survivin-NLS(GFP after the indicated times post treatment with 50 μM MG132, and immunoblots were probed using anti-GFP antibodies. To detect survivin-NLS-GFP at adequate levels for quantitation, a 6-h treatment with MG132 was required. C, quantification of ECL signals in B. Survivin-GFP expression is represented by circles, and survivin-NLS-GFP is represented by triangles. D and E, treatment overnight with 20 μM MG132 (lanes 2) followed by subsequent release into cycloheximide (50 μg/ml) to inhibit protein translation (lanes 3–6) revealed that survivin-NLS(LANA)-GFP degraded more rapidly than survivin-GFP. This experiment was performed twice with similar results. F, quantitation of immunoblots shown in D and E. G and H, to assess the synthesis rate of survivin-GFP (time 0), or survivin-NLS-GFP (time 0), the cells were pulse labeled with [35S]methionine for 2 h, and immunoprecipitation was carried out with anti-survivin antibodies (Novus). Pixel intensities of bands (time 0) were similar: 255246 and 246678, assigned 100% in H. The cells were then subjected to a cold chase before immunoprecipitation as above at 4, 8, or 16 h. Consistent with the immunoblotting experiments, survivin-NLS-GFP turned over more rapidly than survivin-GFP. In H expression at time 0 is taken as 100%. The data graphed are the means and standard deviations from two independent experiments.
GFP or survivin

Strikingly, whereas survivin-GFP was abundantly present in untreated asynchronous cells, survivin

GFP was barely detectable (Fig. 3B). Furthermore, whereas 6 h treatment with MG132 caused only a modest (1.08-fold) increase in survivin-GFP expression (Fig. 3, B and C), survivin

GFP levels rose at steady rate to 3.9-fold (Fig. 3, B and C), demonstrating that the stability of survivin

GFP is proteasome dependent, as is the case for the endogenous protein (Fig. 1A). Similar results were obtained with survivin

GFP. As a control we also compared GFP expression in cells expressing GFP and GFP

GFP. No differences in GFP expression were observed in these lines, indicating that the increased rate of turnover was not an artifact of the tag (data not shown).

To determine the relative stability of these versions of survivin, cells were treated with the translational inhibitor, cycloheximide (Fig. 3, D and E). Because of the rapid clearance of the nuclear forms of survivin, this experiment had to be carried out after pre-treatment with MG132 (Fig. 3E, lanes 1 and 2). Note, 16 h of treatment with MG132 did not affect cell cycle stage as assessed by FACS analysis (data not shown). Over a 16-h time course, survivin

GFP was degraded much more rapidly than survivin-GFP (Fig. 3, D and E), as is evident by the quantitation in Fig. 3F. We also noted that the addition of an NLS to GFP itself did not decrease the stability of GFP (data not shown).

To exclude the possibility that the level of survivin expression was due to changes in the rate of protein synthesis, we next pulse labeled survivin-GFP and survivin

GFP cells with

methionine. First, the cells were treated with MG132 for 4 h, then exposed to

methionine, and incubated for a further 2 h (Fig. 3G). The lysates were then prepared from each population and survivin-GFP or survivin

GFP immunoprecipitated from the extracts using anti-survivin antibodies. As shown in Fig. 3G, survivin-GFP and survivin

GFP incorporated

methionine to similar levels as quantified using a PhosphorImager (pixel intensities of bands 245246 and 246678, respectively). Next, we followed the pulse labeling with a cold chase after the removal of MG132 and the addition of cycloheximide. In accordance with our immunoblotting experiments in Fig. 3 (D–F), the rate of survivin

GFP turnover was more rapid than survivin-GFP (Fig. 3H). Thus these data further indicate that survivin is less stable in the nucleus than in the cytoplasm.

Survivin Is Preferentially Degraded in the Nucleus—Next we made nuclear and cytoplasmic extracts from asynchronous cultures of the stable cell lines of interest and loaded equivalent numbers of cells/lane (Fig. 4). Using tubulin and XRCC1 as markers of cytoplasmic and nuclear fractions, respectively, we found that survivin-GFP, like endogenous survivin, was predominantly cytoplasmic (Fig. 4A), but, consistent with our fluorescent imaging, expression of the NLS fused versions, survivin

GFP (Fig. 4B), and survivin

GFP (Fig. 4C), was extremely low. Moreover, there appeared to be little difference in expression between the two compartments, which was surprising given that survivin

GFP and survivin

GFP were specifically sent to the nucleus. However, upon 6 h of MG132 treatment, the levels of all versions of survivin, wild type and NLS-fused, rose dramatically in the nucleus, further suggesting that survivin is less stable in the nuclear versus cytoplasmic compartment. We noted that the levels of the NLS-fused forms of survivin also increased in the cytoplasmic fraction upon treatment with MG132 (Fig. 4, B and C), illustrating the nucleo-cytoplasmic shuttling activity of the protein.

