DNA topoisomerase (topo) IIα, an essential enzyme for cell proliferation, is targeted to a proteasome-dependent degradation pathway when human tumor cells are glucose-starved. Here we show that the topo IIα destabilization depends on the newly identified domain, GRDD (glucose-regulated destruction domain), which was mapped to the N-terminal 70–170 amino acid sequence. Indeed, the deletion of GRDD conferred a stable feature on topo IIα, whereas the fusion of GRDD rendered green fluorescent protein unstable under glucose starvation conditions. Nuclear localization was a prerequisite for GRDD function, because the inhibition of nuclear translocation resulted in the suppression of GRDD-mediated topo IIα degradation. Further, GRDD was identified as an interactive domain for Jab1/CSN5, which promoted the degradation of topo IIα in a manner dependent on the MPN (Mpr1p/Prd1p N terminus) domain. Depleting Jab1/CSN5 by antisense oligonucleotide and treating cells with the CSN-associated kinase inhibitor, curcumin, inhibited topo IIα degradation induced by glucose starvation. These findings demonstrate that GRDD can act as a stress-activated degron for regulating topo IIα stability, possibly through interaction with the MPN domain of Jab1/CSN5.

The expression of topo IIα is regulated by the state of cellular proliferation, high in proliferation and low in quiescence with a higher cell density or a lower serum concentration. The levels of topo IIα also change within a single cell cycle, peaking in the G2/M phase and declining to a minimal level after M phase completion. However, the levels of topo IIα are relatively constant throughout the cell cycle and in quiescent and proliferating states (5).

The topo II isoforms have been shown to be the molecular target for such clinically important multitumor drugs such as etoposide and doxorubicin (6, 7). The topo II-directed drugs convert this enzyme into a cellular poison by stabilizing the covalent DNA-enzyme intermediates, the so-called cleavable complex. Interestingly, the increase in topo II-cleavable complexes also has been observed in the presence of a number of physiological stressors such as acidic pH, oxidation, and thiol (8, 9). DNA damage resulting from the cleavable complexes is thought to lead to eventual cell death. Thus, any processes leading to a decrease in the number of cleavable complexes may have a positive impact on cell survival. In the case of topo II-directed drugs, cellular resistance can be achieved by both qualitative and quantitative changes in topo II much more frequently with the α-isofrom. Indeed, the resistance often correlates with a decrease in topo IIα expression levels or with mutations in the protein that reduce enzymatic activity (10). C-terminal truncations of topo IIα, which result in the loss of functional NLSes and thus cytoplasmic localization of the enzyme, also have been found to correlate with resistance to topo II-directed drugs (11, 12).

High levels of topo IIα expression have been shown in a wide variety of tumors, partly due to the increased growth fraction (13). The high topo IIα expression may provide a rationale for the use of topo II-directed drugs in clinical settings. However, most of the common solid tumors exhibit resistance to chemotherapy. Malignant cells within solid tumors are often surrounded by conditions such as glucose deprivation, hypoxia, low pH, and other forms of nutrient deprivation (8, 9, 14). These physiological conditions in culture can down-regulate the expression of topo IIα with growth arrest or delay at the G1 phase of the cell cycle (15). What we found to be consistent with the decreased topo IIα levels was tumor cells becoming resistant to topo II-directed drugs when stressed by glucose starvation and related culture conditions (16, 17). These observations suggest that the stress-mediated down-regulation of topo IIα may be an obstacle to successful topo II-directed chemotherapy.

Recently, we have shown that inhibition of proteasome attenuates stress-induced resistance by inhibiting topo IIα depletion (18). Topo IIα restoration is seen only at the protein levels, indicating that topo IIα protein depletion occurs through a proteasome-mediated degradation mechanism.
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tensive-mediated proteolysis is targeted by ubiquitylation of the substrate proteins (19). Consistently, topo IIα can be conjugated with polyubiquitin in a cell-free system with extracts of cancer cells (20). Thus, the ubiquitin-proteasome system appears responsible for topo IIα degradation under conditions of glucose starvation, but the regulatory mechanisms leading to topo IIα degradation are largely unknown.

