DNA topoisomerase (topo I) is an essential nuclear protein and a target for anticancer drug camptothecin derivatives. As a nuclear protein, topo I is concentrated in the nucleolus. However, this nucleolar distribution of topo I is dynamic. It has been shown recently that topo I rapidly moves out of the nucleolus (nucleolar delocalization) in response to topo I inhibitors. In the present study, we demonstrated that nucleolar delocalization of topo I is associated with its conjugation by SUMOs (small ubiquitin-like modifiers) in response to the topo I inhibitor topotecan. Time-course experiments revealed that SUMO-topo I conjugation occurred at as early as 5 min after drug treatment, which was earlier than its observed nucleolar delocalization. Furthermore, heat shock blocked sumoylation of topo I; it also blocked the nucleolar delocalization of topo I fusion proteins. UBC9 is an E2 (ubiquitin carrier protein)-conjugating enzyme essential for sumoylation. Although overexpression of wild-type UBC9 enhanced both sumoylation and nucleolar delocalization of topo I, overexpression of a UBC9 dominant negative mutant attenuated topo I sumoylation and its nucleolar delocalization. Taken together, our results suggest that sumoylation of topo I might serve as an addressing tag for its nucleolar delocalization in response to topo I inhibitors.

DNA topoisomerase I (topo I) is a nuclear enzyme that plays an important role in relieving the torsional strains of DNA resulting from transcription and replication (1). Moreover, the enzyme is a target for such anticancer agents as camptothecin and its clinically important derivatives topotecan (TPT) and irinotecan. Camptothecin drugs are believed to interact with the enzyme to stabilize transient covalent enzyme-DNA complexes (2). After treatment of cells with these agents, single-strand DNA breaks are formed initially, and they are converted to double-strand breaks during DNA replication, which are the lethal signals leading to cell death through apoptosis (2).

Mammalian topo I consists of three functionally distinct domains; they are the N-terminal, core, and catalytic C-terminal domains (3, 4). Although the N-terminal domain is not required for the catalytic activity of the enzyme, it is essential for topo I to function in vivo (5) because some critical nuclear/nucleolar localization signals reside in this region (5). As a nuclear protein, topo I is concentrated in the nucleolus, a site of rRNA synthesis (6). We have shown that amino acids 188–198 at the N terminus of human topo I are important for its nucleolar localization (7). However, the nucleolar distribution of topo I is dynamic because the nucleolus is a non-membrane structure (8), and its formation is dependent upon cell growth (8, 9). For instance, topo I tends to accumulate in the nucleolus of proliferating cells, whereas in leucine-deprived cells, it does not (9). Because the nucleolus is a site for rRNA biogenesis, there appears to be a correlation between the nucleolar accumulation of topo I and the activity of RNA synthesis. Moreover, the notion of this dynamic change of nucleolar distribution of topo I is also supported by the findings that some cytotoxic agents can cause redistribution of topo I in the nucleus (10, 11). Upon treatment with topo I inhibitors or other RNA synthesis inhibitors, topo I has been shown to move out of the nucleolus rapidly (nucleolar delocalization), similar to what has been seen for other nucleolar proteins such as poly(ADP-ribose) polymerase and B23 in response to RNA synthesis inhibitors (11, 12).

Although it has been suggested that this nucleolar delocalization might be related to reduced activity of rRNA biosynthesis in the nucleolus (11), the molecular event(s) that leads to nucleolar delocalization of topo I is not clear.

SUMOs are small ubiquitin-like modifiers that have been shown to form conjugates with the targeted proteins (13–15), a process that is known as sumoylation. UBC9 is an E2 enzyme that has been shown to be essential for sumoylation. Like ubiquitin, SUMOs form a covalent bond at a lysine residue. However, unlike ubiquitination, which leads to degradation of conjugated proteins, sumoylation seems to stabilize the targeted proteins (16, 17). Because the early finding that the RanGAP1, which interacts with nuclear pore complexes, is sumoylated (13), a large number of nuclear proteins have been shown to be sumoylated (18–24). Although the role of sumoylation is not fully understood, two functions for sumoylation have been suggested; they are 1) competing with ubiquitination for protein degradation and 2) serving as an addressing tag to target proteins such that it modulates their functions or activities (25). Of interest, topo I has been shown recently to be sumoylated in response to topo I inhibitors, and it has been proposed that sumoylation of topo I is a possible rescuing mechanism from DNA damage (26). However, it is not clear whether sumoylation of topo I has any impact on its subnuclear distribution. Accordingly, in the present study, we examined the relationship between sumoylation of topo I and its nucleo-
lar delocalization. Our results suggest that sumoylation of topo I might serve as addressing tag for its nucleolar delocalization.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—HeLa cells were obtained from American Type Cell Collection (ATCC, Manassas, VA) and grown in Dulbecco's modified Eagle's medium (BioWhittaker, Walkersville, MD). CEM and CEM/B1 and CEM/VMI–5 cells were grown in Spinner's minimal essential medium (BioWhittaker) as described previously (27). All media were supplemented with 10% fetal bovine serum, 100 units of penicillin/ml, and 100 μg of streptomycin/ml. Cells were incubated at 37 °C in a humidified chamber supplemented with 5% CO₂.

