UV and ionizing radiation (IR) activate DNA damage checkpoints and induce Cdc25A degradation (Mailand, N., Falck, J., Lukas, C., Syljuasen, R. G., Bartek, M., Bartek, J., and Lukas, J. (2000) Science 288, 1425–1429; Falck, J., Mailand, N., Syljuasen, R. G., Bartek, J., and Lukas J. (2001) Nature 410, 842–847). The degradation of Cdc25A is abrogated by caffeine, which implicates Chk1 as the potential mediator (Mailand, N., Falck, J., Lukas, C., Syljuasen, R. G., Welcker, M., Bartek, J., and Lukas, J. (2000) Science 288, 1425–1429). However, the involvement of Chk1 is far from clear, because caffeine is a rather nonspecific inhibitor of the ATR/Chk1 signaling pathway. Additionally, it is not known whether DNA-damaging drugs commonly used in chemotherapy, which may activate different signal transduction pathways than UV or IR, also confer Cdc25A degradation. Herein, we show that camptothecin and doxorubicin, two widely used topoisomerase inhibitors conferring S phase arrest, respectively, cause the degradation of Cdc25A. Using a small interfering RNA that enables the specific elimination of Chk1 expression, we show that the observed proteolysis of Cdc25A is mediated through Chk1. Moreover, Cdc25A overexpression abrogates the Chk1-mediated degradation and overcomes the doxorubicin-induced G2 arrest through dephosphorylation and activation of Cdc2/Cdk1 in a dose-dependent manner. These results suggest that: (a) Cdc25A is involved in the G2/M transition in addition to its commonly accepted effect on G1/S progression, and (b) Chk1 mediates both S and G2 checkpoint and is thus a more ubiquitous cell cycle checkpoint mediator than previously thought.

DNA damage caused by different agents induces cell cycle arrest at G1, S, or G2 thereby preventing replication of damaged DNA or aberrant mitosis until the damage is adequately repaired. These regulatory mechanisms are known as cell cycle checkpoints and involve an intricate network of protein kinase signaling pathways. They are central to the maintenance of genomic integrity and basic viability of the cells. Hence, defects in these pathways may result in either tumorigenesis or apoptosis depending on the severity and particular nature of the defects (3, 4).

Chk11 is a major checkpoint kinase and has been shown to be mainly responsible for the G2/M checkpoint (5). Upon DNA damage, Chk1 is activated by ATR-mediated phosphorylation. Active Chk1 phosphorylates the protein phosphatase Cdc25C at serine 216. This leads to the nuclear export of Cdc25C and its subsequent cytoplasmic sequestration by 14-3-3 protein, which prevents the activation of the downstream target of Cdc25C, the cyclin B/Cdk2 kinase that is responsible for G2/M transition (6, 7). In addition to the role of ATR/Chk1 in G2/M progression, recent experiments using dominant negative ATR showed that the ATR/Chk1 pathway may also play an important role in the S checkpoint induced by topoisomerase I inhibitors (8).

Cdc25 phosphatases are essential cell cycle regulators. By removing inhibitory phosphates from tyrosine and threonine residues of cyclin-dependent kinases (CDKs), Cdc2 phosphatases activate the CDKs and promote cell cycle progression. In fission yeast, Cdc25 is a stimulator of the G2/M transition because of its activation of Cdc2. In mammalian cells, three Cdc25 analogues, Cdc25A, -B, and -C, have been identified (9, 10) that rescue the growth defect of Cdc25-null Schizosaccharomyces pombe cells. In addition to the aforementioned Cdc25C, which promotes the G2/M transition through dephosphorylation of Cdc2, Cdc25B was proposed to act as an initiator of the same process (11, 12). In contrast, overexpression of Cdc25A activates cyclin E-Cdk2 and cyclin A-Cdk2 by inducing Cdk2 tyrosine dephosphorylation. This demonstrates that Cdc25A is involved in S phase (13, 14). Not surprisingly, Cdc25A overexpression causes a shortening of the G1 phase and faster S phase entry (15, 16). Most interestingly, Cdc25A and -B, but not C have been found to be overexpressed in a subset of aggressive human cancers (17–19).