In this study, we have attempted to identify the domain of topo IIα responsible for degradation under glucose starvation. We report herein a novel degradation signal designated glucose-regulated destruction domain (GRDD), which presents within the N-terminal ATPase domain. Our current results show that GRDD functions in the nucleus and can lead to proteasome-mediated degradation of heterologous proteins under stress conditions. We also demonstrate that Jab1/CSN5 binds to and promotes degradation of topo IIα in a GRDD-dependent manner. Consistent with this finding was that specific inhibition of Jab1/CSN5 leads to the stabilization of endogenous topo IIα under stress conditions.

EXPERIMENTAL PROCEDURES

Cell Culture and Treatments—HT1080 and MCF-7 cells were maintained in RPMI 1640 medium (Nissui, Tokyo, Japan), and 283T cells were maintained in Dulbecco’s modified Eagle’s medium (Nissui), each supplemented with 10% heat-inactivated fetal bovine serum and 100 μg of kanamycin/ml. The cells were cultured at 37°C in a humidified atmosphere containing 5% CO2. Glucose deprivation was achieved by substituting glucose-free RPMI 1640 medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum, PST (carbenebonyl-isoleucyl-gamma-t-buty1-glutamy1-t-alany1-t-leucineul) and MG132 were purchased from the Peptide Institute Inc. (Osaka, Japan). Lactacystin and curcinin were from Kyowa Medex (Tokyo, Japan) and Sigma, respectively. These compounds were dissolved in DMSO and added to culture medium so that the final concentration of MeSO was <0.5%. All of the experiments were repeated at least three times using exponentially growing cells.

Expression Plasmids—For expression plasmid construction of full-length topo IIα and its deletion mutants from N or C terminus, the pBlueScript SK+ plasmid containing full-length human topo IIα cDNA (a generous gift from Dr. James C. Wang, Harvard University) (21) was used as a template for PCR amplification with Prime DNA polymerase (Stratagene, La Jolla, CA). To facilitate subcloning, a ClaI and a SalI restriction site was introduced at the 5′- and 3′-ends, respectively. The PCR products first were cloned into the pCR-Script Amp vector (Stratagene) at the ClaI and SalI sites. The sequence corresponding to amino acids 1–1532 was removed from pFLAG-WT topo IIα by BglII/SalI digestion and replaced with a PCR fragment corresponding to amino acids 1288–1532. Further internal deletions within amino acids 1–713 were performed by replacing the sequence between the ClaI and BglII sites of pFLAG-J714–1287 topo IIα with each corresponding PCR fragment. Similarly, the expression plasmids of GFP-NLS2 fusion proteins were constructed by either replacing with or by inserting a GFP gene derived from the pCMV/Myc/nuc/GFP vector (Invitrogen). pFLAG-ND70–170 topo IIα was constructed using two PCR fragments (corresponding to amino acids 1–69 and 161–1532) that were introduced into the NotI-ClaI and ClaI-SalI sites at the 5′- and 3′-ends, respectively. Site-directed mutagenesis including single amino acid substitution, double amino acid substitution, and deletion up to 10 amino acids was carried out using a QuikChange mutagenesis kit (Stratagene).

The pHA expression vector derived from pDNA3 (Invitrogen) was described previously (22). Full-length human Jab1/CSN5 cDNA was generated by PCR from a cDNA library of HT29 cells. The PCR fragment was cloned into the pcHA expression vector at the BamHI/NotI site. A PCR fragment corresponding to amino acids 1–190 of Jab1/CSN5 was also cloned into the pHA vector at the BamHI/NotI site. The plasmid construction of ΔMPN Jab1/CSN5 was carried out using two PCR fragments (corresponding to amino acids 1–54 and 191–344) that were introduced into BamHI-NotI and NotI-XbaI sites at the 5′- and 3′-ends, respectively. The pDNA3-GFP was digested enzymatically (23). The proper construction of all of the plasmids was confirmed by DNA sequencing. All of the plasmids for transfection were prepared using an EndoFree Plasmid Maxi Kit (Qiagen, Tokyo, Japan).

