Overexpression of the dual specificity phosphatase, Cdc25C,confers sensitivity on tumor cells to doxorubicin-induced cell death

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Abstract

Cdc25C is a dual-specificity phosphatase that is involved in induction of mitosis by removal of the inhibitory phosphates from cyclin-dependent kinase 1/cyclin B. In this study, adenovirus-mediated overexpression of Cdc25C sensitizes U2OS tumor cells to doxorubicin-induced apoptosis. U2OS cells that stably overexpress Cdc25C are also sensitized to doxorubicin-induced cell death. These cells show reduced phosphorylation of cyclin-dependent kinase 1 on Tyr15 and impaired up-regulation of p21 in response to treatment with doxorubicin. In contrast to doxorubicin, overexpression of Cdc25C does not confer sensitivity to apoptosis on treatment with 5-fluorouracil or hydroxyurea. This sensitization of tumor cells to doxorubicin-induced cell death by overexpression of Cdc25C is not p53 dependent. Intriguingly, nontransformed MCF10A cells are not sensitized to doxorubicin treatment by overexpression of Cdc25C nor does the lack of Cdc25C affect cell cycle progression or the G2 arrest caused by doxorubicin. These results support the idea that a combination of overexpressing Cdc25C with treatment with conventional genotoxic agents should be given serious considerations as a novel therapeutic approach.

Introduction

Cancer arises due to abnormalities to DNA. The major protective mechanisms against development of cancer are the cell cycle checkpoints (1). Activation of the cell cycle checkpoints in response to DNA damage results in either preventing the cells from entering the next phase of the cell cycle to allow repair or inducing cell death if the lesion is beyond restoration. The two predominant checkpoints include those in the G1 and G2 phases. The former prevents the cells from entering S phase and replicating damaged DNA and the latter inhibits entry into mitosis and propagation of damaged DNA to daughter cells. It has been proposed that the G1 checkpoint is disabled in the majority of cancers as a prerequisite for tumorigenesis (2, 3). The G2 checkpoint, however, appears to remain functional in the majority of cancer cells. Many conventional cancer therapeutic agents exert their effects by causing DNA damage. Thus, retaining a functional G2 is believed to confer resistance to many such therapeutic agents. To circumvent this, attempts have been made to develop abrogators of this arrest in the G2 phase (4). Furthermore, the success of such a strategy would provide the fundamental advantage of selectively targeting cancer cells. Thus, cancer cells unable to arrest at the G2-M border will die in response to treatment, whereas their normal counterparts will still arrest at the G1 border.

At the molecular level, the lack of a G1 arrest in cancer cells is a result of their inability to inhibit cyclin-dependent kinase (Cdk)2, the kinase that is considered to be important for entry into S phase (1, 5). This usually arises as a consequence of a dysfunctional p53 and/or Rb pathway (1, 2). The target for the G2 checkpoint pathway is Cdk1 (1). Cdk1, once activated, ushers the cell into mitosis and subsequent cell division (5). Activity of Cdk1 is regulated at several levels. The final step in activation of Cdk1 is removal of the inhibitory phosphates from Thr14 and Tyr15. In mammalian cells, there are three related dual-specificity phosphatases, Cdc25A, Cdc25B, and Cdc25C, which act to dephosphorylate these residues (6). To arrest the cells at the G2-M border, dephosphorylation of Cdk1 on the two inhibitory residues is prevented by inhibiting the activities of these three phosphatases by various mechanisms (1, 6–13). The kinase Chk1 is a major regulator of the G2 checkpoint, as it is capable of inactivating all three phosphatases via their phosphorylation (1).

Many of the players in the G2 checkpoint pathway including Chk1 have been proposed or tested as means to abrogate the G2 arrest (4). In addition, other approaches have included drugs such as caffeine and UCN-01 (4). The major problems with these candidates have been their involvements in other cellular processes leading to unwanted toxicities. A recent study described a peptide, CBP501, which prevents phosphorylation of Cdc25C on Ser216 in response to DNA damage and thus abrogates G2 arrest (14). Phosphorylation of this site is required for

Received 1/21/08; revised 8/29/08; accepted 9/30/08.