The p53 tumor suppressor mediates DNA damage-induced G1 arrest, but has little or no effect on S or G2 arrest. Consequently, while normal cells mainly arrest in G1 in response to genotoxic stress, p53-null tumor cells are defective in G1 arrest. This leads to increased genetic instability in tumor cells, which further reinforces the tumorigenic process. On the other hand, tumor cells with p53-dependent G1 checkpoint deficiency rely more on the S or G2 checkpoint to repair their damaged DNA and preserve their essential genomic integrity for basic viability when treated with radiation or DNA-damaging drugs. Therefore, by specifically abrogating the G2 checkpoint, normal cells can still arrest in the G1 phase and repair the DNA damage; whereas, tumor cells that lack the G1 checkpoint will undergo mitotic catastrophe and eventually undergo apoptosis. These differences may confer a selective cytotoxicity in the tumor versus normal cells (20, 21). Because of its prominent dependent kinases; PBS, phosphate-buffered saline; FACS, fluorescence-activated cell sorter; BrdUrd, bromodeoxyuridine; scrRNA, scrambled control RNA; siRNA, small interfering RNA; AT, ataxia telangiectasia; ATR, ataxia and Rad3 related.

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role in the DNA damage-induced G2 checkpoint, Chk1 has become an attractive target for this approach, and Chk1 inhibitors are being developed to potentiate the effects of genotoxic chemotherapeutics on p53-null tumors.

Herein we demonstrate that camptothecin and doxorubicin, two DNA-damaging agents that cause S or G2 arrest, respectively, activate Chk1 and cause the rapid proteolysis of Cdc25A. Elimination of Chk1 expression through siRNA not only abrogated the S or G2 arrest, but also protected Cdc25A from degradation. These results suggest that Chk1 mediates the S or G2 phase arrest by targeting Cdc25A for proteolysis following chemically induced DNA damage. This study indicates that a Chk1 inhibitor may potentiate the cytotoxicity not only of DNA-damaging drugs causing G2 arrest, but also of agents conferring S arrest, which significantly broadens its applicability to cancer chemotherapy. Our results additionally demonstrate that Cdc25A plays functional roles not only in the G2/S transition but also in G2/M progression. This later conclusion is supported by our observation that both Cdc25A and Cdc25C overexpression can overcome the Chk1-mediated G2/M arrest induced by doxorubicin in a dose-dependent manner.

MATERIALS AND METHODS

Cell Culture—Human lung cancer cell line H1299 was obtained from ATCC (Manassas, VA). H1299 were grown in RPMI 1640 supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, and 0.45% glucose at 37 °C in 5% CO2 incubator.

Antibodies—Cdc25A was detected with NeoMarker monoclonal anti-cdc25A antibody C-3 (Lab Vision, CA). Chk1 was detected with Chk1 (G-4) monoclonal antibody from Santa Cruz Biotechnology (Santa Cruz, CA). Cdc25C was detected by Cdc25C C-20 polyclonal antibody from Santa Cruz Biotechnology. Phospho-Chk1 (Ser-345) was detected by phospho-Chk1 (Ser-345) antibody from Cell Signaling Technology.

Cdc25A antibody C-3 (Lab Vision, CA). Chk1 was detected with Chk1 antibody (C-20) polyclonal antibody from Santa Cruz Biotechnology. Phospho-Chk1 (Ser-345) was detected by phospho-Chk1 (Ser-345) antibody from Cell Signaling Technology.