**Transfection**—Plasmids carrying the EGFP-topo I gene were introduced into HeLa cells by the calcium phosphate method as described previously (27). Transiently transfected cells were subcultured into 6-well plates with a coverslip per well and allowed to attach and grow overnight before examination under a microscope. Establishment of stably transfected cells was performed by co-transfection with pTK-Hg and selected in the presence of 200 μg of hygromycin/ml (Invitrogen).

**Plasmid Construction**—The EGFP-topo I fusion construct (pTI-5) has been described previously (27). pTI-42 carries the N-terminal domain of human topo I (202 amino acids) fused to DsRed protein (pDsRED-N1, CLONTECH, Palo Alto, CA) and was constructed as follows. The N-terminal 606 base pairs of topo I was first amplified by PCR using the sense primer I-5.1N GAATTCGCCACCATGGTGGGGGACCAC-CTGCACAAC, in which the Kozak sequence (28) was introduced in sense primer I-5.1N GAATTCGCCACCATGGTGGGGGACCAC-CTGCACAAC, in which the Kozak sequence (28) was introduced in

**Treatment of Cells with TPT**—To detect sumoylation of topo I, cells were treated with TPT for 15 min. Treatment of cells with TPT for 30 min before examination under a fluorescence microscope.

**Fluorescence and Confocal Microscopy**—Immunostaining data for the endogenous topo I. This nucleolar localization of topo I because they mimic the endogenous topo I at different stages of the cell cycle. Among various constructs we have made in this laboratory, pTI-5 displayed the most intensive nucleolar localization (Fig. 1). To confirm that this nucleolar localization of EGFP-topo I fusion protein correlates with that of the endogenous topo I, we immunostained the cells with topo I-specific antibody. The nucleolar localization of topo I was essentially identical to that of EGFP-topo I fusion protein (Fig. 1), although some small dots (nuclear body-like structures) were seen in a better resolution in pTI-5-transfected cells. In addition to pTI-5, we made pTI-42, which carries the same topo I fragment as in pTI-5 but fused with pDsRED at the N terminus. Fluorescence microscopy revealed that both pTI-5 and pTI-42 resulted in an identical nucleolar localization pattern of the fusion proteins (Fig. 1).

Because it has been previously shown that nucleolar topo I moved out of the nucleolus upon treatment of topo I inhibitors or RNA synthesis inhibitors (10, 11), we tested whether the fusion protein behaves as the endogenous topo I for its subnuclear redistribution in response to TPT. Sixteen to 24 h after transfection with pTI-5, the HeLa cells were treated with 10 μM TPT for 30 min. As shown in Fig. 1, most of the fusion protein moved out of the nucleolus, which is consistent with our immunostaining data for the endogenous topo I. This nucleolar delocalization seems to be specific for topo I inhibitors because no nucleolar delocalization of topo I was seen for the cells treated with an equitoxic concentration of VM-26, a topo II inhibitor (27). Interestingly, the fusion protein seemed to yield a better resolution than the immuno-staining because in TPT-transfected cells, nuclear body-like structures were clearly seen (Fig. 1). In addition, consistent with the results for pTI-5, we found that the red fusion protein of pTI-42 revealed a similar trend of subnuclear redistribution in response to TPT (Fig. 1). These results indicate that, like the endogenous topo I, EGFP-topo I or DsRED-topo I fusion protein also moves out of the nucleolus after treatment with TPT.