Grant support: National Cancer Institute, Department of Health and Human Services grant CA086001 and Breast Cancer Research Program, Department of Defense grant W81XWH-05-1-0305. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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retaining Cdc25C in the cytoplasm and thereby preventing activation of Cdk1 when DNA is damaged (11, 12). CBP501 selectively enhanced sensitivity of cancer cells to genotoxic cancer therapy drugs in the absence of side effects (14). Thus, Cdc25C is suggested to be a useful target when considering strategies for abrogating G2 arrest. Here, it is proposed that overexpression of Cdc25C may be another means to abrogate the G2 checkpoint. Overexpression or lack of Cdc25C does not affect progression through the normal cell cycle in different cell lines in short-term or long-term assays. Loss of Cdc25C also does not affect the G2 arrest response to doxorubicin, a widely used anticancer drug that exerts its effects by inducing damage to genomic DNA by a variety of proposed mechanisms, notably inhibition of topoisomerase II (15). Nevertheless, excess amount of Cdc25C converts the G2 arrest induced by doxorubicin to cell death in several tumor cell lines. In contrast, overexpression of Cdc25C does not interfere with arrests in other phases of the cell cycle caused by treatment with either 5-fluorouracil (5-FU) or hydroxyurea.

Materials and Methods

Cell Cultures and Drug Treatments

Human U2OS, HCT116-p53–/–, and H1299 cells were grown as monolayers in DMEM (Life Technologies/Invitrogen) supplemented with 10% FCS (Life Technologies/Invitrogen) and 100 units/ml penicillin/streptomycin (Life Technologies/Invitrogen) at 5% CO2 and 37°C. MCF10A cells were grown in DMEM/F12 with 15 mmol/L HEPES, 5% horse serum, 20 ng/ml human epidermal growth factor, 100 ng/ml cholera toxin, 0.5 μg/ml hydrocortisone, and 100 units/ml penicillin/streptomycin. Dose-response curves were done to determine the optimal concentration for each drug and cell line. Exponentially growing cells were treated with various drugs as follows: U2OS, HCT116-p53–/–, and H1299 received 0.2 μg/ml doxorubicin, 400 μmol/L 5-FU, or 1.5 mmol/L hydroxyurea. MCF10A cells received 0.05 μg/ml doxorubicin, 300 μmol/L 5-FU, or 1 mmol/L hydroxyurea.

Construction of Recombinant Cdc25C Adenovirus

The recombinant Cdc25C adenovirus was constructed as described (16). Briefly, the FLAG-tagged open reading frame of Cdc25C was subcloned into the pAdTrack-CMV shuttle vector. It was then linearized by digestion with PacI and transfected into the adenovirus packaging cell line, 293T. High titer of recombinant adenovirus was obtained by several rounds of infection.

Flow Cytometric Analysis

Cells were fixed in ice-cold 70% ethanol followed by rinsing with PBS. DNA was stained with propidium iodide (20 μg/ml; Sigma) in the presence of 1 mg/ml RNase A (Sigma). Cell cycle analysis was done on a FACSCalibur and data were analyzed using CellQuest.

Immunoblotting

Cells were lysed in a buffer containing 50 mmol/L HEPES, 150 mmol/L NaCl, 1 mmol/L EDTA, 1% Triton X-100, 0.1 mmol/L Na3VO4, 10 mmol/L NaF, 80 mmol/L glycercophosphate, 1 mmol/L phenylmethylsulfonyl fluoride, 20 units/ml aprotinin, 5 mg/ml leupeptin, and 5 mg/ml pepstatin. Lysates were clarified by centrifugation (10,000 × g for 10 min at 4°C) and immunoblotted. The following antibodies were used: anti-FLAG M2 was purchased from Sigma. Anti-Cdc25A (F-6), anti-Cdc25C (H-6), anti-Cdk1 (C-19), and anti-pT14/Y15Cdk1 were from Santa Cruz Biotechnology. Anti-poly(ADP-ribose) polymerase was from BP Pharmicon. Antibody to β-actin was from Oncogene Research Products. Peroxidase-conjugated goat antibodies against rabbit or mouse IgG (MP Biomedicals) were used as secondary antibodies. The signals were detected using enhanced chemiluminescence reagents (Amersham Biosciences) and autoradiography films (Labscientific).