Transfections—Human lung cancer cell line H1299 were treated with or without camptothecin and harvested for Western blot analysis of Cdc25A, Chk1 protein levels, and cell cycle profiles. Cdc25A almost completely disappeared after the treatment (Fig. 1A, compare lanes 3 and 4 versus lane 1 and 2), whereas its close homologue, Cdc25C, did not show any change in protein levels. The disappearance of Cdc25A is because of proteasome-mediated degradation, because incubation of cells with MG132, a specific proteasome inhibitor, abrogated this phenomenon (data not shown). Although the amount of Chk1 protein remained constant before and after drug treatment, the ATR-phosphorylated Chk1 species (Chk1 Ser345P) appeared only after camptothecin addition (lanes 3 and 4), indicating Chk1 activation in response to camptothecin-induced DNA damage. We additionally confirmed that cells treated with camptothecin were mostly arrested in S phase (Fig. 3B and data not shown), further suggesting that Chk1 may mediate S checkpoint by targeting Cdc25A for proteolysis.

Doxorubicin Induces Phosphorylation of Chk1 and Cdc25A Degradation, Resulting in G2/M Arrest—We also studied the effect of doxorubicin, a clinically relevant topoisomerase II inhibitor conferring G2 arrest on different cancer cell lines including H1299 cells (Fig. 3A and data not shown). Similar to camptothecin, doxorubicin caused rapid degradation of Cdc25A while leaving Cdc25C mostly intact, which can be correlated to the phosphorylation and activation of Chk1 upon drug treatment (Fig. 1B). Thus, different DNA-damaging agents, which cause arrests at different phases of the cell cycle, lead to simultaneous Chk1 activation and Cdc25A degradation. These observations are not specific to H1299 cells, because similar findings were also obtained from other cell lines including SW620 and HCT-15 (data not shown).

Chk1 Mediates the Proteolysis of Cdc25A in Response to Camptothecin and Doxorubicin—To confirm the direct involvement of Chk1 in the DNA damage-induced proteolysis of Cdc25A, we designed an siRNA that specifically eliminated Chk1 expression. Chk1 siRNA or the scrambled control RNA
(scrRNA) were transfected into H1299 cells that were subsequently subjected to the same camptothecin treatment (Fig. 2A). For scrRNA, we again observed the efficient proteolysis of Cdc25A in response to camptothecin at both 3- and 6-h time points (*lanes 1–3*). In contrast, with transfection of the Chk1 siRNA, the proteolysis of Cdc25A was significantly inhibited (*lanes 4–6*). The identity of the indicated Cdc25A band was confirmed by its exact co-migration with overexpressed Cdc25A protein (data not shown; also see Fig. 2B). The Chk1 immunoblot confirms the effective elimination of the Chk1 protein by the Chk1 siRNA but not the scrRNA. This result established that Chk1 mediates the degradation of endogenous Cdc25A in response to camptothecin treatment.

To further confirm this observation, we ectopically expressed Cdc25A in H1299 cells. When Cdc25A is moderately overexpressed (about 3–5-fold over endogenous level), camptothecin still induces the efficient proteolysis of Cdc25A (Fig. 2B, *lanes 1–3*). However, with Chk1 siRNA, this degradation was essentially abrogated (*lanes 4–6*). In fact, the steady-state level of Cdc25A was greatly increased (by 3–5-fold, compare *lanes 1* and *4*), indicating that under these conditions Chk1 also negatively regulates the level of Cdc25A even in the absence of any DNA damage.

To ascertain whether Chk1 also directly mediates doxorubicin-induced Cdc25A degradation, we treated H1299 cells with doxorubicin at 500 nM overnight to induce the proteolysis of Cdc25A. With Chk1 siRNA transfection, this process remains intact (Fig. 2C, *lanes 3 and 4*). In contrast, the Chk1 siRNA greatly inhibited this clearance (*lanes 1 and 2*). These results suggest that Chk1 targets Cdc25A for degradation in response to both doxorubicin and camptothecin.