Transfection—Transfections were performed using FuGENE 6 transfection reagent (Roche Applied Science) according to the manufacturer’s protocol with minor modifications. HT1080 cells were seeded at 5 × 104 cells/well in a six-well plate 16 h before transfection. After changing to 2 ml of fresh culture medium, 200 μl of transfection mixture containing 2 μg of plasmid DNA and 12 μl of FuGENE 6 reagent in 188 μl of Opti-MEM (Invitrogen) was added to each well and the cells were incubated for 8 h. The cells were washed with phosphate-buffered saline and further incubated for 24 h in fresh medium. After transfection, the cells were cultured in normal or glucose-free culture medium for the indicated periods of time.

Preparation of Nuclear and Cytoplasmic Extracts—Nuclear and cytoplasmic extracts of HT1080 cells were prepared using NE-PER™ nuclear and cytoplasmic extraction reagents (Pierce) according to the manufacturer’s protocol.

Western Blot Analysis—Cells were washed with cold phosphate-buffered saline and lysed in 1× SDS sample buffer (10% glycerol, 5% 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl, pH 6.8) as described previously (17). Protein content of the samples was determined using a Bio-Rad protein assay reagent. Equal amounts of samples were resolved by SDS-PAGE and electrophoretized onto a nitrocellulose membrane (Schleicher & Schuell). Membranes were probed with mouse monoclonal anti-human topo IIα clone (clone KP4, Sigma Genosys), anti-FLAG M2 (Sigma), anti-Jab1/CSN5 (GeneTex, Inc), polyclonal anti-GFP (Clontech, Palo Alto, CA), anti-actin (c-2) horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA), anti-Signalosome subunit CSN1, CSN2, CSN5 (INTI Research), and anti-GRP78 horseradish peroxidase antibodies (BD Transduction Laboratories). The specific signals were detected using an enhanced chemiluminescence detection system (Amersham Biosciences).

Immunofluorescence Microscopy—Cells grown on a 35-mm glass-bottom culture dish with a polylysine-coated glass (MatTek, Ashland, MA) were fixed in 4% formaldehyde for 10 min and permeabilized with methanol for 2 min. The fixed cells were processed for immunostaining with anti-FLAG M2 antibody (1:100 dilution) followed by a fluorescein isothiocyanate-conjugated anti-mouse secondary antibody (1:1000 dilution). The nuclei were counterstained using 0.1 μg/ml 4′,6-diamino-2-phenylindole. The cells were observed using a fluorescence microscope (Olympus IX-70) equipped with a CCD camera (Sony). The imaging system was commercially available from Clontech. The coding region of the N-terminal (aa 1–200 and 1–584) of topo IIα protein was fused in-frame with the GAL4 DNA-binding domain of the pGBK7 vector (Clontech). The resulting bait plasmids were used to screen a human fetal brain cDNA library (Clontech) according to the manufacturer’s protocol. FLAG-tagged topo IIα proteins were expressed in 293T cells by transfection with SuperFect™ reagent (QiaGen). The FLAG-tagged topo IIα proteins were immunoprecipitated using anti-FLAG M2 affinity gel (Sigma) and eluted from the resin with a FLAG peptide according to the manufacturer’s instructions. The immunopurified FLAG-tagged topo IIα proteins (100 ng) were added directly to GST-Jab1/CSN5 fusion proteins immobilized on the GSH beads (100 ng). The mixtures were incubated overnight at 4 °C in a total volume of 1.5 ml of a binding buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA, and 1% Triton X-100. After washing three times with the binding buffer, the protein complex was eluted with SDS-PAGE and subsequent Western blot using anti-FLAG M2 and anti-GST antibodies (Amersham Biosciences).