Immunostaining

Cells were grown on coverslips (Fisher Scientific). After fixation in 3% paraformaldehyde, cells were washed in PBS and permeabilized in a solution containing 0.5% Triton X-100, 3 mmol/L MgCl2, and 0.2 mmol/L sucrose. After washing in PBS, cells were blocked in 3% bovine serum albumin in PBS for 10 min before incubation with the same buffer but containing a 1:1,000 dilution of mouse anti-FLAG antibody (Upstate, Cell Signaling Solutions) for 1 h. The cells were then washed in PBS and subsequently incubated with 1:2,000 dilution of Alexa Fluor 545 goat anti-mouse IgG (Molecular Probes, Invitrogen Detection Technologies) in 3% bovine serum albumin in PBS for 1 h. DNA was stained with 4′,6-diamidino-2-phenylindole (Sigma) at a concentration of 5 mg/ml PBS. The cells were then washed in PBS. The coverslips were mounted on microscope slides (Fisher Scientific) covered with ImmuMount oil (Thermo Electron). The coverslips were sealed with transparent nail polish. The cells were observed using a fluorescence microscope (Nikon Eclipse E800).

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide and Apoptosis Assays

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were done according to the manufacturer’s instructions (Sigma). For detection of apoptotic cells, staining was done with Annexin V-FITC and then analyzed by flow cytometry according to the manufacturer’s protocol (BD Pharmingen).

Small Interfering RNA

Exponentially growing U2OS cells were transfected with SMARTpool reagent containing Cdc25C or Cdc25A small interfering RNA oligonucleotides or control oligonucleotides (Dharmacon, RNA Technologies) using DharmaFECT reagent 1 (Dharmacon) following manufacturer’s instruction.

Results

Overexpression of Cdc25C Sensitizes U2OS Tumor Cells to Doxorubicin-Induced Apoptosis

Phosphorylation of Cdc25C by Chk1 and its subsequent cytoplasmic sequestration or its down-regulation by p53
has been shown to contribute to the G2-M arrest caused by genotoxic stress (11, 12, 17). Thus, the ability of overexpression of Cdc25C to overcome this G2-M arrest was examined. U2OS cells were infected with either two different amounts of a recombinant adenovirus expressing Cdc25C (Ad-25C) 1 day before treatment with doxorubicin (0.2 µg/mL) or empty vector with the same multiplicity of infection as the highest level of Ad-25C (Fig. 1). At this time point, more than 95% of cells were infected as judged by microscopic evaluation of green fluorescent protein expression (data not shown). Uninfected U2OS cells were included as a control. The cell cycle profiles of these cells were examined 2 days after drug treatment by flow cytometry analysis (Fig. 1A and B). Control U2OS cells or those infected with empty adenovirus were able to arrest with a 4N DNA content consistent with a G2 arrest (Fig. 1A and B). U2OS cells overexpressing Cdc25C, however, were unable to maintain the G2 arrest and accumulated with a hypodiploid DNA content (Fig. 1A and B). Interestingly, loss of the G2 arrest was inversely proportional to the amount of the exogenous levels of Cdc25C (Fig. 1C). Levels of p53 and p21 induction in the presence of high levels of Cdc25C appear decreased (Fig. 1C), but it is unclear whether this is a direct effect or related to the use of adenoviral infection. Cells infected with these levels of Cdc25C were able to proliferate until confluency in the absence of drug treatment, indicating that these levels of Cdc25C do not disturb their normal proliferation (data not shown). Accumulation of cells with a hypodiploid DNA content is consistent with cell death by apoptosis. This was further confirmed by examining cleavage of poly(ADP-ribose) polymerase, a known target for apoptosis-associated caspase cleavage (Fig. 1C).