Survivin Is Degraded in the Nucleus in G1—Survivin expression is normally limited to the G2/M stages of the cycle, with a rapid decline in its levels as cells exit mitosis and enter G1. A combination of factors contributes to the reduction in survivin during G1, including transcriptional repression, externalization of the midbody at the end of mitosis, and proteolysis. This prompted us to further investigate survivin degradation during G1, using cells expressing the survivin-GFP, which is not subject to transcriptional repression. The cells were synchronized in G1 using mimosine (Fig. 4D), then treated with 20 µM MG132 (Fig. 4E), and fractionated (Fig. 4F) to assess exogenous levels of survivin-GFP in the cytoplasm versus the nucleus. As with the asynchronous population following MG132 treatment (Fig. 4A), survivin-GFP was selectively up-regulated in the nucleus after 6 h of MG132 treatment in G1-arrested cells (Fig. 4F).

Degradation of Nuclear Survivin Is Mediated by cdh1—Expression of survivin, and its partner protein aurora-B kinase, is known to be regulated by proteolysis as cells exit mitosis (15, 17). Degradation of aurora-B has been demonstrated to be mediated by the APC activated by cdc20 and cdh1 (15); however, how survivin degradation is regulated has not been
addressed. Thus to test whether survivin degradation was cdh1 or cdc20 dependent, we transiently overexpressed Myc-cdh1 or Myc-cdc20 (gifts from Dr. K. Ravid) in cells expressing the survivin constructs of interest. Immunoblotting analysis 24 h post-transfection revealed that cells expressing either Myc-cdh1 or Myc-cdc20 decreased the expression of all forms of survivin, exogenous and endogenous. D, the decrease in survivin expression observed upon overexpression of cdh1 or cdc20 was reversed when cells were treated with MG132 (50 μM for 1.5 h). E, quantification of blots in A–D, showing the level of survivin as a fraction of the control. The data are representative of a minimum of two independent experiments. F, cdh1 was depleted from HeLa cells using predefined small interfering RNA oligonucleotides. Immunoblot analysis revealed a 54% decrease in cdh1 expression. This decrease was not reversed by the addition of MG132 for 1.5 h post-transfection revealing that cells expressing either Myc-cdh1 or Myc-cdc20 decreased the abundance of survivin-GFP, survivin\textsubscript{NLS\textsubscript{LANA}}-GFP and endogenous survivin in asynchronous cells (Fig. 5, A–C). This decrease in survivin levels was prevented by the addition of MG132 for 1.5 h post-transfection (Fig. 5D). (Note also, however, that the transfection efficiency with cdc20 was always lower than for cdh1). Quantitation of survivin expression from Fig. 5 (A–D) is shown in Fig. 5E and plotted as a fraction of the expression in the control cells.

**C**dh1 is a nuclear protein (12), whereas cdc20, whose level is low in G1, is more membranous/cytoplasmic (13). Thus, having established that survivin is degraded preferentially in the nucleus, we next asked whether depletion of cdh1 could increase survivin levels. cdh1 was depleted by small interfering RNA from asynchronous HeLa cells, and protein lysates were prepared 24 h post-transfection and analyzed for survivin expression by immunoblotting. Despite an incomplete knock down of cdh1 (54%), survivin expression doubled under these conditions (Fig. 5F).

**FIGURE 5. cdh1 mediates survivin degradation in the nucleus.** Asynchronous HeLa cells expressing survivin GFP (A), survivin\textsubscript{NLS\textsubscript{LANA}}-GFP (B), or not expressing any construct (C) were transfected with pcDNA3 constructs containing cDNA to cdh1-Myc or cdc20-Myc, and whole cell lysates were prepared 24 h later. To determine the level of survivin-GFP expression and cdh1-Myc or cdc20-Myc expression, immunoblots were probed with anti-survivin and anti-Myc antibodies, respectively. Note that because of the similarity in size between the tubulin and cdh1-Myc/cdc20-Myc, two separate gels were run: tubulin indicates the loading for survivin blots. Overexpression of both cdh-Myc and cdc20-Myc decreased the expression of all forms of survivin, exogenous and endogenous. D, the decrease in survivin expression observed upon overexpression of cdh1 or cdc20 was reversed when cells were treated with MG132 (50 μM for 1.5 h). E, quantification of blots in A–D, showing the level of survivin as a fraction of the control. The data are representative of a minimum of two independent experiments. F, cdh1 was depleted from HeLa cells using predefined small interfering RNA oligonucleotides. Immunoblot analysis revealed a 54% decrease in cdh1 expression. This decrease was accompanied by a 200% increase in survivin levels 24 h post-transfection, when compared with the control (lane C) population treated with a scrambled oligonucleotide. Tubulin indicates equality in loading.