Antisense Morpholino Oligomers—A morpholino antisense oligomer specific for human Jab1/CSN5 as well as a standard control oligomer was purchased from Gene Tools (Philomath, OR). The sequences were 5′-GGCCGCCATCATCGGAGG-3′ and 5′-CCTTCTACTCTTACGTTACATTTATA-3′ for the control. Antisense treatments were performed according to the manufacturer’s “special delivery protocol.” HT1080 and MCF-7 cells were seeded at 6 × 105 and 9 × 105 cells/well, respectively, in a six-well plate 16 h before antisense treatment. Antisense mixtures with the ethoxylated polyethyleneamine (PEI)-DDM10GAGA-2 were added to the cultures, and cells were incubated for 3 h (HT1080) or 6 h (MCF-7). The cells were washed once with phosphate-buffered saline and cultured in normal or glucose-free medium for 18 (HT1080) or 24 h (MCF-7), and the whole cell lysates were prepared for analyzing expression of Jab1/CSN5 and topo IIα.
RESULTS

ATPase Domain and NLS Are Indispensable for Stress-induced Degradation of Topo IIα—When cells are cultured under the stress condition of glucose starvation, topo IIα is degraded in a proteasome-dependent manner. Indeed, degradation of topo IIα, but not topo IIβ, was observed in glucose-starved HT1080 cells (Fig. 1A). Degradation of topo IIα was suppressed by proteasome inhibitor PSI at 3 μM but not by calpain inhibitor, carboxenxyl-leucyl-leucinal (Z-LL-H), at 50 μM. Likewise, wild-type topo IIα tagged with the FLAG epitope at the N terminus was degraded during glucose starvation when it was expressed transiently in HT1080 cells by transfection (Fig. 1C).

To determine which regions of topo IIα were required for the degradation, we constructed a panel of expression plasmids of topo IIα deletion mutants (Fig. 1B) and transfected them into HT1080 cells. Glucose starvation-induced degradation was monitored by Western blot analysis using the anti-topo IIα C terminus and anti-FLAG M2 antibodies (Fig. 1C). Deletion mutants from the N terminus (Δ1–675, Δ1–1007, and Δ1–1287) revealed that the deletion of the ATPase domain led to degradation resistance under glucose starvation conditions. Deletion of the NLS2 region (ΔNLS2) from the C terminus also resulted in stabilization under the same conditions. In contrast, the Δ714–1287 mutant containing the ATPase domain and the NLS2 region was destabilized by glucose starvation, as was wild-type topo IIα. We also found that deletion mutants showed some differences in expression under the normal growth conditions. Although the exact reason is not known, we speculate that these differences are probably a result of alterations in the basal stability of each mutant topo IIα protein.

Subcellular Localization of Topo IIα Deletion Mutants—Immunostaining with anti-FLAG antibody demonstrated that wild-type topo IIα localized in the nuclei of HT1080 cells in a predominantly diffusing manner (Fig. 2A). The Δ714–1287 mutant topo IIα, which was sensitive to the stress-induced degradation, also localized in the nucleus, and the pattern of immunostaining was similar between wild-type and Δ714–1287 mutant topo IIα. In contrast, ΔNLS2 topo IIα, which was resistant to the stress-induced degradation, accumulated mainly in the cytoplasm. A cell fractionation experiment revealed that ΔNLS2 topo IIα was more abundant in the cytoplasmic fractions than the wild type topo IIα (Fig. 2B). The action was in agreement with the fact that the NLS2 sequence is the major functional motif for nuclear translocation of topo IIα (24, 25).
together, these results indicated that nuclear localization of topo IIα was a prerequisite for degradation of this enzyme under glucose starvation conditions. Consistent with these findings, endogenous topo IIα remained in the nucleus of stressed cells when degradation of topo IIα was inhibited by proteasome inhibitors (Fig. 2C).