**Chk1 Mediates Both S and G2 Checkpoints**—To investigate whether Chk1 is involved in the doxorubicin-induced G2 checkpoint, we analyzed the cell cycle profile of H1299 cells in the presence of doxorubicin with or without the elimination of Chk1 via siRNA. When cells transfected with scrRNA were treated with doxorubicin for 24 h, we observed a dramatic increase in the G2/M peak (19–79%), indicating the induction of the G2 checkpoint (Fig. 3A, profiles 1 and 2). However, when Chk1 expression is eliminated via the Chk1-specific siRNA, the extent of G2/M arrest was significantly reduced (28%, Fig. 3A, profile 4), indicating that Chk1 is directly responsible for the doxorubicin-confferred G2 checkpoint. Additionally, we observed a large increase of apoptotic (M1 phase, sub-G0/G1) cells...
Chk1 Mediates Both S and G<sub>2</sub> Checkpoints

A

| Dox (nM) | scrRNA | scrRNA | siRNA | siRNA |
|----------|--------|--------|-------|-------|
| 0        | 500    | 0      | 500   |       |
| Apopt% (M1) | 1       | 1      | 2     | 14    |
| G1% (M2) | 72     | 15     | 76    | 46    |
| S% (M3) | 8      | 4      | 7     | 11    |
| G2/M% (M4) | 19      | 79     | 15    | 28    |

B

No Camptothecin

Mock

| BrdU-positive cells% | 33 | 28 | 27 |

+ Camptothecin

| BrdU-positive cells% | 1  | 1  | 21 |

In doxorubicin-treated Chk1-deficient cells (14% in profile 4 versus 1% in profile 2), suggesting that abrogation of the checkpoint by Chk1 knockdown induced extensive cell death.

In contrast to its role in the G<sub>2</sub> checkpoint, the involvement of Chk1 in S phase arrest is not as widely accepted. To convincingly demonstrate the role of Chk1 in S checkpoint, we carried out a BrdUrd labeling experiment with camptothecin-treated H1299 cells to better characterize the expected S phase arrest. First, in the absence of camptothecin, there were no differences in the labeling profiles among mock transfected cells, cells transfected with Chk1 siRNA, and cells transfected with scrRNA (Fig. 3B, upper panels). With camptothecin treatment, we noticed a dramatic decrease in BrdUrd labeling of the S phase cells in either mock or scrRNA-transfected cells (33 to 1, and 28 to 1%, respectively), consistent with the drug-induced S phase arrest. However, Chk1 siRNA transfection effectively maintained the DNA synthesis rate (27 to 21%) even in the presence of DNA damage, demonstrating abrogation of the arrest and further indicating that Chk1 also mediates camptothecin-induced S checkpoint. Chk1 immunoblot was performed to confirm the elimination of Chk1 protein by the siRNA (data not shown).

The Function of Cdc25A in G<sub>2</sub>/M Transition—So far, we have shown the Chk1 mediates Cdc25A degradation and arrests cells in either S or G<sub>2</sub>/M when induced by DNA-damaging agents. To further confirm that Cdc25A plays a role in G<sub>2</sub>/M transition in addition to its more widely accepted function in G<sub>1</sub>/S progression, we studied the cell cycle profiles of H1299 cells transiently transfected with increasing amounts of expression plasmids encoding either Cdc25A or Cdc25C (Fig. 4A).