**Sumoylation of Topo I in Tumor Cell Lines**—It has been

![Fig. 1. Effect of TPT on the subnuclear localization of endogenous topo I, EGFP- and DsRED-topo I fusion proteins in HeLa cells. Immunofluorescence staining was carried out as follows. Nontransfected HeLa cells were treated with 10 μM TPT for 30 min, after which they were fixed and probed with topo I-specific antibody from scleroderma patients (top panel). For transient transfection, pTI-5 and pTI-42 were separately introduced into HeLa cells by the calcium phosphate method described under “Experimental Procedures.” The cells were then treated with 10 μM TPT for 30 min before examination under a fluorescence microscope. The fusion proteins mimic the endogenous topo I protein in terms of subnuclear localization.](image-url)
were treated with 10 μM TPT for 15 min, and total protein was extracted as described under "Experimental Procedures." A, detection of sumoylation of topo I in HeLa cells. Topo I-specific antibody (TI-1) or anti-SUMO antibody was used as indicated. B, detection of sumoylation of topo I in CEM and its drug-resistant sublines CEM/B1 and CEM/VM1–5. The topo I-specific antibody (TI-1) was used.

shown that topo I is conjugated by SUMO-1 upon treatment with the topo I inhibitor camptothecin (26). Because SUMO-1 binds to many other nuclear proteins such as PML, implying a role for sumoylation in subnuclear distribution of proteins, we sought to determine the relationship between sumoylation of topo I and nucleolar delocalization of the enzyme. First we examined the effect of TPT treatment on sumoylation of topo I in HeLa cells. Western blot analysis detected up to five topo I-specific bands from the HeLa protein extract (Fig. 2 in HeLa cells. Western blot analysis detected up to five topo I-specific bands (Fig. 2A, left panel). These high molecular mass bands (>100 kDa) were SUMO-conjugated because they were recognized by SUMO-1 specific antibody (Fig. 2A, right panel). Next we examined the sumoylation of topo I in other tumor cell lines under the same conditions. Like HeLa cells, T-lineage leukemic CEM cells showed identical multiple topo I-specific bands (Fig. 2B). CEM/VM1–5 was derived from CEM cells by cloning after gradual exposure to increasing concentrations of VM-16 and was 140-fold more resistant to VM-16 than CEM cells (31). However, similar multiple bands with the same intensity were observed (Fig. 2B). CEM/B1 cells were derived from CEM cells by selection of resistance to merbarone, a catalytic topo II inhibitor, but are moderately cross-resistant to topo I inhibitors (32). Although the topo I-specific band patterns in CEM and CEM/B1 are identical, the intensity of the bands in CEM/B1 cells was slightly lower than that of CEM cells, possibly because of its low level of resistance to TPT. Nevertheless, the topo I-specific band pattern is similar among all cell lines tested. Thus, it appears that sumoylation of topo I is a general phenomenon in response to TPT.

Temporal Changes of Sumoylation of Topo I and Nucleolar Delocalization of EGFP-topo I—One of roles for sumoylation has been suggested to serve as an addressing tag for targeted proteins (25). We reasoned that if topo I sumoylation is a tag for its nucleolar delocalization, then sumoylation of topo I should occur earlier than its nucleolar delocalization. Thus, we sought to determine temporal changes of sumoylation of topo I. As shown in Fig. 3A, within as little as a 5-min treatment of TPT, multiple topo I-specific bands were detected. At 10 min, the intensity of sumoylated topo I increased and reached a plateau by 15 min. After 30 min, the intensity of the sumoylated topo I bands started to decrease. Thus, sumoylated topo I seems to be unstable, and sumoylation of topo I is an early event in response to TPT. To determine whether removal of the drug has any effect on the sumoylated topo I, we washed drug-treated cells and incubated them in the absence of the drug for 1 h. Most of sumoylated topo I bands disappeared (data not shown), suggesting that sumoylation of topo I is reversible.

We then examined subnuclear changes of the EGFP-topo I fusion protein (pTI-5) at different time points. For comparison, we treated pTI-5-transfected HeLa cells with the same concentration of TPT (10 μM). We found that although initial nucleolar delocalization of EGFP-topo I fusion protein was seen at 15 min of treatment (Fig. 3B), substantial subnuclear redistribution of EGFP-topo I was not seen until 30 min of treatment. By 45 min, the majority of nucleolar fusion protein moved out of the nucleolus. Therefore, the results clearly indicate that nuclear delocalization of EGFP-topo I occurs later than sumoylation of topo I.