**Analysis of Topo IIα ATPase Domain for Stress-induced Degradation**—The above results suggested that the sequence within the ATPase domain could affect topo IIα stability under glucose starvation stress conditions. To determine the critical region, we constructed deletion mutants within the ATPase domain that were fused with the NLS2 domain for nuclear localization. Stress-induced degradation of the fusion proteins was monitored as described above. As shown in Fig. 3A, aa 1–584, 1–201, 1–170, and 1–140 of ATPase-NLS2 fusion proteins were destabilized by glucose starvation but the aa 1–130 fusion protein remained stable compared with the others. We further examined the N-terminal deletions of the aa

**Fig. 2.** Subcellular localization of wild-type and mutant topo IIα proteins. A, HT1080 cells were transfected with expression plasmids of FLAG-tagged topo IIα proteins as indicated. Twenty h after transfection, the immunofluorescence staining was carried out with anti-FLAG antibody (upper) and 4,6-diamidino-2-phenylindole (DAPI, lower). Merged images (middle) are shown. B, HT1080 cells were transfected with expression plasmids of FLAG-tagged topo IIα proteins as indicated and were cultured for 20 h under normal (+) or glucose starvation conditions (−). Nuclear (Nu) and cytoplasmic (Cy) extracts from the transfected cells were isolated, and the protein levels were determined by immunoblot analysis using anti-FLAG, anti-actin (c-2) horseradish peroxidase, and anti-GRP78 horseradish peroxidase antibodies. Actin and GRP78 protein levels also were determined for a loading control. C, HT1080 cells were cultured for 20 h under normal (+) or glucose starvation conditions (−) and were treated with MG132 or lactacystin during the last 6 h. The protein levels of topo IIα of nuclear (Nu) and cytoplasmic (Cy) extracts were determined by immunoblot analysis using anti-topo IIα C terminus antibody.

**Fig. 3.** Effect of deletion within topo IIα ATPase domain on stress-induced degradation. A, deletion mutants of topo IIα were shown schematically (left), the C-terminal deletion mutants within topo IIα ATPase domain that are fused with the NLS2 domain. B, N-terminal deletion mutants of aa 1–170 ATPase-NLS2 fusion. C, full-length topo IIα proteins. Expression plasmids of the FLAG-tagged topo IIα proteins were transfected into HT1080 cells (A and C). In B, HT1080 cells were co-transfected with the expression plasmid of N-terminal deletion mutants of aa 1–170 ATPase-NLS2 fusion and GFP proteins. After a 20-h culture under glucose-free conditions (−), the expression of each topo IIα protein was examined by immunoblot analysis using anti-topo IIα C terminus and anti-FLAG antibodies (right) as indicated. In B, GFP expression levels also were determined by anti-GFP antibody for loading control.
1–170 ATPase-NLS2 fusion protein by co-transfection with the GFP protein (Fig. 3B). Although stress-induced destabilization still occurred when 1–42 (ΔN1–42) and 1–69 (ΔN1–69) residues were deleted, the 1–124 deletion (ΔN1–124) led to degradation resistance. The N-terminal deletions also were applied to full-length topo IIα (Δ42, Δ69, and Δ124), and essentially, the same results were obtained with regard to the stability under glucose starvation conditions (Fig. 3C).

Interestingly, the 1–69 deletion, both in full-length topo IIα and in the aa 1–170 ATPase-NLS2 fusion protein, decreased its expression level under normal growth conditions as compared with the 1–42 deletion (Fig. 3B and C). The 1–42 deletion itself had little effect on the expression levels under normal conditions (data not shown). Conversely, the 1–124 deletion greatly increased the expression levels in the aa 1–170 ATPase-NLS2 fusion protein, although it had only a marginal effect on the full-length topo IIα expression under normal conditions (Fig. 3B and C). These results indicate that the N-terminal regions can affect the stability of topo IIα under normal growth conditions.