**U2OS Tumor Cells That Are Stably Overexpressing Cdc25C Are Sensitized to Doxorubicin-Induced Apoptosis**

Out of concern that some of the observed effects may be a consequence of adenoviral infection, stable U2OS cell...
lines overexpressing Cdc25C were established. Four control clones were established and shown to behave like the parental U2OS cells when treated with doxorubicin (data not shown). The results from one representative clone at the indicated time points are presented (Fig. 2). Consistent with results obtained from cells infected with Ad-25C, Cdc25C-overexpressing clones were unable to maintain an arrest at the G2-M phases and this occurred in a manner dependent on the levels of Cdc25C. The results of two representative clones expressing different amount of Cdc25C are shown (Fig. 2A and B). Twenty-four hours after drug treatment, only 1% to 2% of the control cells were found with a 2N DNA content representing the G1 phase. The majority of the cells were found to have a DNA content of 4N (Fig. 2A). At the same time point, higher levels of Cdc25C were associated with increases in the number of cells found in the G1 and S phases and consequently less in the G2-M phases (Fig. 2A). At later time points, in a manner that correlates with the level of exogenous Cdc25C, cells overexpressing Cdc25C were unable to maintain their G2 arrest and subsequently died by apoptosis as judged by the increased number of cells with a sub-G1 DNA content (Fig. 2A and B). Excess amount of Cdc25C did not affect other behaviors of these cells that were examined such as proliferation rate or morphology compared with the parental cells even after several passages (data not shown). This suggests that these levels of Cdc25C are tolerated by U2OS cells.

To examine the effects of Cdc25C overexpression on a single-cell basis, immunofluorescence analyses were done using clone 18, which expresses higher levels of Cdc25C (see Fig. 3A). In exponentially growing cells, exogenous Cdc25C was found to be both cytoplasmic and nuclear (Fig. 2C). p53 is a substrate for the ATM and ATR kinases characterized as playing a key role in cell cycle arrest by apoptosis as judged by the increased number of cells with a sub-G1 DNA content (Fig. 2A and B). Higher amount of Cdc25C did not affect other behaviors of these cells that were examined such as proliferation rate or morphology compared with the parental cells even after several passages (data not shown). This suggests that these levels of Cdc25C are tolerated by U2OS cells.

Figure 2. U2OS tumor cells that are stably overexpressing Cdc25C are sensitized to doxorubicin-induced apoptosis. A, U2OS cells stably transfected with a pcDNA3 vector or one containing a cDNA for Cdc25C were treated with 0.2 μg/mL doxorubicin. Cells were harvested at the indicated time points. Representative clone transfected with empty vector and two clones overexpressing different amount of Cdc25C. Cells were fixed and stained with propidium iodide before flow cytometry analyses. B, average cell numbers in different phases of the cell cycle at 4 d from three independent experiments. C, a control clone transfected with empty vector and a clone overexpressing Cdc25C were treated with 0.2 μg/mL doxorubicin. Cells were fixed at the indicated time points and immunostained with antibodies, which recognize either the FLAG epitope (red) or phosphorylated Ser15 on p53 (green). DNA was stained with 4',6-diamidino-2-phenylindole (blue). Arrows, examples of apoptotic cells.