The cells were treated with doxorubicin to induce G<sub>2</sub> arrest; FACS analysis was performed to determine whether Cdc25 overexpression could abrogate the DNA damage-induced G<sub>2</sub> arrest. Control cells in the absence of doxorubicin showed a normal predominant distribution in G<sub>1</sub> phase and a relatively minor G<sub>2</sub>/M population (right profile, panel 1). In contrast, doxorubicin-treated cells exhibited a large increase of the G<sub>2</sub>/M peak, indicating G<sub>2</sub> arrest (left profile, panel 1). With Cdc25C overexpression (panel 2), we observed a dose-dependent abrogation of the G<sub>2</sub> arrest starting at the second dose (100 ng/well transfection), consistent with its published role in promoting G<sub>2</sub>/M phase progression through dephosphorylation of Cdc2 (7). In comparison, Cdc25A overexpression (panel 3) also conferred a similar abrogation of the G<sub>2</sub> arrest in a dose-dependent manner starting with the second dose (100 ng/well transfection), indicating that Cdc25A mediates G<sub>2</sub>/M transition with similar efficacy as Cdc25C. As a control, transfection of cells with increasing amounts of empty vector did not confer any abrogation (data not shown).
To confirm the overexpression of Cdc25A in the above experiment and further investigate the underlying mechanism of the observed abrogation of G₂ arrest, we subjected the same cell samples to immunoblot analysis for Cdc25A and Cdc2/Cdk1 (Fig. 4B). Because Cdc2 is the kinase directly responsible for G₂/M transition, and its dephosphorylation at tyrosine 15 by Cdc25C has been shown to cause its activation and M phase progression (6), we speculated that it might also be the downstream target of Cdc25A. Overexpression of Cdc25A conferred resistance to proteolysis and dephosphorylation of Cdc2. H1299 cells were transfected with 50, 200, and 500 ng of Cdc25A expression plasmid. Cells were then treated with 250 nm doxorubicin overnight. Cell lysates were harvested and analyzed for Cdc25A and Cdc2 Y15P expression profiles. Actin was immunoblotted to control for protein loading. Correspondingly, the 500-ng transfection conferred higher Cdc25A accumulation than the 200-ng transfection, resulting in more complete elimination of Cdc2 Y15P signal (compare lanes 6 and 8), in line with its more complete abrogation of the G₂ arrest (Fig. 4A). As a negative control, transfection of 500 ng of a plasmid expressing Cdc25A C430S, a phosphatase-dead inactive mutant (15), also resulted

Fig. 4. Overexpression of Cdc25A overcomes doxorubicin-induced G₂ checkpoint through decreasing Cdc2 tyrosine 15 phosphorylation. A, overexpression of Cdc25A and Cdc25C abrogated G₂ checkpoint with similar efficiency. H1299 cells were transfected with increasing amounts of Cdc25A or Cdc25C expression vectors (50, 100, 200, 500, and 1,000 ng per well). Cells were then treated with 250 nm doxorubicin (Dox) to induce G₂ arrest. 24-h later cell cycle profiles were analyzed by FACS. B, overexpression of Cdc25C conferred resistance to proteolysis and dephosphorylation of Cdc2. H1299 cells were transfected with 50, 200, and 500 ng of Cdc25A expression plasmid. Cells were then treated with 250 nm doxorubicin overnight. Cell lysates were harvested and analyzed for Cdc25A and Cdc2 Y15P expression profiles. Actin was immunoblotted to control for protein loading. C, overexpression of Cdc25C caused dephosphorylation of Cdc2. H1299 cells were transfected with 50, 200, and 500 ng of Cdc25C expression plasmid. Cells were then treated with 250 nm doxorubicin overnight. Cell lysates were harvested and analyzed for Cdc25C and Cdc2 Y15P expression profiles. Actin was immunoblotted to control for protein loading. DMSO, dimethyl sulfoxide.
in doxorubicin-resistant accumulation of Cdc25A to a level that is even higher than transfection with same amount of wild type construct (compare lanes 9 and 10 and 7 and 8). However, this mutant Cdc25A is unable to dephosphorylate Cdc2 and decrease the Cdc2 Y15P signal, thus confirming that the phosphatase activity of Cdc25A is required for Cdc2 activation. Cdc2 total protein level was also studied for this experiment and found to be mostly constant among the different samples (data not shown).

Fig. 4C shows Cdc25C overexpression-induced dephosphorylation of Cdc2. Similar to Cdc25A, Cdc25C overexpression also led to a decrease in the Cdc2 Y15P signal in a dose-dependent manner, suggesting that Cdc25C abrogates DNA damage-induced G2/M arrest via re-activation of Cdc2 through its dephosphorylation. This corroborates previous reports showing that Cdc2 is a direct downstream target of Cdc25C.