Identification of GRDD, a Novel Transposable Degradation Signal—This deletion analysis implied that the minimal region necessary for the stress-induced degradation was located between 70 and 140 amino acid residues of topo IIα ATPase domain. Indeed, topo IIα became resistant to the stress-induced degradation when the 70–170 residues were deleted (Fig. 4A). It is important to note that ND70–170 of topo IIα localized in the nucleus (see Fig. 2). We designated the 70–170 region of topo IIα as GRDD. To evaluate the GRDD of topo IIα as a stress-specific degradation signal, we constructed GFp fusion proteins that were fused with topo IIα 1–170 or 70–170 residues at the N terminus of topo IIα-NLS2 domain at the C terminus for nuclear localization (Fig. 4B). The GFP-NLS2 fusion protein (not containing GRDD) was expressed at similar levels under normal and glucose starvation conditions, showing that this fusion protein was insensitive to the mechanism (or mechanisms) of the stress-induced topo IIα degradation. By adding GRDD to GFP-NLS2 (aa 1–170 and 70–170), we found that the protein became vulnerable to degradation under glucose starvation conditions as was observed for topo IIα. Thus, the GRDD of topo IIα can act as a transposable stress-specific degradation signal.
Topo IIα has a destruction box-like sequence that is located within GRDD (26). In fact, the amino acid sequence, ENNLISIWN, located between 112 and 120 of topo IIα is similar to the consensus sequence of the cyclin B-type destruction box, RXXLXXIXN. To examine the contribution of the destruction box-like sequence, we used Δ714–1287 topo IIα, which consisted of the entire ATPase domain and the NLS2 region (Fig. 1). The deletion of amino acids 112–120 (ΔDB) within the GRDD of topo IIα had no effect on stress-induced degradation of Δ714–1287 topo IIα (Fig. 4C). We concluded that the destruction box-like sequence in GRDD is dispensable for topo IIα degradation under glucose starvation conditions.

**GRDD-dependent Topo IIα Degradation by Jab1/CSN5**—Jab1/CSN5 was identified as a candidate protein that interacts with GRDD by a yeast two-hybrid screen using the topo IIα ATPase domain (aa 1–200 and 1–584) as bait. To confirm a physical interaction between topo IIα and Jab1/CSN5, we tested the ability of the GST-Jab1/CSN5 fusion protein to bind to immunopurified FLAG-tagged topo IIα. Full-length topo IIα was captured by the GST-Jab1/CSN5 but not by the GST polypeptide alone (Fig. 5A). The in vitro binding between topo IIα and Jab1/CSN5 was dependent on GRDD of topo IIα. Indeed, an 1–584 ATPase-NLS2 and ΔNLS2 topo IIα, which contained GRDD, were captured by the GST-Jab1/CSN5. However, ND70–170 topo IIα and aa 125–170 ATPase-NLS2, which lacked the complete GRDD, were not captured by the GST-Jab1/CSN5 (Fig. 5A).

To determine whether Jab1/CSN5 modulates stability of topo IIα in vitro, we examined the expression levels of FLAG-tagged topo IIα and its deletion mutants in HT1080 cells co-expressing HA-tagged Jab1/CSN5 (Fig. 5, B and C). Similar to glucose starvation, co-expression of Jab1/CSN5 induced the degradation of wild-type topo IIα and aa 1–584 ATPase-NLS2, which interacted physically with Jab1/CSN5 in vitro. However, Jab1/CSN5 as well as glucose starvation did not affect the expression levels of ND70–170 topo IIα, which lacked Jab1/CSN5 binding ability. The combination of glucose starvation and Jab1/CSN5 co-expression had no additive or synergistic effect on the topo IIα expression, and the two treatments did not seem to affect each other under the conditions examined. The Jab1/CSN5-induced decrease in aa 1–584 ATPase-NLS2 expression was suppressed by proteasome inhibitors PSI, MG132, and lactacystin (Fig. 5C). Thus, similar to glucose starvation, Jab1/CSN5 can stimulate proteasome-mediated degradation of topo IIα, and this stimulation occurs in a Jab1/CSN5-topo IIα binding-dependent manner. The Jab1/CSN5-stimulated degradation of topo IIα also was suppressed by the CSN kinase inhibitor, curcumin (Fig. 5C).