1 Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).
Overexpression of Cdc25C in U2OS Tumor Cells Does Not Confer Sensitivity to Apoptosis on Treatment with 5-FU or Hydroxyurea

The role of Cdc25C overexpression in other cell cycle checkpoints was then examined. To investigate the effect of excess amount of Cdc25C on cell cycle arrest in G1-S or S phase of the cell cycle, Cdc25C-overexpressing U2OS clones or control cells were treated with either of two agents that are known to induce arrests at the G1-S-phase border (20). 5-FU and hydroxyurea are inhibitors of  

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Figure 3. U2OS cells that overexpress Cdc25C have reduced phosphorylation of Cdk1 on Tyr15 and impaired up-regulation of p21 in response to treatment with doxorubicin. A and B, a control clone transfected with empty vector and two clones overexpressing different amounts of Cdc25C were treated with 0.2 μg/mL doxorubicin for various indicated times. Cell lysates were prepared and used in immunoblot analyses with the indicated antibodies. β-Actin is a loading control. C, a control clone transfected with empty vector and two clones overexpressing different amounts of Cdc25C were treated with 400 μmol/L 5-FU. At the indicated time points, cells were harvested and used in flow cytometry analyses. Representative experiment. Columns, average cell numbers in different phases of the cell cycle at 2 d from three independent experiments. D, a control clone transfected with empty vector and two clones overexpressing different amounts of Cdc25C were treated with 1.5 mmol/L hydroxyurea. At the indicated time points, cells were harvested and used in flow cytometry analyses. Representative experiment.
pyrimidine synthesis or both purine and pyrimidine synthesis, respectively (20). Flow cytometry analyses of cell cycle profiles of U2OS cells treated with these drugs at the indicated time points are shown in Fig. 4C and D. Cell cycle profile of the control cells in response to 5-FU was consistent with both G1-S and G2 arrests (Fig. 4C). In these cells, ~50% of cells were found to have a DNA content of 4N by 2 days (Fig. 4C). Interestingly, consistent with Cdc25C abrogating the G2 arrest, high levels of Cdc25C caused a reduction in the number of cells with 4N DNA content and a corresponding increase in that of cells with 2N DNA content in a manner that correlated with Cdc25C level of expression (23% in clone 4 and 16% in clone 18; Fig. 4C). Under these conditions, the cells that escaped the G2 arrest did not die but became arrested in the G1 phase.

Figure 4. Sensitization of tumor cells to doxorubicin-induced apoptosis by overexpression of Cdc25C is not p53 dependent. The p53-null tumor cell lines HCT116-p53\(-/-\) (A) or H1299 (B) were infected with the indicated adenoviruses 1 d before drug treatments. Cells were incubated with either 0.2 μg/mL doxorubicin, 400 μmol/L 5-FU, or 1.5 mmol/L hydroxyurea for 2 d. Cells were then prepared for flow cytometry analyses. In each case, cells that were not infected with adenovirus were included as an additional control.
In response to hydroxyurea, the majority of the control cells accumulated with a S-phase DNA content at 2 days following drug treatment (Fig. 4D). This was more predominant in Cdc25C-overexpressing clones in a manner dependent on the levels of exogenous Cdc25C (Fig. 4D). These results show that excess amounts of Cdc25C only override the G2 arrest caused by doxorubicin and 5-FU and have no effect on the G1-S arrest caused by treatment with 5-FU and hydroxyurea. Further, there is no evidence of apoptosis in the Cdc25C-expressing cells in response to either of these agents.

**Sensitization of Tumor Cells to Doxorubicin-Induced Apoptosis by Overexpression of Cdc25C is Not p53 Dependent**

U2OS cells are osteosarcoma cells that express wild-type p53. To determine the role of p53 status in the ability of overexpression of Cdc25C to overcome the G2 arrest, two cell lines, H1299 (lung carcinoma) and HCT116-p53-/− (colon carcinoma), which lack p53 expression, were tested. Both cell lines were treated with similar protocols. Infection of these cells with Ad-25C did not influence cell cycle profiles of these cells as observed by flow cytometry analyses (Fig. 4A and B). The expression from Ad-25C was confirmed by microscopic analyses of expression of green fluorescent protein (data not shown). Infection with Ad-25C, however, abolished the G2 arrest in both cell lines (Fig. 4A and B). As observed in U2OS cells, elevated levels of Cdc25C did not affect the G1-S-phase arrest caused by 5-FU or hydroxyurea (Fig. 4A and B). Thus, excess amounts of Cdc25C are capable of abolishing G2 arrest in cell lines from different origins and regardless of p53 status.