Interestingly, although the nucleolar fusion protein disappeared from the nucleolus during the course of drug treatment, we noticed that the intensity of the fluorescent small particles seemed to increase during this period of treatment (Fig. 3B). Therefore, we followed the subnuclear redistribution of EGFP-topo I for a longer period of time. As shown in Fig. 4B, the increase in intensity of the fluorescent particles persisted through from 1 to 7 h of drug treatment. Although all forms of topo I decreased in the extended period of exposure to TPT, the ratio of sumoylated versus non-sumoylated topo I remained unchanged from 1 to 4 h (Fig. 4A). To investigate the nature of these small particles, we immunostained the cells with anti-PML antibody (red) because PML is predominantly localized in nuclear bodies (33). Although they are structurally similar to nuclear bodies, these green fluorescent particles did not colocalize with PML (Fig. 4C).

Heat Shock Inhibits Sumoylation of Topo I and Nucleolar Delocalization of Topo I Fusion Proteins—It has been shown previously that heat shock inhibits sumoylation of topo I (34). By Western blot analysis, we also found that the heat shock inhibition of sumoylation was complete by 30 min of treatment with 42 °C heat shock (data not shown). Interestingly, although the nucleolar fusion protein was delocalized in response to heat shock, sumoylation of topo I fusion protein was not affected (Fig. 4D). In fact, we observed an increase in the intensity of sumoylated topo I fusion protein in response to heat shock (Fig. 4D). Therefore, heat shock inactivates topo I in the nucleolus but is permissive for sumoylation of topo I fusion protein in the cytoplasm.
inhibited sumoylation of topo I, although heat shock itself led to general sumoylation of other proteins, as seen in Me2SO and heat shock-treated cell extract (Fig. 5A). Thus, we asked if heat shock has any effect on nucleolar delocalization of topo I. When the transfected cells were incubated for 30 min at 43.5 °C and then treated with TPT, we found that the fusion protein was no longer translocated out of the nucleolus, whereas without heat shock, the fusion protein moved out as usual in response to TPT (Fig. 5B). The DsRED fusion protein revealed a better resolution of subnuclear structure (Fig. 5C). Again, heat shock blocked nucleolar delocalization of the fusion protein caused by TPT.

To determine whether heat shock has any effect on the topo I that has already been sumoylated, we first treated HeLa cells with 10 μM TPT for 15 min at 37 °C and then incubated the treated cells at 43.5 °C for 30 min. Although a significant amount of sumoylated topo I was detected before heat shock (Fig. 6A), heat shock treatment reversed this process, i.e. sumoylated topo I bands disappeared, suggesting that de-sumoylation or degradation of topo I occurs upon heat shock. Consistent with this observation, fluorescence microscopy revealed that EGFP-topo I fusion protein re-accumulated in the nucleolus after heat shock (Fig. 6B), although at a less extent compared with Me2SO control.

Overexpression of UBC9-DN Attenuates Sumoylation of Topo I—Because heat shock causes a general stress to the cell and, thus, may affect many other biochemical pathways, inhibition of nucleolar delocalization by heat shock may not necessarily be because of blockage of sumoylation of topo I. To further confirm that sumoylation is an important event for nucleolar delocalization, we overexpressed a UBC9 DN mutant. UBC9 is an E2-conjugating enzyme that is essential to sumoylation (15); on the other hand, UBC9-DN blocks sumoylation (35). Overexpression of UBC9-DN was confirmed by Western blot (Fig. 7A, upper panel). At the same time, we found UBC9-DN reduced the sumoylation of topo I (Fig. 7A, lower panel) in transiently transfected cells.

In addition, we established stably transfected UBC9-DN HeLa cells. Among 24 clones selected, we found 6 of them to express a high level of this recombinant protein (Fig. 7B). As shown in Fig. 7C, reduction of sumoylation of topo I was also detected in these stable cell lines (e.g. clones 4 and 13).