We further examined deletion mutants of Jab1/CSN5 to establish the relationship between degradation and Jab1/CSN5 binding of topo IIα. Jab1/CSN5 has a functional domain known as the MPN domain. Co-expression of ΔMPN Jab1/CSN5 had little effect on degradation of the aa 1–584 ATPase-NLS2 of topo IIα, whereas co-expression of ΔC Jab1/CSN5 containing the entire MPN domain down-regulated it similar to full-length Jab1/CSN5 (Fig. 6A). Consistent with this finding was that GST–ΔC Jab1/CSN5, but not ΔMPN, interacted physically with full-length topo IIα in vitro (Fig. 6B). These results suggested that Jab1/CSN5 stimulated the degradation of topo IIα via direct binding in a GRDD- and MPN-dependent manner.

**Involvement of Jab1/CSN5 in Stress-induced Topo IIα Degradation**—To address whether Jab1/CSN5 mediates degradation of endogenous topo IIα under glucose starvation, we examined the effects of curcumin and Jab1/CSN5 antisense morpholino oligomers. As shown in Fig. 7A, curcumin inhibited stress-induced degradation of topo IIα in HT1080 cells as effectively as the proteasome inhibitors, PSI, MG132, and lactacystin. Curcumin also inhibited topo IIα degradation in MCF-7 cells, and interestingly, it caused a decreased expression of Jab1/CSN5 (Fig. 7C). Applying a Jab1/CSN5 antisense morpholino oligomer, but not scramble control, decreased cellular contents of Jab1/CSN5 by ~65% in both HT1080 and MCF-7 cells without affecting those of a different signalosome subunit, CSN1 or CSN8 (Fig. 7, B and C). The Jab1/CSN5 declined as a result of antisense suppressed stress-induced topo IIα degradation. These results indicated that Jab1/CSN5 is involved in topo IIα degradation under glucose starvation conditions.

**DISCUSSION**

Under the physiological cell conditions of glucose starvation, topo IIα is degraded by the proteasome system. In this study, we identified GRDD as the degradation signal that was located between 70 and 170 amino acid residues in topo IIα. In the presence of the topo IIα NLS2 region for nuclear translocation, GRDD gave the vulnerability to degradation under glucose starvation to GFP as well as various deletion mutants of topo IIα. On the other hand, ΔNLS2 topo IIα did not localize in the nucleus and was not subjected to stress-induced degradation.
even though it contained the entire GRDD region. These results demonstrated that nuclear localization is a prerequisite for GRDD to act as a degradation signal. In addition, biochemical fractionation analysis showed that topo IIα still existed in the nucleus when the stress-induced degradation was inhibited by a proteasome inhibitor (Fig. 2C). Therefore, it is probable that the stress-induced topo IIα degradation occurs in the nucleus. Supporting this notion are previous observations that glucose starvation causes the nuclear accumulation of pro- teosomes, which can affect the efficiency of decreasing topo IIα expression under the stress conditions (27). Our present results suggest that GRDD could be protected in a normal conformation of topo IIα, possibly by the N-terminal region adjacent to GRDD, because the deletion decreases stability of topo IIα under normal growth conditions (Fig. 3C). Taken together, we concluded that GRDD of topo IIα is a stress-activated degron acting in the nucleus.