**DISCUSSION**

Chk1 was proposed to play a role in the S or G2 checkpoints induced by various DNA-damaging agents including UV, IR, and topoisomerase I inhibitors (1, 4, 8, 22). This is supported by the observation that in normal human fibroblasts (MJ90) and also in fibroblasts derived from patients with ataxia telangiectasia (AT), Chk1 is expressed specifically at the S to G phase of the cell cycle at both the RNA and protein levels and its activity is readily detected at the S to M phase of the cell cycle (23). Nonetheless, the direct involvement of Chk1 in these processes have not been convincingly demonstrated and the underlying mechanisms for these checkpoints still remain controversial. We report here that Cdc25A protein undergoes Chk1-mediated degradation in response to various DNA-damaging drugs, and this degradation coincides with and determines the resultant S or G2 checkpoints. Therefore, Chk1 elimination through siRNA leads to abrogation of both Cdc25A proteolysis and the S or G2 checkpoints.

During the final preparation stage of this article, a new report appeared showing that Cdc25A is subject to Chk1-mediated proteolysis in response to double-stranded DNA breaks generated by IR (24). This contrasts a previous study that demonstrated that Chk2, not Chk1, is primarily responsible for mediating IR-induced Cdc25A degradation (25). In this study, we focused on two major topoisomerase inhibitors (camptothecin and doxorubicin), the mainstay of cancer chemotherapy. Camptothecin is the most commonly used first-line and second-line treatment for colon cancer, whereas doxorubicin is widely used to combat breast, stomach, and cervical cancers (26). Because IR and topoisomerase inhibitors confer DNA damage through different mechanisms (see below), they may activate distinct signal transduction pathways. The mechanism of cell cycle arrest by the topoisomerase inhibitors has not been clearly established. This lack of theoretical guidance has seriously impeded the improvement of current chemotherapy and rational design of next generation chemotherapeutics. Our study directly addressed this urgent issue by demonstrating that camptothecin and doxorubicin induce different cell cycle checkpoints (S and G2) through the same process of targeting Cdc25A to degradation through Chk1, and Chk1 inhibition greatly potentiates their cytotoxicity. Therefore, combined with previous studies, we have shown that the checkpoint kinases respond to a wide variety of DNA insults by targeting Cdc25A to proteolysis and triggering checkpoints at different stages of the cell cycle.

**Cdc25A Mediates G2/M Progression in Addition to G1/S Transition**—Cdc25A is usually considered a promoter of G1/S transition through its dephosphorylation and activation of CdK2 kinase (9, 15). However, we have determined that Cdc25A also undergoes rapid proteolysis in response to both S and G2 checkpoints; and disruption of this degradation, through either elimination of Chk1 (Fig. 3) or Cdc25A overexpression (Fig. 4), essentially abrogates the checkpoints. This suggests that Cdc25A functions in both S phase progression and G2/M transition. The latter point is further supported by several previous studies: (a) a potent Cdc25A inhibitor arrests synchronized cells at both G1 and G2/M, and blocked dephosphorylation of both CdK2 and Cdc2/Cdk1 (27); (b) microinjection of Cdc25A antibodies interfere with mitosis (9); and (c) Cdc25A has been found to be active throughout all stages of the cell cycle (13, 15).

Cdc25C is a well known mediator of G2/M transition through its activation of Cdc2 (6, 7). However, it may not be the only mediator of this process because Cdc25C-null mice are viable and do not display any obvious abnormalities. Furthermore, the phosphorylation status of Cdc2, the timing of entry into mitosis, and the cellular response to DNA damage were unaltered in mouse embryo fibroblasts lacking Cdc25C, indicating that Cdc25A and/or Cdc25B may compensate for loss of Cdc25C in the mouse (28). Our current study provides additional evidence that overexpression of Cdc25A could overcome G2 arrest as efficiently as that of Cdc25C through dephosphorylation of Cdc2, strengthening the notion that Cdc2 is also a downstream target of Cdc25A (Fig. 4). Moreover, doxorubicin simultaneously induces G2 arrest and Cdc25A degradation without showing any effect on Cdc25C level (Figs. 1 and 2). These all suggest that Cdc25A may be a more critical and ubiquitous cell cycle regulator than Cdc25C, and the Chk1/Cdc25A pathway mediates both S and G2 checkpoints.