Overexpression of UBC9-DN Inhibits Nucleolar Delocalization of DsRED-topo I Fusion Protein—To better detect the effect of UBC9-DN on subnuclear redistribution of topo I, we co-transfected HeLa cells with pTI-42 (DsRED-topo I) and EGFP-UBC9-DN. For comparison, we also co-transfected HeLa cells with pTI-42 and wild-type UBC9 (EGFP-UBC9). After treatment with 10 μM TPT as above, we found that both wild-type and dominant mutant UBC9 were predominantly in the nucleus, and TPT treatment did not seem to cause any change to the subcellular localization of either wild-type or dominant negative UBC9. However, we observed different effects on the subnuclear localization of DsRED-topo I. For instance, overexpression of UBC9-DN inhibited nucleolar delocalization of DsRED-topo I fusion protein (Fig. 8). In contrast, no such
inhibitory effect was seen after overexpression of wild-type UBC9 (Fig. 8). Because of the high intensity of the red fusion protein, the overlayed pictures were dominated by the red fluorescence in some cells. In addition, we examined subnuclear redistribution of either EGFP-topo I or DsRED-topo I fusion protein in stably transfected HeLa/UBC9-DN cells. A similar inhibitory effect was seen, although at a less extent, presumably because the protein level of UBC9-DN in individual cells from transient transfection is higher than that in those cells from stable transfection due to a high copy number of the gene.

DISCUSSION

Although topo I is a nucleolar protein, its nucleolar distribution seems to be dynamic, presumably due to the fact that the nucleolus is a non-membrane structure (7). This dynamic change of nucleolar redistribution of topo I is reflected not only in different cell growth conditions but in response to various environmental stimuli. Particularly, nucleolar delocalization of topo I occurs in response to the anticancer drugs (topo I inhibitors) such as camptothecin derivatives or RNA synthesis inhibitors (10, 11). Because this dynamic change of topo I may alter the cell response to drugs and provide a way for the cell to cope with drug-induced damage, there is great interest in elucidating the molecular mechanism of its nucleolar delocalization. Accordingly, we have examined several factors that affect nucleolar delocalization of topo I in this study. Our results suggest that sumoylation of topo I by TPT treatment is an important molecular event that is associated with nucleolar delocalization of topo I.

Early studies have suggested that SUMOs play a role in controlling nucleo-cytoplasmic transport of proteins because sumoylation of RanGAP1 seems to facilitate the interaction of RanGAP1 with the nuclear pore complex (36). Recent studies, however, indicate that SUMOs may play a more important role in regulating subnuclear distribution of proteins (25). It has been shown that a number of nuclear proteins are sumoylated, including PML, p53, MDM2, nuclear receptors such as andro-
gen receptor, and heat shock transcription factor-2 (17, 20, 21, 24, 37). Of interest, sumoylation has been shown to be essential for PML to accumulate in nuclear bodies (34). For instance, by recruiting proteins like p53 into the nuclear bodies, PML3 modulates p53 transcription activity (38). Whether SUMOs direct proteins to nuclear bodies or nuclear bodies support sumoylation or both remains to be determined; it appears, however, that sumoylation is required for this subnuclear redistribution. Once in the nuclear bodies, the sumoylated proteins can be either activated or inactivated. In the case of p53, its transcription activity is increased in the nuclear bodies (38); by contrast, Daxx, a Fas-binding protein that acts as a corepressor of transcription (39), no longer represses transcription when it is recruited into nuclear bodies (40).

Although the role for topo I sumoylation is still largely undefined, three lines of evidence presented in this study suggest that sumoylation might serve as an addressing tag for nucleolar delocalization of topo I. First, topo I sumoylation occurs earlier than its nucleolar delocalization. Second, heat shock treatment inhibits topo I sumoylation and counters the nucleolar delocalization of topo I caused by TPT. Although heat shock causes general stress to the cell and has been shown to increase overall sumoylation of proteins including topo II isozymes, sumoylation of topo I is decreased under the same conditions (34). Third, overexpression of UBC9-DN decreases sumoylation of topo I and at the same time reduces nucleolar delocalization of topo I. Based on the data presented in this study, we believe that upon treatment with TPT, topo I is sumoylated and then translocated out of the nucleolus. Although the fate of topo I is not clear after delocalization from the nucleolus, it is unlikely that it moves out of the nucleus because no cytoplasmic topo I is detected. One possibility is that topo I is redistributed in the nucleoplasm; another possibility is that some fraction of the topo I may accumulate in nuclear bodies. We have found that the intensity of fluorescent protein increases in nuclear body-like structures while the nucleolar fluorescent protein is disappearing, although the nature of the nuclear body-like structures is not clear at this moment. The second possibility may partially explain how the cell copes with DNA damage caused by the drug, a possible rescue mechanism. In this regard, we are currently investigating whether any DNA repair enzymes are recruited into the nuclear body-like structures.