We also demonstrated that Jab1/CSN5 mediates topo IIα degradation under glucose starvation. So far, Jab1/CSN5 has been shown to interact with a number of proteins (28). The consequence of the Jab1/CSN5 interaction varies but can be generalized to change protein stability depending on the binding partner proteins. In the case of topo IIα, Jab1/CSN5 can promote proteasome-mediated degradation in a binding-dependent manner. Indeed, the ectopic expression of Jab1/CSN5 was sufficient to induce degradation of exogenously co-expressed topo IIα. This Jab1/CSN5-induced degradation of topo IIα was dependent on GRDD and was inhibitable by proteasome inhibitors as we observed for topo IIα degradation under glucose starvation conditions. In addition, we identified GRDD as an element necessary for physical interaction with Jab1/CSN5 in vitro. However, the binding between Jab1/CSN5 and topo IIα in vivo has not been detected by immunoprecipitation and subsequent immunoblotting (data not shown). Presumably, this was attributed to a transient feature of the in vivo interaction, leading to degradation of topo IIα. At present, the regulatory mechanisms of topo IIα degradation mediated by Jab1/CSN5 remain to be determined. Two major mechanisms of Jab1/CSN5 leading to proteolysis have been described: 1) inducing intracellular redistribution (e.g. cyclin-dependent-kinase inhibitor p27Kip1) and 2) phosphorylation (e.g. tumor suppressor p53).

In the case of p27Kip1, the interaction with Jab1/CSN5 promotes the export of p27Kip1 from the nucleus to the cytoplasm and enhances its degradation by a proteasome (29). The cytoplasmic shuttling of p27Kip1 was shown recently to occur through the nuclear export signal located between 233–242 amino acids of Jab1/CSN5 (30). We noted with great interest that a Jab1/CSN5 deletion mutant that lacked the C-terminal region (amino acids 199–334) containing the nuclear export signal lost the ability to induce p27Kip1 degradation. This observation is quite different from our present finding that AC Jab1/CSN5, which lacks amino acids 191–334, retained the ability to promote topo IIα degradation (Fig. 6). Therefore, it is unlikely that the nuclear export activity of Jab1/CSN5 is involved in topo IIα degradation, further supporting the notion that topo IIα degradation occurs in the nucleus.

Phosphorylation via interaction with Jab1/CSN5 can be mediated by a Ser/Thr kinase known as the CSN-associated kinase that is co-purified with the CSN complex localizing in the nucleus (31–34). In the case of p53, which also binds to Jab1/CSN5 in vitro, CSN-mediated phosphorylation promotes its proteasome-dependent degradation (35). Indeed, the inhibition of the CSN-associated kinase by curcumin or a competitor peptide derived from p53 results in the accumulation of endogenous p53. Consistent with this finding is that topo IIα degradation also is inhibited by curcumin (Figs. 5C and 7), suggesting that the CSN-mediated phosphorylation plays a role in targeting topo IIα for degradation. In fact, topo IIα is a phosphoprotein, and the phosphorylation is involved in the regulation of the activity of this enzyme (4). So far, multiple Ser/Thr phosphorylation sites have been identified, although most of them exist in the C-terminal domain far from the GRDD region. Only one site, Ser-29, has been identified as phosphoryl-
directly. Therefore, it is possible that Jab1/CSN5 functions as ubiquitin ligase complexes (39). These reports indicate that the radiation of topo II abrogated (15, 18). Thus, the GRDD- and MPN-dependent degradation under glucose starvation has been observed in a variety of solid tumor lines, even in cells where the cell cycle-dependent regulation is impaired. Further studies will be needed to elucidate the mechanisms of topo IIα degradation under severe stress conditions. Meanwhile, topo IIα expression in solid tumors. Indeed, microenvironm ental stressors, such as glucose starvation and hypoxia, are not observed in normal tissues, and the Jab1/CSN5-mediated topo IIα degradation pathway appears to be selective in stressed cells. Further studies on the mechanisms of how Jab1/CSN5 promotes topo IIα degradation would provide selective strategies to enhance topoII-directed chemotherapy against solid tumors.

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