**Cdc25A and Cdc25C May Be Both Required for G2/M Progression**—When cells enter mitosis, there is a dramatic increase of cyclin B-Cdc2 kinase activity, which is required for early stage mitotic progression (29). To maintain this requisite level of Cdc2 kinase activity, a sustained pool of Cdc25 phosphatase activity may be necessary. As mentioned previously, among the three Cdc25 members, Cdc25B is regarded as an “initiator” phosphatase that is primarily responsible for creating the first stimulation of cyclin B-Cdc2 kinase activity to set in motion the mitotic process (11, 12). However, Cdc25B is an unstable protein with a short half-life (30), and it is degraded in a proteasome-dependent manner upon M phase entry (31). Thus the burden of maintaining the active cyclin B-Cdc2 kinase falls to the other members of the family, namely Cdc25A and Cdc25C. We hypothesize that the endogenous level of each protein is insufficient to sustain the necessary threshold of cyclin B kinase activity, thus the presence of both are required for proper M phase progression. As shown in Fig. 4, this dual requirement can be clearly circumvented by the transient over-expression of either Cdc25A or Cdc25C.

As discussed above, Cdc25C knockout cells show little mitotic abnormalities (28). This does not mean that Cdc25C normally plays no role in G2/M progression. It only means that Cdc25C is not essential for this process. Apparently other members of the Cdc25 family are able to compensate for its loss. We speculate that in Cdc25C-null cells, Cdc25A may undergo certain kinds of up-regulation, either at the mRNA or protein levels, or at the phosphatase activity level through post-translational modifications, to still maintain a normal pool of Cdc25A phosphatase activity.

**Will Different DNA-damaging Agents, Especially Topoisomerase Inhibitors, All Induce Cdc25A Degradation?**—Previously it was reported that hydroxyurea, an antimetabolite-inducing S phase arrest, also stimulated Cdc25A degradation (32). We showed here that camptothecin, a topoisomerase I poison conferring S arrest, induces the same process specifically through Chk1. It is not clear what distinguishes these two
Chk1 Mediates Both S and G2 Checkpoints

phenotypically different forms of S checkpoint. It will be interesting to know whether the DNA polymerase inhibitor aphidicolin, causing S phase arrest through yet another mechanism, also leads to Cdc25A proteolysis.

IR generates DNA damage through creating double-stranded DNA breaks, this process does not directly involve topoisomerase. In contrast, camptothecin and doxorubicin induce DNA damage through inhibition of topoisomerase I and II, respectively. Topoisomerases regulate DNA architecture by sustaining the correct DNA superhelicity and resolving intertwined DNA strands, particularly during DNA replication. Topoisomerase I generates single-stranded breaks, whereas topoisomerase II creates double-stranded breaks. The cytotoxicity of their inhibitors generally stems from the stabilization of topoisomerase-DNA covalent cleavage intermediates (also known as cleavage complexes). Besides camptothecin and doxorubicin, other clinically effective drugs acting in this manner include topotecan and SN-38 (topoisomerase I) as well as mitoxantrone and etoposide (topoisomerase II) (26). It will be interesting to ascertain whether we can extend our current findings to the other topoisomerase poisons. If yes, this will further broaden the utility of Chk1 inhibitors in the potentiation of cancer chemotherapeutics.

In conclusion, our results showed that the Chk1/Cdc25A pathway mediates both S and G2 checkpoints. This finding defines Chk1 as a more ubiquitous cell cycle checkpoint mediator and hence significantly broadens the application of Chk1 inhibitor in the potentiation of various cancer chemotherapeutics.

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