**Nontransformed MCF10A Cells Are Not Sensitized to Doxorubicin-Induced Apoptosis by Overexpression of Cdc25C**

MCF10A cells are a nontransformed breast epithelial line. These cells were infected with Ad-25C to investigate the effect of overexpression of Cdc25C on cells that were not of tumorigenic origin. Ad-25C infection did not affect the normal cell cycle profile of these cells (Fig. 5A). This same amount of Ad-25C, however, was capable of overcoming the accumulation of cells with a 4N DNA content caused by treatment with doxorubicin showing an increase in cells in the G1 phase and a corresponding decrease in cells with 4N DNA content (Fig. 5A). Notably, these cells showed no evidence of an apoptotic response. These cells were then examined before and after doxorubicin treatment using the MTT assay. Viability of cells infected with either empty adenovirus or Ad-25C for 4 days did not differ significantly from that of control cells (Fig. 5B). Similarly, reduction in cell growth following 3-day doxorubicin treatment was comparable in cells infected with empty adenovirus or Ad-25C and the controls (Fig. 5B). These results suggest that elevated amounts of Cdc25C do not impose a negative effect on viability of MCF10A cells during this time course. Further, these data suggest that MCF10A cells arrest in G2-S rather than G2-M on expression of excess Cdc25C. The Annexin V-PE staining assay was then used as a more sensitive means of detecting apoptotic cells to see whether overexpression of Cdc25C affects death in MCF10A cells. Control and Ad-25C-infected cells were treated with 0.05 μg/mL doxorubicin and harvested at the same time points as described for the MTT assays. Percentages of apoptotic Ad-25C-infected cells at 3 days post-treatment with doxorubicin were comparable with that found in control cells (Fig. 5C and D). This further confirms that overexpression of Cdc25C does not cause enhanced toxicity in nontransformed MCF10A cells. Treatment of either the controls or cells infected with empty adenovirus with 5-FU or hydroxyurea caused a G1-S accumulation that was not affected on infection with Ad-25C (Fig. 5A). Thus, the ability of high levels of Cdc25C to sensitize cells to apoptosis correlates with the transformation phenotype of the cells being examined. This suggests that overexpression of Cdc25C may selectively enhance doxorubicin-induced cell death in tumor cells rather than in nontransformed cells.

**Lack of Cdc25C Does Not Affect Cell Cycle Progression or the G2 Arrest Caused by Doxorubicin**

Mice lacking Cdc25C develop normally and their cell cycle checkpoints appear to be intact (21, 22). To address the effects of loss of Cdc25C expression, Cdc25C was eliminated by the means of a small interfering RNA approach in U2OS cells. Manipulation of the related Cdc25A was included as a control. As shown in Fig. 6A, the majority of Cdc25C and Cdc25A were successfully down-regulated. As judged from flow cytometry analysis, lack of Cdc25A resulted in accumulation of cells with a 4N DNA content with a concomitant decrease of cells with 2N DNA content (Fig. 6B). Lack of Cdc25C, however, did not affect cell cycle progression during the course of study (5 days; Fig. 6B). The phosphorylation status of Cdk1 on Thr14 and Tyr15 by immunoblotting was then examined. Consistent with the flow cytometry analysis, absence of Cdc25C did not affect the levels of phosphorylation on these residues in comparison with control cells (Fig. 6A). Lack of Cdc25A, on the other hand, led to an increase in the phosphorylated form of Cdk1 on these residues (Fig. 6A). Next, the effect of absence of Cdc25C on the response of U2OS cells to doxorubicin was examined. The number of cells accumulated with a 4N DNA content was almost the same as in control cells (Fig. 6C).