**DISCUSSION**

Survivin is a nucleo-cytoplasmic shuttling protein that is predominantly cytoplasmic when overexpressed in cultured cells (2, 9). We and others have recently shown that this subcellular localization is dependent upon CRM1 (β-exportin) and a rev-like NES in the linker region of survivin, between its BIR domain and the C-terminal α-helix (2–4, 6–8).
Here we demonstrate that survivin is preferentially degraded in the nucleus in a cdh1/APC-dependent manner. These findings are consistent with cdh1-mediated degradation of survivin in vitro (14), and the nuclear localization of cdh1 in G1 (12). Somewhat paradoxically, survivin appears to be devoid of destruction motifs recognized by the APC/C. However, the mitotic partner protein of survivin, aurora-B, has three putative D-boxes, a KEN box, and an A-box, and mutation of the cdh1-specific KEN and A-boxes stabilize aurora-B, suggesting that its destruction is mediated preferentially by cdh1 (15, 16). In addition, aurora-B also exhibits accelerated clearance upon overexpression of cdh1, exhibits increased stability upon cdh1 depletion, and co-immunoprecipitates with cdh1 in mitotic extracts (15, 16). Thus, because survivin and aurora-B are both destroyed at the end of mitosis (14–17), it is formally possible that survivin relies on the consensus sequences of aurora-B for destruction. We are currently testing this hypothesis.
Nuclear Survivin Is Rapidly Degraded

In a previous report we found that a mutant form of survivin that accumulates in the nucleus could no longer protect cells against ionizing radiation or TRAIL-induced apoptosis (4). Corroborating data were recently presented by Stauber and coworkers (6–8). However, these experiments raised the question as to whether subcellular relocation alone was responsible for abrogating the anti-apoptotic activity of survivin or whether the effect was mutant specific. Here, we have artificially forced wild type human survivin expression in the nucleus and observed that this relocation prevented survivin from acting as an inhibitor of apoptosis. Furthermore, in some cases we actually noted an increase in sensitivity to apoptotic stimuli, the reason for which is unclear. One possibility may be that the subcellular localization of the exogenous protein influences the localization of the endogenous protein. In a recent study Temme et al. (5) also found that cells were more sensitive to apoptosis when they forced survivin expression in the nucleus, and interestingly they linked this observation to enhanced transcription of p53, and the pro-apoptotic genes, Bad and Bax. Our present data appear to contradict the recent work by Stauber et al. (6), who reported that nuclear sequestration of murine survivin via deletion of the NES increased the stability of the protein, thus suggesting that it is preferentially degraded in the cytoplasm. However, it is possible that deletion of these residues could have affected the folding or stability of survivin specifically rather than increased its stability as a result of its subcellular relocalization. (Note also that our experiments used stable cell lines rather than transiently transfected cells, which could have contributed to the different results). Differential stability caused by subcellular compartmentalization has been noted for a number of proteins including p53, whose localization and stability is altered upon DNA damage (18). Furthermore, the survivin isoform Delta-Ex3, which is nuclear when overexpressed (19, 20), is also cleared from the cell more rapidly than wild type survivin (21) and may explain why endogenous survivin DeX3 is difficult to detect at the protein level (20, 22). Interestingly, it has recently been reported that survivin degradation can also be facilitated by the XIAP association factor, XAF-1, in a proteasome-dependent manner, which suggests that multiple pathways for ensuring the removal of survivin from interphase cells exist (24).

Finally, survivin has a functional NES but no NLS. Thus one outstanding question is how is survivin gaining access to the nucleus? Although the endogenous protein is small enough to enter the nucleus by diffusion even if dimerized, this is unlikely given the behavior of the GFP tagged form. Of the known binding partners of survivin, aurora-B has sequences that correspond to NLSs but appear nonfunctional in a nuclear targeting assay, and INCENP has three functional NLSs (3). However, when overexpressed in MCF cells, neither aurora-B nor INCENP was able to influence survivin localization, which remained predominantly cytoplasmic (3). Another candidate for nuclear targeting is TD60, an RCC1-like protein that has a putative NLS and that co-localizes with the chromosomal passenger proteins (23). However, it should be noted that chromosomal passenger proteins have cell cycle-dependent expression, and whether they are present in interphase cells when survivin is overexpressed is unknown.

In conclusion, we have demonstrated that relocating survivin to the nucleus accelerates its degradation and prevents it from protecting cells against irradiation and inhibiting apoptosis. We have also shown that the presence of an NLS on survivin does not affect its mitotic function. Thus sequestering survivin in the nucleus could be very helpful in cancer therapy because it would re sensitize cells to radiation without affecting the proliferation of noncancerous cells.

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