However, we cannot rule out the possibility that delocalized topo I is degraded, because topo I inhibitors also cause degradation of topo I (30). Time course experiments suggest that sumoylated topo I is unstable because after 30 min of TPT treatment, the intensity of sumoylated topo I bands decreased significantly (Fig. 3); for a longer period of TPT treatment, overall topo I levels (both unsumoylated and sumoylated topo I) were observed to decrease (Fig. 4A). It is possible that sumoylation of topo I has a high turnover, which might be due to translocation of sumoylated topo I, followed by its de-sumoylation or degradation or both.

The mechanism behind sumoylation of topo I is not clear, although DNA damage or conformational changes in topo I caused by topo I inhibitors has been suggested (26). However, because TPT itself is an RNA synthesis inhibitor, it is also possible that topo I inhibitors, like RNA synthesis inhibitors, interfere with transcription. Because topo I preferentially binds to supercoiled DNA that is actively transcribed, particularly rDNA (41), once RNA synthesis is inhibited by these drugs, the torsional stress on the DNA region is no longer built up. Under these conditions, a decrease in affinity of topo I for DNA could be expected and, thus, would be released from the nucleolus. Furthermore, we found that sumoylation of topo I was reversed by removal of TPT, and this correlates with nucleolar delocalization of topo I fusion proteins. Thus, any conformational change of the DNA structure, when bound by topo I, might also cause sumoylation of topo I, leading to nucleolar delocalization of topo I.

In addition to binding of topo I to the transcribed DNA, topo I also physically interacts with many other nuclear proteins. In this context, it is worth mentioning that the N terminus of topo I, which is essential for in vivo function of topo I, is also a major domain that interacts with several nuclear proteins (42–44). Whether interaction of these proteins with the N-terminal domain regulates its nucleolar accumulation remains to be determined; however, any interference with the formation of the topo I-containing nucleolar complex may cause its nucleolar delocalization. Therefore, it will be informative to determine whether sumoylation of topo I causes any change to such interactions. Nucleolin is a major nucleolar protein and has been shown to physically interact with topo I. Although binding to topo I by nucleolin does not seem to affect its catalytic activity (42), it will be interesting to test if such interaction facilitates the formation of nucleolar complexes.

The consensus sequences for protein sumoylation have been proposed to be YKXE, where X can be any amino acid, Y is mainly a hydrophobic residue, and K is the residue that is covalently linked to SUMOs (45). According to this consensus, we have found five potential sumoylation sites in human topo I: amino acids 102–104 (IKKE), amino acids 116–119 (IKDE), amino acids 152–155 (IKTE), amino acids 327–330 (IKKE), and amino acids 434–437 (IKGE). This prediction seems to be consistent with our data in that we detected at least five sumoylated topo I bands (Fig. 3). Unlike ubiquitination, where polyubiquitination usually occurs, SUMOs have been shown so far to be formed as a single molecule (25). Interestingly, three of the five potential sumoylation sites on topo I are located at the N terminus. In addition, it has been shown that a nuclear localization signal is required for sumoylation (46); we have found that the N terminus of topo I is also involved in interacting with UBC9,2 thus, highlighting an importance of this N terminus for its sumoylation.

So far, UBC9 has been shown to be the sole E2 enzyme for sumoylation. Cells overexpressing the UBC9-DN are interesting because they not only reveal attenuation of sumoylation of topo I and its nucleolar delocalization, but they also seem to be more sensitive to topo I inhibitors,2 a result that is similar to the finding that yeast expressing temperature-sensitive UBC9 are hypersensitive to camptothecin (26). Although the mechanism behind this phenomenon might be complex because overexpression of UBC9-DN may affect other genes, it is nevertheless reasonable to assume that inhibition of sumoylation by the UBC9-DN may contribute to this sensitivity. Furthermore, UBC9 has been implicated in DNA repair, and it physically interacts with DNA repair enzymes such as Rad51 (47). Therefore, further investigation of the UBC9-DN-overexpressing cells is needed to determine the role of UBC9 in drug responsiveness and DNA repair.

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Nucleolar Delocalization of Topo I

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