**Discussion**

Evidence provided in this study supports overexpression of Cdc25C as a bona fide abrogator of the G2 checkpoint. It is noteworthy that there is little effect of high levels of Cdc25C on the G1-S and S-phase checkpoints (4). Here, it is shown that excess amounts of Cdc25C abrogates the G2 arrest caused by two common chemotherapeutic agents, doxorubicin and 5-FU, whereas it does not affect the G1-S- and S-phase arrests caused by 5-FU and hydroxyurea in several cell lines. Interestingly, these observations were made in cell lines derived from different tissues. This suggests that Cdc25C may have utility to abrogate the...
G2 arrest in cancer cells from various origins. Of great interest is the observation that the same amount of Cdc25C that is capable of overcoming the G2 arrest does not affect the normal cell cycle progression of the examined cell lines including one that is nontumorigenic. To date, there is no reported evidence of overexpression of Cdc25C in human tumors in contrast to the other two related phosphatases, Cdc25A and Cdc25B (23). Furthermore, as observed here, Cdc25C needs to be overexpressed at considerably high levels (when endogenous Cdc25C is not detectable) for it to overcome the G2 arrest. This strongly suggests minimal toxicity caused by overexpression of Cdc25C even in a nontumorigenic line such as MCF10A. Importantly, Cdc25C did not sensitize this latter nontumorigenic cell line to doxorubicin-induced cell death. In this case, cells failed to arrest in G2-M at the examined time points, further confirming the role of Cdc25C in the G2 checkpoint. This is a desirable result as proliferation of cells harboring DNA damage following chemotherapy is an unwanted outcome. Loss of a G2 arrest has been reported to lead to a phenomenon called mitotic catastrophe, which results in formation of large micronucleated cells with decondensed chromatin (24). Examination of MCF10A cells overexpressing Cdc25C and treated with doxorubicin revealed no morphologic changes consistent with mitotic catastrophe. This, however, does not exclude death as a result of apoptosis or mitotic catastrophe in other types of nontransformed cells when exposed to elevated levels of Cdc25C and doxorubicin.

In this study, lack of Cdc25C did not affect cell cycle progression of U2OS cells or their response to doxorubicin. Furthermore, mice lacking Cdc25C develop normally and

Figure 5. Nontransformed MCF10A cells are not sensitized to doxorubicin-induced apoptosis by overexpression of Cdc25C. A, nontransformed breast epithelial MCF10A cells were infected with the indicated adenoviruses 1 d before drug treatments. Cells were incubated with either 0.05 μg/mL doxorubicin, 300 μmol/L 5-FU, or 1 mmol/L hydroxyurea for 2 d. Cells were then prepared for flow cytometry analyses. Uninfected cells were included as an additional control. B and C, MCF10A cells were infected with either empty adenovirus or Ad-25C 1 d after plating. One day later, one set of cells was treated with doxorubicin (0.05 μg/mL). Three days later, viability and apoptosis of all cells were measured by MTT (B) and Annexin V-PE (C) staining assays, respectively.
the integrity of the DNA damage checkpoints of their embryonic fibroblasts remains intact (21, 22). Based on these observations and a lack of alteration of Cdc25C in many human tumors, it is reasonable to postulate that Cdc25C is unlikely to be involved in any other cellular processes and, if so, it is likely to be a redundant player.

Two major obstacles encountered with many chemotherapeutic agents include their adverse side effects and the resistance developed against them. In addition, they often need to be used in combination for a positive therapeutic outcome. Often, however, this further increases undesirable side effects. Thus, it is expected that a combination of high levels of Cdc25C and such drugs would provide the opportunity for reducing the level of drug actually used and hence toxicity. These results support the idea that a combination of overexpressing Cdc25C with treatment with conventional genotoxic agents should be given serious considerations as a novel therapeutic strategy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
We thank Drs. Stuart Aaronson, Zhen-Qiang Pan, and Lois Resnick-Silverman for advice.
References

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Overexpression of the dual specificity phosphatase, Cdc25C, confers sensitivity on tumor cells to doxorubicin-induced cell death

Shohreh Varmeh and James J. Manfredi


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